

Scotland's Rural College

How do housing structures in free-range production affect laying hen welfare?

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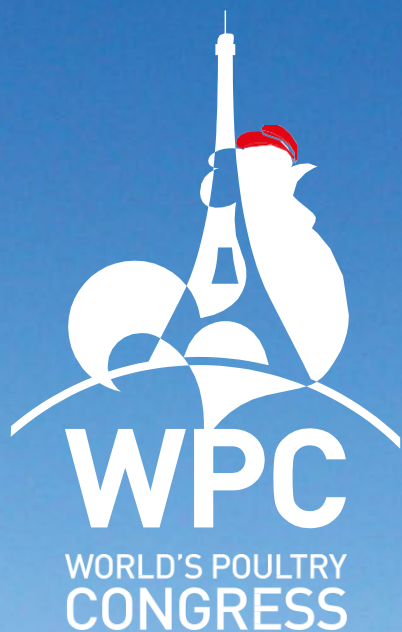
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26th World's Poultry Congress Book of abstracts 2021



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Abstracts submitted in 2019 and accepted in 2020

26th World's Poultry Congress

Organized by

French Branch of the World's Poultry Science Association

The 26th World's Poultry Congress - 07-11 August 2022

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Editors

Dr Michèle TIXIER-BOICHARD, chair of 26th WPC

Dr Michel DUCLOS, Chairman of the Scientific Committee of 26th WPC

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Dear participants to the 26th WPC, dear readers,

In the context of the COVID-19 pandemics, which emerged in 2020 and is still present, the highest priority of the French Branch of WPSA and the organizing committee of the 26th World's Poultry Congress has been to organise a successful congress, avoiding health hazards and welcoming participants from all continents. As a consequence, the event planned in 2020 had to be postponed to 2021 and again to 2022.

Nevertheless, the Organising Committee and the board of WPSA decided to celebrate the centenary of WPC with an on-line event on August 10, 2021 opened to all WPSA members. This event can be viewed as replay on the WPSA website.

Furthermore, it was also decided to value the abstracts received in 2019 and accepted in 2020, by organising monthly webinars from September 2021 to June 2022, to bridge the gap between the centenary of the WPC and the 26th WPC in August 2022. These abstracts are presented in the following 'Centenary book', published as an online document by the WPSA.



Let's celebrate!



Dr Michèle TIXIER-BOICHARD, chair of 26th WPC

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The organizing committee will publish regular updates on <https://wpcparis2022.com>

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OBJECT ORIENTED SESSIONS

ADAPTING POULTRY PRODUCTION TO CLIMATE CHANGE

ID : 226

DETERMINATION OF THE HEAT SHOCK RELATED FACTOR EXPRESSION PATTERN AFTER HEAT TREATMENT OF TWO-DAY-OLD CHICKEN

R. Toth*(1), N.Tokodyne szabadi(1), B.Lazar(1), K.Liptoi(3), A.Korosine molnar(2), E.Patakiné varkonyi(4), E.Gocza(1)

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Nowadays, the drastically increased average temperature has a significant environmental effect on agriculture and animal husbandry. The high temperature is a negative stressor for the animals. In this case, the milk-, meat- and egg production of the animals dramatically decrease. HSP superfamily members are the essential genes in the stress response. Heat stress induces the increase of the expression level of heat stress-related factors (HSFs and HSPs).

In our research, we wanted to develop a new method that helps in the adaptation to the higher temperature at adult age in poultry. It is known if the birds are keeping on the first few days after hatching in higher temperatures than the optimal, they develop a higher tolerance to the increased environmental temperature. It was published that HSP70 family members were highly expressed in the brain, gonads after heat stress.

Transylvanian necked neck chickens were used at our experiment. We incubated the two days old chicks at 38.5o C for 12 hours. We collected the samples (brain, liver, gonads, and muscle) in RNA later immediately after the heat-treatment and on the 14th week after hatching. We used as a control group the non-heat treated two days old Transylvanian necked neck chickens.

We isolated RNA from the collected samples. As the first step, we diluted the isolated RNA samples to 100 ng/μl concentration. As the next step, we pooled these diluted RNA samples belonging to the same group (according to the treatment and organs). We determined the expression level of heat-shock related factors using real-time PCR. We used GAPDH as a housekeeping gene and chicken embryonic fibroblast (CEF) as the plate control sample.

We found that in heat-treated female chickens, the expression level of all examined factors (HSP70 HSP90, HSF1, HSF2, HSF3, HSF4) decreased in gonads at adult age compared to the control group. In the muscle, we found elevated expression levels at all of the heat shock factors immediately after heat treatment compared to control, but these differences disappeared at adult age. In the case of the brain, we determine increased expression in the HSP70 level only at a matured age.

We think that an optimal heat treatment method will help to develop tolerance to heat stress that occurs in the hot summer.

ID : 482

HOW CHRONIC HEAT STRESS AFFECTS IN VIVO AND POST-MORTEM OXIDATIVE PARAMETERS OF BROILER CHICKENS?

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Heat stress is one of the major environmental stressors in intensive poultry production since it can adversely affect behaviour, immune response and productivity including meat quality. The objective of the present research was to evaluate the effect of chronic heat stress on oxidative status of broiler chickens, comparing the in vivo response (blood parameters) to the meat oxidation. A total of 300 1-d-old male chicks (Ross 308) were divided in two experimental groups (6 replicates/group of 25 chickens/each): Thermoneutral (TN) raised under conventional environmental conditions for the whole rearing cycle and Heat Stress (HS) subjected to chronic heat stress conditions (30°C for 24 h/d) from 35 to 41 d. Chickens were fed the same standard diet and the water was supplied ad libitum. At slaughtering (41 d), blood was collected (n=12 animals/group) and plasma/serum obtained by centrifugation. Twelve breasts were also collected for each experimental group and stored for oxidative parameters (tocopherols, retinol, carbonyls, TBARS) and fatty acids profile evaluations. A linear model was applied and the temperature was considered as the fixed effect. T-test was used for groups comparison ($P<0.05$). Results showed that HS chickens had slightly lower plasma antioxidants (mainly α -tocopherol) and worse in vivo oxidative status (5.40 vs. 1.77 nmol/mg proteins and 31.38 vs. 28.50 nmol MDA/mL, for carbonyls and TBARS respectively; $P<0.05$) than TN ones. On the contrary, the oxidative profile of meat was not affected by the higher temperature. Similarly, the fatty acids profile of meat did not change between groups, whereas the concentration of MUFA (mainly oleic acid, C18:1n-9), showed a lower proportion in HS serum than TN (12.7 vs. 15.6 %; $P<0.01$). As for meat oxidation, TN breasts exhibited significantly higher carbonyl content (1.77 vs. 1.53 nmol/mg of protein; $P<0.05$) than HS group, coupled with a lower amount of TBARS (4.70 vs. 5.23 mg MDA/kg of meat; $P<0.001$). Although apparently contrasting, these results might be explained by considering that, aside from MDA, lipid oxidation can result in different reaction-products not quantifiable with this method. In conclusion, the chronic heat stress conditions tested in this study did not negatively affect the oxidative status of meat although slightly influenced the in vivo one. Thus, other insights are need to clearly define the oxidative mechanisms elicited by heat stress conditions in fast-growing broilers.

ID : 491

FUNCTIONAL AMINO ACIDS ARE EFFICIENT TO RESTORE PERFORMANCE AND PHYSIOLOGICAL TRAITS OF BROILERS SUBJECT TO A HEAT-STRESS CHALLENGE

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Heat stress (HS) is associated with decreased performance and livability of chicken broilers. Published literature provides evidence that arginine or glutamine supplementation could be useful in birds facing this challenge. In addition, branched-chain and sulfur amino acids support immunity and oxidative stress defense, parameters which are impaired in this context. The current study aimed to compare the efficiency of these different amino acids to restore performance and other physiological traits in HS broilers.

Seven hundred sixty-eight newly hatched male chicks (Ross 308) were fed common starter and grower diets until 21 days of age. After this period, they were allocated for another 21 day-period to 6 treatments. Basal diets were formulated following Ajinomoto recommendations. For all except one treatment (CTRL), chicken broilers faced a HS (average temperature: 31.06°C, average humidity: 77.27%). Among HS birds, one treatment was not supplemented (CTRL-HS) while the others received one of the following supplementations: 0.5% arginine (ARG), 0.25% glutamine (GLN), 1.0% branched-chain amino acids (BCAA) or 0.25% cystine (CYS2). Performance and livability of animals were followed. The effect of HS on gene expression in jejunum was assessed using an Affymetrix® Chicken Gene 1.1 ST Array Strip. At the end of the trial, jejunum morphology and plasmatic oxidative stress markers were measured. All data were subjected to an ANOVA.

HS decreased body weight gain (-11.7%), feed intake (-5.0%), villous height in jejunum (-20.7%) and increased feed conversion ratio (+7.0%) with no effect on plasmatic oxidative stress markers or livability. Transcriptomic analysis revealed that adaptation to HS was associated with a process similar to tissue healing in the jejunum. The supplementation of ARG, GLN, BCAA significantly improved performance of HS broilers from d21 to d42, ARG being the most efficient amino acid. During this same period, CYS2 increased numerically performance. No effect of the supplementations was noticed on plasmatic oxidative stress markers or livability but the supplementation of CYS2 increased villus height in jejunum.

This study showed that amino acids are capable of partially restoring performance in HS broilers when supplemented over the requirement for growth. While the effect of ARG, GLN AND BCAA could rely on their ability to support tissue healing, CYS2 could be beneficial via the improvement of gut morphology.

ID : 504

ASSESSING THE SUSTAINABILITY OF SLOW GROWN BROILERS

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We conducted a study to evaluate the sustainability of slow-growing broiler chickens. The same poultry company produces slow-growing and conventional broilers. The management practices used in the production of these birds were the same. We estimated the greenhouse gas emissions from all the farms in the complex using the Poultry Carbon Footprint Calculation Tool (PCFCT). This spreadsheet was designed to calculate emissions from broiler grow-out, pullet and breeder farms. We calculated the emissions from information we collected from each farm. This information included the farm data (number and size of houses, type of birds, number of birds, number of flocks grown each year and the number of days it took to grow each flock), the activity data and the manure management. The activity data consisted of information we collected from farm records of: electricity use, fuel use (diesel or liquid petroleum gas) from the year 2018. This information was from all broiler farms in a broiler complex. There was a total of 571 regular broiler houses and 952 slow-growing houses. The activity data was then used to calculate the emissions from mechanical sources (heaters, etc.), non-mechanical sources (manure, etc.), and electricity use.

More square footage was required to raise the slow-growing birds, leading to an additional 381 houses. An additional 17.2 days was required for slow-growing broilers to reach an average market weight of 7.5lbs. The company placed more slow-growing birds during the period than conventional birds, with 3.07 million more broilers placed in slow-growing houses. The livability for both flocks was 96%. Slow-growing broilers had 25% more total greenhouse gas emissions than conventionally grown broilers.

ID : 727

EFFECTS OF SEQUENTIAL FEEDING ON PERFORMANCES OF BROILERS IN HOT AND HUMID CLIMATE

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Fast-growing broilers are still recording poor growth performance in tropical conditions. Thus, this experiment was conducted to evaluate the suitable feeding program in order to improve their performances in tropics. A total of 770 10-day-old Cobb500 broilers were assigned to 7 treatment groups having 5 replicates of 22 birds each. Group CONT (control) fed complete diet with constant energy (Estandar) and constant protein (Pstandar) levels. Sequential groups were fed two types of diet alternated in morning and afternoon: group B fed with Estandar and low protein level (Plow) diet and thereafter with Estandar and high protein level (Phigh), group C fed diet with Pstandar and low energy level (Elow) diet and subsequently with Pstandar and high energy level (Ehigh), group D received Elow Plow diet and thereafter with Ehigh Phigh diet; group E, F and G received diet with Elow Phigh and subsequently with Ehigh Plow respectively in 24H, 12H and 48H cycles. Feed was provided two times daily: 6.30 AM and 6.00 PM in all groups except group F which was served diets 7.30 AM and 1.00 PM. Broiler chickens were conducted during 5 weeks. Temperature and relative humidity were recorded daily. At 45 days of age, a sample of 6 chickens per replicate were slaughtered for carcass evaluation, ultimate pH (pHu) and water holding capacity (WHC) of meat. Results showed that temperature and relative humidity in the morning were significantly ($p < 0.05$) lower than that in evening. Feed intake in morning was significantly ($p < 0.05$) across the treatment groups. However, body weight and WHC were significantly improved and abdominal fat significantly reduced ($p < 0.05$) in group C birds. It was concluded that alternate low energy and high energy with constant protein level diet in broilers could improve their growth performance under tropical conditions.

Keywords: Sequential feeding, broilers, meat quality, tropical climate.

ID : 815

CYCLIC HEAT STRESS IN COMBINATION WITH A CHALLENGING DIET REDUCES BROILER PERFORMANCE AND MODIFIES ANIMAL PHYSIOLOGY

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Heat stress (HS) is a major concern in poultry production. Cyclic HS (cHS) impairs performance, welfare and behavior, increases risks of leaky gut, oxidative stress (OS) and inflammation. This study aimed to evaluate the effects of cHS in combination with a challenging diet on broilers performance and welfare parameters. A total of 2,112 day-old Ross 308 male broilers were distributed in a randomized block design with 2 temperature conditions x 2 diets (24 pen replicates per treatment combination). Temperature conditions were thermoneutrality (TN) or cHS from d 22 to d 42. TN was set at 22°C with relative humidity (RH) of 60-75 %. cHS was set at 35°C for 10 h and 28°C for 14 h with RH of 50-60 %. Diets D1 and D2 were iso-metabolizable energy and iso-digestible amino acids and fed from d 0. The challenging diet (D2) was higher than the control diet (D1) in soluble fiber (0-21 d: +96%; 22-42 d: +112 %) and total protein (0-21 d: +11 %; 22-42 d: +10 %). Results were analyzed by ANOVA and linear regression. At d 21, body weight (BW) and feed intake (FI) were reduced by D2, whereas feed conversion ratio (FCR) was increased ($p < 0.05$). From 21 to 42 d, cHS vs TN and D2 vs D1 increased FCR by 10.2 % and 4.6 % respectively ($p < 0.05$). Final BWs were 3,539 g, 2,992 g, 3,190 g, and 2,851 g for TND1, cHSD1, TND2 and cHSD2, respectively, and were significantly different for main effects and the interaction. Broilers fed D2 had a lower cHS-induced reduction of BW than broilers fed D1 ($p = 0.40$). At 22 d, cHS significantly increased rectal temperature (RT) by 0.99°C ($n = 12$). Later, at d 29, 36 and 42, RT remained higher in cHS group with +0.65°C on average ($p < 0.05$), showing an adaptation of broilers subjected to cHS after d 22. Accordingly, HS increased blood glucose (6.9 %) and pH (0.94 %) at d 22 ($p < 0.05$) but at d 36 measurements of glucose stress response and blood alkalosis were not significant ($n = 6$). More biomarkers (blood gases, hematology, OS and inflammation) will be analyzed and presented at the congress. In conclusion, the cHS model reduced broiler performance and modified their growth and thermoregulatory response. However, the challenging diet have alleviated broiler sensitivity to HS, by lowering BW gain and FI, a result that will have to be compared with welfare parameters.

ID : 856

CENTRAL TAURINE CONTRIBUTES TO THERMOREGULATION AND FOOD INTAKE IN CHICKS

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Our recent findings reported that intracerebroventricular injection of neuropeptide Y (NPY) increased the plasma taurine concentration and lowered the body temperature in chicks (Eltahan et al., 2017). Moreover, our group previously reported that developing embryonic brain concentrations of taurine and gamma-amino butyric acid (GABA) were higher than those in the muscle (Sato et al., 2009). The aim of this study was to examine the central effects of taurine on body temperature and food intake in neonatal chicks and to investigate the central regulatory factors of taurine-dependent thermoregulation and food intake. In Experiment 1, 6-days-old Julia layer chicks (n = 12) were intracerebroventricularly injected with different doses (0, 1.25, 2.5 and 5 $\mu\text{mol}/10\ \mu\text{l}$) of taurine under control thermoneutral temperature (CT). In Experiment 2, 6-days-old Julia layer chicks (n = 12) were intracerebroventricularly injected with 0 or 5 μmol of taurine and exposed to either a high ambient temperature (HT: $35 \pm 1^\circ\text{C}$ for 60 min) or a CT ($30 \pm 1^\circ\text{C}$ for 60 min) in temperature-controlled chambers. Rectal temperature was recorded at 0, 30 and 60 min after the treatment and food intake was recorded at 0 and 60 min. Chicks were properly anesthetized by isoflurane before collection of the blood and brain (diencephalon). Experimental data were subjected to a Thompson rejection test to eliminate outliers and the remaining data were used for statistical analysis by the Stat View version 5.0. The results showed that central taurine induced dose dependent hypothermia and reduced food intake under CT. In contrast, central taurine attenuated food intake reduction and induced hyperthermia under HT. It was further found that central taurine significantly decreased the 3-methoxy-4-hydroxyphenylglycol in the brain which is a major metabolite of norepinephrine (NE), while the brain serotonin, dopamine, epinephrine, 3,4-hydroxyindoleacetic acid and homovanillic acid were not changed by the central taurine. Moreover, plasma glucose, uric acid and glutamic oxaloacetic transaminase as well as the diencephalic mRNA expressions of NPY, GABAA and GABAB receptors were not significantly changed in taurine injected chicks. In conclusion, central taurine significantly decreased the body temperature and food intake of chicks under CT, which might be linked with the central NE metabolism. In addition, brain taurine may work against food intake declining under heat stress and contribute to hyperthermia.

ID : 916

EFFECT OF A COATED DIETARY BETAINES AND ANTIOXIDANTS SUPPLEMENTATION PRODUCT ON GROWTH PERFORMANCE, MEAT YIELD AND QUALITY OF BROILERS UNDER WARM CONDITIONS

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High environmental temperature can decrease growth performance of broilers by reducing feed intake and breast meat yield. Meat quality can also be impaired as a result of oxidative stress. Dietary betaine may reduce such negative effects thanks to its osmoprotective properties. In addition, antioxidants may protect against oxidative stress. This trial compared under warm conditions the effect of supplementing broiler diets with free betaine or with a coated blend of active ingredients (Metalixir S; vectorization technology). The trial took place at IRTA in Spain. A total of 828 days-old Ross 308 male chicks were randomly allocated to three dietary treatments, each replicated 16 times. Dietary treatments were: Negative control (NC, no additive), free betaine (FB, NC + 1 kg/t of anhydrous betaine), Metalixir S (MS, NC + 750 g/t of Metalixir S). From hatching till 21 d, housing temperature was set according to standard practices. From 21 d to 40 d, housing temperature varied every day to simulate high summer temperatures (08:00-11:00: 28°C, 11:00-18:00: 30-32°C, 18:00-8:00: 24°C). Growth performance was measured throughout the experiment. At 40 d, 18 birds per treatment group were sacrificed. Breast meat yield, footpad dermatitis and meat quality were measured. Throughout the period, there was a significant improvement of bodyweight gain (+2.6 g/d; $P < 0.05$) and FCR (-4 pt; $P < 0.05$) for birds fed MS compared to NC and FB. There was no significant difference in growth performance for birds fed FB compared to NC. Both MS and FB increased numerically breast meat yield compared to NC (+0.3 pt). Birds fed MS tended to have lower white stripping scores than birds fed NC and FB diets ($P < 0.1$). No difference was found among treatment groups concerning footpad dermatitis. This trial suggests an additional effect of coated dietary betaine and antioxidants (MS) on the growth performance and meat quality of broilers compared to betaine (FB) alone.

ID : 937

ROLE OF NATURAL VITAMIN C ON GROWTH PERFORMANCE, ANTIOXIDANT DEFENSE AND IMMUNE RESPONSES IN BROILERS UNDER HEAT STRESS

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Introduction

Vitamin C participates in important biological processes & preventing oxidation. Because poultry can synthesize Vitamin C, it is not considered as required dietary nutrient for poultry. However, when chicken is subjected to environmental stressors, Vitamin C supplementation is necessary to satisfy growth requirements. C Power (natural supplement containing Vitamin C equivalent) exhibits high antioxidant activity. Thus, objective of study was to evaluate comparative effects of Vitamin C & C Power on growth performance, antioxidant defense & immunity in heat stressed broilers.

Experimental design

A total of 480 1d old Cobb500 broiler chicks were randomly allocated to 4 treatments (6 repx20 birds/pen); Control T1: were given corn/soybean meal diet without vitamin C; T2@200g/ton Vitamin C (35%); T3@100g/ton C Power; and T4@200g/ton C Power. Birds were given exposure under heat-stress for 8h per day for 42d, body rectal temperature was recorded as stress indicator using thermocouple rectal before & during heat exposure. Body weight (BW), body weight gain (BWG), feed intake (FI), & feed conversion ratio (FCR) were measured weekly. At 5wk, liver samples were collected for gene expression of pro-inflammatory cytokines, heat stress apoptosis markers & antioxidant properties. At 6wk, 4 birds per pen were randomly selected to measure body composition using DEXA. Experimental data were analyzed statistically by one-way ANOVA using SAS software & differences between means were determined using Duncan's Multiple Range test ($P \leq 0.05$).

Results

In starter phase, Vitamin C (T2) & low concentration of C Power (T3) exhibited a higher BW at end of 1 wk ($P=0.011$) & 2wk ($P=0.006$) when compared to control group (T1). T2 showed higher BWG ($P=0.014$) & FI($P=0.026$) than T1 at 1wk, whereas there was no difference between T2&T3. T3 improved FCR ($P=0.043$) at 2wk compared to other groups. At end of 6 wk, BW was significantly higher (2650.7g) in T4 than Control (2579.1g) and there was no significant difference between T2&T3. FCR was significantly better in T3 & T4 than T1 & T2. At 4 wk, body temperature of T2&T3 was significantly reduced than T1. No differences in body composition & gene expression among treatments were recorded.

Conclusion

It is demonstrated that under heat stress, broiler growth performance was improved by Vitamin C or C Power. C Power has the potential to benefit poultry production under heat-stressed environment.

ID : 938

PROTECTIVE EFFECTS OF A NEW PHYTOGENIC FEED ADDITIVE ON GROWTH PERFORMANCE VIA MODULATION OF HYPOTHALAMIC FEEDING- AND DRINKING-RELATED NEUROPEPTIDES IN CYCLIC HEAT-STRESSED BROILERS

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Identification of alternatives to antibiotics in livestock and poultry is necessary. Fueled by consumer preferences, phytogenic feed additives are increasingly used in the food system, however their mode of action is not well defined.

Here, we used broiler chickens to determine the effect of the phytogenic feed additive "Comfort" (PFA-C) as well as its underlying molecular mechanisms on growth performance under chronic cyclic heat stress (HS). Three week-old male Cobb500 broilers (n=600) were maintained in environmentally controlled chambers, fed three diets (control or PFA-C at 250 and 400 ppm), and subjected to two environmental conditions (12h/d cyclic heat stress, HS, 35°C vs. thermoneutral condition, TN, 24°C) for 3 weeks in a 3 × 2 factorial design. Feed and water intakes were recorded daily and body weights were measured weekly. Core body temperature was recorded continuously using thermologgers. At the end of the experiment, blood was collected to measure gas, hematology, electrolytes, and circulating metabolites using iSTAT portable system. Hypothalamic samples were harvested for target gene expression analyses. HS significantly increased birds' core body temperature, water intake, and the hypothalamic expression of HSP70, while it decreased feed intake, body weight, and woody breast (WB) incidence. PFA-C supplementation down regulated the hypothalamic expression of HSP70, reduced the core body temperature, increased feed and water intake, and improved body weight in heat-stressed broilers. At molecular levels, the effect of PFA-C on growth performance seemed to be mediated via modulation of hypothalamic expression of melanocortin receptor 2 (MC2R), vasopressin (AVP), aquaporin 2 (AQP2) and ATPase Na⁺/K⁺ transporting subunit beta 1 (ATP1B1) polypeptides. In summary, PFA-C supplementation ameliorates heat stress productivity losses via a potential cytoprotective effect, reduction of hypothalamic intracellular stress, and modulation of hypothalamic feeding- and drinking-related polypeptide expression.

ID : 940

HEAT LOAD UPREGULATES THE EXPRESSION OF INTESTINAL HEAT SHOCK PROTEINS AND ALTERS TIGHT JUNCTIONS AND MITOCHONDRIAL DYNAMICS IN BROILER CHICKENS.

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Unusually warm and longer season temperatures have increased markedly over the past years and predicted to be even larger during the next decade. Global warming and heat stress (HS) are already affecting animals, insects and crops. In broilers, that play a key role in worldwide meat production, HS alters gut health, however the underlying molecular mechanisms are not well defined. The present study aimed to determine the effects of heat stress on gut integrity and to define the fundamental signaling pathways. One-day old Cobb 500 broiler chicks were randomly allocated into 12 environmental chambers (2 pens/chamber and 20 birds/pen) and reared under standard conditions. The rearing temperature was gradually decreased from 32°C for days 1 to 3, 31°C for days 4 to 6, 29°C for days 7 to 10, 26°C for days 11 to 14, and 24°C thereafter. On day 28, the ambient temperature was increased within 10 minutes to 35°C in half of chambers to induce HS, with the remaining chambers maintained at 24°C. Two hours after the onset of HS, birds were euthanized by cervical dislocation and intestinal segments were collected, snap frozen in liquid nitrogen and stored at -80°C for molecular analyses. The porcine jejunal (IPEC-J2) cells were cultured and exposed to HS (45°C) for 2 hours. Control cells were maintained at 37°C. Leaky gut syndrome was assessed by FITC-d method. The expression of target genes and proteins were measured by real-time qPCR and immunoblot, respectively. The tight junction integrity and structure were assessed by TEER and immunofluorescence staining. HS significantly increased core body temperature and circulating FITC-d levels in broilers and reduced TEER and increased FITC-d levels in the basolateral chamber in IPEC-J2 cells, indicating a leaky gut. HS upregulated the expression of HSP60/70/90 in both in vivo and in vitro studies. Biochemical analysis and immunofluorescence staining showed that HS altered the tight junction protein expression and structure confirming a leaky gut syndrome. Electron microscopy, showed elongated mitochondria under HS condition which suggest an enhanced fusion and absence of fission. These data has been confirmed by an upregulation of MFN1 gene. Together, this is the first evidence showing that heat load induced intestinal stress and alters mitochondrial dynamics and gut integrity resulting in leaky gut syndrome.

ID : 1079

ASSESSMENT OF AFLATOXINS CONTAMINATION IN POULTRY FEED AND FEEDSTUFFS IN THE THREE REGIONS OF MYANMAR

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Aflatoxins contamination in poultry feed and feedstuffs are a widespread occurrence in countries with high temperature and humidity. Since Myanmar has hot and humid climate condition, this study was conducted to investigate the prevalence and level of aflatoxins contamination in poultry feed and feedstuffs by the ELISA technique (Romer Labs®). Sampling was done from poultry farms, poultry feed shops and feed mills from Yangon, Mandalay and Nay Pyi Taw regions. Totally 135 samples, each of 15 samples of corn, groundnut cake and complete feed, were collected from each region. The data obtained were analyzed by Chi Square test and $p < 0.05$ is considered as significant. Based on types of feed, prevalence of aflatoxins contamination in groundnut cake (100%) and complete feed (95.56%) was significantly higher than ($p < 0.05$) in prevalence of aflatoxins contamination among Nay Pyi Taw (93.33%), Yangon (80%) and Mandalay (75.56%). The mean aflatoxins level in groundnut cake ($148.25 \mu\text{g/kg}$) were significantly higher ($p < 0.001$) than those of corn ($36.25 \mu\text{g/kg}$) and complete feed ($31.21 \mu\text{g/kg}$) in detected regions. The prevalence of aflatoxins contamination with exceeding level of ASEAN regulatory limit based on types of feed were (35.56%, 35.56% and 88.89%) in corn, complete feed and groundnut cake, respectively. Based on regions, it was highest in Nay Pyi Taw (64.44%), followed by Mandalay (51.11%) and Yangon (44.44%). By categorization of aflatoxins level into low, medium and high, (71.11%) of groundnut cake was contaminated at high level whereas (64.44%) of corn and complete feed were contaminated at low level. Highest prevalence, highest mean level and highest percentage with exceeding ASEAN regulatory limit of aflatoxins were occurred in groundnut cake samples in comparison with corn and complete feed samples in the detected regions of Myanmar. Overall, this study indicates that aflatoxins contamination in most of groundnut cake samples from the three regions of Myanmar were higher than ASEAN regulatory limit and still need to maintain quality of the feedstuff and finished feed using groundnut cake.

ID : 1126

INFLUENCE OF CHRONIC AND ACUTE HEAT STRESS MODELS ON GROWTH PERFORMANCE, CARCASS TRAITS AND GENE EXPRESSION IN BLOOD OF SOME STRESS MARKERS OF BROILERS.

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Heat stress (HS) is an important environmental stressor that decreases bird performance and causes significant economic losses for the global poultry industry. The objective of this study was to compare the effects of an acute HS model and two chronic HS models (constant and cyclic) on performance, carcass characteristics and stress-related gene expression in the blood of broiler chickens. A total of 720 Cobb 500 male chicks were allocated to 12 environmentally controlled chambers divided into two pens of 30 broilers and were reared under thermoneutral temperatures until d20. From d20 to d41, 4 chambers were set to a thermoneutral temperature of 24°C with half of the birds pair-fed to equalize feed intake with constant HS birds (PF) and the other half fed ad-libitum (CTRL). Four other chambers were set to 35°C (constant HS), and the remaining 4 chambers were set to 35°C for 12h and 24°C for the next 12h (cyclic HS). At d41, a total of 16 CTRL birds were randomly selected (4 birds per pen) and exposed to acute HS (35°C for 2 hours). Standard starter (d0 to d13), grower (d13 to d27) and finisher (d27 to 41) diets were fed throughout the experiment. Feed intake and BWG were measured weekly and carcass traits were determined at the end of the experiment. Blood samples were collected on d41, and for the cyclic HS groups, samples were taken before and after 6h of HS (35°C). Gene expression of HSP70, TNF α , IL-6 and IL-18 was determined on 4 birds per treatment. At d41, BWG and FCR of constant and cyclic HS birds were decreased compared with CTRL birds ($P < 0.001$), and these reductions were greater with constant HS than with cyclic HS ($P < 0.001$). Performance of PF birds was better than constant HS birds but lower than CTRL broilers ($P < 0.001$). A higher fat yield was obtained with constant and cyclic HS birds compared to the CTRL ones ($P < 0.001$) and a lower breast and tender yield was measured ($P < 0.001$). Gene expression of blood samples revealed that HSP70 was upregulated in all treatments compared to CTRL birds. TNF α and IL-6 were upregulated in PF and constant HS birds and IL-18 was upregulated in constant HS birds. These analyses bring a better understanding of metabolic responses and different stress and inflammation marker levels involved under different HS models.

AFRICAN POULTRY NETWORK

ID : 251

ANTI-MYCOTOXIN AGENT AS A FEED ADDITIVE TO IMPROVE PERFORMANCE OF LAYER CHICKENS IN TOGO

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Mycotoxins are a great threat for animals' health as they affect feed intake and efficiency, immune system and reproduction. According to Hanvi et al. (2016), the most frequent occurring mycotoxins in maize samples from Togo were fumonisins (88%, 101- 1838 µg/kg) and aflatoxin B1 (38%, max. 256 µg/kg). One of the methods to reduce mycotoxin exposure is the usage of in feed anti-mycotoxin agents. However, there are many and their efficiency is highly variable. The objective of this study was to investigate the effect of a specific anti-mycotoxin agent (Excential Toxin Plus, Orffa Additives BV) on laying hen performance. Birds were housed in cages (3 layers per cage) and followed for 12 weeks (first 4 weeks = adaptation). Feed and water were provided ad libitum. The trial was performed as a randomized complete block design with two dietary and two age treatments (each treatment consisted of 6 replicates with 35 birds/replicate). Dietary treatments were: control diet without (TE) and with 1.5 kg/ton of anti-mycotoxin (TB). Age treatments were: 49-week-old laying hens (young) and 65-week-old laying hens (old). Production performance, egg quality and haematological parameters were measured. The collected data was subjected to one-way ANOVA analysis using Graphpad prism 5.0 software. Egg production of the old hens fed the anti-mycotoxin agent (old TB) was significantly higher ($P < 0.05$) than that of the control group (old TE). A similar, non-significant, result was seen in the young hens. Feed conversion ratio of birds fed the anti-mycotoxin (TB) was improved compared to the control group. Interestingly, daily feed intake of hens of old TB and young TB was lower than those of old TE and young TE, respectively. Egg weight in old TB was higher than that of old TE. Moreover, a similar trend was recorded in the young hens. Albumen and shell weight of the eggs of old TB were significantly higher ($p < 0.05$) than that of old TE. The same trend, but non-significant, was observed in young hens. The anti-mycotoxin agent slightly improved the yolk colour of both young and old hens. Haugh units, haematocrit value and mortality were statistically similar in all treatments. It can be concluded that incorporation of the anti-mycotoxin agent has a beneficial effect on laying hen performance (e.g. egg production). Regardless of the age of the hens, the anti-mycotoxin was effective in influencing egg weights.

ID : 253

EFFECT OF ANTI-MYCOTOXIN AGENT SUPPLEMENTATION ON PERFORMANCE OF GROWER-FINISHING BROILERS IN SENEGAL

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Mycotoxins are common contaminants in animal nutrition and produced by certain fungi. Exposure to mycotoxins can affect health and performance of farmed animals, resulting in lower growth, reduced immune response and even death (Leeson et al., 1995). The most common mycotoxins in cereals (and arachidic meal) used in poultry feed in tropical Africa are aflatoxins. One way to prevent chronic exposure to aflatoxins in chickens consists of supplementing feed with anti-mycotoxin agents. In Senegal, very few studies have been conducted on the effect of anti-mycotoxin agents on broiler performance.

The objective of this study was to analyse the effect of a specific anti-mycotoxin agent (Excential Toxin Plus, Orffa Additives BV) on the zootechnical performance of broilers. 600 non-sexed chicks (Cobb500) were fed for 10 days on a maize/arachidic meal/wheat bran/fish meal-based diet. Pelleted feed and drinking water were provided ad libitum. At day 11, chicks were randomly assigned to 12 pens and divided into 3 treatments according to the content of anti-mycotoxin agent in the feed (0, 1 or 5 kg/ton). For the preparation of experimental rations, arachidic meal containing 160.4 µg aflatoxin/kg was used. Weekly measurements of body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were performed. At the end of the rearing period (42 days), carcass and organ weights were determined. The collected data were subjected to one-way analysis of variance. Incorporation of the anti-mycotoxin agent into the feed at a rate of 1 kg/ton resulted in a 2% improvement in BW at 42 days ($p < 0.096$) and an improvement in FCR of 5% compared to the control. Incorporation of the anti-mycotoxin agent into the feed at a rate of 5 kg/ton did not significantly change the end BW. No significant effects were observed either on carcass weight or carcass yield nor on the ratios of the weight of different organs to the carcass weight.

It can be concluded that incorporation of the anti-mycotoxin agent at 1kg/ton of feed has a beneficial effect on performance. These results are, however, below expectations probably because of the low aflatoxin contamination of the arachidic meal used. The level of aflatoxin contamination from which toxic effects are observable vary according to the authors (Suganthity al., 2011; Yunus et al., 2011). To better appreciate the effect of the anti-mycotoxin agent, commercial aflatoxin could be used in future trials.

ID : 397

EFFECT OF VINEGAR ON CARDIOVASCULAR RISK IN FAST AND SLOW GROWING CHICKENS

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Exotic chicken that have been bred for fast growth have shown certain health issue like cardiovascular risk. Independently, vinegar has been shown to reduce cholesterol in species other than chickens. In view of this, a research study was conducted to evaluate the effect of vinegar on growth and cardiovascular risk on Exotic chickens and Local chickens to determine whether the observed effects were dependent on genotype. A total of ninety-six (96) day old birds consisting of forty-eight (24 Anak, 24 Arbor acre, twenty four (24) Noiler and 24 improved Yoruba chickens were randomly assigned to dietary treatment group, each having 12 replications of the same breed of the bird in a factorial layout within a complete randomized design such that birds in each genotype were randomly allocated to two diet that are defined by diet 1 (control), diet 2 (normal feed mixed with 4% vinegar). During the experimental period of 8weeks, growth performance traits and linear measurement including body weight, body length, body girth, wing length, thigh length, drumstick length, shank length, keel length were recorded weekly. Blood haematology and serum chemistry at age 8 weeks were also analyzed. At the end of the analysis, it was discovered that vinegar has a transient effect on Body Mass Index at week 6. It was shown that vinegar reduced cardiovascular risks through a reduction in Lipidemia (Total cholesterol and blood triglyceride). It also helps in the improvement of Insulin secretion and action. Vinegar had no effect on other cardiovascular risk factors including inflammation as measured by White blood cell count (WBC) and blood rheological risk indices including RBC, PCV.

ID : 561

FIRST TIME USE OF NEXT-GENERATION SEQUENCING TECHNOLOGY TO MONITOR VACCINE VIRUS REPLICATION IN EGYPT

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This study is aimed to evaluate the first attempts of using Next-Generation Sequencing Technology to monitor rHVT-ND-IBD vaccine replication in Egypt and correlate these results with hatchery vaccination audit, serological results and field performance parameters. For this purpose, a total of 160,000 commercial broilers vaccinated subcutaneously at a day of old with rHVT-ND-IBD were observed from a day of old till slaughter age. These birds were obtained from two different breeder farms with two different breeds (Ross and Cobb) and hatched in the same hatchery and reared in the same farm under the same management with different houses. The hatchery vaccination process including vaccine storage, preparation and administration were evaluated and recorded using a digital platform. For vaccine take evaluation; feather pulps from randomly selected 20 birds per house at 25 days were placed onto FTA cards to investigate vaccine take evaluation and to correlate the NSG results with serology, 25 blood samples per house were collected at slaughter age from the same two houses to evaluate antibodies titers against NDV using HI test and IBDV using two different commercial specific ELISA kits. Finally, field performance parameters were evaluated for the whole flock. The hatchery vaccine evaluation for vaccine storage, preparation and administration was 100% for Ross birds and for the Cobb birds vaccine storage and administration was 100% while vaccine preparation was 95%. The results of Next-Generation Sequencing showed that 16 from 20 were positive for rHVT ND-IBD in Ross birds and 13 from 20 in Cobb birds. For serological evaluation; the Mean ND HI test was 5.6 and 4.9 for Ross and Cobb house, respectively. For mean antibody titer against IBDV was 9569 (Ross) and 9922 (Cobb) using the commercial Synbiotics, BD+ ELISA kit, while 4903 (Ross) and 3278 (Cobb) using the commercial IDvet, ID Screen® IBD VP2 ELISA kit. Field performance parameters for the whole flock demonstrated 96.99 livability % with average body weight 2,597 gm with 1.76 FCR and 309.56 EPI. In conclusion; the new technology of Next-Generation Sequencing able to evaluate vaccine administration accuracy and program effectiveness.

ID : 706

AVIAN LEUKOSIS VIRUS SUBGROUPS A/B AND J ANTIBODY PROFILES OF LAYERS FROM FARMS WITH CASES OF AVIAN NEOPLASTIC DISEASES IN KADUNA AND PLATEAU STATES, NIGERIA.

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Avian neoplastic disease has been identified as one of the major diseases affection poultry production in Nigeria. The avian neoplastic diseases of importance in poultry are Marek's disease, avian leukosis and reticuloendotheliosis. Available literature shows that there is an increase in outbreaks of avian neoplastic diseases in Kaduna and Plateau States, most of which have been attributed to MD despite vaccination of commercial chickens. Previous reports indicate high seroprevalence of avian leukosis virus (ALV) p72 antigen in layers from farms with cases of avian neoplastic diseases tentatively diagnosed as Marek's disease, in Kaduna and Plateau States. The specific subgroups responsible for ALV infection in layers in Kaduna and Plateau States is still unknown, hence the need for this study. Therefore, the objective of this study was to determine the ALV A/B and J antibody profiles of layers from farms with cases of avian neoplastic diseases in Kaduna and Plateau States, Nigeria. Seven and 16 layer farms with cases of neoplastic diseases in Kaduna and Plateau States respectively, were screened for the presence of antibodies to ALV subgroups A/B and J using IDEXX enzyme linked immunosorbent assay (ELISA) kits. Out of the seven farms screened in Kaduna State, antibodies to ALV subgroup A/B was detected in six (85.7%) of the farms, while antibodies to ALV subgroup J was detected in only one (14.3%) farm. Antibodies to both ALV subgroups A/B and J were detected in one farm (Z5), which suggest co-infection of the two subgroups in one farm. Out of the 16 farms screened in Plateau State, antibodies to ALV subgroup A/B were detected in 15 (93.8%) of the farms, while antibodies to ALV subgroup J were detected in six (37.5%) farms. Antibodies to both ALV subgroups A/B and J were detected in five (31.3%) of the farms screened. The high detection of antibodies to ALV A/B suggests that ALV infection in layers is mostly due to ALV subgroup A/B in the study areas. A detailed study to determine the source of ALV infection in layers so as to effectively control the infection and reduce associated production problems is recommended. To the best of our knowledge, this is the first report of concurrent detection of antibodies to ALV A/B and ALV-J in layer farms in Nigeria.

Keywords: Avian neoplastic disease, antibodies, avian leukosis virus.

ID : 1106

EVALUATION OF IMMUNE RESPONSE OF TANZANIAN FREE-RANGE LOCAL CHICKENS FOLLOWING CHALLENGE WITH A LENTOGENIC STRAIN OF NEWCASTLE DISEASE VIRUS

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Free range local chicken (FRLC) keeping is the major form of chicken production in Africa. The chickens show relative adaptability to disease, a low plane of nutrition, and tropical climate. Given the wide phenotypic and genotypic diversity exhibited in these birds, assessing innate resistance to Newcastle disease (ND) was the focus of our investigation. Our research focuses on three major ecotypes of local chickens in Tanzania, namely Morogoro-medium, Kuchi and Ching'wekwe. This study aimed to evaluate immune response following challenge with a lentogenic strain of ND virus (NDV) among three chicken ecotypes as a measure of protection against ND. In total, 623 Morogoro medium, 417 Kuchi, and 562 Ching'wekwe, were raised from parent breeding stocks. At 28 days of age, chicks were challenged with a lentogenic LaSota NDV through oculo/nasal route. At 2 and 6 days post infection (dpi), tear samples were collected from each chicken for NDV RNA isolation and subsequently analyzed using qRT-PCR to measure ND viral clearances. At 10 dpi, blood samples were collected, sera isolated and tested to determine ability of the FRLC types to raise anti-NDV antibodies by enzyme linked immunosorbent assay (ELISA) using the IDEXX® NDV test kit and reading absorbances by spectrophotometer. Univariate analyses were done in linear model to compute viral clearances and antibody titres for the ecotypes as least square means (LSM) considering chicken ecotypes, replicate numbers as main effects. Ching'wekwe ecotype had highest viral clearance rate (0.26) compared to Kuchi (0.22) and Morogoro-medium (0.22) which had similar viral clearance rates. The viral clearance rates were only significantly different between Ching'wekwe and Morogoro-medium and between Ching'wekwe and Kuchi ecotypes. The LSM antibody level differences were significant between Kuchi (3.54) and Ching'wekwe (3.53) and between Morogoro-medium (3.55) and Ching'wekwe. The findings imply that there are some NDV clearance rates and immunological differences among the ecotypes when challenged with NDV; this may reflect differences in ability to mount an adaptive immune response following infection and relative susceptibility to the virus among chicken ecotypes. This study was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Feed the Future Innovation Lab for Genomics to Improve Poultry (cooperative agreement number AID-OAA-A-13-00080).

ID : 1335

MORBIDITY AND MORTALITY OF TANZANIAN FREE-RANGE LOCAL CHICKEN ECOTYPES WITH AND WITHOUT NDV VACCINATION AFTER VELOGENIC NDV NATURAL EXPOSURE CHALLENGE

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Free range local chickens (FRLC) important resources for livelihoods of rural communities in Tanzania. They provide meat, eggs and income for poor families through sales of poultry and poultry products. FLRC are adapted to harsh weather and believed to be resilient to infections and parasite infestations. This study aimed to compare the severity of post-mortem (PM) lesions between chickens pre-challenged with LaSota Newcastle disease Virus (NDV) strain and naïve chickens. The experiment had two groups; the first group of 763 chickens was pre-challenged with 200µl of 1*10⁷ titer of LaSota vaccine strain of NDV and after three months was naturally exposed to virulent NDV (vNDV) strains at 28 days of age (doa). The second group of 1258 naïve chickens was exposed naturally to vNDV strain at 28doa. In natural exposure trials in both pre-challenged and naïve groups, seeder birds were prepared by mixing with positive vNDV by Polymerase Chain Reaction (PCR) market birds to amplify vNDV. The infected seeder birds of the same age with group of naïve birds at 4 days post exposure (dpe) to vNDV were mixed with the experimental birds; and mortalities monitored for 21 days and survivors were euthanized. From the dead and euthanized chickens, PM lesion scores recorded from the trachea, proventriculus, intestines and caecal tonsils and scored 0 to 4 according to the extent of haemorrhagic lesions. Data analysis was done in MS excel and R statistical software; linear model to compute mortalities percentages, PM lesion score for the chickens and the least square means (LSM) respectively considering the chicken experimental groups, the pre-challenged and naïve as main effects. Twenty-percent of pre-challenged chickens died by 18dpe while the remaining survived to end of the experiment. In naïve group, 99% died by 14dpe and the rest survived to the end of experiment. The mean PM lesion score among birds that died during the natural exposure trial were significantly higher in naïve birds (5.06) compared to the pre-challenged birds (4.39). On the contrary, among survivors of the trial, PM lesion scores were higher in the pre-challenged birds (2.63) relative to naïve birds (1.82). The differences in survival and severity of lesions are likely due to some birds being pre-challenged and age differences during exposure to vNDV, are might have offered some degree of protection to vNDV infection which contributed to the differences in morbidities and mortality between the two groups.

DIVERSITY OF PRODUCTION SYSTEMS AND SERVICES DELIVERED TO HUMANS

ID : 331

A SERIOUS GAME TO MAKE FARMERS AWARE OF THE SERVICES PROVIDED BY FREE-RANGE POULTRY SYSTEM

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The outdoor run, an essential component of free-range poultry systems, can be managed and implemented in different ways by farmers. Chiron et al. (2019) develop a conceptual framework to evaluate the services provided by free-range poultry systems in five dimensions: resource and value production, life quality, relationships between farmers and society, territorial integration, and environmental quality. To raise awareness about the diversity of services provided, and in fine, help farmers/technicians to take into account those services when planning the implementation and the management of the outdoor run, a serious game was developed. Such an approach was found the most effective one to share knowledge among stakeholders, as demonstrated by previous experiences in agriculture (e.g. Martin et al., 2011). The development of the serious game took place in three steps. The first one was to establish the objective and main constraints of the game. The second one aimed at establishing the game mechanics and rules. The last one was the test of the game with actual operators. The objective of the game is to design an outdoor run that maximizes the provision level and the diversity of services, while considering socio-technical constraints (such as the pedoclimatic context) and limited resources. The game is collaborative as the design is made within a team of 3-4 players in about 90 minutes. A board representing a simplified outdoor run was designed. On this board, players can choose different options to organize the outdoor run (e.g. planting trees or hedges, while choosing the location and species). Each choice will have direct impacts on foraging behaviour of the birds and the provision (or not) of services. For instance, sowing honey plants could be attractive for birds, and it will have positive implications for local biodiversity and economic performance. However, actions are limited because of their cost in labour time and money. The game was then tested with groups of farmers and technicians. The playful approach was found effective to introduce complex notions such as “services” to farmers. Furthermore, it offers a support for discussion between players, allowing them to share their own experiences regarding outdoor runs. Therefore, this game could be used as progress tool for farmers and technicians involved in free-range poultry systems.

Chiron et al. (2020). World Poultry Congress

Martin G. et al. (2011). Environmental Modelling & Software

ID : 389

EVALUATING SERVICES PROVIDED BY FREE-RANGE POULTRY SYSTEMS: THE “BOUQUET” APPROACH.

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In free-range poultry production, outdoor run is a key element for the multiperformance of the farming system. However, managing the outdoor run can be seen more as a constraint when its potential to provide more global agroecological solutions and meet society's expectations, beyond animal welfare, is not perceived. Thus, it is necessary to provide tools for farmers, adapted to different knowledge levels and context, allowing them to think about an optimized management of the outdoor run or to better take into account the diversity of services it provides. The objective of this study was to develop and test a generic method to assess the services provided by different free-range poultry systems (i.e. species, location, or outdoor run characteristics). First, a conceptual framework was developed through a participatory approach by experts before being challenged by futures users (farmers, advisors, outdoor run management structures...) in three poultry production regions (West, South-East and South West of France). The conceptual framework finally considers of 13 services, being allocated in five different service categories (production of resources and value, farmer's life quality, farmer-consumer-citizen interconnection, territorial integration and environmental quality). For each service, the experts also selected relevant indicators to assess each service in an easy, quick, and cheap way in commercial farms. These indicators are based on either on-farm surveys (e.g. labour time or economic performance) or in situ measurements (e.g. assessing animal welfare). In order to validate the feasibility and genericity of the method in practical conditions, 21 commercial houses were studied. These farms differed in their location (West, South-East and South West of France), the production (chicken meat, eggs, duck meat) and the shape, size, and design (trees, bushes, woods...) of the outdoor run. The first results indicates that the method is enough sensible to highlight different bundles of services according to the outdoor run characteristics. Therefore, this method could be used by farmers and their advisors, to evaluate the actual provision of services by a free-range poultry system, and further discuss about the most adequate management of the outdoor run for a maximal provision of services.

ID : 1033

JAPANESE QUAIL FARMING IN INDIA

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At present quail have become the third largest avian species in number only next to chickens and ducks in the country. Quail are very suitable for commercial production of both meat and eggs. Quail can adopt all types of climate and environment and Indian climate is very suitable for raising quails commercially. One sq.ft. floor space sufficient to rear five broiler quails as well as less fixed investment for housing and equipment. In quail rearing, there is a good return on investment in a short period of five weeks and they are more disease resistant than chicken and do not require any vaccination as of date. The ICAR-CARI has come out with few strains namely CARI UTTAM, CARI BROWN, CARI SUN-HERI, CARI UJJAWAL (White breasted quail), CARI SWETA (White feathered quail) and CARI PEARL (White egg shell line). Japanese quail was introduced in Tamil Nadu in the year 1983. The Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai has released three meat type strains of Japanese quail viz: Nandanam Japanese quail –I, Nandanam Japanese quail –II, Nandanam Japanese quail –III, during 1993 - 2004 with gradually improved weight gain and feed efficiency and broiler type breeder quail in 2013. Veterinary College and Research Institute, TANUVAS, Namakkal, has released a meat-type Japanese quail hybrid strain, Namakkal quail –I and Namakkal gold quail for the benefit of farming community. GADVASU, Ludhiana released three strains of Japanese quail as Punjab – I, II and III while CPDO, Northern region and CPDO, Western region have also released new strains of Japanese quail. Japanese quail farming is making rapid strides in Tamil Nadu that meets the huge demand in South India. Japanese quail farming for eggs is popular in Kerala. About 30 million meat type broiler quail are produced in Tamil Nadu and 50 million Japanese quail eggs in Kerala annually. Japanese quail will consume around 500 g of feed and FCR is 2.5 to 3. Female body weight (250 g) will be always higher than male body (185-190 g) weight. The age at marketing is 28 to 30 days. Female Japanese quail will start lay at the end of 6th week and egg production is 200- 220. A sex ratio of 1: 3 (Male: Female) will give optimum fertility rate and hatchability is 75- 80 % and 65 to 70 % respectively. The average selling price of Japanese quail meat is \$ 0.5 per quail. Hence, rearing of Japanese quail will provide employment opportunities for the jobless youth.

EARLY MANAGEMENT OF BROILERS

ID : 101

EARLY FEEDING MANAGEMENT OF TWO DIFFERENT STRAIN OF BROILER STARTER PHASE

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Early feed restriction program to improve performance of broilers have been limited due to the lack of consistent results. These inconsistent results occur because many factors, such as restriction type applied, the age of application, and strain. Different strain of broiler chickens may have different body characteristics that will lead to different carcass yield and abdominal fat. This study was conducted to investigate the effect of early feed restriction on the performance of two commercial broiler strains. Three hundreds (300) broiler chicks were allocated to 6 treatments with 5 replicates of 10 chicks/replicate. The experiment used a factorial design with 2 strains, and 3 feeding methods. The 3 feeding methods were as follows: 1) ad libitum consumption defined in Cobb 500 guidelines, 2) quantity restriction (60% of the daily ad libitum consumption defined in Cobb 500 guidelines), 3) time restriction (standard feed offered for 9 hour/day). Chicks were exposed to feed restriction from 7-14 day of ages. Following the restriction period, the chickens were fed ad libitum. The research was terminated at 35 days of age. Chickens were provided with regular pelleted starter diet until 3 weeks of age. From 3 to 5 weeks of age, all chickens were given finisher diets. All of the finisher diets contain 20% crude protein and EM 3150 kkal/kg. Chickens from each strain were standardized to a similar weight. Data were subjected to One-Way ANOVA procedures of SAS software for analysis of variance. The results show that weight gain and carcass yields of broilers of both strains fed restricted diet was significantly lower than those fed ad libitum. However, feed restriction program at an early age improved feed efficiency for both strain A and B. On 14-21 days of age feed restriction increases feed intake. The higher feed intake can be related to the hypertrophy of the gastrointestinal tract that occurs after the restriction period, when the chickens are fed ad libitum. However, on 14-21 days of age chickens were not able to compensate for the weight lost during the restriction program. Abdominal fat of chickens restricted by quantity was the lowest. Performance of strain A broiler were significantly better than strain B. The better performance of strain A would be expected as it was the strain selected for higher body weight, and faster growth. Quantity restriction was most severe for strain B than strain A.

ID : 275

GROWTH PERFORMANCE OF BROILER STARTER BIRDS FED DIETS SUPPLEMENTED WITH TURMERIC (CURCUMA LONGA)

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Introduction: Success in poultry production is mainly the nutrition, breeding and management as well as the optimal development of birds immune status. Antibiotics were the utmost regularly used as feed additives but the use of antibiotics is not only restricted but also their practice in livestock and poultry industry have been prohibited in many countries due to modification of natural gut microbiota and drug resistance in microorganism and human. This can be stopped by supplementing the use of these antibiotics with the use of non-conventional herbs like turmeric. Turmeric is noted to be rich in minerals and it has the ability to stimulate the digestive system, increase the secretion of intestinal lipases, amylase, trypsin and chymotrypsin enzymes hence enhancing digestibility (Rajput et al., 2012). In this way, the modification of natural gut microbiota and drug resistance in microorganism and human will be greatly reduced. The objective of the study was therefore to evaluate the growth performance of broiler starter birds fed diet supplemented with turmeric (*Curcuma longa*). **Material and Methods:** Sixty day old abor acre broiler chicks were used to address the effect of supplementing turmeric in broiler starter birds on the growth performance after 4 weeks of feeding trial. The birds were divided into four groups of 15 birds each, each group was further replicated 3 times with 5 birds per replicate in a completely randomized design (CRD). The feed offered to the birds were 0%, 0.25%, 0.50% and 0.75% turmeric supplementation. At the 4th week of the experiment, growth performance indices (feed intake, weight gain & FCR) of the birds were determined. Highest feed intake was observed in T3 0.50% turmeric as it achieved significantly ($P < 0.05$) higher values than other treatment. There is also a corresponding significantly ($P < 0.05$) higher values in weight gain in the birds in T3 0.50% turmeric. However, the FCR in different dietary treatment during the 28 day feeding trial was not statistically significant ($P < 0.05$). **Conclusion:** It may therefore be inferred that supplementation of turmeric rhizome powder at 0.50% supplementation yielded better results in the growth performance. **Keywords:** turmeric, broilers, feed, growth.

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ID : 334

DEVELOPMENT OF A BACTERIAL AND FUNGAL BLOOM MODEL SIMULATING COMMERCIAL HATCHING CONDITIONS TO EVALUATE FORMALDEHYDE FUMIGATION ALTERNATIVES

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Microbial blooms that emerge during hatch consist of apathogenic and pathogenic organisms, including *Escherichia coli*, *Enterococcus faecalis*, and *Aspergillus fumigatus*. Commercially, horizontal transmission of these organisms occurs throughout hatch. Objectives of the present study included development of a novel multi-pathogen laboratory challenge model to mimic commercial conditions and optimization of sampling methods to quantify bacterial and/or fungal presence within the hatch cabinet. The pathogen challenge mix (PM) was recreated based on bacterial or fungal isolates recovered from infertile egg homogenates. Selected isolates included *Enterococcus faecalis* ($\sim 10^8$ CFU/egg), *Staphylococcus aureus* ($\sim 10^8$ CFU/egg), *Staphylococcus chromogens* ($\sim 10^8$ CFU/egg), *Aspergillus fumigatus* ($\sim 10^6$ spores/egg), and two *Escherichia coli* ($\sim 10^8$ CFU/egg) isolates. Challenge (100 μ L of pathogen mix) was administered to a 28mm area on the blunt end of the eggshell on d19 of embryogenesis (3 hatchers/trt, n=225/hatcher). Microbiological data, such as environmental hatcher samples (open plate method), fluff samples, and post-mortem gastrointestinal tract (GIT) samples were collected to evaluate PM circulation within the hatch cabinet and colonization of GIT in three experiments. There were significantly ($P < 0.05$) more presumptive Gram-negative bacteria, *Enterococcus*, and *S. aureus* recovered from day-of-hatch GIT samples and fluff samples of the PM group compared to the non-challenged group in all experiments. The PM group had a marked ($P < 0.05$) increase in *A. fumigatus* recovered from fluff samples compared to the control in two out of three experiments. Additionally, the PM hatchers had an average cumulative increase of 2200 colony forming units (select bacteria and fungi) from d20 of embryogenesis to day-of-hatch indicating that application of the PM to eggshells results in a measurable microbial bloom under laboratory conditions. These data suggest this innovative multi-pathogen challenge model could be utilized to evaluate fumigation alternatives to carcinogenic formaldehyde.

ID : 409

CHICK VOCALIZATIONS : DEVELOPMENT OF A RECORDING AND ANALYSIS METHOD

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Genetic selection on the production performances leads to more efficient animals but also more demanding and less rugged against disturbances in their environment. In this context, the chicks's quality control at start-up period, is a major lever. Its evaluation is based on visual indicators, partly subjective and time-consuming and does not account for the multiple components of chicks's quality. Sound indicators of vocalizations emitted by the chicks at hatching could be a good candidates to characterize, in a complementary way, the chicks's quality. Indeed, they are early indicators, not invasive, easy and quick to measure directly on animals alone or in groups. It becomes possible to obtain a result in real time allowing punctual and/or continuous monitoring. This study aims to develop a method for recording and analysing chicks vocalizations emitted during the first 3 days of life (D0 to D3). This study, part of a project named Chick'Tip, received financial support from the French Agriculture Ministry (CAS DAR). First, a bibliographic review identified vocalizations categories emitted by chicks (comfort, distress, snuggle, fear or pleasure trills) and identified their sound characteristics. Then, two programs developed under Matlab, made it possible (1) to automate the recording of sound sequences and (2) to detect audio events and identify comfort and distress vocalizations, through a time-frequency analysis of their signature. The study defined optimal sound recording, under laboratory conditions, allowing an optimal analysis of sound signals: a group of ten chicks, omnidirectional microphones, 2-minute recording sequences. Between D0 and D3, chicks emit short sounds with a limited frequency range (2000 - 5000 Hz). The program developed is functional to characterize the following sound indicators: the frequencial gravity center, spectral spreading and instantaneous frequency. Work must be continued to test the method on a wider variety of recordings (genetic strains, different environments), and to characterize other categories of vocalizations (fear or pleasure trills) so that they can also be detected. It now remains to assess whether a link can be made between the frequency of more or less marked appearance of comfort or distress vocalizations and the visual or physiological quality parameters of the chick.

ID : 951

EFFECTS OF POST-HATCH FAST OF CHICK ON DIGESTIVE TRACT DEVELOPMENT AND GROWTH PERFORMANCE ACCORDING TO DIET AND REARING ENVIRONMENTAL CONDITIONS

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In commercial conditions, chicks underwent a post-hatch fast (PHF) between 24 and 72h, and about 24h when hatchery and farm are in the same region. Effects on digestive tract development (DTD) and animal growth are controversial due to several factors such as definition of bird age (from hatching in most studies, or from farm arrival), animal genetics, or dietary and environmental conditions (DEC). Here, the effect of PHF was studied on body weight (BW) and DTD of Ross PM3, during a first experiment (48h fast), with age of birds determined as the age at farm arrival with access to feed and water. To study the effect of DEC, birds were reared either in optimal DEC, or damaged DEC (low quality diet and/or low quality rearing environment) in a 2x2x2 factorial design (6 pens/treatment ; 54 birds/pen of 2.3 m² of useful area). In a second experiment, the effect of a shorter PHF (24h) was studied on chick BW and DTD.

In the first study, PHF showed effects on DTD, BW and animal health, differing according to DEC. At the farm arrival, PHF chicks had lower BW than direct fed (DF) chicks (-3.5 g), due to lower yolk sac (YS) (2.0 vs 5.1 g), but similar BW without YS. The relative weight (rW) of the segments of the DT (expressed relative to BW without YS) was higher in PHF chicks (proventriculus : +37% ; gizzard : +33% ; small intestine (SI) : +27% ; caeca : +79%). At 3 weeks of age (w), PHF showed no effect on the DT, but, at 5 w, a lower rW of gizzard (-14%) was observed in PHF chickens, irrespective of DEC. No difference in SI morphology was observed at 3 and 5 w, irrespective of DEC, as well as for dry matter ileal digestibility at 3 w. In optimal DEC, PHF led to beneficial effect on BW as well as at 3 w (+5%) and 5 w (+3.5%). Damaged DEC led to a higher decreased BW in PHF than in DF chickens, -12% and -10.5% respectively at 3 w, and -16% and -10% at 5 w. This led to a lower final BW in PHF compared to DF chickens (-3.5%). These damaged DEC led to higher footpad dermatitis in PHF chickens at 3 w, but no difference at 5 w. In the second study, a decrease of chick BW after PHF was also observed (-2.6 g), due to lower YS (3.2 vs 6.0 g), as well as an increase of the rW of the segments of the DT (proventriculus : +21% ; gizzard : +25% ; SI : +23% ; caeca : x 2.75).

In conclusion, PHF (24 or 48h) has a positive effect on the chick DTD. These PHF seem beneficial on bird growth in optimal, but not in damaged DEC. This period of live needs to be managed according to DEC.

ID : 1250

THE EFFECT OF ENRICHED PRE-STARTER DIETS COULD LEAD TO BETTER BROILER PERFORMANCE AT MARKET AGE DISREGARDING THE STOCKING DENSITY

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Broiler early nutrition is becoming an important topic due to the positive correlation between early growth rate and market weight. A variety of early nutrition strategies reported that gut and the muscle development, immunity and overall growth can be improved by lowering Ca levels, supplementing spray dried porcine plasma (SDPP) and/or feeding extra amino acid levels. Additionally, recent works suggested that stocking density represents the main restriction for modern chickens to express their full growth potential at market age. The current study aimed to investigate whether providing low Ca, SDPP and extra amino acid levels in a pre-starter period could result in better growth at market weights and if these strategies could interact with the stocking density. A total of 1504 male day-old Ross 308 broilers were allocated across 48 collective pens and randomly assigned to experimental treatments. The study followed a 2 pre-starter programs (Standard vs. Enriched) x 3 stocking densities (Low: 27 kg BW/m²; Medium: 33 kg BW/m² and High: 39 kg BW/m²) factorial arrangement treatments. Birds received the different pre-starters (Standard or Enriched) from placement up to 4th day and, subsequently, all birds received a common 3-phase feeding program (4-10d, 10-28d and 28-42d) based on the CVB recommendations. Birds under Low stocking densities showed higher body weight (BW) and better feed conversion ratio (FCR) compared to those under High stocking density ($P < 0.05$) after 28 days. On the other hand, birds fed Enriched pre-starter led to greater daily weight gain (DWG) in the 0-4 d period thereby resulting in a higher BW at 4 days ($P < 0.0001$). The latter effects on BW were maintained over the complete production cycle which resulted in a 2.4% higher BW at market age ($P < 0.05$) and heavier carcasses (+2.2%; $P < 0.05$) compared to birds fed the Standard pre-starter diet. No interaction between pre-starter nutritional strategy and density level was identified for BW, DWG, daily feed intake, FCR, uniformity or carcass yield. However, birds fed the Enriched diet led to a lower incidence of lameness compared to the Standard pre-starter diet, being this effect only evident when High stocking densities were used ($P < 0.05$). Based on these results, there was a positive correlation between early growth and market weight using Enriched pre-starter diets disregarding the stocking density used.

ID : 1326

EFFECT OF INCREASED TEMPERATURE AT THE BEGINNING OF INCUBATION ON DAY-OLD CHICK QUALITY IN BROILERS

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The significance of conditions during incubation period for broiler chickens has been recognized to be critical due to the fact that broilers spend up to 40% of their total life inside the egg during incubation. Thus, anything that can optimize posthatch growth and development may have great scientific and practical interest. The present study was therefore conducted to elucidate the effects of increased temperature during first 36 hours of incubation on embryonic development, hatchability and day-old chick quality. The experiment was conducted using Ross 308 fertile eggs that had been stored for 4 days, broiler breeder's age was 55 weeks. For the experiment totally 1200 hatching eggs were used in 20 repetitions. The temperature in hatching machine at first 36 hours of incubation for control group (CG) was 100.8°F. For experimental group (EG) the temperature at first 36 hours of incubation was 102.5°F.

There was no significant difference in fertility between the groups, however the hatchability was higher in CG ($P < 0.05$). Hatchability of fertile eggs was 92.2% in CG and 90.4% in EG. There was not significant difference between CG and EG in early and middle stage embryonic mortality.

But mortality in the last third of incubation was significantly higher ($P < 0.05$) in EG. In CG the late mortality was 2.47%, in EG 4.17%. In average day-old chick weight neither yolk sac weight, percentage of yolk sac from the weight nor triiodothyronine (thyroid hormone T3) activity there was not significant difference between the groups.

Temperature setting change in hatching machine at first 36 hours of incubation from standard 100.8°F to 102.5°F had negative impact on day-old chick hatchability.

GENETICS X ENVIRONMENT INTERACTIONS AND EPIGENETICS

ID : 145

TRANSCRIPTOMIC PROFILE OF EARLY WHITE LEGHORN PGCs*

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In vertebrates crucial epigenetic reprogramming events occur during germ cell development and early embryogenesis. Primordial germ cells (PGCs), as the precursors of reproductive cells are important tool in the epigenetic studies and in study on reproduction of vertebrates. However, the epigenetic regulation of gene expression during PGCs migration in vivo has been rarely studied due to limited cell number. Especially, little is known about the comprehensive transcriptome dynamics in the chicken PGCs during early developmental stages. The establishment of the precise methods of isolation of competent and high quality avian PGCs as well as the method of RNA isolation from a single animal cell, offers a unique system for the study of an early germ cell differentiation. In the current study the transcriptomes of White leghorn PGCs at three developmental stages: 4.5, 8 and 12 day of embryo incubation were investigated. PGCs were collected and RNA was isolated with commercial kit for single cells application (GeneElute Single Cell RNA Purification Kit, Sigma Aldrich). The quality and quantity of obtained RNA was controlled by 2100 Bioanalyzer instrument (Agilent). High quality and purity RNA was intended for microarray analysis (One-color microarray-based gene expression analysis, Agilent). Microarray analysis was carried out according to the manufacturer's protocol. After scanning and feature extraction, obtained data were analysed by GeneSpring GX software (Agilent).

The number of differentially expressed genes (DEG) between 4.5 and 8 days of embryo incubation was 403 DEG, while between 8 and 12 days was 5304 DEG. These data may indicate that the highest biological activity occurs between 8 and 12 days of embryo development. Unique DEG undergoing gradual increase in expression from 4.5 to 12 days (141 DEG) are mostly involved in pathways related to multicellular organism process, cell communication, signalling or signal transduction, system development and anatomical structure morphogenesis.

These results help us to understand the chicken PGCs development and conduct the future studies to understand the nature of transcriptome changes in PGCs isolated from chicken donor, cultured in vitro, injected into recipient embryos and subjected to interaction between donor/recipient PGCs.

*The research was supported by the National Science Centre, grant no. UMO-2017/27/B/NZ9/01510In vertebrates crucial epigenetic reprogramming events occur during germ cell development and early embryogenesis. Primordial germ cells (PGCs), as the precursors of reproductive cells are important tool in the epigenetic studies and in study on reproduction of vertebrates. However, the epigenetic regulation of gene expression during PGCc migration in vivo has been rarely studied due to limited cell number. Especially, little is known about the comprehensive transcriptome dynamics in the chicken PGCs during early developmental stages. The establishment of the precise methods of isolation of competent and high quality avian PGCs as well as the method of RNA isolation from a single animal cell, offers a unique system for the study of an early germ cell differentiation. In the current study the transcriptomes of White leghorn PGCs at three developmental stages: 4.5, 8 and 12 day of embryo incubation were investigated. PGCs were collected and RNA was isolated with commercial kit for single cells application (GeneElute Single Cell RNA Purification Kit, Sigma Aldrich). The quality and quantity of obtained RNA was controlled by 2100 Bioanalyzer instrument (Agilent). High quality and purity RNA was intended for microarray analysis (One-color microarray-based gene expression analysis, Agilent).

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*The research was supported by the National Science Centre, grant no. UMO-2017/27/B/NZ9/01510

ID : 1043

GENE EXPRESSION, DNA METHYLATION AND GLUTATHIONE LEVELS OF MEAT-TYPE CHICKENS FED DIETARY METHIONINE FROM DIFFERENCES SOURCES AND SUBJECTED TO HEAT STRESS

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Heat stress (HS) generates reactive oxygen species and causes oxidative stress in tissues. Most of the cellular studies on HS have been reported using liver tissue. There are studies that suggest that dietary methionine which is a precursor to glutathione, a major antioxidant in the body can be used to ameliorate the negative effects of HS. We therefore tested the efficacy of methionine from two sources on the molecular expression and DNA methylation of genes in the transsulfuration pathway, and, reduced (GSH) and oxidized glutathione (GSSG) level ratio in the Pectoralis (P.) major. The genes studied were cystathionine beta synthase (CBS) and cystathionine gamma lyase (CTH). For this study, Cobb500 broiler chickens were subjected to thermo-neutral temperature (TN) (25°C) or HS (35°C) from day 7 to 35. Additionally, birds in both treatments were fed recommended diets supplemented with molar equivalent of either DL-Methionine (DL-Met) or Methionine Hydroxy Analogue (MHA) at either requirement. The P. major was sampled at day 10, 21 and 35 for both cellular and molecular assays. mRNA expression differences were done using the $\Delta\Delta C_t$ method with DLM as the control and β -actin as internal control. Under TN conditions, CBS was upwardly expressed about 30 fold in the MHA group at day 10, and about 2.5 fold at day 21, but was undetectable at day 35 when compared with the group fed DLM. However, under HS, CBS was upregulated two fold at day 10, and 34 fold at day 21, but was undetected at day 35 when compared with the group fed DLM. However, CTH was downwardly expressed in the MHA group at day 10, significantly upregulated at day 21 and undetectable at day 35 compared to the DLM group under TN conditions. However, under HS, CTH was upregulated about 80 fold in the MHA group, significantly downregulated at day 21 and undetected at day 35 when compared with the DLM group. The differences in gene expression from the different methionine sources did not translate into significant differences in glutathione levels across the days measured. This may be due to differential DNA methylation in the CBS gene with respect to the different methionine sources. It could be concluded that difference sources of dietary methionine caused differences in gene expression in the transsulfuration pathway, however, under both thermo-neutral and heat stressed conditions, the GSH/GSSG ratios were not significantly different putatively due to methylation.

ID : 1164

TRANSGENERATIONAL INHERITANCE OF FATTY LIVER IN CHICKEN MEDIATED BY DNA METHYLATION

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In the chicken, liver is the core organ for lipid metabolism. Disorders of lipid metabolism in liver can cause fatty liver syndrome (FLS) in chicken. Studies have shown that FLS has a predisposition to transgenerational inheritance, which may be related to an epigenetic modification. DNA methylation plays the most extensive form of epigenetic modification, while the role in the mechanism of transgenerational inheritance of FLS is limited. Aiming to study the potential mechanism, we designed the experiments as follows:

1. Establishment of chicken FLS model. The 3×3 two-factor experiment was conducted, both male and female chickens from three breeds (White Leghorns, Jingxing-Huang (JXH), Beijing-You chicken) and three diets (basal diet, high-fat diet (HFD), methionine-deficiency diet) were used. The HFD may cause liver damage with higher alanine aminotransferase. And serum triglyceride (TG) and total cholesterol (TC) of JXH were increased significantly. Unsurprisingly, the combination of JXH × HFD were found to be highest incidence (41.94%) of FLS.

2. The test for transgenerational inheritance model of FLS. The combination of JXH (F0) × HFD was used in this section. The paternal group (cocks with FLS × healthy hens), maternal group (healthy cocks × hens with FLS), and control group (healthy cocks and hens) were created and mated to produce offspring (F1). Serum TG and TC content were no difference between maternal and control group, but significantly increased in paternal group compared to other two groups in F1. Expectedly, the incidence (41.5%) of FLS in paternal group is significantly higher than that in maternal (20.0%) and control group (18.75%). Therefore, the male chickens with FLS and healthy female chickens were used to produce offspring (F1-F3).

3. Analysis of the mechanism of transgenerational inheritance of FLS mediated by DNA methylation. The great difference of expression and methylation profiles was detected between fatty liver and normal liver by RNA-seq and WGBS in F1 and F3. Key gene IGF2BP1 was found the same differential tendency of two levels in F1 and F3. Ca signaling pathway, Wnt signaling pathway, and MAPK signaling pathway were enriched in both F1 and F3.

In conclusion, we constructed an ideal model to study the mechanism of transgenerational (paternal) inheritance of FLS; the DNA methylation played a crucial role in this process, and the expression and methylation difference were found to persist in at least 3 generations; the gene IGF2BP1, as well as Ca signaling pathway, Wnt signaling pathway, and MAPK signaling pathway were regulated by DNA methylation in F1 and F3, which were effective in the transgenerational inheritance of FLS. Our results would further the study of transgenerational inheritance of FLS in chicken.

GENETICS X NUTRITION INTERACTION ON THE DIGESTIVE MICROBIOTA

ID : 180

INTEGRATION OF PROTEOME, TRANSCRIPTOME, AND METAGENOME ANALYSES PROVIDES INSIGHTS INTO THE FISHY ODOR OF DUCK EGGS

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Though the consumption of duck eggs increased gradually, the fishy odor restricts the development of the duck egg industry to some extent. Base on the result that trimethylamine (TMA) caused the intense fishy odor in duck eggs, the main purpose of this study was to reveal the genetic basis of the fishy odor of duck eggs.

The study population included 492 female Jingjiang ducks pure line (60-72 wks old). Ducks with high and low levels of TMA were killed after choline addition, and samples were collected for subsequent experiments and analysis. The results showed that the TMA content in serum, cecum, and eggs of ducks had significantly positive correlations (PP

Livers from 6 ducks at the top and 6 ducks from the tail of the TMA deposition distribution were sampled for transcriptomic sequencing, proteomic expression profiling, and FMO3 enzyme activity detection. RNA-Seq sequencing results revealed that 712 transcripts, and 499 transcripts down-regulated and up-regulated TMA deposition, respectively. The FMO3 transcript significantly down-regulated the TMA content in duck eggs (FDR = 6.68E-19). Meanwhile, the weighted gene co-expression network analysis (WGCNA) showed that the module with FMO3 transcript was strongly associated with the TMA content in duck eggs (R = 0.61). Proteomic results showed that 112 proteins, and 106 proteins down-regulated and up-regulated TMA deposition, respectively. FMO3 protease significantly down-regulated the TMA content in duck eggs (P = 0.00876). The GO entries of differentially expressed proteins mainly included oxidoreductase activity pathways. Furthermore, the TMA content of duck eggs decreased with the increase of FMO3 enzyme activity. Besides, The 16S V4 amplicon sequencing results showed, that Mucispirillum and Akkermansia were positively and negatively correlated with the TMA level, respectively.

In summary, the TMA content was affected by Mucispirillum and Akkermansia in the cecum of ducks. Then, TMA reaches the liver via intestinal absorption and blood circulation. The TMA oxidation was affected by FMO3 mRNA expression, FMO3 protein expression, and FMO3 enzyme activity in the liver. All these parts above jointly regulate the TMA metabolism in ducks, which resulting in different levels of fishy odor in duck eggs.

ID : 302

THE EFFECT OF GENOTYPE, SEX AND FEED MANAGEMENT ON THE MICROBIAL SAFETY OF CHICKEN MEAT PRODUCTION

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In terms of both welfare and economic traits of meat production, the developed countries tend to focus on production of poultry meat from optimised conditions. Aside from the production itself, a critical point in production of poultry meat is, of course, its microbial safety. One of the examples of the aforementioned trends is the use of various genotypes (medium-fast or slowly growing chicken) and feed restriction. The aim of the presented study was to evaluate the effect of the genotype and feed restriction on the microbial parameters of poultry meat production.

The experiment was carried out on three genotypes of chicken: Ross308 (standard broiler chicken meat genotype, fast growth), Hubbard (JA) (medium-fast growth) and Isa Dual (slowly growing chicken). We used males and females separately, both either ad libitum, or with restricted feeding (30% quantitative feed restriction). After the euthanasia (Ross308 in 31 days, Hubbard in 45 days and Isa Dual in 77 days) the microbiological analysis of the caecum content was performed. We enumerated the number of total bacteria, *Escherichia coli*, salmonellas, bifidobacteria and lactobacilli. Regarding to genotype, total count, bifidobacteria and *Escherichia coli* were significantly ($P \leq 0.05$) the lowest in Dual (9.69, 8.77 and 5.85 Log₁₀ CFU/g, respectively) and with respect to sex, males had more ($P \leq 0.05$) total counts of bacteria (9.96 in males vs. 9.76 Log₁₀ CFU/g in females, all genotypes). The significant interaction of all factors (ie sex, genotype and feed restriction), was observed in total counts ($P \leq 0.001$) and *Bifidobacterium* spp. ($P \leq 0.05$), with a higher variability among groups in JA in comparison with Ross and Dual. In particular, the counts of total bacteria in JA varied from 10.6 Log₁₀ CFU/g in males with feed restriction to 9.39 Log₁₀ CFU/g in females with feed restriction. In terms of bifidobacteria, the highest counts of 9.78 Log₁₀ CFU/g were found in males with feed restriction, while the lowest were 8.68 Log₁₀ CFU/g in females with feed restriction.

These results indicate that the choice of sex, genotype and feed management can be crucial for the hygiene and safety of animal products. The results can be used for further decisions concerning the safety of chicken meat production.

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ID : 645

MULTI-OMICS APPROACHES TO DECIPHERING THE COEVOLUTION OF HOST AND GUT MICROBIOTA

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Although the emergence of the hologenome theory has broadened our perspectives for studying host-microbe interactions, there is a void in its application in higher organisms. Chickens from lines that had undergone long-term (56 generations) bidirectional selection for high (HW) and low (LW) 56-day body weight from a common founder population provided an ideal model to further explore the coevolution of the holobiont under selection pressure. Using chickens from these lines, we applied multi-omics technology and observed that the selection pressure on the holobiont dramatically altered the intestinal DNA methylation and gene expression profile of host as well as the composition of gut microbiota. On the microbiome side, five genera from the Ruminococcaceae family and two genera from the Lachnospiraceae family increased in the HW. Energy metabolism-related pathways, such as glycolysis/gluconeogenesis and citric acid cycle, were enriched in the microbiome of HW. On the other side, after long term bidirectional selection, there were 4,779 different methylation regions (DMR) and 327 differentially expressed genes (DEGs) were identified between the gut of HW and LW. Of them, Insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) was the most down-regulated genes in the gut of LW. Its expression was likely regulated by both a DE miRNA, gga-miR-2128 that was up-regulated in LW, and a DMR that was involved in CpG island whose distance to the transcription start site of IGF2BP1 was only 388bp at LW genome. Integrated analysis of host and gut microbial data revealed that DEGs could link to gut microbes. For instance, expression level of IGF2BP1 remarkably correlated with the abundance of several microbes, such as Veillonella, Lactobacillus, and Methanocorpusculum, which were highly abundant in gut of HW, suggesting an adaptive interaction may have between host and gut microbiota. Overall, these results provided evidence for the hypothesis that there is a coevolution relationship between the host and gut microbiome and that both could contribute to the fitness of the holobiont.

ID : 889

THE EFFECT OF MINERAL SORBENT ON THE INTESTINAL MICROFLORA OF EGG LAYERS AND THE EXPRESSION OF GENES ASSOCIATED WITH IMMUNITY

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Since the presence of mycotoxins requires time for them to be identified, preventive doses of sorbents are regularly added into egg-laying hens feed by modern manufacturers of poultry products. Due to the fact that modern enzyme-based sorbents are rather expensive, commercial egg producers are willing to use natural mineral sorbents based on bentonite, clay or carbon. Recently, the carbon-based sorbent (Shungit) in its fullerene-like form C60 has started to be actively used in the Russian Federation.

A feed experiment was launched to study the effect of the mineral sorbent on the intestinal microflora of egg-laying hens as well as the expression of the genes associated with immunity. The studies conducted were based on birds from 26 to 29 weeks of age, in the vivariums of the laboratory of genetics and genomics of birds, at the Academy of Veterinary Medicine and Biotechnology named by K.I. Scriabin. Before the start of the research, the productivity of the birds at the age of 20-25 weeks was estimated and the groups of the same egg-laying intensity were formed. The birds of the control group have been fed for 28 days in accordance with the recommendations for commercial layers. In the diet of the experimental group, the studied sorbent was added in an amount of 1500 g per ton of feed. The intensity of egg production, egg number per bird and egg weight were determined. At the end of the study, ten hens from each group were randomly chosen for sampling tissues and contents of selected blind gut processes. The contents of the blind processes were studied for a microbial profile by NGS sequencing of microbial DNA on the Gene Studio S5 instrument (ThermoFisher scientific, Inc). Sequencing results were analyzed using Ion Reporter to determine the composition of the microbiota (<https://ionreporter.thermofisher.com/ir>). The expression of genes associated with immunity (β -defensin-9, proencephalin, Galinacin-10, interleukin-8) was evaluated on RNA which was isolated from the tissue of the blind processes of the intestine. In total, the production of poultry of the experimental and control groups did not differ significantly, yet there was a decreasing egg weight trend for the hens treated with sorbent as compared to the control group. The tendency to a decrease or the increase deceleration in egg weight may be associated precisely with the removal of part of the nutrients in the feed. It was found that the content of beneficial bacteria of the family Lactobacillaceae was reduced to 62,6%. Furthermore, the cellulolytic bacteria of the Ruminococcaceae family was increased to 72,3%. Along with this, there was a significant 600% increase in pathogenic and undesirable microorganisms, the increase was due to the bacteria of the Enterobacteriaceae family. The effect of the sorbent on the expression of genes related to productivity has been studied as well. Since mycotoxins have an immunosuppressive effect, the impact of the sorbent is expected precisely on the genes associated with immunity rather than productivity. The expression of the β -defensin 9 gene has decreased by more than 5 times, the proenkephalin and galinacin-10 gene has decreased by 4 times and interleukin-8 has decreased by 1.75 times. The data obtained illustrate an overall decrease in inflammatory processes in the body, which is apparently associated with the adsorption of mycotoxins and their removal from the body.

Thus, the Mustala mycotoxin adsorbent used for preventive purposes can affect the absorption of nutrients by changing the profile of the microbiota (reducing lactobacilli and increasing cellulolytic bacteria), while there is no doubt about its positive effect on the immunity of poultry.

ID : 992

THE KICK-STARTER EFFECT OF ARABINOXYLAN-OLIGOSACCHARIDES (AXOS) ON ARABINOXYLAN DIGESTION IN THE YOUNG BROILER

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With the ban of prophylactic antibiotics in 2006, the use of prebiotic oligosaccharides as tools to maintain and promote broiler's health and performance responses has attracted more and more interest. In addition, the beneficial effects observed upon endoxylanase supplementation of broiler feed were recently explained by their ability to create such prebiotics, in this case arabinoxylan-oligosaccharides (AXOS), in the hindgut of broilers. However, the prebiotic function of AXOS in its classical sense can be questioned, as the performance effects observed cannot be explained in full by the augmented selective microbial fermentation and subsequent short-chain fatty acid production. The objective of this study was hence to assess and understand the possible additive effects of dietary AXOS addition on the age-related arabinoxylan (AX) digestion in the hindgut of broilers. Therefore, a feeding trial was performed on 480 one-day old chicks (Ross 308) receiving a wheat-based diet supplemented with or without AXOS (0.5%), containing no endoxylanases. Digesta samples from the ileum and caecum, and feces were collected at six different broiler ages: d 5, d 10, d 15, d 21, d 28 and d 35 and examined for AX content and digestibility values, intestinal viscosity and microbial AX-degrading enzyme activities. Data were analyzed using two-way ANOVA and the post-hoc Tukey's HSD test to further identify significantly different means. Higher intestinal viscosity values were observed for AXOS fed broilers compared to control fed broilers ($P < 0.01$). This remarkable observation could be explained by the faster acquired AX solubilizing capacity of the AXOS fed intestinal microbiome ($P < 0.05$). When 0.5% AXOS was fed from hatching, higher total tract water-extractable AX and total AX digestibility coefficients were observed, especially for broilers younger than 15 d of age ($P < 0.04$). The impact of AXOS on hormonal changes delaying transit time and/or the emergence of a microbial community with enhanced AX degrading capacity are probably at the basis of these observed enhanced AX digestibilities at young broiler ages. Our results hence indicate that the provision of AX-derived oligosaccharides has the potential to kick start dietary fibre digestion in the hindgut of young broilers. It enables a greater functional value to be extracted from the dietary fibre fraction from young broiler ages onwards.

INNOVATIVE STRATEGIES TO ANSWER NEW EXPECTATIONS FROM THE SOCIETY

ID : 237

INNOVATIVE, NON-INVASIVE, FAST AND HIGHLY ACCURATE VISIBLE-NEAR-INFRARED IN OVO SEXING METHOD ON BROWN CHICKEN EGGS

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Today, 6 billion chicks from layer lines are yearly hatched with only 50% raised as laying hens for egg production. Whereas the remaining 50% are males with inefficient growth for meat production. Billions of healthy animals are killed at birth, raising substantial ethical concern. Therefore, an alternative method is required to pre-select males during embryonic development. This method is called in ovo sexing and allows more humane male culling. A robust and non-invasive in ovo color sexing technique was developed in this research. Thereby, visible-near-infrared (vis-NIR) point spectroscopy was used, which has the advantage over state of the art hyperspectral imaging in terms of accuracy and cost.

Three independent experiments were each conducted on a batch of 600 Isa Brown eggs. These eggs were individually illuminated on a daily basis by a halogen lamp and transmitted light over 180° was measured in a 300-1145 nm range. Every sample was measured five times with a specific exposure time between 500-1000 ms. An optimal preprocessing strategy was subsequently performed on the average signals. After preprocessing, the dataset was randomized and divided in a 60% calibration set and a 40% validation set. A partial least squares discriminant analysis (PLS-DA) with cross-validation was then used together with forward interval partial least squares (FiPLS) for variable reduction for determination model construction. Finally, only data was used from eggs with hatched chicks that were color and vent sexed without mismatches.

Results on the validation set demonstrated that the highest accuracies of 98.54% at day 13 and 99.52% at day 14 were achieved on the full wavelength range in experiment 3. This accuracy is lowered to 83.45% at day 12 since the colored feathers were insufficiently developed. On the other hand, growing embryos absorbed more light which resulted in a lower prediction of 93.62% at day 18. Furthermore, a variable reduction in experiment 2 at day 14 resulted in a smaller range with a 98.46% prediction, proving potential use of smaller spectrometers towards industrial application. Finally, model robustness was proven by correctly classifying with 98.63% the full dataset of experiment 3 at day 14 with this reduced model of experiment 2.

In conclusion, non-invasive vis-NIR in ovo sexing on brown eggs is highly accurate and has the potential for cost-efficient and high throughput application in hatcheries after two weeks of incubation.

ID : 289

GROWTH AND SLAUGHTERING PERFORMANCE OF MUSCOVY DUCK FED WITH INSECT LIVE LARVAE AS ENVIRONMENTAL ENRICHMENT

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Introduction. Pecking is an important problem in Muscovy duck (*Cairina moschata domestica*) production but few works investigated this phenomenon, probably related to redirected foraging. The aim of the present work was the evaluation of growth and slaughtering performance of Muscovy duck fed with *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) live larvae as environmental enrichment.

Material and methods. A total of 126 female Muscovy ducks (3d-old, average live weight, LW: 79.8±3.3 g) were allotted in 18 pens (6 replicate/treatment, 7 birds/pen) and assigned to 3 experimental treatments. The C group (control) was fed with commercial feed, while HI and TM groups were fed with commercial feed supplemented with HI and TM live larvae (provided as 5% of expected daily feed intake, DFI), respectively. The LW, average daily gain (ADG), DFI, and feed conversion ratio (FCR) were evaluated for starter period (3-31d-old), grower-finisher period (31-55d-old) and for the whole experimental period (3-55d-old). Larvae consumption time was recorded daily during the whole trial. At the end, 12 birds/treatments were selected for average LW and slaughtered. Hot and chilled carcass weight were recorded, as well as organs (spleen, liver, heart, bursa of Fabricius) and abdominal fat weight. Breast and thigh muscles were excised, weighed and the corresponding yields were then calculated. Larvae consumption time was analyzed by Generalized Linear Mixed Model (GLMM), while all the other data were analyzed using one-way ANOVA (P0.05). The larvae consumption time was affected by the age of the birds (P0.05). Hot and chilled carcasses weight were not affected by dietary treatment, as well as the weight of the organs and the abdominal fat content (P>0.05). Breast and thigh yields were similar among groups (P>0.05; 16.9±1.5 g and 22.9±1.2 g on average, respectively).

Conclusion. The supplementation of 5% of HI and TM live larvae in Muscovy duck did not affect the growth and the slaughtering performance of the birds. The age of the birds influenced the larvae consumption time but the animals did not have preference between the two insect species.

ID : 398

LOW-INPUT OUTDOOR AND ORGANIC POULTRY PRODUCTIONS: HOW TO IMPROVE ANIMAL WELFARE IN THESE SYSTEMS?

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Although low-input outdoor and organic poultry production systems allow natural expression of poultry behaviour and are expected to support a high level of animal welfare, health and welfare issues are still observed (van de Weerd and al. 2009). In order to improve animal welfare in these systems, they must first be characterised and qualified because the diversity of practices applied in these systems can lead to a range of welfare problems. Our methodology enabled us to highlight the major animal welfare challenges and concerns in low-input outdoor and organic poultry production systems at the European level. Firstly, standards, regulations and labelling schemes imposing practices in these systems were gathered and described. Secondly, relevant peer-reviewed articles, congress proceedings and on-going research projects were reviewed to ensure state-of-the-art information on practices and their impacts on animal welfare. Thirdly, interviews with key-informants were conducted in Italy, Finland, France and the United Kingdom to acknowledge any gaps in identification of the animal welfare challenges and related solutions. The study identified close to 50 systems, which differed in their characteristics. For instance, variations in space allowance, application of mutilations and free range access differed substantially between identified systems. The screening of grey literature and articles enabled to check innovative solutions related to ethics, behaviour, health and management issues such as the use of genetic strains, phytobiotics and enrichment of the range. The results of the interviews enlighten the perception of stakeholders regarding animal welfare in these systems. In fact, items such as “the 5 freedoms” and “Animal needs” or “Consumers perception” were systematically raised whereas “Regulation” was qualified as a component of animal welfare definition only by stakeholders from Finland and Italy. Besides providing a shortlist of systems, our study allowed us to inventory the issues and the barriers and levers related to them in a database. A comprehensive inventory of current and prospective production practices in poultry low-input outdoor and organic systems helps us to understand the diversity of farming systems and the barriers and levers to animal welfare observed in these systems.

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Reference: Van de Weerd, H. A., R. Keatinge, and S. Roderick. 2009. “A Review of Key Health-Related Welfare Issues in Organic Poultry Production.” *World's Poultry Science Journal*.

Key-Words: poultry, low-input outdoor, organic, practices, welfare

ID : 694

EFFICACY OF 1% FLURALANER AQUEOUS SOLUTION IN THE TREATMENT OF INFESTATIONS OF MENACANTHUS CORNUTUS ON LAYING HENS

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Infestations of poultry by lice have generated economic losses for the egg industry including drop in egg production, skin irritation and it can adversely affect animal welfare. 1% fluralaner aqueous solution was evaluated under controlled conditions as a treatment for laying hens infested with *Menacanthus* (M.) *cornutus*. The study was designed to evaluate the efficacy of 1% (10 mg/mL) fluralaner aqueous solution intended for treatment of M. *cornutus* infestation in laying hens in Brazil. Ninety birds were divided into two groups of 45 birds: one shed was assigned to the Control Group (CG) that is untreated and another one was the Treated Group (TG). In the TG the hens received the treatment via drinking water on Study Day (SD) day 0 and 7, at a dose of 0.5 mg fluralaner per kg of body weight (BW) (equivalent to 0.05 mL product/kg BW). Hens were individually examined for the level of M. *cornutus* infestation on Day -5 (before treatment), and on Days 7, 14, 21 and 42 after treatment, following the criteria: Level 0 - no lice; Level I - Low - Presence of lice in the cloaca and/or ventral region; Level II - Moderate - Presence of lice in the cloaca and wings; Level III - Strong - Presence of lice in the cloaca, wings and back; Level IV - Extra Strong - Presence of lice on cloaca, wings, back and neck. During the study, the level of M. *cornutus* infestation was checked using a scale from 1 to 4 in both groups. The average score on day -5 was 1.8 in both TG and CG. After the administration of 1% of fluralaner solution, no lice were identified in the TG between day 7 and day 42. After treatment the mean infestation scores in the CG on days +7, +14, +21 and +42 were 2.4, 2.1, 1.8, and 2.6 respectively. There were no abnormal health observations after treatment. As a systemic acaricide given in the drinking water, fluralaner provides a combination of potent efficacy, bird safety, and user convenience due to a ready-to-use aqueous solution for water administration with zero egg withdrawal time for laying hens. Under the described experimental conditions, 1% (10 mg/mL) fluralaner solution caused no adverse effects in treated birds and was highly effective in eliminating the infestation of M. *cornutus* from day 7 up to the study end (42 days after the first treatment administration). The present study followed local regulations. It was submitted for evaluation by an Ethics Committee on Animal Use under the number 2019/10-03 before the study started.

ID : 796

THE MEAT PRODUCTION OF DIFFERENT CROSSBRED GENOTYPES OF YELLOW HUNGARIAN CHICKEN BREEDS IN TWO KEEPING SYSTEM

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The demand for poultry products has increased, therefore products from alternative keeping system became more important. The aim of this study was to investigate the meat production parameters of crossbred genotypes of Yellow Hungarian chickens. 3 crossbred genotypes (A: 50 % Yellow Hungarian + 50 % TETRA HB, D: 25 % Yellow Hungarian + 25% TETRA HARCO + 50 % TETRA HB, E: 25 % TETRA H + 25 % Yellow Hungarian + 50 % Tetra HB) were established as F2 generations and were compared to pure breed Yellow Hungarian (YH) as a control group. Two types of pens were used (indoor: 2.5 × 3 m and free range: 2.5 × 5 m). Totally 320 birds (4 genotypes, 2 types of pen, 40 birds/ pen) were reared in the same condition (deep litter). The outdoor yards were completely covered with forage. Outdoor access from these pens was provided after 35 days of age during daylight hours through a single doorway (30 × 30 cm) in the free range groups. The animals were slaughtered at the age of 98 days. The live weight and feed consumption were measured every two weeks.

In the free range system, the E genotypes reached the highest live weight (3044 g), it was significantly higher than that of D (2628 g) and A (2222 g) groups, all of them were significantly higher than the live weight of purebred YH (1516 g). The ratio of carcass did not differ between groups (A: 70.2%, D: 69.8%, E: 71.6% and YH: 66.9 %). The feed conversion values were 2.9 kg/kg (A group), 2.2 kg/kg (D group), 3.0 kg/kg (E group) and 2.5 kg/kg (YH group). In the indoor systems, the A (2440 g), D (2441 g) and E (2525 g) groups had significantly higher live weight than the pure breed YH (1498 g). There were no significant differences in the ratio of carcass between genotypes. The feed conversion was A: 2.3 kg/kg, D: 3.2 kg/kg, E: 2.8 kg/kg, YH: 4.1 kg/kg. The effect of keeping systems was demonstrated only in one case, the live weight of free range E (3043 g) was significantly higher than the indoor E (2525 g). According to the results, there was one outstanding crossbred genotype (E: 25 % TETRA H + 25 % Yellow Hungarian + 50 % Tetra HB), which could be used for the meat production sector.

ID : 1152

PRODUCTION PERFORMANCE OF TWO DUAL-PURPOSE CHICKEN BREEDS IN A MOBILE STABLE SYSTEM

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Debates on culling day-old male egg-type chicks are constantly growing. Besides the in-ovo sex determination and the fattening of male chicks of layer lines, the use of Dual Purpose Breeds is considered as a strategy to avoid killing of day-old male egg-type chicks. The current study investigated the performance of two Dual Purpose Breeds in a mobile production system under organic conditions. 509 Lohmann Dual (LD) and 505 Lohmann Dualexperimental (LDex) day old chicks were reared in two mobile stable barns as hatched. The cockerels were slaughtered on day 85, the hens remained in their respective mobile barn for a prolonged laying period (74 weeks, including molting). Body weight (BW) development, group feed consumption and mortality was recorded at regular intervals. At time of slaughter of the males, randomly selected birds (n = 30 / group and genotype) were dissected to determine slaughter weight (SW) and carcass composition. Additionally, laying performance (quantitative and qualitative) of the hens was recorded and evaluated in frequent intervals during the laying period. Average BW and SW of cockerels at d 85 was 2,650 (SD: 262.1) and 1,786 g (SD: 185.3) in LD and 2,176 (SD: 263.9) and 1,449 g (SD: 179.3) in LDex, respectively. Over the fattening period, average daily weight gain was 34.6 g in LD and 29.7 g in LDex. Percentage of breast muscle (16.3 %) and legs (32 %) did not differ between genotypes. In 68 weeks of production, average laying performance (excluding molting) was 62.2 % in LD and 66.9 % in LDex; an average LD hen produced 289 eggs whereas it was 307 eggs in LDex. Average egg weight during 68 weeks of production was around 60.5 g in both genotypes, whereas LD hens had a higher feed consumption per kg egg mass (X:1) when compared with LDex (2.9 kg vs. 2.4 kg). The performance of both Dual Purpose Breeds is not comparable with those of specialized hybrids. The use of such breeds may still be sufficient if the products realize higher prices at the market as their production is more expensive and less efficient when compared to specialized hybrids. However, when taking animal welfare and ethical aspects into account, the use of Dual Purpose Breeds may still be considered as one potential strategy to avoid culling of day olds.

INTEGRATED STRATEGIES TO CONTROL MEAT QUALITY DEFECTS

ID : 270

NEW GENES OF INTEREST IDENTIFIED FOR MEAT QUALITY IN CHICKEN

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Spectacular gains in the productive capacities of meat-type chickens have been accompanied by important changes in their morphological and metabolic characteristics, but also by the appearance of increasing meat quality defects, such as acid or DFD meat conditions, and more recently white striping (WS) and wooden breast (WB) muscular disorders. While many omics studies have been conducted during the last decade in order to identify the underlying biological pathways, they do not allow distinguishing consequences from causes. Genetic studies can contribute to identify candidate genes at the origin of the defects as well as polymorphisms that could be used for marker-assisted selection. Two chicken lines divergently selected for the breast meat ultimate pH (pHu) were used in order to map the genomic regions controlling this trait as well as WS defect. Results indicated a polygenic inheritance of meat defects but suggested a few outstanding regions and candidate genes involved in human glycogen storage diseases. This was the case for PPP1R3A, SLC37A4, GAA, and PHKA1, which were evidenced within selection signatures or QTL (Quantitative Trait Loci) regions controlling pHu. A few WS-QTLs were also identified, whose gene content analysis suggested positional and functional candidates, such as LRSAM1 at the origin of a form of Charcot-Marie-Tooth hereditary neuropathy, characterized by progressive distal muscle weakness and atrophy in human. Other genes such as MYH1F and MYH15, involved in muscle development and fibre regeneration, appeared as other promising candidate genes. Although divergent selection for breast meat ultimate pH had led to a correlated response on susceptibility to WS (with an increased incidence in pHu+ line), no common QTL controlling both pHu and WS phenotypes was evidenced. Nevertheless, the genetic control of WS appeared to be in part dependent on the energy status of the muscle, since the WS-QTLs identified in the pHu+ line were not recovered in the pHu- line. This highlights that complex gene by gene interactions could contribute to the establishment of this muscle physiopathology.

In conclusion, these studies suggested several candidate genes whose variability in expression or functionality could predispose to meat quality defects in chicken. They provide a first basis for future works aimed at validating their causal role and identifying candidate mutations by taking advantage of the sequence data already acquired on the pHu lines.

ID : 300

FEEDING HIGHER DOSES OF L-HISTIDINE FOR DECREASING BREAST MUSCLE MYOPATHIES IN BROILER CHICKEN: A USEFUL APPROACH?

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INTRODUCTION

The high demand for white meat has led to modern broiler chicken reaching the slaughter weights in shorter times. Now the industry is confronted with several muscle defects like white striping (WS), woody breast (WB) and spaghetti meat (SM) leading to losses of high-quality meat for the market. Several studies describe high metabolic stress and excessive formation of reactive oxygen species in affected muscles. In a previous experiment, we showed that feeding high amounts of L-Histidine (His) can increase the concentration of the natural antioxidant carnosine in skeletal muscles of broilers. In this study, the effect of feeding different dosages of His to the outcome of meat quality defects in broiler breast filets was investigated.

MATERIALS AND METHODS

One-day-old male Ross 308 broilers (n = 440) were randomly divided into five treatment groups. Birds were fed ad libitum and four feeding phases were scheduled. The feeding groups were given different ratios of His:Lys (41, 44, 47, 50 and 54). Performance was determined at the end of each feeding phase and all birds were slaughtered at 38 days of age. Breast myopathies (WS, WB, and SM) were graded (normal, moderate or severely affected) and pH was measured on all right filets directly after slaughter and after 24 h. Pale-soft and exudative (PSE) and dark, firm and dry (DFD) meat was also recorded. Drip loss was measured at Day 1, 2, 4, 6 and 8 of storage at 2 °C by using the left filets of two randomly selected birds of each pen. Data were analyzed with Minitab18© by using the χ^2 -test for grading and Kruskal-Wallis-test or one-way ANOVA for all other data. Significance was declared at $p \leq 0.05$.

RESULTS

Performance and drip loss were not different between the feeding groups. At a His:Lys ratio of 50 PSE meat and lower pH were more frequently observed than in all other treatments 24 h after slaughter. WS and WB were present in 45 % and 72 % of the filets, respectively. Both myopathies showed a trend to lower incidences by feeding a ratio of 44 compared to all other groups, whereas a ratio of 47 showed the highest incidence.

CONCLUSION

Feeding His can impact meat quality of broiler breast filets. A moderately higher amount of His tended to decrease the incidence of WS and WB. However, the negative impact by feeding a His:Lys ratio of 47 must be considered. Including higher His levels than currently recommended in broilers' diets while balancing the His:Lys ratio could help to manage quality problems.

ID : 317

MOLECULAR PHENOTYPING OF WHITE STRIPING AND WOODEN BREAST DEFECTS

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White striping and wooden breast defects are two myopathic syndromes whose occurrence increases in modern fast-growing broilers. The impact of these defects on the quality of the meat is very important as they greatly affect its visual aspect, nutritional value and processing yields. The research conducted to date has improved our knowledge of the biological processes involved in the occurrence of these meat defects, but for now, no solution has been identified to reduce significantly their incidence without affecting growing performance. This study aims to follow the evolution of molecular markers linked to fast growing and appearance of defects in order to identify potential biomarkers for diagnostic purposes but also to improve our understanding of physiological dysregulation involved. This was done through enzymatic, histological and transcriptional approaches by considering slow- and fast-growing breast muscles, affected or not by WS and WB. Mitochondrial activity and ROS production were respectively lower and higher in the fast growing muscles compared to slow growing ones. Among the markers linked to muscle remodeling, the immunohistochemical staining of several genes involved in fibrosis and muscle fiber regeneration or necrosis was higher in fast growing compared to slow-growing muscles. For three of them, their amount also increased lineary with the presence and severity of WS and WB defects, making them potential biomarkers to accurately assess their presence and severity. The muscular expression of several genes implicated in the development of connective tissue and in the regulation of the myogenesis process was also positively linked to the presence and severity of the defects. Our results demonstrate a metabolic dysregulation and oxidative stress in fast-growing chickens and muscle remodeling associated to the defects in a quantitative manner. They also suggest several molecular biomarkers, whose potential for the development of diagnostic tests have now to be validated in other genotypes and experimental contexts.

ID : 420

RELATIONSHIPS BETWEEN MUSCLE WATER PROPERTIES AND TEXTURE CHARACTERISTICS IN RAW INTACT BROILER PECTORALIS MAJOR WITH THE WOODY BREAST CONDITION

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Abstract Relationships between muscle water properties and texture characteristics were investigated in raw intact broiler breast fillets (pectoralis major) with the woody breast (WB) condition. Raw intact broiler breast fillets were collected from a commercial plant. Tactile characteristics of fillets were determined with both subjective scores (3-point scale) based on palpable hardness and rigidity and blunt Meullenet-Owens Razor Shear (BMORS). Water properties were determined with time-domain nuclear magnetic resonance (NMR). Muscle water properties or specific water components were determined using an inverse Laplace algorithm (CONTIN). Spearman correlation was used to estimate relationships between WB scores and NMR measurements and Pearson correlation for relationships between BMORS measurements and NMR measurements. Three water components, hydration water (T2b), intra-myofibrillar water (T21), and extra-myofibrillar water (T22) were identified in raw intact chicken fillets. There were significant relationships between NMR measurements ($P < 0.001$; $r > 0.45$) and WB scores and between NMR measurements and BMORS measurements ($P < 0.05$; $r \geq 0.24$) regardless of water component. The greatest Spearman correlation coefficients were found for relative contents of T21 and T22, which were -0.78 and 0.77, respectively, indicating that the extra-myofibrillar water (T22) may contribute to subjective tactile characteristics, palpable hardness and rigidity, of the fillets with the WB condition. The greatest Pearson correlation was noted for T21 relaxing time (0.72), indicating that the greater mobility of intra-myofibrillar water may contribute to the greater BMORS measurements of broiler fillets with the WB condition. These results demonstrate that muscle water properties may contribute to the specific texture characteristics of broiler pectoralis major with the WB condition.

Key Words: BMORS, intra-myofibrillar water, extra-myofibrillar water, NMR, wooden breast

ID : 521

MITOCHONDRIA-RELATED GENES AND MORPHOLOGICAL CHANGES OF MITOCHONDRIA IN CHICKEN PECTORALIS MAJOR WITH WHITE STRIPING AND WOODEN BREAST

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In recent years, broilers have suffered from muscle degeneration and fibrosis, known as white striping (WS) and wooden breast (WB). This degeneration has been suggested to result from hypoxia in the pectoralis major. In this study, we focused on muscle fiber mitochondria, the first organelles to be affected by ischemic stress. Samples collected from the pectoralis major were classified by fibrosis grade and analyzed morphologically and genetically.

Thirty-six 55-day-old broiler superficial pectoral muscles were sampled and graded according to the rate of tissue fibrosis as “normal” (n = 4), “mild” (n = 8), “medium” (n = 11) or “severe” (n = 13). Observations of muscle fibers were performed with optical microscope and transmission electron microscope (TEM). Quantitative polymerase chain reaction was used to analyze expression of genes that control mitochondrial fusion and fission - mitofusin-1 (MFN-1), MFN-2, optic atrophy-1 (OPA-1), and dynamin-1 related protein 1 (DRP-1). Expression of the apoptosis-related gene, caspase-3 (CASP3), was also analyzed.

As fibrosis grade increased, muscle fiber swelling, degeneration, and regeneration were observed. Muscle fibers with many small vacuole degenerations were observed at all fibrosis grades. TEM observation showed normal myofibrils and mitophagy in vacuolar muscle fibers. Mitochondria-related gene expression tended to decrease with increasing fibrosis grade, and significant expression differences between the normal and severe group were observed in all genes, except MFN-2. No significant difference was found in CASP3 expression.

Because mitophagy of muscle fibers is also seen in normal chicken muscle fibers, we propose that this is a physiological phenomenon particular to fast-growing broilers. Broiler muscle fibers affected by early ischemic stress are repaired by dynamic changes in mitochondria and mitophagy. However, when the ischemic state persists, repair mechanisms appear insufficient. With persistent ischemia, expression of mitochondrial-related genes decreases, muscle fiber necrosis and fibrosis rapidly progresses, and severe fibrosis occurs. This study suggests that ischemic stress leads to WS and WB. Suppression of ischemic stress during initial rearing of poultry could be a means to prevent the development of fibrosis.

ID : 961

BIOCHEMICAL CHANGES IN SPAGHETTI MEAT CHICKEN BREAST DURING REFRIGERATED STORAGE

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The aim of the study was to provide insight on the postmortem evolution of Spaghetti Meat (SM), as no data are currently available on the effect of SM on the proteolytic processes occurring during meat aging. During two trials, 30 severe SM and 30 unaffected (N) fillets were subjected to 7 days of refrigerated storage. At days 0, 3 and 7, myofibrillar proteins profiles, free calcium concentration, calpain activity as well as the degradation patterns of desmin and troponin T (TnT) were assessed on 5 different breasts per group. A two-way ANOVA was carried out considering group (G: N and SM), time (T: 0, 3, 7) and their interaction $G \times T$ as fixed effects and trial as a random effect. Overall, the SM myopathy had only a minor impact on the myofibrillar proteins profiles, while free calcium concentration, calpain activity as well as desmin and troponin-T proteolysis were not affected by the occurrence of the pathologic condition. As expected, postmortem time exerted a strong effect on all the measured traits. As the concentration of free calcium increased from day 0 to 7 ($d0 = 43.6 \mu\text{M}$; $d3 = 40.5 \mu\text{M}$; $d7 = 54.5 \mu\text{M}$; $P = 0.0392$), casein zymograms demonstrated a progressive decrease in the proportion of native μ/m calpain ($d0 = 70.1\%$; $d3 = 48.0\%$; $d7 = 29.4\%$) concomitant with the accumulation of its autolyzed form ($d0 = 29.9\%$; $d3 = 52.0\%$; $d7 = 70.6\%$; $P < 0.0001$). Concurrently, the electrophoretic profiles of myofibrillar proteins from both muscle conditions exhibited a similar progression of protein degradation. Western blots for desmin and TnT from both N and SM exhibited bands corresponding to intact proteins and degradation products, and their relative abundances were quantified. As a result of storage, the abundance of the intact desmin decreased ($d0 = 52.7\%$; $d3 = 41.6\%$; $d7 = 34.4\%$), coupled with the accumulation of its 39-kDa degradation product ($d0 = 47.3\%$; $d3 = 58.4\%$; $d7 = 65.7\%$; $P = 0.0001$). As for the degradation pattern of TnT, the bands ascribed to the protein intact form (41.7 – 39.9 kDa) reflected a tendency to decrease over time; at the same time, the 32.5 and 30.2 kDa fragments ascribed to TnT degradation products tended to accumulate ($P = 0.0559$). Overall, the lack of significant interaction effects between muscle condition and storage time suggested that the proteolytic processes taking place during the postmortem period are similar in SM and N breast meat.

ID : 1174

EFFECTS OF MAGNESIUM SUPPLEMENTATION ON BREAST QUALITY OF HEAVY BROILERS

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Wooden Breast (hardness of broiler fillet) is a myopathy occurring in heavy broiler breast. Even if it doesn't represent a food safety issue, it results in huge economic losses to the industry as it affects meat quality (hardness of the meat, lower water holding capacity). Magnesium (Mg) is known for its implication in multiple biological functions and particularly in muscle metabolism. Nevertheless, this element is not taken into account in poultry diets as their content exceeds the nutritional requirements. The aim of this study was to evaluate the effect of Mg supplementation using a commercial product (Optibreast®) on the occurrence of myopathies. A total of 400 broilers (Ross308-males) were randomly assigned to two groups, control and treatment group. The basal diet fed was similar for the two groups, except for the Mg supplementation for the treatment group. Magnesium supplementation was provided during growing and finishing periods by addition of 0.15% of Mg²⁺ of the basal diet. Growth performances were periodically registered. Magnesium supplementation had no significant effect on final bodyweight (average 3683g) and performances. In a precedent study, the occurrence of Wooden Breast was significantly lower with Mg supplementation (data not shown). Recently, it has been proven that using instrumental compression force to assess fillet hardness is a reliable tool to identify Wooden Breast. Regarding meat quality, at day 45, 40 broilers from each group were randomly selected, measuring fillet tenderness one day after slaughter (by physical method using a compression machine, 3 repeats per fillet). Compression force for control and treatment group was 9.93N and 8.13N ($p=0.054$), respectively. Hardness for control and treatment group was 3.35N/mm and 2.74N/mm ($p<0.05$), respectively. We can conclude that Mg supplementation had a significant positive effect on breast quality of heavy broilers.

INTERPRETATING THE CITIZEN'S AND CONSUMER'S EXPECTATIONS

ID : 116

OUTLINE OF RECENT BREEDERS' VIEW ON JAPANESE DOMESTIC FOWLS

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Through the breeding process in Japanese fowls, the eager selection based on the breeders' preferences for their fowls, was successful in creating the various morphological characteristics. It is expected that the relationships between the breeders' consideration and the characteristics of breeds are helpful to comprehend the process of domestication. However, the reason why some characteristics attracted the breeders, and the background of motivation for breeding, remain unclear. To understand the real relationships between preferences for the breeds and the characteristics of each breed, it is important that the outline of breeder's view on Japanese breeds are clarified. In this study, we attempted that the Japanese breeder's view on their breeds is elucidated by using the sociological methods.

To grasp the image of breeders' view and to determine the key question items, we conducted the pre-interview survey. Subsequently, the main interview survey was performed in 23 prefectures in Japan. Semi-structured interview was adopted with using the open-ended interview questions and designed 7 key questions. The study participants were Japanese fowl breeders who displayed their fowl in competitive exhibition. The conversation between the participants and the interviewer was recorded by video and the contents were dictated. The dictated sentences were analyzed by using text mining method, and the non-parametric multivariate methods were performed for comparing the breeders' opinion from the extracted words. Because the breeders provided care for their family's fowls from childhood, they had been wanting to breed their own fowls. On the other hand, the competitive exhibition was the opportunity to the breeders for cultivating the potential new breeders. The principal purpose of breeding was to admire the appearance (75%), however, some breeders showed the certain opinion for preservation of rare and region-specific breeds. Most of breeders value the shape of breeds in breeding (p

The recent breeders' opinions indicate the importance of the correspondence between the shape and significance in some breeds, and the conflict between their convictions and the predetermined constraints.

ID : 1442

STAKEHOLDERS' PERCEPTIONS OF LAYING HEN WELFARE: EMPIRICAL EVIDENCE FROM SERBIA

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Improvement of the laying hen welfare legislation in developed countries so far has been mainly driven by citizens' expectations and market demands. However, successful transition to housing systems with improved welfare has been achieved only in those countries where support of all relevant stakeholders is provided. In the non-EU countries, the animal welfare legislation applying to egg production systems is overall less comprehensive and detailed than the legislation in the European Union. Even though Serbia, being a candidate country for the EU accession, makes serious efforts to gradually align national legislation with the EU entry requirements no significant achievements in this sense have been done so far. Even though from the 1st of January 2021, keeping laying hens in battery cages will be banned in Serbia, the vast majority of laying hens is still in classical, non-enriched cages. Therefore, the transition to permitted systems needs support of different egg sector stakeholders. Yet, little is known about their perceptions of animal welfare. The main aim of this paper was to examine the main egg sector stakeholders' perceptions of laying hens welfare. For this purpose, the quantitative research has been done through a survey with 545 different stakeholders: 57 farmers, 157 poultry specialists i.e. veterinarians and extension agents, and 331 citizens. Since observed variables are categorical, to determine whether there are statistically significant differences in stakeholders' perceptions of laying hen welfare a Chi-Square test has been used. The results obtained show that the welfare of laying hens is of great importance for the majority of respondents (87%). However, the willingness to support welfare by paying a higher price for eggs produced in non-cage systems is significantly less. On average, 67% of respondents are willing to pay a higher price. Testing of differences amongst different stakeholders groups has shown that there are no statistically significant differences, both in terms of importance of animal welfare ($\chi^2(2, n=545) = 0,793, p = 0,673$ Cramer's $V = 0,038$) as well as in terms of their willingness to support animal welfare through higher eggs prices ($\chi^2(2, n=545) = 0,106, p = 0,948$ Cramer's $V = 0,014$).

MANAGEMENT OF BROILER BREEDERS

ID : 27

MODELLING LIFE-TIME ENERGY PARTITIONING IN BROILER BREEDERS WITH INCREASED BODY WEIGHT AND DIFFERING REARING PHOTOPERIODS

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Understanding energy partitioning in broiler breeders is needed to provide efficiency parameters for breeding goals. This study compared 4 models partitioning metabolizable energy (ME) intake to body weight (BW), average daily gain (ADG), and egg mass production (EM) and described effects of BW and photoperiod on energy partitioning. Ross 708 broiler breeders (n = 180) were kept in 6 pens, controlling individual BW of free run birds with precision feeding stations. Half of the birds in each chamber were assigned to the breeder-recommended target BW curve (Std) or to an accelerated target BW curve reaching the 21-week BW at week 18 (High). Pairs of chambers were randomly assigned to 8L:16D, 10L:14D, or 12L:12D rearing photoschedules and photostimulated to 16L:8D at week 21. Hens were palpated daily to identify the presence of hard-shelled eggs to determine age at first egg and egg production. Eggs matched with individual hens were weighed. Model [1] was: $MEI = a \cdot BW^b + c \cdot ADG \cdot BW^d + e \cdot EM + \epsilon$, where MEI = daily ME intake (kcal/d); BW = BW (kg); ADG = ADG (g/d); EM = EM (g/d). Models [2-4] were the same as model [1], except that individual [2], age-related [3], or individual and age-related [4] variation was separated from other sources of random variation by including 1 [2,3] or 2 [4] normally distributed random term(s) associated with maintenance requirements (a). Model parameters were estimated with SAS PROC NLMIXED and treatment differences with PROC MIXED. The mean square error was 2,111, 1,532, 1,668, and 46 for models [1-4] respectively, inferring extra random variation was explained by incorporating 2 random terms. Standard deviation was 20.1 kcal/kg^{0.58} associated with age and 10.9 kcal/kg^{0.58} with the individual, indicating twice as much variation was explained by age compared to the individual bird. Estimated ME partitioned to maintenance was 130.6 ± 1.15 kcal/kg^{0.58}, and the ME requirement for ADG and EM were 0.63 ± 0.03 kcal/g/kg^{0.54} and 2.42 ± 0.04 kcal/g, respectively. Maintenance estimates were 123.4 and 137.8 kcal/kg^{0.58} for Std and High BW treatment (P < 0.001), and 134.4, 132.2, 125.3 kcal/kg^{0.58} for the 8L:16D, 10L:14D, or 12L:12D treatments, respectively (P < 0.001). Although hens on the Std treatment with a 12L:12D rearing photoschedule were most energetically conservative, their reproductive performance was the poorest. The proposed model provided a biologically sound estimation of life-time energy partitioning in broiler breeders.

ID : 205

EFFECTS OF DIET DENSITY AND FEEDING FREQUENCY ON PERFORMANCE OF BROILER BREEDER PULLETS

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Broiler breeders need to be fed restricted to prevent health and production problems, especially during the rearing period. However, little information is available on production performance during the pullet phase. Therefore an experiment was conducted to evaluate the effects of diet density and feeding frequency on performance of broiler breeder pullets. In total, 960 female pullets (Ross 308) were randomly allotted to 24 floor pens (40 pullets/pen) to 4 different treatments within a 2 × 2 factorial completely randomized block design. From 3 weeks onwards, pullets received either control diets (CON) or diluted diets (DIL) containing oat hulls. Besides the diluted diets, pullets were fed once (FO) or twice (FT) a day. The FO pullets were fed at 0815 h (100% daily amount of feed) and the FT pullets at 0815 h (60% daily amount of feed) and 1215 h (40% daily amount of feed). Pullets were fed a 5-phase feeding program. The pens contained plastic slats (25%), 2 feeding troughs and 7 drink cups above the slats. Birds of all treatments were fed to reach the same target body weight (BW). To meet target BW, DIL-pullets received a 16% higher amount of daily feed. Body weight was measured weekly and BW uniformity (CV) at 10 and 20 weeks of age. Water intake was measured weekly and cumulative water, feed intake and water/feed ratio were calculated. Litter quality (friability and wetness) and dry matter (DM) content were measured at 10, 15, and 20 weeks of age. At 20 weeks of age litter and manure (below slats) production was determined. The data were analyzed using ANOVA. Pullet BW and uniformity were not affected by diet density at 10 and 20 weeks of age. The similar water intake and higher feed intake resulted in a lower water/feed ratio (2.11 vs. 2.50 mL/g; $P = 0.032$) for the DIL-pullets, resulting in a higher DM content (751 vs. 699 g/kg; $P < 0.001$), improved litter quality and 20% higher manure production. The FT-pullets showed a lower BW CV (11.5 vs. 14.2%; $P < 0.001$) at 10 weeks of age, however, no effect was found at 20 wk of age. Feed intake, water intake, water/feed ratio, DM of the litter, litter quality and manure production were not affected by feeding frequency. In conclusion, diluted diets during the rearing period resulted in a higher feed intake, lower water/feed ratio, improved litter quality and higher manure production. Furthermore, twice a day feeding improved BW uniformity halfway the rearing period.

ID : 235

UTILIZATION OF "DILUTED SPLITFEEDING SYSTEM" IN ADULT BROILER BREEDER HENS

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Broiler breeders hens are feed restricted during all their live to ensure their health and performance. However, it has a negative effect on bird welfare as birds could be chronically hungry. On the other hand, broiler breeders hens are fed once a day mainly during the morning and this approach might not be ideal. The called Splitfeeding system in laying hens provides an optimal supply of nutrient requirements using two different diets throughout the day, according to egg formation phases, improving eggshell quality and sustainability. This study was conducted to assess whether the concept of Splitfeeding combined to a lower quantitative feed restriction might be utilized in broiler breeders as a potential strategy to alleviate hunger feeling and improve performance in the last phase of production cycle. A total of 2560 breeder females 53 weeks aged were allocated in 20 pens for 10 weeks. Ten of this pens received a single control diet (165 g/henxday; 11.4MJ ME/kg, 14% CP, 3.2% Ca, 0.28 %dP) at 6:00 am whereas the other 10 pens followed a diluted splitfeeding program (DSF) which had two meals, one with a morning diet given at 6:00 am (95 g/henxday) and an afternoon diet supplied at 13:00 pm (95 g/henxday). DSF was formulated with a mixture of fiber sources to provide less AME (-1%), CP (-2%), Ca (-2%) and dP (-11%) than the control diet. Animals who received DSF had a greater egg production, hatching eggs and chicks produced compared to the control diet, without negatively affecting egg shell quality. The higher total feed intake and the lower fasting period with DSF increased time spent eating and reduced stereotypic object pecking, which may indicate a reduced hunger feeling and frustration of feeding motivation. Finally, the use of DSF affected positively to economics, decreasing the production cost per chick hatched up to 10.5%.

ID : 525

PREFERENCE FOR DIFFERENT TYPES OF RESTING AREAS IN BROILER BREEDERS

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Chickens prefer to sleep on an elevated area during the night. In addition, chickens also use elevated resting areas for fleeing, comfort behaviour and thermoregulation during the day. Current national regulations do not require elevated resting areas for broiler breeders during rearing. However, increasing concerns about animal welfare are pushing towards including perches in broiler breeder husbandry, although hardly any research has been done on broiler breeders' preferences for resting areas. We observed the preference for particular resting areas in female Ross 308 broiler breeders, housed in 24 floor pens (40 pullets/pen at d0) with 4 feeding treatments in a 2x2 factorial design. From 3 weeks of age onwards, pullets received either control diets (CON) or diluted diets (DIL) containing oat hulls and they were fed once (FO) or twice (FT) a day. The FO pullets were fed at 0815 h (100% amount of feed) and the FT pullets at 0815 h (60% amount of feed) and 1215 h (40% amount of feed). The pens contained elevated plastic slats (25% of the floor area), 2 plastic mushroom shaped perches (2m), wood shavings as litter material, 2 feeding troughs and a drinking line (7 cups) above the slats. From 16 to 40 weeks of age, the number of animals per resting area (litter, slats, perches, drinking line and nest (from 22 weeks of age)) were counted biweekly, half an hour after lights went off. Data were analysed with analysis of variance as a randomised block design. During rearing (until 21 weeks of age) 51% of the pullets rested on the slats, 33% on the perches, 9% on the drinking line and 7% on the litter. During production, more hens tended to rest on the litter (10%; $p=0.09$) and less hens rested on the perches than in rearing (25%; $p<0.001$). This shift is probably due to use of the nests as an additional resting place. The number of hens resting on the slats (49%) and drinking line (9%) was similar to the rearing phase. Interestingly, in both rearing and production, FT animals rested more on the slats than FO animals (45 vs 52%; 36 vs. 65%, $p=0.002$). During rearing, this effect tended to be enhanced by diluting the diet ($p=0.067$). As a consequence, FT animals rested less on the perches than FO animals ($p<0.001$) in both phases (29 vs 20; 43 vs. 23%; $p=0.014$). In conclusion, broiler breeders preferred to rest on elevated slats compared to perches and litter. Feeding management seems to influence the preferred resting area.

ID : 1296

IMPACT OF AGE AND BODY WEIGHT OF BREEDING HENS ON EGG QUALITY TRAITS IN BROILER BREEDERS

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Several factors can affect the poultry egg characteristics, including hen age, environment, nutrition and genetics. Breeding dam's body weight and age has strong association and impact on hatching egg quality. A study was conducted to see the impact of broiler breeder age (30 versus 60 weeks) and body weight (standard, low and high weight) on egg quality traits in ROSS-308 broiler breeders. One thousand and fifteen hundred Ross308 females and one hundred and fifty males (male to female ratio 1:10) were kept with females of standard body, under-weight and over-weight females were kept with 500 females in each group. All birds were selected and managed separately in the same shed. Birds were fed according to management guide book of Ross308(Aviagen).

All hens were weighed at the start of the trial. Egg production was recorded on daily basis. Eggs were collected 8 times in a day. The collected eggs were classified as 'normal' or 'damaged'; the letters included the following: broken eggs and cracked eggs. To measure egg quality traits, 250 eggs from each treatment groups (50 from each replicate) were used at second day of collection. Egg quality traits like egg mass, chick weight, shape index, albumen weight, albumen height, yolk weight, yolk weight, yolk diameter, yolk index, color index, Haugh unit, shell weight, shell thickness, egg length and egg diameter were measured according to the prescribed scientific method. The data was gathered for investigation of variance (ANOVA) with Completely Randomized Design with the assistance of SPSS-16.00

Data showed that the age of breeding hens has significant effect on egg traits as compare to body weight of hens. Egg weight, chick weight, shape index, yolk height, yolk weight, albumen weight, yolk index, yolk color, Haugh unit, shell weight, shell thickness, egg radius and egg diameter has significant ($P<0.05$) effect on all internal and external egg traits in all breeding hens. It is concluded that the age of breeding hens has significant impact on egg quality and chick quality traits.

ID : 1384

OMEGA-3 FATTY ACID SOURCES FED TO BROILER BREEDERS: IMPACT ON GROWTH PERFORMANCE OVER HATCHES

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Enriching broiler breeder (BB) diets with omega-3 fatty acids such as α -linolenic acid (ALA) and docosahexaenoic acid (DHA) may impact progeny (PR) growth performance. The effects of BB age and enrichment of diets on PR growth performance parameters were examined. A total of 588 BB pullets (Ross 708) and 60 cockerels day-old were placed in separate rearing pens (28♀ and 20♂/pen) and fed three diets: 1) corn and soybean meal control (CON), 2) CON + source of DHA or, 3) CON + source of ALA. Birds were fed (♀ pens; n=9 for CON & n=6 for DHA/ALA) to wk 22, moved to a laying house and grouped based on rearing diets (60♀ and 10♂/pen). Birds reared on CON were split into: CON-CON, CON-DHA and CON-ALA. Birds reared on DHA and ALA either continued these diets or switched to CON. Fertile eggs (210/diet) were collected and incubated at wk 34, 44 and 54. Chicks were placed in cages (8/cg) based on BB diet, sex and BW. PR diets were (BB rearing-BB laying-PR diet): CON-CON-CON, CON-DHA-DHA, CON-DHA-CON, CON-CON-DHA, CON-CON-ALA, CON-ALA-CON, CON-ALA-ALA, DHA-DHA-DHA, DHA-DHA-CON, DHA-CON-DHA, DHA-CON-CON, ALA-ALA-ALA, ALA-CON-CON, ALA-CON-ALA and ALA-ALA-CON. Feed intake (FI) and BW data was collected on d0, 10 and 42. Body weight gain (BWG) and mortality corrected feed conversion ratio (FCR) were calculated for d 10 and 42. On d 42 a subsample of birds were sampled for breast yield. Data was analyzed on a factorial arrangement of diet, maternal age and progeny sex. There was no ($P>0.05$) interaction between BB age, diet and PR sex on measured variables. An interaction between BB age and diets was such that BWG of all progeny from wk 54 BB were significantly heavier regardless of diet. Males from wk 54 BB were the heaviest at hatch ($P<0.001$). However, progeny from 44 wk BB were heaviest at d 10 and 42 with no diet effect ($P<0.001$). There were no diet effects for any measured parameters. Progeny from wk 44 BB had the lowest FCR at d 10 and progeny from wk 54 BB had the lowest FCR at d 42 ($P<0.001$). Males were heavier than females at d 0 and 10, but no differences were seen by d 42 ($P<0.001$). Progeny from wk 44 and 54 BB had heavier breast muscle yield than progeny from wk 34 BB ($P<0.001$). Lastly, females had heaviest breast muscles at d 42 (g/kg, $P=0.027$). These data indicated that BB age and PR sex consistently influenced PR growth performance. Enriched diets for the BB and/or PR with ALA and DHA did not have consistent effects on BW gain, FCR or FI.

ID : 1386

OMEGA-3 FATTY ACIDS SOURCES FED TO BROILER BREEDERS THEIR PROGENY: IMPACT ON LESION SCORES AND IMMUNE ORGAN WEIGHT IN BROILER CHICKENS UPON COCCIDIOSIS CHALLENGE

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Enriching broiler breeder (BB) diets with omega-3 fatty acids such as α -linolenic acid (ALA) and docosahexaenoic acid (DHA) may impact progeny (PR) immune response. The effects of BB age and enrichment of diets on PR response to coccidiosis challenge were examined. A total of 588 BB pullets (Ross 708) and 60 cockerels day-old were placed in separate rearing pens (28♀ and 20♂/pen) and fed three diets: 1) corn and soybean meal control (CON), 2) CON + source of DHA or, 3) CON + source of ALA. Birds were fed (♀ pens; n=9 for CON & n=6 for DHA/ALA) to wk 22, moved to a laying house and grouped based on rearing diets (60♀ and 10♂/pen). Birds reared on CON were split into: CON-CON, CON-DHA and CON-ALA. Birds reared on DHA and ALA either continued these diets or switched to CON. Fertile eggs (210/diet) were collected and incubated at wk 34, 44 and 54. Chicks were placed in cages (8/cg) based on BB diet, sex and BW. PR diets were (BB rearing-BB laying-PR diet): CON-CON-CON, CON-DHA-DHA, CON-DHA-CON, CON-CON-DHA, CON-CON-ALA, CON-ALA-CON, CON-ALA-ALA, DHA-DHA-DHA, DHA-DHA-CON, DHA-CON-DHA, DHA-CON-CON, ALA-ALA-ALA, ALA-CON-CON, ALA-CON-ALA and ALA-ALA-CON. Chicks were challenged with a mixed *Eimeria* culture on d 10. Duodenal and jejunal lesion scores were assessed on d 15. Organ (liver, spleen and bursa) weights were assessed on d 15 and 42. Data was analyzed on a factorial arrangement of diet, maternal age and progeny sex. There was no ($P>0.05$) interaction between BB age, diet and PR sex on measured variables. Diet effect was noted for d 15 liver weights with DHA-CON-CON treatment having heavier ($P=0.032$) liver than ALA-CON-CON and DHA-DHA-CON diets. ALA-ALA-CON treatment had heavier ($P=0.001$) bursas than CON-ALA-CON and DHA-CON-DHA at d 42. An interaction between BB age and sex was male PR reporting heavier bursa than female PR from 34 and 44 wk old BB. At d 15, female PR from 54 wk BB had the heaviest spleens and female PR from wk 44 BB had the lightest ($P=0.044$). An interaction ($P=0.043$) between BB age and diet was observed on jejunal lesion scores with the greatest ($P<0.001$) lesion scores observed in PR from 54 wk old BB. The PR from 54 wk old BB showed heavier ($P<0.05$) d 42 spleen and liver. These data indicated that BB age and PR sex influenced PR responses challenge. Enriching diets for BB and/or PR with ALA and DHA did not have consistent effects on intestinal and immune organs response in broiler chicks challenged with coccidiosis.

MEDITERRANEAN POULTRY NETWORK

ID : 243

REPRODUCTIVE PERFORMANCES IN FOUR ITALIAN LOCAL CHICKEN BREEDS: LIVORNO, SICILIANA, MUGELLESE AND VALDARNESE

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In recent years the interest in saving biodiversity and local breeds has grown and become a matter of great importance (Franzoni et al., 2018). The aim of this study was to characterize, evaluate and compare the reproductive performances over the first breeding season of five Italian local chicken populations of four different local breeds: black Livorno (BL), white Livorno (WL), Siciliana (S), Mugellese (M) and Valdarnese (V). The sex ratio for each population was 1:8.

Birds were housed in outdoor pens (2,5 m²/bird) and fed ad libitum with a commercial breeder diet. A total of ten incubations were performed for each breed during the reproduction season from 39th to 57th week of age. Eggs were collected twice a day, labeled and stored for maximum 7 days in an environmental controlled room (16°C, 75% RH). A batch of minimum 25 eggs for each considered population was incubated following good hatchery practices.

At 7 and 18 days of incubations two candlings were performed. All the removed eggs were open to record true fertility (TF) and the exact age of death in days for each embryo, thus to assess early mortality (eM, 1-4 days), early middle (emM, 5-10 days) and late middle (lmM, 11-17 days) mortality. At the end of each incubation, hatchability (H) and late mortality (IM, 18-21 days), assessed according to the same procedure, were determined.

All the recorded data were analyzed by Chi-squared test using the statistical analysis software JMP 9.0.1 (SAS institute Inc., 2009, Cary, North Carolina, USA), for evaluating the age effect within each population and comparing populations at the same age. Differences (p

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ID : 600

EFFICACY OF BIVALENT RHVT-ND-IBD VECTOR VACCINE AGAINST CHALLENGE WITH VELOGENIC NEWCASTLE DISEASE VIRUS GENOTYPE –VII.1.1 IN COMMERCIAL BROILERS

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Newcastle disease virus (NDV) is a major threat to the poultry industry worldwide, with a diversity of genotypes associated with severe economic losses in all poultry sectors. Class II genotype VII.1.1 NDV are predominant in Middle East and Asia, despite intensive vaccination programs using conventional live and inactivated NDV vaccines. In Egypt, the disease is continuously spreading causing severe economic losses in poultry. In this study the protective efficacy of commercial bivalent herpes virus of turkey-Newcastle disease and infectious bursal disease (bivalent rHVT-ND-IBD) vector vaccine against challenge with velogenic NDV strain (Chicken/USC/Egypt/2015) was evaluated in commercial broiler chicks. Four vaccination regimes were used; live NDV genotype II (clone 30) vaccine on day 1 and 14 (G1, G2, G3, G4) with either inactivated NDV genotype II vaccine on day 7 (G1) or bivalent rHVT-ND-IBD vector vaccine on day 1 (G3) or both vaccines on day 1 and 7 (G2), in addition non-vaccinated group (G5). All groups were challenged on day 28. The protection conferred by all vaccines regimes was evaluated after experimental infection based on mortality rate, clinical signs, gross lesions, seroconversion and the mean lesion scores (MLS) of histopathological changes in brain, trachea, spleen, kidneys, bursae and thymus at 14 days post-challenge (dpc). The results showed that the mortality rates were 2.5%, 0%, 15% and 42.5%, respectively, in G1, G2, G3 and G4 vs 100% in G5. Moreover, the MLS were 1.3, 1, 1.8 and 2.2, respectively, in G1, G2, G3 and G4 vs 3.0 in G5. It was concluded from this study that a complete clinical protection against challenge with genotype VII.1.1 NDV was revealed in commercial broiler chickens vaccinated with live NDV genotype II and both the inactivated NDV genotype II and bivalent rHVT-ND-IBD vaccines. Upon that the combination of bivalent rHVT-ND-IBD with live and inactivated ND vaccines in commercial broilers is recommended to obtain optimum clinical protection against genotype VII.1.1, especially in endemic countries.

PLURIDISCIPLINARY APPROACHES TO REACH THE ONE HEALTH OBJECTIVES

ID : 128

EFFECT OF DIETARY INDIGESTIBLE CRUDE PROTEIN SUPPLY ON THE DEVELOPMENT OF FOOTPAD DERMATITIS IN BROILERS OVER TIME.

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There is interest in substituting soybean meal (SBM) in European broiler diets by homegrown protein sources such as rapeseed (RSM) and sunflower meal (SFM) and in reducing nitrogen (N) excretion to ameliorate broiler farm environmental impact. Although, footpad dermatitis (FPD) development is augmented by increased CP content, effects are confounded by changes in electrolyte balance (DEB), increased weight gains, imbalances in EAA:Lys ratios and variable grain source soluble NSP content. Herein, we offered diets with similar digestible CP (dCP) but different indigestible CP content (inCP), whilst accounting for these factors. We hypothesized that at decreasing inCP levels, broilers would achieve similar ADG but show reduced FPD development and improved litter quality.

1800 male Ross 308 day old chicks were housed in 48 pens and were offered diets with low (L), medium (M) or high (H) levels of inCP with increments of $\approx 0.5\%$ inCP, over the grower (d10-21), finisher1 (d21-30) and finisher2 (d30-42) periods. M diets were SBM based, whilst RSM and SFM partially replaced SBM in L and H diets, respectively. We assessed FPD on d21, 29 and 41, litter N content, and temperature (T) on d21 and 29, and litter DM content on d21, 29 and 41. Data were analysed with diet as a factor and pen as the replicate with the GLM procedure.

Diet significantly affected ($P<0.01$) ADG, ADFI and FCR over finisher2 and overall growing periods; H diets resulted in significantly greater FCR and smaller ADG than both L and M diets, whilst ADFI was significantly higher for L diets than both H and M diets. FPD was significantly lower ($P<0.05$) for L than M diets and both M and H diets at d29 and 41, respectively. Litter DM content was significantly greater ($P<0.05$) for L in comparison to H diets and was intermediate for M diets, at all dates. Litter N content and T were unaffected.

In conclusion, formulating diets low in inCP content ameliorated FPD severity as early as 29 days of age. Furthermore, they improved performance during the finisher2 and overall growing periods. This may be due to a negative impact of excessive inCP on gut health, an effect of FPD severity on performance, or both. Future studies should investigate other primary ingredients within the same concept and to elucidate to what extent effects on performance are attributed to the level of systemic and local inflammation.

ID : 242

BIOSECURITY IN POULTRY FARMING: A PARTICIPATORY APPROACH TO PROMOTE COMPLIANCE WITH BIOSECURITY MEASURES

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Biosecurity is defined as all measures taken to prevent both the introduction and the spread of disease agents on the farm and between them. Consecutive to the influenza avian outbreaks in France, a reinforcement of legislation occurred, requiring poultry keepers to apply strictly biosecurity measures. However, prescriptive approaches could have limitations as regards their effective compliance due to a lack of understanding, but most of all to the difficulties encountered in implementing them. The project PartAge aims to propose an alternative approach using participatory methods, for a sustainable compliance.

Two groups of 11 farmers each were included in the project. Qualitative interviews were carried out during the first two steps: an initial diagnosis in 2018 and a midterm evaluation in 2019, with a participatory meeting between the two steps. For the first step, the farmers' interview guide included the own farmer's perception of biosecurity and sanitary risk, his implemented practices, and the description of improvement paths possible on the farm. For the second step, the interview guide included a feedback on the participatory meeting, the impact of this meeting on knowledge, attitudes and practices, and the expectations of farmers for a next participatory meeting.

Initial diagnosis showed two different farmer's views about "biosecurity": "a common sense, normal practices", vs "standards, constraints that could be useful". The main motivation to implement biosecurity measures was the belief on their efficiency to avoid contaminations. However, farmers were uncomfortable with checking of external stakeholders practices, which was a real constraint for compliance at farm level. The levers and barriers to change appeared essentially material (financial, work organization). At the end of this first step, knowledge and working comfort appeared as interesting levers, which it seems possible to address during a participatory meeting. Then, the midterm evaluation revealed that the first participatory meeting had an overall but limited impact on knowledge, had changed the attitude of more than a third of the farmers and the practices of the majority of them. This is consistent with the plebiscite for sharing ideas between peers.

For the end of the project, two meetings scheduled in 2020 will permit to continue the exchange between peers. Partners aim to provide tools to implement participatory methods to improve biosecurity in poultry farming.

ID : 327

DUST EXPOSURE LEVELS IN DUCK OVERFEEDING SYSTEMS AND POTENTIAL RISK TO WORKERS' HEALTH

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Duck housing systems during overfeeding have changed considerably over the past 4 years, in line with animal welfare regulation. These new housing systems impact working conditions of farmers, who show greater respiratory discomfort due to 1. more aerial dust due to greater freedom for animal's movement and 2. drier ambient air that would favor the suspension of these dusts in the air. In this context, we are conducting a study (project AGAtE) on dust exposure levels and protective measures to prevent respiratory diseases induced by inhalation of particles. A part of the study consists in measuring the dust exposure levels in commercial overfeeding farms, during different working tasks. Thirty-one overfeeding farms and their workers were followed in 2018-2019 during 4 working tasks: animal arrival (A), feed preparation and overfeeding during the first 4 days (B), during the last 4 days (E), animal leaving (L). Measurements were repeated at two periods (November to February; April to August), i.e. 8 campaigns of measurement per farm. The farms produced ducks only and had a minimum of 600 overfeeding places in a closed building. Nine were located in the West and 22 in the South West of France. For each campaign, we recorded data related to: 1. quantities of inhalable (particles < 100 µm) and alveolar (< 5 µm) dust, using two CIP10 (TECORA®) carried by the worker at the level of the respiratory tract, 2. workers' activities during the measurements 2. indoor/outdoor temperature and hygrometry, 3. configuration of the building and settings of equipment, 4. animals' performances and their body condition.

We observed very variable levels of exposure between working tasks and, to a lesser degree, between farms within the same working task. The task A records the highest dust levels. For inhalable dust, the concentration ranges from 0.97 to 104.72 mg/m³ and for alveolar dust from 0.08 to 6.76 mg/m³. For this task, the variability among farms could probably be explained by great differences between farmers' activities (some push carts, others catch ducks, others take cages, ...). Staff from poultry catching companies, who carries out this task frequently, is thus particularly exposed to dust; these workers should be included in the prevention approach. Data on the measured dust quantities and the farm equipment will be analysed in order to propose preventive and corrective measures.

ID : 380

ANTIMICROBIAL RESISTANCE TRANSMISSION IN ANTIBIOTIC-FREE POULTRY PRODUCTION PYRAMID

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The transmission of antimicrobial resistant (AMR) bacteria from food-producing animals to humans is an important public health issue. The aim of this project is to characterize the vertical and horizontal transmission as well as the persistence of AMR in the broiler production pyramid without antibiotic selective pressure, considering all stages over three successive generations of animals and their associated environments, to identify new levels of risk control.

AMR monitoring was realized in an experimental poultry farm integrating all steps of the broiler production chain (from broiler breeder females to chicken at slaughterhouse). The commercial chicken line chosen was bred to study the quality of the meat. Fourteen representative parent layers and progeny during 3 generations were sampled including 2 sibling batches at each generation, one for reproduction purpose so reared in protected houses and one for meat production purpose so in standard conditions (group in floor pen). Fecal and caecal samples, incubated eggs, neck skin, and meat were collected as well as environmental samples (drinking water, feed, houses surfaces, water bath before plucking). AMR Gram-negative bacteria were selected on MacConkey agar supplemented with different antibiotics. Their antimicrobial susceptibility phenotype was determined by the disk diffusion method.

Fecal carriage of *Escherichia coli* and *Proteus mirabilis* with different multi-drug resistance (MDR) phenotypes was found in all flocks/generations of broilers. *E. coli* isolates with identical MDR phenotypes were found in successive generations suggesting vertical transmission while several others were associated to only 1 flock. More diverse MDR phenotypes were observed in broilers production flocks than in reproduction ones indicating an important role of the rearing conditions. Sequencing of representative MDR isolates and plasmids is ongoing to determine their genetic relationship. Environmental MDR bacteria identified in the experimental farm might contribute to transmission of resistance genes by mobile genetic elements. Moreover, metagenomic sequencing will be performed to analyze global caecal resistomes.

Our first results indicate that MDR bacteria are widespread and persist in the experimental poultry production pyramid, in spite of the non-use of antibiotics for a decade. Further investigations will be conducted to decipher the transmission of AMR bacteria and mobile genetic elements identified in this system.

ID : 492

THE EFFECTS OF D. VS. L-METHIONINE SUPPLEMENTATION ON GROWTH PERFORMANCE AND INTESTINAL HEALTH OF BROILER CHICKENS AFTER A CHALLENGE WITH EIMERIA SPP.

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The aim was to study the effects varying total sulfur amino acid (TSAA) levels supplemented with either D. vs. L-Methionine (Met) on the performance and intestinal health of broilers during the recovery phase after a single challenge with *Eimeria* spp. A total of 720 d-old-male Cobb 500 chicks were randomly distributed in a factorial arrangement 5 by 2 (6 replicates/12 birds). The factors were diets and challenge (CHA) or not (UCH) with *Eimeria* spp. The diets were negative control (NC – TSAA and Met deficient), 85% of TSAA supplemented with either D or L-Met (85D and 85L), and 100% of TSAA supplemented with either D or L-Met (100D and 100L). At d 14, the CHA group received orally 12,500 *E. maxima* and *E. tenella*, and 62,500 *E. acervulina* sporulated oocysts. At d 26 (14 dpi), BWG, FI, and FCR were measured, and intestinal samples were collected (1 bird/replicate) for morphology. Two-way ANOVA was performed, and means were compared by Tukey's test using SAS ($P < 0.05$). Considering the overall period (14-26d), interactions between treatments were found for FI and FCR ($P < 0.001$), whereas no interactions were found for BWG ($P = 0.456$). The FI of UCH birds fed 100% of TSAA (D and L-Met) was lower than the intake of UCH birds fed 85% of TSAA, regardless Met isomer. Additionally, birds fed NC in the UCH group had higher intake than the birds fed the same diet in the CHA group. The FCR of UCH birds fed 100% of TSAA (D and L-Met) was lower than that of birds fed 85L and NC in the UCH group, and lower than birds fed NC, 85% of TSAA (D and L-Met) and 100D in the CHA group. The BWG was affected by both challenge and diet. The UCH birds showed the highest weight gain ($P < 0.001$), and birds fed NC showed the lowest BWG ($P < 0.001$). No interactions were found for intestinal morphology traits. The duodenum of UCH birds showed higher villus height ($P = 0.007$) and crypt:villi ratio ($P = 0.011$) than CHA birds. The jejunum of UCH birds showed higher villus height ($P = 0.002$), lower crypt depth ($P < 0.001$), and higher villi:crypt ratio ($P < 0.001$) than CHA birds, whereas the ileum of UCH birds had lower crypt depth ($P = 0.006$) than CHA birds. In conclusion, the *Eimeria* spp. challenge reduced the performance and intestinal health during the recovery (14 dpi) phase. Additionally, the use of 100% of TSAA, regardless Met isomer, led to better growth and intestinal development, whereas no differences were seen between D and L-Met, suggesting that both isomers are equivalent after the challenge.

ID : 506

ANTIMICROBIAL RESISTANCE IN FECAL E. COLI AND SALMONELLA ISOLATES OF SMALL POULTRY FLOCKS IN ONTARIO, CANADA

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Small flocks have become increasingly popular in urban and rural areas of North America. Small flocks can pose a health risk to their owners by exposing them to antimicrobial resistant zoonotic pathogens. The objective of this study was to determine the AMR patterns of fecal E. coli and Salmonella enterica isolates of chickens, turkeys, waterfowl, and game birds from Ontario small flocks submitted for laboratory diagnostic testing between October 2015 and September 2017 because of production issues, morbidity, or mortality. From each submission, a pooled cecal sample was collected and tested for the presence of fecal E. coli and Salmonella. Three isolates from each positive E. coli sample and one isolate from each positive Salmonella sample were tested for susceptibility to 14 antimicrobials using a broth microdilution method. A total of 433 E. coli isolates (358 chicken, 27 turkey, 24 duck, and 24 game bird) and 5 Salmonella isolates (3 chicken, 1 turkey, 1 duck) were recovered.

For E. coli, a moderate to high proportion of isolates were resistant to tetracycline (43% chicken, 81% turkey, 42% duck, and 38% game bird isolates), streptomycin (29% chicken, 37% turkey, and 33% game bird isolates), sulfisoxazole (17% chicken, 37% turkey, and 21% duck isolates), and ampicillin (16% chicken and 41% turkey isolates). Multidrug resistance was found in 37% of turkey, 20% of chicken, 13% of duck, and 8% of game bird E. coli isolates. Streptomycin-tetracycline-sulfisoxazole-trimethoprim-sulfamethoxazole was the most commonly identified resistance pattern in the chicken E. coli isolates. The Salmonella isolates were most frequently resistant to streptomycin, tetracycline, and sulfonamides. For E. coli and Salmonella, there was a very low frequency of resistance to cephalosporins, carbapenems, macrolides, and quinolones — antimicrobials important in human medicine.

Escherichia coli isolates were frequently resistant to antimicrobials commonly used to treat poultry bacterial infections, which highlights the necessity of judicious antimicrobial use to limit the emergence of multidrug resistant bacteria. Results can be used to educate veterinarians and small flock owners about issues surrounding antimicrobial resistance to improve flock health and mitigate public health risks.

ID : 510

ANTIMICROBIAL RESISTANCE IN CAMPYLOBACTER ISOLATES OF SMALL POULTRY FLOCKS IN ONTARIO, CANADA

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Small flock farming has become increasingly popular in North America. Small flock poultry are used as a source of food and are also kept as pets, which can pose a health risk to flock owners through exposure to antimicrobial resistant zoonotic pathogens. The objective of this study was to determine the AMR patterns of Campylobacter isolates of chickens, turkeys, waterfowl, and game birds from Ontario small flocks submitted for laboratory diagnostic testing between October 2015 and September 2017 because of production issues, morbidity, or mortality. From each submission, a pooled cecal sample was collected and cultured for Campylobacter. Three isolates from each positive sample were tested for susceptibility to nine antimicrobials using a broth microdilution method. Cluster and multiple correspondence analyses were used to explore relationships among antimicrobials in terms of their similarity in Campylobacter resistance in the isolates from chicken samples.

A total of 176 isolates were recovered (141 chicken, 21 turkey, 6 duck, and 8 game bird). A high proportion of the *C. jejuni* isolates from chickens (77%) and turkeys (100%), and the *C. coli* isolates from turkeys (50%) and game birds (40%), were resistant to tetracycline. The odds of resistance to tetracycline were higher in the *C. jejuni* isolates (OR = 3.54, $P \leq 0.01$) than the *C. coli* isolates. There was a low frequency of resistance to quinolones and a very low frequency of resistance to macrolides. Antimicrobial susceptibility testing results for ciprofloxacin and nalidixic acid were clustered together. The high frequency of tetracycline resistance emphasizes the importance of prudent antimicrobial use in small flocks. Although resistance to macrolides and quinolones was uncommon, these are used to treat severe cases of campylobacteriosis in humans, highlighting the need for proper food safety and infection control practices by small flock owners to prevent exposure to antimicrobial resistant Campylobacter. Results can be used to educate veterinarians and small flock owners about issues surrounding antimicrobial resistance to mitigate public health risks.

ID : 548

DEVELOPMENT OF A METHOD FOR ASSESSING THE BIOACTIVITY OF PLANT EXTRACTS ON THE ANTIOXIDATIVE STATUS AND INNATE IMMUNITY IN CHICKEN

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The potential of plant extracts to strengthen the antioxidative status and innate immunity in chickens could lead to a reduction in the use of chemical antimicrobial drugs in poultry farms. The assessment of plant extract (PE) efficacy is generally directly tested in chickens using various *in vivo* protocols with lipopolysaccharide (LPS) challenge. The objective of this study was to develop a relevant, robust and as minimally as possible method to reproduce the oxidative stress and inflammation induced by LPS. An *ex vivo* method was evaluated using chicken whole blood stimulated *in vitro* with LPS and compared with a method based on blood sampled after LPS injection *in vivo*. Blood sampled from 20 days-old broilers was cultured for six hours in contact with LPS or not. *In vivo*, the chickens were injected with LPS and two blood samples were taken, one before the injection and one six hours later. Biochemical and molecular markers of redox balance and innate immunity were tested by biochemical and qRT-PCR assays and analyzed by ANOVA or Mann-Whitney or Kruskal-Wallis non-parametric analyses. Glutathione peroxidase activity and the oxidized/reduced glutathione ratio were only modified *in vivo* after LPS injection indicating the induction of an oxidative stress. The other biochemical indicators tested (uric acid concentration, superoxide dismutase activity, total antioxidant status, thiobarbituric acid reactive substrate, haptoglobin-like activity) were not modified whatever the method. IL1-b, IL-6, IL8(L2) cytokine and iNOS gene expressions were significantly increased by LPS for both methods, the range of gene expression being higher *ex vivo* than *in vivo*. This *ex vivo* method can be used for assessing the capacity of plant extracts to modulate the antioxidative status and innate immunity of chickens. It will be used in a project aiming to define a methodology to select plant extracts from the literature, to screen their safety and biological activities *in vitro* and *in vivo* on chicken performance, health and welfare.

Funding: this study is a part of a CASDAR project (MEXAVI) funded by the French Ministry of Agriculture.

ID : 564

EFFECT OF AN ESSENTIAL OILS BASED FEED ADDITIVE ON IMMUNE RESPONSE GENES EXPRESSION IN LOHMANN WHITE LAYING HENS

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The «BIOTROF+» Ltd and All-Russian Research Veterinary Institute of Poultry Science (ARRVIPS) teams, have carried out the experiment on 40 Lohmann White adult laying hens in vivarium of ARRVIPS. The Experimental group received the feed additive based on essential oils mix from the first day of the experiment, while the Control group received standard feeding diet. 3 weeks after the start. Half of the hens of each group were infected with an epizootic strain of *S. enteritidis* in the amount of 5×10^8 CFU and afterwards further parting into groups was performed: Control (C), Infected Control (CI), Experimental (E), Infected Experiment (EI). A day and a week after infection, a planned slaughter of hens with sampling was executed.

The gene expression analysis was performed in molecular-genetic laboratory of Biotrof Ltd (Saint-Petersburg, Russia). Analysis of the level of relative expression of immunity genes was performed by qPCR that requires preliminary steps of RNA extraction and cDNA synthesis. Total RNA from the samples was isolated using the Aurum™ Total RNA Mini Kit (Bio-Rad, Hercules, CA, mUSA) according to the manufacturer's instructions. Using the iScript™ Reverse Transcription Supermix (Bio-Rad) kit, a reverse transcription reaction was performed to produce cDNA using RNA template. The amplification reaction with the gene primers was performed using the SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad) kit according to the manufacturer's protocol. The relative quantification (RQ) of gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. As a reference gene, the β -actin protein (ACTB) gene was chosen.

One day after infection, a decrease of IL-6 gene expression level was noted in the CI group compared to the C group by more than 2 times. At the same time, in the EI group the IL-6 gene expression significantly increased in comparison with the E group, and was also 1.78 times higher than the C group level. This trend continued even after 7 days after infection.

The AvBD-10 gene expression a day after infection in all groups lower, the C group level, which could be more likely due to an increase in the expression level of the C group. But the tendency of AvBD-10 expression ratio among the CI, E and EI groups correlated with IL-6 gene expression indices. A week after infection, an increase in AvBD-10 gene expression in all groups in comparison to the C group was noticed.

The IL8L1 gene expression a day after infection in the CI group was 7 times higher than in the C group. The IL8L1 expression in the E group was 14 times higher than in the C group and the expression in the EI group was 15 times higher than in the CI group. 7 days after infection, an equalization of IL8L1 gene expression level among CI, E, EI groups was noticed.

In the CI group, the level of bactericidal activity of blood serum a day after infection decreased, while in the EI group the level of bactericidal activity increased. In the CI group a week after infection the level decreased even more, while in the EI group it raised even more.

In conclusion, it was found that addition of an essential oil based feed additive to the feed ration of hens led to an increase in the immune activity.

ID : 609

EFFICACY OF AN ATTENUATED COCCIDIOSIS VACCINE IN COMBINATION WITH DIFFERENT FEED ADDITIVES ON PRODUCTION PERFORMANCE AND INTESTINAL LESIONS IN BROILERS CHALLENGED WITH NECROTIC ENTERITIS

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INTRODUCTION

The combination of vaccination against coccidiosis and the supplementation of the diet with feed additives could be a composite approach to the control of necrotic enteritis problems triggered by *Eimeria* spp. infestation in broilers. Therefore, the objective of this study was to test the efficacy of an attenuated coccidiosis vaccine in combination with different feed additives in preventing a loss of production performance and intestinal lesions in broilers challenged with NE

MATERIALS&METHODS

Healthy day-old Ross 308 male broiler chicks (960 chickens in 48 groups of 20 chicks) were reared in cages on wood shavings as the bedding material. These were then equally distributed between 6 experimental groups.

NE was triggered with a primary infestation with *Eimeria maxima*, a secondary infection with *Clostridium perfringens* and by feeding birds during the growing phase with a predisposing diet. Groups 3-6 were vaccinated at one day of life against coccidiosis with an attenuated coccidiosis vaccine for broilers. Groups 1 and 2 were then reared without feed additives in the diet, whereas the rest of groups were reared with Short Chain Fatty Acids (SCFA), Medium Chain Fatty Acids (MCFA) or Phytogenic Feed (PFA) additives respectively in the diet. NE was then experimentally induced in groups 2-6, whereas group 1 was sham-infected. Data were analysed by ANOVA using the Fisher's post-hoc Least Significant Difference (LSD) to test for treatment effects. Statistical analyses were based on two-sided tests implemented in GenStat®

RESULTS

Vaccine administered alone or in combination with feed additives in the diet significantly decreased the intestinal lesion score associated with *C. perfringens* and slightly but not significantly the mortality compared to the untreated group. MCFA group was the only one among those fed with additives in the diet, which showed a synergistic effect with the vaccine.

Vaccinated birds converted feed better than the non-vaccinated group as shown by the higher BWG and lower FCR. MCFA group was the one among those with a diet supplemented with additives which converted the feed more efficiently.

CONCLUSIONS

Vaccination with an attenuated coccidiosis vaccine for broilers, was shown to be able to reduce intestinal lesions associated *C. perfringens* compared to non-vaccinated birds and as a consequence benefits in productive performances were observed as well. MCFA were shown to be able to further decrease intestinal lesion scores associated with *C. perfringens* than vaccination alone

ID : 670

SUPPLEMENTATION WITH SAPONIN RICH PLANT EXTRACTS AS EFFICIENT AS MONENSIN SUPPLEMENTATION TO MAINTAIN BROILERS' ZOOTECHNICAL PERFORMANCES UNDER COCCIDIOSIS CHALLENGE.

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Introduction:

For decades, ionophores such as monensin have been used successfully to prevent coccidiosis in broilers flocks. However, the emergence of resistant strains of *Eimeria* and issues linked to residues in meat¹ led poultry producers to find efficient alternatives.

Saponin rich plants, mainly *Yucca schidigera* (YS), were shown to be effective to control coccidiosis in broilers². However, few studies assessed the effect of combination of saponins from different plants and compared their effects to usual coccidiostats.

The objective of this study was to assess the effect of Norponin XO2 (NPXO2), a combination of saponins from YS and *Trigonella foenum-graecum* (TFG) on zootechnical performances and intestinal lesions of broilers artificially challenged with coccidiosis.

Materials and methods:

192 broilers (males, ROSS 308) were housed in cages and allocated to the following groups: 1. uninfested untreated, 2. infested untreated, 3. infested, monensin treated 4. infested, NPXO2 treated. Infested birds received at D16 orally and individually 805000 sporulated oocysts (52000 *E.acervulina*, 17500 *E.tenella*, 10000 *E.maxima* and 1000 *E.mitis*). Zootechnical performances and intestinal lesions were assessed at D22. Data were analyzed using linear mixed regression models. In all models, treatment groups were compared to the group 2 as reference.

Results:

Group 1 birds had a higher body weight at the end of the experiment. Birds of group 3 have no difference, however group 4 animals tend to have higher body weight ($P=0.08$). Even if FCR of birds of group 1, 3 and 4 was numerically lower, no significant difference was observed. Intestinal lesion of *E.acervulina*, and *E.tenella* were reduced in group 3 and 4 significantly. Lesion due to *E.maxima* were reduced only in group 3. No significant difference was observed between group 3 and 4 in zootechnical performances and intestinal lesion.

Conclusion:

According to these results, feed supplementation with saponin rich plants, YS and TFC (NPXO2), is as efficient as monensin supplementation to maintain zootechnical performances of broilers under artificial coccidiosis challenge.

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ID : 705

CHARACTERISATION, THE CORNER STONE OF A SUSTAINABLE USAGE OF BOTANICALS IN POULTRY:
CASE STUDY OF CITRUS EXTRACT SUPPLEMENTATION

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Introduction

For a long time, antibiotics have been used as growth promoters for preventing diseases and improving zootechnical performances. For the one health purpose, this application has been banned in Europe, giving way to alternatives such as botanicals. Among botanicals, citrus extract have already shown beneficial effect on broiler performances and health¹. However, all citrus extracts are not the same and can vary a lot in terms of composition and concentration of active compounds². This trial has been carried out in order to compare the effect of a Characterised Natural Citrus Extract (CNCE) with a Citrus Peel Extract (CPE), in terms of composition and effect on performances.

Experimental design

A solution of each extract has been prepared in methanol and characterised by HPLC-UV-DAD-DEDL, in order to compare their composition. In the meantime, 540 Arbor Acres one day old birds were divided into 3 groups. Each group contained 180 birds divided into 6 replicates:

- Group 1: CTL group: a standard diet without supplementation;
- Group 2: CNCE group: a standard diet supplemented with 250 ppm of CNCE (Nor-Spice AB, Nor-Feed SAS)
- Group 3: CPE group: a standard diet supplemented with 250 ppm of CPE

Birds were reared until day 46. Heat stress (30°C/4h) was applied, from day 26 to day 30. Zootechnical performances were weekly recorded.

Results

HPLC analysis show differences between the composition profile of CNCE compared to profile of CPE. Regarding zootechnical performances, birds from CNCE group were heavier (2312g ±196) than birds from CPE group (2097g ±92) and CTL group (2114g ±120). Moreover, birds from CNCE group had a better carcass yield (77.16 ± 15 %) than birds from CPE (72.57 ± 1.7%) and CTL groups (73.11 ± 3.1%) (p

Conclusions

Citrus extract effect on animal's performances differs between CNCE and CPE. This difference may be linked to their composition. These results showed that botanicals may be efficient alternatives to AGPs as long as they are characterised. Indeed, characterisation gives the possibility to determine their composition and understand their mode of action, making it the corner stone of a sustainable usage of botanicals.

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ID : 756

ALKYL ESTERS OF MEDIUM CHAIN FATTY ACIDS ENHANCE BROILER GUT HEALTH AND PERFORMANCE

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The level of intestinal microbial activity affects animal growth efficiency. The microbial community in the gut competes for nutrition with the host, accelerates enterocyte turnover rates resulting in lower efficiency in nutrient absorption and decreases fat digestibility by deconjugating bile acids (Yadav and Jha, 2019). Higher diversity of microbial genera has been associated with higher resistance and better production performance in broilers and other species (Stanley et al 2016).

An alkyl ester is a chemical compound derived from an acid in which at least one hydroxyl group is replaced by an alkoxy group. Hydrolysis of alkyl esters of medium chain fatty acids (MCFA) by pancreatic lipase is 10-50 times slower compared to triglycerides (McKay, 2007). Consequently, the level of 'free' MCFA is higher in the lower parts of the small intestine. Multiple R&D studies showed that alkyl esters positively affect total microbial counts and diversity and related growth performance compared to the 'free' form of lauric acid.

An in vivo study with 776 female broilers fed a corn-soybean meal based diet with or without 1.5kg/t alkyl ester C12 and exposed to a dietary challenge compared microbiota counts. The total bacterial count in the jejunum of broilers fed the alkyl ester C12 diet was significantly ($p < 0.05$) reduced compared to the negative control at 21 days of age. The phylogenetic representation of jejunal samples showed that alkyl esters help to increase diversity in jejunum by 22%.

In an in vivo study performed with 936 female broilers fed a corn-wheat-soybean meal based diet, birds were exposed to a negative control (NC), C12 (1 kg/t) and alkyl ester of C12 (1 kg/t) in the diet. A tendency ($p = 0.079$) to reduce *Clostridium perfringens* in jejunum compared to NC and C12 was found for the C12 alkyl ester diet. A 2% lower FCR was found for the alkyl ester diet compared to NC in the first 3 weeks of age ($p = 0.01$).

In a re-used litter challenge study with 192 broilers, birds fed diets with alkyl esters of C12, had a tendency ($p < 0.1$) for higher body weight at 42 days compared to a standard diet.

Alkyl esters of MCFA's reach lower parts of the gastrointestinal tract compared to free MCFA and triglycerides. This results in improved microbial diversity and lower microbiota counts which is reflected in improvements in zootechnical broiler performance.

ID : 944

EFFECTS OF THE DIETARY SUPPLEMENTATION WITH GRAPE POMACE AND CHESTNUT EXTRACTS ON GROWTH PERFORMANCE AND INTESTINAL MUCOSA OF BROILER CHICKENS

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During the last years, poultry production has been moving towards antibiotic-free production systems in which tannins can be used as feed additives to improve animal immune response and health, due to their anti-microbial, anti-oxidant, and anti-inflammatory activities. Results widely vary according to tannins source and structure, however. Thus, the present study assessed the effects of the dietary supplementation in broiler chickens with two vegetal extracts containing tannins, from grape pomace (GP) or chestnut (CN), on growth performance and morphology and immune response of intestinal mucosa. A total of 864 chickens (both sexes) in collective pens (24 birds/pen, 12 pens/group) were assigned to three experimental groups fed a control diet (C) or the same diet added with 0.2% CN extract (diet CN) or 0.2% GP extract (diet GP). At 14 d and 34 d of age, 12 chickens per diet were slaughtered to sample jejunum. Serial sections of 4 µm were stained with hematoxylin/eosin for morphometric evaluation and with antibodies against intraepithelial CD3+ T-cells and CD45+ leukocytes (only samples of the first slaughtering) to evaluate the anti-inflammatory activity. Data were submitted to ANOVA using a mixed model with diet as the main effect and pen (growth data) or animal (intestinal mucosa data) as a random effect. The diet GP significantly increased daily weight gain (DWG) in the whole trial (70.5 g/d vs. 69.4 g/d and 67.1 g/d; $P<0.01$) and final live weight (3148 g vs. 3099 g and 3084 g; $P<0.01$) compared to diets C and CN, with no effect on feed intake (on average 111 g/d), feed conversion ratio (1.59), and mortality (3.4%). Indeed, the diet GP promoted DWG in the second (+1.7%, $P<0.001$; 14-28 d) and in the third period of growth (+2.1%, $P<0.05$; 28-45 d) compared to diet C and, mostly, diet CN. The diets CN and GP decreased villi height compared to diet C (954 µm and 934 µm vs. 1033 µm; $P<0.01$), whereas crypt depth and villi-to-crypt ratio did not change. Regarding intestinal immune status, chickens fed diet GP showed higher densities of both CD3+ (2302 vs. 2116 cells/mm²; $P<0.001$) and CD45+ (2198 vs. 2040 cells/mm²; $P<0.05$) compared to those fed diet CN, whereas chickens fed diet C exhibited intermediate results. In conclusion, GP dietary supplementation improved chicken performance and promoted immune response in intestinal mucosa. Further insights are required to define the action mechanisms at the intestinal level.

ID : 1021

BACILLUS PROBIOTIC AND YEAST PARIETAL FRACTION TO REDUCE COLONIZATION OF SALMONELLA ENTERITIDIS IN LAYERS

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Frequent high occurrence of Salmonella in poultry flocks can lead to major public health issues, hence be controlled. 2 in vivo trials were setup to investigate the potential, of a Bacillus probiotic and a parietal yeast fraction prebiotic to reduce Salmonella Enteritidis colonization in laying pullets and hens. In the first trial, sixty 9-week-old Hy-Line Brown pullets were randomly allocated to individual cages in 15 replicate blocks. Animals were either supplemented the basal control diet, or the basal diet containing 500g/ton yeast parietal fraction, 500g/ton Bacillus spp probiotic or 1kg/ton yeast culture. On day 3 of the trial all birds received 3×10^7 CFU of a nalidixic acid resistant Salmonella Enteritidis by oral gavage. Seven days after inoculation all birds were humanely euthanized, and the ceca aseptically removed for CFU determination. In the second trial sixty Hy-Line Hens aged 56 weeks were placed in individual cages and fed a mash diet. The treatments were negative control, 500g/ton Bacillus spp. probiotic, 500g/ton yeast parietal fraction and a combination of yeast parietal fraction and Bacillus spp. probiotic. At 60 weeks of age all hens were challenged orally with 7×10^7 CFU of Salmonella Enteritidis. One week later, birds were humanely euthanized, and the ceca aseptically removed and analyzed for Salmonella Enteritidis prevalence and enumeration by the Most Probable Number (MPN) method. Results of the first trial show that the mean quantity of Salmonella Enteritidis detected in the ceca expressed in log₁₀ MPN/g was 2.52 in the control, 2.49 in the yeast parietal fraction treatment, 1.73 in the probiotic treatment, and 1.66 in the yeast culture treatment. In the second study, the control birds had 3.35 log₁₀ MPN/g of Salmonella. Enteritidis in the ceca. The Probiotic group had 1.90 MPN/g, a reduction of 1.44 ($p < 0.05$) and the yeast cell wall group had 1.84 MPN/g a reduction of 1.51 ($p < 0.05$). The combination had 2.56 MPN/g a numerical reduction of 0.79 ($p = 0.14$). These studies confirm that both, the Bacillus spp. probiotic, the yeast parietal fraction or yeast culture could reduce the Salmonella Enteritidis colonization in the ceca of laying pullets and hens.

ID : 1023

PROBIOTIC STRATEGIES TO REDUCE SALMONELLA INFANTIS COLONIZATION IN BROILERS

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With a recent growing push from consumers for poultry administered no antibiotics, feed additives such as parietal yeast fractions have become a potential option of microbial intervention that has gained interest from producers. Although previous research tends to indicate a trend toward their influencing Salmonella populations in chickens, more in vivo studies are needed to effectively evaluate their effects on Salmonella. The objective of this study was to evaluate the efficacy of a commercially available parietal yeast fraction on the reduction of Salmonella Infantis in the ceca of broilers and its translocation into other organs. In this trial, 240 Cobb 500 off-sex male chicks were obtained from a commercial hatchery on day of hatch and randomly distributed into stainless steel battery pens in groups of 5 birds per pen with 8 pen replicates per treatment (48 pens total). Treatment groups of interest consisted of a control diet, a parietal yeast fraction at 500g/ton, and a Bacillus spp probiotic at 500g/ton. At 3 days of age, all birds were orally gavaged with 1.25×10^7 CFU's of Salmonella Infantis. On days 14 and 21, chicks were humanely euthanized, and organ samples (liver, spleen and ceca) were taken for detection of translocation between organs by enumeration and prevalence. Organ samples were analyzed for Salmonella Infantis enumeration by direct plating on XLT-4 agar treated with Nalidixic acid and Novobiocin, and for prevalence by direct plating after 24 hours of incubation in Rappaport Vassiliadis broth. The parietal yeast fraction showed reductions in Salmonella prevalence in the liver at day 14, and a reduction in prevalence in all three organs at day 21. Although not significant, at Day 14 the Challenged Control treatment showed 2.15×10^5 CFU's of Salmonella in the ceca compared to 8.08×10^3 CFU's in the parietal yeast fraction, & 1.44×10^5 in the bacillus treatment, providing a reduction of approximately 1.4 logs of Salmonella in the ceca of birds fed yeast fraction. Cecal Salmonella load enumeration for bacillus treatment was reduced at day 21 to 4.55×10^4 though not significantly different. Further reductions were observed in birds fed parietal yeast fraction, as loads were significantly reduced from 1.04×10^5 CFU's in the control treatment to 3.50×10^3 CFU's in the parietal yeast fraction, a reduction of over 1.5 logs (p

ID : 1100

CHARACTERIZATION AND ASSESSMENT OF POSTBIOTIC METABOLITES ON BACTERIOLOGICAL QUALITY OF LAYER EGGS: AN INNOVATIVE APPROACH

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The microbiological quality of chicken eggs is of zoonotic importance for consumer's welfare. The intrinsic relationship between the bird and the gut microbiota has crucial implications on their health and products quality. Probiotics have been an attractive alternative to antibiotic growth promoters in food animals for maintenance of gut performance but recent studies indicated that these selected organisms could transfer resistance genes to inhabitant pathogens. Postbiotics, the active metabolites of probiotic organisms can perform a similar function without any concern on resistance transmission and its study on layer chicken are much limited. Laboratory isolated probiotic *Lactobacillus plantarum* (LGFCP4) was incubated in selective broth for 24 hours and its metabolites were collected and stored at 4°C. The postbiotics were in vitro characterized and there was a linear reduction ($p < 0.05$) in media pH, with an increase in osmolarity in comparison to the uninoculated media. Assessment of its anti-inflammatory activity against hemolysis of erythrocytes demonstrated a significant ($p < 0.05$) protective effect which was comparable to that of ibuprofen. A biological experiment was carried out with 48 laying hens (45 weeks of age) in six replicates to study the effect of postbiotics on gut microbes and in turn the bacteriological quality of eggs. One group of hens were fed with 0.2% of postbiotics for a period of 7 days and another group served as control under t-test distribution. The faecal samples from individual birds were collected at 0, 3 and 9 days of postbiotic feeding. Representative samples of egg contents and egg shell rinse were collected at the end of postbiotic feeding trial. The total coliform count showed significantly ($p < 0.01$) reduction in postbiotic group (6.75×10^5 CFU/g) vs. control (8.95×10^5 CFU/g) and the period effect showed a non-significant decrease. However, lactobacillus count remained unaltered by the supplementation of postbiotics but exhibited period increase ($p < 0.01$) with 8.10×10^5 CFU/g at day 9. There was significant ($p < 0.05$) increase in lactobacillus colonies of egg shell rinse due to postbiotic supplementation (4.27×10^5 CFU/g) over control (3.57×10^5 CFU/g) but the coliforms or lactobacillus in egg contents were non-detectable. The study indicated that postbiotics could work similarly to probiotics in manipulating the gut environment and improving product quality.

ID : 1108

CHICKEN CONSUMPTION INCREASES PREVALENCE OF POLYCYSTIC OVARY SYNDROME: A MYTH IN PERSPECTIVES OF PAKISTAN

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Polycystic ovary syndrome (PCOS) is common endocrine disorder in women of reproductive age, across the world. In this modern era food intake is mainly progressing towards fast foods and soft drinks. These unhealthy eating patterns as well as lack of exercise lead to PCOS. It is a common perception in Pakistan that junk foods especially made of chicken are the main cause behind PCOS.

The objective of this study was to find out the percentage of prevalence of PCOS in Faisalabad region and relationship of dietary habits of females with PCOS.

To fulfill this purpose, 500 female PCOS patients were chosen from private clinics and public hospitals of Faisalabad, Punjab, Pakistan. A Performa was developed to record information regarding subject's socio demographic, clinical record (i.e. hirsutism, acne etc), dietary habits on weekly basis, physical activity and menstrual history.

The findings of present study showed that highest number of females that was about 59.20% suffered in PCOS at the age of 21-30 years. However, lowest number of females about 10.40% had PCOS at the age of 15-20 years. About 30.40% PCOS patients were working women while 69.60% were non-workers. Regarding the marital status, PCOS prevalence was high as 63.40% in married women. On the base of BMI, highest percentage of PCOS was recorded in obese women (43%). The current study showed that 32%, 18%, 10%, 55% and 12% PCOS females had thin, dry, lusterless, easily plucked out and dandruff loaded hairs, respectively. About 55% PCOS females were having facial hairs and 45% were having neck hairs. Regarding the skin problems, dry, purplish spots, itchy and acne skin types were found in 20%, 18%, 22% and 40% PCOS females, respectively. About 42% PCOS females were facing irregular menstrual cycle. About 70% PCOS females were not exercising in their routine habits. Dietary habits data revealed that there was more consumption of fast foods, soft drinks, cereals, sweets and chocolates by PCOS women. However reduced intake of poultry, fish, fruits and vegetables and dairy products was observed.

It can be concluded from the results that PCOS women have unhealthy life style pattern including unhealthy eating habits which is responsible for worsening this condition. There was no solid link found between the chicken/poultry consumption with the incidence of PCOS. Excess of everything is bad so it has been proved that people have wrong conception about chicken intake and hormonal imbalance in young females.

ID : 1115

EFFECT OF DIFFERENT ADDITIVES IN THE PROTECTION OF BROILERS CHALLENGED EXPERIMENTALLY WITH CAMPYLOBACTER

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The major source of human campyobacteriosis is considered undercooked and cross-contaminated broiler meat. The society is demanding to reduce antibiotic use and alternatives to fight against bacterial infections in farms. In this context, the aim of the trial was to assess the effect of different additives on caecal colonization of Campylobacter in broilers. A total of 288 day-old chicks (negative to Campylobacter) were distributed in 4 treatments among 24-floor pens (n=6). The treatments were: T1, Control feed without additives; T2, GUSTOR N'RGY (2.7Kg/t of feed), based on sodium butyrate (NaBut) protected with sodium salts of palm fatty acids distillates (Active ingredient, AI=56% C4); T3, DICOSAN (4Kg/t), based on sodium salts of medium-chain fatty acids distillates (NaMCFA, AI=38% C8+C10+C12); and T4, DICOSAN+ (2.3Kg/t), based on NaBut protected with NaMCFA (AI=65% C4+C8+C10+C12). All additives were supplied by NOREL and were dosed to achieve the same quantity of active ingredient in the final feed. At d11 of rearing, 20% of birds were orally challenged with Campylobacter jejuni (6 log₁₀ CFU/mL). From each treatment 18 chicks were euthanized for caeca sampling at 1, 2, 3 and 4 weeks post-inoculation (WPI). Data were analysed by one-way ANOVA using the software package SPSS 24.0. The results showed that no treatment was able to prevent the contamination by Campylobacter 3 days post-inoculation. All additives got a significant reduction on Campylobacter counts along the rearing period.

By week, a significant logarithmic reduction of Campylobacter counts at 4th WPI were achieved by T2, T3 and T4 (1.63, 1.23 and 1.26 Log R, Pst and 2nd WPI. However, at 3rd WPI, T4 tended to have the lowest counts (6.75y, 6.59xy, 6.86y and 6.06x, for T1, T2, T3 and T4, respectively). At 4th WPI, T2 and T3 had significant lower counts of Campylobacter (6.66b, 5.91a, 6.03a and 6.24ab, for T1, T2, T3 and T4, respectively).

In conclusion, all treatments achieved a significant reduction of caecal colonization by Campylobacter. However, DICOSAN+ at 32d of rearing with 1.4 log₁₀CFU/g reduction and GUSTOR N'RGY at 42d of rearing with 1.6 log₁₀CFU/g reduction were the most effective additives. It has been reported that Campylobacter reductions around 2 log₁₀ CFU/g is estimated to reduce human campylobacteriosis cases attributable to broiler meat by at least 76%.

ID : 1169

EFFICACY OF SYNERGISTIC MIXTURE OF ORGANIC ACIDS AS AN ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTERS IN COMMERCIAL BROILERS RAISED UNDER OPEN-SIDED HOUSING SYSTEM IN MYANMAR

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The use of antibiotic growth promoters (AGPs) in commercial broiler production in Myanmar is currently abundant and a common practice applied by virtually all feed millers. With the increasing concern of antimicrobial resistance, alternatives to the use of AGP in livestock production are tried worldwide. Therefore, two field trials were conducted to evaluate efficacy of replacement of in-feed AGPs with synergistic mixture of modified organic acids (OAs) (Selko pH, Trouw Nutrition) by drinking water in commercial broilers under open-sided housing system in Myanmar. One-day-old commercial broilers, 6120 Ross308 and 7602 Arbor Acres Plus in the first and the second trial, respectively, were equally divided into two groups, AGP group and OA group with the same housing system. The broilers from AGP group, with two replicates, were provided the same commercial feeds with in-feed AGPs, Chlortetracycline, Zinc Bacitracin and Tylosin. The broilers from OA group, with two replicates, were provided the same commercial feeds without AGP but synergistic mixture of organic acids (Formic Acid, Acetic Acid, Ammonium formate and Copper) in the drinking water. The pH of drinking water for OA group were adjusted to get between 3.6-4.1 by adding the OA mixture. Mortality rates, feed consumption, live weights at the market age are recorded in both experiments. Broiler performance indices (BPI) and uniformity of live weight were evaluated at 41-day old and the data obtained were analyzed by Student t test. The BPI of 344.017 and 351.332 in the first trial and 407.555 and 411.889 in the second trial were observed in AGP and OA group, respectively. The uniformity of live weight observed were 86.56% and 87.41% in the first trial and 91.25% and 88.60% in the second trial in AGP and OA group, respectively. However, there were no significant differences ($p>0.05$) in BPI and uniformity of live weight between the two groups. The results of the two field trials clearly indicate that the use of in-feed AGP can be replaced by the drinking water organic acids synergistic mixture in commercial broilers raised under open-sided housing condition in Myanmar.

ID : 1182

A DIETARY COMBINATION OF MICROENCAPSULATED ORGANIC ACIDS AND ESSENTIAL OILS REDUCES SALMONELLA ENTERICA SV. TYPHIMURIUM IN CHALLENGED BROILER CHICKENS

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Contamination of poultry meat with foodborne pathogens, such as Salmonella, remains an important public health issue. Therefore, ensuring the microbial safety of poultry meat products is crucial for the poultry industry. We hypothesized that organic acids and essential oils (EO) could reduce Salmonella in chickens digestive tract because of their reported antimicrobial effects.

In this context, a trial of 21 days was performed to test the efficacy of two targeted-release blends of organic acids (Blend A: Calcium butyrate, Blend B: Calcium butyrate, fumaric acid and lauric acid), combined with EO, on growth performance and Salmonella colonization in experimentally infected broilers (d0, 107 S. Typhimurium). A total of 255 one-day-old male broiler chicken were distributed into 3 dietary treatments: negative control (NC) and NC supplemented with either 0.7g/kg of blend A or 2g/kg of blend B, with 5 replicas per treatment and 17 chicks per replica. Birds were weighed and feed disappearance was determined at d3, 7, 14 and 21 on a pen basis to evaluate growth performance. On the same days, 3 birds per replicate were euthanized to collect cecum and spleen samples for Salmonella quantification. Blood samples were collected from the 3 chickens euthanized per replicate, at the end of the experiment, to analyse acute phase proteins. One-way ANOVA followed by a Tukey's multiple comparison test was used to analyse the significance of the differences among treatment groups. For the count of positive spleens a chi-squared analysis was performed. The significance threshold was set on 0.05.

On d21, all chickens presented lowest performance than the ROSS standards. However, birds supplemented with the blend B showed higher body weight than those of the NC ($P=0.04$) as a result of higher average daily gain ($P=0.030$). These birds also presented reduced $\alpha 1$ -acid glycoprotein concentrations compared to those on blend A and NC ($P< 0.001$). On d21, the supplementation of blend A reduced Salmonella concentration in the ceca ($P=0.02$) compared to the rest of dietary treatments. As for spleen, difference among treatments was observed only on d3 where blend B showed the lowest positive spleens ($P=0.03$) compared to blend A and NC.

In conclusion, a protected blend of calcium butyrate and EO did not affect growth performance of challenged birds. However, it decreased Salmonella concentration in the cecum, which may suggest that this blend exerts some antimicrobial effect on S. Typhimurium.

ID : 1252

THE EFFECT OF DIFFERENT DIETARY RATIOS OF LYSINE AND ARGININE IN DIET OF HIGH OR LOW METHIONINE LEVEL ON OXIDATIVE AND EPIGENETIC DNA DAMAGE, GENE EXPRESSION OF TIGHT JUNCTION PROTEINS AND SELECTED INDICES OF METABOLISM IN CLOSTRIDIUM PERFRINGENS CHALLENGE

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Essential amino acids are dietary components with immune-enhancing functions, therefore their proper dietary ratio may help birds to cope with the bacterial infections. Two experiments were run to investigate effect of different ratios of arginine (Arg) to lysine (Lys) in diets of low (30% Lys; experiment 1) and high (45% Lys; experiment 2) methionine levels on selected indices of metabolism, oxidative and epigenetic damage of DNA, and mechanisms determining gut integrity in turkeys subjected to *C. perfringens* infection. In each experiment, 108 one-day-old Hybrid Converter female turkeys were placed in 6 pens (18 birds per pen) and reared for 42 days. At days 34, 36 and 37 of life, half of the birds was subjected to *C. perfringens* challenge. A 3 x 2 factorial design with three levels of Arg relative to Lys (90, 100 and 110%; Arg90, Arg100 and Arg110, respectively) and *C. perfringens* infection (-, +) was employed. Challenging birds with *C. perfringens* increased lipids oxidation and oxidation and methylation of DNA of gut mucosa as well as it did downregulate the activities of DNA-repairing enzymes. Markers of liver function and metabolism did not differ neither of dietary treatments nor of challenge. Feeding diets of Arg110 and high methionine level evoked DNA oxidation and methylation while, in birds fed diets of Arg90 they were significantly downregulated. Results indicate that diets of high methionine level and Arg90 has a beneficial action on gut-associated indices of integrity in turkeys subjected to necrotic enteritis. Although, the obtained results indicating interactive actions of the investigated amino acids ratios and host gut integrity-maintaining systems, such dietary intervention appear likely supported birds for coping with necrotic enteritis.

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ID : 1275

COMPARISON OF DIFFERENT IBD VACCINATION PROGRAMS UNDER FIELD CONDITIONS.

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Infectious bursal diseases (IBD) is a viral poultry disease characterised by immunosuppression and huge economic losses. IBD control is a crucial point of antibiotic reduction in broiler, and the only effective control system is vaccination, with live attenuated vaccines or with turkey herpesvirus construct vaccines expressing IBDV VP2 protein (rHVT-IBD). The main advantages of this innovative vaccine are absence of vaccine reactions and absence of interference with maternal antibodies.

The objective of this field monitoring was to compare serology for IBD and performances of birds vaccinated with traditional vaccine (at 21 days, according to Deventer formula, group A) and with rHVT-ND-IBD (an rHVT-IBD construct expressing also F protein of Newcastle disease virus) half dose (group B) and full dose (group C).

In a broiler integration weekly serological monitoring program, 13 farms were selected, 3 for group A, 5 for B and 5 for C, all birds came from same breeders and the level of maternal antibodies was alike. Serological titers for IBD of the three groups were compared at each time point using one way ANOVA test, and t Student test, significance was set at $p < 0.05$.

Production data of the same integration coming from 3 months of production for each vaccination plan were compared.

Group A had a drop in serological titers from week 1 to 3, from week 4 the serological titers began to raise. Group B had a similar trend for weeks 1 and 2 (no significant difference vs group A), but at week 3 the titers started to increase (significant difference vs group A). Group C never had a drop in serological titers (significantly higher titers vs groups A from 1 and to 2 weeks, and vs group B at 1 and 2). From 5 weeks of age all groups showed a strong seroconversion.

Performances were similar for all vaccination programs, but the use of antibiotics seemed to be lower with rHVT-ND-IBD vaccine.

The group vaccinated with live vaccine (A) had a period of susceptibility to IBD, with birds negative to serology, whereas groups vaccinated with rHVT-ND-IBD (B and C) never had negative samples. These findings confirm that rHVT-ND-IBD provides protection against IBD since the beginning of the cycle without showing periods of susceptibility that usually affect traditional vaccination schemes.

Among the rHVT-ND-IBD groups the onset of immunity was quicker in the group vaccinated full dose. This finding supports the use of full vaccine dosage in order to have an early and uniform immunity.

ID : 1310

THE INTERACTIVE ACTION OF TWO STRAINS OF PROBIOTICS ON PERFORMANCE AND EXPRESSION OF SELECTED GENES DETERMINING TIGHT JUNCTION PROTEIN IN CHICKENS SUBJECTED TO NECROTIC ENTERITIS

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Microbial feed supplements are applied into poultry feed to protect birds from pathogen infections and to fortify the gut barrier function. Spore-forming bacteria seem to be particularly well-suited for use in feeds, as they are metabolically dormant and resilient to environmental stresses, including feed processing. The objective of this study was to evaluate the actions of a two strains of commercial probiotics (GalliPro®Tect and GalliPro®Fit containing a spore-forming strains of probiotic bacteria).

A total of 432 male day-old broilers Ross 308 were divided into group cages of 9 birds. The 6 dietary treatments were allocated to T1 - T6, so that each treatment was applied to 8 cages of 9 broilers; T1 standard commercial diet (C), T2 – C + GalliPro®Tect, T3 – C + GalliPro®Fit, and treatments T4-T6 were as T1-T3, respectively but assigned birds were challenged with

C. perfringens in a subclinical dose at days 15 and 16 of age. At day 29 of life, samples of jejunum were collected from 8 birds of each treatment. Differences between groups were compared by two-way ANOVA as a 2 × 3 factorial design: challenged vs. not challenged with *C. perfringens*, and dietary supplementation: (i) without additives, (ii) with GalliPro®Tect, (iii) with GalliPro®Fit.

The body weight (BW) at day 21 ($P=0.018$), BW gain (BWG) for the period 12-21 and 0-21 days ($P<0.02$), and FCR for the period 12-21 and 0-35 days ($P<0.05$) were compromised due to the challenge. The most efficient feed conversion ratio for the period 12-21 days was found in the control and GalliProFit groups while, for the same period but in challenged birds it was the best in birds fed GaliPro®tect. *C. perfringens* challenge and probiotic supplementation significantly increased expression levels of zonula occludens-1, occludin and claudin-3 genes in the ileal tissue, which was also associated with the significant interactions ($P<0.001$).

Our findings suggest that beneficial action of GalliPro®fit and GalliPro®tect depends on the physiological status of birds. In normal conditions (without challenge) GaliPro®fit was better than GaliPro®tect in maintaining birds performance whereas opposite was true in birds applied to challenge. The results regarding expression level of genes determining tight junction of the gut indicating an interactive actions of the investigated additives and host gut integrity-maintaining system however, such dietary intervention appear likely supported birds for coping with necrotic enteritis.

ID : 240

PERCEPTION OF HUMAN HEALTH RISKS RELATED TO AIR QUALITY AND PREVENTIVE BEHAVIOURS IN DUCK OVERFEEDING SYSTEMS

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Duck housing systems during overfeeding have evolved significantly over the past 4 years, in line with animal welfare regulation. This change has impacted working conditions of farmers who complain of greater respiratory discomfort due to 1. more aerial dust due to greater freedom of movement for animals and 2. drier ambient air. In this context, the project AGAtE is underway on dust exposure levels and preventive measures to prevent respiratory diseases induced by inhalation of particles. A part of the project addresses the risk perception of duck producers linked with their preventive behaviors, to improve arguments and communication methods on prevention.

The survey guidelines used to carry out a qualitative study (14 interviews), include perception of working conditions (motivations, constraints, health, general knowledge about risks), awareness about risk linked to air quality in duck houses (air quality perception, farmer-specific risk evaluation, sources of information), and preventive behaviors. A longitudinal analysis and a typology have been performed.

Most of the duck producers feel very concerned about the respiratory risks linked with dust inhalation. Risks awareness is mainly ensue from visible indicators, odors, respiratory or ocular discomfort, but also from the social network (e.g. exchanges between peers). Nevertheless, half of them, older and more experienced, describe a “quite good” air quality in duck houses, mainly arguing about their technical results. Wearing a protective mask is a largely widespread behavior, and it seems to be a professional standard. Nevertheless, young and less experienced farmers, who have no occupational pathology, seem to wear protective masks less longer than other (e.g. only at the beginning of the rearing period when particles are most perceptible). Finally, we identified four different profiles based on their air quality perception and preventive behaviors. Experience, probably associated with a large social network rather than person's age and occupational pathology appear as the mains motivations for wearing a protective mask when handling ducks.

Exchange meetings between peers or videos may be constitute a good way to inform on respiratory risk among duck producers. Moreover, it seems import to alert and train technicians about respiratory health risks, because they could be a good vector to inform young farmers at the beginning of their professional life.

POULTRY PRODUCTION FOR INSURING FOOD SECURITY

ID : 341

FEED THE FUTURE INNOVATION LAB FOR GENOMICS TO IMPROVE POULTRY: INCREASING FOOD SECURITY IN AFRICA BY ENHANCING RESISTANCE TO NEWCASTLE DISEASE AND HEAT STRESS IN CHICKENS

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Homestead and small-scale poultry production has tremendous potential for alleviation of malnutrition and poverty in climate-stressed rural communities in Africa. Poor animal health and husbandry practices limit village poultry production. Newcastle disease (ND) is the number one constraint of raising poultry in Africa, causing high mortality among village flocks. This research and development program seeks to improve poultry production by households and small farmers, and thereby improve food security, nutrition, and livelihoods in Africa -- key goals of the USAID Feed the Future Program. Genetics plays a significant role in resistance of poultry to NDV infection. Our program is applying advanced genetics and genomics to sustainably enhance innate resistance to ND and heat stress in chickens adapted to African environment. In the past six years, we identified the most robust genes and markers for genetic resistance through an integrated analysis of NDV challenge experiments and genomic analyses of well-characterized chicken lines under strictly controlled environmental conditions by RNA-seq and genome-wide association analysis (GWAS) using chicken 600K SNP panel, and of six African indigenous chicken ecotypes by GWAS. Our results confirmed the polygenic control of resistance to NDV and suggested that viral load and antibody titer following infection are important parameters for evaluating disease resistance. We estimated heritabilities for these traits in African chicken to be moderate to high (0.14 – 0.55), which indicates that selection to improve these ecotypes for resistance to ND is feasible. In addition, natural exposure trials of about 3,000 birds of African ecotypes with velogenic NDV strains were conducted to identify associations of markers with viral shedding, survival time, and anti-NDV titers. Based on our results, a low-density 5K SNP panel for genetic resistance to NDV was developed to select and breed local ecotypes with enhanced resistance to ND. We are currently assessing correlations of disease resistance traits with crucial production traits, such as egg production and growth rate. We are also conducting value chain assessment and developing a business plan to enable distribution of improved chicken lines to rural farmers with a focus on women who would benefit the most of an increase in resistance and productivity.

ID : 896

SUPPLEMENTARY FEEDING ENHANCES INCOME OF FARM WOMEN IN EXTENSIVE SYSTEM OF POULTRY PRODUCTION

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Family poultry production has been recognized globally to alleviate poverty, hunger and malnutrition in developing countries. It offers advantages over other agricultural sectors and is an entry point for promoting gender balance in rural areas. Vanaraja, a dual purpose bird developed by ICAR-Directorate of Poultry Research, Hyderabad, India is gaining popularity across India for family poultry farming. The present study was conducted to study the effect of supplementary feeding on production performance of Vanaraja laying hens and income of farm women reared under extensive system of production. A total of 300, four weeks Vanaraja chicks were distributed to 20 selected farm women (15 female chicks) in Chanrapada and Denua basta, Nimapada block of Puri district, Odisha, India. The farm women reared the chicks by allowing them scavenge outside during day time and during night they were provided night shelter (low cost house), constructed using locally available bamboo and paddy straw with provision of drinking water. At 16 weeks of age, supplementary feeding @ 35g/bird/day was given to one group (birds reared by 10 farm women). Egg production was recorded daily. Egg weight was taken at 32 and 40 weeks of age. Supplementary feeding @ 35g/bird/day during 16-40 weeks of age resulted in significantly higher egg production compared to those of without supplementation. The birds of supplementary fed group produced more eggs with higher egg weight compared to non supplementary one. Calculating the feed cost at Rs.20/kg, the net return was higher by Rs. 1790 per beneficiary due to supplementary feeding (around Rs. 128/ bird). From the results of the present study, it is concluded that supplementary feeding @ 35g/bird/day to Vanaraja laying hens in extensive system of production resulted in higher egg production and economic return.

ID : 1085

VILLAGE CHICKENS: OPTIONS TO IMPROVE NUTRITION AND HEALTH OF HIV AND AIDS AFFECTED INDIVIDUALS IN MARRACUENE DISTRICT, MOZAMBIQUE

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The prevalence of HIV is high in Mozambique. In young women, aged 15 to 24, the estimated HIV prevalence rate is 10.7%, compared to a prevalence rate of 3.6% among young men in the same age group. Nearly half of the people with HIV living in poor urban areas have a high prevalence of food insecurity. Village chickens can provide a scarce resource – high quality protein and micronutrients – in the form of meat and eggs, and can be sold or bartered to meet essential family needs such as medicine, clothes and school fees. Two hundred and forty vulnerable households comprising women, orphans and vulnerable children and HIV-affected individuals were selected in collaboration with local leaders and relevant government agencies in Maracuene District, trained in low-cost improvements to village chicken production, and each received a chicken 'starter pack' (three indigenous hens and one rooster) to improve their nutrient intake and household food and nutrition security. Over a period of 9 months, 98 hens had hatched 1 210 chicks. With the increased availability of chickens, farmers started selling more, but home consumption remained low during the first year. The number of chickens consumed per family over a 3 months period, increased slightly from 1.02 to 1.51 and the average number of chickens sold per family in three months period, increased from 0.38 to 2.53 chickens. Consumption of eggs remained low with households preferring to use them for hatching. In collaboration with the District Veterinary Services, thirty-six (21 male and 15 female) community vaccinators were trained and they vaccinated 18 056 chickens against Newcastle disease (ND) in 1 957 households of the District, including people affected by HIV and AIDS. The average flock size in the households that vaccinated regularly increased from 8.5 to 10.54 after two vaccination campaigns. No reports of mortality of chickens with clinical signs of ND were made in the areas where vaccination was taking place.

KEYWORDS

HIV - affected individuals, nutrition; Village chicken; Newcastle disease.

ID : 1276

FACTORS AFFECTING EMPOWERMENT OF SMALL HOLDER WOMEN POULTRY FARMERS IN SEMBABULE DISTRICT, UGANDA

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Introduction

This research was part of the bigger project “Hearing their voices: Action research to support women’s agency and empowerment in livestock vaccine distribution, delivery and use in Rwanda, Uganda and Kenya”

Indigenous chicken production is a preserve for women in Sembabule District but traditionally a cock belongs to a man.

Objectives

To determine the factors that affect women empowerment in small holder poultry production in Sembabule District, this research assessed;

- i. Gender dynamics in small holder livestock keeping households.
- ii. How gender dynamics affect poultry production in small holder households.
- iii. The effect of gender dynamics on livestock vaccine adoption at household level.
- iv. The effect of women empowerment level on livestock vaccine adoption at household level.

Methodology

The five USAID domains of gender analysis and qualitative methodologies were used in data collection during the months of November and December.

Women keeping a maximum of 80 chickens were targeted as research participants. Field work comprised of 6 jar voices, 4 focus meals, 6 focus group discussions (FGDs) and 15 key informant interviews which explored local understanding of empowerment, the livestock vaccine value chain and how these relate to rearing of indigenous chicken.

Results

Generally in Sembabule, all resources in a home belong to a man; including a woman. In remote areas, men usually take dominance over women in resource control and distribution. Whereas in trading centres are unmarried women control their own resources.

Women can rear chicken on family land but marketing of chicken remains a man's duty. Conflicts in households mainly arise when chicken multiply and a man realizes his wife is generating significant income. Such a man demands a part of the income claiming the wife is farming on his land.

Where women controlled some resources, those homes were happier and better than where men were dictators.

Women are more involved in group associations; through these groups, they easily move out of home, access credit and own livestock with little interference from their husbands.

There was little vaccine use due to lack of cold chain and a knowledge gap on vaccines.

Conclusions

This research identified the following intervention areas that can help empower women in Sembabule;

- i. Training women on commercial poultry production.
- ii. Creating opportunities for women to participate in the vaccine value chain.
- iii. Involving men in the empowerment process.

ID : 1424

VALORIZATION OF CRICKET FLOUR AS A SUBSTITUTE OF FISH MEAL ON QUAIL PRODUCTION PERFORMANCES IN THE SUDANO-GUINEAN ZONE OF CAMEROON,UC

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Fish meal is one of the relatively expensive ingredients that contribute to making the food nearly 70% of the cost in poultry farming (NRC, 1994; INRA, 1989). About 27% of marine fishing products are processed into fishmeal or fish oil without meeting farming needs (Cashion et al., 2017). The use of local resources like cricket (*Acheta domesticus*) flour could help to remedy these shortcomings.

The objective of the study was to increase the profitability of poultry farming in general.

To assess the effect of fish meal substitution by cricket flour on quail production performances, a study has been conducted from July to September 2018 in Ngaoundéré, Cameroon. For this purpose, 192 four weeks old quails were divided into 12 comparable batches of 8 females and 8 males. Each of the four experimental diets (T00, T15, T30 and T45), formulated on the basis of the level (0, 15, 30 and 45% respectively) of substitution of basic diet (Crude Protein: 20,18% and ME: 3013,78Kcal/Kg) fish meal by cricket flour was randomly assigned to 03 batches in a completely randomized design corresponding to 04 treatments repeated 03 times each. Data were collected on growth, carcass and some reproductive parameters. One way ANOVA and Duncan test at 5% significance level were used for data analysis.

Main results show that diet containing cricket flour has increased body weight in males (204.32 ± 5.69 g for T45) and regardless of sex (226.72 ± 29.45 g for T30) compared to respectively 184.17 ± 3.11 g and 214.55 ± 32.77 g recorded with the control group. In females, substitution at 30% increased the body weight (253.80 ± 6.48 g) and 45% induced a reduction in body weight (216.67 ± 6.49 g) compared to the control group value (244.92 ± 6.07 g). Carcass yield and proportion of different parts were not significantly affected by experimental diets. The significantly higher liver proportions were recorded at 15% and 35% incorporation of cricket flour compared to that of 45%. First songs and laid eggs were recorded at 07 weeks with T15 which also led to an improvement of laying performances in contrast to other treatments. In T45 females, ovaries were poorly developed compared to others.

It has been concluded that cricket flour could be used to replace fish meal in the diet of quail at the finisher and reproduction phases at the level of 15 and 30%

Key words: storage time, external, internal, chemical characteristics, battery eggs.

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REDUCING THE ENVIRONMENTAL IMPACTS OF POULTRY PRODUCTION

ID : 107

EFFECT OF INCLUSION OF BACILLUS SUBTILIS TO ENERGY AND PROTEIN REDUCED DIET ON GROWTH PERFORMANCES, NUTRIENT DIGESTIBILITY AND GAS EMISSION IN BROILERS

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816 day-old Ross 308 broiler chicks were used in a 6-wk feeding trial. Birds were randomly allotted to 1 of 6 dietary treatments (8 replicate pens/treatment with 17 birds/replication pen). The trial design is a 3×2 factorial arrangement with 3 levels of metabolizable energy (ME) and crude protein (CP), (100% ME and CP (1+2), 98.8% ME and CP (2+3), and 97.6% ME and CP (4+5) with constant amino acid levels. Treatments 2, 4, 6 were supplemented with 500 g/ton of a Bacillus subtilis-based probiotic. Performance results of this study showed a significant reduction ($P < 0.05$) in Body Weight (BW) and a degradation of Feed Conversion Ratio (FCR) in relationship with diet density reduction. Birds fed probiotic supplemented diets during the growing and finishing periods were heavier ($P < 0.05$) than those fed diets without probiotic. FCR improved ($P < 0.05$) in Bacillus-based probiotic supplemented groups during these periods, but Feed Intake (FI) remained unaffected when comparing nutrient densities and probiotic inclusion. Concerning nutrient digestibility, Apparent Total Tract Digestibility (ATTD) of nitrogen (N) and energy were significantly lower ($P < 0.05$) in ME- and CP-reduced diets than the control diet. Dry matter (DM) ATTD, ME- and CP-reduced diets tended ($P = 0.051$) to be lower compared with the control. Overall, DM, energy and nitrogen AATD improved in correlation with the supplementation of a Bacillus-based probiotic. The ATTD of nitrogen (N) and energy was significantly lower ($P < 0.05$) in ME and CP-reduced diets than the control diet. A decreasing trend ($P = 0.059$) of NH₃ gas emission from the excreta was observed for birds fed ME- and CP-reduced diets compared with control diets. Birds fed Bacillus-based probiotic supplemented diets showed reduced ($P < 0.05$) NH₃ emission compared to those fed a diet without probiotic. Inclusion of a Bacillus-based probiotic to ME- and CP- reduced diets improved zootechnical performances and reduced ammonia emission from the excreta, which could be due to improved intestinal health associated with microbial balance and lower levels of protein in the diet. Thus, inclusion of B. subtilis (DSM 17299) in nutrient-reduced diets could contribute to a more efficient feed to meat transformation, with lower nutritional safety margins and reduced NH₃ emissions. These combined effects could contribute to more sustainable poultry production.

Keywords: Bacillus subtilis, broilers, nutrient metabolism, digestibility, gas emission

ID : 216

REDUCTION IN PHOSPHORUS EXCRETION USING BUTTIAUXELLA SP. (META-ANALYSIS) AND POTENTIAL REDUCTION OF INORGANIC PHOSPHATES USE IN BROILER DIETS

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Buttiauxella sp. phytase has high activity at low pH and a wide pH range, meaning faster and more complete breakdown of phytate in the early gastrointestinal tract. This can result in more efficient release of phytate bound P, reduced dependency on inorganic phosphates, and can lead to reduced P concentration in excreta. To calculate P excretion reduction using Buttiauxella sp., data from ten studies were analysed (through meta-analysis) with 510 data points. These studies were conducted in different locations including UK, The Netherlands, USA, Canada and New Zealand. Commercial broiler chickens were used in these studies till the age of 21 days. Birds were fed ad libitum. The diets were mainly wheat or corn based with soybean meal as a protein source. Nutrient requirements (except Ca and P) were met using breed recommendations. All trials had a positive control (PC) with inorganic phosphate, a negative control (NC, without phytase and reduction of available P and Ca vs. PC) and graded levels of Buttiauxella sp. phytase added on to the NC (250, 500, 750, 1000 and 2000 FTU/kg). At day 21, excreta samples were collected to determine apparent total tract digestibility of P. Increasing phytase dose led to decrease in total P excretion in exponential manner ($P < 0.001$ for scale and growth rate, JMP Pro statistical program). At phytase dose of 2000 FTU/kg, the P excretion reduced by 50.7% and 63.4% respectively vs NC and PC. Based on the total broiler feed produced in 2018, in the EU (55 million tons, MT) and globally (274.7 MT), P excretion with inorganic phosphate would be 173,000 T in EU and 864,000 T globally respectively. P excretion can be reduced to 96,600 T and 482,000 T by using phytase at 500 FTU/kg in the EU and globally. This can further be reduced to 63,400 T and 316,500 T in the EU and globally with the higher phytase dose of 2000 FTU/kg. Similarly based on the ileal digestible P contribution, usage of inorganic phosphates (Monocalcium phosphate used for these estimations) in broiler feed can be reduced. Without use of Buttiauxella sp. vs inclusion of 500 and 2000 FTU/kg respectively, MCP inclusion can potentially be reduced from 2.5 MT to 0.63 MT and to 0.22 MT globally. The reduction in MCP usage at the EU level is from 0.51 MT to 0.13 MT and 0.04 MT at 500 and 2000 FTU inclusion respectively. In conclusion, inorganic phosphates and P excretion can be immensely reduced with the use of Buttiauxella sp. phytase in meat producing chickens.

ID : 345

ATTAPOULGITE AND OREGANO OIL IN FEED INFLUENCES ENVIRONMENTAL MICROBIAL COUNT IN POULTRY HOUSES

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It has been reported that increased density of birds in the poultry houses results in the accumulation of various pollutants, including bacteria and their toxins, toxic gases and other volatile compounds. Contamination levels in the houses, increase steadily in proportion to the birds age and especially towards the end of the rearing process. The increased microbial loaded the air is associated with microbial contamination of the birds. Pathogenic bacteria have been found within three kilometers of poultry farms.

The aims of this study were to examine the microbial load of air in broiler chicken houses and to examine the possible beneficial effects of dietary attapoulgite and oregano essential oil on the air microbial load.

In this trial, three identical experimental houses were used (14m x 58m), the first as control, where broiler chickens were fed commercial diets. In the second house, attapulgit (4000 ppm) was supplemented in the diets throughout the rearing period. In the third house, both attapulgit (4000 ppm) and oregano essential oil (300ppm) were supplemented in the diets. The outside environment of the poultry houses was also evaluated. The experiment was conducted in the months of September and October. Sampling was performed two days before slaughter to determine the total mesophilic flora (TMF). Samples were taken with the air sampler "Air Ideal" (bioMerieux S.A, France) at one location in each chamber, near its center and at a distance of 1.5m from the ground. After incubation in Plate Count Agar at 35°C for 48 hours, colonies were counted with the «Scan 100 automatic colony counter» (InterScience). In the control house, mean TMF was 3.40×10^5 cfu/m³ of air. In the second and third houses, mean TMF was 2.45×10^5 cfu/m³ and 2.23×10^5 cfu/m³ of air, respectively. In the outside environment air samples TMF was 4.80×10^4 cfu/m³. The predominant bacteria encountered indoors were Staphylococcus, Micrococcus and in lower counts Streptococcus, Bacillus and Enterobacteriaceae, whereas in the outdoor measurements Staphylococcus, Bacillus and Aerococcus were the most abundant.

The results of this trial could be used for the improvement of chicken housing conditions and microbial growth.

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ID : 355

THE EFFECT OF FEED FORM AND CRUDE PROTEIN CONTENT ON NITROGEN DIGESTIBILITY IN BROILERS

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Nitrogen (N) excreted by poultry is converted to ammonia (NH₃) which presents an environmental risk and a health risk to the farmer and animals. A reduction in dietary crude protein (CP) can effectively reduce N excretion in broilers provided that amino acid (AA) requirements are met. Feed form influences gastrointestinal tract development, feed and water intake and therefore the N and moisture level of the excreta. The aim of this study was to investigate the extent to which feed form and CP content influence N digestibility broilers. In total, 72 30-day-old Ross 308 male birds were assigned to 36 digestibility cages. Birds received diets with 19.5% CP (control), a 1.5 and 3.0% reduction in CP, while digestible AA:Lysine ratios were maintained. Each treatment was fed in mash and pellet form, which resulted in a 3×2 (CP level x feed form) experimental design with 6 treatments, each with 6 replicates. The study consisted of an adaptation and excreta collection period of 4 days each. Excreta was analysed for dry matter (DM), total N, total ammonium N (TAN) and uric acid (UA). Feed and water intake were also recorded. After the collection period, treatment diets containing 0.4% titaniumoxide (TiO) as an indigestible marker were fed for 3 days. To calculate apparent ileal N digestibility (AID), ileal digesta samples were analysed for total N and TiO. Data were analysed with linear mixed models using R 3.5.1 for Windows. Results were expressed as lsmeans and SE of those means. Pellets led to a higher feed ($P<0.001$) and water intake ($P=0.018$) compared to mash, while water to feed intake ratio was higher for control than for reduced CP treatments ($P=0.003$). Excreta moisture content did not differ between treatments, while CP ($P<0.001$) and UA ($P<0.001$) decreased with decreasing dietary CP. Faecal TAN was higher for pellets than for mash ($P=0.002$), while for UA the opposite was observed ($P=0.001$). Faecal CP digestibility (corrected for UA) was higher for mash than for pellets ($P=0.006$). Compared to the control, AID decreased with the 1.5 and 3.0% reduced CP treatments ($P<0.001$). N retention increased with a 3.0% CP reduction and was higher for mash than pellets ($P=0.034$). Although mash diets resulted in better N digestibility and retention compared to pellets, these results suggest that a dietary CP reduction within both mash and pelleted diets increased N retention and decreased excreta UA in broilers, resulting in lower levels of NH₃ emitted from broiler litter.

ID : 463

SUSTAINABLE BROILER FARMING: INCREASING BUTTIAUXELLA SP. PHYTASE DOSE TO 2000 FTU/KG LINEARLY REDUCED NITROGEN EXCRETION BASED ON META-ANALYSIS OF 10 TRIALS

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One of the strategies to increase sustainability of broiler production is to improve feed efficiency and reduce nutrients excretion such as nitrogen (N) to the manure. Among others, feed enzymes such as phytase and protease can improve crude protein (CP) and amino acids (AA) digestibility and can lead to reduced N excretion. Phytate is the major storage form of phosphorus (P) in plant-based feedstuffs and it has not only low P availability, but also can bind to AA and increase endogenous AA losses. Phytase breaks down phytate, increases availability of P and reduces the anti-nutritional effect of phytate and improves the digestibility of protein and AA. This study evaluated the impact of increasing a Buttiauxella sp. phytase dose level to 2000 FTU/kg on N excretion based on meta-analysis of data from 10 in vivo trials. These studies were carried out at Universities or Research Institutes globally in the last 10 years. In these studies, the phytase was added in a range from 250 to 2000 FTU/kg to a negative control diet that was deficient in P and calcium, but without reduction in dietary CP and without other enzymes. In each study, a positive control (PC) diet formulated to meet the nutrient requirements of broilers was used as a reference. Total tract excreta samples were collected in these studies to evaluate N excretion at 21 days of age (after 4 days collection). Diets were mainly based on corn and soybean meal or wheat and soybean meal with analysed CP level in the range of 19.6 to 23.0% (mean value of 21.5%), in mash or pelleted form and fed ad lib. In total, 513 data points were used in the analysis. Dose response was analysed for linear, quadratic or exponential curve using JMP 14. A significant linear ($P < 0.001$) response was observed on dietary digestible CP and total CP excretion. At phytase dose level of 1000 and 2000 FTU/kg, CP excretion decreased by 6.3 and 12.6 g/kg diet, compared to the nutritional adequate PC. It was estimated that 274 million MT broiler feed was produced in 2018. Based on this analysis, supplementation of the Buttiauxella sp. phytase at dose level of 1000 and 2000 FTU/kg could contribute to a great reduction in N in the manure (276,000 and 552,000 MT reduction in N excretion, respectively). In addition, these studies were carried out without application of CP and digestible AA matrix values, when applying these matrices, the N excretion could be further reduced

ID : 1458

PHYSICOCHEMICAL AND MICROBIAL PROFILING OF POULTRY LITTER OVER TIME POST-HARVEST

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Whilst experimental exposure to used litter often aims to induce reduced performance arising from anticipated litter-derived challenges (1,2), performance may be improved instead (3,4). Such variation may be associated with differences in litter quality between studies, e.g. arising from variation in pathogen load and duration between litter collection and usage. Here we investigated changes over time in microbial profiling and quality of litter, derived from a 35d broiler trial. Litter was kept at room temperature at trial end (d0), 200 g samples were collected weekly in sterile plastic bags at d0, d7, d14, d21 and d28, and stored at -80 °C pending analysis of pH, moisture and microbial profiling. The latter included quantification of 16S ribosomal DNA for total bacterial counts, 18S ribosomal DNA for total fungal counts and PCR screens for selected pathogens. DNA extracted from litter-derived bacterial pellets (5) using the DNeasy® PowerSoil® kit, was used to quantify total bacteria and fungi, *Salmonella* spp., avian pathogenic *E. coli* O1:K1:H7 (APEC) and *Clostridium perfringens* through qPCR, with outcomes converted into log gene copy number per g litter (cpg). Analysis of variance showed that pH gradually reduced from 8.42 at d0 to 8.09 at d28 (s.e.d. 0.066; P=0.002). Moisture levels were stable between d0 and d14 at ~33.7%, and then gradually reduced to 12.5% at d28 (s.e.d. 2.28%; P<0.001). Total bacteria increased from 8.06 log cpg at d0 to 8.25 log cpg at d7, then gradually reduced to 7.71 log cpg by d21 and then increased to 8.23 log cpg at d28 (s.e.d. 7.44 log cpg; P<0.001). Total fungi gradually reduced from 6.85 log cpg at d0 to 6.30 log cpg at d28 (s.e.d. 6.49 log cpg; P=0.013). PCR results revealed that this litter was negative for *Salmonella* spp and *Clostridium perfringens*. However, APEC gradually reduced from 7.00 log cpg at d0 to 6.30 log cpg at d28 (s.e.d. 5.67 log cpg; P<0.001). Thus, microbial profiles and quality of litter may vary over time post-harvest. Future work employing reused litter methods may benefit from detailed litter characterisation to optimise study design, to define what constitutes a sufficient pathogenic load, and to assist explaining subsequent outcomes on broiler performance and gut health.

1 Kidd et al 2003. J App Poult Res 12, 115-123

2 O'Reilly et al 2016. Proteome Sci 15, 10

3 Kennard et al 2017. Poult Sci 30, 47- 54

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ROBOTICS AND BIG DATA FOR PRECISION FARMING

ID : 212

PRECISION NUTRITION STRATEGIES TO IMPROVE GROWTH PERFORMANCE AND ECONOMIC PERFORMANCE OF BROILER CHICKENS.

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Near infrared reflectance spectroscopy (NIRS) is a rapid technique to evaluate the nutrient profile of feed ingredients. A more advanced precision nutrition system such as NutriOpt has incorporated NIRS, extensive nutrient databases and broiler modelling programs to improve animal and economic performance. The objective of the study was to compare the various precision feeding solutions to optimize growth and economic performance of broiler chickens.

In total 1350 male Ross 308 broiler chickens were used. Five treatments were applied, with 18 replicate pens of 15 birds each per treatment. In treatment 1 (T1) birds received a standard European diet. The raw ingredients used in T1 were analyzed via NIRS. Diets optimizations were done using NIRS results only (T2), NIRS + Trouw Nutrition nutritional database (TNB) (T3), NIRS + TNB + NutriOpt Broiler model for margin (T4) and NIRS + TNB + NutriOpt Broiler model for feed conversion ratio (FCR) (T5). All diets were fed in 3 phases, a starter (D0-14), grower (D14-28) and finisher (D28-42). The growth performance was measured per pen at the start and end of each feeding phase. On D42 two birds per pen were sampled for carcass measurements. Economic performance was calculated based on the growth performance results. All results were statistically analyzed via ANOVA (SAS).

During the entire study, T1 and T2 showed the lowest body weight. Until D28, T5 (1.58 kg) outperformed ($P<0.05$) the other treatments, followed by T4 (1.51 kg) which was heavier ($P<0.05$) than T1 (1.23 kg), T2 (1.18 kg), and T3 (1.41 kg). The FCR was the lowest ($P<0.05$) in T4 and T5 in the starter phase, and in T5 in the grower phase. An unexpected heat wave during the finisher phase caused that the overall FCR was highest for T4, which was similar to T5 and T1, but higher than T2 and T3.

The margin calculations based on the overall growth performance were not significantly different. Until day 28, before the heat wave, the margin of the treatments were in the following order: $T1 \& T2 < T3 < T4 < T5$ (0.68, 0.66, 0.80, 0.88, and 0.92 euro/bird, respectively). There was no effect of the treatments on carcass yield, thigh yield or wing yield. The breast meat yield was significantly highest in T5 (23.7%) and lowest in T2 (21.0%).

In conclusion, using NutriOpt precision feeding strategy comprising of NIRS + global database + broiler model results in an optimal growth and economic performance compared to the traditional formulation approach.

ID : 227

SUPPORTING NORMAL FUNCTION OF THE GASTRO-INTESTINAL TRACT WITH EFFECTIVE PROBIOTICS

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It is well-known and thoroughly documented that effective probiotics support many of the normal functions of the gastrointestinal tract, specifically, the digestive, absorptive, barrier and immune functions. For decades, those investigating the efficacy of probiotics in poultry have reported improvements in variables of economic importance, such as feed conversion, average daily gain, morbidity, and mortality. It is our hypothesis that these types of differences measured in well-controlled, challenge-type studies or in field observation trials are due to a greater percentage of the birds in the probiotic-fed group simply being normal. That is to say, as the percentage of normal, healthy birds increases in one group versus another, then all variables of economic importance should likewise improve. Concomitantly, variation among the birds in the probiotics-fed group should decrease. To test this hypothesis, we worked with a major global broiler integrator and fed 1.8M Ross birds from 72 flocks from the same complex, placed as hatched (males and females mixed); half receiving a daily feeding of GalliPro Fit (1.6×10^6 cfu/g of feed) and other half only on the standard diets. Average age at market was 35,7 and 35,2 days respectively. For feed conversion, livability, and condemnation, the distribution of observations from the probiotics-fed group compared to the control group was shifted in an economically-positive direction and variation was reduced. As a consequence, average performance for each variable was significantly improved (FCR: 1.617 ± 0.074 vs 1.651 ± 0.106 ; livability: $96.12\% \pm 1.70\%$ vs $95.73 \pm 2.33\%$; and condemnation: $0.64\% \pm 0.39\%$ vs $0.86\% \pm 0.64\%$; GalliPro Fit-fed vs Control, respectively). Daily feeding of an effective probiotic enables commercial broilers to digest and absorb nutrients in a normal manner, to maintain a balanced microbiome, and to achieve as much of their genetic potential as their environment allows.

ID : 403

DEVELOPMENT OF AUTOMATED MEASURES OF INDICATORS OF WELL-BEING AND HEALTH OF POULTRY: SPECIAL CASE OF AVIAN INFECTIOUS BRONCHITIS

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Guaranteeing consumers a production process that respects animal welfare is one of the civil societal demands, especially for fast-growing poultry production (1). EBENE® method, developed by ITAVI, coupled with the new image and sound technologies must allow for finer and additional analysis than those carried out by man, and even better to manage health of poultry. From the video-surveillance methods/tools are proposed to measure general variations in the activity of chickens but none of them can be used to measure the animal welfare principles proposed by the OIE. One objective of the project is to demonstrate the feasibility to associate group-wide sound measurements signing the state of well-being and health of poultry in the course of a respiratory pathologies. This project (E-broiler-track) received financial support from French ministry of agriculture (CAS DAR). The project will thus lead to technical specifications that will serve as a basis for the development of an infectious diseases control tool for poultry breeders. First part of this study was to collect sound data after infectious induced disease under experimental controlled conditions. Different protocols have evaluated welfare with EBENE® method and clinical symptoms were measured during avian infectious bronchitis (IBV). After infection, all infected chickens (30/30) for each chicken line (EOPS PA12 and ROSS 308) developed respiratory clinical signs. 100 percent of tracheal swabs were positive IBV at D4 post-infection (p.i.). Tracheal lesions of IBV (7/7 chicken after necropsy at D7 p.i) confirmed the IBV infection, as compared to the control group. Chicken rales, coughs and sneezes have therefore been characterized and analysed using an algorithm developed here. The 2/3 of the registered sound were coughs and sneezes and 1/3 were rails in infected chickens. These initial results made possible to better characterize the pathophysiology of IBV from zootechnical, behavioural, acoustic and histological points of view. The acoustic approach is innovative and has made possible the development of new algorithms and sound analysis to differentiate healthy from diseased chickens. Those results gave us relevant data for early predictive information of a potential infection. In short, this approach will aim to develop a useful tool for poultry farmers to monitor their farms.

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ID : 411

MEASURING CARBON DIOXIDE CONCENTRATIONS IN BROILER HOUSES

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European directive 2007/43/CE establishing standards for the protection of broilers, sets a limit of 3000 ppm of carbon dioxide (CO₂) concentration not to be exceeded at animal's level, over the entire duration of the flock. Since then, CO₂ concentration sensors are developing in French poultry buildings. The purpose of this article is to provide methodological advices for continuous measurement of CO₂ concentrations in a broiler house, by looking at CO₂ commercial sensors' technical specifications and their optimal position in barn. This study, part of a project named GestCO₂, received financial support from the French Agriculture Ministry (CAS DAR). Various CO₂ sensors were compared in a commercial barn. Analysis of accuracy, response time and drift in time of each sensors showed that two sensors over five were not suitable to be used for CO₂ monitoring and/or control in poultry houses. The position of the CO₂ sensor at 80 cm +/- 20 cm of height, between pipets and feeders appeared to be the best compromise to measure, not only a representative CO₂ concentration at animal's level but also of the whole house. However, at the end of the flock, this height can underestimate CO₂ concentrations at animal's level in case of high CO₂ productions by animals and litter. In the observed commercial barns, the horizontal heterogeneity of CO₂ was higher at the beginning of the flock than at the end. These results suggest using more than one CO₂ sensors for continuous measurements in poultry barns to characterize this heterogeneity. According to the sensors's tests, first level investment should be in high-performance sensor and in its maintenance than purchasing an additional sensor.

ID : 78

FROM MEASURING AVERAGE BODY WEIGHT OF THE FLOCK TO PRECISION FEEDING IN BROILERS: A MODELLING APPROACH TO ADJUST DAILY FEED COMPOSITION

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Precision feeding in broiler production could reduce feed cost and nitrogen and phosphorus excretion. However, in order to adjust daily feed composition, daily requirements of birds according to their actual growth performance must be evaluated. In commercial farms, body weight (BW) is now frequently measured with automatic weighing scales. We propose here to use the data provided by these devices to 1) forecast BW gain to come, 2) estimate associated metabolizable energy (ME), digestible lysine (dLys) and available phosphorus (avP) requirements, and 3) recompose a full diet using two types of pellets with different nutritional characteristics. Firstly, BW data collected during the previous days are used to fit a quadratic function ($BW=f(\text{age})$). Using this function, $BW(d)$ and $BW(d+1)$ are predicted, and "most probable" BWG for the next day ($BWG(d+1)$) is then calculated as $BW(d+1) - BW(d)$. This first model was validated by comparing BW and BWG predictions (Y) to measured data (X, $n = 814$). BW is very well predicted ($Y = 1.04 X - 0.03$, $R^2 > 99\%$), while BWG prediction quality is lower but still acceptable ($Y = 0.76 X + 0.02$, $R^2 = 56\%$). Secondly, lipid and protein depositions according to actual $BW(d)$ and predicted $BWG(d+1)$ are estimated with allometric relationships developed using literature data. Requirements for ME and dLys are then estimated from these daily depositions. For avP, we considered an equation from the literature, directly using $BW(d)$ and $BWG(d+1)$ values. The requirements sub-model was validated by comparing predictions (Y) to measurements of ME, dLys and avP daily intake (X, $n = 325$). ME predictions are very good ($Y = 1.04 X$, $R^2 = 96\%$) while the predictive quality for dLys and avP is slightly lower but still acceptable ($Y = 0.93 X + 139$, $R^2 = 75\%$ and $Y = 0.99 X + 31$, $R^2 = 71\%$ respectively). Thirdly, 1001 blend of A/B pellets are computed (increase of %A in the blend from 0 to 100% with a step of 0.1%), with A being poorer in ME and richer in dLys and avP than B, respectively. For each blend, assuming that feed intake is regulated on an energy basis, daily feed intake is estimated using ME requirement and blend content. dLys intake is then calculated and compared to dLys requirement. The best blend is the first one where dLys intake is above daily requirement. In practice, this blend could be easily prepared and distributed with a commercial weighing/mixing hopper.

ID : 462

BIRD-E: A NEW DEVICE TO MEASURE THE FEED INTAKE, FEEDING BEHAVIOR AND GROWTH IN POULTRY

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In poultry production, feed represents more than 60% of the total production cost. So, it's important to find out strategies to improve feed efficiency through better nutrition and appropriate genetic selection. Apart from the economic impact, a better feed efficiency is also associated to a lower environmental impact.

Calculation of feed efficiency implies to record feed intake and growth. Until recently, to obtain these individual measures, animals had to be reared in cage, not allowing them to express their natural feeding and social behaviors. Electronic feeders are thus essential tools to record individual intake of animals raised in group and on floor, i.e. in conditions comparable to usual rearing conditions, especially for organic or label rouge systems. Several devices have been developed, but none include all the required characteristics, i.e. feed intake and body weight measures, from hatch to slaughter, without a corridor for the access to feed. INRAE, together with ITAVI and SYSAAF thus designed BIRD-e (Bird Individual Ration Dispenser-electronic), a feed station meeting all these needs.

BIRD-e is a circular feeder with 8 free accesses to the feed. It can feed 100 animals as soon as they hatch. One access is composed of a tray and a feed tube ending on a manger. Both are fixed on scales, giving respectively the weight of the animal and the feed weight. The presence of an animal is detected thanks to a microchip recognized by an antenna attached above each access. All data obtained with scales and antennas are synchronized and transmitted to an internal computer.

We used this feed station to test the adaptation of two genotypes of chickens (slow-growing chickens and rapid growing chickens) to sustainable diets. Apart from performances showing that animals were able to adapt to alternative diets without major effects on performances (detailed results on performances are presented in Berger et al. abstract), daily results showed that in some cases, transitions between starter, grower or finisher diets should be moved forward, as performances start to decrease several days before the transition.

As our new tool also give us access to information such as number, length or intensity of meals per day and intervals between meals, we can finely study transition phases and thus detect whether some behavioral profiles of birds are connected to differences in adaptation to transitions, even with similar final performances.

ITAVI: Institut Technique de l'Aviculture

SySAAF: Syndicat des Sélectionneurs Avicoles et Aquacoles Français

TOWARDS LONGER CARRIERS IN LAYERS

ID : 186

HOW DO HOUSING STRUCTURES IN FREE-RANGE PRODUCTION AFFECT LAYING HEN WELFARE?

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Free-range laying hens in the EU are housed with either single-tier ('flat deck', FD) or multi-tier (MT) housing structures in the shed. MT allows a greater number of hens per square meter of shed space, because each tier adds to the useable space. As a result, their use is growing in popularity, but with what welfare consequences? A total of 42 commercial free-range laying hen flocks were visited once near end-of-lay (FD: mean age 73 weeks, median age 72 weeks; MT mean age 75 weeks, median age 74 weeks) in the UK. Production data were collected from farm records, and welfare measures (e.g. feather condition, as an indicator of feather pecking, using a 0-5 scoring method of Savory and Mann 1999; keel bone fracture (KBF) and deviation (KBD), using a binary system devised by Casey-Trott et al. 2015; and foot condition, using a 0-2 scoring method from Welfare Quality 2009) were taken from up to 5 hens in 6 different zones of the shed and range (zones 1-4 inside the shed; zones 5-6 on the range, where zone 5 was within 5 m of the popholes, zone 6 was 5-10 m from the shed. Data were transformed where necessary for normality, and analysed by linear mixed models or generalised linear mixed models in Genstat. Mean values shown are original means. Average flock size was larger with MT (n=17 flocks, 14,671 hens) than FD (n=25 flocks, 8,896 hens). Mortality was not significantly different due to high variation (P=0.189, FD 7.5%, MT 4.8%). Mean feather condition was low (i.e. good) with both structures and did not differ (P=0.533, MT 0.90, FD 0.74) but was significantly lower in zone 6 compared to any zone inside the shed (P

ID : 228

BETAINE AND ITS EFFECT ON BONE STRENGTH.

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Introduction: Laying hens can suffer from osteoporosis (OP). Genetics plays a role in susceptibility to OP and bone fracture^{1,2}. Cystathionine Beta Synthase (CBS) was identified as a strong candidate gene at a QTL for bone strength (BS)³. Poor BS was associated with high homocysteine (HC), the substrate for CBS. HC can be methylated into methionine with betaine (BT), a common feed additive⁴. Phytase (PY) breaks down phytate from grains to increase the availability of phosphorus, an important factor in bone quality⁵. This study aims to evaluate the effects of BT and superdosing PY on bone quality, plasma HC and egg production.

Methods and materials: 1920 LSL-lite laying hens were reared following the Lohmann management guide, split into 4 treatment groups with 24 replicates. A 2x2 design, 1000mg/kg BT was provided both alone and with PY. The control was 300 PY units (FTU), and the superdosing level was 1000FTU. Plasma and bone (keel, humerus and tibia) were collected at 45 and 70 weeks of age (Wk) from 400 birds each time. Measurements of plasma HC level and bone radiodensity were taken. Humerus and tibia were subjected to 3-point bending and maximum load was recorded. Statistical analysis was carried out using linear mixed models with the main effects BT, PY and age as fixed effects and the replicate as a random effect.

Results: At 45Wk, HC levels was lower ($p=0.0440$) in birds fed BT ($13.8\pm0.470\mu\text{M/L}$) and those not fed BT ($14.7\pm0.441\mu\text{M/L}$); no difference at 70Wk. At 45Wk, tibia BS ($p=0.0250$) was higher in birds fed BT ($288\pm3.25\text{N}$) than in birds not fed BT ($280\pm3.92\text{N}$). At 70Wk, tibia BS ($p=0.0360$) was higher birds fed BT ($281\pm3.82\text{N}$) than in birds not fed BT ($274\pm4.24\text{N}$). There was no difference in tibia density (D) at 45Wk, but at 70Wk tibia D ($p=0.0160$) was higher in birds fed BT (2.04 ± 0.0127) than in birds not fed BT (2.00 ± 0.0127). There was no effect of PY. No difference was seen with keel and humerus. There was no effect of BT or PY on bird performance or on egg quality parameters.

Conclusion: The addition of dietary BT successfully reduced plasma HC level and improved tibia BS without compromising production. BT could reduce the effect of OP and improve laying hens' welfare. Ongoing investigation includes tibia histology. Future studies with different BT concentrations and relevant factors may be valuable.

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ID : 305

EFFECT OF HERBAL CALCITRIOL - IN LAYING HENS ON PERFORMANCE AND EGG QUALITY IN THE LATE LAYING PERIOD

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Decreased laying performance and lower egg quality of high-performing laying hens are often reducing egg producers profits. One of the reasons for this issue is the changing metabolization capacity of vitamin D3 in the birds, as a result of aging, physiological changes and production disorders. The objective of this study was to evaluate the effect of an additional herbal active vitamin D3 on performance parameters and egg quality in the late production period in laying hens.

73-week-old Novogen Brown (NB) laying hens were randomly divided into two groups of 225 birds per group (with 3 replicates per group) for a trial duration of 8 weeks. The birds were kept in floor pens and had ad libitum access to water and feed. Both groups received a wheat and corn-based diet. 450mg/kg of herbal calcitriol product was added to the treatment group on top of regular vitamin D3 (3000 IU D3/kg in both groups), whereas the control group received no herbal-calcitriol product. The egg count and animal losses were recorded daily. Sorting and egg weight recording was carried out once a week. Feed consumption by back weighing in a 28-day rhythm. The egg quality (breaking strength, egg weight, haugh units etc.) was determined at the start, after 28 days and after 56 days on the basis of a sample of 50 eggs per compartment.

In the study, plant-based calcitriol numerically improved laying performance (+4.8%), total egg mass (4.8%) and reduced feed conversion rate (-3.3%). The herbal calcitriol product increased the albumen consistency significantly improved the eggshell breaking strength by 4.08 N ($p \leq 0,05$ +/- 0,999). No significant effects were seen on egg weight and mortality. Furthermore, herbal calcitriol product showed no effect on number of cracked or dirty eggs.

In conclusion, the results of the study indicated that the herbal-calcitriol product tended to improve laying performance in older laying hens. Moreover, the product had a positive influence on the persistence of eggshell stability. A supplementation of the active form of vitamin D seems to be an efficient supplement to maintain performance in aged laying hens.

ID : 336

THE EFFECT OF GENOTYPE AND AGE ON LAYING PATTERN AND EGG WEIGHT OF LAYING HENS

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The study evaluated laying pattern, egg weight and oviposition time of seven different laying hen genotypes at the beginning and end of laying cycle. In order to fulfil the study purpose, an experiment was conducted on Moravia BSL, Bovans Sperwer, ISA Sussex, ISA Dual, Moravia Barred, Dekalb White and Bovans Brown. The same birds were kept at the beginning (20-26 weeks of age) and at the end of laying cycle (64-70 weeks of age). Total number of 105 laying hens were individually housed in cages (550 cm² per bird). Egg production, oviposition time and egg weight data were recorded daily. The mean rate of lay, mean sequence length and the mean internal cycle length were then calculated. The data were analysed using ANOVA two-way analysis of variance PROC GLM in SAS. The study results indicated an interaction between hen genotype and age for the rate of lay (P

ID : 349

HOW TO FEED NON-BEAK TRIMMED LAYING HENS?

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Since 2018, it is not allowed to beak trim laying hens in the Netherlands. It was expected that an intact beak would result in more feed selection and feather damage. The aim was to test which feed structure would be beneficial for performance and feather condition in non-beak trimmed (NBT) Dekalb White laying hens. The experiment was carried out as a randomised block design with 6 dietary treatments and 2 beak treatments (NBT vs. beak trimmed by laser (BT)). Diets differed in feed structure: coarse mash, fine mash, crumble, pellet, and pellet + wheat (similar in ingredient and nutrient levels; 2800 kcal/kg, 6.8 g dLys/kg). A sixth dietary treatment was a coarse mash with an energy level of 2600 kcal/kg and dLys level of 6.3 g/kg. These diets were fed to either BT or NBT birds. Each Diet x Beak treatment was tested with 3 aviary pens (= replicates) with 330 laying hens each. Production performance (feed intake, laying rate, egg weight, egg mass, feed conversion ratio (FRC), mortality rate) was measured from 25-72 weeks of age. Feather condition (5 birds per pen; scale 0-5; 0 = intact feathers, 5 = completely denuded) was determined every 8 weeks starting from 24 weeks of age onwards. Experimental data were analysed by ANOVA with Diet, Beak and interaction as factors.

Significant interactions were found for feed intake (P

To conclude, non-beak trimmed birds should be fed a fine mash diet to have best production performance and feather condition.

ID : 733

SKELTAL QUALITY IS RELATED TO KEEL BONE DAMAGE, BUT IS NOT EXPLAINED BY VARIATION IN EGG PRODUCTION

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The physiological adaptations for egg laying make hens susceptible to osteoporosis. There is also a welfare paradox; cage free production is associated with better bone quality but also with greater incidence of bone damage, often the keel. The 'Long Life Layer' has great benefits for sustainability, but long production could increase the risk, it is often stated that poor bone quality is due to selection for increased egg production. However, we do not believe the evidence for a negative relationship between the numbers of eggs laid and bone quality is strong. In this study, we examine the hypothesis that there is negative genetic correlation between egg production and bone quality and demonstrate that keel bone damage is related to general skeletal quality.

Heritability and genetic correlations between egg production and bone quality in a White Leghorn (WL, 53 weeks of age n=956) and a Rhode Island Red (RIR, 68 weeks of age, n=973) pure line were calculated using ASREML. Early egg numbers, related to age at first egg, and the remaining periods (egg number) were analysed separately. Tibia and humerus breaking strength and the radiographic density of the tibia, humerus and keel bone were measured. In the WL the keel bone was given a numerical fracture grade (0-5), with higher graded keel bones having more damage.

Heritability estimates for bone quality traits are between 0.19 ± 0.07 and 0.59 ± 0.09 . Heritability of keel bone density was near zero. Genetic correlations between egg number and bone quality were not significant. In the WL there was a negative genetic correlation between bone quality and early egg number (-0.50 ± 0.11). When bone quality in grade 0 and grade 5 keels were compared, tibia breaking strain was 217.4 ± 3.3 and 181.2 ± 14.6 ($p < 0.001$) respectively.

There was no evidence for a relationship between egg production and bone quality. However, there was an effect of age at first egg (WL). Longer laying periods may not effect bone quality, but the onset puberty needs to be considered. The confounding effects of damage, as fracture callus is radio dense, may explain the lack of heritability for keel bone density. Results also show an inverse relationship between keel bone grade and tibia and humerus breaking force - higher graded fractures have lower breaking force. This demonstrates that keel bone grade is related to skeletal health and that bone quality overall can be improved by genetic selection.

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ID : 781

IS THERE A NEED TO REDUCE THE ENERGY AND/ OR PROTEIN LEVEL IN THE FEED OF AGED LAYING HENS?

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Very little information is available on the nutritional requirements of aged laying hens (>70 wks). However, considering the tendency towards extended production cycles, gaining insight in these demands is of utmost importance. Feeding laying hens to meet their needs is essential to fully exploit the genetic potential on production and simultaneously maintaining good bird health. It can be assumed that the energy and protein demand of aged laying hens decreases. Nevertheless specific guidelines are lacking.

An experiment was conducted to determine whether there is a need to reduce the energy and/ or protein level in the feed of aged laying hens. A total of 288 ISA Brown laying hens (74- 91 wks) were distributed over 4 dietary treatments with 8 replications and 9 birds per experimental unit (enriched cage). The hens of the control group (T1) were fed a standard laying hen diet containing 2759 kcal/kg ME and 15.5% CP. In dietary treatment 2 the CP-level and limiting amino acid levels (meth, cyst, lys, and thr) were reduced by 10% (ME 2759 kcal/kg; CP 13.95%). Treatment 3 contained a reduced energy level (1%) based on a reduction of starch (ME 2601 kcal/kg; CP 15.5%). Whereas for dietary treatment 4 both the energy and protein level (+limiting AA's) were reduced (ME 2613 kcal/kg; CP 13.95%). All four diets were corn- soy based with a stable 5.6% crude fat. Heterogeneity, common in older flocks, results in large within-groups variation (e.g. body weight). Therefore 32 ISA Brown laying hens were individually monitored from 97 to 101 weeks of age as well. Performance traits, egg production and egg quality (mean egg weight, dynamic stiffness, breaking strength, Haugh Units, yolk color and shell thickness) were measured for each 28-day period. Liver health associated parameters (lactate dehydrogenase, aspartate transaminase, liver weight, abdominal fat content, liver haemorrhagic and color scores) and plumage condition were determined at 74, 87 and 91 weeks (n=8/ treatment) of age. Performance traits for the individually kept laying hens were measured every 7-day period.

Both energy and protein level did not significantly influence feed intake, feed conversion ratio, or other performance traits. Egg production nor egg quality were affected by dietary energy or dietary protein level. No significant differences were found for liver health associated parameters, plumage condition was no different for hens fed a reduced energy and/ or protein level. It can be concluded that the energy and protein content in the feed of aged laying hens can be reduced by 1%, and 10% respectively without compromising performance traits or egg quality. Hopefully the results of the digestibility trial will explain the absence of effect on mean egg weight, hen weight and abdominal fat content. These results are currently being analyzed.

ID : 927

POSITIVE IMPACT OF PREBIOTICS AND ANTIOXIDANTS ON EGG QUALITY AT THE END OF THE LAYING HEN PRODUCTION CYCLE

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Laying hens are confronted with different stressors (heat stress, parasites, mycotoxins...) which leads to a higher production of free radicals that affect layer health and egg quality. Indeed, oxidation decreases the eggs quality. CalSEAGrow® contains pectic oligosaccharides, which have a prebiotic effect and chlorogenic acid which has an antioxidant effect. The current study aimed to evaluate the efficiency of this combination on egg quality and laying performance at the end of the production period (from 69 to 79 weeks-old). Indeed, when hens get older, egg producers face a decrease of egg quality and performance.

A total of 576 laying hens were assigned to 1 out of 2 treatments as follow: T1 – Control (Basal Diet), T2 – Basal Diet + tested product (1.0kg/ton of feed). Each treatment is divided into 12 cages of 24 hens during production period. Experiment is divided into 2 periods:

- 1st period (14 days) Hens receive the same feed during this period in order to test comparability of the initial groups,
- 2nd period (70 days) is the experimental phase.

Production data (feed consumption and egg production) were collected during the entire period of the trial. Egg quality was measured at 67 and 79 weeks old through Haugh Units and vitelline membrane strength. Along with the ageing of the hens, these criteria can be deteriorated. Kirunda et al. (2001) demonstrated a positive impact of vitamin E (antioxidant effect) on vitelline membrane strength.

Data were analyzed with Anova (with fixed effect: treatment and block) on SAS software.

First period results analysis showed no difference between the two groups concerning performance and egg quality. Thus, comparability of initial groups is admitted.

Second period analysis shows significant difference for T2 group that present higher Haugh Units: 80.0 ± 9.6 (T2) > 77.6 ± 10.3 (T1), $p = 0.014$; and better vitelline membrane rigidity (N/mm): 8.6 ± 1.0 (T2) > 8.4 ± 1.2 (T1), $p = 0.036$. Although statistical tests can't assure significant difference for feed conversion ratio, it also seems better for T2 group: 2.20 ± 0.10 (T1); 2.18 ± 0.10 (T2), $p = 0.463$; while maintaining egg mass (g/hen/week) T1 = T2 (390 ± 0.02), $p = 0.427$.

This study reported that the tested product enhances laying hens egg quality and performances, notably through its antioxidant properties that contribute to maintain the oxido-redox balance, thus reducing the negative consequences generated by oxidation like degradation of egg quality.

ID : 1258

EFFECTS OF A GLOBAL ENZYME SOLUTION ON PERFORMANCE, NUTRIENT DIGESTIBILITY AND BONE METABOLISM OF EGG LAYING HENS FROM 18 TO 84 WEEKS OF AGE

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Exogenous enzymes allow for dietary nutrient density reductions. The objective was to determine the efficacy of a multicarbohydrase and phytase complex (MCPC) in laying hens. The hypothesis was that MCPC added to nutrient-reduced diets would support productivity equivalent to that of the positive control (PC; no nutrient reduction). Single-comb White Leghorn hens were housed in 56 cages of 4 birds for each of 5 dietary treatments from 18 to 84 weeks of age (woa). The PC diet met the primary breeder nutrient specifications; negative control 1 (NC1) was reduced in apparent metabolizable energy (ME) and digestible amino acids (dAA) by 5%, available P (avP) and Ca reduced by 0.18% of the diet; NC2 was reduced in ME and dAA by 7%, and avP and Ca reduced by 0.18% of the diet; and each of NC1 and NC2 supplemented with 1,250 xylanase units, 860 b-glucanase units and 1,000 FTU of phytase/kg of feed (NC1+MCPC and NC2+MCPC, respectively). Daily egg production was recorded, and on a 4-week basis, hen body weight (BW), % production, egg weight, specific gravity, shell breaking strength, and feed conversion ratio (FCR; kg feed:kg eggs) were determined. Apparent ileal digestibility (AID) of crude protein and P, apparent ileal digestible energy (AIDE) and bone ash were determined at 36, 56, and 84 woa. The cage was the experimental unit; data were analyzed by ANOVA for diet and age main effects and their interaction, with differences significant at P 0.05. From 20 to 84 woa, BW, egg weight and shell quality were not influenced by diet, but egg production and average daily egg mass were reduced by NC1 (P = 0.02 and 0.03, respectively) and NC2 (P = 0.03 and 0.008, respectively) relative to PC; MCPC restored performance of each NC diet (P = 0.24 and 0.26, respectively, for egg production and daily egg mass). FCR was lower for the PC hens than either NC diet (P

ID : 1461

TOWARDS THE IDENTIFICATION OF THE MOLECULAR PATHWAYS UNDERLYING THE ALTERATION OF CALCIUM METABOLISM IN HENS AT THE END OF LAY

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The current objective of the egg industry is to increase the hen's productive carrier up to 100 weeks. However, numerous studies have reported that egg quality and hen's bone strength deteriorate with age, because of altered calcium (Ca) homeostasis. This study was designed to make an integrative description of Ca homeostasis pathways in laying hens at peak and end of lay (23 and 90 weeks old). Brown laying hens aged 20 or 87 weeks were reared in individual cages to record oviposition and fed a commercial layer's diet containing 3.5 g/kg CaCO₃ (1/3 fine, 2/3 coarse particles). At 23 or 90 weeks of age, hens were euthanized at 0-1h, 9-10h or 18-19h post ovulation (PO) prior to the collection of plasma and tissue samples (6 hens per stage PO). Plasma samples served for the assay of iP and vitamin D metabolites. RNA were prepared from parathyroid gland (PG), liver, jejunum, medullary bone (MB) and kidney for a quantitative study of candidate gene expression. All data were analyzed using a linear model by robust regression. The effects of the main factors PO stage, age and the interaction were tested. In case of significant effects in the model ($P < 0.05$), all pairwise comparisons were performed using the least-square means method and a Tukey adjustment. Parathyroid hormone (PTH) gene expression in the PG did not vary with age, while the expression of several Ca transporters and vitamin D receptor (VDR) in the jejunum, and that of the transient receptor potential channel subfamily V member 5 (TRPV5), mediating Ca reabsorption in the kidney, decreased with age ($p < 0.05$). This could explain the previously reported reduced Ca retention in older hens. The higher expression of the Carbonic Anhydrase 2 (CA2) gene ($p < 0.05$) and the lower expression of collagen type I alpha 1 chain (COL1A1) gene ($p < 0.05$) in the MB also suggested exacerbated bone resorption and impaired bone accretion in the older hens. An increased expression of Fibroblast growth Factor 23 (FGF23) was also observed in the MB. This likely triggered the decreased plasma levels of 1.25(OH)₂D₃ and expression of target genes under its regulation. Our data highlights the molecular mechanisms underlying the osteoporotic syndrome previously documented in aged laying hens, thus providing new perspectives for future dietary interventions.

UNDERSTANDING NUTRITION X GENETICS INTERACTION TO OPTIMIZE FEED EFFICIENCY

ID : 175

EFFECT OF LEAF MEALS AND THEIR COMPOSITE ON THE PERFORMANCE, ANTI-OXIDANT, SERUM AND HAEMATOLOGY PROPERTIES OF TWO BROILER CHICKEN BREED

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One of the reasons for the inclusion of leaf meals (LMs) in broiler diets is to take the advantages of their bioactive components to improve the growth performance (Sugiharto et al., 2019). Thus, the present study seeks to assess the efficacy of Bamboo Leaf Meal (BLM), Avocado Leaf Meal (ALM) and their Composite (mixed at 1:1) as phytogetic growth promoters in broiler chicken diets. A basal diet was formulated to contain 21% crude protein and 3000 kcal/kg metabolizable energy and used as straight diet from day-old to 42 days. The basal diet was divided into four portions such that the first portion was without LM (control) and the remaining 3 portions were supplemented with 5g/kg of BLM, ALM and their Composite (CLM), respectively. One hundred and twenty eight (128) each of Cobb and Arbor acre were purchased and randomly distributed into the four (4) treatments arranged in 2x4 factorial (2 breeds x 4 treatments) in Completely Randomised Design. Each treatment had four (4) replicates with eight (8) birds per replicate. Leaf Meal supplementation significant ($p < 0.05$) affected. The glutathione (GSH), superoxide dismutase (SOD) and catalase were not significant ($p > 0.05$) for supplement effect, breed effects and their interaction. However, broilers fed supplemental LM diets recorded lower SOD and catalase but higher GSH than those fed control diet. Haematological indices measured were not significantly ($p > 0.05$) affected by the supplemental LMs except for MCV, MCH, Lymphocytes and monocytes. Although, breed effect was not significantly ($p > 0.05$) different for all haematological indices measured, the interactive effects of breed and supplemental LM were significant (p

Keywords: Leaf Meals, Anti-oxidants, Blood, Broiler Chicken, Phytogetic.

Reference

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H.I. and Sartono, T.A. (2019), Recent advances in the incorporation of leaf meals in broiler diets. *Lrrd* 31(109). http://www.lrrd.org/lrrd31/7/sgu_u31109.html

ID : 321

ADAPTATION OF SLOW- AND RAPID-GROWING BROILERS TO ALTERNATIVE DIETS

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To refine feed efficiency selection of broilers, we developed an electronic feed station allowing a continuous record of body weight and feed intake of animals reared in group on floor. Using this station, we compared slow-growing Label Rouge (LR) and fast-growing standard (S) chickens fed either with a corn-soybean diet (CD) or with an alternative diet (AD) including DDGS, fava beans, sunflower and rapeseed meals. Within a genotype, diets were isoproteic, isoenergetic and fulfilled the need of the animals during the three phase of their growth (S birds : 2850, 2900, 2950 kcal.kg⁻¹ and 21.5, 20, 18.5 % CP; LR birds: 2750, 2850, 2900 kcal.kg⁻¹ and 20, 18, 16.5 % CP).

For each genotype, the diet effect was estimated by an ANOVA on daily data of body weight, feed intake and feed efficiency, and on carcass composition and meat quality traits at slaughter (84 and 35 d for LR and S, respectively). The potential effect of the diet on performance homogeneity was checked through an ANOVA on the coefficient of variation including the effects of the period (starter, grower, finisher), the diet and their interaction. Correlations between slaughter traits and daily data were calculated by strain and diet.

LR chickens fed with AD were 4 to 9% heavier and had a 6 to 12% lower FCR during the starter period, but no effect was observed on feed intake nor meat characteristics. For S chickens, AD had no impact on the daily BW and feed efficiency but led to a slightly less acidic (+1.7% for pH), less yellow meat (9.7% for the b* index) and leaner carcass (-14% for abdominal fat yield). Unexpectedly, groups fed with AD were more homogeneous for all traits than with CD. For example, CV was respectively 20 and 22% lower for FCR in LR and S chickens.

Correlations between daily and slaughter traits differed between genotypes and diets. For example, for the group fed with the alternative diet, cumulated FCR was correlated with breast yield in LR chickens (-0.23), but not in S chickens (-0.13). Moreover, these correlations showed that some traits recorded during the starter period could be used as precocious indicators of slaughter traits. For example, bodyweight during the starter phase was positively correlated with fat yield in LR chickens fed with AD.

These first results indicate that animals can adapt to alternative feedstuffs, even those with a rapid growth rate. A complementary study is in progress on pedigree birds from a heavy line of broilers fed with the alternative diet, in order to suggest new criteria of selection related to feed efficiency and adaptability.

ID : 144

CONVENTIONAL VS. SLOW-GROWING BROILER CHICKENS: APPARENT RETENTION OF NUTRIENTS, CECA DIGESTA MICROBIAL METABOLITES, JEJUNAL HISTOMORPHOLOGY, AND TIBIA ASH WITH OR WITHOUT A DIETARY MULTI-ENZYME SUPPLEMENT

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Emerging market differentiation for broiler meat production in terms of conventional (C) and slow-growing (SG) strains is necessitating comparative research on various production aspects of these strains. We investigated the effects of a dietary multi-enzyme supplement (MES) on apparent retention (AR) of components, ceca digesta short chain fatty acids (SCFA), jejunal histomorphology, and tibia ash in C and SG strains. A total of 640 d-old chicks of 4 strains (C and 3 SG: F6, J10, and N14) were housed in metabolic cages (10 birds/cage). The strains differed by time they took to reach 2.2 kg BW corresponding to 37, 43, 47, and 50 d for C, F6, J10, and N14, respectively. An antibiotic-free corn soybean meal-basal diet was formulated for two-phase program (starter and grower). Half of the birds per strain received basal diet with 0.04% MES containing phytase, protease, cellulase, and β -glucanase. Diets were allocated by strain, creating a 2x4 factorial arrangement of treatments (8 treatments and 8 replicates/treatment). Birds were allocated equal amount of feed in each phase (starter, 476 g; grower, 1,360 g) and had free access to water. Excreta samples were collected at the last 3 days of trial and pooled within cages. Birds were euthanized at the end (upon the consumption of allocated feed) for cecal digesta, jejunal tissue, and left tibia samples. There was no interaction ($P>0.05$) between strain and diet on all parameters with exception of ceca digesta concentration of acetic acid ($P<0.01$). There was strain effect ($P<0.01$) on AR of nitrogen (J10 and N14 had the lowest retention) and fat (N14 had the lowest retention). Dietary MES improved the AR of dry matter (DM), ash, nitrogen (N), fat, NDF, and gross energy (GE; $P<0.05$). Inclusion of MES improved the AR of DM (3.4%), ash (22.0%), N (2.7%), fat (4.1%), NDF (6.2%), and GE (2.4%) compared to the birds fed without MES. There was strain effect on the concentration of lactic, propionic, and iso-butyric acid ($P<0.05$). We did not observe any effects on jejunal histomorphology. Tibia ash (g/kg BW) weight was higher ($P<0.01$) for strain J10 compared with strain C and F6 whilst strain N14 was intermediate. The data indicated that the MES improved AR of components independent of strain, indicating effects of feed enzymes are not influenced by genetic background of broiler chickens. The SG strains had higher tibia ash content, indicating higher bone mineralization compared to conventional strains.

ID : 325

EFFECT OF REARING CONDITIONS AND NUTRITIONAL CONCENTRATION ON GROWTH PERFORMANCE AND MEAT QUALITY OF FAST- AND INTERMEDIATE-GROWING BROILERS BREEDS

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There is a growing consumer demand for higher poultry welfare and meat quality in Europe. Such demand may be met by using slower growing broiler breeds. Nutritional concentration and rearing practices may vary across farms or countries. Hence, the aim of this study was to evaluate the effect of nutritional concentration (Reco ; recommended vs Dec; -3% metabolizable energy and -5% crude protein and amino acids) and rearing conditions on the performance and meat quality of fast (Fast; Ross 308) or intermediate (Int; JA 957) growing broiler breeds. Rearing conditions were either optimal (Opt) or suboptimal (SOpt). Birds in SOpt conditions were housed in higher density (18 vs 15/m²), on a less absorbent litter (straw vs sawdust) and were fed diets with higher byproducts inclusion rates and without xylanase compared to their Opt counterparts.

2088 day old chicks were randomly allocated into a 2x2x2 factorial design with breed (Int or Fast), nutritional concentration (Reco or Dec) and rearing conditions (Opt or SOpt) as experimental factors. There were 6 replicates per group. Feed intake and bodyweight gain were measured at 0, 10, 21, 35 and 40 days. Footpad dermatitis, myopathies and breast meat yields were measured at 41 day for 24 birds/group. A three-way ANOVA was performed to compare groups.

Compared to their Int counterparts, Fast birds showed a higher bodyweight gain (2.3 vs 1.8 kg, $p<0.001$) and tended to have a lower feed conversion ratio (1.64 vs 1.67, $p<0.10$) when fed recommended diets and reared under optimal conditions. Hence, Fast birds appeared more sensitive to dietary or environmental interventions than Int birds. In addition, Int birds had lower breast meat yields, footpad dermatitis and myopathies scores than Fast birds ($P<0.05$) irrespective of the other experimental factors. Also, footpad dermatitis scores were significantly affected by rearing conditions ($P<0.05$).

Overall, this study suggests that Int birds may be able to demonstrate higher welfare outcomes than Fast birds under various experimental factors, as measured by lower footpad dermatitis and myopathies scores. Fast birds have, however, a higher economic profitability through superior bodyweight gain and breast meat yield. Hence, future improvements in broiler welfare are partly conditioned by the consumer's willingness to spend more money in supporting improved rearing conditions or by purchasing slower growing birds.

ID : 127

THE DISCOVERY OF HUNDREDS OF NOVEL MEMBERS OF THE CHICKEN CAECAL MICROBIOTA USING METAGENOMIC SEQUENCING

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The microbes which inhabit the chicken caeca play an important role in chicken nutrition, principally by converting dietary fibre into short chain fatty acids which can be used as an energy source by the host. They also play an important role in pathogen protection and the development of the immune system. By utilising sequencing data we are able to construct microbial genomes from these communities, allowing us to gain a greater understanding of their diversity and function. In this study we extracted DNA from the caecal contents of 24 chickens, 12 of which belonged to a slower-growing breed (Ranger Classic) and 12 of which belonged to a faster-growing breed (Ross 308). Birds were fed either a vegetable-only diet or a diet containing dietary fish meal. DNA was sequenced using shotgun sequencing on an Illumina NovaSeq. Reads were assembled then binned to construct metagenome assembled genomes.

Using 1.6T of sequencing data we constructed 469 high-quality draft bacterial genomes, which included 283 novel species (5 of which were *Lactobacillus*) and 42 novel genera. We then mapped our genomes to metagenomic data generated by a previous study of commercial chicken farms across nine EU countries, demonstrating that our genomes were abundant in chickens from all nine of the countries studied. We also compared the abundance of our genomes and their carbohydrate-active enzymes between our samples, finding significant differences between birds from different lines and on different diets, as well as a core microbiome shared between all groups. In conclusion, we have substantially increased the amount of chicken microbiota derived genomes present in public databases through the construction of metagenome assembled genomes. Our data can be used as a reference for future studies of the structure and function of the chicken microbiota.

ID : 229

THE EFFECT OF A MULTI-CARBOHYDRASE ENZYME AND YEAST-DERIVED PRODUCT ON INTESTINAL MICROBIOME STRUCTURE AND GUT FUNCTION OF TURKEYS

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The aim of this study was to determine the effect of a multi-carbohydrase enzyme (E) and its combination with an enzymatically-modified yeast cell wall (Y) on the abundance of ileal and cecal microbiota and gut function of turkeys. The experimental diets, including the control (C), C+E, and C+E+Y, were fed to 7 replicate pens of 4 birds each from 22 to 56 days of age. In comparison to C, consecutive effects of E and E+Y on gut microbiome were observed. The addition of E+Y resulted in a decrease in relative abundance of the phylum Firmicutes (from 86.9 to 61.7; $P=0.010$), including the family Lactobacillaceae (from 57.8 to 23.2; $P=0.074$), and was followed by a significant increase in the number of Actinobacteria (from 2.5 to 14.5; $P=0.036$), including the family Bifidobacteriaceae (from 2.2 to 15.6; $P=0.003$), Cyanobacteria (from 2.9 to 15.1; $P=0.065$), and Proteobacteria (from 1.1 to 3.0; $P=0.026$) in the ileal digesta. A significant decrease in the abundance of Firmicutes was not followed by the order Clostridiales (26.1 vs. 30.3) with the family Ruminococcaceae, the butyrate-producing bacteria, showing somewhat, although not significant ($P>0.05$), increase in their relative abundance (2.8 vs. 6.9). This coincided with an increased concentration of butyric acid in the ileal digesta ($P=0.023$), as well as the proportion of butyric acid within the total SCFA content ($P=0.022$). As opposed to the ileum, the addition of E or E+Y did not affect the abundance of Firmicutes in the ceca. Within this phylum, however, the E+Y diet increased the relative abundance of Lachnospiraceae (from 11.2 to 17.5; $P=0.049$) and Blautia (from 5.9 to 11.1; $P=0.027$). Furthermore, a trend towards increased abundance of Proteobacteria ($P=0.066$) and Enterobacteriales ($P=0.067$) was observed. The addition of E+Y increased the activities of bacterial α -arabinopyranosidase, α -arabinofuranosidase, β -xylosidase and β -cellobiosidase. The SCFA content of the cecal digesta was much higher than that of ileum, which would reflect differences in microbial activities in both compartments as well as possible increased rate of absorption of SCFA, including butyric acid, from the ileum. In this context, butyrate is the preferred energy source for the epithelial intestinal cells and has been implicated in the prevention of intestinal inflammation. Overall, it appears evident that the positive effects of enzyme and yeast supplements on bacterial communities would be more pronounced in the small intestine.

ID : 348

**GUT HEALTH OF BROILER CHICKENS REARED WITH INSECT LARVAE AS ENVIRONMENTAL ENRICHMENT:
FOCUS ON INTESTINAL MORPHOLOGY AND MICROBIOTA MODULATION**

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Introduction. Insect larvae have recently been tested as feed additive for broiler chicks [1], but their use as environmental enrichment to improve animal health and welfare represents a novel approach. This study investigated the effects of *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) larvae as environmental enrichment on gut morphology and microbiota of broiler chickens.

Material and methods. A total of 180 4-day-old male broiler chickens were randomly allotted to 3 experimental treatments (6 pens/treatment, 10 birds/pen): a) control group (C, commercial feed), b) HI group (commercial feed + HI live larvae) and c) TM group (commercial feed + TM live larvae). The insect larvae were daily provided as 5% of the expected daily feed intake and feces were collected at 0, 7, 14 and 38 days. The animals (3 birds/pen) were slaughtered at 38 days of age and gut samples and cecal contents were collected. Small intestine morphology was evaluated by morphometric analysis, while fecal and cecal microbiota were assessed by 16S rRNA amplicon based sequencing. Data were analyzed by R software ($P < 0.05$). Fecal microbiota showed an increase in α - and β -diversity measures as a function of the sampling time ($P < 0.05$). At genus level, HI- and TM-fed broilers showed a decrease in *Butyricoccus* and *Ruminococcus* (short chain fatty acids [SCFAs]-producing bacteria [2]), an increase in *Clostridiales* (common fecal bacteria) and an increase in *Lactobacillus* (beneficial bacteria) and *Staphylococcus* (potential pathogenic bacteria, $P < 0.05$). At genus level, *Clostridium*, TM7, *Victivallaceae*, *Eubacterium* (the latter two being SCFAs-producing bacteria [3]) and *Collinsella* (modulator of virulence and pathogenicity of potential enteric pathogens [4]) were characteristic of insect larvae-fed broilers ($P < 0.05$).

Conclusion. The utilization of HI and TM larvae as environmental enrichment caused no negative effects on gut health of broiler chickens in terms of unaffected gut morphology and partial, positive cecal microbiota modulation. The controversial results of fecal microbiota analysis may be attributed to its temporal fluctuations, as it consists of microbial mixtures originating from different gastrointestinal segments.

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UNDERSTANDING THE MICROBIOME FOR AN IMPROVED MANAGEMENT OF HEALTH AND WELFARE

ID : 382

A PRECISION GLYCAN MICROBIOME MODULATOR REDIRECTS CORE METABOLIC PATHWAYS OF THE MICROBIOME LEADING TO IMPROVED GASTROINTESTINAL FUNCTIONALITY AND GROWTH PERFORMANCE

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The strong motivation to eliminate antibiotic growth promoters (AGPs) from animal production provides an enormous opportunity to rethink the conventional approach to animal performance, health and welfare. Most current alternatives to AGPs focus on the different microbial species present in the gut. Rather, we reconsider the microbiome as an organ whose collective metagenome is responsible for thousands of metabolic functions that strongly influence the host. Although the microbiota themselves can vary significantly between farms and pens, our approach targets core metabolic pathways and functions that are highly conserved across microbiomes.

Here we present a precision glycan microbiome modulator (M2-1) and demonstrate a controlled shift in the functional metagenome of the gut microbiome with a consequential benefit on host biomarkers and performance.

Broiler caecal microbiomes sampled from various locations, with significant underlying variation in the phylogenetic composition, were exposed to M2-1 under anaerobic ex vivo conditions. Targeted metabolomic analysis demonstrated a consistent increase in the production of short chain fatty acids via the induction of the C3 and C4 microbial pathways and a decrease in ammonia.

A second study was performed with broiler chickens over 42 days to confirm the effects in vivo. Cobb 500 broilers were fed in two treatment groups with 21 replicates per treatment and 40 birds per replicate. Control birds received a standard corn-soy diet. Treated birds received the control diet supplemented with 500 ppm M2-1. Body weight gain, feed intake and weight-corrected feed conversion (cFCR) were measured. From 10 birds per treatment; caecal microbiome samples were analysed by functional metagenomics shallow shot gun sequencing and ileum tissue samples were analysed by gene expression microarrays.

Induction of C3 and C4 microbiome pathways was confirmed in vivo. Additionally, the nitrogen utilization pathway of the microbiome was downregulated in broilers fed M2-1 compared to control. In the ileal tissue KEGG pathways cytokine-cytokine interaction and nitrogen utilization were significantly altered by feeding M2-1, indicating a host response to the metabolites produced by the microbiome. Treated birds exhibited an improvement of 6.9 points in cFCR versus control.

In conclusion, M2-1 redistributes metabolic fluxes into core metabolic pathways of the microbiome, aiding gastrointestinal functionality and improving animal performance.

ID : 383

A PRECISION GLYCAN MICROBIOME MODULATOR CONSISTENTLY IMPROVES BROILER GROWTH PERFORMANCE

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One of the largest global trends influencing how animals are produced is a decreased reliance on AGPs. This trend is motivated by the need to control the risk of developing antibiotic microbial resistance and by an ever-increasing consumer pressure. Most current alternatives to AGPs focus on the promotion of specific bacteria within the gastro-intestinal tract, underestimating the strength of the microbiome as a whole and its thousands of metabolic functions that strongly influence the host animal.

We present a precision glycan microbiome modulator (M2-1) that has been selected from over 200 possible glycan feed ingredients. M2-1 was selected because it targets core metabolic pathways and functions that are highly conserved across microbiomes, leading to improved gastrointestinal functionality. Here, we demonstrate the selection of M2-1 for consistent broiler performance across multiple growth conditions through a meta-analysis of 15 in vivo studies involving candidate glycans. We then highlight a subset of these studies focused on M2-1.

A total of 20,820, 1-day old male broilers were used, (Ross 308, Ross 708, and Cobb), and fed either a corn soybean-meal or a wheat soybean-meal based diet, from 0 to 35 or 42 days of age. The experimental treatments were: T1, control; T2, T1 + M2-1 included at 500 ppm. All diets contained 1,000 FYT/kg Ronozyme HiPhos. Feed intake (FI) and weight gain (BWG) were measured at the end of each study. Mortality was recorded daily. Corrected feed conversion ratio (cFCR) was calculated by considering the weight of dead birds and corrected with a common body weight. The data was analysed by means of a linear mixed model with treatment group as categorical fixed variable, control group as reference level, and study ID as random intercept. Trial-specific variances were allowed by the model, which also weighted the data proportionally to the inverse of the study variance. All analyses were performed in R (Version 3.4.3). At the end of the growing period, M2-1 had significantly improved cFCR by 4.9 points on average ($P < 0.001$) versus the control group, with improvements between 2.7 points and 6.9 points. This was driven by a significant increase in BWG, with an average improvement of +1.9%.

In conclusion, the M2-1 microbiome modulator, selected through a strong screening platform, consistently increased performance of broilers regardless of locations and diets. This has the potential to close the gap created by the AGP removal.

ID : 385

A PRECISION GLYCAN MICROBIOME MODULATOR REDIRECTS CORE METABOLIC PATHWAYS OF THE MICROBIOME LEADING TO IMPROVED RESILIENCE TO ENTERIC DYSBIOSIS AND INFLAMMATION

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The removal of antibiotic growth promoters (AGPs) opens the potential for health challenges, formerly suppressed by AGPs, to re-emerge in the broiler industry. For example, AGPs reduced intestinal inflammation by broadly suppressing the gut microbiota. Our view is that AGP removal provides an opportunity to rethink how we achieve resilience to enteric dysbiosis and inflammation. We consider the microbiome as a beneficial organ, whose collective metagenome is responsible for thousands of metabolic functions that strongly influence host animal health.

Here, we present a novel precision glycan microbiome modulator (M2-1) that improves broiler resilience to enteric dysbiosis and inflammation by stimulating protective pathways in the gut microbiome, despite natural variations in phylogenetic composition between animals. The effects of M2-1 on health were assessed by an in vivo inflammation challenge study in broilers.

Ross 308 broilers were housed in battery cages for 28 days. Unchallenged control birds were fed a standard corn-soy diet. Challenged birds received a dysbiosis component (day 14 administration of Paracox-5 at 10x commercial dose) as well as a nutritional pro-inflammatory component (16% potato protein starting on day 7). Treated birds were subjected to the challenge model supplemented with 1000 ppm of M2-1. Results were analysed using one-way ANOVA (GraphPad 5.0).

Ileal histology showed reduced villi damage in M2-1 treated birds compared to challenged birds ($p < 0.01$), as quantified by Alcian blue staining. M2-1 birds demonstrated increased gene expression associated with ileal nutrient absorption (FABP2, MGAM and SLC34A2) versus challenged birds ($p < 0.05$) and increased expression of the SCFA transporter SLC5A8 ($p = 0.02$). Proinflammatory genes NOS2, IL1B and IFNG tended towards decreased expression in the M2-1 group versus challenged birds. In the Peyer's patches the percentage cytotoxic T cells was increased in the challenge group, while less T helper cells were observed; M2-1 supplementation attenuated this. Intestinal permeability was improved in M2-1 fed birds as shown by claudin 5 expression ($p = 0.05$). Liver histology showed improved phenotypes in the M2-1 treated group, which also exhibited a decrease in plasma hepatic acute phase Alpha-1-acid glycoprotein levels.

In conclusion, M2-1 supports gastrointestinal functionality via its effects on core metabolic pathways of the microbiome, making birds more resilient to enteric dysbiosis and inflammation.

ID : 433

EVALUATION OF PROBIOTICS EFFECT ON GUT MICROBIOTA OF CHICKENS BY ANALYZING FRACTAL STRUCTURES

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INTRODUCTION

Gut microbial communities in chickens are self-organized to implement joint biochemical processes and extract dietary components from substrates. As a result of adaptation to birds' natural environment the gut microbiota was able to self-organize into fractal structures that enable complex biochemical transformations of plant substrates with minimal energy costs. The microbial biosystem is regularly adjusted depending on the feed entering the gut evenly distributing the enzymatic load between the microbial components. Calculating the index of the fractal structures is proposed to assess the qualitative and quantitative parameters of these processes.

MATERIALS AND METHODS

The experiment investigated the microbial communities in the intestines of chickens with different diets and two types of probiotics. The method is based on fractal analysis of the frequency spectrum of the operational taxonomic units (OTUs) in the microbial communities obtained by molecular genetic method. The initial OTU spectrum is converted into a reduced spectrum of OTU groups with each group combining OTUs that occur at a similar frequency. A two-dimensional fractal portrait of the microbial community is constructed where each OTU group is represented by a point with coordinates that depend on this group's frequency. A fractal portrait is necessary to conduct a topological analysis of the point location and isolate fractal structures in the microbial community. The analysis uses triangles with vertices at the OTU groups points as a fractal measure. The smallest triangle heights are chosen as the fractal measure scale. Their share in the microbial community determines the index of fractal structures (IFS) which also takes into account the uniform enzymatic load distribution between OTUs. The obtained frequency-taxonomic spectra were used to calculate the index of fractal structures.

RESULTS

An analysis demonstrated that probiotics affect the self-organization of microbial communities (IFS up to 0.7–0.9) when grain (barley, wheat) is included in the feed. In other cases the probiotic effect on the self-organization of the microbial gut communities was low (IFS about 0.06). The chicken gut microbiota had not yet adapted to individual diet components and had formed an efficiently functioning microbial biosystem for digesting a new substrate.

CONCLUSION

The experiment with chicken diets demonstrated that self-organization level of the gut microbial communities depends on the diet.

ID : 471

ORGANIC AND MEDIUM CHAIN FATTY ACIDS SUPPLEMENTS FOR BROILER CHICKENS - EFFICIENT POULTRY PRODUCTION AND MEAT QUALITY BASED ON MICROBIOTA MODIFICATION

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The ban of antibiotic growth promoters in European Union (EU) has resulted in a need for new strategies to modulate the microflora metabolism in chickens (Buclaw M., 2017). Many different feed additives were developed, but effect of them depend on many factors including management conditions, stock density, litter quality, age and gender of broiler chickens, microbial ecology of digestive tract and etc. (Hajati H. 2018). In this study, the impact of organic acid (OA) and medium chain fatty acids (MCFA) on broilers caecal microbiota, productivity, and meat quality under industrial conditions were analyzed. The comparison study was conducted on 60 000 broiler chickens allocated to two groups (fed with OA and fed with MCFA supplements). Basal diet of the above mentioned two groups was supplemented with (I) MCFA (caproic, caprylic, capric and lauric acids) at the level of 15 g/kg and (II) with OA (formic, lactic, propionic, butyric, acetic acid, citric, sorbic acids) at the level of 20 g/kg. Chickens were slaughtered at the 42 day of age, and the caecum microbial profiles, short chain fatty acids (SCFA) and meat quality characteristics (carcass yield, physical, chemical and technological parameters) were evaluated. In statistical analysis differences were classified by Tukey test and results were considered statistically significant at $p \leq 0.05$. OA supplement showed higher effectiveness, in compare with MCFA, on increasing quantity and variety of beneficial bacteria strains (*Lactobacillus* and *Bifidobacterium*) in caecum of broilers. Also, MCFA showed significant influence on SCFA increasing, including acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. As well MCFA increases breast meat yellowness and water holding capacity (WHC), however, higher carcass yield (leg and abdominal fat yield) and better growth performance (higher body weight and lower mortality) of the group fed with OA were established. Finally, both tested supplements showed desirable influence, however, the OA supplement was more efficient on beneficial bacterial profile, growth performance, and carcass traits, and the MCFA was more appropriate in increasing amount of SCFA and breast meat technological parameters.

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ID : 536

CHICKEN GUT MICROBIOTA AS A POTENTIAL SOURCE OF THE OVIDUCT MICROBIOTA

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The mechanism in which the poultry gut microbiota is established in the newly hatched chick is unknown. It is well known that Salmonella, a poultry pathogen, can transfer from the chicken's intestines to the oviduct and be incorporated into the developing egg¹. This mechanism might be employed by commensal bacteria and facilitate their transfer from hen to chicks. Recently, diverse bacterial DNA has been found in the oviduct, which suggests the existence of an oviduct microbiota and the possibility of bacterial vertical transmission².

The objective of this study was to compare the oviduct microbiota and the gut microbiota, and to provide evidence of viable bacteria in the oviduct and the existence of a functioning oviduct microbiota.

We collected cecum, jejunum and oviduct samples from 10 broiler breeders (Cobb; 37 weeks). We analyzed and characterized the bacterial composition in each sample using 16S rDNA sequencing. Samples were also plated in anaerobic conditions to determine the viability of the bacteria.

We found that the similarity between the oviduct microbiota and the cecum microbiota ($22.42 \pm 17.23\%$) was significantly higher than the similarity between the oviduct microbiota and the jejunum microbiota ($5.55 \pm 3.07\%$). Overall, 353 OTUs were shared between ceca and oviducts, representing 52% of the cecum OTUs (making up 90% of total cecum abundance) and 62% of the oviduct OTUs (making up 60% of total oviduct abundance). Finally, preliminary trials of culturing bacteria from the oviduct demonstrate that there are viable bacteria in the oviduct, belonging to the families Lactobacillaceae and Enterobacteriaceae.

The high similarity between the gut microbiota and the oviduct microbiota, and the fact that the oviduct microbiota is similar throughout its length, indicate that one of the sources of the oviduct microbiota is the gut microbiota. It can be assumed that material from the gut reaches the top of the oviduct. Our ability to culture at least some of the oviduct bacteria establishes the existence of this microbiota and refutes the main claim against 16S data which only prove the existence of bacterial DNA and not necessarily of viable bacteria.

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ID : 566

THE EFFECT OF ESSENTIAL OILS BASED FEED ADDITIVE ON BACTERIAL PATHOGENS IN CAECUM OF LAYING HENS WHILE S. ENTERITIDIS INFECTION

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The effect of a feed additive based on a mix of essential oils on caecum microbiome of laying hens was observed.

The experimental part of the study was carried out on 40 Lohmann White adult laying hens in All-Russian Research Veterinary Institute of Poultry Science vivarium.

The Experimental group received the feed supplement based on essential oils from the first day of the experiment, while the Control group received standard feeding diet. 3 weeks after the start of the experiment, half of each group hens was infected with an epizootic strain of *S. enteritidis* in the amount of 5×10^8 CFU, afterwards further parting into groups was performed: Control, Infected Control, Experimental, Infected Experimental.

The metagenomics analysis was performed in molecular-genetic laboratory of Biotrof Ltd (Saint-Petersburg, Russia). Metagenomic analysis was performed on a MiSeq genomic sequencer (Illumina, Inc., USA) with a MiSeq Reagent Kit v3 (Illumina, Inc., USA) sequencing of the variable V3 and V4 regions of the 16S rRNA gene. The reference database was RefSeq Ribosomal Database Project (RDP) 16S v3 May 2018 DADA2 32bp.

It was noticed that the introduction of essential oils-based feed additive into the hens' diet resulted in significant decrease of total pathogenic bacteria proportion in the caecum content. The part of pathogenic microorganisms in caecum samples was 5% -16% lower than the group with the standard diet.

Infection of a hens by *S. enteritidis* did not lead to its detection in the caecum content with 16S metagenomics method, but already a day after infection it caused an increase in the content of other pathogenic microorganisms: in the Control group, the content of pathogenic bacteria after infection increased by 31.5%, and in the Experimental group by 65%. It should be noted that the number of pathogenic bacteria in the Control Infected group was the highest and higher than in the Experimental Infected group by 6.5%.

A week after infection, the content of pathogenic bacteria in the studied groups decreased among infected hens by 30%, which indicates a gradual normalization of the pathological process. Still in samples from an Experimental Infected group, the content of pathogenic bacteria was 5.5% lower than in Control Infected group.

Uninfected groups had a lower part of pathogenic bacteria in comparison with infected analogues: in the Control group it was 35% lower than in the Control Infected group, and in the Experimental group it was 32% lower than Experimental Infected group.

In conclusion, it should be noted that the content of pathogens was highest in samples from Control Infected group hens, and the lowest - in samples from Experimental group hens.

ID : 584

CHARACTERIZATION OF GUT MICROBIOTA IN CHICKENS WITH EMBRYONIC DEVELOPMENT

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Greater attention has been given to gut microbiota in poultry industry, due to its important roles. However, there are limited reports on gut microbiota development of chicken embryo with time. To bridge this gap, we examined the gut microbiota composition in the chicken embryos across different stages of development.

Fifty-four samples, collected in Beijing Fatty, Shiqiza and Bobcork (Specific Pathogen Free) breeds including whole embryos that were incubated for three days, guts from chickens embryo that were incubated for 12th and 19th days for the study .DNA extraction was conducted immediately and16S rRNA gene Sequencing was performed. Furthermore, QIIME, a general bioinformatics pipeline was used for microbiome analysis. KEGG helped in the alignment of the predicted genes and their functions. Microbiota compositions among different groups were further analyzed using LEfSe.

96 genera such as Ochrobactrum, Phyllobacterium, and Amycolatopsis were core microbiota in three stages of development. Secondly, 94 genera of microbes were observed to change significantly between E3 and E12, 143 genera significantly differed between E12 and E19 of chicken embryo ($P < 0.05$). Ochrobactrum and Amycolatopsis reduced with growth changes of E3 (30.4%), E12 (25.1%), E19 (13.6%) and E3 (11.5%), E12 (7.4%) and E19 (5.6%) respectively. Contrarily, Phyllobacterium rate increased to 47.9% at E19, indicating the growing trend of microbial diversity among the embryo's development. Moreover, the Principal Component Analysis showed a high level of similarities between the E3 and E12 compared with E19 while the alpha analysis showed more diversity of gut microbiota at E19. Furthermore, the functional predictions showed that metabolic pathways such as energy metabolism, genetic information processing were significantly enriched on day 3 and day 12 in our study, suggesting their strong influence on growth, development and immunity of chicken embryo.

Our results indicated time as a cogent factor in gut microbiota composition, and a significant change in the gut microbiota population as they developed with time was observed. Our findings indicate the growing trend of microbial diversity among the embryo's development, also establish a background for understanding the distribution of gut microbiotas in the chicken embryo, across different developmental stages.

Keywords: Gut Microbiota, Embryo Development, 16S rRNA gene sequencing, Chicken

ID : 626

EFFECTS OF ORAL SUPPLEMENTATION WITH LIQUID WHEY AND ACIDAL IN DRINKING WATER ON GUT PH AND MICROFLORA AND PRODUCTIVE PERFORMANCE IN LAYING HENS.

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Whey, a liquid remaining from cheese production, seems to act as a natural prebiotic in birds. The objective of this study was to evaluate the effect of liquid whey (LW) and an organic acid (ACIDAL) administered in the drinking water on improving production performance and gut health in hens. Seven hundred and fifty Isa Brown hens' chicks were randomly assigned to five treatments groups (n=150) and each group divided in five replicates. The animals were reared for 48 weeks. Treatments were administered in the drinking water at dosages: 250 mL/L of LW (Lacto 25) and 500 mL/L of LW (Lacto 50), and 1 mL/L of ACIDAL (Aci). A positive control group (T+) was treated with 500 mg/L of Tétracolivit (antibiotic). The negative control group (T-) did not receive any treatment in the drinking water. At weeks 8, 12, 24, 36 and 48, 10 birds from each treatment were sacrificed, and crop, proventriculus, ileum and caecum pH were measured. Fecal samples were taken from ileum and caecum contents and analyzed for Lactobacillus, Escherichia coli, total coliforms and total streptococcus. Age and weight of hens at first lay as well as weights of the first eggs were recorded. Egg production was collected daily. Egg weight, egg component ratio, Haugh units and yolk color intensity were recorded weekly. Administration of LW and ACIDAL in the drinking water reduced significantly ($P<0.05$) the gastrointestinal pH, the number of total coliforms and Escherichia coli, and improved Lactobacillus counts compared to the negative control group. Birds of Lacto 50 and Aci groups laid their first eggs earlier. Weight of birds at first lay and first egg weight in the four treated group was higher than those of control. Furthermore, egg production was increased by 10.44% in Lacto 25 but the weight and quality traits were unaffected, while the egg shell ratio was higher in Aci group compare with the rest of the groups. In conclusion, LW and ACIDAL were as effective as antibiotic on improving gut health, without the inconveniences of antibiotic bacterial resistance.

ID : 656

IMPACT OF STRUCTURAL POLYSACCHARIDE IN COHERENCE WITH A PROBIOTIC ON HENS CAECUM MICROBIAL COMMUNITY

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The paper presents the study of effect of a structural polysaccharide in coherence with a probiotic on hens caecum microbial community. The most effective ways to regulate energy metabolism in laying hens is limited feeding, but it can lead to stress. To prevent stress, it is recommended to use feeds with high fiber content. As a source of fiber there was used granular alfalfa grass meal.

To help the bird absorb hardly hydrolyzable ingredients of grass meal there was added Cellobacterin-T (biologically active additive that functions as feed enzyme and a probiotic). The studies were carried out on the basis of the Collective Use Center "Genetic Collection of Rare and Endangered Chicken Breeds" in All-Russian Research Institute of Agricultural Animals Genetics and Breeding. The hens of Amrock breed were kept on the floor in aviaries. At 112 days of age the hens were divided into 3 equal groups of 23 hens and 6 cocks in each group.

I Group - Control, II Group - Experimental 1, III Group - Experimental 2.

Starting from 126 days of age during the entire experiment (84 days) the hens of group I were fed by standard diet. In the Group II the diet was modified by addition of 10% alfalfa grass in the form of granules, and in the Group III in addition to alfalfa grass there was added "Cellobacterin-T" (Biotrof Ltd - St. Petersburg, Russia).

During the post-mortem examination the samples of caecum contents were taken to determine the different groups of bacteria inhabiting the digestive tract of hens using the T-RFLP analysis.

The metagenomics analysis was performed in molecular-genetic laboratory of Biotrof Ltd (Saint-Petersburg, Russia). An analysis has shown that in the caecum of hens a significant part of the microorganisms was represented by uncultivated bacteria. In birds of Group III, amount of uncultivated bacteria was 33.4% higher than in the Group I. The number of Lactobacillus sp. in birds of Group III increased by 2.8%, the number of class Actinobacteria decreased from 8.9% to 4.2%, and family Enterobacteriaceae from 4.7% to 2.7%. The number of family Fusobacteriaceae has decreased from 1.7% to 0.96%, Peptococcus sp. from 1.6% to 0.3%, Staphylococcus sp. from 0.12% to 0.07%.

The egg production in 60 days in Groups II and III was 4% and 9.8% higher, compared with the Group I.

Thus, the use of alfalfa grass with probiotic "Cellobacterin-T", contributed to increase the beneficial microflora and to a decrease in the undesirable bacteria in the caecum of hens.

ID : 657

THE EFFECT OF THE BACILLIUS SUBTILIS ON POULTRY'S GASTROINTESTINAL TRACT MICROBIOME AND THE EGGS QUALITY

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The goal of the research was evaluation of the effect of the *Bacillus subtilis* strain (part of the "Liquipro" water-soluble probiotic) on poultry's gastrointestinal tract microbiome and the eggs quality. High incubation properties of eggs depend on the balance and ratio of the nutrients in parent stock diet. Improper feeding leads to a lack or absence of the vital components, proteins, vitamins, minerals necessary for the normal development of the embryo in the egg.

The studies were carried out in a poultry farm of Voronezh Region. The Hisex Brown hens of 343 days age were observed. The hens were divided into 2 groups, in the amount of 25558 hens in the Experimental group (Group I) and 57275 hens in the Control group (Group II).

Throughout the experiment (65 days), the hens of Group I were fed with a standard diet with the addition of water-soluble feed additive "Liquipro" (Biotrof Ltd - St. Petersburg, Russia), the hens of the Group II were fed with a standard diet without additions.

During the post-mortem examination of hens, samples of caecum contents were taken to determine the different groups of bacteria inhabiting the digestive tract of hens using the T-RFLP analysis. The analysis was performed in molecular-genetic laboratory of Biotrof Ltd (Saint-Petersburg, Russia). An analysis of the data showed that in the caecum of hens, a significant part of the microorganisms was represented by cellulolytic bacteria. In birds of the Group I, amount of cellulolytic bacteria was 4.3% higher than in the Group II. The number of family Lactobacillaceae in the Group I birds increased by 0.7%, the number of family Pasteurellaceae decreased from 2.6% to 1.3%, and *Fusobacterium* sp. from 3.1% to 0.6%.

Group I also showed an increase in egg protein mass of 1.4 g and an egg shell thickness of 0.03 cm as compared to the Group II.

In conclusion, the use of water-soluble feed additive "Liquipro" in the poultry diet led to the increase of beneficial microflora, decrease in the number of opportunistic and pathogenic bacteria in hens' gastrointestinal tract, and improved the quality of eggs.

ID : 692

EFFECT OF TWO NOVEL PLANT EXTRACTS (CITRUS AND CUCUMBER) ON BROILER CHICKEN PERFORMANCE AND GUT MICROBIOTA

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The need to find good alternatives to antibiotic growth promoters is a major challenge for the broiler industry. The presence of active molecules such as pectins, limonene, carotenoids and polyphenols in novel plant extracts makes them potential candidates due to their reported but unconfirmed modulation of the gut microbiota, metabolism and immune system of chickens (1)(2)(3). The aim of this study was to test the hypothesis that a baseline diet (diet 1) supplemented with citrus (diet 2) or cucumber extract (diet 3) can improve gut health of broiler chickens through the modulation of the gut microbiota and performance.

A dietary trial (12birds x 4replicates x 3dietary treatments) was performed in the experimental poultry facility at University of Glasgow. Feed intake, body weight gain (BWG) were recorded twice a week from day 0 until day 28. The cumulative feed intake and body weight of birds was used to estimate the feed conversion ratio (FCR). To study the microbiota 12 birds per treatment were culled at day 14 and 28 and the DNA of the ceca and jejunum was extracted and amplified by a 16S specific-PCR, sequenced using Illumina Sequencer and analysed with QIIME 2™ at Polyomics, Glasgow.

Birds performance data was analysed by two-way ANOVA. The birds fed the citrus diet showed a significantly higher Feed intake and FCR compared to those on the cucumber and basal control diets while those fed the cucumber diet were similar to the basal diet. However, no differences in treatments were revealed on the BWG basis. Alpha and beta diversity analysis showed that the citrus and cucumber diets did not significantly affect the entire bacterial taxonomy. However a significant difference in the microbial composition was found between the two different tissues (ceca versus jejunum). Bird age also had a small effect (day 14 versus day 28). Ongoing work using LEFSE (Linear discriminant analysis Effect Size) determined that citrus and cucumber were able to modulate the growth of some bacterial strains. The results from this study form part of the European Joint Doctorate in Molecular Animal Nutrition (MANNA) (www.phd4manna.eu) and will be used to search for novel biomarkers of health status and develop new dietary plans that would enhance bird growth, maximize feed efficiency and protection from diseases.

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ID : 719

TEMPERATURE, HUMIDITY AND AMMONIA ARE IMPORTANT ENVIRONMENTAL PARAMETERS MODULATING THE GUT MICROBIOME OF BROILERS UNDER “RAISED WITHOUT ANTIBIOTICS” PRODUCTION

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The chicken gut microbiome consists of all microorganisms including bacteria, viruses, protozoa, and fungi, and their collective genetic material present in the gastrointestinal tract (GIT). The gut microbiome plays key roles in nutrition absorption, development of immunity, and disease resistance. Gut microbiome may have positive or negative effects on feed efficiency, productivity, and health of chickens. However, we do not know how the poultry house environmental parameters (temperature, ammonia, humidity) modulate the bacterial community residing in the GIT. In this study, we raised six hundred Cobb-500 broiler chicks in floor pens with clean pine shavings in two houses (300 birds/house; 25 per pen) for 49 days under environmental conditions reflecting those of commercial “Raised Without Antibiotics (RWA)” broiler production. We performed 16S rRNA gene sequencing on the meconium of day-old chicks, on the ceca when the birds were 14, 28, 42 and 49- days old and on weekly litter samples. Furthermore, we tested the effect of an 8-hour feed withdrawal period on the microbiome of 240 birds (120 birds/house). We also monitored the temperature, relative humidity and ammonia levels in the house and the moisture, pH and culturable *E. coli* in the litter. The bacterial community in the meconium, ceca and litter were significantly different throughout the study ($p < 0.05$), and the ceca had the highest alpha diversity. The top genera in the meconium and ceca were *Enterococcus* and *Bacteroides*, respectively. For the litter, the top genera consistently changed from day 7 to day 49. The bacterial community in the ceca and litter were significantly different between houses (house 1 and 2) from day 28 – day 49 ($p < 0.02$). Temperature and morning (7 – 10 am) relative humidity had a significant effect on the β -diversity of the ceca. In addition to house temperature, relative humidity and ammonia, the pH and moisture of the litter were the important parameters affecting the β -diversity of the litter. An 8-hour feed withdrawal before slaughter had no significant effect ($p > 0.05$) on the bacterial community of the ceca. Together, our results indicate that the environmental conditions in the farm house significantly affected the gut microbiome of broiler chickens. As a consequence, broilers in house 1 had significantly different ceca/litter microbiome and higher live weights than broilers in house 2 upon harvest.

ID : 724

HEPATOTOXIC EFFECT OF FUMONISIN - B1 IN BROILER CHICKEN

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Fumonisin-B1 (FB1), a mycotoxin extracted from *Fusarium moniliforme*, has been implicated in a variety of clinical syndromes in several animal species including human beings. FB1 was fed experimentally to one hundred and twenty five healthy, unsexed, day old Vencob broiler chicks, randomly divided in to five groups of twenty five each @ 0 (control), 50,100,200 and 400 ppm per kg feed. The effect was dose dependant and showed hepatotoxicity characterized grossly by severe enlargement with yellowish discoloration. Microscopically, mild to moderate congestion, cellular swelling, nodular infiltration of mononuclear cells, various degrees of necrosis, hepatitis, fatty changes, mild to moderate fibroplasia with formation of pseudolobules in an acinar pattern giving adenomatous appearance (prominent in high dose group), stenosis of bile duct, degenerative / necrotic changes and or hyperplasia and hypertrophy of bile ductular lining epithelium was noticed. The hepatic lesions described in the experimental birds are suggestive of precancerous stage and hepatotoxic nature of Fumonisin-B1.

Key words: Fumonisin-B1, Broiler chicks, hepatotoxicity, adenomatous appearance, precancerous stage

ID : 766

MICROBIAL ESTABLISHMENT AND DYNAMIC CHARACTERISTICS OF PIGEON MILK

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The breast milk of mammals is rich in microbes which can be transmitted to their offspring. Like mammals lactate for their offspring, the parent pigeons vomit the pigeon milk out of craw and feed the squabs mouth to mouth. However, there is little research on the microbiota of pigeon milk. In this study, high-throughput sequencing technology was adopted to detect and analyze the diversity and composition of microbiota. A total of 24 pigeon milk samples were collected, 12 parent pigeons and 12 squabs at different ages (4 each at 0, 5 and 10 days). Based on the results of operational taxonomic units, 8 phyla and 106 genera of microbiota in pigeon milk were annotated. Four major phyla dominated the pigeon milk Firmicutes (67%), Actinobacteria (20%), Proteobacteria (5%), and Bacteroidetes (5%). At the genus level, the microbiota of pigeon milk of parent pigeons were dominated by *Lactobacillus*, which was the most abundant genus with a proportion of 42% followed by *Enterococcus* (9%), *Veillonella* (9%), and *Bifidobacterium* (8%). While, the proportion of *Lactobacillus* (61%) and *Bifidobacterium* (15%) were significantly higher in squabs than parent pigeons. *Lactobacillus* exhibited an increasing trend with age which suggested that it may be an essential microbe in squabs. The *Veillonella* and *Prevotella* in squabs showed a gradual decline from day 0 to 10 days after hatch. In addition, we found that the predominant gut microbiota in pigeon were *Lactobacillus* and *Turicibacter*. The gut microbiota of squabs are more abundant than parents, but the parents have high gut microbial diversity. Our findings provide evidence to support the hypothesis that part of the microbial colonizers harbored in squabs were derived from parents and beneficial microbiota may be preferentially colonized in squabs.

ID : 769

MICROBIOME GWAS UNCOVER NOVEL GENETIC MUTATIONS OF THE HOST ASSOCIATED WITH INFECTION OF SALMONELLA PULLORUM

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The aim of this study was to explore the effect of relationship between host genome and the gut microbiota on salmonella pullorum disease. Here, we used composition of their gut microbiota as the informative intermediate trait of host. The tool of microbiome GWAS was employed to perform the association of genetic variation and beta diversity of gut microbial communities. Two sets of data are required for this approach: one is 366,189 high-quality host genomic variations obtained from reduced-representation genome sequencing (Personal Biotechnology Limited Company, Shanghai) in our previous study. The variations were from 135 positive and 140 negative pullorum infected individuals, for whom microbiome was profiled for the present study. The other is pairwise microbiome distance matrix of weighted UniFrac calculated using their stool microbiome data. The mutations with adjusted P-value that was corrected by skewness and kurtosis less than 10^{-5} were considered as significantly associated with microbial communities. After that, we found that 425 significant mutations located on 141 annotated genes. Pathway analyses and GO keywords enrichments of these genes were conducted by DAVID. Interestingly, 26 genes of them, including GDF6, TH, SOX9, VPS33B, TGFB1, were significantly involved in the keywords of disease mutation ($P=0.02$). Additionally, several pathways related to immune function were remarkably enriched, such as inflammatory mediator regulation of TRP channels, TGF-beta signaling pathway, and Rap1 signaling pathway. In this study, using Unifrac, a widely used distance metric, as the intermediate trait of chicken with pullorum positively and negatively infected, we identified novel disease-related genetic variants that are associated with gut microbiome composition. They may affect gene transcription and activity to further influence the chicken immunity against pullorum infection. Overall, our results contribute to understanding the effect of host genetics and their gut microbiota on salmonella pullorum infected chicken.

KEYWORDS: microbiome GWAS; host genetics; microbiome; salmonella pullorum

ID : 976

A MACHINE LEARNING APPROACH EXPLORING CHARACTERISTIC HOST-MICROBE MOLECULAR INTERACTIONS IN THE GUT OF TWO LINES OF DIVERGENTLY SELECTED WHITE LEGHORNS

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For forty years, two lines of White Leghorn chickens have been selected for high (HAS) or low (LAS) antibody response to a low dose injection of sheep erythrocytes. These lines have become an important model for understanding avian immunogenetics though currently only about 40% of the observed phenotypic differences can be explained. Their gut is home to billions of microorganisms and the largest number of immune cells in the body. Better understanding host-microbe interactions may further explain the antibody response differences of HAS and LAS. Previously, our group has sequenced the RNA and the 16S microbiome of the jejunum of six birds from each line. Sequencing data was further analyzed using machine learning to determine the microbes most predictive of line followed by the differential gene expression in the jejunum most predictive of high, medium, or low abundance of those microbes. Using the machine learning software Waikato Environment for Knowledge Analysis (University of Waikato, New Zealand), we ran four machine learning algorithms: support vector machines (SVM), artificial neural network (ANN), random forest (RF), and J48 decision trees (J48). RF and J48 performed best for microbe-line predictions while all four performed variably for gene expression-microbe abundance predictions depending on microbe. The most predictive taxa were the order Streptophyta (no lower classification), and genera Enterococcus, Lactobacillus, and SMB53, all with >89% predictive performance. The lists of top predictive genes (>91% for one or more algorithms) for each of the four microbes were subject to pathway analysis to identify molecular targets in the gut where the microbes may be interacting with the host. Microbial abundance was associated with gene enrichment for pathways involved in the complement system, antigen presentation, pattern recognition receptors, DNA repair, and metabolism of amino acids, lipids, carbohydrates and inositol phosphates. Host microbe interactions can be extremely challenging to elucidate, however by utilizing machine learning to identify host genes predictive of microbial abundance we identified molecular pathways where commensal microbes may be more likely exerting an effect. This information will be useful in directing future research focusing more intensely on ways the microbiome may be influencing the phenotypic differences in antibody response via the identified pathways and in turn, accelerate scientific discovery.

ID : 1168

EFFECT OF ESSENTIAL OILS BASED ADDITIVE ON IMPLEMENTATION OF ILEAL MICROBIOME OF BROILERS WITH METAGENOMICS ANALYSIS

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Under the pressure to reduce antibiotics in poultry production, emerging alternatives like essential oils are increasingly used to improve gut health and performances. This study had the objective to understand how the gut flora implementation of broiler chickens is impacted by an essential oil blend, initially developed for its bacteriostatic effect on *E.coli* (Girard et al., 2019). This trial was conducted at the Faculty of Veterinary Medicine, University of Montreal with 2x90 1day ROSS broilers till 21 days old.

A negative control was compared to a treated group that received essential oils supplementation in an usual broiler feed (0.05% "Phyto CSC", Phytosynthese equal to 55ppm of volatile molecules). At 7, 14 and 21 days 15 birds/group were euthanized; their ileal contents were collected and stored in liquid nitrogen to perform a MiSeq 16S rRNA gene sequencing according Thibodeau et al., 2017 method. α -diversity was described by Shannon Index and analysed with a Wilcoxon test; β -diversity was analysed with Bray-Curtis similarity index associated with Permanova-Adonis test. Body weight of euthanized bird was analysed with Student test.

Results indicated α -diversity of microbiomes were significantly higher at D14 in ileum for treated group. α -diversity of ileal microbiome tended also to be higher at D7 for treated group ($p=0.1$). β -diversity of ileum were significantly different at D7 and 14 ($p<0,001$). Moreover inside each groups, β -dispersion of Bray-Curtis index tended to be smaller before 14 days for the treated group ($p=0.09$ at D7 and $p=0.03$ at D14). This result suggests the treatment could lead to better homogeneity of gut flora between chicks before 14 days. Recorded body weights were not significantly different between control and treated groups with respectively: 137 vs. 133 at D7; 426 vs. 422 at D14 and 816 vs. 849 at D21.

To conclude, the addition of this essential oils supplementation in broilers feed is not delaying the implementation of ileal flora according the α -diversity analysis. The microbiome community structures were influenced by the supplementation until D14 in ileum. Further studies could measure how this gut flora modulation impacts the performances and disease resistances in field conditions with numerous bacterial challenges.

ID : 1342

IN OVO FEEDING OF CLOSTRIDIALE STRAINS MODULATE GUT MICROBIOME

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The use of live microorganisms to manage enteric health is becoming a feasible practice, especially when administered early in a broiler's productive life. Strains within the Clostridiales order, including many butyric acid-producing anaerobes, are found in the intestines of healthy animals that have been shown to promote growth performance and improve gut health. White Dog Labs (New Castle, DE) has developed a novel biologic called BioTyton™ consisting of unique Clostridiales strains. The objective of this study was to examine the effect of BioTyton™ delivered by in ovo feeding (IOF) on gut health. Two experiments were conducted to measure the stimulation effect of BioTyton™ on the gut microbiome of broiler chickens. Viable eggs of equal weight (± 0.5 std.) were segregated for different experimental treatments. At ~17 day of incubation, 100 μ L of either 101, 103, 105, 107 or 109 CFU BioTyton™/mL was administered into the embryonic amnion. In both studies, a saline control was also injected, and a sham control (i.e., no injection) was used in one of the studies. Cecal material from 21 d old chicks was obtained and microbiome metabolites were subjected to analysis using GC for quantification of acetate, lactate, and butyrate. Analysis for the microbiome profiles showed a significant difference for both 105 CFU/mL and 107 CFU/mL from the saline control. The 105 CFU/mL dosage had a greater proliferation of the BioTyton™ strains in the cecum. Butyrate was detected in samples analyzed for the 105 CFU/mL dosage with an average concentration of 0.14 mg/g cecum material. These data suggest that BioTyton™ influencing the profile of the cecum in broiler chickens. In conclusion, in ovo administration of 105 CFU/mL Injection of BioTyton™ into the amnion of the chicken embryos can modulate the development of the gut microbiome that may positively affect subsequent gut health. BioTyton™ is not yet for sale at this time.

ID : 1430

INFECTION OF LAYERS WITH HISTOMONAS MELEAGRIDIS HAS SEVERE CONSEQUENCES ON CAECAL MICROBIOTA

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Histomonas meleagridis is the etiological agent of histomonosis or blackhead disease in various galliform birds. Clinical appearance of the disease in turkeys as primary host is well described but there is substantial lack of knowledge in chickens. The parasite is frequently noticed in layers in coincidence with reduced performance which was also demonstrated experimentally (1). Furthermore, colibacillosis often appears together with blackhead disease in chickens but the pathomechanism is not known (2).

In the actual study, the composition and structure of caecal microbiome as well as the colonization of a bioluminescent labelled pathogenic strain of *Escherichia coli* in birds with or without *H. meleagridis* were investigated. For this, a total of 48 commercial layers were equally allocated in 3 different groups. Birds in group 1 were infected at 23 weeks of age with *H. meleagridis*. At 2, 4 and 6 days post *H. meleagridis* infection (dpi), birds of groups 1 and 2 were inoculated with tagged-*E. coli*, whereas the third group was kept as negative control. Four birds/group were necropsied at 7, 10, 14, 28 dpi for sampling. The composition of caecal microbiota in birds from all three groups were analyzed after sequencing the v3-v4 hypervariable regions of 16S rRNA gene with illumina MiSeq platform. As a result, the biggest changes in gut microbial community, even at genera levels were prominent in birds of group 1, in coincidence with macroscopic and histological lesions. Species richness and evenness were lower in co-infected birds at all four time points compared to birds infected with *E. coli* only and the negative control. The relative abundance of *E. coli*-*Shigella*, *Helicobacter* and *Bacteriodes* was higher in birds of group 1 than in birds from other two groups, in coincidence with decreased proportion of *Lactobacillus*. Upon quantification of the tagged-*E. coli* in caecal tissues, birds in group 1, compared to other groups, contained significantly higher bacterial load at every time point of sampling, demonstrating a supportive effect of the parasite.

In conclusion, the co-infection of birds with *H. meleagridis* and *E. coli* and the appearance of blackhead disease had severe consequences on the caecal microbiota, characterized by significantly reduced species diversity and increased colonization of *E. coli*, which might influence the pathobiology of colibacillosis.

1Liebhart et al. 2013. Avian Pathology. 42, 79-84

2Paudel et al. 2018. Avian Diseases. 62, 300-306

WORKING GROUP

EF 1

ECONOMY AND MARKETING

ID : 422

CURRENT STATUS OF POULTRY PRODUCTION IN THE REPUBLIC OF CROATIA

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Croatian poultry production has a long tradition and a solid present, as well as a good base for further development, and covers almost all forms of poultry production, of which the most important are fattening of chickens and turkeys and production of eggs. They are accompanied by modern slaughterhouses and meat processing and egg sorters. In addition, breeding and keeping of parent flocks, hatcheries, pullets rearing and animal feed factories are present. The rearing of other poultry such as ducks and geese is seasonal and occurs mainly on family farms for personal use or rarely for the market. Similar is the case with the breeding of Croatian original poultry breeds - Zagorje turkey and Hrvatica hens which are exclusively grown on small family farms also for personal needs or only rarely for sale on the market. With the accession of the Republic of Croatia to the European Union in 2013, there were significant changes in poultry production, especially in the production of eggs. Currently, the registered facilities have the capacity to accommodate 1,944,000 laying hens, of which 72% are in enriched cages, 25% are in the floor mode, 2.9% are in the free range mode and only 0.1% are in organic production. The consumption of eggs per capita is 172 pieces, and self-sufficiency is estimated at 95%. The production of poultry meat is 85,000 tonnes, with a share of 78% of chicken meat and 15% of turkey meat, while 7% are duck and goose meat. At the same time, large imports of poultry meat are still recorded, amounting to 26% of domestic production, while only 10% of domestic production is exported. Self-sufficiency in poultry meat production and consumption in Croatia is 89%, and per capita poultry meat consumption is 21 kg. The global problem of securing sufficient quantities of feed for poultry feeding, alternative sources of protein in poultry feeding, as well as increasing attention to poultry welfare, and the growing vocation of abolishing cages in the production of table eggs, will certainly affect poultry production in Croatia. The challenges and expectations of Croatian poultry production in the coming period are likely to be under the auspices of events and trends primarily in other EU Member States, surrounding countries and of course the world. It is believed that Croatian poultry production will continue to be the leading stock of livestock production in the total agricultural production of the Republic of Croatia.

ID : 599

COST-BENEFIT ANALYSIS FOR EGGS PRODUCERS

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Introduction: The supply and variety of feed additives for laying hen feeds is increasing from yearly, which is why growers are debating whether the increase in cost is worthwhile in terms of added cost versus the expected increase in revenue as a result of improved performance.

Aim: To examine the cost of various additives in laying hen feeds compared to the benefit of improving the performance by those additives and their financial gain.

This is an effective and useful tool for farmers and nutritionists giving them an opportunity to achieve optimal economic decisions.

Materials & methods: The model examines the interaction in the following current flock parameters: Feed price, average egg weight, average daily feed consumption, hen-day production, percentage of cracked eggs and percentage of dirty eggs, and changes in these parameters on a monthly basis.

Any cost modification due to feed prices, average daily feed consumption and/or a variation in income as a result of a change in the hen-day production, egg weight, percentage of cracked eggs and percentage of dirty eggs are taken into consideration.

The model calculates a current flock revenue versus monthly expenses, with reference to the current layer flock performance, as well as taking into account upcoming fluctuations in feed additive cost against the expected additional revenue. The calculated balance is per layer hen for the coming month.

Results:

For example: (prices in €)

Eggs price: XL= 0.1393, L= 0.0928, M= 0.0817, S= 0.0611, Cracked & Dirty eggs = 0.05.

Current Flock Data:

Feed Price = 270 €/ton, Average Eggs Weight =64 gram, Average Daily Feed Consumption = 120 gram/day/hen, Hen-Day Production= 90%, Percentage of Cracked Eggs =2% and Percentage of Dirty Eggs=2%.

If the price of the feed increases by 7 €/ ton as a result of adding an additive to the feed, it will cost the farmer 0.026 € per month per hen. In order to offset this added cost, hen's performance needs to be improved.

Egg weight must increase by 0.6 grams, or average daily feed consumption must decrease by 3 grams. The model can combine the parameters on demand.

Conclusion: The model examines the different interactions between the above-discussed parameters and allows the farmer the opportunity to make an optimal judgment concerning the contribution of feed additives or other decisions related to the interaction of parameters.

ID : 1006

CURRENT STATE OF POULTRY EGGS AND MEAT PRODUCTION IN UKRAINE

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Poultry farming in Ukraine remains the leading branch of animal husbandry. This is evidenced, first of all, by the number of poultry, which increased by 3.3% on all farms in 2018, while the number of cattle, sheep and goats decreased compared to the previous year.

Thus, according to the State Statistics Service of Ukraine, as of January 1, 2019, the number of poultry of all species in poultry farms of Ukraine of all categories was 211614.7 thousand heads (SSSU, 2019). As of January 1, 2019, the number of poultry amounted to 118,812.9 thousand heads in agricultural farms, which is 5.6% more than in the previous year. The main species of the poultry is chickens (115280.8 thousand heads). There are also turkeys (778.5 thousand heads), geese (260.4 thousand heads), ducks (3633, thousand heads), quails (758.6 thousand heads) and poultry of other species.

The largest number of birds is concentrated in the holdings of Vinnytsya (32588.6 thousand heads), Kyiv (28389.2 thousand heads), Cherkasy (26031.7 thousand heads) and Dnipropetrovsk regions.

Households also tend to increase in the number of poultry.

In 2018, there has been an increase in the production of poultry eggs of all species. For example, in January-December 2018, farms of all categories produced 16138.5 million eggs, which is 4.1% more than in 2017, including the production of eggs in the agricultural farms amounted to 8900.4 million units (by 6.4 %), and in households – 7238.1 million units (by 1.4 %).

As for the production of poultry meat, in January-December 2018 agricultural farms sold 1426.7 thousand tonnes of poultry in live weight, including of broilers – 1343.9 thousand tonnes, which is by 7.0 and 6.7% more than in the previous year. In 2018, the first place for the production of poultry meat was taken by the Vinnytsya region, and the second – Cherkasy, at the expense of poultry farms belonging to the holding "Myronivskyi khlіboproduct".

In 2018, 750.5 million eggs of poultry of all species were incubated by poultry and hatchery enterprises of Ukraine, which is 2.6% more than in the previous year. In total, 604.6 million heads of healthy young were incubated, that is 4.5 % more than in 2017.

Thus, the statistics for number of poultry and poultry production in 2018 show that, despite the global problems that continue to exist in the country, the poultry industry is developing rapidly.

References

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ID : 1032

LAYING HENS - HUSBANDRY SYSTEMS ON DIFFERENT WELFARE LEVELS – AN ECONOMIC COMPARISON

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Due to stricter animal protection's regulations worldwide, Germany seeks to get its pioneering role in this area. One of the most important milestones, among others is the abolition of cage farming for laying hens. However, the question remains: is everything on track? Most of the current farming systems show some weaknesses which are not yet adequately covered.

Towards a better future, together with farmers and consultants, the Thünen Institute developed criteria for objective evaluation of the current housing systems in a comparative way. In the first step, biological and technical production figures were compared for each farming system in order to figure out the causes of the discrepancies in total costs. The assessment of the production costs in the investigated housing systems was carried out on the basis of branch evaluations at farm level, which provided valuable information. The evaluations showed that none of the farming systems currently being used can be completely convincing.

Although barn husbandry enables low production costs (7.5 ct./egg), however, the animals suffer limitations regarding space availability and exercise space outside. Providing a veranda could help in this context, but at the same time it might impair the ventilation system.

With free-range system, animals are given the opportunity to use the outdoor area, but often stay close to the barn to escape birds of prey, especially when there are no shelters. This can lead to high nitrogen emissions and infection risks. Furthermore, if free-range farming has to be temporarily banned due to an outbreak of avian flu, the conditions in the free range-barn are the same as in the barn husbandry without outdoor access.

In principle, this problem also arises with mobile barns; however the damage caused by predators is even worse. Moreover, it can also be difficult to ensure the mobility of the barns and thus sufficient nutrient distribution in winter and rainy conditions. The production costs are more than twice as high as for barn husbandry, mainly because of the high labour input. In terms of direct costs (especially feed), smaller flocks can also incur cost disadvantages if rebates can only be obtained for higher purchase charges.

The process of optimizing current husbandry systems has started.

KEYWORDS: laying hens, animal welfare levels, free range, cost comparison

ID : 1137

INDIAN POULTRY INDUSTRY: WAY FORWARD

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The aim of the present study is to analyse the current status, practical challenges and opportunities of the Poultry industry in India. The data were collected from the official websites of national and international agencies. India ranks the 3rd largest egg producer and the 4th largest poultry meat producer in the world. As per the latest census (2019), the total poultry population is 851.81 million which indicates an increase of 16.81 per cent over the previous census period (i.e., 2012). A total of 45.78 per cent increase in backyard poultry is witnessed; the total commercial poultry in the country is 534.74 Million in 2019, increased by 4.5 per cent over previous Census. Indian poultry industry is contributing Rs.698.88 billion to the total national GDP of Rs.1,24,486 billion in 2015-16 (0.56 %). The total egg production and meat production in the country was 95.20 billion and 3.76 million tonnes respectively during 2018. Annual per capita availability of eggs and chicken meat increased from 10 eggs and 146 grams in 1970s to 74 eggs and 3.6 kg respectively during 2018, while National Institute of Nutrition recommends at 180 eggs and 11 kg per annum. Welfare of commercial layer is given more importance and the government of India has issued new guidelines for egg rearing of birds. Increasing population, increasing per capita income and changing food habits, wider market for chicken than beef or pork and contract farming system are some factors that helped in increasing production and efficiency. Reduced marketing margins led to keep the consumer price under check. Emerging and reemerging diseases, abnormal rise in cost of feed ingredients, over dependence on maize and soya, lack of attempts for processing and further processing of egg and meat, institutions not keeping pace with the growth of industry and increase in demand for skilled manpower at all levels of value chain, lowering of import duties on poultry products are the challenges facing Indian poultry industry. Mass based production and continued performance to buy from wet market hamper introduction of technology and improve quality of products sold in local markets. The scope of export promotion for poultry products is also discussed.

ID : 359

HOW TO INCREASE FEED PROTEIN SELF-SUFFICIENCY OF THE FRENCH POULTRY SECTOR?
DEVELOPMENT OF A ROLE-PLAYING GAME TO UNDERSTAND THE RELATIONSHIPS BETWEEN
OPERATORS OF CROP AND POULTRY PRODUCTIONS

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French poultry production is relying on large amounts of imported soybean meal. This dependence is associated to many economic, environmental and societal concerns, urging for the improvement of protein self-sufficiency at national scale. Yet, this issue not only concerns the poultry sector but also the crop one. To better understand the relationships between these sectors, we developed a role-playing game (RPG) approach. Among all the operators involved in crop and poultry sectors, five key roles were identified and considered in the RPG: Crop farming (FARM), grain collection and storage (STOR), grain transformation e.g. for oilmeals production (TRANS), feed and broiler production (FEED-BRO), and finally, chicken meat retailing (RETAIL). For each role, a fictive profile was created (e.g. name, farm/company characteristics...) and a specific Excel simulator was developed and calibrated with realistic parameters, allowing the calculation of relevant economic indicators. For instance, FARM semi-gross margin could be calculated according to crop rotation, annual yields and price context. Actual operators were invited to play their own role in the RPG. RETAIL launched the game by asking the two FEED-BRO for chicken fed with French vegetal feedstuffs. Operators were then free to interact and decide, but they had to record their decisions (e.g. sale contract). They were also observed by the RPG animators. Interactions were only observed between two roles at a time, in a classical seller/customer relationship. Specific demands toward the crop production sector were effectively observed to meet RETAIL's demand, mainly by increasing rapeseed/sunflower grains/meals production. Immediately after the RPG, a debriefing was organized in order to get the collective feedback. The main findings were that i) differences in management timescales (e.g. years for FARM vs. days/weeks for RETAIL) are perceived as a limiting factor to an optimal cooperation between operators, and ii) that the economic value, potentially generated by new labels, is difficult to assess and should be more fairly distributed. One solution could be the contractualization between operators, but many factors such as timescale differences, yield/price uncertainty, or bargaining power of each operator have to be considered. The importance of RETAIL, the only operator directly connected to consumers' expectations, was also highlighted in order to help in structuring new supply chains.

EF2

NUTRITION

FEEDSTUFFS

PROTEIN NUTRITION

FEED TECHNOLOGY

MINERAL NUTRITION

PHYSIOLOGY OF NUTRITION

INGESTION AND DIGESTION

FEEDING IN ALTERNATIVE PRODUCTION SYSTEMS

METABOLISM

FEEDSTUFFS

ID : 117

EFFICACY OF CARBOHYDRASE ENZYME SUPPLEMENT DEPENDS ON SOLUBLE ARABINOXYLAN CONTENT

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A study was undertaken to evaluate the impact of carbohydrase enzymes (Rovabio® Advance containing xylanase, β -glucanase and arabinofuranosidases) in diets containing different amount of soluble arabinoxylan (sAX). Experiment 1 employed a $2 \times 3 \times 2$ factorial arrangement of treatments. Factors were: enzymes - 0 or 0.005%, sAX - normal (21% of total AX), low (15%) or high (27%) and run - 1 vs. 2. Six iso-energetic wheat-SBM based-diet treatments were composed of 8.5% non-starch polysaccharide (NSP) and 20.9 crude protein (CP) for normal sAX diets, 10.6% NSP and 21.9% CP for low sAX diets, as well as 8.9% NSP and 20.1% CP for high-sAX diets. Five and 8 replicates per treatment were realised in experiment 1 and experiment 2, respectively. Experiment 1 used closed respiratory chambers (two birds/chamber) with treatment diets fed from d19 to 28 initially for 4 d adaptation. Data for metabolisable energy (ME) and net energy (NE) calculation were recorded from d25 to 28. Experiment 2 used floor pens with 12 birds/pen and treatment diets were fed from d19 to 35. Weight of feed and birds/pen were recorded on d28 and 35 to calculate feed intake (FI) and weight gain (WG). On d35 all birds were euthanised and opened for sexing and sex was used as covariate during data analysis. In both experiments, data were analysed using the General Lineal Model of Minitab. Results from experiment 1 showed higher ($P < 0.05$) respiratory quotient and ME value in the high-sAX diets supplemented with the enzymes than unsupplemented high-sAX diets. The enzymes also increased NE value ($P = 0.01$) in all supplemented diets. In the floor pen feeding experiment, enzymes only reduced ($P < 0.01$) FCR in birds fed low-sAX diets from d19 to 28 and d19 to 35. The enzymes also reduced ($P < 0.001$) ME intake (MEi)/WG in the low-sAX birds from d19 to 28. The application of the enzymes increased ($P < 0.001$) energy intake (MEi and NEi) per metabolic body weight (MBW) and per WG in birds fed high-SAX diets from d19 to 35. As expected, the FCR was highly correlated ($P < 0.001$) to MEi/WG ($r = 0.755$) and NEi/WG ($r = 0.702$) from d19 to 35. A weak correlation ($P < 0.05$, $r = 0.362$) was observed between MEi/MBW and FCR from d19 to 35 but NEi/MBW only tended to correlated with FCR from d19 to 35 ($P = 0.078$, $r = 0.260$). Overall, the supplemental enzymes released more energy in high sAX diets and a lower FCR and energy intake per WG was observed in the supplemented low sAX diets.

ID : 130

EVALUATION OF SCENT LEAF (OCIMUM GRATISSIMUM) PHYTOGENIC FEED ADDITIVES ON THE GROWTH PERFORMANCE AND ANTIOXIDANT CAPACITY OF BROILER CHICKENS

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A study was carried out using five hundred and forty Cobb 500 broiler chickens to evaluate the effect of Ocimum gratissimum leaf meal as phytobiotics on the growth performance and antioxidant capacity of broiler chickens. The birds were allotted to four dietary treatments with three replicates comprising of forty five birds each in a completely randomized design. Ocimum gratissimum was added at 0, 400 and 800g while the last treatment contained Oxytetracycline at sub-therapeutic level as growth promoters. Data were collected on growth performance and antioxidant capacity. All data collected were subjected to analysis of variance and significant differences among treatment means were compared using Tukey procedure of SAS. The result obtained showed that birds fed diets containing Oxytetracycline and 800g of Ocimum gratissimum had significantly ($P < 0.05$) higher final weight and weight gain, best feed conversion and least feed cost per kg gain. Result for antioxidant capacity showed higher values ($P < 0.05$) for glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the treatment groups fed dietary supplement of the experimental materials. GPx activity was significantly ($P < 0.05$) higher for birds fed diets containing Ocimum gratissimum when compared with the control and AGP groups. Malondialdehyde (MDA) was significantly reduced ($P < 0.05$) by the addition of Ocimum gratissimum. It is concluded therefore that addition of Ocimum gratissimum at 800g per 100kg diet improved the growth performance and antioxidant capacity of broiler chickens. Ocimum gratissimum leaf meal as phytobiotics can therefore be added to the diet of broiler chickens at 800g/100kg as a replacement for antibiotic growth promoters for improved growth performance and higher antioxidant capacity of birds.

ID : 136

GLYCAEMIC RESPONSE OF CHICKENS TO HIGH-TEMPERATURE SHORT-TIME EXTRUDED LEGUME GRAIN DIETS

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This study was conducted to evaluate the glycaemic response of broiler chickens to high-temperature short-time (HTST) extruded legume grain diets under different extrusion temperatures. Broiler starter diets (NRC, 1994) were formulated to contain 15% of either African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranea*) or Pigeon pea (*Cajanus cajan*) as energy-protein sources and extruded on a single screw extruder at either 80, 100 or 120°C at 25% moisture, while unextruded diets served as the controls.

A total of five hundred and forty 1-d old broiler chicks were neck-tagged, weighed and randomly allotted to the twelve diets in 3 replicates, 15 chicks/replicate for 21 days. On day 21, 5 chickens per treatment were fasted for 12h preceding, and basal blood glucose was measured for each chicken using a glucometer (ACCUCHEK active, Roche Diagnostics GmbH, Mannheim, Germany), with ~100µL blood drawn from the wing vein. Diets were then offered to each experimental group for 30 mins, and blood glucose concentration measured at 30, 60, 90, 120 and 180 mins postprandial. Glucose response curves were plotted for each diet and used to estimate peak glucose concentration (mg/dL), time at peak glucose concentration (mins), max. glucose increment (mg/dL), area under the glucose concentration curve ($\times 103 \text{ mg} \cdot \text{dL}^{-1} \cdot 180 \text{ min}^{-1}$), area under the incremental glucose concentration curve ($\times 103 \text{ mg} \cdot \text{dL}^{-1} \cdot 180 \text{ min}^{-1}$), and glycaemic index normalized against the corresponding unextruded diets. Data obtained were subjected to ANOVA ($P < 0.05$) and preplanned contrasts, elucidating the effect of extrusion (unextruded vs extruded; 80, 100 and 120°C) and extrusion temperatures (80°C vs 100°C, 80°C vs 120°C and 100°C vs 120°C).

Significant effects of extrusion cooking were observed for the area under the glucose concentration curve (36.65 ± 1.16 vs 40.58 ± 2.42 , $P = 0.00$), area under the incremental glucose concentration curve (0.84 ± 0.55 vs 2.96 ± 2.25 , $P = 0.01$) and glycaemic index (100.0 ± 0.00 vs 110.84 ± 7.67 , $P = 0.00$) of chickens on the African yam bean diets and on max. glucose increment (79.40 ± 29.96 vs 54.86 ± 18.28 , $P = 0.04$) for chickens on the Bambara groundnut diets, however, no effect of extrusion was observed for pigeon pea diets. Also, the effect of extrusion temperatures on glycaemic response indices was not significant ($P > 0.05$) for all legume grain diets.

This result affirms that the effect of extrusion cooking on the glycaemic response of chickens differ in the legume grains, however, variations in extrusion temperature ranging from 80-120°C do not impact on glycaemic responses of chickens.

Keywords: Glycaemic index; Extrusion cooking; Pigeon pea; Bambara groundnut; African yam beans

ID : 143

GROWTH PERFORMANCE, ANTIOXIDANT CAPACITY AND LIPID PROFILE OF BROILER CHICKENS FED DIETS CONTAINING CURRY LEAF (OCIMUM CANUM) AS PHYTOGENIC FEED ADDITIVES

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Five hundred and fifty two Cobb 500 broiler chickens were used in a study to determine the effect of Ocimum canum leaf meal as phytobiotics on the growth performance, antioxidant capacity and lipid profile of broiler chickens. The birds were allotted to four dietary treatments with three replicates comprising of forty six birds each in a completely randomized design. Four experimental diets were formulated containing Ocimum canum at 0, 400 and 800g while oxytetracycline in sub therapeutic doses as recommended by the manufacturer was used as a positive control. The diets and water were fed ad lib to birds throughout the period of the experiment. Data were collected during the starter and finisher phases on growth performance, antioxidant capacity and lipid profile of the broiler chickens. All data collected were subjected to analysis of variance and significant differences among treatment means were compared using Tukey procedure at $P < 0.05$ level of significance. The result obtained showed that birds fed diets containing Oxytetracycline and 800g of Ocimum canum had higher ($P < 0.05$) final weight and weight gain, best feed conversion ratio and least feed cost per kg gain at both the starter and finisher phases. Mortality was also reduced by the inclusion of the experimental material. Result for antioxidant capacity showed higher values ($P < 0.05$) for glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the treatment groups fed dietary supplement of Ocimum canum at 400 and 800g. GPx activity was significantly ($P < 0.05$) higher for birds fed diets containing Ocimum canum when compared with the control and AGP groups. Malondialdehyde (MDA) was not significant across the treatment groups ($p > 0.05$) while catalase (CAT) content was significantly ($P < 0.05$) improved by the additive. Broilers fed with diets containing Ocimum canum had significantly ($P < 0.05$) lower cholesterol, triglycerides and high density lipoprotein compared to the control treatments. It is concluded therefore that addition of Ocimum canum at 800g per 100kg diet improved the growth performance and antioxidant capacity of broiler chickens. Ocimum canum leaf meal as phytobiotics can therefore be added to the diet of broiler chickens at 800g/100kg diet for improved growth performance and the development of higher antioxidant capacity by broiler chickens.

ID : 147

STIMULATORY FEED COLOR EFFECTS ON PERFORMANCE OF HIGH GROWTH RATE BROILERS

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This research study was conducted to determine color of feed effects on average body weight, body weight gain, cumulative feed consumption, and adjusted feed conversion of high growth rate broilers. Previous feed color research from this lab assessing effects of red, green, and blue on broiler performance has been presented. Treated diets were colored using food grade powdered dyes. Four treatments [control (complete broiler starter diet), orange (#f59b54 hex color code), yellow (#e5c92a hex color code), and purple (#6b5669 hex color code)] with 4 replicates (60 birds/treatment) were fed to 240 male Cobb 500 broilers over a 21 d battery cage grow out. Bird weights and feed consumption data were collected on 7 d intervals. Data were analyzed as a completely randomized design, with battery cage representing the experimental unit, using the ANOVA procedure. Overall, there were no significant treatment effects for average body weight at d 7 ($P=0.4575$), 14 ($P=0.1468$), and 21 ($P=0.7228$); cumulative feed consumption between d 0 to 7 ($P=0.2422$), 0 to 14 ($P=0.1645$), and 0 to 21 ($P=0.2612$); and adjusted feed conversion between d 0 to 7 ($P=0.5357$), 0 to 14 ($P=0.6661$), and 0 to 21 ($P=0.9175$). There were no significant treatment effects for body weight gain between d 0 to 7 ($P=0.4097$) and 0 to 21 ($P=0.2298$). However, there were significant treatment effects for body weight gain between d 7 to 14 ($P=0.0125$) and 0 to 14 ($P=0.0258$). Birds on purple had a significantly higher body weight gain than birds on orange or yellow between d 7 to 14. Body weight gain between d 0 to 14 for purple (489.15 g/bird) was significantly more than control (468.99 g/bird; $P=0.0572$), orange (463.13 g/bird; $P=0.0197$), and yellow (452.48 g/bird; $P=0.0044$). Additionally, there were no observed treatment differences for mortality anytime during the 21 d grow out ($P>0.05$). These results are indicative that inherent stimulatory color spectral effects for orange, yellow, and purple did not influence most high growth rate broiler performance parameters. Therefore, based on the results of this study there appears limited economic value in coloring commercial broiler feed orange, yellow, or purple as a method to increase feed consumption. However, further research on purple colored feed effects on broiler performance may be warranted from the early body weight gain effects observed in this study.

ID : 167

COMPARATIVE GROWTH PERFORMANCE OF BROILER CHICKS AND TURKEY POULTS REARED UNDER CROSS-SPECIES HUSBANDRY CONDITIONS

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Two experiments utilizing 192 birds each (male turkey poults or male broiler chicks) were done simultaneously to compare growth performance response to enzymes supplementation in broilers and turkeys raised under cross-species rearing conditions. In one experiment, 96 male broiler chicks and 96 turkey poults were reared under management condition (brooding temperature and day light provisions) specific for Cobb 500 broilers. In the second, the same number of broilers chicks and turkey poults were reared in conditions specified for BUT 6 turkeys. In each experiment, each species received control diets formulated to meet the specification for the breed except marginal reduction in metabolizable energy and non-phytate phosphorus levels. The control diets were supplemented with xylanase or phytase individually or in combination. Birds and feed were weighed and excreta were collected on days 7 and 21. Data for each experiment were analyzed for the main effects of xylanase (2), phytase (2) and husbandry type (2) as a 2x2x2 factorial. Broilers, although having lower ($P < 0.05$) initial body weight, gained more weight and consumed more feed ($P < 0.05$) than turkeys, irrespective of husbandry conditions. For broilers, phytase supplementation, irrespective of husbandry condition, improved ($P < 0.05$) FCR on days 7 and 21. There was phytase \times xylanase \times husbandry interaction for day overall weight gain with birds receiving individual phytase and xylanase supplementation having lower weight gain when raised under turkey husbandry condition. There was significant husbandry \times phytase interaction ($P < 0.05$) on day 21 broiler body weight, with phytase improving weight gain when birds were raised under broiler, but not turkey, husbandry condition. There were minimal enzyme effects on turkey poults raised under both husbandry conditions. Overall, the only significant effect was the main effect of husbandry condition, with d 0 to 7 FCR being lower ($P < 0.05$) when poults were raised under turkey husbandry condition even though weight gain for turkey was consistently greater when raised under broiler, not turkey, husbandry condition. It is concluded that the husbandry condition influences growth performance response of broiler chicks and turkey poults and this needs to be considered when executing comparative species studies or carrying out horizontal comparison responses across studies.

ID : 182

EFFECT OF A-TOCOPHEROL AND TANNIN EXTRACT SUPPLEMENTATION TO LINSEED OIL-ENRICHED DIETS ON INTESTINAL MORPHOLOGY OF BROILER CHICKENS

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It has been demonstrated that high level of n-3 polyunsaturated fatty acids (PUFA) could cause negative effects for epithelial cells and even induce the mucosa apoptosis and reduce the villus height. This is why it is very important to supplement linseed-oil enriched diets with certain level of antioxidants. Current scientific evidence suggests that tannins had multiple biological activities, including anti-inflammatory properties attributed mainly to their antioxidant and antiradical activity that might exert positive effects on gut health.

The aim of the experiment was to determine the effect of supplementation of linseed oil enriched diets with vitamin E or tannin extract (Farmatan®, Tanin, Slovenia) on production results and intestinal morphology of broiler chickens. 300 day-old male chickens (Ross 308) were allocated to 3 treatment groups with 5 replicates of 20 male birds. From the day 21 to day 42 the 5 % of linseed oil was added to the experimental basal diets supplemented with: no additives (control group, C), 200 IU vitamin E/kg (E) or 500 mg of tannin extract/kg of feed (T). At the age of 40 days, 12 animals per group were sacrificed and their tissue samples were taken from the mid-section of the jejunum, washed with saline solution, placed in 10% formaldehyde and after histological procedure stained with hematoxylin and eosine. Jejunal histology parameters were determined using light microscope and software for image analysis (IM1000 Image Manager, Leica). Obtained data were analyzed by one-way ANOVA, using general linear models. Results were considered significant when $P < 0.05$.

The results showed that there were no differences in production results between control and experimental groups (body weight, feed consumption and feed conversion ratio). However, significant influence of tannin extract on morphology of jejunum has been demonstrated. Tannin group had significantly higher villus height (1,940.82 μm (C), 2,027.60 μm (E) and 2,207.66 μm (T)), lower crypt depth (439.90 μm (C), 485.87 μm (E) and 426.23 μm (T)), higher villi/crypt ratio (4.53 (C), 4.37 (E) and 5.35 (T)) and significantly higher villus area compared to C and E groups (0.35 mm^2 (C), 0.40 mm^2 (E) and 0.47 mm^2 (T)). The results confirmed positive effect of tannin extracts on jejunal morphology of broiler chickens, while the addition of vitamin E in oil-enriched diets didn't show significant effect on gut morphology parameters.

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VISCOSITY (IN VITRO) AND PROXIMATE ANALYSIS OF UK WHEAT HARVEST OVER FIVE YEARS

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Wheat is quite widely used as a cereal in poultry diets in the UK and other parts of Europe. Many factors affect the quality of harvest including seed quality, soil composition, fertilizer and weather conditions. Wheat contains crude protein (CP), oil, starch, sugars and variable amount of crude fiber (including neutral detergent fiber (NDF), Hemi-cellulose (HC). Although, attention is paid towards starch, oil and protein contents for least cost formulation purpose, NDF and HC draw less attention and viscosity is even less reported and discussed. In vitro analysis for measuring viscosity (cP, centipoise as units), has been developed inhouse as published elsewhere (Bedford and Classen 1992). Wheat samples were collected across the UK from 2014 till 2019 (except 2018 due to unforeseen circumstances). Near-infrared scan measurements were done for CP, oil, starch, sugars, NDF and HC on dry matter basis. Viscosity was measured as mentioned above on fresh basis. The average (20 samples + SD) results of 2019 wheat harvest was compared with average of four years ($n = 89 + SD$). Observed values (in % except where stated otherwise) for dry matter were ($85.7 + 0.9$ vs $87.0 + 0.3$), CP ($12.6 + 0.1$ vs $13.3 + 0.2$), oil B ($1.4 + 0.1$ vs $2.5 + 0.1$), starch ($68.9 + 0.9$ vs $65 + 1.1$), sugars ($1.85 + 0.7$ vs $2.9 + 0.5$) NDF ($8.5 + 0.3$ vs $10.8 + 0.1$), HC ($5.8 + 0.3$ vs $7.9 + 0.4$) and viscosity (cP $7.5 + 1.9$ vs $8.4 + 1.3$). Interesting to note CP, oil B, sugars, NDF and HC seems to be lower for 2019 compared to previous years. Starch contrary to sugars, appears to be higher than previous years. It should also be noted that although viscosities for 2019 harvest were lower, the values show more variability than previous years. In conclusion, wheat quality looks quite good for 2019 harvest. However due to variable viscosity and higher starch content, attention should be paid when it comes to feeding wheat to birds. Exogenous supplementation of xylanase, beta-glucanase and amylase may help to improve nutrient digestibility in poultry (Kiarie et al., 2013).

Reference: Bedford and Classen. An in Vitro assay for prediction of broiler intestinal viscosity and growth when fed rye-based diets in the presence of exogenous enzymes. Poultry Science (1993). 72:137-143.

Kiarie. E, Romero. Luis F. and Nyachoti. M.C. The role of added feed enzymes in promoting gut health in swine and poultry. Nutrition Research Reviews (2013), 26, 71–88.

ID : 245

THE PRODUCTIVE PERFORMANCE IN BROILER CHICKS FED DIETS WITH ENZYMATIC HYDROLYSATES OF KERATIN- AND COLLAGEN-CONTAINING MATERIALS

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The slaughter and processing of poultry result in substantial amounts of inedible wastes (feathers, bones, tendons, etc.) which can be converted into protein feed additives for animals and poultry by an adequate processing technology. The aim of the study presented was the evaluation of effects of substitution of such protein additives produced by the two-stage processing of keratin- and collagen-containing poultry wastes (short thermal treatment with subsequent enzymatic hydrolysis) for fishmeal in diets for broiler chicks (cross Ross-308, four treatments). Control treatment 1 was fed standard broiler diet with fishmeal; experimental treatments were fed the same diet with the substitution of fishmeal by hydrolysate of keratin-containing material (feathers; treatment 2); hydrolysate of feathers additionally supplemented with a probiotic to improve digestibility and assimilation of dietary nutrients (treatment 3), and a mixture of hydrolysates of feathers and collagen-containing material and a probiotic (treatment 4).

It was found that at 38 days of age live bodyweight and feed conversion ratio (FCR) were better in experimental treatments. Live bodyweight in treatment 2 was significantly higher in compare to control by 6.7% ($P \leq 0.01$) while FCR was better by 5.6%. In treatment 4 live bodyweight was higher by 5.4%, FCR better by 5.1% in compare to control ($P \leq 0.01$). The best growth efficiency was found in treatment 3 (hydrolysate of feathers plus probiotic): live bodyweight was significantly higher by 8.6% in compare to control ($P \leq 0.001$) while FCR was better by 6.7%. The conclusion was made that protein feed additives based on the hydrolysates of keratin- and collagen-containing poultry wastes can significantly improve the productive performance in broiler chicks.

The study was financed by the Russian Science Foundation, grant No 17-16-01028.

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PREMIUM YEAST FRACTION REDUCES SALMONELLA TYPHIMURIUM COLONIZATION IN BROILERS

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Salmonellosis is an important food-borne zoonosis, with poultry and poultry products as the main transmission vector. The occurrence of antimicrobial resistance in non-typhoidal *Salmonella* is considered an additional major public health concern. With the increasing consumption of poultry products, new intervention strategies are necessary along the food chain to reduce *Salmonella* prevalence during poultry production. Yeast fractions (YF) rich in mannans and β -glucans can modulate the bird's immune system but also to adhere to type-1 fimbriae of pathogenic bacteria including *Salmonella*, making it more difficult for them to colonize the intestinal epithelium.

The objective of this study was to evaluate the effect of the supplementation of a premium yeast fraction (PYF) obtained from a *Saccharomyces cerevisiae* strain and containing at least 20% of mannan and 20% β -glucan (1,3 and 1,6) on *Salmonella* colonization in broilers.

One-day old broiler chicks were divided over 3 isolators, each representing one treatment group; the unchallenged control (UC), the challenged control (CC) and premium yeast fraction supplemented (250g/ton) and challenged group (PYF). At day 2, all animals in the CC and YF group were orally inoculated with 108 CFU of *Salmonella* Typhimurium ATCC 14028. Performance data were recorded at days 3, 7, 14 and 28 and statistically evaluated with SPSS22 software using one-way ANOVA with SNK post-hoc test ($P \leq 0.05$). Mucosal flagellin antibodies and *Salmonella* Typhimurium loads in ceca were determined at day 28 and statistically analyzed using a Kruskal Wallis test ($P \leq 0.05$) with SPSS22 software.

At D7 ($p=0.014$) and D14 ($p=0.023$) of the trial, challenge with *Salmonella* Typhimurium reduced body weights in the CC group (136 ± 8.02 and 338 ± 28.06) compared to that of the UC (162 ± 4.19 and 432 ± 10.80), while administration of the PYF to challenged birds reversed the effect *Salmonella* challenge on the birds performance on both days (154 ± 4.45 and 397 ± 24.06). Significant decreases in *Salmonella* Typhimurium numbers were seen in the caecum ($p \leq 0.001$) of the PYF supplemented group at day 28 (2.68 ± 1.76) compared to the CC (9.52 ± 1.36), while the UC group remained negative for *Salmonella* throughout the experiment.

Additionally, the detection of higher geometric mean flagellin IgA antibody titers ($p \leq 0.001$) in the supplemented group (600 ± 2.49) compared to the UC (150 ± 2.10) and CC (82 ± 1.28), shows the positive effect of the PYF on the immune system.

Consequently, administration of a PYF can reduce the negative effects *Salmonella* challenge on birds and food safety.

ID : 254

IMPACT OF BACILLUS COCKTAIL ON PERFORMANCE OF BROILERS EXPOSED TO NECROTIC ENTERITIS CHALLENGE

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Use Bacillus probiotics has gained a lot of interest in the animal industry. Their effects on animal health, with improvement of intestinal health and pathogen reduction, have made them very attractive especially since the reduction/ban of antibiotics. Different Bacilli were screened based on diverse functionalities, leading to the construction of a probiotic cocktail containing 3 different Bacillus species (*B. pumilus*, *B. amyloliquefaciens* and *B. licheniformis*). To validate this cocktail, a necrotic enteritis challenge dose response trial was carried out. 2100 Cobb 500 chicks were divided over 7 groups with each 10 replicates of 30 birds. Treatment groups were negative control (NC); positive control (PC); Bacillus cocktail at Log4, Log4.5, Log5, Log5.5 or Log6 cfu/g feed. All animals were fed at libitum and on D17 and 18 all birds, except those belonging to the NC, received an oral gavage with 2ml *Clostridium perfringens* culture. The culture was grown overnight in cooked meat broth and later incubated for 12h in a sterile nutrient broth. Mortality was recorded daily, Necrotic enteritis (NE) lesion scores were evaluated on D21 and zootechnical performances were evaluated on days 14, 28 and 42. Parameters were analyzed by ANOVA and means separated by Tukey's test ($P \leq 0.05$) using Statistix 10 analytical software.

P-values of the NE scores ($p=0.028$) showed a significantly higher NE lesions in the PC (2.17) compared to the NC (0.59) and the Bacillus log 4 group (1.17), while other Bacillus groups performed intermediate. On day 42 of the trial, a tendency for a significant difference ($p=0.059$) could be observed for the body weights. Body weights of the PC only reached 1.580 kg, while those of the NC were 1.850kg. All the Bacillus supplemented groups acquired a final body weight that was intermediate between those of the PC and NC and were ranging from 1.681 to 1.745 kg. Statistically significant differences were observed for FCR ($p=0.001$), the PC had significantly higher values compared to the NC and all the Bacillus supplemented groups, except for the log 4.5 group who was performing intermediately. The PC (18.06%) also exhibited a significantly higher overall mortality ($p=0.013$) compared to the NC (4.44%) and the log 4.5 (8%) and log 5 (7.67%) Bacillus supplemented groups. All other Bacillus supplemented groups decreased the mortality intermediately compared to the PC. Consequently, administration of a Bacillus cocktail to birds under necrotic enteritis challenge, leads to enhancement of birds' performance.

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XYLANASE CAN IMPROVE METABOLISABLE ENERGY OF THE WHEAT-MAZE DIET AND INCREASE CAECAL CONTENTS OF SHORT-CHAIN FATTY ACIDS WHEN FED TO BROILER CHICKEN

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The use of xylanase is well-accepted practice in poultry farming but recently, research is focused on the effects of the interrelationships between xylanase, feed ingredients, retention variables and the concentrations of short-chain fatty acids (SCFAs) in the caecal content. The current study examined the effect of xylanase on apparent metabolisable energy (AME), dry matter (DMR) and nitrogen (NR) retention in relation to the SCFAs. The study used three hundred and sixty Ross 308 male broiler chickens which were allocated in 18 floor pens (20 birds per pen). The feed, provided in mash form, was wheat-maize based and the study was comprised of two phases – starter (from day 0 to 21) and finisher (from day 22 to 35). The main ingredients in the diets were wheat 29%, corn 31.27% and soybean meal 33.30% for the starter phase and 39%, 35.40% and 19.70% respectively for the finisher phase. After splitting the basal diet in two batches one was used as a control diet and the other was supplemented with Econase®-XT (AB Vista, Marlborough, UK) at 100 g/t, providing 17100 BXU/kg units of xylanase. The retention data was obtained from analysing the faeces collected at the end of each growth phase and for caecal contents of SCFAs, gas-chromatography was used. The statistical analysis was done with Genstat software using a 2 x 2 factorial design. The results of the study showed that in comparison to the control diet, xylanase supplementation showed an increased retention of AME (12.32 vs 12.13, SEM=0.10, $P < 0.05$), NR (0.758 vs 0.745, SEM=0.006, $P < 0.05$) and DMR (0.689 vs 0.674, SEM=0.01, $P < 0.05$). Xylanase supplementation increased caecal acetic acid (78.5 mM vs 60.6, SEM=11.43, $p < 0.05$), volatile fatty acids (96.2 mM vs 75.1, SEM= 14.29, $P < 0.05$) and SCFAs (100.9 mM vs 82.4, SEM=15.56, $p < 0.05$). These results suggested that xylanase can improve metabolisable energy and part of this may be through influencing fermentation processes in the caeca through mechanisms which can be the subject of further research.

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COMBINATION OF XYLANASE AND XYLOOLIGOSACCHARIDES IMPROVES FEED EFFICIENCY IN BROILERS

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Currently, some combinations of enzymes and different compounds which can be potentially beneficial for the bird's performance and health are being extensively studied. The use of the xylanase alone has been proven to be beneficial for broiler chickens and it is expected that if it is combined with some prebiotic-like substances it will further increase its effectiveness. The aim of the current study was to examine the effect of combination of xylanase and prebiotics (xylooligosaccharides - XOS) supplemented to a maize-based diet. The main ingredients in the diet, which was formulated to meet commercial recommendations (Aviagen Ltd), were maize, soybean meal and soya oil and their respective quantity for the three dietary phases were as follow: starter diet - 56.44%, 35.95% and 2.66%; grower diet – 65.96%, 26.86% and 2.13%; and finisher diet – 69.64%, 23.72%, and 1.99%. Each basal diet was split further into two batches – one was used as a control and the other was supplemented (50g per tonne) with Signis (AB Vista, Marlborough, UK) which provided a combination of xylanase and xylooligosaccharides (35% XOS, degree of polymerization from 2 to 7 xyloses). Two hundred and forty male Ross 308 chicks were obtained from a commercial hatchery and assigned to the experimental diets following randomisation. Birds were reared in 12 floor pens (20 birds each) and the feed (crumbs for the starter diet; pellet for the grower and finisher) and water were provided at libitum. The performance variables feed intake (FI), weight gain (WG), and feed conversion ratio (FCR_m, mortality corrected) were measured at the end of each dietary phase of bird's growth – 10, 24 and 35 day old. The data were analysed by ANOVA using repeated measurements for the dietary phases and the results are presented as overall for the entire study period. Although the impact on FI and WG was only numerical ($P > 0.05$), birds fed Signis had an improved (lower) FCR_m (1.33 vs 1.35, SEM = 0.012; $P < 0.05$). The combination of xylanase and XOS can be beneficial for the growth performance of birds through improved feed efficiency of maize-based diet.

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EFFICACY OF A COMBINED XYLANASE AND XYLO-OLIGOSACCHARIDE IN SUPPORTING THE PERFORMANCE OF BROILERS

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Efficacy of a combined Xylanase and xylo-oligosaccharide in supporting the performance of broilers
Introduction

The mode of action for exogenous xylanase (Xyl) has been debated widely, with a general consensus that Xyl aids the degradation of non-starch polysaccharides present in viscous grains, thus increasing diffusion of nutrients in the intestine. Recently it has been suggested that Xyl may also have beneficial effects on the gut's microbiome due to provision of xylo-oligosaccharides (XOS) as a nutrient source for microbiota. This study looked at the effect of a combined xylanase and XOS product on digesta viscosity and the performance of broilers to 35 days.

Materials and Methods 240 Ross 308 male, day-old broilers housed in 48 pens, 5 birds per pen, and 16 replicates per treatment for 35 days. Birds were randomly assigned to one of 3 treatments manufactured from a wheat/soy mash basal; Control, Control +0.1g/kg Xyl and XOS, and Control +0.2g/kg Xyl and XOS. Diets were made in 3 phases, starter, d0-21 and finisher d21-28 and finisher D28-35. Birds and feed were weighed weekly to calculate body weight gain (BWG), feed intake (FI) and FCR. On day 35, 1 bird per pen was euthanised. Jejunal digesta supernatant was analysed for viscosity using a cone and plate viscometer (DV Viscometer, Brookfield, USA). Data was analysed using IBM SPSS Ver 26) Significance was declared at $p \leq 0.005$

Results

There was a significant increase in BWG ($p=0.002$) for both treatments vs Control and a significant improvement in FCR ($p=0.003$) for diet C (0.2g/kg Xyl + XOS) from d0-35. Although there was no significant difference in weekly FI, from d0-35 there was a significant increase in the FI for both Xyl + XOS treatments. There was no significant difference in digesta viscosity between treatments ($p=0.103$).

Conclusion

Whilst the viscosity results were numerically reduced in line with Xyl supplementation, these results seem to indicate that the performance gains seen are due to more than a simple reduction in digesta viscosity. However, Xyl and XOS supplementation does effectively improve broiler performance with greater improvements with double the recommended dose.

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EFFECT OF A FEED SUPPLEMENT CONTAINING BACILLUS SPP. ON PERFORMANCE, INTESTINAL MORPHOLOGY AND INTESTINAL MICROBIOTA OF BROILER CHICKENS FED DIETS WITH NORMAL OR LOW ENERGY LEVELS

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In recent years, numerous new feed additives are examined in broiler nutrition to support their health and welfare and to enhance their performance. The main aim of this study was to test the efficacy of a new probiotic *Bacillus* strain on broilers performance, intestinal morphology and intestinal microbiota. A second objective was to test the possible energy sparing effect of this probiotic.

640 day-old male chicks (Ross-308) were allocated into 4 equal treatments with 10 replicates each (16 chicks per cage). The L1 (control) treatment was fed the basal diets (starter 1-10 d, grower 11-24 d, and finisher 25-35 d). The L2 treatment was fed diets containing 4% lower Metabolisable Energy, compared to the control. The L3 and L4 treatments were fed the diets of the 1st and 2nd treatment, respectively, further supplemented with a novel probiotic strain of *Bacillus* spp (1×10^9 CFU/kg feed). All diets were provided ad libitum in mash form. Weight gain (WG), feed intake (FI) and FCR were evaluated on days 10, 24 and 35. Intestinal morphometric analysis (villus height (VH), crypt depth (CD) and goblet cells) and microbiological analysis (conventional and RNA methods) were performed in samples collected on day 35. Statistical analysis was performed using two-way ANOVA (probiotic x feed energy level) at 0.05 significance level.

For the overall experimental period, lower dietary energy decreased the final body weight and WG ($P=0.002$), but did not affect FI and FCR. The probiotic supplementation increased the final weight and WG ($P=0.003$), decreased FCR ($P=0.002$) but did not affect FI. Intestinal morphology was affected by both the probiotic supplementation ($P<0.001$ for VH/CD ratio), and energy level of the feed ($P<0.001$ for ileum VH and villus goblet cells). Probiotic supplementation resulted in higher ileum and cecum *Lactobacillus* ($P<0.001$) and *Bacillus* ($P<0.001$) counts and lower cecum *E. Coli* ($P<0.001$), while lower dietary energy increased cecum *C. perfringens* ($P=0.023$). Significant interaction between probiotic supplementation and energy level was noted for FI ($P=0.026$), FCR ($P=0.016$), intestinal morphology ($P\leq 0.001$) and microbiological analyses ($P<0.05$), showing that this probiotic was able to reduce the negative effects induced by lower dietary energy.

The results of the trial showed that probiotic supplementation can support growth performance, provide an energy sparing effect, and improve intestinal health status of broilers.

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DRIED BOVINE COLOSTRUM POWDER DECREASES MORTALITY IN BROILER CHICKENS

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In broiler production, the first week of life is a relevant period and the foundation for a successful grow out period. Therefore, supporting chicks in the first days of life have long-lasting positive effects.

Bovine colostrum offers immunoglobulins which may provide cross protection against different agents that share the same neutralizing sites, even when used in a different species. Additionally, colostrum contains growth factors and various immune factors, which might further add to a beneficial use in poultry. A previous study indicated that feeding bovine colostrum to broiler chickens improved growth performance and immune responses (Qureshi et al., 2004).

The aim of the study was to evaluate the effect of bovine colostrum on zootechnical parameters in broiler chickens in two different studies.

Experiment I used 320 male Ross 308 broiler chickens for a trial duration of 35 days. Birds were kept in floor pens and received ad libitum access to feed and water. Feed was based on corn, soybean meal, and wheat. Chicks were randomly distributed to two treatments (8 replicates): NC-I: Negative Control (basal diet, no supplements); C-I: Colostrum (1.125 mg Immune Milk / ml water; applied from d 0 - 7). Colostrum was offered in bell drinkers (500 ml colostrum / water mix) for the first four hours after a dark period.

Experiment II used 288 off-sex male Cobb 500 broiler chickens for a trial duration of 35 days. Birds were kept in battery cages. Feed was based on corn and soybean meal. Birds were picked up directly at the hatchery and were randomly distributed to two treatments (8 replicates): NC-II: Negative Control; C-II: Colostrum (337.5 mg Immune Milk / chick; gavaged once at the hatchery). Afterwards, all birds were denied access to feed and water for 24 hours to mimic transport stress.

In both studies, final body weight was numerically higher in colostrum supplemented birds (2634 and 2654 g; 1502 and 1563 g, respectively; $p > 0.05$). Furthermore, numerically decreased mortality was observed in both trials for the overall period (2.5 and 0.625 %; 7.86 and 1.39 %, respectively; $p > 0.05$). No effects were seen on FCR or feed intake. The results of the studies indicate that providing chicks at an early age with colostrum can have positive effects on the overall grow out period, despite different genetics, husbandry, or feed composition.

Qureshi, M.A. et al. (2004): Immunmilk feeding increases growth and immune responses in broiler chickens. Int. J. Poult. Sci. 3: 305-312.

ID : 308

THE EFFECTS OF A GEL CARRIER CONTAINING MICROALGAE AND A PROBIOTIC ON THE EFFICIENCY OF COCCIDIA VACCINATION IN BROILERS

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Objective

The discussed ban of coccidiostats and arising resistances increase the necessity for more frequent and efficient application of coccidia vaccines. The objective of the study was to investigate the efficiency of a patented gel carrier containing microalgae in combination with a probiotic as a suitable carrier for coccidia vaccines applied in the hatchery.

Material and methods

In this floor-pen trial, 552 day-old chicks (ROSS 308) were randomly allocated to 3 treatment groups (n=8; 23 chicks per pen, ♂). Chicks in control group (CON) received no coccidia vaccination (CV), whereby the other groups received a commercial CV (Huveguard MMAT) at d1 either sprayed on feed (FEED) or via gel application (GEL) using microalgae-based gel (HatchGel® SP, Biochem) and including a probiotic product (B.I.O.Sol, Biochem). The gel was dropped on chicks while still in crates before placing in pens to simulate hatchery application. In GEL the probiotic was additionally supplemented via water from d1 to d3. All treatment groups were fed a commercial broiler diet free of coccidiostats. Fecal samples were pooled penwise from d5 to d9 for counting of oocysts per gram feces (OPG). On d7, fecal samples of 4 animals per pen were collected and OPG was counted individually (n=32). Data was analyzed with R-software. Differences in species specific and total OPG were analyzed with linear regression models with group as fixed effect. In case oocyst counts were low or absent, differences were not statistically analyzed. To evaluate uniformity of oocyst shedding, percentage of birds shedding per group was studied using a logistic regression model with group as categorical fixed effect. Differences at $P < 0.05$ were considered as significant.

Results

In CON no marked shedding of oocysts was observed from d5 to d9, whereby in FEED and GEL significant numbers of oocysts were counted compared to CON ($P < 0.01$). Furthermore, a higher total and species-specific OPG from d5 to d9 and a one-day earlier peak in shedding was observed in GEL compared to FEED. Percentage of birds shedding oocysts tended to be higher in GEL compared to FEED on d7 (78.1% vs. 56.3%, $P = 0.066$).

Conclusion

Vaccination was successful and oocysts were shed from d5 to d9 after vaccination. Although differences in amount of oocyst shedding and percentage of birds shedding were not significant, the GEL group did have a higher OPG count and a higher percentage of shedding birds indicating a higher success rate of vaccination.

ID : 312

EFFECTS OF SELECTED HERBS AND A NATURAL FLAVORING COMPOUND IN FEED ON BROILER PERFORMANCE, BREAST MEAT YIELD AND QUALITY UNDER MILD HEAT STRESS CONDITIONS

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This study was conducted to investigate the effect of selected herbs in combination with a natural flavouring compound on broiler growth performance, breast meat quality and breast meat yield under mild heat stress conditions. A total of 315 one-day old Cobb 500 male broiler chickens were randomly assigned to three treatment groups with seven replicates: control (T1 no supplementation), T2 (300 ppm herbal premix supplementation) and T3 (600 ppm herbal premix supplementation). The feed was of standard composition and identical for all three groups besides the addition of herbal premix. The herbal premix consisted of: 18% grape pulp (*Vitis vinifera*); 75% rosemary (*Salvia rosmarinus*), fenugreek seeds (*Trigonella foenum-graecum*), olive leaves (*Olea europaea*) and quillaja bark (*Quillajaceae*); 2.5% natural flavouring compound and a carrier. From day 21 to 35, room temperature was 28°C to induce mild heat stress in broilers. Body weight gain, feed intake and feed conversion ratio were determined between day 1-14 (starter feed) and day 15-35 (grower feed), and also for each 7-day period. Data were analysed using the software package SPSS (IBM SPSS Version 25) based on Oneway Anova. Body weight gain on d 14 was significant (p

ID : 346

EFFECT OF THE COMBINED USE OF A MIXTURE OF AROMATIC PLANTS, ESSENTIAL OILS AND TRIBUTYRIN ON BROILER CHICKENS' PERFORMANCE AND GUT MICROFLORA

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Nowadays, a main effort of the animal nutrition science is to support high levels of animal health and performance with limited use of therapeutics. In this effort, the management of intestinal microflora plays a significant role.

In this 37 days trial, a total of 360 one-day-old male broiler chicks (Ross-308) were randomly allocated to 4 groups with 6 replicates (of 15 chicks), all housed in floor pens with rice hulls litter. Commercial breeding, management and vaccinations procedures were employed. Group A (Control) was fed basal diets (based on maize and soybean meal) in mash form, which did not contain anticoccidials or antibiotics. The diets of the other groups were further supplemented: Group B with a mixture of dried aromatic plants and essential oils (3 g/kg); Group C with a premix containing tributyrin (1 g/kg); Group D with the mixture of dried aromatic plants and essential oils (3 g/kg) and the tributyrin premix (1 g/kg). Intestinal samples from the jejunum and caecum (N=96) were collected at slaughter to evaluate microbial counts. Appropriate agars were used for the isolation, enumeration and identification of total aerobes and total anaerobes, Enterobacteriaceae, Escherichia coli, Lactobacilli and Clostridium perfringens. Identification of all bacterial isolates was performed by MALDI-TOF Biotyper (Bruker Daltonics). Data were analyzed with the ANOVA and Kruskal-Wallis methods, using SPSS v20 software. The combined use of the feed additives (group D) resulted in improved (PE. coli (5.22 vs 3.53 log CFU/g) and Enterobacteriaceae (5.42 vs 3.83 log CFU/g), compared to the control, whereas groups B, C and D had lower (PC. perfringens counts compared to the control (2.56; 2.47; 2.30 vs 2.88 log CFU/g). In the ceca, group D, compared to the groups A, B and C, had lower (PE. coli (6.18 vs 7.44; 7.17; 7.43 log CFU/g), Enterobacteriaceae (6.53 vs 7.56; 7.29; 7.66 log CFU/g) and C. perfringens (3.77 vs 4.87; 4.82; 5.37 log CFU/g), while group D, compared to group B, had lower (P

Based on these findings, the combined use of the two feed additives had the best results in improving broiler performance and lowering intestinal populations of potentially harmful bacteria.

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ID : 377

THE IMPACT OF THE LENGTH OF THE COLLECTION PERIOD ON THE ESTIMATE OF DIETARY METABOLISABLE ENERGY IN BROILERS

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The aim of the study was to evaluate the effect of three collection periods (24, 48 and 96h) on apparent metabolisable energy (AME) implementing the total collection technique when using two dietary treatments with different energy density fed to female and male chicks. A high energy diet containing 539 g/kg of maize and 387 g/kg soybean as the major ingredients was prepared alongside a low energy diet where 50 g/kg wheat bran was substituted for maize. The diets were fed as pellets and did not contain any coccidiostat, antimicrobial growth promoters or prophylactic additives. Six-hundred-forty Ross 308 (50% females and 50% males) were obtained from a commercial hatchery, allocated to 32 floor pens, 16 pens females and 16 pens males, and were fed the experimental diets following randomisation. At 7d age, 5 birds from each pen were chosen by random and allocated to 32 raised floor pens and fed the same diets. The birds were fed the experimental diets for 7 days prior to their allocated collection periods of 24, 48 or 96 hours. Water and food were supplied ad libitum throughout the study. At the start of the collection period, birds were 8 d age and all excreta were collected daily for 24, 48 or for 96h, respectively, immediately dried at 60°C and then milled. Feed intakes were also measured for the same period as excreta collection. Data were statistically analysed by ANOVA using a 3 x 2 x 2 factorial arrangement of treatments. The main effects were the collection period, the diet and the sex of the birds. The AME values for 24h collection period were higher ($P<0.05$) as the 48h collection period (12.14 vs 11.96 MJ/kg), and there was no difference ($P>0.05$) between 48h and 96h collection periods. There was a difference in daily feed intake (FI) ($P<0.05$). There were no interactions ($P>0.05$) between the length of the collection, dietary energy content and the sex of the birds. The results demonstrated that the length of the collection period has an impact on the estimate of dietary AME values.

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THE EFFECT OF DIETARY FIBRE AND SUPPLEMENTARY XYLANASE ON GROWTH PERFORMANCE VARIABLES AND GASTROINTESTINAL TRACT DEVELOPMENT IN MALE AND FEMALE BROILERS

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With the increased scarcity of grain cereals, the poultry industry may be required in the future to use an increasing amount of cereal by-products in their feed formulations. A study was conducted to investigate the response of male and female Ross 308 broiler chicks on feed intake (FI), weight gain (WG), feed conversion ratio (FCR) and gastrointestinal tract development (GIT%) as per cent of body weight (BW) fed two levels of dietary fibre, with and without xylanase (XT; ABVista, UK). A positive control (PC) contained 539 g/kg maize and 387 g/kg soybean meal as the main ingredients, and with a calculated 12.59 MJ/kg AME, 228 g/kg CP and 26.1 g/kg fibre content were mixed. The negative control (NC) contained 50 g/kg wheat bran at the expense of maize; and with a calculated 12.28 MJ/kg AME, 232 g/kg CP and 28.5 g/kg fibre content. Both PC and NC were then further split into two and one part was supplemented with xylanase (16000 BXU/kg XT), resulting in 4 diets, or 8 treatments in total including 4 diets and 2 sexes. Each diet was fed to 12 floor pens, 20 birds in each, from day old to 21d age following randomisation. Room temperature and lighting regime met commercial recommendations (Aviagen Ltd, Edinburgh, UK). At day 21, a bird from each pen was dissected and the GIT development was assessed. Data were analysed by ANOVA following 2 x 2 x 2 factorial design (including 2 fibre levels, with and without enzyme, and two sexes). Overall, feeding NC reduced final weight gain (Pvs 1891g) and male birds were heavier than females (Pvs 34.9 g/b/d). Male birds ate more feed (Pvs 55.7 g/b/d) and grew faster (Pvs 52.0 g/b/d) compared to females. There was fibre by xylanase interaction (Pvs 1.411), but not the low fibre diet (1.379 vs 1.348). Female birds had a relatively heavier pancreas and spleen (as % of the body weight) compared to males (P vs 0.34 and 0.09 vs 0.07, respectively), although no differences in the overall GIT% was observed between treatments (P>0.05). This experiment has confirmed the expected growth performance effects of diets that differ in fibre contents and xylanase supplementation when fed to male and female birds at this age. The results support the view that feeding xylanase in high fibre poultry diets is a tool to improve feed efficiency in broilers.

ID : 384

RESIN ACID CONTAINING TALL OIL FATTY ACID REDUCED MORTALITY AND OOCYST SHEDDING IN BROILERS CHALLENGED WITH EIMERIA

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Resin acids have well documented anti-inflammatory and tissue healing effects. An experiment was conducted to evaluate the effect of resin acid composition (RAC), containing bioactive resin acids and free fatty acids from coniferous trees, on broiler health and performance under an Eimeria challenge. A total of 1760 Ross 308 one-day-old chicken were housed in a trial facility. The birds received one of the 2 dietary treatments during the 30 days experiment: control (CTRL), or RAC supplemented diet (1.5 g RAC/kg of feed) (RAC). There were in total 11 pens in each treatment; 3 pens of females, 3 pens of males and 5 pens with 50% of each sex. Each pen housed 80 chicken. The pens were divided in 3 rooms, sexes and dietary treatments were balanced within the rooms. On day 16 all chicks were challenged with a 10-fold dose (0,25 ccm/chick) of Eimeria vaccine (Huveguard, Huvepharma, Belgium). Growth, feed intake, feed conversion ratio (FCR) were recorded per pen, and shedding of Eimeria oocysts (OPG) at days 7, 14, 20, 21, 22, 23 and 28 per dietary treatment. The results were subjected to ANOVA or when response variables were not normally distributed, to two-sided X2-test with R software (R i386 3.6.1).

In both treatments there were no oocysts found in excreta samples taken at day 7, 14 or 20. At d21, total oocyst counts were high (> 600 000 OPG) in both treatments, but 10% lower in treatment RAC than in CTRL. Furthermore, RAC gave better reduction of OPG from d21 to d23 compared to CTRL (-86% vs. -60%), and from d21 to d28 (-50% vs. -37%). The birds receiving RAC diet had a lower mortality in comparison to CTRL (2-sided X2-test, $p < 0.05$). There were no statistically significant differences between the diets in growth or FCR.

In current trial, RAC gave protection against the Eimeria challenge when compared to the control, denoted by the lower mortality and improved reduction of OPG. RAC seems to be a useful tool in supporting gut health and performance in coccidiostat-free broiler production.

ID : 412

EFFECT OF PHYTASE ENZYME LEVEL AND SOURCE ON BROILER PERFORMANCE AND TIBIA ASH FROM 1 TO 21 DAYS

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Diets fed to broilers are mainly composed of corn and SBM in which phosphorus is partially unavailable in a phytate form. Phytases breakdown the phytate molecule, which reduces feed costs and phosphorus excretion. This study was conducted to evaluate the effect of phytase source and inclusion level on broiler performance, breaking strength, and tibia ash from 1 to 21 d. The treatments consisted of a combination of two phytase sources (coated - Optiphos and uncoated- Optiphos+, which is a new intrinsically heat stable form of Optiphos), positive control (PC), negative control (NC), and NC + 0, 250, 500, 1000 or 1500 FTU kg⁻¹ of phytase. The positive control (PC) was formulated to contain 1.00% calcium and 0.40% available phosphorus and the negative control (NC) was formulated to contain 0.80% calcium and 0.20% available phosphorus. A total of 640-day-old male broilers were placed in battery cages and randomly distributed among 10 treatments with 8 replicates per treatment and 8 birds/cage. Feed intake and BW were determined at 7, 14 and 21 d and FCR adjusted by adding the weight of the mortality to the BW of live birds. On d 21, 3 birds per cage were euthanized by CO₂ asphyxiation and used to evaluate bone breaking strength and tibia ash. The left tibia was removed and cleaned for bone breaking strength measurement using an Instron texture analyzer. Bones were ashed to measure tibia ash content. Data were statistically evaluated using ANOVA procedure and means were separated by Tukey's HSD test. Broilers fed the NC without phytase had the lowest (845 g; $P < 0.05$) BW and lowest feed intake (991 g; $P < 0.05$) at 21 d. However, the addition of coated and uncoated phytase enzymes to the NC from 250 to 1500 FTU linearly increased BW at 21 d of age. Birds fed diets with 1500 FTU of coated phytase and 500 FTU of uncoated phytase had higher feed intake than the NC (1,071 and 1,099 g vs. 991 g; $P < 0.05$), but similar feed intake than the PC (1,071 and 1,099 g vs. 1,087 g; $P > 0.05$). Broilers fed the NC had the lowest (29.42%; $P < 0.05$) tibia ash and bone breaking strength (20.09 N; $P < 0.05$). The addition of either coated or uncoated enzyme linearly increased tibia ash and bone breaking strength of broilers, when diets were deficient in available phosphorus. Compared to the coated product across all levels, uncoated Optiphos+ yielded improved 21-day BW (942 vs. 892 g; $P < 0.05$), FCR (1.205 vs. 1.231 g/g; $P < 0.05$), tibia ash (36.02 vs. 34.22%; $P < 0.05$) and bone breaking strength (33.70 vs. 29.92 N; $P < 0.05$). The results of this experiment indicated that phytase source and level have an impact on broiler performance and bone characteristics from 1 to 21 d of age.

ID : 437

A SURVEY OF LIMESTONE QUALITY FROM THE EUROPEAN CONTINENT: IMPLICATIONS FOR BROILER AND LAYER NUTRITION

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Limestone (LS) is the main source of Ca in feeds for broilers and laying hens, contributing of more than 50% and 90% of dietary Ca respectively. In recent years, several research groups have shown digestibility of Ca to vary dramatically depending on the solubility and particle size of the LS. In the light of this recent research, our objective was to conduct a survey of LS used in European feed mills to better understand the variation in LS quality used commercially. A total of 255 LS samples were collected from feed mills in 16 countries on the European continent. Of these, 192 samples were fine LS with an average geometric mean diameter (GMD) of 1000 μm . All samples were analysed for moisture, and 9 minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn). LS particle size was determined on a 100g sample using a set of 14 sieves plus the base pan and GMD of LS particles by mass (dgw) calculated. Solubility of LS was determined in duplicate using the dynamic solubility assay recently published by Kim et al., (2019). For fine LS, solubility was determined at 5, 15, and 30 minutes and LS grit at 30, 90, and 150 minutes. Analyzed Ca was 37.82 \pm 1.93% and 38.26 \pm 1.27 for fine and grit LS, respectively. The average GMD of fine LS was 248 μm , \pm 223 μm with a CV of almost 90%. Over 48% of the fine LS had a GMD below 150 μm , and 30% below 100 μm , reflecting the fine nature of LS used in Industry. LS grit had an average GMD of 1797 μm \pm 478 μm . There was a significant correlation between GMD particle size and solubility of the fine LS at 5 minutes and grit LS at 30 min. However, GMD particle size could not explain all of the observed variation in solubility at the first time-point. For example, two LS from Ukraine and Poland with GMD of 299 and 285 μm had solubility at 5 minutes of 87% and 45%, respectively. In a similar manner, two LS samples from different quarries in Germany had GMD particle sizes of 46 μm and 250 μm but both reached 94% solubility at 5 minutes. Similarly, LS grit samples from Germany and Turkey had respective GMD particle size of 2523 μm and 2587 μm , solubilizing to 73% and 42% at 30 minutes. This suggests that in addition to particle size, geology of LS rock can alter the rate of solubility. The large variation in solubility of LS used in Europe should be considered when selecting LS for broilers and layers since this can potentially alter Ca and P digestibility, and phytase efficacy.

ID : 451

EFFECTS OF DIETARY FRESH LEMON ON THE PRODUCTION PERFORMANCE, EGG QUALITY, SERUM PARAMETERS AND IMMUNE STATUS OF JINGFEN LAYING HENS DURING THE LATE LAYING PERIOD

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INTRODUCTION

The prolonged use of antibiotics may lead to the development of bacteria resistant to drugs, which can be transferred to humans; thus, alternate approaches to replace antibiotics in poultry production are urgently needed. Lemon (*Citrus limon* L.), the third most important Citrus species, is rich in phenolic compounds as well as vitamins, minerals, and carotenoids and are beneficial to the health of animals. Although the composition and byproducts of lemon have been reported to have potential uses in animal feed, few studies have investigated the use of fresh lemon (FM) as an additive in poultry feed. The aim of this study was to investigate the effect of dietary fresh lemon, citric acid (CA) and flavomycin (FLA) on the Jingfen laying hens from 79 to 96 weeks of age.

MATERIALS AND METHODS

Female Jingfen chickens (n=540) were randomly distributed into the following 5 dietary treatments with 36 replications of 3 birds raised in cages: 0 (control), 20 (2%), and 40 g/kg (4%) FM, citric acid (CA) and flavomycin (FLA). Average daily feed intake (ADFI) and feed to egg ratio (FER) were recorded daily. Body weight, shank length, shank girth and egg quality traits were measured biweekly. Serum biochemical indices and antibody titers were detected every 4 weeks from 84 to 96 weeks of age. The data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) in SAS version 9.3. Tukey's multiple comparison was used to test the significance of the differences between treatment means; significance was declared at $P < 0.05$.

RESULTS

Compared with the control diet, the 2% FM, 4% FM, CA and FLA diets increased the laying rate ($P < 0.05$). FM and CA improved antioxidant enzyme activities ($P < 0.05$) and reduced serum HDL and LDL concentrations. Serum TG and T-CH concentrations were lower in all treatment groups than in the control group, indicating favorable effects on lipid metabolism. Antibody titers against H5N1, H9N2 and NDV were higher in the 2% FM and 4% FM treatment groups than in the control group during most experimental periods ($P < 0.05$), while the effect of CA and FLA on the immune performance of chickens was weak.

CONCLUSIONS

In conclusion, FM enhanced the immune and antioxidative status of laying hens during the late laying period and improved their production performance and albumen quality; thus, FM can be used as a new feed additive and as a potential substitute for organic acid and antibiotics during the late laying period.

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GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF JAPANESE QUAILS FED DIETS CONTAINING VARYING INCLUSION LEVELS OF GINGER WASTE MEAL (ZINGIBER OFFICINALE) AS A PARTIAL REPLACEMENT FOR MAIZE

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A study was carried out to investigate the growth performance, nutrients digestibility and carcass characteristics of Japanese quails (*Coturnix coturnix japonica*) as responses to varying inclusion levels of ginger (*Zingiber officinale*) waste meal as partial replacement for maize in their diets. 288 two weeks old mixed sexes Japanese quails were purchased from National Veterinary Research Institute (NVRI) Vom, Jos, Plateau State Nigeria with average initial weight of 30.88 g. They were randomly allotted to four dietary treatments in which ginger waste meal replaced maize at 0, 10, 20 and 30 % designated as T1, T2, T3 and T4 respectively, in a completely randomized design experiment. Each treatment had four replicates with 18 birds/replicate, feed and water were given ad libitum. The parameters measured included initial weight, average feed intake, average weight gain, final weight, feed conversion ratio, nutrients digestibility and carcass characteristics. All data generated were subjected to one way analysis of variance (ANOVA) using SPSS 2017 and where differences occurred, Duncan Multiple Range Test was used to separate the means. The results showed that there were no significant ($p>0.05$) differences in all the parameters measured except in average feed intake, crude fibre and nitrogen free extracts digestibilities where significant ($p<0.05$) differences were observed across treatment groups. Japanese quails on 30 % inclusion level had the highest value for average feed intake, crude fibre and nitrogen free extract digestibilities. Therefore, it was concluded that dietary maize could be replaced with ginger waste meal up to 30 % in the diets of growing Japanese quails without adversely affecting growth performance, nutrients digestibility and carcass yield.

ID : 529

EVALUATION OF THE EFFECT OF LIVE YEAST ON BROILERS PERFORMANCES IN DIFFERENT CONDITIONS:
A MULTI-ANALYSIS

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The use of live yeast *Saccharomyces cerevisiae* boulardii CNCM I-1079 (LY) to improve performances of broiler chickens has been documented in several environments: well controlled research institutes trials as well as field evaluation. The objective of this multi-analysis is to combine the results of all these trials and evaluate the overall effect of the supplementation of LY on broilers performance. A total of 24 trials were included in this analysis: 12 institute and 12 field studies. In all trials, a Control group (receiving a standard broiler feed (NC)) was contemporaneous compared to a probiotic group receiving the same Control diet supplemented with 1×10^9 CFU/kg feed of LY. In 6 different trials, animals from control and treated groups were submitted to a challenge with micro-organism(s) commonly encountered in poultry production. To allow comparison between different experimental conditions the performance results of each trial were expressed as % of the corresponding genetic standard values (Cobb or Ross as applicable) at the appropriate age. Growth performances of each replicate were recorded (final body weight (BW), average daily gain (ADG), feed conversion ratio (FCR) and mortality). Average trial data were analysed using a Mixed Model with treatment as fixed effect and study as a random effect. The trial was the experimental unit. Overall results (n=24) show that the addition of the LY to the feed significantly increased growth performances: +1.75% final BW ($P < 0.05$). FCR improved by 4 points. Mortality was reduced in LY group by -18.7%, compared to NC. When a challenge was applied to the animals (n=6), the differences between NC and LY were even more pronounced (+6% BW, -7points of FCR and -42% mortality rate). From the results of this multi-analysis, it can be concluded that the addition of the live yeast *Saccharomyces cerevisiae* boulardii CNCM I-1079 to broiler chickens feed is beneficially affecting their growth performances.

ID : 549

APPLICATION OF PHYTOGENIC FOR SUSTAINABLE EGG PRODUCTION IN LAYING HENS DURING THE LATE LAYING PERIOD

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Improved chickens performance and sustainable egg production can be accomplished by phytogetic as feed supplements. The present study was conducted to investigate the effect of dietary Moringa oleifera leaves supplementation on laying performance, ammonia production, nutrient digestibility, egg quality, economic prospects and serum biochemical profile. A total of one hundred and fifty Bovans Brown laying hens (66-week-old), were assigned to five treatment diets including dry moringa leaves at 0, 3, 6, 9, or 12 g/kg, respectively, for 12 weeks. Each treatment had six replicate pens with five hens. All production variables were determined for each replicate. Thirsty eggs were randomly collected per treatment to determine egg quality parameters. Twelve birds from each treatment were randomly selected for serum biochemical profile. The statistical analysis was performed using a completely randomized design and orthogonal polynomial contrasts were used to determine the linear and quadratic effects of the increasing levels of inclusion. Over the course of the trial, incremental dietary moringa leaves significantly increased ($P < 0.001$) egg weight, egg production, egg mass and feed intake from 66–70, 70–74, 74–78 and 66–78 weeks of age. Moreover, feed conversion ratio was linearly improved ($P < 0.001$) with increasing levels of moringa in laying hens diet. Concerning ammonia concentrations were ($P < 0.05$) decreased in hens consumed diet supplemented moringa compared with control group. The inclusion of 9 g/kg moringa resulted in overall best productive performance. Eggshell thickness and Haugh unit of hens fed diets supplemented with moringa leaves were greater ($P < 0.01$) than the control diet. Digestibility of crude protein, ether extract and crude fiber linearly increased ($P < 0.001$) with increasing levels of supplementation. Interestingly, serum cholesterol, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, urea and creatinine linearly decreased with increasing moringa levels in the diets. The economic efficiency of hens fed diets supplemented with moringa leaves was greater ($P < 0.01$) than that of hens fed the control diet. It can be concluded that Moringa oleifera leaves can be used as an effective feed additive to improve productive performance of laying hens during the late laying period.

ID : 556

EFFECTS OF ENZYME-HYDROLYZED POULTRY BY-PRODUCT MEAL ON PERFORMANCE AND BLOOD CHARACTERISTICS IN BROILER CHICKENS

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The search for an alternative protein source has been an issue of major importance in the feed industry. Some agricultural waste or byproducts have a big potential to be used in livestock feed. As the poultry industry grows, the production of byproducts from poultry processing plants has been increasing. Although the animal protein sources have a higher protein level and balanced amino acids, the high protein content of feedstuff does not always guarantee the efficient utilization of protein in the body. There have been various methods, such as hydrolyzation, to process the poorly digested protein into a more digestible form. This study was conducted to investigate whether enzyme-hydrolyzed poultry byproduct meal (EHPBM) is more effective as a protein source than poultry byproduct meal (PBM) and soybean meal (SBM) for broiler chickens.

A group of 300 one-day-old broiler chicks were randomly allocated to three treatments with five replicates (20 birds/replicate) for five weeks. The treatments were: 1) Basal diet containing soybean meal (SBM), 2) basal diet containing poultry byproduct meal (PBM), and 3) basal diet containing enzyme-hydrolyzed poultry byproduct meal (EHPBM). Feed intake and weight gain were measured every week. At day 35, a total of 75 birds were selected for blood and internal organ sampling. Duncan's Multiple Range Test was used for significant differences at the 5% level.

The EHPBM-fed group ($1,853 \text{ g} \pm 125.60$) showed the highest final body weight when compared to PBM-fed group ($1,723 \text{ g} \pm 76.81$) and SBM-fed group ($1,545 \text{ g} \pm 62.31$) ($P < 0.05$). The feed conversion ratio of the EHPBM treatment group is considered to be effective in improving productivity when the feed conversion ratio by weight is considered. The feed conversion ratio of the EHPBM-fed group was 1.740, which was significantly higher than the PBM group (1.674) and SBM group (1.653) ($p < 0.05$). There were no significant effects on internal organ weight, but in blood biochemical characteristic analysis, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were higher in the EHPBM group and PBM group ($P < 0.05$), which is considered to be due to rapid growth.

In conclusion, EHPBM may partly replace conventional dietary protein sources such as soybean meal or poultry byproduct meal and can be used to improve the productivity of broilers. However, for practical uses considering economic feasibility, more studies should be conducted to identify the effective and optimum use of EHPBM.

ID : 631

EFFECT OF PROBIOTIC ON GROWTH PERFORMANCE, MORPHOMETRY INTESTINAL, HAEMATOLOGICAL PARAMETERS AND CAECAL MICROFLORA OF GUINEA FOWL BROILERS.

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Following the ban of antibiotics used as growth factor promoter in poultry production, several alternatives such as prebiotics and probiotics have been explored. Stabilized products of sorghum enriched with lactobacilli (SPSL): *Lactobacillus casei*, *Lactobacillus fermentatum*, *Lactobacillus acidophilus* and *Enterococcus faecium* would be a justified alternative. The objective of this study was to evaluate the effect of SPSL on growth performance, morphometry intestinal, haematological parameters and caecal microflora of Guinea fowl. A total of 630 guinea fowl broilers with average body weight of 33.02g were assigned into 3 treatments with 6 replicates (35 birds/replicate). The 3 treatments were: 1) basal diet (T-), basal diet supplemented with TetracolivitND (antibiotic) in water at 1g per liter 5 consecutive days per month (T+) and basal diet supplemented with the SPSL at the dose of 3% (T3). During experimental period (12 weeks), feed intake, body weight and mortality were recorded weekly. At the 4, 8 and 12 weeks, 54 birds per treatment were used to collect blood samples and caecal digesta respectively for haematological and microbiological analyses. Results showed that at wk 12 the birds fed with diets containing SPSL (T3) had higher (1506 ± 10.6) body weight, compared with birds of T+ (1401 ± 8.36) and T- (1398 ± 9.22), ($p=0.008$). At the 12 week, a significant increase was observed in T3 group (117 ± 1.35) compared to T+ (109 ± 1.25) and T- (106.5 ± 2.01) in terms of small intestine length ($p + (16.78 \pm 0.72)$ compared to T- (19.63 ± 1.30) at 12th week. It's similar for the White blood cells where the birds in T3 (205 ± 1.85) and T+ (206.6 ± 3.23) recorded the lower level ($p=0.0128$) compare to T- (210.4 ± 2.33) at 12th week. At the 4 weeks, total coliforms bacteria was high ($p=0.0245$) in the T- (6.05 ± 0.31) and T+ (6.35 ± 0.45) than T3 (4.09 ± 0.68). It's similar for the *Escherichia coli* were the T3 (3.26 ± 0.59) and T+ (3.79 ± 0.43) recorded the lower level ($p=0.0481$) compare to T- (5.08 ± 0.57). It can be concluded that the SPSL has significantly improved the growth performance and health status of Guinea-fowl.

Key words: guinea fowl broilers, growth, SPSL, antibiotic, caecal microflora.

ID : 652

THE EFFECTS OF SOURCE, PARTICLE SIZE, AND LEVEL OF CALCIUM ON CROP- AND GIZZARD-EMPTYING RATES AND BLOOD PARAMETERS IN LAYING HENS

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Laying hens need to take a large amount of calcium (Ca) from feed during their egg production, it is therefore important to select appropriate feed additive as a Ca source for hens. It is believed that the retention time of ingested feed in the crop affects Ca digestion and absorption in the gastrointestinal tract of chickens, but the relationship between the retention time and the Ca sources added in the feed has not been known. The present study examined whether crop- and gizzard-emptying rates were affected by the difference of the Ca sources which are commercially available in Japan.

Fifty seven laying hens at 180 days of age were used. The hens were given free access to water and a commercial basal diet. The hens were fasted for 16 hours and then fed 9 test diets which contained the different source, particle size, and level of Ca as follows: (1) control (fed a basal diet, n = 7); (2) 1% addition of fine limestone (0.8-1.7 mm in particle size, n = 7) to the basal diet; (3) 2% addition of fine limestone (n = 6); (4) 1% addition of large limestone (1.7-4.0 mm in particle size, n = 6); (5) 2% addition of large limestone (n = 6); (6) 1% addition of fine oyster shell (1.7-4.0 in particle size, n = 6); (7) 2% addition of fine oyster shell (n = 7); (8) 1% addition of large oyster shell (4.0-7.0 mm in particle size, n = 6); (9) 2% addition of large oyster shell (n = 6). Three hours after feeding, the blood samples and total contents of the crop and gizzard were collected. Crop- and gizzard-emptying rates were calculated by the formula which is equal to dry weight of feed emptying from the crop and gizzard per dry weight of ingested feed. Plasma was used for measurement of glucose, NEFA, Ca, and inorganic phosphorus concentrations. For data comparisons, we performed one-way ANOVA, and the significance of the differences was assessed using a Tukey-Kramer test.

One-way ANOVA revealed that crop-emptying rate in the hens fed 2% large oyster shell-added feed significantly decreased as compared to that in the hen fed control feed ($P < 0.01$). Crop-emptying rate in the hens fed 2% large limestone-added feed tended to decrease as compared to control ($P < 0.1$). There were no significant differences in gizzard-emptying rate between the experimental groups. There were also no significant differences in blood parameters between the experimental groups. These results suggest that addition of 2% large oyster shell to feed is appropriate as a Ca source for laying hens.

ID : 695

CASSAVA ROOT-BASED COMPOSITE MEAL IN POULTRY NUTRITION: EFFECTS OF BLENDING MATERIALS AND ENSILING PERIOD.

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Cassava root meal is underexploited in poultry nutrition in Nigeria because of its low protein value, dustiness and high hydrogen cyanide content. The purpose of this study was to develop a composite cassava-based meal by ensiling a blend of peeled or unpeeled cassava root meal, wet brewers' grain and palm kernel meal, and determine their proximate compositions and feeding values in broiler diets. Peeled and unpeeled cassava root meals were blended with wet brewers' grain and palm kernel meal or wet brewers' grain in a ratio of 1.0:1.5:0.5 or 1.0:1.5 and ensiled for 0, 7 and 14 days in a black polythene bag under room temperature. The sun dried composite meals were analyzed for their proximate compositions and incorporated into broiler diets at 50% replacement of maize in different experiments. The design was a complete randomized design. Protein content (DM) was 10.90–11.89, 10.54–13.03, 12.02–12.56 and 12.45–13.16% for peeled cassava-brewers' grain, peeled cassava-brewers' grain and palm kernel meal, unpeeled cassava-brewers' grain, unpeeled cassava-brewers' grain and palm kernel meal blends, respectively. Ensiling decreased the protein values for unpeeled cassava-based products but increased those of peeled cassava-based products at 14 and 7 days respectively. Gross energy value was 4672.94–4942.94, 5113.94 – 5315.64, 4562.45–4705.46 and 4948.26–5224.80kcal/kg for peeled cassava-brewers' grain, peeled cassava-brewers' grain and palm kernel meal, unpeeled cassava-brewers' grain and unpeeled cassava-brewers' grain and palm kernel meal blends, respectively. Except for the peeled cassava-brewers' grain product, the gross energy values increased with increasing period of ensilage. The enhanced protein and energy values of the composite meals relative to cassava root meal and reduced dustiness is noteworthy. Weight gain and feed conversion ratio were similar in starter broiler chickens fed diets containing 50% peeled cassava-based products irrespective of the combinations and ensilage period. Broiler chickens fed unpeeled cassava-based product had significantly ($p<0.05$) lower weight gain and poorer feed conversion ratio, lower abdominal fat and drier litter moisture than the control irrespective of ensilage period. Carcass yield organ weights and mortality were unaffected. Nutritionally improved and energy rich composite meal can be prepared from a blend of cassava root meal, wet brewers' grain and palm kernel meal.

ID : 728

EFFECT OF MINERAL SUPPLEMENTS BASED ON SHUNGITE ON LAYING HENS.

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INTRODUCTION.

In industrial environment birds are exposed to significant functional load, which often weakens the natural defence and negatively affects the health and growth rates. Mineral additive from shungite rock is of great interest. Fossil shungite rock is an ancient Precambrian carbonaceous formation with a specific structure, properties and high adsorption capacity. Shungite carbon has a fullerene-like structure characterized by high chemical and physico-chemical activity.

MATERIALS AND METHODS.

The research was conducted on "Hisex brown" hens aged 56-64 weeks in commercial poultry farm. Mineral feed additive based on shungite was grinded with the use of innovative technologies and sort by fractions of different size. The size of particles was determined by regulatory requirements for poultry feeds. The trial used biochemical, molecular genetic and zootechnical approaches to assess the impact of feed additive based on shungite on birds condition. The total number of bacteria in the intestinal was analyzed by quantitative PCR and NGS. The effect of feed additive on the expression of productivity and resistance genes was evaluated. Mineral additive based on shungite was added into the diets in the amount of 1 kg/t of feed.

RESULTS.

The mineral feed additive based on shungite has been tested for the ability to bind mycotoxins. The true sorption of mycotoxins: aflatoxin B1- 99.8%, ochratoxin A - 99.7%, T-2 toxin - 59.6%, zearalenone - 98.7%.

It was observed an increasing production performance of laying hens with feeds supplemented with shungite in the form of grits with a particle size of 2-3 mm diameter. During the experiment the average egg production per hen increased and reached the target values. Average body weight of laying hens in the experimental group increased by 1.5%, which indicates a good physiological condition. It was observed raise in overall grade of eggs and increas of total eggmass. The yield of high grade eggs (7.6%) was significantly increased.

It was shown that the number of uncultivated bacteria throughout the entire period of growing broilers ranged from 28.9 to 48.2 %. Entering a feed additive based on shungite changes the structure of microbiota, reducing the relative numbers of uncultivated bacteria phylotypes. The number of useful cellulolytic microorganisms was rather high throughout the entire broilers rearing period. The number of lactobacilli was higher on 27% in variant with shungite compared with control.

CONCLUSION.

The use of a mineral supplement based on shungite in a preventive dose can neutralize the negative impact of mycotoxins on birds and keep their productivity at the target level. Feed additive based on shungite tested for lack of binding capacity in relation to vitamins, trace elements and amino acids in the balance trial. Thus, the feed additive based on shungite when used in poultry feeds increase productivity and has the potential to improve feed conversion ratio. It practically does not affect the metabolism of vitamins, amino acids and trace elements.

ID : 750

RESIN ACID CONTAINING TALL OIL FATTY ACID PRODUCT IMPROVED BROILER PERFORMANCE

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Tall oil fatty acid, originating from coniferous trees is comprised of free long-chain fatty acids (90%) and bio-active resin acids (8.5%). In previous trials tall oil fatty acid has consistently shown positive effects on animal performance. An experiment was conducted with sodium salt of tall oil fatty acid (STOFA) to evaluate its effects on broiler health and performance under challenging housing conditions, and with or without in-feed coccidiostat (maduramicin). The experiment had 3x2 factorial design with three levels of STOFA; 0, 1.0, and 2.0 g/kg feed (equating 0, 0.5, and 0.1 g of tall oil fatty acid/kg feed), and two levels of coccidiostat (maduramicin); 0 and 5 mg/kg feed.

A total of 7680 Ross 308 mixed sex one-day-old chicken were randomly allocated into 6 experimental treatments with 8 replicate pens in each treatment. In each pen maximal allowed animal density was maintained along the trial with extra fencing. The house temperature was reduced by 3 centigrade lower than the recommendation after day 2. The broilers were weighed at days 14, 20, 28 and 34. Feed intake and FCR were calculated for each period and for the entire trial period. The results were subjected to two-way ANOVA for mean effects and interaction effects with R software (R i386 3.6.1). Post-hoc comparisons were made if the differences were statistically significant ($p < 0.05$).

Diet significantly affected the final body weight of the broilers. There was a significant interaction between the effect of coccidiostat and the effect of STOFA; STOFA improved birds body weight more in coccidiostat free diet group than in group with in-feed coccidiostat ($p < 0.05$). Birds on coccidiostat-free STOFA diets reached similar average body weight as birds on coccidiostat diets, while birds without STOFA and in-feed coccidiostat had the lowest body weight. Dietary treatments had no statistically significant effects on feed conversion ratio.

Based on the current trial showing significantly improved final body weight, sodium salt of tall oil fatty acid with bio-active resin acids supports broiler growth performance. The effect was more pronounced in broilers on coccidiostat-free diet.

ID : 783

CITRULLUS LANATUS ESSENTIAL OILS INCLUSION IN DIETS ELICIT NUTRACEUTICAL EFFECTS ON EGG PRODUCTION, EGG QUALITY AND PHYSIOLOGICAL CHARACTERISTICS IN LAYER HENS

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Use of natural feed additives such as phytogetic plants with medicinal benefits has been suggested as possible alternatives to the commercial feed additives (Karori et al., 2007; Boskurt et al., 2014; Omonijo et al., 2017). The current study evaluated the effects of C.lanatus essential oil inclusion in diet on egg production, egg quality and physiological parameters in layer hens. A total of 72 white leghorn point-of-lay hens at 18- weeks were randomly allocated to three (3) dietary treatments: 1) Commercial layer diet (control), 2) Commercial diet + 1g C. lanatus essential oil/ kg feed and 3) Commercial diet + 2g C. lanatus essential oil/ kg feed. Each treatment was replicated 8 times arranged in completely randomized design. The general linear statistical model (SAS, 2010) was used for analysis of variance to determine the effects of essential oil inclusion in diets on performance of the layer hens. From the results, an increase in total weight gain, average daily feed intake (ADFI) and average daily gain (ADG) was observed with inclusion of the C. lanatus essential oil. In addition, the inclusion of C. lanatus in diet improved the egg mass and feed conversion ratio (FCR). Hens fed C. lanatus containing diets had higher (P C. lanatus containing diets also had lower (P C. lanatus oil appeared to reduce shell weight and shell ratio, while increasing albumen height and haugh unit (HU). Diets containing C. lanatus had significantly higher (P PUFA), total n-6 fatty acids (14.81 ± 0.59) and also had the highest (P C. lanatus essential oils in diets. Moreover, tibia bone parameters were also significantly improved with inclusion of C. lanatus essential oils in diets. It can be concluded that C. lanatus essential oil positively affected egg production and quality and health of layer hens and can be used successfully as a natural feed additive in layer hens.

ID : 819

EFFECT OF RAPESEED MEAL OR TOTAL SULFUR CONTENT IN THE DIET ON PERFORMANCE AND MINERAL METABOLISM OF THE BODY IN BROILER CHICKENS

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Studies show that in birds fed with the addition of rapeseed meal are more likely to have leg problems. Probably, this is due to the high concentration of sulphur in rape products, which in some way negatively affects the calcium economy of the body. The aim of the study was to investigate the effect of total sulphur or rapeseed meal contained in feed on broilers chicken performers and Ca digestibility.

Study was conducted with 480 1 day old ROSS 308 chickens. Birds were randomly assigned to 5 dietary treatments (12 rep / 8 birds). The experiment lasted 35 days and was divided into two periods: starter (1-11d) and grower (12-35d). The first group was the control group and the main protein component was soybean meal. In the second and third group, there was addition of rapeseed meal 5 and 10% in starter diets and 10 and 20% in grower diets, respectively. In the 4th and 5th groups, the diet from the control group and the addition of sulfate ions were used so that the total sulfur content was equalized to the level from groups 2 and 3. On day 35 of the experiment, the birds were sacrificed and intestinal content and tibia bones were collected for analysis.

During the starter and grower period, both 10% addition of rapeseed meal and addition of sulfur aligned to the same level (groups 3 and 5) caused a statistically significant increase in FCR (P

The results of these studies clearly suggest that the high sulfur content (served as ions or derived from rapeseed meal) in the diet for chickens negatively affects the production results and calcium digestibility. This suggests that sulfur affects the mineral balance of the birds' body.

ID : 828

EFFECTS OF GUANIDINO ACETIC ACID SUPPLEMENTATION TO CORN, SOYBEAN MEAL BASED DIETS AND ENERGY LEVELS ON PERFORMANCE, INTESTINAL MORPHOLOGY AND CARCASS QUALITY OF BROILERS

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Creatine has a key role in cellular energy metabolism and could be synthesised from Guanidinoacetic acid (GAA) however, its de novo synthesis is not able to fulfil the demand of energy metabolism in modern broilers. Vegetable based broiler diets which are free of creatine should be supplemented with an exogenous source of creatine such as GAA. So, the research was carried out in Beypiliç Broiler Company in Turkey to evaluate the energy contribution of GAA (CreAMINO®, 96% GAA) in reduced energy diets on performance, intestinal development. 10260 one-day-old Ross-308 chicks were randomly allocated to 6 dietary treatments with 9 replicates. Dietary treatments were arranged as 3*2 factorial arrangement with three levels of ME (ross recommended, 50 and 100 kcal/kg reduced) and two levels of GAA supplementation (0.00 and 0.06%). Diets were based on corn, soybean meal and wheat produced as pellet form in Beypiliç Feed Mill. Feed intake (FI), body weight (BW), carcass yield and ileum morphological measurements were determined on pen basis. Statistical analysis was performed by one-way Anova for main effects and interaction, followed by Tukey's test (P

Dietary treatments showed no significant effects on FI, carcass parameters and mortality. However GAA supplementation significantly improved final BW, BWG and FCR compared to unsupplemented groups. The lowest FCR was obtained from the birds received regular (recommended by Aviagen) ME diet supplemented by GAA. BW and FCR was improved 1.63% and 1.43 respectively in the GAA supplemented treatments compared to unsupplemented.

ME level did not have significant effects on the final BW and, interestingly 50 and 100 kcal energy reduction from control diet did not cause any significant impairment on BW, but FI and FCR were significantly increased as both reduced ME level.

There was a significant interaction effect on FCR such that within the ME levels, FCR increased in 100 kcal reduced ME treatment with GAA supplementation, while no significant change observed in treatments without GAA. The interaction effect was also found for BW where 100 kcal lower ME reduced the final BW in the treatment with GAA, while no impairment was observed in the birds received no GAA.

This results supported by ileal development as measured villus height (VH), crypt depth (CD), villus width (VW) and villus surface area (VSA). Carcass and breast meat yield were not significantly affected by either dietary energy level or GAA supplementation.

It might be concluded that 0.06% GAA supplementation may improve BW and FCR and save 50 kcal/kg ME

ID : 830

EFFECTS OF ORANGE PEEL OIL AS A DIETARY SUPPLEMENT ON EGG PRODUCTION AND OXIDATIVE STABILITY OF EGG YOLK IN LAYING QUAIL

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In recent years, essential oils have been used as feed additives instead of antibiotics for poultry nutrition. Besides, the effects of essential oils on poultry products are the subject of research. In fruit juice industry , after orange fruit processing, orange peel are obtained as waste. In this way, significant amounts of orange peels are obtained each year in fruit industries throughout the world. D-limonene is one of the most common terpenes in nature and it is mostly found in the peels of orange

The objective of this study was to investigate the effects of dietary orange peel essential oil supplementation on performance and egg yolk antioxidant status in quail (*Coturnix coturnix japonica*). A total of 120 twenty-week old quails were allocated randomly to 1 of 3 dietary treatments. Basal diet and basal diet supplemented with 200 or 300 mg of orange peel essential oil /kg of diet. Each treatment comprised 4 replications with 4 cages (10 quail per cage), amounting to 40 quail per treatment group. Diets (in mash form) and water were provided for ad libitum consumption. To investigate the effect of diet with orange peel essential oil on lipid oxidation of shell eggs during refrigerated storage, four eggs from each replicate collected during the last week of the feeding trial, totalling 16 eggs from each dietary treatment were placed in a refrigerated cabinet at 4 °C to be analyzed for yolk malondialdehyde content at 0, 15, 30 days of storage. Dietary supplementation of orange peel essential oil significantly increased egg production compared with control ($P < 0.05$). Moreover Egg production was the highest in 200 mg/kg orange peel essential oil treatment group. There were no significant differences in feed consumption between treatments, whereas feed conversion ratio was significantly improved by 200 mg/kg orange peel essential oil supplementation. There were no differences between the treatment groups with regard to egg weight. Supplementation of orange peel essential oil to diet had positive effect on oxidative stability of eggs during storage. The extent of lipid oxidation in shell eggs differed among the dietary treatments. Lipid oxidation was higher in the control group compared to other groups. The results of this study showed that supplementing the diet with orange peel essential oil have beneficial effect on egg production and feed conversion ratio. Orange peel essential oil could be considered as a potential natural feed additive in poultry diets.

ID : 831

RESPONSE OF EGG-TYPE PULLETS TO DIETARY INCLUSION OF HEAT TREATED GUAR SEEDS (CYAMOPSIS TETRAGONOLOBA) AS PROTEIN SOURCE FORTIFIED WITH ENZYME

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Dietary inclusion of non-conventional feedstuffs for poultry could be used as a valuable source of nutrients and efficient strategy to reduce the production cost. A (2 × 3) factorial arrangement was used in a completely randomized design to study the effect of different four levels of boiled guar seeds (0, 5, 10 and 15 %) and two levels of commercial multienzyme (Natuzyme) supplementation (0 and 500 mg/kg) on egg-type pullets. A total of 240 one day-old (Hy-Line W-98) pullets were allotted to 8 treatments and each treatment was replicated three times with 10 birds each. The experimental diet without guar and multienzyme was considered as the control treatment. The experimental diets were iso-energetic and iso-nitrogenous to meet or exceed the requirements of pullets' growing phases (starter, grower and developer). Feed consumption (FC), weight gain (WG), and feed conversion ratio (FCR) were recorded weekly and at the end of each growing phase. Blood samples were collected for determination of some constituents and hematological profile. Results showed that guar phytate and tannin content were reduced by boiling processing. During the entire growing period, WG and FCR were deteriorated as the dietary level of guar increased ($p \leq 0.01$). The WG and FCR were improved when diets were supplemented with Natuzyme ($p \leq 0.05$). Feed consumption was not affected by experimental treatments. At the end of developer period, protein, cholesterol, calcium and phosphorus were not influenced by Natuzyme. However, cholesterol and calcium were affected by incorporation of guar. Hematological parameters at 18 week of age were not affected by experimental treatments. The only exception was the white blood cells count which was affected by dietary guar. Ages at onset of egg were postponed as the level of guar increased. Birds fed on diets supplemented with multienzyme had earlier age of onset of egg production. Based on the present results, it can be stated that high levels of boiled guar in egg-type pullet diets may cause significant adverse effects on the performance. Dietary supplementation of Natuzyme improved pullets' performance.

ID : 833

HYDROLYSABLE TANNINS RICH PHYTOGENIC REDUCE THE INCIDENCE OF KEEL BONE DAMAGE

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Phytogenic substances rich in hydrolysable tannins reduce the incidence of keel bone damage in hens. A growing number of studies have shown the extraordinary bioactive properties of hydrolysable tannins rich extracts used in small concentrations of, which improved are a source of improved health status, nutritional and production efficiency in the monogastric domestic animals. The purpose of this study was to determine the influence of phytogenic substances rich in hydrolysable tannins on reducing the incidence of keel bone damage in different parts of the keel bone in commercial laying hens. The hens were traditional Slovenian breeds of Prelux-G housed in a deep litter system. Animals included in the experiment, we randomly divided into two groups (n = 18 birds per group) and three pens per group. The control group (K) had a standard complete feed mixture for laying hens while the test group (HTBCO) had an additive comprise of 75% hydrolysable tannins, 15% simple sugars and encapsulated calcium butyrate (Tanin Sevnica, Slovenia). HTBCO concentration in feed was 250 g/t from week 20 to 30 and 500 g/t from week 30 until the end of breeding. The pens differ in the number (1-3) and level of the perches (one or two levels). They had 1 or 2 perches in one level or three perches in two levels, respectively, according to recommendations. In each pen 6 individually marked hens the same age was followed. During the laying period, we performed the observations from the 20th to the 34th week of age. At the end of the weeks 25th, 30th, 34th we had control day when we performed a palpation and ultrasound analysis of keel bone, egg productivity, the physical parameters of the eggs and behavior characteristics. For statistical analyses, we used SPSS 25.1 for Windows. The χ^2 test we used for analyses of the effect of HTBCO on keel bone injuries incidence and the characteristic behavior traits of the hens. The t-test we used to analyses the effect of HTBCO between pens with the same furnishing type on the physical parameters of the eggs and egg productivity. Results indicated that the behavior of hens and the physical parameters of eggs were not affected by HTBCO as well as the rearing system. However, we found a significant (P

ID : 839

EGG PRODUCTION, EGG QUALITY AND IMMUNE STATUS OF LAYING HENS FED DIETS CONTAINING SYNTHETIC AFLATOXIN B1 AND CALCIUM BENTONITE CLAY AS A MYCOTOXIN BINDER

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Corn is a major ingredient of layer feed and may be contaminated with aflatoxins, which potentially decrease the productive performance of laying hens and can cause significant depression on immune response. This experiment was conducted to determine the effect of synthetic aflatoxin B1 and calcium bentonite clay as a mycotoxin binder in the laying hen diet on egg performance, egg quality and immune response to vaccination. A total of 90 Lohmann brown-classic hens (22 weeks old) were divided into 3 groups of 6 replications with 6 hens each. This experimental design was completely randomized design and there were 3 experimental diets: (1) Positive control diet (2) Positive control diet +200 ppb aflatoxin B1 (negative control) and (3) Positive control diet +200 ppb aflatoxin B1 and +0.25% calcium bentonite clay. All hens were vaccinated with ND and IB vaccine. At the end of the 24 weeks of feeding trial, dietary treatments did not affect the egg production, egg mass, feed conversion ratio (FCR) and IB titer of laying hens; however, aflatoxin B1 significantly decreased final body weight ($P<0.05$) and tended to decrease the eggshell color score ($P=0.05$). Moreover, the ND titer was reduced by aflatoxin B1 at 8th and 12th weeks of feeding trial ($P=0.05$ and <0.05 ; respectively). Interestingly, supplementation of 0.25% calcium bentonite clay in feed improved body weight, eggshell color and ND titer of laying hens similar to those fed the positive control diet. Supplementation of calcium bentonite clay as a mycotoxin binder improves the egg quality and immune response of laying hens without the negative effect on egg production during 24 weeks feeding trial.

ID : 858

EFFECTS OF POMEGRANATE SEED PULP AND SUMAC SEED POWDER ON PERFORMANCE AND SOME BLOOD INDICES IN BROILER CHICKENS

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This study was conducted to evaluate the effect of medicinal plants- of pomegranate seed pulp and sumac seed powder on performance traits such as- feed intake, weight gain, feed conversion ratio, carcass and internal organs weight and blood parameters such as cholesterol, triglyceride, glucose, Albumin, total protein, urea, aspartate aminotransferase and alanine aminotransferase were performed using 280 broiler from Ross 308 bred. Treatments were including control, treatment 1, 2 and 3% of pomegranate seed pulp, treatment of 0.25, 0.5 and 1% sumac seed powder. Data were analyzed by SAS statistical software and GLM procedure. The results showed that during the whole period of growth (1-42 days), growth period (14-28 days) and the end period (28-42 days), feed intake were increased significantly in 1% sumac treatment compared to control treatment ($0.05 > P$). The amount of weight gain during growth period was significantly different between treatments of 2% pomegranate seed pulp with 0.25% sumac powder treatment. Feed conversion ratio did not showed any significant difference between treatments ($P > 0.05$). There was no significant difference between the treatments in relation to the oral organs (thigh, breast, wing, back and neck, gizzard, heart). The percentage of carcass was significantly higher in treatment of 1% sumac powder than other treatments ($P < 0.05$). There was no significant difference at the percentage of weights of non-femoral internal organs (liver, spleen, pancreas, bursa, duodenum, jejunum, ileum, preventricle and abdominal fat) in experimental treatments. The amount of glucose at 0.5 and 1% sumac powder treatments were increased compared to the control treatment significantly. The total protein concentration in treatments of 0.5 and 1% sumac powder was significantly higher than 1, 2 and 3% pomegranate seed pulp Also, the amount of urea in treatments of 0.5 and 1% sumac powder was significantly different with control and 1, 2 and 3% pomegranate seed pulp ($P < 0.05$). The treatment of 1% sumac powder showed the highest amount of urea (9.25 g/dl). In general, treatment of 1% sumac powder showed a significant effect on feed intake and carcass weight, that can be considered to future studies.

ID : 865

EVALUATION OF CARAWAY AND LEMON BALM WASTE ON CARCASS YIELD AND MICROBIAL POPULATION OF BROILERS

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The use of antibiotic growth promoters because of the risk of bacterial resistance, as well as transfer of manufacturing products to consumers is limited. Medicinal plants with antimicrobial effects as well as decreasing serum lipids as viable alternatives to antibiotics have been proposed. In this study, 280 chicks from Ross in the form of a completely randomized design with 6 treatments and 1 control group and 4 replicates and 10 chicks were used in each iteration. The experimental diets based on corn - soybeans, according to all requirements of broilers based company advised Ross (2014) was used and herbs Lemon Balm 0.3, 0.6 and 0.9 and plantain respectively values of 0.2 and 0 / 4 and 0.6 percent in the diet in the form of powder was added to the diet. Cholesterol, triglycerides, HDL, LDL serum test kits Pars Commercial and enzymatically - Kali meters were measured as well as the carcass was examined in each of the groups. The results showed that there was no significant difference between treatments on feed consumable. Body weight at 42 days was significant ($0.05 > P$). So that the treatment Lemon Balm showed the highest body weight. FCR 42 days in between treatments was significant ($0.05 > P$). So that the highest and lowest FCR was belong to Lemon Balm 0.3 and plantain 0.9, respectively. Cholesterol and triglyceride levels were also affected by treatments ($0.05 > P$). The highest and lowest amount of cholesterol in order for the treatment evidence and Lemon Balm 0.3.

ID : 870

THE EFFECT OF DIFFERENT LEVELS OF POULTRY BY PRODUCT MEAL (PBPM) AND ACIDIFIER ON GROWTH PERFORMANCE AND BLOOD PARAMETERS IN BROILERS

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A trial was conducted to evaluate the effect of different levels of poultry by product meal (PBPM) and acidifier on growth performance and blood parameters in broilers. 200, one day-old broiler chickens (Ross 308) were used in a completely randomized design with 5 treatments of 4 replicates/treatment and 10 birds/replicate. Five treatments including (control), %5 PBPM and % 0.1 acidifier, %8 PBPM and % 0.1 acidifier, %5 PBPM and % 0.2 acidifier and %8 PBPM and % 0.2 acidifier with four replicates were added to the basal diets and fed to birds for a period of 42 days. The variables measured at the end of 10, 24 and 42 days, include body weight gain, feed intake and feed conversion ratio. Blood samples were collected to measure blood parameters at the end of experiment. The broilers were slaughtered according to health instructions and their body quality was evaluated. The results show that increasing the levels of PBPM to the extent of %8 with %2 acidifier has no significant effect on body weight, carcass weight, feed intake and conversion ratio. Mortality in treatment without acidifiers were higher than other treatments. The effect different treatments on blood cholesterol ($P=0.00$) and Ca ($P=0.037$) was significant, but it did not on high density lipoprotein blood P and Newcastle titer significantly ($P=0.05$). The results of experiments showed that in broilers fed with PBPM, acidifier can on growth performance and Mortality in broilers.

ID : 984

EFFECT OF SODIUM BUTYRATE PROTECTED WITH SALT OF MEDIUM-CHAIN FATTY ACIDS ON GASTROINTESTINAL MICROBIOTA AND MORPHOLOGY IN BROILER CHICKENS

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Butyric acid is associated with antimicrobial activity and plays an important role in the development of the intestinal epithelium. The use of medium-chain fatty acids (MCFA) as a protection for sodium butyrate may widen the spectrum of action to promote gastrointestinal health. So, the aim of this study was to evaluate the effect of a protected butyrate with sodium salts of MCFA from coconut distillates (BUT+) on microbial population and morphology of jejunum and ileum in broilers at 10 and 39d of age. A total of 192 one-day-old female broilers Ross 308 were randomly distributed into 4 dietary treatments (6 cages / treatment). A basal diet (CTR) was supplemented with increasing levels of BUT+: 0.5, 1 and 2 kg/t of BUT+. Diets were provided ad libitum: a started feed until day 21 and a grower-finisher feed from day 22 to 39. Feed intake and live body weight were monitored for each period and during the whole experiment. At 10d and 39d of age, digestive content was collected to analyse microbiota population (lactic acid bacteria (LAB) and total Enterobacteriaceae (E) to calculate LAB/E ratio and total coliforms) by traditional count. To characterise histomorphometry, tissue from the posterior jejunum and the middle of ileum were removed. Ileum analysis was carried out at both ages; jejunum data were only from 39d animals. Data was analysed by one-way ANOVA. Tukey's multiple range test was performed to determine whether means were significantly different ($P < 0.05$). There were no significant differences on performance or in ileum microbiota counts. However, in jejunum content at 39d of age, the highest dose (0.2 %) tended ($P = 0.082$) to increase Enterobacteriaceae, so that LAB/E ratio was significantly reduced in opposition to the others doses (0.05% and 0.1%) that had the highest ratio ($P = 0.025$). Focusing on histological parameters, the supplementation of BUT+ did not modify villi high and crypt depth at any age. However, the animals receiving the highest dose of BUT+ also had higher mitosis count in jejunum than the animals that received non-supplemented diet ($P = 0.027$). In the ileum, the highest dose of BUT+ had the lowest number of goblet cells at 10d ($P = 0.005$) and reduced the goblet cells in comparison with BUT+ at 0.5 kg/t at 39d ($P = 0.025$) that had the highest goblet cells counts. Results confirm the potential activity of this butyrate protected with sodium salt of MCFA on gut barrier modulating jejunum microbiota and mucus layer from ileum in 39d broilers.

ID : 999

A BROILER DIET OF HIGH-OLEIC PEANUTS ENHANCES THE UNSATURATED FATTY ACID PROFILE OF THE MEAT PRODUCED

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Early feeding trials using peanut meal prepared from normal-oleic peanuts helped to identify peanuts as a suitable alternative feed ingredient for poultry. Yet, no studies to date have examined the use of high-oleic peanuts (HO-PN) as a feed ingredient for broiler meat-type chickens. Therefore, this study aimed to determine the effect of feeding whole unblanched HO-PN on the fatty acid profile of the meat produced from broilers. At hatch male chicks were randomly placed in raised wire cages, in 10 replicate pens per treatment with 10 chicks per pen, and fed with one of the 3 isocaloric, isonitrogenous diets ad libitum for 42 days: (1) conventional control of soybean meal + corn (2) 10 to 12% HO-PN and corn diet, or (3) control diet spiked with $\approx 6.0\%$ oleic acid oil. All body weights (BW) were collected, and broiler selection for processing was determined by individual BW within one-half a standard deviation of the experiment 42-D mean BW, with one bird selected per pen (10 replicate pens per treatment, 3 treatments, 10 birds selected per treatment, yielding a total sample size of 30 birds). Performance was determined weekly and breast samples were analyzed for fatty acid and amino acid profile. All data was analyzed using analysis of variance, with t-test mean comparisons at $p < 0.05$. Body weights were similar between broilers fed the HO-PN and control diets, while the feed conversion ratio of broilers fed the HO-PN diet were significantly higher at week 2, 4 and 6 relative to the other treatments ($p \leq 0.03$). HO-PN broilers had reduced hot carcass and cold carcass weights, and pectoralis major breast weights relative to the other treatments. Chicken breast produced from broilers fed the HO-PN diets had significantly reduced saturated and trans-fatty acid content relative to the controls ($p \leq 0.0002$). While additional studies must be conducted, this study suggests that feeding whole unblanched high-oleic peanuts to broiler chickens may serve as a means to enrich the meat produced with unsaturated fatty acids.

ID : 1003

ALLERGENIC PROTEINS PEANUT AND/OR SOY POULTRY FEED INGREDIENTS ARE NOT DETECTED IN THE MEAT AND/OR EGGS PRODUCED

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Previous studies have demonstrated that allergenic feed proteins from peanuts in the diets of layer hens are not detected in the eggs produced. Hence, in this study we aimed to determine if soy and/or peanut proteins in poultry feed rations of broiler chickens or layer hens would be transferred or detectable in the meat or eggs produced. To meet this objective, 99 layer hens and 300 broiler chickens were equally divided into treatment groups and fed one of three experimental diets, (1) control soybean meal and corn diet(2) whole unblanched high-oleic peanut and corn diet (HO PN), or (3) a control diet spiked supplemented with oleic acid oil (OA). At termination, broiler chickens were processed and chicken breast samples of the left pectoralis muscle were collected, and pooled egg samples were collected from layers. Total protein extracts from pooled egg samples and chicken breast samples were subjected to ELISA methods and immuno-blotting analysis with rabbit anti-peanut agglutinin antibodies and rabbit anti-soy antibodies for detection of peanut and soy proteins. Peanut and soy proteins were not detected in all pooled egg samples and individual chicken breast meat samples using immuno-blotting techniques with rabbit anti-peanut agglutinin and rabbit anti-soy antibodies. Moreover, quantitative ELISA allergen detection methods determined that all pooled egg samples and individual meat samples as “not containing” peanut or soy allergens. Therefore, this study helps to evaluate the risk associated with the potential transfer of allergenic proteins from animal feed to the products produced human consumption.

ID : 1007

STARCH DIGESTIBILITY IN BIRDS FED AMYLASE SUPPLEMENTED DIET OVER A 42-DAY STUDY

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The application of amylase has been driven by the idea that newly hatched chicks may be deficient in this enzyme. It could be hypothesized that exogenous amylase supplementation will improve starch digestibility in birds.

The aim of the study was to assess individual variation on starch digestibility of broiler chickens at different ages, with and without exogenous amylase supplementation.

The study was conducted with 120 day-old ROSS 308 chickens raised in individual cages. Birds were randomly and equally assigned to a control treatment (CON) and a amylase-supplemented treatment (CON+AMY). Excreta were collected individually, three times per week (16 collections in total) and at the end of the experiment (day 42nd), birds were killed and ileal digesta was collected.

Amylase supplementation increased ($P<0.05$) amylase activity in duodenal digesta (from 18.7 to 51.6 U/g). The CON+AMY birds were characterised by higher BWG ($P<0.05$) and lower FI and FCR ($P<0.05$). Total tract starch digestibility was improved after amylase supplementation ($P<0.05$) and the average improvement was 0.88 percentage points ($P<0.05$). Irrespective of amylase supplementation, we found individual birds' effect ($P<0.05$) and age effect ($P<0.05$) in starch total tract digestibility. Surprisingly, total tract starch digestibility decreased, as birds got older ($P<0.05$). There was a tendency for that the same birds constantly (from day 7 to day 42nd) exhibited low starch digestibility and this was more obvious for unsupplemented birds (CON). Additionally, we observed lower variation in total tract starch digestibility in birds fed diets supplemented with amylase (CON+AMY).

The results of the trial, suggest that starch digestion is incomplete in birds and could be improved by amylase supplementation. Additionally, it seems that starch total tract digestibility reaches a maximum at day 10, and slowly decreases with age.

ID : 1068

FEEDING BACILLUS SUBTILIS QST713 COULD BE AN EFFECTIVE ANTIBIOTIC ALTERNATE GROWTH PROMOTER FOR BROILER CHICKEN: AN INDIAN CONTEXT STUDY

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The use of antibiotics as growth promoters is one of the major concerns for the Indian poultry industry. Accordingly, the purpose of the experiments was to evaluate the efficacy of *Bacillus subtilis* QST713 (Grobig® BS) as a growth promoter in comparison to two conventional antibiotic agents. The effect on performance, gut health, and immunity in broilers were studied. A total of 400 chicks were randomly divided into four groups of four replicates with each having twenty-five birds. Four treatments are respectively T1 (Basal diet), T2 (*Bacillus subtilis* QST713 1×10^{10} CFU/g @ 50g/ton), T3 (Bacitracin Methylene Disalicylate @250g/ton), T4 (EnramycinHCl @63g/ton). During the first three-week weekly weight gain of the probiotic (T2) group was less than the antibiotic (T4) group but at the end of the sixth week, the bodyweight of T2 was significantly higher than the other groups. The T2 group had the highest weight 1855.20 ± 18.36 g compare to control (1633.46 ± 11.93 g) and antibiotic T3 (1710.09 ± 2.36 g) as well as T4 (1686.56 ± 10.57 g) group. The feed efficiency in *Bacillus subtilis* QST713 (T2) group (1.78 ± 0.06) was significantly (P<0.05) higher than the other groups. *Bacillus subtilis* QST713 in the broiler diet resulted in better production performance, immunity and gut health by favoring the growth of beneficial microbes leading to the development of intestinal morphology. Hence, *Bacillus subtilis* QST713 (Grobig® BS) @ 50g/ton could be an effective alternative to antibiotic growth promoter in broiler chickens.

Reference-

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ID : 1130

DO THE EMULSIFIERS AND CARBOHYDRASES ACT SYNERGICALLY ON PERFORMANCE TRAITS AND INTESTINAL MICROBIOTA ACTIVITY IN BROILERS FED RAPESEED MEAL DIETS?

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Intensive broiler production under sustainable conditions of development enforces greater feed conversion. Successful usage of enzymes in poultry diets is well known, however, no data are available in the literature about the use of emulsifiers and carbohydrase in birds. Presumably, improved fat digestibility decrease the fat content in digesta and, consequently, enhance carbohydrate availability for microbial enzymes.

The objective was to evaluate the possible synergic effect of emulsifiers and carbohydrases on performance traits and intestinal microbiota activity of broilers fed rapeseed meal diets.

During 42 days of a trial, 384 individuals were divided into 4 groups (12 repetitions/group, 8 individuals/cage). Each group was fed by diets containing 5%, 7%, 15% of rapeseed meal (starter, grower, finisher period, respectively) without the addition of enzyme or emulsifier (control group), with the addition of emulsifier or enzyme, and with the addition of both. Bodyweight gain (BWG) and feed intake (FI) data were calculated at the end of each trial period to obtain the feed conversion ratio (FCR). After 36 days of trial 25 birds from each group were euthanized for cecum digesta collection to determine volatile fatty acids (VFAs) content and microbiota enzyme activity.

A greater (P1) than in group with emulsifier and carbohydrases (1,55 [U/g]1), however none of both differ significantly from the control group.

Current trial revealed positive effect of emulsifiers and carbohydrases and their mixture in rapeseed meal diets on performance traits and intestinal microbiota activity in broilers.

ID : 1176

ANTIOXIDANT STATUS OF BROILER CHICKENS IN RESPONSE TO GRADED LEVELS OF DIETARY LEVELS OF MYO-INOSITOL.

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Levels of reactive oxygen species (ROS) above the antioxidant capacity of a cell can cause the oxidation of lipids, proteins and DNA. myo-Inositol (Ins) has been linked to improving the antioxidant status of fin fish and humans. The objective of the experiment was to assess different levels of free Ins in diets on the activity of superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), and the contents of lipid peroxidation (malondialdehyde (MDA)), and protein oxidation (protein carbonyl) in blood plasma in order to understand the interactions Ins and the antioxidant status of broiler chickens. One day old broilers (Ross 308) were randomly allocated to four treatment groups supplemented with different levels of dietary Ins (0 g/kg, 1.5 g/kg, 3 g/kg or 30 g/kg). Each diet was fed to 10 pens as mash from 0-21 days of age, with eight birds per a pen. At day 21, one bird per pen was electrically stunned and blood collected. Antioxidant status of plasma samples were analysed using premade colorimetric kits according to manufacturer's recommendations. Data were analysed using one-way ANOVA with a protected Fisher's least significant difference test to separate means. Graded levels of Ins tended to effect SOD activity in plasma ($P=0.08$), with the highest level of Ins (30 g/kg), significantly reducing the activity compared to the unsupplemented diet ($P<0.05$). There was no significant effect on levels of CAT ($P=0.556$), MDA ($P=0.1$), GR ($P=0.756$) or protein carbonyl ($P=0.544$) due to the treatment diets. In addition, there was a tendency of a numerical decrease of MDA with increasing Ins levels suggesting that Ins may play a role in reducing lipid peroxidation and can protect the lipid membrane of cells from ROS. Further investigation into the role of dietary Ins to improve antioxidant status of broiler chickens is warranted and high inclusion rates of Ins reduce some enzyme activities.

ID : 1178

THE EFFECTS OF MYO-INOSITOL AND PHYTASE SUPPLEMENTATION ON THE BODY WEIGHT OF BROILER CHICKENS REARED TO 21 DAYS AND PRE-CAECAL DRY MATTER AND NITROGEN DIGESTIBILITY.

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The effects of super-dosing phytase (PHY) in poultry studies is well researched, whereby there is an increase of free Ins into the ileal digesta of broiler chickens. Understanding what effect free Ins has on the nutritional value of the feed is important. The aim of the study was to quantify the growth performance to feeding graded levels of myo-inositol and phytase to broiler chickens on the body weight of birds and pre-caecal dry matter (DM) and nitrogen (N) digestibility. The basal mash diet was split into six parts, with each part supplemented either with two levels of PHY (1,500 or 4,500 FTU/kg; Quantum® Blue, ABVista, UK), or with three levels of Ins at 1.5 g/kg, 3 g/kg or 30 g/kg, with one part left un-supplemented. Each diet was fed to 10 pens from 0-21 days, with eight birds per a pen. Four birds per pen were killed by cervical dislocation and ileal digesta removed and dry matter and nitrogen digestibility determined. Results were analysed using one-way ANOVA with a Tukey test to separate means. The effect of diets on body weight of broiler chicks at day 7 and 14 was not significant ($P>0.05$). By day 21, the diets did have a significant effect ($P<0.001$), with the diet containing 4500 FTU/kg having a significant ($P<0.05$) improvement compared to 3 g/kg and 30 g/kg Ins. Inclusions of 30 g/kg of Ins in diets significantly differed from the addition of 3 g/kg Ins, reducing overall ileum DM digestibility by 1.6 % compared to the basal diet. Ins included at 3 g/kg improved DM digestibility compared to the control and there was an overall significant effect of treatments on DM ($P<0.05$). The treatment with 1.5 g/kg of Ins was significantly different ($P<0.05$) from the other treatments, reducing N to 30.8 %. Based on the findings the nutritional value of myo-inositol is based on its inclusion level.

ID : 1215

NUTRITIONAL EFFECTS OF DIETARY INCLUSION OF MANIHOT ESCULENTA CRANTZ LEAF ON BROILER'S GROWTH PERFORMANCE AND HAEMATOLOGICAL PARAMETERS

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Insufficient supply, high prices and competition with the human food and biofuel industries means there is a continuous demand for alternative sources for poultry. As a result, cassava leaves is becoming an increasingly important ingredient in poultry diets, largely due to its high availability (Verot, 1994). This study investigated the effect of Manihot esculenta leaf (MEL) on growth performance and haematological parameters of Cobb500 broilers. A total of three hundred (300) Cobb500 chicks, were used and equally divided into 3 dietary treatments with 4 replications of 25 birds each reared on deep litter system. Treatments diets were :[(FM0 (0% MEL), FM2.5 (2.5% MEL) and FM 5% (5% MEL). During the experimental period, feed intake, feed conversion ratio as well as body weight and weight gain were recorded weekly. The blood samples for Red Blood cell (RBC), and White Blood cell (WBC) analysis were taken in 16 birds (4/replicate) at 6th weeks of age. Statistical analysis was performed by using Graph Pad Prism5. One -way ANOVA was used to evaluate the effects of cassava leaves on growth performance and blood haematological values. Tukey multiple range test was used for testing difference of means. Results showed that feed intake (FI) were higher ($p<0.05$) for FM2.5 and FM5 as compared negative control. Birds in the FM5 group showed the highest feed conversion ratio value ($p<0.05$). Average body weight and weight gain of birds in the FM0 and FM2.5 groups were similar and statistically higher ($P0.05$). The liver, gizzard, heart and intestine weight showed not significant differences among treatments. Except abdominal fat in the FM2.5 and FM5 group who were respectively lowest compared negative control. Hematocrit (%) and lymphocyte rate (%) increased significantly ($p<0.05$) in birds fed 2.5% and 5 % of MEL. While Hemoglobin volume was higher in the group FM5 compared that negative control. In conclusion, Manihot esculenta leaves (MEL) have proven to be a good alternative source of food ingredients for broilers, which can be used up to 2.5% to improve poultry yields.

Key words: Manihot esculenta, broilers, growth performance, haematological

ID : 1230

FIBROUS FEEDS ALTERNATIVES TO OAT HULLS AS A SOURCE OF FEED STRUCTURE IN PELLETTED BROILER FEEDS

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Unground oat hulls (OH) are often added to pelleted broiler feeds to alleviate the lack of physical structure that effectively stimulate gizzard development and, subsequently, improve gut functioning and feed efficiency. Due to its relatively poor availability in certain markets the current study aimed to identify potential fibrous feedstuffs alternatives to OH. A total of 4160 day old Ross 308 chicks were randomly allocated across 80 collective pens (52 birds/pen). Birds underwent a 3 phase feeding program (0-14d; 14-28d; 28-36d) based on a wheat-soya diet. The study followed a complete randomized block design with collective pens as experimental replicates (n=10). The control treatment was formulated following CVB recommendations, whereas for the other dietary treatments 3% of wheat in the control treatment formula was replaced by the corresponding feedstuff (i.e. OH, soya hulls [SH], carob bean [CB], beet pulp [BP], wheat straw [WS], rice hulls [RH] or wheat bran [WB]) thereby applying a certain nutrient dilution relative to control treatment. At the end of each feeding phase, one bird per pen was killed to determine proventriculus and gizzard weights relative to body weight (P+G/BW). At 14 d, 6 birds from each pen were transferred to individual cages and after a 7 d acclimatization a composite excreta sample was analyzed for digestibility determination. Most treatments with fibrous feed supplementation grew to a comparable level than the control treatment despite the dilution applied to the diet. However, BP led to a lower ($P<0.05$) body weight relative to control (2799 g vs. 2858 g) mainly due to a 3 g/d lower daily feed intake ($P<0.05$) compared to control. Regarding feed conversion ratio (FCR), OH resulted in lower ($P<0.05$) and CB in higher ($P<0.01$), FCR than the rest of dietary treatments including control. Also, BP and OH led to a heavier P+G/BW ($P<0.05$) compared to control. Digestibility assessment revealed that the use of OH led to a greater ($P<0.05$) starch digestibility and BP greater ($P<0.05$) fat digestibility than the control treatment. In summary, OH seemed to provide the best performance above that found among the rest of the fibrous feeds tested. However, in light of these results the use of OH, SH, WS, RH and WB partially replacing wheat led to similar or better animal performances than the control treatment and, therefore, can be potentially considered to alleviate the lack of feed structure in broiler feeds.

ID : 1237

IMPACT OF PHYTATE FROM CORN ALONE OR CORN AND SOYBEAN MEAL ON ILEAL INOSITOL
HEXAPHOSPHATE DIGESTIBILITY IN THE PRESENCE OR ABSENCE OF PHYTASE

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Work was done to determine the impact of limestone (LS) and phytate source on ileal inositol hexaphosphate (IP6) digestibility (IP6dig) in the presence or absence of *Buttiauxella* sp. phytase in broilers. A total of 3 treatments (TRT) without phytase were formulated as follow: A) corn and corn germ-based diet without limestone (0.02% Ca, 0.23% phytate-P, PP); B) same as A but with 0.8 mm LS (0.71% Ca, 0.23% PP); C) corn-SBM-based diet with the same 0.8 mm LS (0.77% Ca, 0.23% PP of which 72% came from SBM). Phytase at 1000 FTU/kg was added to diets A-C to produce TRT D, E, and F, respectively. TRT A, B, D and E were used to determine LS and phytase effects, whereas TRT B, C, E and F were used to determine the impact of phytate source (corn/corn germ vs. corn/SBM) and phytase on IP6 digestibility. Broilers were fed the mash diets ad lib for 36 h from 20 to 22 d of age (4 birds/rep, 7-14 reps/TRT based on pre-planned contrasts). At the end of the trial, distal ileal digesta were collected from all birds and pooled by pen. Impacts of LS, phytase source and phytase on IP6dig were analysed by MIXED procedure (SAS 9.4). There was an interaction between phytase and LS ($P<0.05$). In the absence of phytase, 93% reduction on IP6dig was observed with LS inclusion (88.5 vs. 6.5%; $P<0.05$). With 1000 FTU phytase/kg, IP6dig was 98.43%, indicating almost complete degradation of IP6 by the distal ileum when Ca from LS was not included in the diet, compared to 85.1% when LS was added ($P<0.05$). There was no interaction between phytate source and phytase. Phytate source did not impact the IP6dig regardless of phytase. In the presence of Ca from LS IP6dig for both corn (6.5%) and corn/SBM (3.16%) TRT was low without phytase. Phytase significantly improved the IP6dig from 4.8 to 83.3% across the two different phytate sources ($P<0.05$). In summary, inclusion of LS negatively affected IP6dig, with a greater impact on IP6dig when phytase was not included. Phytase at 1000 FTU improved IP6 dig irrespective of phytate source (more focus on phytase effect than phytate source effect)

ID : 1246

IMPACT OF PHYTATE CONCENTRATION FROM CORN ON ILEAL INOSITOL HEXAPHOSPHATE DIGESTIBILITY IN THE PRESENCE OR ABSENCE OF PHYTASE

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Work was done to determine the impact of increasing concentration of inositol hexaphosphate (IP6) from the same source (corn) on ileal IP6 digestibility (IP6dig) in the presence or absence of *Buttiauxella* sp. phytase in broilers. Treatments (TRT) were based on a 4x2 factorial arrangement of 4 IP6 levels (0.57, 0.83, 1.02 and 1.22% (0.16, 0.23, 0.29, and 0.34% phytate-P, respectively), and 2 phytase (0 or 1000 FTU/kg) levels. The TRT were fed for 36 h from 20 to 22 d of age (4 birds/pen, Ross 708 male broiler). By analysis, the total P was 0.20, 0.30, 0.37, and 0.45%, respectively for the 0.57, 0.83, 1.02 and 1.22% IP6 TRT. Unequal replications (reps) of TRT were used based on planned-contrasts. Diets only contained corn, soy oil, vitamins and oils. Different concentrations of corn and corn germ meal were mixed to achieve desired concentration of IP6 from the same grain source. The same limestone (LM) with a geometric mean diameter of 0.8mm was added to contribute 0.67% Ca, so the final diets all contained 0.7% total Ca. Distal ileal digesta were collected from all birds and pooled by pen. Digesta were analyzed for Ca, P, IP6 and Ti (marker). Digested (DigIP6) and undigested IP6 (UndigIP6) were determined based on Ti, ileal digesta, and diet IP6 concentrations. Impacts of IP6 concentration and phytase on digestibility were analysed by MIXED procedure (SAS 9.4). There were interactions between phytate and phytase for IP6dig, DigIP6 and UndigIP6 ($P < 0.05$). In the absence of phytase, IP6dig was 1.22, 6.50, 43.47 and 67.05% for TRT containing 0.57, 0.83, 1.02 and 1.22% IP6, respectively ($P < 0.05$). As a result, of the IP6 in the specific treatments, 0.56, 0.78, 0.58 and 0.40% was undigested with IP6 level increased from 0.57 to 1.22% ($P > 0.05$). Because of the difference in TRT IP6 concentration, even though there was no difference in IP6dig with 1000 FTU phytase/kg, the resulting DigIP6 was increased from 0.48 (0.57% IP6) to 1.05% (1.22% IP6; $P < 0.05$). In summary, with 0.7% dietary Ca, of which, 0.67% was derived from limestone, IP6 digestibility varied considerably depending on dietary IP6 concentration (1.22 to 67.05%) in the absence of phytase. The *Buttiauxella* sp. phytase at 1000 FTU/kg was capable of degrading an average of 84% IP6 irrespective of dietary IP6 concentration.

ID : 1347

EFFECT OF DIFFERENT ADDITIVES ALONE OR IN COMBINATION ON PERFORMANCE PARAMETERS OF BROILERS

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Since the ban of antimicrobials as growth promoters in 2006, several alternatives like feed additives have been used. Among those, short-chain fatty acids, including butyric acid and its salts, and essential oils (EEOO) have been used alone as health and performance boosters in poultry production. The aim of this trial was to evaluate the effect of sodium butyrate, EEOO and their combination on broilers performance. A total of 3000 one day old chicks were distributed in 5 treatments (n=6). The treatments were: T1, Control feed without additives nor antibiotics; T2, Control feed with antibiotics; T3, GUSTOR N'RGY (1 Kg/t of feed), based on 70% sodium butyrate protected with sodium salts of palm fatty acids distillates; T4, EO-Fit Poultry (1Kg/t), based on essential oils combination; and T5, Combination of GUSTOR N'RGY (0.75 Kg/t) + EO-Fit Poultry (0.25 kg/t). All additives were supplied by NOREL, SA. Performance parameters were recorded weekly. On day 21 and 35, one bird in each treatment was euthanized for carcass and intestinal analysis. Data were analysed by one-way ANOVA using the software package SAS 9.0. No significant differences were observed neither in feed intake nor in feed conversion ratio. However, there were significant differences in body weight at 21 days, being the animals in T4 the heaviest (997g a, 1018g ab, 1017g ab, 1020g b and 1001g ab, for T1, T2, T3, T4 and T5, respectively, P=0.028). At 35d, although differences were not significant, animals in T3 and T5 were heavier than the others (2173, 2155, 2188, 2175, 2199, for T1, T2, T3, T4 and T5). The accumulated mortality at 35 days showed also significant differences among treatments (4%a, 6.2%b, 6.0%b, 4.8%ab, 3.0%a, for T1, T2, T3, T4 and T5, P=0.006). Those differences were also reflected in the European Production Efficiency Factor (EPEF) where the combination of the additives achieved the highest EPEF (416xy, 387x, 421xy, 398x, 434y, for T1, T2, T3, T4 and T5, P=0.061). On the other hand, there were no significant differences in carcass weights. At 21d, the length of jejunum and large intestine was longer for T3 followed by T5 (P=0.012 and P<0.001, respectively). At 35d, no differences were found. Both feed additives, GUSTOR N'RGY and EO-FIT POULTRY, and their synergistic combination, have demonstrated improvements on performance, even better results than treatment with antibiotics, that could be partially explained by the better development of the gut in the early stages of life.

ID : 720

EFFECT OF FEEDING THE SACHA INCHI SEED POWDER ON THE OMEGA-3 FATTY ACID CONTENT OF GUINEA FOWL MEAT AND EGG YOLK

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To increase the concentration of omega-3 unsaturated fatty acids in the thigh and breast meat, and in eggs of the guinea fowl, layer feed with 5% sachai inchi seed powder was fed ad libitum to the test group. Measurement of fatty acid content was entrusted to Japanese food research laboratories. The samples were analyzed by gas chromatography after saponification, either by acid decomposition or chloroform-methanol mixed liquid extraction, and methyl esterification. The test birds were slaughtered and dissected after 2 weeks of being fed the sachai inchi feed, and the fatty acid concentration in the meat was measured. Total fatty acid content in the experimental group decreased to 78% in the thigh meat and to 61% in the breast meat compared to that in the control. Saturated fatty acids decreased to 72% in the thigh meat and to 55% in the breast meat. Omega-6 unsaturated fatty acid decreased to 73% in the thigh meat. In contrast, the content of omega-3 unsaturated fatty acids increased by 3.8 times in the thigh meat and by 5 times in the breast meat. The ratios of omega-6 to omega-3 (n-3/n-6 ratio) fatty acid in the experimental groups were 2.6 and 0.3 in the thigh meat and the breast meat, respectively. These ratios were 3.9 times higher in the thigh meat and 7.6 times higher in the breast meat compared to those in the control group. The concentration of fatty acids in the egg yolk was measured after feeding the test group with sachai inchi-supplemented feed for 8 days. The concentration of unsaturated fatty acid was about 1.3 times higher than that of the control group, and the concentration of omega-3 unsaturated fatty acids was about 3.3 times that of the control group. The n-3/n-6 ratio of fatty acids was 0.18 in experimental group and was 3 times higher than that of the control group. In particular, the contents of the omega-3 fatty acids alpha-linoleic acid and docosahexaenoic acid were 6 and 2 times higher than those in the control, respectively.

The above results showed that the addition of sachai inchi seed powder in feed of guinea fowl increased the omega-3 fatty acid content of its egg and meat. Higher omega-3 fatty acid content in guinea fowl products is a factor that would appeal to health-oriented consumers.

ID : 190

EVALUATION OF FEED INTAKE, GROWTH PERFORMANCES, SURVIVAL RATE AND CARCASS TRAITS IN SILKIE CHICKENS WITH ARTEMISIA AND PLECTRANTHUS LEAF POWDER SUPPLEMENTATION IN DIET

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Phytobiotics are regarded as substitutes for antibiotic growth promoters or immune booster in animal feeding. Also, there are a variety of herbal medicines that can be as supplementation in animal feeds, and their effects vary with different species, environments and growth periods. The purpose of this study was to evaluate growth performances and carcass traits in chickens with Artemisia and Plectranthus leaf powder supplementation in the diet. The silkie chickens weighted at hatch and were divided into the control diet group which basic on corn and soybean meal, 2% Artemisia leaf powder supplementation group, 2% Plectranthus leaf powder supplementation group, 1% Artemisia and 1% Plectranthus leaf powder supplementation group or 50 ppm tylosin supplementation group. Each group consisted of four repeats and each repeat included 12 chickens for a total of 320 chickens with ad libitum feed and water. Body weight, feed intake, survival rate, and feed conversion efficiency were collected and measured during the feeding period. Carcass traits, breast meat color, and skin color were determined at sixteen weeks of age. The results showed that there was no significant difference among groups for body weight, feed intake, feed efficiency, heart part ratio, testis part ratio, abdominal fat part ratio, and a value in the breast skin and meat. The survival rate of each group was, respectively, 93.75%、91.67%、83.33%、100% and 97.91%. The control diet group and the 1% Artemisia and 1% Plectranthus supplementation group, which compare to the 2% Plectranthus supplementation group and tylosin supplementation group, had higher dressing rate and L value in the breast skin color and had lower b value in the breast meat color ($P < 0.05$). Taken together, the diet with 2% Artemisia or 2% Plectranthus supplementation had no adverse effects on the performance of growth and carcass traits. Furthermore, a diet with 1% Artemisia and 1% Plectranthus supplementation had beneficial effects on dressing rate and survival rate. The results could be used as a reference for the diet supplementation strategy of silkie chickens.

ID : 703

EFFECT OF DIETARY SODIUM BUTYRATE SUPPLEMENTATION ON PERFORMANCE, THE INTESTINAL MORPHOLOGICAL STRUCTURE AND GUT FLORA IN BROILERS

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Uncoated-buffered synthetic sodium butyrate (SB) can have effect on the upper part of the digestive tract of the broilers. The positive influence of SB on intestinal cells of small intestine could improve the intestinal health of this part of the GIT, feed digestibility and performance. The objective of the present study was to evaluate the effect of dietary supplementation SB on the performance, on the jejunum histomorphology and on the coliform and lactic acid bacteria populations in the proximal part of the GIT of broilers. Day-old male Cobb chicks (144) were allocated in cages in a completely randomized block (8 replicates/treatment). A starter (0-14 d) and a grower (15-28 d) diets were used for dietary treatments: with (SB) or without (CT) the inclusion of 600 ppm of SB additive (54% SB of Dietaxion SAS, France). Birds and feed were weighed weekly individually and by cage, respectively, and the mortality recorded daily. At 14 and 28 days of age, one bird per cage was selected and euthanized. The content of the last part of duodenum was used to examine microbial population (total coliforms and lactic acid bacteria were analyzed as log₁₀ cfu/g). The jejunum was fixed with buffered formalin for histomorphological examination. Villus height (VH) and crypt depth (CD) of each animal was the mean of 5 determinations. Data were analyzed as a 2-way ANOVA. Model included block, SB and their interaction. Cage was considered the experimental unit. There were not significant differences in performance traits, colony counts of total coliforms and of lactic acid bacteria at any time ($P>0.05$). Histomorphological traits VH and CD also were not different between treatments ($P>0.05$) and the same happened at 14 days for the ratio VH/CD (VCR), but at 28 days supplemented broilers obtained a higher VCR than the CT ones ($P<0.05$): 9.42 vs. 7.17 ± 2.06 . These results suggest the evaluated SB could be a good therapy to improve the development of the upper part of the GIT during the first 28 days of growth. Tendency is consistent with a better digestibility of feed, but this must be confirmed in future experiments.

PROTEIN NUTRITION

ID : 102

EFFECTS OF LYSINE SUPPLEMENTATION IN LOW PROTEIN DIETS ON THE BROILERS PERFORMANCE IN THE TROPICAL REGION

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The experiment was conducted to evaluate the effect of lysine supplementation in low crude protein (CP) diets on performance of broiler chickens finisher. Five test diet series were used: 1) the 19% CP diet; 2) the 18% CP diet; 3) the 18% CP diet plus additional amino acids (18.18% total CP) to provide at least as much of the essential amino acids as present in the 19% CP diet; 4) the 17% CP diet and 5) the 17% CP diet plus additional amino acids (17.81% total CP) to provide at least as much of the essential amino acids as present in the 19% CP diet. For each of the five test diets series, additional Lys was added to provide total lysine levels of 1.10%, 1.20%, 1.30%. This resulted in a total of 15 final experimental treatments in a 5 x 3 factorial arrangement. Each treatment was fed to 5 replicate pens of 5 broilers in floor pens from 22-42 days of age. Chicks fed diet containing 17%CP without additional lysine had significantly (P

ID : 291

DIETARY PROTEIN WAS SUCCESSFULLY REDUCED IN TURKEY FEED WHILE BLENDING DIETS WITH WHOLE WHEAT IMPAIRED PERFORMANCE AND ECONOMICS

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Introduction and Objective:

Minimising nitrogen emissions from turkey production systems is a major priority for both the industry and turkey breeding sector. Nitrogen was reduced in diets for male BUT Big 6 turkeys applying various strategies.

Materials and Methods:

Day old turkeys were grown on a six phase feeding program. In phases 1 (P1, weeks 1,2) and 2 (P2, weeks 3-5) all birds received the same feed while in phases 3 to 6 (P3 to P6, weeks 6-9, 10-13, 14-17, 18-21) dietary crude protein (CP) was reduced to varying degrees. CP levels of the control (TRT1) represented German recommendations. CP was reduced by 1% in absolute terms in different phases; for TRT2 in P3 and P4 feeds, TRT3 in P5 and P6 feeds, TRT4 in P3 to P6 feeds while maintaining first order amino acids (AA) lysine, threonine, tryptophan, methionine+cysteine and valine levels across all treatments at levels of TRT1. TRT5 was the same as TRT4 but also isoleucine and arginine were balanced to the same levels at TRT 1. TRT6 was based on TRT5 with unground wheat added at 5, 10, 20, and 15% resulting in additional CP and AA dilution.

Results and Discussion:

After 21 weeks final bodyweights of birds fed TRT 1, 2 and 5 diets were similar while, although not significant ($p>0.05$), bodyweights of TRT's 3 and 4 relative to TRT1 suggesting that ignoring lower order AA is counterproductive in a nitrogen (N) reduction strategy. TRT6 had the lowest bodyweights ($p>0.05$) suggesting further dilution of AA density by addition of wheat limits growth. Breast meat yield (BMV) of TRT 4 and 6 was lower ($p<0.05$) compared to TRT 1 while BMV decreased gradually from TRT 1 to TRT 4. Interestingly, AA balancing in TRT 5 did not allow for complete restoration of meat deposition to the level of TRT 1. Further adjustment of the AA profile might be needed. Foot pad lesion scoring was worst for TRT 1 and 2 while scores for TRT 3 to TRT 5 were better, TRT 6 was better than all other treatments. N-excretion of TRT 6 and TRT 5 was lower than that of TRT 1 and 2 ($p<0.05$).

Conclusion:

Reducing the CP-level of turkey diets resulted in a trend towards lower liveweight and worse FCR compared to birds fed diets with recommended dietary CP however redressing the AA balance, through supplementation with low order AAs, provided a similar response to the control. Reducing CP levels in feeds reduced N excretion. Diluting compound feed with whole wheat impaired performance and economics while it was beneficial for foot pad health.

ID : 370

NITROGEN AND AMINO ACID DIGESTA FLOWS IN BROILERS FED HEAT DAMAGED PROTEIN INGREDIENTS

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Due to an increasing feed-food competition for protein, less digestible protein is expected to be fed to broilers in the future. However, pre-cecal indigestible protein may lead to hind-gut protein fermentation (PF) and the production of potentially health detrimental metabolites. To evaluate effects of PF on gut health, an experimental contrast in ileal N flow is required.

Often, such a contrast is realized by replacing a highly digestible protein source with a less digestible one. However, this does not always increase PF metabolites in the ceca, as other nutrients, such as fibers, may confound the effects of PF. Therefore, our objective was to develop a model that creates a contrast in PF by reducing protein digestibility within ingredients. To this end, we used severe toasting of protein sources and evaluated the effect on ileal nitrogen (N) and amino acid (AA) flows.

Pre-cecal ileal content, excreta and ceca content were collected from 240 broilers fed one of six diets (8 replicate pens) for 30 days. Diets contained 20% soybean meal (SBM), high protein sunflower meal (SFM) or dehulled rapeseed meal (dRSM) as is, or heat damaged by secondary toasting at 136°C for 20 minutes (tSBM, tSFM or tdRSM). TiO₂ (1g/kg) was included as inert marker in diets for the determination of digesta flows. The effect of toasting was evaluated per ingredient separately, using the GLM procedure of SAS 9.4.

Toasting SBM increased the ileal flow of N (851 vs. 744 mg/d) and most of the AA (mean increase 35 mg/d). The daily excreted N (excl. uric acid N) did not differ significantly between SBM and tSBM diets, but excretions of some AA did increase (mean 34 mg/d).

Toasting SFM did not affect ileal flow or excretion of N and AA. Toasting dRSM increased the ileal flow of N (862 vs. 665 mg/d) and all AA (mean 56 mg/day), as well as daily excretion of N (999 vs. 761 mg/d, excl. uric acid N) and AA (mean increase 71 mg/d).

Toasting dRSM, and to a lesser extend SBM, increased the flow of PF substrate into the hindgut of broilers, making these potential models for examining PF. The increase in N and AA from ileum to excreta might be the result of microbial growth. Microbe quantification will be needed to elucidate whether this is the case.

ID : 467

INFLUENCE OF DIETARY METFORMIN ON MUSCLE WEIGHT AND GLYCATION IN TWO TYPES OF CHICKENS.

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Glycation is a non-enzymatic reaction binding amino acids and proteins with glucose and α -dicarbonyl compounds. Glycated amino acids lost the capability to synthesize peptide bonds with other amino acids since these compounds lack the α -amino group. Because chickens have a high blood glucose level compared to mammals, their amino acids might be more converted to glycated amino acids. Glycation in chicken might reduce the efficiency of amino acids utilization because glycated amino acids could not be a precursor of protein synthesis. The attempts to improve the efficiency of amino acids utilization by inhibiting glycation have not been investigated. Here we show that metformin (MF), which is an anti-glycation reagent, feeding increase the muscle weight in layer and broiler chickens. Dietary MF increased the mass of the pectoralis major muscle in both layer and broiler chickens. The plasma N ϵ -(carboxymethyl)lysine (CML), as an index of glycation, levels were decreased in layer chickens but were not changed in broiler chickens. The plasma concentration of precursors of CML, α -dicarbonyl compounds, were increased in layer chickens fed the diet including MF but were not affected in broiler chickens. In addition, dietary MF supplementation significantly increased the plasma concentrations of amino acids in layer chickens. On the other hand, there were no significant differences observed in the plasma concentrations of amino acids among any groups of broiler chickens. Our results suggest that anti-glycation reagent might have the potency to increase muscle production in chickens. However, it has not been clarified the mechanism how MF increases muscle weight. Further investigation should be conducted in the future.

ID : 688

COMBINATION OF FUNCTIONAL AMINO ACIDS CAN RESTORE PERFORMANCE OF BROILERS FACING COCCIDIOSIS

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In broiler chickens, coccidiosis is caused by the infection of *Eimeria* species which colonize different parts of the intestinal tract. Coccidiosis condition decreases feed intake and growth and is a predisposing factor of necrotic enteritis further impairing performance. Literature reports that amino acids such as arginine, threonine, glutamine and glutamate, based on different modes of action (increased immunoglobulins and mucins, decreased inflammation, reduced oocyst shedding) could exert beneficial effects in broilers facing this challenge. In addition, glycine and tryptophan seem to be particularly critical for growth and stress response in broilers chickens, respectively. However, the effect of the supplementation of these amino acids, when combined, has been poorly investigated in this context.

Nine hundred sixty newly hatched male chicks (ROSS 308) were allocated to six different treatments (10 repetitions of 16 birds per treatment) for 35 days. At day 14, all birds were artificially challenged with a Paracox vaccination 10x dose by oral gavage to induce coccidiosis. Basal diet was formulated following Ajinomoto recommendations. For all except one treatment (Infected Untreated Control – IUC) birds received one of the following supplementation: 80 g/ton of the anticoccidial drug Maxiban G160 (Infected treated Control – ITC) or 4 kg/ton Glutamine + Glutamate + Arginine (MIX) or MIX + 1 kg/ton Threonine (MIX+THR) or MIX + 1 kg/Ton Glycine (MIX+GLY) or MIX + 0.5 kg/ton Tryptophan (MIX+TRP). Performance was followed. Oocyst counting and classification in feces were performed at day 21. All data were subjected to an ANOVA and differences among mean were detected by multiple range tests.

All treatments, except the one receiving Maxiban, exhibited high counts of *Eimeria tenella* in feces. Compared to ITC birds, IUC birds had a lower body weight gain (-3.7%), a higher FCR (+2.6%) with a similar feed intake during the trial. The supplementation of the amino acid combinations increased performance to the level of ITC birds without affecting the counts of *Eimeria*, MIX+THR being the most efficient combination of amino acids.

This study reported that supplementation of a selected combination of functional amino acids restored fully performance of coccidiosis-challenged birds, at the same level as the coccidiostat drug. This mode of action does not rely on a direct effect on oocyst and remains to be determined.

ID : 707

IS THE BROILER RESPONSE TO DIETARY VALINE MODULATED BY THE SAME BRANCHED-CHAIN AMINO ACIDS AS IN PIGLETS?

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Branched-chain amino acids (BCAA), that include valine (Val), leucine (Leu) and isoleucine (Ile), share unique catabolism pathway, leading to strong metabolic interactions. Excess of dietary Leu was first shown in piglets to stimulate the degradation of all BCAA including Val and Ile, with negative consequences on feed intake and growth. The aim of this study was thus to quantify and compare the response of broilers and piglets to dietary Val and the impact of others BCAA on this response thanks to a meta-analysis approach. The databases were composed of 23 articles published between 1999 and 2018 including 44 experiments and 239 treatments for broilers, and 16 articles published between 2001 and 2018 including 23 experiments and 126 treatments for piglets. Multiple regression models were fitted with the MIXED procedure of Minitab software with experiment as random effect. The Y variables were average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). For broilers, the Y variables were expressed in percentage of the highest-level intra-experiment to make the values comparable between experiments (different ages and genetic lines). The main X variable was the dietary standardised ileal digestible Val level (ValSID) and the other ones were dietary SID Leu and Ile (LeuSID and IleSID, respectively). The response of ADG and ADFI to ValSID was linear and quadratic for broilers and piglets (Broilers, ADG: $P < 0.001$, $R^2=0.65$, ADFI: $P < 0.001$, $R^2=0.50$; Piglets, ADG: $P < 0.001$, $R^2=0.93$, ADFI: $P < 0.001$, $R^2=0.97$). Results showed that increasing dietary LeuSID reduced ADG and ADFI in broilers and piglets ($P < 0.05$) but also that the response to ValSID was stronger with high LeuSID levels for these 2 species (Interaction ValSID×LeuSID: Broilers, ADG and ADFI: $P < 0.001$; Piglets, ADG and ADFI: $P < 0.05$). The response of ADG and ADFI to ValSID was not modified by IleSID. The FCR responded linearly ($P < 0.001$) and quadratically ($P < 0.001$) to ValSID, while LeuSID and IleSID have no effect on this response for both species. In conclusion, the current study showed that ADG and ADFI but not FCR response to ValSID is modulated by dietary LeuSID level. These results indicate that Leu is the most important regulator of BCAA metabolism that works similarly in broilers and pigs. This quantification will facilitate the decision-making on amino acid formulation constraints and the implementation of low protein diets for these two species.

ID : 782

EFFECTS OF L-ARGININE AND L-CITRULLINE SUPPLEMENTATION IN BROILERS FED REDUCED PROTEIN DIETS UNDER CYCLIC WARM TEMPERATURE

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This study aimed to determine the effects of L-arginine (Arg) and L-citrulline (Cit) supplementation on performance, carcass traits and gut permeability of broilers fed reduced protein diets and housed at thermo-neutral or cyclic warm temperature. One-day-old Ross 308 male birds (n = 720) were fed a common starter diet until d 7, and treatment diets during grower (d 8 to 21) and finisher (d 22 to 35) periods. On d 8, birds were weighed and randomly allocated to one of four dietary treatments with 12 replicates of 15 birds per pen. Dietary treatments were: normal protein (NP), reduced protein (RP) deficient in Arg, RP supplemented with Arg, and RP supplemented with Cit. Equivalent levels of Arg and Cit were added in grower and finisher diets at 0.29% and 0.28%, respectively. The difference in crude protein levels between NP and RP diets was approximately 25 g/kg. From day 22 to 35, the temperature was cycled up to 33°C ± 1°C for 6 hours per day in one room while the other room was continuously set at 24°C. On d 28, two birds per pen were selected, marked and inoculated with 1ml dose of Fluorescein isothiocyanate dextran (FITC-d), a leaky gut marker, to measure the gut permeability. Data from d 22 to 35 were analysed to show the main effect of temperature, treatment and their interaction. Dietary treatment × temperature effect was not observed for performance variables (P > 0.05). Regardless of temperature, birds fed Arg-deficient RP diets showed reduced weight gain and 2.3 points higher FCR compared to those fed NP diets (P < 0.05). Feeding Arg-deficient RP diets resulted in lower thigh and drumstick, and higher fat pad yields at d 35 compared to NP diets (P < 0.05). Supplementation of Arg in RP diet improved body weight gain and feed efficiency whereas Cit only improved feed efficiency (P < 0.05). Reduced protein diets supplemented with Arg or Cit improved carcass traits which were similar to those fed NP diets (P < 0.05). Regardless of the dietary treatment, birds exposed to the cyclic warm temperature showed reduced feed intake, body weight gain, and impaired gut permeability (higher serum FITC-d level) compared to those housed at thermo-neutral temperature (P < 0.05). In conclusion, broilers perform poorly in Arg deficient reduced protein diet and the supplementation of Arg or Cit in reduced protein diet helps to improve the performance of broilers. Further research on the effect of Arg and Cit during heat stress (temperature > 35°C) is warranted.

ID : 812

VALINE REQUIREMENT OF FEMALE BROILERS IN STARTER PHASE

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Valine is the 4th limiting amino acid in growing chicks, and deficient of the amino acid in diet, impair growth performance. So, the purpose of this study was estimate the digestible valine to lysine ratio in practical diets of starter female Cobb-500 broiler chicks. A total of 540 female broiler chickens of the Cobb-500 from 8 to 21 days of age, were distributed in a completely randomized design with 6 treatments and 6 replicates of 15 birds each. A basal diet (based on corn-soy bean meal) was formulated to provide of all nutrients according to Rostagno et al. (2011), with the exception of valine level. The basal diet contained 2,991 kcal AMEn/kg, and L-glutamic acid was used to make it equal in CP at 19% of the diet. To avoid excessive lysine, 90% digestible lysine (1.05%) was used. To obtain the different valine to lysine ratio in treatment diets, crystalline L-glutamic acid in basal diets was gradually replaced by crystalline L-valine, and six different ratios of digestible valine (% of lysine) of 70, 75, 80, 85, 90, and 95% were evaluated. To evaluate valine requirement of female broiler chicks based on performance parameters, body weight and feed intake were recorded at the beginning and end of the experiment, and the FCR was calculated. The result of this experiment showed that body weight gain and FCR were significantly affected by the valine ratios in basal diet (P

ID : 824

EFFECT OF AMINO ACID LEVELS DURING BROILER BREEDER PULLET REARING ON REPRODUCTIVE PERFORMANCE AND FEATHERING UP TO 65 WK

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Dietary amino acid (AA) concentration during pullet rearing affects growth, development, and egg production. This experiment was conducted to determine the optimum AA levels for broiler breeder pullets from 5 to 24 wk of age to obtain the best egg production, hatchability, fertility, and feathering condition. Four AA levels were evaluated in the grower (2,700 kcal/kg ME) and developer (2,800 kcal/kg ME) phases. These diets contained balanced protein with digestible (dig) Lys of 0.48, 0.54, 0.60, and 0.66% for grower, and 0.51, 0.57, 0.63 and 0.69% for developer. Diets were formulated according to Cobb recommendations and ideal protein profile for each feeding phase. A total of 1,360 Cobb-500 slow-feathering pullets and 288 Cobb MV males were placed in an enclosed fan-ventilated 32-pen litter floor rearing house with 16 pens for females (85 pullets/pen) and 16 pens for males (18/pen). All pullets and males were fed one common starter diet in crumbles up to 4 wk. Grower diets were fed from 29 d up to 15 wk and developer mash feeds from 16 wk to 5% egg production. Feed amounts varied slightly (± 3 g/d) among treatments in the developer phase to maintain BW close ($\pm 2\%$) to Cobb guideline (2018). Females and cockerels were fed in a 6/1 and 5/2 feed restriction program. At 21 wk, 7 males and 65 females were moved to 16 slat-litter floor laying pens, photostimulated with 14 h of light, and fed separately. After 5% egg production all hens were fed two common mash layer diets. Feed increments and reduction post-peak were made according to egg production by treatment. Egg production and mortality was recorded daily. From 27 to 65 wk, 180 eggs/pen were incubated every 2 wk, and 30 eggs/pen used for egg quality evaluation. Data was analyzed in a completely randomized design with 4 AA levels and 4 replicate pens each. ANOVA and regression analyses were conducted. Dietary AA levels during rearing had a consistent quadratic effect ($P < 0.05$) on hen-wk (%) and total hen housed egg production indicating that the optimum level of dig Lys for grower and developer phases were 0.58% and 0.61%, respectively. The best average hen housed egg production observed up to 65 wk was 176.5 eggs and the worst 153.7 eggs. Eggshell strength and elasticity were affected ($P < 0.05$) by treatments up to 46 wk of age. Fertility was not affected ($P > 0.05$) by treatments, but hatchability increased linearly ($P < 0.05$) as AA during rearing increased up to 50 wk and decreased linearly during the last 15 wk of egg production. Feathering got worst after 52 wk and AA during rearing affected feathering and fleshing scores during lay in a quadratic ($P < 0.05$) manner. Feathering was related more to aging and fleshing than to AA levels during rearing. In conclusion, the optimum AA levels for the grower and developer phases are related to balanced protein with 0.58% and 0.61% dig Lys, respectively.

ID : 898

EFFECT OF ENERGY AND PROTEIN LEVEL UNDER STANDARD OR HEAT STRESS CONDITIONS ON GROWTH PERFORMANCE AND THE INCIDENCE OF MEAT MYOPATHIES

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Energy and amino acids (AA) are the two nutrients with the largest contribution to the broiler feed price, animal performance and ultimately meat myopathies occurrence. However, the response of these nutrients on broiler performance is influenced by environmental conditions such as temperature and humidity. Therefore, a trial was conducted to determine the effect of dietary energy and digestible AA on growth performance, carcass yield and meat myopathies of broilers raised under recommended or heat stress (HS; high temperature and humidity) conditions. A total of 4160 broiler Cobb 500 were randomly assigned to 80 pens (52 broilers/pen, 50:50 males and females). Twelve treatments were arranged in a completely randomized block design using a 2 x 3 x 2 factorial arrangement with 2 dietary energy densities (AMEn; high vs. low), 3 energy to digestible lysine (dLys) ratios (low vs. medium vs. high) and 2 environmental conditions (Control vs. HS) and their interactions as main effects. Broilers were fed during 4 feeding phases (d0 to d 15; d15 to d29; d29 to d37; and d37 to 48d). Dataset of growth performance, carcass and breast meat yield and meat myopathies were analyzed using MIXED and GENMOD procedure of SAS, respectively. Broilers raised under HS had 6% lower feed intake (FI) and 5% lower average daily gain (ADG; $P<0.05$) while feed conversion ratio (FCR) was not affected. Broilers fed with high AMEn/dLys (low Energy to dLys ratio) increased market weights ($P<0.1$), reduced FI ($P<0.1$), and improved FCR ($P<0.05$). Broilers fed high levels of energy had lower FI and better FCR ($P<0.05$). Carcass and breast meat yield increased with high dLys levels ($P<0.05$). Broilers growth under Control or HS responded similarly to energy and protein changes. Broilers fed low energy diets tended to reduce incidence of severe scores of white stripping whereas increased the incidence of poor cohesion meat. Wooden breast incidence was the highest with low AMEn/dLys (high dLys). It was concluded that the growth performance of the broilers raised under HS conditions performed worse than the birds raised in standard conditions independently of the energy and dLys level tested. . In addition, the best growth performance, as well as the highest carcass and breast meat yield were obtained at low AMEn/dLys, disregarding the environment. Meat myopathies were affected by the energy and amino acids level being their incidence somewhat predictable.

ID : 1113

THE RESIDUAL ANTINUTRITIVE POTENTIAL OF SOYBEAN PRODUCTS AFFECTS THE ESTIMATION OF STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY BY MODULATING ENDOGENOUS PROTEIN SECRETION

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Soybeans are rich in trypsin inhibitors (TI), which impair protein utilization and thus animal performance. Hence, TI have to be reduced by heat and pressure. But a recent study has shown, that different processing techniques result in vast differences in qualities (Hoffmann et al., 2019). In this study, we examined how these quality differences in terms of trypsin inhibitor activity (TIA) affect the estimation of endogenous amino acid (AA) losses by linear regression extrapolation and, hence, the estimation of standardized ileal AA digestibility from these products.

For this experiment 45 soybean cakes (SBC) with a vast range of TIA (0.3- 23.6 mg/g) were created using four different processing techniques (thermal, hydrothermal, pressure and kilning. These soybean cakes were then merged into a basal diet at two inclusion levels (15%, 30%). In this way 91 experimental diets (one basal diet as control) were designed. All diets contained 0.5% of titanium dioxide as indigestible marker. 5,490 1-day-old male broiler chickens (Ross 308) were used in four consecutive experimental trials. From d1 to d14 the birds were fed a commercial starter diet. On d15 the birds were allocated to pens with 10 birds each and the 91 experimental diets were fed ad libitum. On d22 the broilers were euthanized and the digesta of the terminal ileum was collected for determination of amino acid digestibility. For each SBC a digestibility coefficient (DC) was calculated. DC were analyzed using linear regression models defining TIA as main factor.

DC of every single AA declined significantly ($p < 0.001$) in a linear way with increasing dietary TIA. The depressive effect of TIA differed between single AA. Arg was the least affected AA with DC ranging from 44.8% - 96.5%. Digestibility of sulfur-containing AA was impaired most. Each increase of dietary TIA by 1mg/g reduced DC of Met and Cys by 2.36% and 2.31%.

This experiment showed that TIA has a linear effect on protein utilization, which means the presence of anti-nutritional substances must be considered using linear regression approach when estimating standardized ileal AA digestibility.

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ID : 1147

EFFECTS OF DIETARY ENERGY AND PROTEIN LEVELS ON BROILER PERFORMANCE, CARCASS QUALITY AND WATER INTAKE

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Dietary protein and energy are the most expensive nutrients in broiler diets and their optimal supply must be continuously updated for modern broiler genetics. In addition, lowering dietary crude protein (CP) is well recognized to improve nitrogen efficiency, animal health and welfare.

In total, 1248 Ross 308 male broilers were allocated to six dietary groups varying in metabolizable energy (ME) and CP content, with 5 to 6 replicates per group. Birds were fed a common starter diet from 0 to 10 days. The control group (T1) contained 3000 kcal/kg ME, 20% CP and 1.05% digestible Lysine (dLys) in grower feed (10-21d) and 3050 kcal/kg ME, 18.5% CP and 0.98% dLys in finisher feed (21-35d). For T2 and T3, dietary ME was increased (3100 & 3200 kcal/kg in grower; 3150 & 3250 kcal/kg in finisher) with the same levels of dietary CP and digestible amino acids (dAA). From T4 to T6, the level of CP was decreased by 1% point per group in grower and 0.8% point per group in finisher (19, 18 & 17% in grower; 17.7, 16.9 & 16.1% in finisher) with the same levels of ME and dLys than T1, and with dAA kept above recommended levels for optimal performance. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were recorded. Broilers were slaughtered at 35 days to measure carcass yield (CY), breast meat yield (BMY) and abdominal fat yield (AFY) for 40 broilers per group. Water intake was recorded and water to feed ratio calculated.

Increasing ME supply reduced ADFI from 0-35d, especially for T3 (111; 112 & 109 g/d for T1, T2 & T3) and in turn improved FCR (1.470, 1.457 & 1.428) but linearly reduced BMY and linearly increased AFY. No effect of dietary ME on ADG, CY or water intake was observed. Broilers fed lower dietary CP showed similar ADG, ADFI and FCR whatever the dietary CP content. Severe dietary CP reduction (T6) resulted in lower CY (72.0 & 71.7 in T1 & T6) and BMY (24.0 & 22.5 in T1 & T6). Dietary CP reduction also linearly increased AFY and linearly reduced water intake and water to feed ratio, an indicator of litter quality. The present study confirms that broiler may benefit from higher supply of energy and lower supply of protein, and that it is dependent on production criteria to optimize: performance, yields or welfare indicators.

ID : 1202

THE INCLUSION RATE OF FREE AMINO ACIDS IS NOT A LIMITATION TO DECREASE THE PROTEIN LEVEL IN BROILER DIETS

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The reduction of dietary crude protein (CP) is a major issue for sustainable poultry production from economic, environmental, and societal reasons. This requires the use of free amino acids (AA) added to diets to ensure a sufficient supply of indispensable AA. However, broiler performance is not systematically maintained when the CP content in the diet is drastically reduced, even when the indispensable AA requirements are apparently met. The aim of this study was to investigate whether the form of the provision of AA (i.e. as protein or as free AA) would limit performances in growing-finishing broilers. Thirty percent of the dietary CP fraction was provided by a highly digestible protein (casein) and/or a mixture of free AA whose digestible amino acid profile was exactly the same as that for casein, using graded levels of free AA inclusion in replacement of casein. Chickens (ROSS PM3) received one of the seven experimental diets (n=30 per treatment) during the whole experimental period (from d14 to d32) and were slaughtered at d32. Diets were formulated to be sub-limiting in digestible lysine (dLys) at 0.9%. The other AA were provided at levels to meet or exceed the recommendations, so that no AA other than Lys would limit growth. The AA digestibility and apparent metabolisable energy content did not differ between treatments. Increasing the level of free AA from 0 to 30% of the total CP content (i.e. 0.15 to 5.4% inclusion of free AA in the diet) resulted in reduced growth only in the diet with 30% free AA. The FCR was also degraded when the AA incorporation rate was greater than 20% free AA (i.e. 3.8% free AA in the diet). Chickens were able to adapt their digestive physiology (morphology and transporter expression) to increasing provision of free AA, at least for levels lower than 3.8% in the diet. Beyond this level, the absorption capacity may be affected, limiting the use of nutrients by the animal, which could partly explain poorer performance. In practice, the use of AA in free form should not be a limiting factor for the reduction of the protein content since the quantities for formulating low CP diets do not exceed 2% free AA in broilers.

ID : 1304

EFFECT OF NUTRITIONAL MANIPULATION ON THE JUVENILE LOCAL KEETS (*NUMIDA MELEAGRIS*)
RAISED IN TOGO

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Traditional guinea fowl farmers use grains, agricultural by-products and termites without limit in the diets of the birds. This practice is inadequate for sustainable guinea fowl production as it provides insufficient or excess energy and protein. Reproductive performance in poultry is strongly linked to juvenile development. The most crucial and exigent period for rearing guinea fowl with energy and protein intake is early stage of growth (Lombo, 2018). The purpose of this study was to assess the effects of variation in energy and protein intake on local keets. A total of 600 local keets were allocated to 5 treatment groups (control, low-protein, high-protein, low-energy and high-energy) with 4 replicates of 30 keets each. Feed was offered ad libitum and feed intake was recorded daily. The birds were weighed weekly and mortalities were recorded. At 8 week, blood sample were collected for blood count and biochemical analysis. The data were processed by Graph Pad Prism 5.00.288 software and the ANOVA test was used to analyze the results. The means were compared by the Tukey HSD test and the probability of $P < 0.05$ was considered as the significance threshold. The results showed that the birds fed high-energy diet consumed less feed at all ages than those in other treatment groups. At 5 weeks onwards, body weights of keets fed low-energy and high-protein diets increased ($p < 0.05$) more rapidly than those in control, high-energy and low-protein diets. Keets fed high-energy diet had a lower average daily gain than those in control, low-energy, high-protein and low-protein diets. Feed conversion ratio was similar across treatment groups. Keets fed high-protein and low-energy diets had less mortality. Blood parameters were not affected ($p < 0.05$) by feed treatments. However, there was a significant difference ($p < 0.05$) in the hematocrit according to feed treatments. This experiment showed that low-energy and high-protein diets improved body weights, average daily gain and reduced mortality of the birds.

Key words : Protein, energy, juvenile, local keets, Togo.

ID : 1372

INTERACTIVE EFFECTS OF DIETARY METHIONINE AND SELENIUM SOURCES ON BROILER GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND MEAT QUALITY

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Broiler meat quality has shown to be affected by dietary methionine (Met) and also selenium (Se). However, the combined effect of both dietary Met and Se sources on broiler meat quality has not been previously investigated. Hence, this research focused on the effect of DL-Methionine (DL-Met) and DL-methylthio-butanoic acid (DL-HMTBA) in combination with Na selenite and 2-hydroxy-4-methylselenobutanoic acid (HMSeBA) on broiler growth performance, carcass characteristics and fresh breast and thigh meat quality and Se content. A total of 320 day-old, straight-run Cobb 500 broiler chicks were randomly allotted to 4 treatments following a randomized complete block design arranged as a 2 × 2 factorial. Factors were Met source (DL-Met and DL-HMTBA) and Se source in the mineral premix (Na selenite and HMSeBA). There were 8 replicate cages per treatment with 10 broilers in each cage. Broilers and feed refusals were weighed to calculate for ADG, ADFI and F/G. Meat quality of fresh breast and thigh meat were evaluated for pH, objective color, water-holding capacity, lipid oxidation, and Se content. The data were analyzed using the MIXED Procedure of SAS with cage as the experimental unit. There was a significant Met × Se source interaction for water-holding capacity (WHC) wherein the WHC of thigh meat was greater ($P=0.016$) if HMSeBA is used in DL-Met diets but lesser in DL-HMTBA diets. Broilers fed diets with DL-Met had greater feed/gain ratio (F/G) than those fed DL-HMTBA diets ($P=0.009$). Deboned thigh yield was greater by 5% ($P=0.031$) in broilers fed DL-Met diets than with DL-HMTBA. Consistently, the DL-Met fed birds had greater breast meat L^* ($P=0.011$) and thigh meat pH 0h ($P=0.027$) and thigh meat pH 5h post mortem ($P=0.002$) than the DL-HMTBA. Na selenite elicited a 14% increase ($P=0.037$) in abdominal fat than the HMSeBA. Breast meat color, b^* was greater ($P=0.025$) in HMSeBA fed broilers than the Na selenite. Lastly, selenium content in breast and thigh meat was greater ($P<0.001$) by 62% and 37%, respectively in HMSeBA fed broilers than Na Selenite. In conclusion, the dietary Met and Se source had independent effects on growth performance, carcass characteristics and meat quality of broilers. The WHC of thigh meat was greater if HMSeBA was used in diets containing DL-Methionine. Na selenite can be used as the Se source in DL-HMTBA diets without affecting the WHC of meat. HMSeBA improved the Se content of both the breast and thigh meat from broilers.

ID : 1406

EFFECT OF SUPPLEMENTAL AMINO ACIDS TO LOW PROTEIN DIETS ON PRODUCTION PERFORMANCE, EGG QUALITY AND SERUM BIOCHEMICAL INDICES OF VANARAJA LAYING HENS

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Among the nutrients needed by the bird, protein is one of the most important nutrient, which has a major influence on growth, feed utilization and production performance. The present study was conducted to study the effect of supplemental amino acids to low protein diets on production performance, egg quality and serum biochemical parameters of Vanaraja laying hens (32-48 weeks). A total of 120, Vanaraja laying hens were randomly distributed into four treatment groups with three replicates of 10 birds in a complete randomized design. Four different diets were formulated containing different levels of protein (13.5, 15, 16.5 and 18% CP) with similar energy (2600 ME kcal/kg). Lysine (0.75%) and methionine (0.35%) levels were kept constant in all the diets. Each dietary group were offered a measured quantity of feed daily. The egg production was recorded daily on individual pen basis. The eggs laid by the bird on individual pen basis during the last five days of each four weeks period were collected to measure the egg weight. Twelve eggs were randomly chosen from each dietary treatment from the eggs laid on 44th week to measure egg quality. At 48 weeks of age, about 2 ml of blood was collected from the wing vein of 12 birds (four birds from each replicate) per treatment. Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance and means of different treatments were compared with Duncan's multiple range tests. The hen-housed egg production, egg weight, egg mass per day and feed efficiency were not influenced by variation in CP contents of the diet. There was no influence of dietary protein levels on egg quality parameters like albumin %, Yolk %, Haugh unit and eggshell thickness except egg shell %. Significantly higher egg shell percentage was noticed in 16.5% and 18% CP diet ($P < 0.05$) compared to either 13.5 or 15% CP diet. Serum biochemical parameters like total protein, globulin, cholesterol and creatinine, alkaline phosphatase (ALT) concentrations were not influenced by dietary protein levels. It is concluded that diet containing 13.5 % CP, 0.75 Lysine and 0.35% methionine is adequate for optimum production performance and egg quality of Vanaraja laying hens during 35 to 46 week of age.

ID : 151

OPTIMAL METHIONINE PLUS CYSTEINE REQUIREMENT IN BROILER STARTER DIETS SUPPLEMENTED WITH L-METHIONINE

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Methionine (Met) is the first limiting amino acid in broilers and together with cysteine (Cys) are two crucial amino acids to be met correctly. Methionine is supplemented in feed in powder form i.e. DL-Met and L-Met, or in liquid form (DL-Hydroxy analogue). The sulphur amino acid (SAA) requirements of broilers are defined in DL-Met supplemented diets. The objective of this trial was to investigate the optimal SAA requirement of broilers in the starter phase (first 10 days of life) in diets supplemented with L-Met. A basal diet containing 1.28% standardized ileal digestible (SID) Lys and 0.60% SID L-Met plus Cys (50% Met and 50% Cys; deficient in SAA) was created. L-Met was added to the basal diet to reach 0.65, 0.70, 0.75, 0.85, 0.95 and 1.05% SID L-Met plus Cys (6 replicates per treatment and 15 animals per replicate). The body weight (BW), daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) were measured. Additionally, feed samples of the test diets were analysed to determine major nutrients including amino acids which appeared to be at or close to the formulated values. After 10 days on the test diets, significant differences ($P < 0.05$) were observed in all measured parameters. The optimal L-Met plus Cys requirements were determined using linear broken line and exponential asymptotic models. Model fit was evaluated based on the R^2 of individual observations and graphical interpretation. The linear broken line model showed the best fit for BW, DWG and DFI. The models based on FCR showed a poor fit. Based on the fitted models, the optimal SID L-Met plus Cys level was found to be 0.69-0.70%. This new value is 20% lower compared to the recommendations of NRC and Ross 308 (0.90% and 0.94% SID Met plus Cys, respectively), which are defined based on DL-Met. Performance of broilers based on Ross 308 at day 10 of age is 321g BW. Herein, chickens at the requirements for the maximum performance needed 20% lower SID Met plus Cys while performing similarly (326 g BW). In conclusion, the lower Met plus Cys requirements might be due to the higher bioavailability of L-Met compared to DL-Met. Moreover, nowadays broilers are receiving lower crude protein diets, no AGPs, and have no access to animal protein sources. Hence, these changes in broiler feeding might be associated with the lower Met plus Cys requirements in trials based on L-Met as compared to the conventional requirements which has been based on DL-Met.

ID : 154

OPTIMAL METHIONINE PLUS CYSTEINE REQUIREMENT IN BROILER GROWER DIETS SUPPLEMENTED WITH L-METHIONINE

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Methionine (Met) is the first limiting amino acid in broilers. The requirement of Met together with cysteine (Cys) must be met correctly. Most of sulphur amino acid (SAA) requirement studies are defined with DL-Met supplemented diets. The objective of this trial was to determine the optimal SAA requirement of broilers in the grower phase (10-23 days of age) in diets supplemented with L-Met. A basal diet containing 1.15% standardized ileal digestible (SID) Lys and 0.55% SID Met plus Cys (50% Met and 50% Cys; deficient in SAA) was created. L-Met was added to the basal diet to reach 0.60, 0.65, 0.70, 0.80, 0.90 and 1.0% SID Met plus Cys (6 replicates per treatment and 15 animals per replicate). All birds were fed the same commercial starter diet during the first ten days of life meeting Ross 308 recommendations. The body weight (BW), daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) were measured. Additionally, feed samples of the test diets were analysed to determine major nutrients including amino acids which appeared to be at or close to the formulated values. The optimal Met plus Cys requirements were determined using linear broken line and exponential asymptotic models. Based on the R² of individual observations, the linear broken line model showed the best fit for BW, DWG and FCR. The models based on DFI showed a poor fit. The optimal SID Met plus Cys level was found to be 0.66% based on BW and 0.62-0.63% based on FCR and DWG. These new requirement values are more than 20% lower than the recommendations of NRC and Ross 308 (0.90% and 0.87% SID Met plus Cys, respectively), which are defined based on DL-Met. The suggested body weight of broilers for Ross 308 at day 23 of age is 1142g. In the present study, the comparative performance of the broilers (1191g BW) at day 23 of age was achieved with 20% lower Met plus Cys as compared to the standard recommendation. Herein, L-Met supplemented diets fed to broilers in grower phase caused a lower SID Met plus Cys requirements compared with the NRC and Ross 308 recommendations which are defined mostly using DL-Met based literature. Nevertheless, authors cannot exclude other confounding factors impacting the SID Met plus Cys requirement in broilers.

FEED TECHNOLOGY

ID : 51

IN VITRO INHIBITORY EFFECT OF A NEW THREE-STRAINS SELECTED PROBIOTIC ON SEVERAL SEROVARS OF SALMONELLA

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Bacillus are known to be capable of producing antimicrobial substances including e.g. bacteriocins which may be able to inhibit Gram-negative bacteria such as Salmonella (Abriouel et al., 2011). Reduced Salmonella colonization in liver and cecum has also been observed in Bacillus subtilis fed birds challenged with Salmonella Heidelberg (Hayashi et al., 2018) and Salmonella Enteritidis (Knap et al., 2011). The objective of the study was to evaluate the in vitro inhibitory effect of a new three-strain selected bacillus-based probiotic for poultry (GalliPro® Fit) on several Salmonella serovars using two different methods. The Salmonella enterica serovars tested were Albany, Braenderup, Enteritidis, Gallinarum, Heidelberg, Infantis, Kentucky, Mbandaka, Minnesota, Muenchen, Schwarzengrund, Stanley, Tennessee and Typhimurium.

The first method was an agar plate-based method. An agar plate was poured with pathogens on it. Wells were created into the plate. The bacillus-based probiotic was added to selected wells. After 24 hours of incubation, a zone of inhibition of up to 2 mm could be measured digitally.

The second method was a feed matrix-based methodology. The feed was autoclaved, then the probiotic was added at 10^5 CFU/g of feed. The pathogens S. Heidelberg or S. Typhimurium were added at 10^3 CFU/g of feed. During the 24 hours' time of incubation, samples were taken at different time points (4, 8 and 24 hours). Samples were added to plates, incubated and a counting of pathogens was conducted.

In these in vitro experiments the new bacillus-based probiotic was able to inhibit the growth of all Salmonella serovars belonging to different serogroups. The new bacillus-based probiotic inhibited type B, C, C1, C2, D Salmonella serogroups. When testing the probiotic vs. Salmonella spp. in an agar-based method, a radius of the inhibition zones of 1 to 2 mm was observed for all of them. When testing the probiotic vs. Salmonella spp. in a feed matrix, it inhibited the growth of S. Heidelberg (1.3 log inhibition after 4 hours, 4.6 log inhibition after 8 hours) and S. Typhimurium (1.6 log inhibition after 4 hours, 2.2 log inhibition after 8 hours). Both in-vitro methods confirm the growth inhibition activity on numerous Salmonella serovars by this new three-strain probiotic.

ID : 68

EFFECT OF AZOLLA ON EGG PRODUCTION PERFORMANCE AND CHOLESTEROL CONTENT OF EGG YOLK OF LAYING HEN

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Abstract

Azolla pinnata is a small aquatic fern which flows on the surface of the water, rich in protein, essential amino acids (lysine, leucine, arginine, and valine), vitamins (Vita A, Vita B12 and Beta-carotene), chlorophylls and minerals; Ca, P, Fe, Mg, Cu, Zn, Na, etc. It may be used in poultry diet as an abundant unconventional plant protein source which reduces the production cost and cholesterol and increases yolk color and profitability. Therefore, the present study was planned for assessing the effect of Azolla on egg production, profitability, egg yolk color, and cholesterol content of egg for producing quality, safe and profitable chicken eggs.

A total of 70 ISA-brown ready to pullets was distributed into 3 dietary groups; D1 (control-no Azolla) with 46 pullets, and D2 (diet with 4% sun-dried Azolla meal) and D3 (diet with 8% sun-dried Azolla meal) with 12 pullets each to investigate egg production performance and egg yolk color. Six experimental (2 eggs/diet) and 2 commercial farming (D4) eggs were tested to measure egg yolk cholesterol. The pullets were reared in the individual cage management system and fed iso-nitrogenous (iso-lysine) and an iso-energetic diet containing 17% CP and 2750 KcalME/kg up to 44 weeks of age. The collected data were analyzed using the Statistix10 computer package program. Cholesterol content of egg yolk was analyzed using a t-test.

Feed intake, FCR (Feed/kg egg), production cost, net-profit, and yolk color significantly differed among dietary groups (p3 showed the highest egg mass, and the lowest feed intake and FCR (Feed/kg egg), followed by D1 and D2, respectively. Statistically similar egg, hen-day, and hen housed egg production were obtained among dietary groups. The lowest amount of production cost and the highest amount of net-profit was observed in D3, followed by D1 and D2, respectively. The deepest yellow yolk color was measured in D3, followed by D2 and D1, respectively. Therefore, Azolla significantly increased egg yolk color compared to the control diet. However, 8% of Azolla was much better than 4% Azolla in terms of increased yolk color. The lowest amount of cholesterol was determined in D3, followed by D2, D1, and D4, respectively (p

Therefore, Azolla may be considered as a novel and beneficial feed item for producing quality, safe and profitable eggs.

KEYWORDS: Azolla; cholesterol; egg production; hen; yolk color

ID : 206

THE IMPACT OF CONDITIONING TIME, STEAM PRESSURE AND SPEED OF COOLING ON THE THERMOSTABILITY OF A NOVEL HEAT STABLE 6-PHYTASE IN A LAYER FEED AFTER PELLETING.

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The aim of the study was to investigate the impact of steam pressure, conditioning time and speed of cooling on the thermostability of a novel *E. coli* phytase (OptiPhos® Plus CT, Huvepharma NV). A wheat/corn based layer feed was supplemented with phytase to yield a final concentration of ± 1000 FTU/kg. The feed was prepared in a vertical mixer (700 litres) and conducted to a conditioner unit. The conditioner was a fast rotating paddle mixer with a capacity of 20 litres in which steam is added. This steam is produced via an electric steam generator at 9 bar and is dewatered and reduced to a working pressure of the desired bar before entering the conditioner. The residence time is controlled manually. The pellet mill is a Simon Heessen Labor Monoroll type (Adritz Sprout, Netherlands) with a 4x50 mm die. The conditioning was performed at 85°C, but with different residence times and steam pressure: 30 sec. & 1.5 bar saturated steam (steam temperature: 127°C), 30 sec. & 3 bar saturated steam (steam temperature: 140 °C), 200 sec. & 1.5 bar saturated steam (steam temperature: 127°C). Cooling and drying was performed by an ambient flow through air cooler in 2 ways: instantly or slowly. Instant cooling means each subsample (10) is cooled instantly after leaving the die, while slow cooling means the subsamples (10) are first pooled during 2 to 3 minutes of production and then brought in the cooler. In both cases the residence time in the cooler was equal to 15 minutes. The recovery was determined as (phytase in supplemented pellet – phytase in blanc pellet)/(phytase in supplemented mash – phytase in blanc mash). Results showed that condition time had no impact on heat stability of the phytase, yielding 94.9 % and 92.9 % at 30 sec conditioning with respectively 1.5 and 3 bar steam pressure, and 93.2 % at 2 min. conditioning and 1.5 bar steam pressure. Speed of cooling had a large impact on the recovery as slow cooling brought recoveries down to 86.8 and 82.6 % at 30 sec conditioning and 1.5 respectively 3.0 bar steam pressure, while recovery was 76.5 % at 2 min. conditioning and 1.5 bar steam pressure. It can be concluded from this trial that conditioning time and steam pressure did not have much impact on the heat stability of the phytase, while cooling had a large impact. Therefore it is also advisable in pelleting studies to detail better, or to uniform, the cooling of (sub)samples.

ID : 223

EFFECTS OF PRONUTRIENTS (BOTANIC MOLECULES) ON PERFORMANCE AND COMPARISON WITH BUTYRATE IN BROILERS

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Pronutrients are active molecules from plant extracts that naturally improve the physiology of their target organs.

An experiment was conducted to determine the effects on performance and carcass yield of pronutrients, butyrate and the combination of both in broilers. Pronutrients and butyrate are described as natural growth promoters and can replace AGPs.

320 male Vencobb broilers were distributed into four treatment groups with 80 birds each: control (T1); a group receiving pronutrients in feed at 0.5 kg/t (T2); a group receiving butyrate in feed at 0.3 kg/t (T3); and a group receiving the combination of pronutrients + butyrate at the mentioned doses (T4). Birds received the treatment continuously from day 1 until day 35 of age (end of trial). All diets were fed ad libitum. Data were statistically analyzed by multi-variate ANOVA in GLM of SPSS (v 26.0). Results showed that pronutrients (T2) improved body weight (BW), whether alone (+3%) or in combination with butyrate (+3.9%), compared to the control (T1). Butyrate alone (T3) showed no positive effect on BW. Feed conversion (FCR) was improved in all treatments (T2, T3 and T4), compared with the control, and pronutrients (T2) improved it significantly (-4.9%, P

In conclusion, pronutrients in feed can be used to improve productive performance in broiler chickens, as it showed a trend to increase BW, decrease mortality, and significantly improved FCR. It was more effective than butyrate. Additionally, no interaction was observed between pronutrients and butyrate, so they can be added together to broiler diets.

ID : 283

GROWTH PERFORMANCE, INTESTINAL MORPHOMETRY, AND BLOOD SERUM PARAMETERS OF BROILER CHICKENS FED DIETS CONTAINING INCREASING LEVELS OF WHEAT BRAN WITH OR WITHOUT EXOGENOUS MULTI-ENZYME SUPPLEMENTATION

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Wheat bran (WB) is an alternative ingredient that could be utilized as an energy and protein source partial replacement of corn and soybean meal in poultry feed formulation. The objective of the study was to investigate the effects of multi-enzyme supplementation (XGP) on the growth performance, intestinal morphometry, and blood-serum parameters of broilers fed diets containing increasing levels of WB. A total of 720 Cobb 500 seven-day-old chicks were assigned to 1 of 8 treatments to obtain 15 chicks per replicate and 6 replicates per treatment. A 2×4 factorial arrangement with 2 levels of multi-enzymes (0 or 360 g/ton) and 4 levels of WB (0, 4, 8, or 12%) were used. The multi-enzyme premix consisted of Xylanase, β -Glucanase, and Phytase (XGP). Bodyweight and feed intake were determined at 14, 28, and 42 d and used to calculate ADG, ADFI, and FCR per pen. There was a WB \times XGP interaction ($P < 0.01$) for the overall BW and ADG of broilers. Broiler ADFI was not affected by dietary treatments. Increasing the WB level linearly increased ($P = 0.003$) the overall FCR. Besides, WB \times XGP interaction ($P < 0.01$) was observed for villi height (VH) and villi height to crypt depth ratio (VH: CD). XGP increased ($P < 0.01$) high-density lipoprotein cholesterol (HDCL) but decreased cholesterol (CHOL). Feeding broilers diets containing increasing levels of WB with or without XGP resulted in similar BW, ADFI, and FCR as broiler control diets. Increasing the WB level linearly decreased overall BW and made FCR of broilers poorer. Broilers fed XGP had increased VH, VH: CD, and HDCL but decreased and CHOL.

ID : 395

WATER ACIDIFIER ENHANCES FEED EFFICIENCY AND BREAST YIELD OF BROILERS

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Water acidifier enhances feed efficiency and breast yield of broilers

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ABSTRACT

The current trend in the global poultry industry is towards a reduction in antibiotic use during production. To maintain the same level of zootechnical performance achieved with antibiotic growth promoters (AGP), several feed additives are being marketed as suitable AGP alternatives. This study was designed to investigate the efficacy of water acidifier (SPH) based on a synergistic blend of free and buffered short-chain fatty acids on growth performance and carcass yield of broiler chickens as AGP replacement. One-day-old male broiler chicks (n=1300) were randomly assigned to one of four treatments with 13 replicate pens of 25 birds each. The treatments included a negative control (NC, a basal diet without AGP), a positive control (AGP, as NC + Bacitracin methylene disalicylate (BMD; 500 g/T in phase 1-3) and Virginiamycin (45 g/T in phase 3); water acidifier (SPH, as NC + 2L SPH/1000L water), and water acidifier + AGP (SPH+AGP, as PC+SPH). All chicks were reared in floor pens with a deep litter system in an open-sided poultry house and were provided with a three-phase corn-soy based diet. The body weight (ADG) and daily feed intake (ADFI) were recorded at d1, 14, 28, and 42 and the feed conversion ratio (FCR) was calculated. The carcass yield (percentage of breast weight (without skin) over the hot carcass weight) and dressing percentage were evaluated and the feed cost per kg live weight was calculated (based on the cost of the product and market conditions at the time of the study) on d42. Data were analyzed using the GLM procedure in SAS and Tukey's range test was used to determine significance between treatment means (P

In all parameters examined, no significant difference was observed between the AGP, SPH and the combination of SPH+AGP treatments (P>0.1).

In contrast to the NC, the FCR reduced significantly by -7.2 points with the supplementation of SPH+AGP (1.697 vs 1.615, P=0.02), while the effect of SPH (1.650) or AGP (1.664) was similar and intermediate between SPH+AGP and the NC (P>0.05). The breast yield increased significantly by +1.9% compared to the NC (26.32 vs 24.39%, P=0.04). The dressing percentage, livability and cost to produce kg LW were not affected by the treatments (P>0.05).

In conclusion, the study demonstrated that water supplementation with SPH or in combination with AGP (SPH+AGP) is as effective as AGP in enhancing breast meat yield and improving feed efficiency of broiler chickens.

Keywords: additives, antibiotic-free environment, antibiotic resistant

ID : 431

EFFECTS OF NATURALLY CONTAMINATED DIETS WITH FUSARIUM TOXINS AND ADDED ANTI-MYCOTOXIN AGENT ON PERFORMANCE PARAMETERS AND LIVER HEALTH OF LAYING HENS

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T-2 is a mycotoxin classified as trichothecene, produced by *Fusarium* molds infecting cereal crops, and found frequently in raw materials used for animal feeds. The toxic effects of DON and T-2 on poultry are shown mainly in the liver, the gastrointestinal tract and the immune system. Liver function is crucial for egg production and when liver function is impaired egg quality can be detrimental and when the exposure is chronic egg production can also be affected. 90 Lohmann Brown laying hens were assigned randomly to three groups (10 repetitions - 3 hens each) to evaluate the effects of 12 weeks' exposure to multiple *Fusarium* toxins through feed, and its amelioration by an anti-mycotoxin agent. The groups included: Control (C) fed a standard diet, very low mycotoxin load: DON ~100ppb; Fumonisin B1+B2 ~400ppb; Zearalenone ~20ppb; Low (L) fed a contaminated diet with T-2 ~90ppb, DON ~100 ppb, Fumonisin B1 ~1000ppb, Fumonisin B2 ~250ppb and Zearalenone ~50ppb & another one fed with the contaminated diet adding 0.15% of an anti-mycotoxin agent (LM). Performance was measured daily, feed intake and egg quality weekly. Egg quality included weight, Haugh units, eggshell strength, eggshell thickness, shape index, and relative albumen, yolk and eggshell weights. Blood samples were taken for Ca, P, liver health and stress indicators. Liver samples (five birds per group) were semi-quantitatively scored on a scale of 0-5 for color, presence of haemorrhages (macroscopic); tissue was taken for histopathology. The data was fitted by nonlinear growth models within R environment, by R "nlseasy" package. There were no significant differences between the fitted growth curves for the treatments, although in the last 6 weeks, L showed a tendency for lower feed intake and laying smaller eggs. No differences in liver health indicators (AlbG, AlkP, ALT and AST, GGT and GLDH), stress indicators (H/L ratio), blood Ca and P levels, and liver macroscopic were found. Histopathologic examination showed mostly scant to mild multifocal interstitial and perivascular infiltrates of lymphocytes in all liver tissue samples. It can be concluded that the mycotoxin challenge was not able to reduce welfare, health performance and egg quality of laying hens during the trial period but longer exposure to mycotoxins challenge without binders can result in reduced feed intake and smaller eggs.

ID : 509

THE EFFECT OF PRE-PELLETING WHOLE CORN INCLUSION ON GROWTH PERFORMANCE, PROCESSING YIELD, AND ORGAN DEVELOPMENT OF BROILERS

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Previous research suggested that whole grain inclusion can reduce grinding costs and improve broiler performance. Two studies were conducted to evaluate the effect of whole corn inclusion pre-pelleting on growth performance, processing yield, and organ development of broilers. Both experiments consisted of 4 dietary treatments with 10 replicate pens per treatment and 25 broilers per pen. In first experiment treatment diets consisted 0, 2.5, 5, and 7.5% whole corn that replaced ground corn and was provide from 1 to 42 d of age. In second experiment, 0, 3, 6, and 9% whole corn was included from 14 to 42 d of age, following a common starter diet from 1 to 13 d. The starter diets were fed in crumbled form and the grower and finisher diets in pelleted form. In both studies, feed intake and BW were determined at 14, 28, and 42 and FCR adjusted by adding the weight of the mortality to the BW of live birds. At 43 d, 10 birds/pen were processed for yield determination. In the second study two-broilers per pen were euthanized by CO₂ asphyxiation and used to determine the weight of crop, proventriculus, gizzard, liver and ceca and expressed as a ratio of total live body weight. Data were statistically evaluated using ANOVA procedure and means were separated by Tukey HSD procedure. In first experiment whole corn inclusion significantly improved FCR from 28 to 42 d of age (1.94 vs. 2.00, P<0.05). Birds fed diets with 5% of whole corn had higher breast meat weight (292 vs. 284 g, P0.05) from 14 to 42 d of age. However, birds fed diets with 9% whole corn had higher carcass yield (P0.05). But the relative proventriculus weight decreased with increasing level of whole corn inclusion (P=0.036, 0.302 vs 0.346). Results of these studies indicated that 5% whole corn can be included in starter feed and 9% in grower and finisher feeds without a negative effect on broiler performance.

ID : 578

SUPPLEMENTATION OF PROTEASE IN A LOW PROTEIN DIET ON GROWTH PERFORMANCE IN BROILERS FOR 35 DAYS

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Research on exogenous enzymes is being actively conducted on broilers to improve the protein utilization and subsequent reduction of nutrients in the animal waste generated from the feed. Endogenous proteases synthesized and secreted in the gastrointestinal tract (GIT) are known to be sufficient for the protein utilization in the diet (Nir et al., 1993). However, reports have demonstrated that considerable amounts of protein pass through the GIT rather than completely digested in broilers. Undigested proteins in the GIT can improve the digestibility by endogenous proteases. Therefore, the objective of this study was to evaluate the effects of the endogenous protease on growth performance with low protein and amino acid diet.

A total of 448 one-day-old Ross male broiler chickens were allocated according to completely randomized design into 64 pens. Eight dietary treatments were practiced and each treatment consisted eight replications with seven birds in each cage. Dietary treatments were: positive control with standard diet (PC), PC + protease (Kemin; 150g/ton; PC-150), negative control with CP 0.5% and AA 2% down from PC (NC1), NC1 + protease (150g/ton; NC1-150), NC1 + protease (300g/ton; NC1-300), negative control with CP 1.0% and AA 4% down from PC (NC2), NC + protease (150g/ton; NC2-150), NC2 + protease (300g/ton; NC2-300). Corn and soybean meal control diets were formulated to meet the Ross 308 nutrition specification. Diets were provided on an ad-libitum base in a mash form. The protease was top-dressed onto the diets in order to make eight treatments. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were measured for 5 weeks. Data were subjected to analyse using one-way ANOVA in SPSS.

Results revealed that the supplementation of protease into low CP, AA density diets improved ($P<0.05$) the daily gain (PC-150: 2.2% than PC; NC1-150: 1.7%, NC1-300: 3.8% than NC1; NC2-150: 1.4%, NC2-300: 7.2% than NC2) and feed efficiency (PC-150: 7.2% than PC; NC1-150: 2.2%, NC1-300: 3.9% than NC1; NC2-300: 3.8% than NC2) of the chickens compared to the chicken fed with NC1, NC2 diets from hatch to 35 days.

In conclusion, our results indicated that the supplementation of protease in to the reduced CP, AA diets has the ability to improve the growth performance of broiler chickens by compromising the impact of low CP, AA in the diets.

Nir I Z Nitsan and M Mahagna. 1993. Br. Poult. Sci. 34:523–532.

ID : 748

NATURAL FEED ADDITIVE FROM AGRICULTURAL BIOMASS WASTE: MICROENCAPSULATED PYROLIGNEOUS ACID POWDER FOR BROILER REARED IN THE TROPICAL CLIMATE AREA

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One approach to utilize agricultural biomass waste is carbonization, which generates organic charcoal as the main product and pyroligneous acid (PA) as a by-product. The microencapsulated powder of PA prepared from the carbonization of agricultural biomass waste so-call natural feed additive (NFA) was developed. It composes with rich phenolic compounds and short and medium chain fatty acids as the main components with a concentration of 72.94-94.91 mg/g and 63.18 mg/g, respectively. The stability testing of the encapsulated powder shown more stable for animal feed industrial applications. The effect of NFA supplement on growth performant and healthy improvement in broilers was investigated. 400 one-day-old male broiler chicks (Ross 308) were allocated into 4 treatment groups including (1) negative control (no supplemented NFA) (2) supplemented NFA 1,500 mg/kg (3) supplemented NFA 3,000 mg/kg (4) supplemented NFA 4,500 mg/kg. Each treatment was divided into 5 replicates of 20 chicks each (20 males). The results found that it was not improving the growth performance in broilers. However, stress and inflammatory levels in broilers feed with NFA treatments were suppressed by which the blood cortisol hormone and IL-1b, IL-8, IL-10, TLR-4 and IFN-g in an intestinal tract were significantly lower than that the control treatment ($P<0.05$). Besides, it also increasing volatile fatty acid content in ileum and caeca in broiler with statistical significance when compared with control treatment. Moreover, it stimulated immunological respond by which the IgG, IgM, and IgA in plasma of broilers feed with NFA have a significantly higher concentration than that the control treatment ($P<0.05$). The intestinal histology showed that male chickens fed diets supplementing 1500-4500 mg/kg NFA had significantly higher villus height and villus height: crypt ratio of duodenum and jejunum as compared to the control group ($P<0.05$). The gut microbiota analysis showed that supplement the NFA can improving the actinobacteria in the intestinal tract for boiler. It can be implied that the NFA prepared for this study has the prebiotic characteristic. Overall, it could be concluded that the feed additive product prepared from agricultural biomass waste in microencapsulated powder has the potential for application as a natural feed additive for boiler with integrated functions.

ID : 921

EFFECT OF YEAST FRACTION SUPPLEMENTATION ON INTESTINAL ENVIRONMENT IN BROILER CHICKS WITH DEXTRAN SULFATE SODIUM

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It is known that yeast fraction (YF) works as prebiotics and improves gut health by adsorption of harmful bacteria and maintenance of barrier function in animals. The objective of the study was to investigate the protective effects of YF added to diet on the intestinal environment in broiler chicks challenged with dextran sulfate sodium (DSS), an agent to cause gut inflammation. Chicks were separated in two groups: one control group fed with a basal diet and another group fed with the basal diet supplemented with 0.025% YF. At 14 days of age, half of the birds in the birds in the control group (CC) and half of the birds in the yeast fraction group (YC) were given saline (0.1 mL). The other half of control group (CD) and yeast fraction group (YD) were orally gavaged with DSS (1 mg/0.1 mL). The number of chickens in each group was n = 6. After 6 h, all chicks were hyperanesthetized, and their jejunum tissues and cecal contents were collected. Samples were stored at -80°C prior to the RNA or DNA isolation. As for the gene expressions in jejunum tissue, significant increases were found on mucin-1, claudin-5, and IL-8 in YF group compare to other groups. Same effects were measured in YD group with the mRNA levels of mucin-1, and IL-8. Regarding the cecal microbiota, ratio of Bacteroides in CD group tended to be higher than other groups. These findings suggest that YF may work against the substance, which has the ability to disturb the intestinal environment, and helps to recovery any damage induced by a harmful material in broiler chicks immediately.

ID : 967

EFFECT OF COMBINATION OF HYDRATED SODIUM CALCIUM ALUMINOSILICATE (HSCAS) AND CLINOPTILOLITE AS ENDOTOXIN BINDER ON BROILER PERFORMANCE AND INTESTINAL HISTO-MORPHOLOGY

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Poultry is being exposed to endotoxins released by the death of Gram-negative bacteria present in the normal intestinal flora and the environment. Sub-therapeutic antibiotic usage as a growth promoter is one of the causes of the increased release of endotoxin from these bacteria (Shinozuka et al. 2007). In the current research, a combination of hydrated sodium calcium aluminosilicate (HSCAS) and clinoptilolite was evaluated as an endotoxin binder (EB) in broilers. The EB was used alone and along with an antibiotic (AB) to assess its effect on broiler growth performance, intestinal histo-morphology and techno economics of EB inclusion.

Two hundred day-old Vencobb-400 broiler chicks were divided into five groups with four replicates of ten chicks in each. The experimental treatment consisted of G1-fed with basal diet (BD) + 0.5 kg/ton AB, G2-BD + 2.0 kg/ton EB, G3-BD + 2.5 kg/ton EB, G4-BD + 1.0 kg/ton EB + 0.5 kg/ton AB and G5-BD + 1.5 kg/ton EB + 0.5 kg/ton AB. The daily feed intake and weekly body weight were recorded and six birds from each group were sacrificed at every phase (pre-starter, starter and finisher) for intestinal studies. The data obtained was subjected for Duncan one-way ANOVA using SPSS-IBM 24.0 version software. Fourier Transform Infrared spectrum of EB was analysed.

The resulted body weight gain (BWG) and feed efficiency ratio (at 42nd day) compared between EB alone and EB with AB groups demonstrated that either EB at 2 kg/ton or EB at 1.0 kg/ton along with AB were significantly (Pnd day was significantly (P

ID : 975

USE OF A PHYTOGENIC BLEND OF CINNALMALDEHYDE AND THYMOL ALONE OR IN COMBINATION WITH A C-BACILLUS PROBIOTIC IMPROVES PERFORMANCE OF BROILERS UNDER HIGH CHALLENGE SITUATION

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The current study looked at the effects of including a phytogenic blend (EO), alone or in combination with a Bacillus based probiotic (EOPRO) or an AGP in broilers fed a diet containing a phytase/carbohydrase combination with associated full matrix applied under challenged conditions. Total of 2160 day-old Lohman Indian River chicks was randomly allocated to 5 dietary treatments with 12 replicates (36 birds/pen). Birds were fed test diets in 2 phases: starter (D1-21) and grower/finisher (D22-35). Control diets were formulated to meet the birds nutrient requirement allowing for nutrient matrix of enzymes. All diets contained Buttiauxella phytase (Aextra® PHY) at 1000 FTU/kg of feed and a combination of xylanase, amylase and protease (Avizyme® 1505X) at 200 g/tof feed. One group of birds were fed the control diet and given no challenge. Challenged birds (CC) were inoculated with C. perfringens and E. coli. The CC were fed either unsupplemented or supplemented with Antibiotic Growth Promotor (BMD) at 50g/t, Phytogenic blend (EO) at 100 g/t, or a combination (EOPRO) of EO at 100 g/t and a 3 strain Bacillus probiotic at 60 g/t of feed. Gut samples were taken on days 21 and 35, from 24 birds/treatment. Lesions were scored in duodenum (E. acervulina), ileum (E. maxima) and caeca (E. tenella). Results confirmed the applied enzyme matrix as the unchallenged birds fed the enzyme containing control diet hit their production targets. Supplementing the diets of CC with EO and EOPRO treatments resulted in numerically improved performance. Average daily gain was numerically improved with EO and EOPRO compared to the CC by 1.31% and 2.45%, respectively. FCR was numerically improved by 3 points with both EO and EOPRO treatments vs. CC and was not significantly different to the UC birds. In contrast the AGP treatment did not result in any performance improvements in the CC, with both the EO and EOPRO treatments numerically improving performance versus the AGP. Broiler Index was numerically improved by 4.36% and 5.23% by EO and EOPRO treatments compared to the CC. Gut health scores did not differ between treatments. Results showed that full matrix implementation for combination of Phytase (Aextra PHY) and mixed enzymes (Avizyme) at 126 kcal/Kg ME was the proper level based on the diets' specifics. The EO and EOPRO treatments proved to be effective options to replace AGP and maintain or improve performance under challenged conditions.

ID : 1002

EFFECT OF GRAIN SORGHUM PARTICLE SIZE, NUTRIENT LEVEL, AND PHYTASE DOSING ON THE PERFORMANCE OF BROILER CHICKS TO 18 DAYS OF AGE

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Broiler diets require cereal grains to be finely ground to improve pellet quality, which optimizes feed intake and growth rates. This grinding decreases the work necessary by the gizzard resulting in reduced physical exertion and atrophy of gizzard mass. Grain sorghum is more apt to produce crops in dryland farming but must be properly processed to optimize nutrient retention when fed to broilers. Because the gizzard functions to grind and mix digesta for processing in the small intestine, a study was conducted to test the effect of combinations of grain sorghum particle size (PS), nutrient density and phytase enzyme levels on broiler chick performance. Whole grain sorghum was ground using a hammer mill to PS of 378 nm (small) and 747 nm (large). Control rations were formulated to meet the recommended nutrient requirements of Ross male chicks (Positive control, PC) with rations made using either small or large sorghum particles then fed in mash form. A set of negative control rations (NC) were formulated to 3% less energy and protein than the PC and with the available P (aP) reduced to 0.4%. Each NC diet was manufactured with small or large PS sorghum and supplemented with 0, 500 or 1500 FYT of phytase (DSM Ronozyme HiPhos 2700). The battery trials were conducted with 6 chicks per pen and 6 replications of each treatment. Performance data and gizzard weights were recorded at 18d. Data were analyzed as a factorial arrangement using main effects of diet (PC vs NC), PS (small vs large) and phytase level (0, 500 or 1500 FYT) using SAS (9.4) with significance levels set at $P \leq 0.05$. The results showed a significant main effect of PS to increase total body weight gain (BWG), gizzard weight (GW) and improve feed conversion (FC) when fed diets manufactured with large sorghum PS. These parameters were significantly reduced by diets limited in nutrients and aP but improved with increasing phytase. When the NC rations were supplemented with phytase, BWG, GW, and FC were significantly improved ($P \leq 0.05$) with increasing enzyme levels with the optimum performance observed for the group fed the large PS at 1500 FYT of phytase. The results indicate that maintenance of gizzard mass is important to optimize the BWG and FC of broiler chicks when feed rations are supplemented with increasing levels of phytase enzyme. Although pellet quality improves by reduction of PS, research is needed to determine optimal PS for the maintenance of desired gizzard function in broilers.

ID : 1225

EVALUATION OF DIETARY SUPPLEMENTATION OF A NOVEL MURAMIDASE TO WHEAT-SOYA DIETS ON BROILER PRODUCTION EFFICIENCY IN THE PRESENCE OF FEED ENZYMES AND COCCIDIOSTAT

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Muramidase enzymes can hydrolyse peptidoglycan, the main structural polymer of bacterial cell walls. When released into the gut during natural bacterial turnover, peptidoglycan in bacterial cell debris may detriment gastrointestinal functionality. Indeed, supplementation of a novel microbial muramidase (Balancius™) has been shown to benefit growth performance and gastrointestinal functionality in broilers (1,2,3).

Here, we evaluated the effect of Balancius™ in practical diets, supplemented with phytase, xylanase and a coccidiostat on growth performance, litter quality and footpad dermatitis of male broilers. Control wheat-soya bean meal based diets (T1) were supplemented with muramidase at 25,000 (T2), 35,000 (T3) or 45,000 (T4) LSU(F)/kg feed. The four dietary treatments were allocated to 96 pens, each with ten day-old Ross 308 male broilers in a randomized block design (n=24), using a three phase feeding strategy with starter (d0-10; crumbs), grower (d10-21; pellets) and finisher (d21-35; pellets) diets. From d14 to d18, feed was removed for 4 h/d from 8 am to induce a mild gut health disturbance. Analysis of variance showed that muramidase supplementation increased final bird weight from 2685 to 2772, 2804 and 2848 g (s.e.d. 32 g; P<0.001) and average feed intake from 3746 to 3816, 3887 and 3913 g (s.e.d. 41 g; P<0.001) for T1, T2, T3 and T4, respectively. This resulted in an improved mortality corrected feed conversion from 1.420 to 1.401, 1.414 and 1.401, respectively (s.e.d. 0.005; P<0.001). These beneficial impacts on broiler production efficiency were achieved largely over the first 21 days, and most pronouncedly during the grower phase, when the mild gut health disturbance was induced. In this study, muramidase supplementation did not affect litter score, pH, dry matter or nitrogen content at d35, which averaged 2.1±0.03, 8.28±0.050, 56.9±1.91% and 1.9±0.05%, respectively. Footpad dermatitis incidence at d35 was low and did not differ between treatments. We concluded that in the presence of a mild gut challenge but otherwise good rearing conditions, dietary supplementation of Balancius™ on top of feed enzymes and a coccidiostat significantly improved broiler production efficiency during the starter and grower phase, which carried through to five weeks of age.

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ID : 1403

EVALUATION OF PARTICLE SIZE, FEED FORM AND PELLET DIAMETER ON BROILER PERFORMANCE AND PROCESSING YIELD FROM 1 TO 39 DAYS OF AGE

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Particle size and feed form manipulation have emerged as alternatives to improve poultry performance. The objective of the study was to elucidate the effect of particle size and feed form on broiler performance and processing yield from 1 to 39 d of age. A total of 1800 d old male Cobb 500 birds were randomly assigned to 9 dietary treatments with 8 replicate pens per treatment and 25 birds per pen. The experiment consisted of a 3 × 3 factorial arrangement of 3 corn particle sizes (750, 1150 and 1550 µm) and 3 feed forms (mash, 3.0- and 4.0-mm pellets) provided from 1 to 39 d. Feed intake and BW were determined at 17, 27 and 39 d of age and FCR was calculated by using the weights of mortality. On d 40, 10 birds per pen were processed and on d 41, carcasses were deboned to determine meat yield. Data were statistically evaluated as a 3 × 3 (feed form × corn particle size) factorial arrangement in a randomized block design. Broilers fed 3.0- and 4.0-mm pellets had higher BW, FI and lower FCR ($P<0.05$) compared to broilers fed mash at 39 d of age. Broilers fed diets with 750 µm corn particle size had higher BW ($P<0.05$) than broilers fed diets with 1550 µm at 39 d of age, while broilers fed diets with 1150 µm had an intermediate BW. Broilers fed diets with 750 µm corn particle size had higher FI ($P<0.05$) than broilers fed diets with 1150 and 1550 µm at 39 d of age. Particle size of corn did not influence FCR at 39 d of age. Broilers fed 3.0 mm pellets had the heaviest ($P<0.05$) carcass and breast weights, followed by broilers fed 4.0 mm pellets and the lowest weights by broilers fed mash. Tenders and wings weight were similar among broilers fed 3.0- and 4.0-mm pellets, but higher ($P<0.05$) than broilers fed mash. Broilers fed diets with 750 µm corn particle size had heavier carcass and breast weight ($P<0.05$) than broilers fed diets with 1550 µm, with 1150 µm having an intermediate carcass and breast weight. Furthermore, broilers fed diets with 750 µm corn particle size had higher tender weight and yield ($P<0.05$) than broilers fed diets with 1150 and 1550 µm. These results showed that feed form and particle size influence broiler performance and processing yield of Cobb 500 broilers.

ID : 804

EFFECTS OF CORN PARTICLE SIZE AND FORMIC ACID ON PRODUCTIVE AND PROCESSING PERFORMANCE OF BROILERS

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Previous research has reported that inclusion of coarse corn particle size in broiler diets can improve gastro-intestinal tract (GIT) function. Formic acid decreases the feed pH which could create a less favorable environment for bacteria and alter buffering capacity which may improve the efficacy of digestive enzymes. However, research evaluating the interactive effects between coarse corn particle size and dietary levels of formic acid throughout the production period of broilers is sparse. The objective of this study was to evaluate the effects of corn particle size and formic acid on broiler growth performance and carcass characteristics. A total of 2,034 male Ross YPM × 708 broiler chicks were randomly distributed among 6 dietary treatments with 12 replicate pens/treatment and 32 birds/pen, which constituted a 3 × 2 factorial arrangement of 3 corn particle sizes (395 µm fine corn (FC), 850 µm coarse corn (CC) obtained with greater inclusions of 1642 µm corn (CC-Hi) or lesser inclusions of 2290 µm corn (CC-Lo) when blended with FC and 2 dietary levels of formic acid (0.0% and 0.4%) fed from 11-49 d of age. Feed intake and BW were determined at 10, 21, 35, 42 and 48 d of age and feed conversion (FCR) was calculated and adjusted for mortality. At 43 d, 2 birds/pen were processed for the determination of carcass characteristics and hot carcasses were deboned to determine total breast meat yield (pectoralis major and minor muscles). Data were statistically evaluated as a 3 × 2 (corn particle size × formic acid) factorial arrangement in a randomized complete block design. Although there were no significant differences in FI attributed to the inclusion of formic acid ($P > 0.05$), birds fed CC-Hi diets had a lower FI compared with those fed CC-Lo diets at ($P < 0.05$) at 48 d. At 35 d, birds fed FC diets with 0% formic acid had a lower BW compared with those fed FC diets with 0.4% formic acid. Birds fed CC and 0.4% formic acid exhibited an improved FCR compared with those fed diets with FC and 0% formic acid at 48 d. Dietary inclusion of formic acid increased pectoralis minor yield at 43 d ($P < 0.05$). These data indicated that up to 0.4% of formic acid can be fed with a coarse corn particle size without negatively affecting broiler performance or carcass characteristics.

MINERAL NUTRITION

ID : 134

IMPACT OF AN E. COLI 6-PHYTASE ON IN VITRO PHYTATE BREAKDOWN OF DIFFERENT FEEDSTUFFS

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The aim of this study was to estimate the effectivity of different levels of a E. coli 6 phytase (OptiPhos®, Huvepharma NV) on the in vitro degradation of phytate in six feed ingredients (soybean meal, rapeseed meal, sunflower seed meal, barley, maize, and wheat). The in vitro approach was based on an incubation which simulated the conditions in the stomach and the small intestine. The incubation was conducted in triplicate according to the following procedure: 0.8 g of feed ingredient and 20 ml of pepsin-HCl solution (pH 3.0), with or without phytase, was incubated during 1.5 hours at 37°C. After neutralisation, 20 ml acetate buffer (pH 5.5) was added and the mixture was incubated (2.5 h, 37°C). The incubation was stopped and the supernatant and residue was separated by centrifugation (4000g, 15 min). Samples of the supernatant were analysed on P while also the P and phytate-P content in the feed ingredients and the amount of P in the residues were analysed. Based on the amount of inorganic P into solution at the end of the trial of the phytase and non-phytase treated samples, it could be calculated how much P per gram of feed materials has been transferred into soluble inorganic P when supplementing different levels of OptiPhos®. This corresponds to the breakdown of mainly soluble phytate-P due to the action of OptiPhos®. Results indicated that adding OptiPhos® increased significantly the amount of total P release (mg per g feed material) strongly at 500 FTU/kg, while increasing the levels of OptiPhos® resulted in extra P release ($p > 0.05$). Of the three protein sources, the improvement (%) in phytate-P degradation vs the control was highest for sunflower seed meal (79, 81 and 86 %) and rapeseed meal (70, 69 and 70 %) compared to soybean meal (54, 56 and 59 %) at 500, 1000 and 1500 FTU/kg respectively. Of the three cereals, the phytate-P breakdown was highest in corn (70, 72 and 76 %) and less in barley (39, 46 and 49 %) and wheat (50, 52 and 52 %) at 500, 1000 and 1500 FTU/kg respectively. It could be concluded that OptiPhos® (a) led to a higher inorganic P release from phytate-P in raw materials with higher phytate-P levels, (b) had even a more efficient phytate breakdown (%) in feedstuffs containing high phytate-P levels (sunflower seed meal and rapeseed meal) than in feedstuff containing lower phytate-P levels and (c) that from the 3 cereals, phytate breakdown by OptiPhos® was higher in corn compared to barley and wheat.

ID : 160

DIETARY ZINC-L-SELENOMETHIONINE IMPACTS PROGENY OF BROILER BREEDER HENS

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Selenium is one the most controversial trace elements. Although toxic at high doses, deficiency is related to increased disease susceptibility of both animals and humans, and decreased productive and reproductive performance of farm animals. A study was conducted to evaluate the effect of inorganic Se, zinc-L-selenomethionine, and Fe supplementation on broiler breeder hen performance. A total of 132 Cobb 500 broiler breeder hens at 20-weeks-of-age (woa) were placed in individual cages. Hens were randomly assigned to 11 treatments of Se and Fe trace mineral supplementation: Control with 55 ppm Fe as FeSO₄ + 0.30 ppm sodium selenite (Se-S; T1). Treatments 2 through 9 (T2-T9) included Se-S at 0.30 ppm and increasing levels of Se as zinc-L-selenomethionine (ZnSeMet) at 0.15, 0.20, 0.30, and 0.40 ppm, with either FeSO₄ at 55 ppm or Fe amino acid complex (Fe-AA) at 40 ppm. Treatment 10 included 0.30 ppm Se as ZnSeMet + 55 ppm Fe as FeSO₄, and T11 had 0.30 ppm Se as ZnSeMet + 40 ppm Fe as Fe-AA. Hens were adapted from 20 to 26 woa. Data collection was completed in 3 periods of 28 days each, from 27 to 38 woa, with one incubation per period. All data were analyzed using proc MIXED of SAS, and means compared using the Tukey-Kramer test, where differences were considered significant at $P < 0.05$. Dietary treatment did not impact overall hen productive performance or incubation, except for hatchability of settable eggs, which was higher in T11 compared with Control ($P < 0.05$). In T3, T4, T5, and T7, chick length was improved over the Control ($P < 0.05$). Groups with higher Se generated lower chick leg score abnormalities than groups fed lower Se in partial replacement ($P < 0.05$). Selenoprotein-W (SelW), which can serve as an antioxidant in different cell types, did not differ among treatments at d 14 of embryo incubation development (E14; $P > 0.05$), nevertheless it had a significant difference at E18 and E21 ($P < 0.0001$), where SelW gene expression decreased with increasing Se levels compared to Control ($P < 0.05$), except for T6 at E18. This result may indicate that SelW could be compensated due to higher Se available provided by breeder diets. In conclusion, ZnSeMet supplementation promotes improvement in hatchability of settable eggs, chick length and score, and lower SelW expression.

Keywords: broiler breeder, chick, leg score, selenoprotein-W.

ID : 161

PARTIAL AND TOTAL REPLACEMENT OF INORGANIC TRACE MINERALS BY AMINO ACID COMPLEXED MINERALS IN DIETS OF BROILER BREEDER HENS

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Feeding organic trace minerals (OTM) to broiler breeders is common practice in industry, however, totally replacing inorganic trace minerals (ITM) with reduced OTM levels is innovative. This study evaluated the effect of partial and total replacement of inorganic TMs (sulfates of Zn, Mn, Cu, Fe and Se) with amino acid complexed minerals (AACM) in broiler breeder diets. Eighty-four Cobb 500 broiler breeder hens at 20 wks of age (woa) were placed in individual cages, with treatments provided from 27 to 38 woa after a 6-wk adaption period. Experimental diets were composed of 7 treatments: ITM Full (Zn: 100/0, Mn: 100/0, Cu: 20/0, Fe: 60/0, Se: 0.30/0, and I: 2/0 ppm); ITM Reduced (Zn: 60/0, Mn: 60/0, Cu: 13/0, Fe: 40/0, Se: 0.15/0, and I: 2/0 ppm); ITM Plus (Zn: 120/0, Mn: 120/0, Cu: 30/0, Fe: 70/0, Se: 0.40/0, and I: 3/0 ppm); AACM Partial Replacement (Zn: 60/40, Mn: 60/40, Cu:13/7, Fe: 40/20, Se: 0.2/0.15, and I: 0/2 ppm); AACM Plus Replacement (Zn: 60/60, Mn: 60/60, Cu: 13/12, Fe: 30/40, Se: 0.2/0.2, and I: 0/3 ppm); AACM Reduced (Zn: 0/40, Mn: 0/40, Cu: 0/7, Fe: 0/20, Se: 0/0.15, and I: 0/2 ppm); and AACM Extra (Zn: 0/60, Mn: 0/60, Cu: 0/12, Fe: 0/40, Se: 0/0.2, and I: 0/3 ppm). All data were analyzed using proc MIXED of SAS and means compared using the Tukey-Kramer test, with differences considered significant at $P < 0.05$. Dietary treatments did not impact hen productive performance. Eggshell thickness increased with AACM supplementation ($P < 0.05$), and eggshell breaking strength was higher in total replacement compared with ITM treatments ($P = 0.055$). Egg weight prior to incubation was lower for partial replacement treatments, compared to ITM sources and total replacement treatments ($P < 0.05$). These differences were maintained for chick weight. Chick length was higher in partial replacement treatments of AACM, compared to ITM forms ($P < 0.05$). Percentage of leg score 1 (normal legs/toes), and 2 (signs of inflammation/redness in the legs) were higher and lower, respectively, in chicks from hens fed with total replacement treatments of AACM sources compared to ITM sources ($P < 0.05$), showing less leg abnormalities. There were no differences in navel button score of hatchings among treatments. In conclusion, diets supplemented with AACM led to improvements in eggshell thickness and shell breaking strength, along with hatching chick length and leg score, indicating better chick quality.

Keywords: broiler breeder, leg score, eggshell thickness, trace mineral

ID : 274

GROWTH PERFORMANCE RESPONSES OF BROILER CHICKENS TO PHYTATE PHOSPHORUS AND PHYTASE CONCENTRATIONS DURING DAYS 1 TO 11 OR 12 TO 23 POST HATCHING

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Common feed ingredients for broiler chickens have phosphorus (P) bound in the unavailable form of phytate complexes. However, phytase enzyme releases some of this P necessary for growth in broiler chickens. This study evaluated the effect of phytate P (PP) and phytase concentrations on broiler chickens during days 1 to 11 (phase 1) or 12 to 23 (phase 2) post hatching using growth performance as response criteria. Adequate P positive control (PC) diets in phase 1 or 2 contained 4.4 or 3.9 g non-phytate P (nPP)/kg, respectively; low-P negative control (NC) diets in phase 1 or 2 contained 2.3 or 1.7 g nPP/kg, respectively (without inorganic P, but with added meat & bone meal). Broiler chicks were randomly assigned to 6 replicate cages in a 3 × 5 + 1 factorial arrangement of treatments with 3 low-PP NC [2.3, 2.8, or 3.3 g PP/kg], 5 phytase levels (0, 500, 1,000, 2,000, or 4,000 FTU/kg) and a P-adequate PC. The PP was from raw feed materials while the phytase is a next generation biosynthetic bacterial 6-phytase. In phases 1 and 2 respectively, 1,152 and 768 male broiler chicks were used. In both phases, birds fed the NC had lower (P

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DOSE RESPONSE TO AVAILABLE PHOSPHORUS IN TWO STRAINS OF AGED WHITE LAYING HENS

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The eggshell quality continues to be a major concern of the egg industry. Despite the numerous studies on phosphorus (P) requirements in the laying hen, the optimum dietary level of P remains unknown in aged laying hens. Unfortunately, P is an expensive nutrient, which might have adverse consequences on the environment. Moreover, requirements are affected by genetic which is also constantly changing. Consequently, a trial was performed to evaluate P requirements in two white hens strains.

At 76 weeks of age, 480 hens of each strain (A and B) were allocated in 5 groups (4 or 5 replicates of 20 animals), consisting of increasing levels of available P (0.13, 0.20, 0.27, 0.34 and 0.41%) and the same level of calcium (3.80%), for 16 weeks. The performance (egg production, weight of hens and feed consumption) and quality of the eggs were recorded weekly. Two-way ANOVA was performed to compare strains and P levels. Broken-line model was used to find the optimum P level.

The results showed that the increase of the available P concentration led to an increase of the feed consumption from 101.5 to 107.1 g on average for both strains ($P < 0.05$) and limited weight loss from -140 g to -55 g ($P < 0.05$) between 76 to 92 weeks of age. Following these results, the optimal dose of available P using broken-line analysis was between 0.27 and 0.33%.

In terms of strain, the feed efficiency was improved by 0.13 point for the strain A. Consequently, at 92 weeks, hens of the strain A weighed approximately 83 g more than strain B ($P < 0.001$). There was no significant difference between the two strains for feed consumption or rate of lay. In terms of egg quality, the egg mass was greater for strain A compared with strain B (A : 66.7 g vs B : 61.9 g, $P < 0.001$). However, eggs of strain A were more downgraded (+ 5.1 points; $P < 0.001$) with more broken and dirty eggs. Moreover, the eggs of the strain B had a greater shell static stiffness: +3.5 N / mm ($P < 0.05$).

Also, no statistical difference was observed for the eggshell index, eggshell breaking strength, bone mineralization of the tibias, regardless the strains and the levels of available P.

Thus, despite an extended range of P, the quality of the eggshell was only slightly improved by increased P intake. However, the strain was preponderant on the egg quality. This type of dose-response trial is a good tool for studying the impact of a nutrient on the performance of laying hens.

ID : 407

EFFECT OF DIFFERENT TRACE MINERAL PROGRAMS ON PERFORMANCE OF BROILER BREEDER GRANDPARENTS

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Several organic trace mineral (TM) forms have been developed as supplements to animal feed. The type of chelate, such as amino acid (AA) or organic acid, and its binding properties are key factors differentiating organic TMs and effects in livestock performance are expected. Minerals complexed to AA (AACM) is the result of a soluble metal salt chelation to a single AA; hence, being absorbed via AA pathway. This study investigated the effects of supplementing zinc (Zn), manganese (Mn), copper (Cu), and iron (Fe) to broiler breeders as inorganic sulfates, chelate of methionine hydroxy analog (MHA), or as AACM on hen performance. A total of 1080 Hubbard A-7 Line D grandparent (GP) hens were assigned to 1 of 3 treatments: T1) Control, sulfates of Zn (80 ppm), Mn (80 ppm), Cu (17 ppm), Fe (40 ppm); T2) MHA, Zn (50 ppm), Mn (50 ppm), Cu (10 ppm), Fe (65 ppm, as FeSO₄); T3) AACM, AACM of Zn (40 ppm), Mn (40 ppm), Cu (7 ppm), Fe (40 ppm) + sulfates of Zn (40 ppm), Mn (40 ppm), Cu (10 ppm). All diets had equal methionine supplementation levels. Each treatment had 15 repetitions, and each replicate contained 6 cages with 4 hens per cage. Females and males were fed the same experimental diets from 35- to 59-weeks-of-age (woa). Data were analyzed using ANOVA and c2 tests. The AACM breeders had greatest egg quality (Haugh unit and albumen height at 40 woa, $P = 0.006$), followed by MHA or Control hens. Likewise, hatchability of eggs set at 40 woa tended to improve ($P = 0.13$) and late embryo mortality [>14 day of incubation (doi)] tended to be reduced ($P = 0.10$) with AACM supplementation, followed by MHA and Control. Early embryonic mortality ($P = 0.086$). A total of 500 grade-A male chicks from each treatment at the 59 woa hatching were placed in floor pens (10 pens/treatment \times 50 chicks/pen), and body weight and mortality rate were measured at d 7. Progeny from hens fed organic TM had greatest body weights in comparison to Control ($P = 0.004$). In conclusion, the feeding program with a partial replacement of sulfates by AACM improved egg quality and reproductive performance of Hubbard A-7 Line D GP broiler breeders, with positive carry-over effects in progeny during first week of growth.

ID : 452

ADDRESSING ENVIRONMENTALLY SUSTAINABLE POULTRY PRODUCTION WITH REDUCED COPPER SUPPLEMENTATION IN BROILERS REARED UNDER 'NON-ANTIBIOTIC EVER' CONDITIONS CHALLENGED WITH CLOSTRIDIUM PERFRINGENS

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Environmentally sustainable poultry production starts with a reduction in excess environmental nutrient excretion, while maintaining flock performance and health status. Although diseases such as necrotic enteritis (NE) undermine this strategy, they also intensify the search for more efficacious nutrients to address them. For this, two experiments were carried out with the objective of evaluating the effect of reduced copper (Cu) supplementation on performance and Cu excretion in broilers reared under a 'non-antibiotic ever' (NAE) system. In Experiment 1 (Exp1), 1,296 one-day-old Cobb 500 chicks, vaccinated for coccidiosis at hatch, were randomly assigned to 1 of 3 treatments: 1) 125 ppm Cu as tribasic copper chloride (TBCC); 2) 21 ppm Cu as copper amino acid complex (AAC); and 3) 21 ppm Cu as AAC in a step-down program (AAC SD). In Experiment 2 (Exp2), 1,728 chicks were vaccinated against coccidiosis at hatch, followed by an in-feed challenge with *Clostridium perfringens* (CL-15, Type A strain, 1X109/bird) at 17 days-of-age, were randomly assigned to 1 of 4 treatments: 1) 125 ppm Cu as TBCC (TBCC-Cp); 2) 21 ppm Cu as AAC (AAC-Cp); 3) 21 ppm Cu as AAC in a step-down program (AAC SD-Cp); and 4) 14 ppm Cu as AAC in a step-down program (AAC LowSD-Cp). Data obtained was analyzed by two-way ANOVA, using the GLM procedure of SAS. Means were separated by the Tukey test when $P \leq 0.05$. In Exp1, there were no significant differences in performance or mortality at d 42, nor in footpad scores at d 35. There was significantly lower excretion of Cu for both AAC and AAC SD treatments at 18 days ($P < 0.0001$), and for AAC at 42 days ($P < 0.0001$) compared to TBCC. Liver Cu was higher in treatments supplemented with AAC at both 21 ($P < 0.06$) and 35 days ($P < 0.0001$) compared to TBCC. In Exp2, there were no differences in performance or NE mortality at 42 days. At days 21, 28, and 35, intestinal lesion scores presented no differences between treatments. At d 35, treatments also showed no differences in footpad scores. In summary, there were no relevant differences in performance and health status of birds in either of the studies when Cu supplementation was reduced. This data suggests that decreasing overall Cu supplementation through the use of AAC Cu can maintain production performance while reducing Cu excretion and improving environmental sustainability.

ID : 485

ZINC AMINO ACID COMPLEX IMPROVES INTESTINAL HEALTH PARAMETERS IN BROILERS REARED UNDER CHRONIC CYCLIC HEAT STRESS CONDITIONS

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Heat stress (HS) not only impairs animal health and performance, but also negatively impacts intestinal morphology and gut barrier integrity in broilers. The objective of this study was to evaluate the effect of supplementing zinc (Zn) as ZnSO₄ or Zn amino acid complex (ZnAA) on intestinal health in broilers subjected to chronic cyclic HS (32°C ± 2°C and relative humidity of 50 to 65% for 6 h daily) applied in the finisher period. It has been suggested that vitamin E (Vit E) may help alleviate negative effects of HS, and therefore an increased inclusion level of Vit E was used as a positive control (100 IU/kg). A total of 1224 day-old male Ross 308 broilers were randomly distributed among 4 dietary treatments (9 replicates of 34 birds per treatment), organized in a 2×2 factorial arrangement: two Zn sources (60 ppm Zn as either ZnSO₄ or ZnAA) combined with two levels of Vit E (50 or 100 IU/kg) were added to a wheat/rye-based diet. A 3-phase feeding scheme was applied: Starter (d 0 to 10), Grower (d 10 to 28) and Finisher (d 28 to 36). Intestinal health was evaluated by measuring villus length (VL) and crypt depth (CD), along with infiltration of CD3+ T-lymphocytes in the propria mucosae in duodenum sections, and ovotransferrin leakage in ileum content. Statistical analysis was performed using R for Windows (v3.5.1) utilizing a General Linear Model (GLM)) with 'Zn source' and 'Vitamin E level' as fixed factors and block as a random factor (factorial analysis; pen as experimental unit). A main effect of zinc source on intestinal health parameters was observed, however, no interactions or main effect of Vit E was observed. Supplementation with ZnAA, independent of Vit E level, increased VL (d 10: +16%, P < 0.001; d 28: +15%, P = 0.028; d 36: +7%, P = 0.009). Likewise, at the end of the Finisher phase (d 36), infiltration of CD3+ T-cells in duodenum was decreased (11.2 vs 13.1 area %, P = 0.023) and ovotransferrin leakage in ileum content was lower (15.99 vs 20.12 µg/g, P = 0.094) in broilers fed ZnAA, independent of Vit E level. In conclusion, replacing ZnSO₄ with ZnAA in diets of broilers subjected to heat stress improved intestinal morphology and mitigated intestinal inflammation.

ID : 534

THE EFFECT OF LIMESTONE SOLUBILITY AND PHYTASE DOSE ON PHOSPHORUS DIGESTIBILITY IN BROILERS

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This study evaluated the effect of two limestones from a single source, with high and low solubilities and phytase doses on phosphorus (P) digestibility in broilers. Male Ross 308 broilers were reared in floor pens on starter (0-10d) and grower diets (11 to 19 or 23d) formulated to breed recommendations. At 19d and 23d, broilers were weighed individually, and allocated to 80 battery cages, with 6 birds per cage, based on similar BW per cage. The trial design was a 2x5 factorial arrangement with 2 limestone particle sizes/solubilities (0.123 and 0.722 mm geometric mean diameter (GMD) with 96 and 26% solubility at 5 min respectively), and 5 dose levels (0, 250, 500, 1000 and 2000 FTU/kg) of Buttiauxella sp. phytase expressed in T. reesei. The 10 test diets were randomly assigned to 8 replicate cages and fed for 34h in two blocks from 19–21d or 23–25d. Diets were based on corn, soybean meal, full-fat soya, canola, and sunflower oil cake and contained 0.4% Cr₂O₃ as a marker, fed ad lib in mash form. Limestone Ca was analyzed and added to the basal diet that contained no added inorganic P to achieve 0.75% Ca in test diets. After 34h of feeding test diets, the distal half of the ileum digesta was collected for apparent ileal digestibility (AID) of P measurement. Data were analysed as a two-way ANOVA using fit model in JMP (JMP 14) at a significance level of $P < 0.05$. No interaction was found between limestone solubility and phytase dose. Low solubility limestone increased AID P. Increasing phytase dose resulted in step wise increase ($P < 0.05$) in AID P with each incremental phytase dose. The AID P increased with increasing phytase dose from 0 to 2000 FTU/kg from 21.2 to 75.1%, and 35.5 to 83.5% with high and low soluble limestone respectively. Phytase at 2000 FTU/kg released 1.97 (0.87 to 3.08) and 2.21 (1.46 to 3.42) g/kg digestible P, respectively for low and high soluble limestones, calculated as digestible P above the non-supplemented diets. The measured digestible P content in NC was lower than the calculated value (1.49 g/kg, CVB) with high soluble limestone, which may lead to risk of P deficiency. These data confirm previous findings that a rapidly soluble fine limestone results in lower P digestibility in broilers. Increasing phytase to 2000 FTU/kg resulted in incremental steps in AID P and can be used as a strategy to reduce negative effects of limestone on P utilization and thereby improve the environmental sustainability of broiler production.

ID : 594

EFFECTS OF PHYTASE SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY AND BLOOD PARAMETERS IN LAYING HENS FED ON DIFFERENT LEVELS OF NONPHYTATE PHOSPHORUS

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An experiment was conducted to investigate the effects of various levels of nonphytate phosphorus with phytase supplementation on performance, egg quality, and some blood biochemical parameters in laying hens. A total of five hundred and forty 61 week-old Hy-line Brown laying hens were randomly allotted to six dietary treatments with five replicates and eighteen hens in each replicate. Three isocaloric and isonitrogenous diets were formulated to contain 2.0, 2.5 and 3.0 g NPP/kg diet with the level of 45 g Cakg-1 diet and supplemented with or without phytase 1000 FTY per kg diet (Ronozyme HiPhos-L, *Aspergillus oryzae* 6-phytase). All laying hens were subjected to the same management practices during the experimental period. Productive performance and egg quality were measured every four weeks for the entire eight-week experiment from 61 to 68 weeks of age. The Serum biochemical parameters were assessed at the end of the experiment. Data were analyzed in a completely randomized design using the GLM procedure of SAS software (SAS 9.1, 2009). The results showed that no significant differences were seen in hen-day egg production, feed intake, egg weight, egg mass and feed to egg mass ratio of laying hens at 64 and 68 weeks of age, however, egg production was tended to be numerically higher in phytase supplemented groups at 68 weeks of age as compared to the without phytase added groups. Egg quality including albumen height, Haugh unit, yolk color, shell color and egg shell breaking strength were not influenced by dietary treatments whereas egg shell thickness was significantly higher in phytase supplemented groups than that of unsupplemented at 68 weeks of age. Moreover, there was no influence effect of dietary treatments on albumin, total protein, glucose, cholesterol, high-density lipoprotein, aspartate aminotransferase, alanine aminotransferase and triglyceride concentrations in serum. In conclusion, dietary nonphytate phosphorus with supplemented phytase can improve egg production and egg shell thickness in laying hens.

ID : 834

CUO NANOPARTICLES INFLUENCE ON THE QUAILS EGG PARAMETERS AND GROWTH PERFORMANCE

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It has been becoming increasingly important to produce micronutrients of the size of nanometers. The aim of the research was to synthesize a nanoform of a trace mineral feed additive. The nanoform of trace mineral feed additives could be applied in poultry to improve the absorption of trace minerals and to decrease the amount of trace minerals in premixes. The research was carried out in order to investigate the influence of CuO nanoparticles on growth performance and egg parameters of Japanese quails (*Coturnix coturnix japonica*).

CuO nanoparticles were synthesized applying a wet chemical sol-gel method. For the synthesis of CuO, CuSO₄ • 5H₂O, NaOH and citric acid were used. Cu content in eggs was determined using the inductively coupled plasma emission spectrometry. The quails were grown in cages from the first day of age to 12 weeks of age. For the trial, the quails were divided into three groups. The first group was a control group, and the quails were fed with standard feed. The feed for the quails in the second group was enriched with the inorganic CuSO₄ • 5H₂O additive. The feed for the quails in the third group was enriched with the CuO nanoparticles additive. The following parameters were investigated: the amount of Cu in eggs yolk and albumen, the influence of the CuO nanoparticles additive on the growth of quails, laying intensity, and an average egg weight.

The experiment and the analysis of the study showed: the highest level of Cu in the egg albumen was observed in the quails at 12 weeks of age in the second trial group (2.941 µg/g), i.e. it increased by 25.74 percent in comparison with the control group (p increased from 206.73 g to 218.12 g, i.e. it increased by 5.51 percent in comparison with the control group (p

Keywords: nanoparticles, copper, trace element, egg parameters, quails.

ID : 905

PERFORMANCE AND MINERALIZATION IN BROILERS: WHAT ARE THE BEST DIETARY LEVELS OF AVAILABLE PHOSPHORUS AND CALCIUM, REGARDING CALCIUM TO AVAILABLE PHOSPHORUS RATIO?

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Low phosphorus (P) diets may support sustainability in broiler production. In fast growing broilers, it is, however, observed that low P diets leads to inconsistent bone mineralization and welfare outcomes. This may be due to differences in available P (aP) utilization. The latter is conditioned by dietary calcium (Ca) supply. Currently, little is known about aP and Ca:aP requirements of broilers. Thus, a trial was performed to study the effect of dietary aP and Ca:aP ratio on the growth performance, carcass yield and tibia characteristics. A total of 384 day old male Ross 308 broiler chicks were randomly assigned to six dietary groups, each replicated 16 times, for 42 days (d). Dietary groups consisted in different feeding sequences with low (L), medium (M) and high (H) aP levels in the starter (L:0.45; M:0.50, H:0.55), grower (L:0.35, M:0.40, H:0.45) and finisher feeds (L:0.27, M:0.32, H:0.37) with low (1.8) or high (2.2) Ca:aP ratios. Groups were arranged as follows: LLL2.2, MMM2.2, HHH2.2, HHL2.2, LLL1.8 and HHH1.8. Orthogonal contrasts were performed for statistical analysis. Dietary treatments did not differ significantly in growth performance during the starter and grower phase. In the finisher phase, FCR and ADG tended to improve linearly with aP level at the highest Ca:P ratio ($P < 0.1$). A decrease in growth performance was observed with low aP in finisher phase, suggesting a high aP requirement during this phase. Concerning bone characteristics, no effect was observed at 21 d. At 42 d, the decrease of aP in HHL2.2 led to lower tibial breaking strength (TBS) and stiffness (TS), resulting in values similar to LLL2.2. In contrast, HHH2.2 resulted in a higher tibial TBS and TS compared to HHL2.2 and LLL2.2 ($P < 0.05$). LLL1.8 resulted in the lowest tibial TBS and TS values of all groups. HHH1.8 did not differ from HHH2.2 for those parameters. Concerning carcass traits, LLL2.2 had a significantly higher breast meat yield than LLL1.8 (+1.1 pt; $P < 0.05$). In conclusion, this trial demonstrates that decreasing aP in the finisher phase negatively impacts growth performance. Low aP with low Ca:aP ratio seems to be the worst sequence. In contrast, high aP seems to be mandatory to achieve the best bone strength, irrespective of Ca supply. Overall, high aP with high Ca seemed to be the best strategy for both criteria. Future trials could investigate the effect of reducing aP and Ca during the starter and grower phases, e.g. with LLH2.2 or LLH1.8 sequences.

ID : 928

EFFECT OF A SODIUM ELECTROLYTIC ACIDIFIER ON LAYING HENS PERFORMANCE AND EGG SHELL QUALITY

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The dietary inclusion of acidifiers can contribute to the gut health and improve the absorption of some minerals and others nutrients by a decrease in pH and a stimulation of the villus height. The aim of this study was to evaluate the effect of the dietary addition of a sodium electrolytic acidifier on the performance of laying hens under typical production conditions. The trial was carried out at the facilities of a commercial egg producer to compare two treatments (T1: Control and T2: Idem to T1 plus acidifier) during 4 periods of 28 days. Two repetitions per treatment were used. A total of 4202 laying hens, were allocated in two barns (500 cm² of cage per hen), one with 1868 Hy-Line W-80 (81 weeks old) and another with 2334 Lohmann LSL-Lite (47 weeks old). A completely randomized block design was used. Each barn was considered as a block and divided longitudinally conforming two experimental units (lots). Control diets based on corn and soybean meal were formulated according to the lines recommendations and hens feed intake. The same diets were used in T2 replacing part of sodium chloride and corn by the acidifier (1.5 kg/MT). Each kg of acidifier replaced 600 g of sodium chloride. The rest of nutrients were the same for both treatments. The acidifier used is based on a mix of natural extracts (polyphenols from *Schinopsis* sp. and citrus pectins) and sodium bisulfate (AES®, Porfenc SRL). Egg production (%), feed intake (g/day), egg weight (g), feed conversion ratio (g/dozen eggs) and egg shell quality (mg/cm²) were analyzed at the end of each period, and body weight at the end of the 4th period. Data were analyzed by a 2-way ANOVA. No differences in feed intake and egg weight were observed between treatments throughout the four experimental periods ($P>0.05$), while egg production was higher along all the trial when AES was used (Control: 78.2% vs. AES: 80.8%, $P<0.05$; means of the 4 periods), so feed conversion ratio was also better with AES (Control: 2.00 kg of feed/dozen eggs vs. AES: 1.92, $P<0.10$). Hens from Control treatment were heavier than those from the AES treatment (Control: 1679 g vs. AES: 1634 g; $P<0.10$). On the other hand an improvement in egg shell quality was observed (Control: 82.1 mg/cm² vs. AES: 83.9 mg/cm²; $P<0.10$). This result was associated with a reduction in the excreta calcium content. In conclusion, the inclusion of AES in laying hens diets improved the egg production, feed conversion ratio and the egg shell quality.

ID : 971

OPTIMIZE PHYTASE DOSE BASED ON PHYTATE LEVELS IN BROILER FEED FORMULATION

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Phytic acid [inositol (1,2,3,4,5,6) hexakisphosphate] or phytate (salt of phytic acid) is the major phosphorus (P) storage in plant-based ingredients. A large portion (60-80%) of the phosphorus in the plant-based ingredients occurs in the form of phytate. Mono-gastric animals have limited capability of utilizing the phytate-bound P. To improve the availability of plant-based phosphorus, phytase is commonly applied in poultry diets. When first launched, phytase was commonly used at 500 FTU/kg with the main purpose of reducing the P excretion to the environment. Recently, with more efficient and cost effective phytases in the market the phytase inclusion rate has increased to up to 5000 FTU/kg based on literature. However, optimal dose of phytase is related to the level of the phytate substrate in the diets. Phytate content of major feed ingredients could be derived from literature or feed tables but this does not account for variability in different plant-based feedstuffs, not only due to plant type, but also depending on the growing conditions and soil types and contents. This study reported the analyzed phytate-P content in 37 major feed ingredients sourced globally and analyzed by DuPont lab in the last 10 years. IP6 levels were determined by HPLC and then Phytate P calculated. In total more than 1000 samples were analyzed from more than 40 countries. The average Phytic acid, Phytate P content, variation, minimum and maximum levels were determined. The data showed there was a large variation in phytate content for the same feed ingredient, such as corn, the phytate-P content is in a range of 0.14 to 0.30% with a mean value of (256 samples). For SBM, phytate-P content is in a range of 0.28 to 0.54% (214 samples), and the range for wheat (97 samples) and barley (64 samples) is from 0.18 to 0.31% and 0.14 to 0.34% respectively. For ingredients with high phytate content, the variation is even bigger, for example, phytate-P content is in the range of 0.58 to 1.10% and 1.34 to 2.15% for wheat bran (61 samples) and rice bran (40 samples) respectively. Understanding the substrate and phytase ratio is important to determine the optimal phytase dose in feed formulation. Previous research has demonstrated that substrate level will impact the magnitude of response seen with phytase supplementation in terms of the extra-phosphoric effects (energy and amino acid sparing) and therefore knowing the accurate phytate level can be used to identify the optimal dose for feed cost savings for a given diet.

ID : 989

EFFECT OF DIFFERENT SOURCES OF CU ON ANTIMICROBIAL RESISTANCE IN BROILER CHICKENS

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Copper (Cu) requirement for broilers is 8 mg/kg diet. The use of higher doses (125 to 250 mg/kg) has a growth promoter effect but may increase the antimicrobial resistance in broilers. Therefore, the objective of this trial (part of Project E! 11780) was to study the effect of 150 mg/kg of Cu as Cu sulfate (CuSO_4), or as dicopper oxide (Cu_2O , CoRouge®) on the antimicrobial resistance in *Enterococcus* spp. and *Escherichia coli* (*E. coli*). A total of 300 one-day-old male broilers (Ross 308) were distributed into 3 experimental treatments (10 pens/ treatment and 10 birds/ pen): negative control (NC), with no Cu addition, and NC with 150 mg/kg of Cu supplemented as CuSO_4 or Cu_2O . Broilers were challenged by reusing an old litter with high concentrations of *Clostridium* spp. At d 16, 28 and 42 fecal samples were collected from one broiler per pen. Samples were analyzed for microbiological identification of *Enterococcus* spp. and *E. coli*. A phenotypical antimicrobial resistance test was performed to determine the resistance of *Enterococcus* spp. and *E. coli* isolates against thirteen antimicrobial agents. Additionally, extracted bacterial DNA was used to detect the antimicrobial resistance genes of vancomycin (*vanC1* and *vanC2*), tetracycline (*tet M*), erythromycin (*erm B*), copper (*trcB*) and zinc (*czcA*). Data were analyzed using the chi-squared test. There were no differences among treatments on the phenotypic antimicrobial resistance on d 16 and 28. However, on d 42 the isolated *Enterococcus* spp. from broilers fed the NC diet had higher ($P < 0.05$) resistance to enrofloxacin, gentamicin, and chloramphenicol compared to isolates from birds fed 150 mg/kg of Cu as CuSO_4 and Cu_2O . By contrast, in the isolated *E. coli* from broilers fed 150 mg/kg of Cu as CuSO_4 and Cu_2O had higher ($P < 0.05$) resistance to streptomycin (78 and 56%, respectively) and chloramphenicol (56% on average) compared to isolates from birds fed the NC diet (11 and 0%, respectively). The percentage of resistant strains in *Enterococcus* spp. was high ($> 80\%$) for all the studied genes, treatments, and days. However, in the isolated *E. coli* the frequency of resistant strains was lower ($< 20\%$) for *VanC1* and *VanC2* in all treatments and days, but for *TcrB* gene resistance increased over time in all treatments. To conclude, the antimicrobial resistance is affected by time and by bacterial species and likely by the microbiota background, but the effect of Cu source remains unclear yet, and more research is required.

ID : 1251

EFFECTS OF DIETARY SLENIUM SOURCES ON PRODUCTIVE PERFORMANCE, HATCHING TRAITS AND ANTIOXIDANT STATUS OF COMMERCIAL BROILER BREEDERS

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Objective of the experiment was to evaluate the effects of different dietary sources of selenium on productive performance, hatching traits, antioxidant capacity and immune responses of commercial broiler breeders (Ross-308). A total of 600 female and 180 male, 50-wk-old Ross-308, broiler breeder birds were randomly allotted to 4 treatments, having 5 replicates with 30 females and 9 male birds each, with a 14-d pre-treatment and 98-d trial period. Birds were fed a basal corn-soybean diet supplemented with inorganic i.e. Sodium Selenite (SS; control) and other three groups were fed diet supplemented with. Selenium enriched yeast, L-Seleno-methionine (L-Se-Meth) or Seleno-hydroxy-methionine (OH-Se-Meth), respectively, at 0.3 mg/kg of diet. Egg production and quality parameters, fertility and hatchability were measured as performance indicators. After incubation, chicks of each replicate were individually weighed and graded separately. Glutathione peroxidase activity, total antioxidant capacity and antibodies titer against Newcastle disease virus was also measured. Data collected were analyzed using GLM Procedures of Minitab Statistical Software 18. Means were compared using Tukey's Test. Dietary selenium sources did not affected egg production and their quality parameters, fertility rate, hatchability percentage and antibodies titers against Newcastle disease virus at any phase of experiment. Glutathione peroxidase status, total antioxidant capacity and chick quality were improved by inclusion of organic selenium in the diet of broiler breeders. It was concluded that organic selenium supplementation improved chick quality and antioxidant status, however, did not affect body weight, flock uniformity, egg production and their quality, and immune responses in commercial Ross-308 broiler breeders.

Keys words: Selenium sources, egg production, egg quality, antioxidant status, immune response, broiler breeders

ID : 1261

EFFECT OF WHEAT MALTING ON ZOOTECHNICAL PERFORMANCE OF SLOW GROWING BROILER CHICKENS

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In conventional production systems, exogenous phytase is used as feed additive to increase phosphorous (P) digestibility. However, this is not allowed in European organic farming systems. Earlier studies suggest that a germination (malting) is accompanied by significant hydrolysis of phytic acid (Lemmens et al., 2018). The aim of this experiment was therefore to investigate the effect of malted wheat on the zootechnical performance of slowly growing meat chickens under the terms of practical feeding according to the rules of organic farming. Four different diets were formulated for this experiment containing either wheat or malted wheat in combination with and without phosphorus (P) supplementation from 1.5% Monocalcium-phosphate, respectively (diet A: malted wheat + low P; diet B: malted wheat + high P; diet C: wheat + low P; diet D: wheat + high P). 2.000 1-day-old broiler chickens (both sexes) were used for this experiment. From d1 to d16 the birds were fed with an organic starter diet. On d17 the equal number of birds from both sexes were randomly allocated to the four experimental diets (5 replicates per diet, 100 birds per pen) comprising a completely randomized design. Live weight (LW), feed (FC) and water consumption (WC) was recorded pen wise on a weekly basis. Statistical analyzes comprised ANOVA (wheat variant, phosphorus and interactions). From d28 on, birds fed with wheat had a significantly higher LW (760 ± 40 g) than birds fed with malted wheat (700 ± 50 g; $p = 0.0081$). Surprisingly, P content had no significant effect on LW and FC during the whole experimental period. But groups fed with wheat had also a significant higher FC (89 ± 5 g vs. 81 ± 5 g) from d28 onwards (p

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ID : 132

EFFECT OF A NOVEL 6-PHYTASE AT 250 AND 500 FTU/KG ON PERFORMANCE AND BONE ASH IN BROILERS

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A broiler trial was set up with 480 male ROSS 308 broilers distributed over 24 pens to investigate the effect of a novel thermostable 6-phytase (OptiPhos® Plus, Huvepharma NV) on performance and tibia bone ash content. All birds were fed a P sufficient starter diet (day 1 to 5) after which different grower feeds (day 5-21) and a finisher feeds (day 22-35) were provided. The starter feed contained 21.5 % CP, 0.90 % Ca and 0.45 % available P (aP). The grower feed contained 20.5 % CP, 0.75 % Ca and 0.34 % aP and the finisher feed contained 19.4 % CP, 0.7 % Ca and 0.31 % aP. A negative control (NC) grower and finisher feed was produced by reducing 0.10 % Ca and 0.15 % aP from the PC. To this NC, 250 and 500 FTU/kg of phytase was added. Feeds were pelleted at $\pm 80^{\circ}\text{C}$ (starter was crumbled). Technical performance was measured after every feeding phase. At day 21, 2 birds per pens were selected with a weight close to the pens' average weight and the right tibia was removed, pooled to one sample and analysed for ash on fat free dry matter. The basic statistical models employed were one-way ANOVA, whereby $P \leq 0.05$ was considered statistically significant.

Reducing Ca and P from the PC led to reduced end weight (2004 g for the NC vs 2425 for the PC, $P < 0.05$) while FCR was non-significantly higher (1.47 vs 1.44). Adding phytase brought performance up to 2293 g for 250 FTU/kg (sign. different from PC and NC) and 2323 g at 500 FTU/kg (sign different from NC, but not from PC). The FCR were not significantly different from PC and NC (1.46 and 1.45 for 250 and 500 FTU/kg). Tibia ash dropped from 49.4 % (PC) to 41.6 % for the NC ($P < 0.05$), while the addition of the phytase brought bone ash levels up to 45.5 and 46.5 % for 250 and 500 FTU/kg respectively ($P < 0.05$ vs NC). It can be concluded from this trial that OptiPhos® Plus already at low inclusion level of 250 and 500 FTU/kg to a P deficient diet could increase performance of the broilers to the same level as broiler fed a P sufficient diet, while bone ash was also increased.

ID : 133

EFFECT OF A NOVEL E. COLI 6 PHYTASE ON BROILER PERFORMANCE, BONE ASH AND ILEAL DIGESTIBILITY OF PHYTATE, PHOSPHORUS AND PROTEIN AT LEVELS UP TO 1000 FTU/KG

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A broiler trial was set up (98 floor pens, 17 male ROSS 308 broilers per pen) to investigate the effect of a novel thermostable 6-phytase (OptiPhos® Plus, Huvepharma NV) on performance, bone mineralization and ileal digestibility of total phosphorous (P) and crude protein (CP). All birds were fed a P sufficient starter diet (day 1 to 5) after which different grower feeds (day 5-21) and finisher feeds (day 22-35) were provided. The starter feed contained 21.5% CP, 0.90% Ca and 0.45% available P (aP). The grower feed contained 20.5% CP, 0.80% Ca and 0.32% aP and the finisher feed contained 19.4% CP, 0.75% Ca and 0.30% aP. A negative control (NC) grower and finisher feed were produced by reducing 0.15% and 0.15% Ca and 0.17% and 0.15% aP from grower and finisher, respectively. To this NC, 0, 250, 500, 750 and 1000 FTU/kg of phytase was added. Feeds were pelleted at $\pm 80^{\circ}\text{C}$ (starter was crumbled). Feed contained an indigestible marker (0.4% TiO₂) in order to calculate digestibility. Technical performance was measured after every feeding phase. At day 21, 2 birds per pens were selected close to the pens' average weight and the right tibia was removed, pooled to one sample and analysed for ash on fat free dry matter. At day 35, 9 birds of each pen were killed to collect ileal material (from terminal ileum to 10 cm before the ileo-cloacal junction) for the determination of P, CP, and Ti. The body weight (BW) and feed conversion (FCR) for the NC were 1.58 kg and 1.57, respectively. Adding phytase increased the BW and FCR in a dose-responsive way reaching 2.30 kg and 1.52, respectively at 35 days ($p < 0.05$ vs phytase treatments). Similarly, bone ash was increased from 36.9% to 45.9% at 0 and 1000 FTU/kg phytase ($P < 0.05$ for all phytase levels vs NC), while the PC reached 47.9%. Protein digestibility in the PC and NC were 69.8 and 73.5%, respectively, while protein digestibility increased to 78.9, 79.2, 77.8 and 77.6% for 250, 500, 750 and 1000 FTU/kg phytase, respectively ($p < 0.05$ vs NC and PC at all phytase levels). The addition of phytase to the NC feed increased dig. P by 1.00, 1.12, 1.54 and 1.53 g/kg feed at 250, 500, 750 and 1000 FTU/kg, respectively. It can be concluded from this trial that the addition of OptiPhos® Plus at increasing levels to a P deficient diet increased the performance, bone ash formation and digestibility of P.

ID : 318

TOWARDS PRECISION FEEDING IN LAYING HENS: UPDATE AND VALIDATION OF A MATHEMATICAL MODEL TO PREDICT DAILY CALCIUM AND PHOSPHORUS FLOWS

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In laying hens, precision feeding could consist in providing an optimal calcium supply (level, source, and distribution kinetics) to ensure the well-being and longevity of animals (i.e. limiting osteoporosis and bone fractures) while maintaining eggshell quality. During eggshell synthesis (occurring mostly by night), hens can mobilize calcium from bones stored in a specific structure called the medullary bone. The calcium stock is then rebuilt after oviposition. However, the animal cannot always fully compensate for a supply deficiency in calcium from feed. An intense and repeated mobilization / reconstitution of the medullary bone can thus lead to long-term and severe lesions affecting the animal's skeleton. To better understand and predict daily calcium requirements, and minimize bone mobilization, an existing mathematical model [1] describing calcium and phosphorus fluxes in the different compartments of the animal (i.e. digestive tract, blood, bone, and egg) was implemented and adapted. The main adaptation of the existing model was to include the ability to simultaneously consider different calcium sources (i.e. meal vs. particles) with different associated solubilization rates. An experimental trial involving 288 Lohmann Tradition laying hens from 19 to 33 weeks of age was conducted. Six different dietary treatments were tested (4 pens of 12 hens/treatment). They differed according to the form of calcium carbonate (i.e. meal, two different particle sizes, or a mix of meal and particles) and to the relative amount of feed distributed in the morning and in the afternoon (30:70 or 70:30). Three sampling series at 27, 30, and 33 weeks of age were performed to measure ionic calcium and inorganic phosphorus blood concentrations over 24 hours (3 to 6 samples per hen). The model was then calibrated and subsequently validated according to these measured concentrations. In its current state, the model can be seen as a decision support system (DSS) for the precision feeding in laying hens, giving to the user an insight on what could be the best feeding strategies to maintain animal longevity, increase well-being or even reduce feed cost, at the scale of an individual hen. In the future, this model could also be implemented in a new tool, taking into account the variability among birds (e.g. oviposition time), to identify the best feeding strategies at the flock level.

[1] Kebreab et al. (2009). Poultry Science, 88 (3), 680–689.

ID : 697

EFFECT OF SUPPLEMENTING CALCIUM PIDOLATE AND PHYTASE IN DIETS WITH LOW LEVELS OF CALCIUM AND PHOSPHORUS ON BROILER PERFORMANCE

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The calcium–phytate complex may reduce calcium (Ca) absorption and may also reduce the activity of phytase. Decreasing dietary Ca may improve phosphorous (P) utilization, while an excess of Ca may aggravate a P deficiency. Therefore, different researchers have shown that a moderate reduction on dietary Ca had no deleterious effects on broiler performance. Consequently, we tested the hypothesis that a decrease in the level of Ca and P may not affect broiler performance if molecules to improve mineral retention were included in the diet. The aim of this study was to evaluate the effect of Ca pidolate and phytase dietary supplementation on the broilers performance fed low Ca and P diets. A total of 486 day-old male chicks (Cobb-500) were allocated in floor pens in a completely randomized block design (9 replicates/treatment). Pre-starter (1-7 d), starter (8-21 d), grower (22-28 d) and finisher (29-41 d) diets were used for dietary treatments. Control diets (CTRL) were formulated according to Cobb recommendations (2018). Low Ca and P diets (LCP) were formulated to obtain a 20% reduction in total Ca and available P, and no differences in metabolizable energy and the main digestible amino acids. Another set of LCP diets were formulated to include Ca pidolate and phytase (LCP+PP diets). Ca pidolate (PIDOLin PCa, Dietaxion) was included on-top between 0-21 d at 300 ppm. Phytase matrix was used for the formulation of the LCP+PP diets (750 FTU/Kg feed, Aextra PHY, Dupont). Birds and feed were weighed weekly individually and by pen, respectively, and the mortality recorded daily. Data were analyzed as a 2-way ANOVA. The pen was considered the experimental unit. Although there were not significant differences ($P>0.05$) in feed intake at the end of the experiment, CTRL broilers had a higher feed intake than LCP and LCP+PP ($P<0.05$). Also for feed conversion ratio there were not significant differences between treatments ($P>0.05$). Using the performance results at the end of the experiment and the cost of each diet, the feeding cost per kg was calculated and were (USD/kg): 0.271, 0.289 and 0.299 for LCP+PP, LCP and CTRL, respectively ($P<0.05$). It can be concluded that total Ca and available P concentrations can be reduced by 20% in broiler diets with no effects on their performance. If done in combination with Ca pidolate and phytase allowed to lower the feeding cost.

ID : 1341

EFFECTS OF IN OVO INJECTION OF NANO SELENIUM AND NANO ZINC OXIDE AND HIGH EGGSHELL TEMPERATURE DURING LATE INCUBATION ON HATCHABILITY AND ANTIOXIDANT CAPACITY AND GENE EXPRESSION OF HEAT SHOCK PROTEIN 70 OF BROILER HATCHLINGS

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Introduction

With the onset of pulmonary respiration after internal pipping in the chick embryo, the presence of reactive oxygen species (ROS) and oxidative stress is higher, while natural antioxidant reserves have not reached an adequate level for innate protection (Surai, 2007). Also, it is reported that heat stress cause oxidative stress (Elnesr et al, 2019). Therefore the effect of in ovo injection of Nano-Se and Nano-ZnO and high incubation temperature was evaluated.

Materials and Methods

On 17 day of incubation (doi), 750 fertile eggs assigned to 5 experimental treatments of 5 replicates including non-injected eggs incubated at normal incubation temperature (37.8°C) or high incubation temperature (38.9°C) and eggs injected with 0.5 ml 0.9% NaCl solution or 40 µg Nano-Se or 500 µg Nano-ZnO in 0.9% NaCl which are incubated at high incubation temperature. Eggs of heat stress group were exposed to high temperature from 19 to 21 doi. Blood samples of 10 hatchlings per treatment were collected and plasma malondialdehyde (MDA) and total antioxidant capacity (TAC) were determined. Total RNA of liver samples were extracted and Real-time PCR was used to evaluate the liver HSP70 gene expression. Data were subjected to the GLM procedure for ANOVA. Means were compared using Duncan's test.

Results

Percentage hatchability was not affected by treatments. Egg exposure to high incubation temperature reduced TAC and increased MDA of hatchlings plasma. In ovo injection of Nano -ZnO and Nano-Se restored the TAC plasma and reduced MDA concentration. Although embryonic heat stress during late incubation increased gene expressions of HSP70, in ovo injection of the nano minerals ameliorated the adverse effects of heat stress.

Conclusion

It is concluded that in ovo injection of Nano-ZnO and Nano-Se could reverse negative effects of high incubation temperature during late incubation on oxidative status and liver HSP70 gene expression in broiler hatchlings.

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PHYSIOLOGY OF NUTRITION

ID : 30

COMBINING ALGAL EXTRACTS TO IMPROVE BROILER PERFORMANCE THROUGH INFLAMMATION REDUCTION IN THE GUT

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The poultry industry is continually under pressure to reduce the use of antibiotic/antimicrobials in feed. Macro algae contain unique sulphated beta-glucan polysaccharides that have shown to be anti-inflammatory and antibacterial when used in animal feed of pigs and poultry (O'Doherty et al., 2010), (Yan et al., 2011). However, their use in poultry is currently limited due to variations in purity, different extraction methods and difficulty in assessing the ratio of the extracts when used together (Fitton, 2011).

The present study was conducted to investigate effects on inflammation markers and performance of broilers. The marine algal extracts, fucoidan (500PPM) and laminarin (300PPM) were fed separately and in combination (500PPM and 300PPM respectively) with a control diet to 1,200 Ross 308 males, half of the birds were then challenged with *Salmonella typhimurium* at day 8 resulting in 8 treatments. Weight gain analysis for the period 7-14 days, the days immediately post challenge, show that when *S. typhimurium* challenged birds were fed the fucoidan extract during this time no difference in weight gain to non-challenged birds was observed, where all other treatments had significantly ($P=0.045$) lower weight gain for the same period compared to the non-challenged treatments. When investigating the inflammation response, adding fucoidan increased calprotectin levels by only 52% for fucoidan alone and 46% for the fucoidan and laminarin combination when compared to the control without challenge, however, the laminarin only treatment significantly ($P=0.042$) increased calprotectin levels by 72% after the *S. typhimurium* challenge compared against the unchallenged control. These results suggest a difference in mode of action in response to the *S. typhimurium* challenge between the two test materials.

These recent works show the potential of high quality marine sulphated polysaccharides, when used in broiler feed, to improve performance through a reduction in inflammation after infectious challenge and via inflammatory responses in the gut.

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ID : 40

GROWTH HORMONE, INTESTINAL MORPHOLOGY AND JUVENILE GROWTH OF BROILER CHICKENS FED DIFFERENT LEVELS OF CURCUMA LONGA UNDER HOT HUMID TROPICAL CLIMATE

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The performance of broiler chickens in the tropical environments in the absence of temperature-controlled housing facilities is suboptimal as a result of thermal stress. Nutritional strategy has been proposed as a viable means of mitigating the adverse effect of thermal stress on the birds to reduce economic losses. This study, therefore aimed at evaluating the juvenile growth, growth hormone and intestinal morphology of broiler chickens fed different levels of curcuma longa under hot humid tropical climate. Two hundred and forty broiler chicks were randomly allocated to four dietary treatments of basal diets supplemented with 0 (CT), 4 (TG), 8 (FT) and 12g (SG) of turmeric powder/Kg of diet, having 4 replicates of fifteen birds each, in a Completely Randomized Design for 8 weeks. Data were collected weekly on feed intake and body weights at the juvenile age. Blood samples were collected from 8 birds per treatment at week 3 (starter phase) and week 8 (finisher phase) of the experiment for the determination of plasma growth hormone. At day 56 of age, 8 birds per treatment were slaughtered and dissected. 2cm segments of duodenum, jejunum and ileum were collected for the measurement of villi width, villi height and crypt depth and villi height: crypt depth ratio. Data obtained were analyzed using one-way ANOVA using SAS (2008). Results showed that the weight gain of the chicks in SG and TG were however comparable to that of CT but the weight gain of the chicks in FT was higher than that of CT. The feed intake of the birds was similar across the treatment group at week 2. FCR of the birds in CT was similar to those of TG and SG but higher than that of FT. Plasma growth hormone was similar at starter and finisher phases. Duodenal villi width of the birds in SG, TG and CT was similar but lower than that of FT. The ileal crypt depth of chickens in FT was lower than those of CT but similar to those of the other treatment groups. There was no difference in the crypt depth of jejunum. The ratio of villi to crypt depth ratio in the duodenum was in the order of FT > SG > TG > CT. The ratio in ileum was higher in FT than that of TG which was higher than SG whose value was higher than that of CT. It was concluded 8g/Kg diet Curcuma longa improved juvenile growth and nutrient absorption through enhanced intestinal morphology of broiler chickens under hot humid tropical climate.

ID : 41

THE EFFECT OF FUMONISIN CONTAMINATION ON BROILERS IMMUNE RESPONSE AFTER VACCINATION

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There is a market request for feed additives that help to overcome immunosuppression by mycotoxins after vaccination. To determine immunosuppression by fumonisins (FUM) after vaccination and to screen for a solution, a 3x2 full factorial design was setup. In total, 330 Ross-308 birds (5T*6R*11) were randomly allocated to: (1) a control diet; (2) a contaminated diet with 20 ppm FUM; (3) a contaminated diet with 100 ppm FUM; (4) a contaminated diet with 20 ppm FUM supplemented with Elitox® at 0.25% or (5) a contaminated diet with 100 ppm FUM supplemented with Elitox® at 0.25%. At d7, 2 birds per pen were subcutaneously vaccinated against Newcastle disease with a killed vaccine (Poultvac, Zoetis). Blood samples were taken from 2 vaccinated and 2 non vaccinated birds per pen at d28 to determine the humoral immune response (major histocompatibility complex II (MHCII) expression, Immunoglobulins and Antigen Presenting Cells (APC)), and cell-mediated immune response by interferon gamma (IFN- γ). Vaccination resulted in higher percentages of APC ($p=0.0262$) and monocytes ($p<0.0001$), but lower levels of MHC II expression by APC ($p=0.0026$). Whereas, increasing levels of FUM resulted in decreasing levels of APC ($p=0.0456$) and monocytes ($p=0.2012$). This clearly indicates the adverse effects of mycotoxins on the humoral immune response after vaccination. Low levels of FUM (20 ppm) resulted in decreased levels of MHC II expression, whereas contamination with higher levels (100 ppm FUM) resulted in increased MHC II expression by monocytes ($p=0.0041$) and APC ($p<0.0001$). This suggests that more energy is allocated towards immunity in the highly contaminated birds, as confirmed by the numerically worse performance. The amount of IFN- γ was also significantly increased in blood of birds receiving 100 ppm FUM ($p=0.0001$), indicating that FUM at such high levels promotes inflammation with its repercussion on performance. Supplementation of Elitox® not only resulted in numerically heaviest animals but also restored contaminated birds humoral and cell-mediated immune response after vaccination, by reducing the increased IFN- γ at 100 ppm ($p=0.0001$) and by increasing MHC II expression at 20 ppm FUM ($p=0.0089$). In general it can be concluded that FUM had an inverse effect on vaccination and that Elitox® could prevent the losses induced by FUM contamination.

ID : 98

DIETARY SUPPLEMENTATION WITH A MICROENCAPSULATED BLEND OF ORGANIC ACIDS AND BOTANICALS ALTERS METABOLIC PATHWAYS IN THE JEJUNUM TO ENHANCE GROWTH AND FEED EFFICIENCY IN BROILERS

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A microencapsulated feed additive containing citric and sorbic acids, thymol, and vanillin (AviPlus®P) has been shown to increase growth rate and feed efficiency in broilers; however, the mechanisms associated with these benefits are not fully understood. The objective of this study was to identify key metabolic signaling pathways activated by dietary supplementation with this blend of organic acids and natural botanicals. Day-of-hatch by-product breeder chicks were randomly assigned to a control diet or a diet supplemented with 500 g/metric ton AviPlus®P (n=2 replicate pens/group). At 15-d-of-age, a section of jejunum was collected for kinome analysis using an immuno-metabolism peptide array to compare control and treated tissues (n=5 birds/pen [10 birds/treatment were used for the analyses]). The data was analyzed using PIKA2 peptide array analysis software and fold-change between control and treated groups determined. The P value was calculated by conducting a one-sided paired t-test between treatment and control values for a given peptide. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using Search Tool for the Retrieval of Interacting Genes (STRING; <https://string-db.org>). Changes to key metabolic pathways include: Insulin, PI3k-Akt, AMPK, FoxO, mTor, and adipocytokine signaling pathways. Additional analysis of the AMPK and insulin signaling pathways revealed numerous peptides with increased ($P<0.05$) levels of phosphorylation. Specific proteins that were associated with these peptide changes in the AMPK (5' adenosine monophosphate-activated protein kinase) pathway included PFK and G6Pase which impact glycolysis; ACC and CPT1 which are involved in fatty acid oxidation; or CyclinD which can result in growth arrest. The Insulin signaling pathway had increased phosphorylation of IRS, PDK1/2, TSC2 and 4EBP1 which can lead to protein synthesis and reduced cell growth. The changes in these signaling pathways provide insight into how supplementation specifically impacts energy use and production in a segment of the gut. Collectively, the kinome analysis showed key differences in the jejunum sample from the supplement-fed birds compared to those on the control diet. The data provide insight into the mechanisms and pathways associated with increased growth rate and feed efficiency in broilers following supplementation with a microencapsulated feed additive containing organic acids and natural botanicals.

ID : 103

BIOCHEMICAL BLOOD PARAMETERS IN LAYING HENS FED DIETS SUPPLEMENTED WITH DIFFERENT VEGETABLE OILS

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The experiment was performed on 10 Hisex White hens (28-46 weeks of age) fed a diet optimized according to the recommendations for this. The birds were allotted to four treatments: control treatment 1 was fed the basic diet supplemented with 2.6% of sunflower oil; the latter was substituted in the experimental treatments 2-4 by soybean, rapeseed, and flaxseed oils, respectively. The blood was sampled (2-3 ml) from the axillary vein after 14 hours of starving, the solution of sodium citrate was added. The samples were centrifuged at 5000 rpm for 5 min. The activities of the digestive enzymes in serum were determined on semiautomatic biochemical analyzer BS-3000P (Sinnova, China) and automatic analyzer ChemWell 2900 (T) (USA) using reagent kits Human (Germany). The tryptic activity was determined with benzoyl-DL-arginine p-nitroanilide (BAPNA) as a substrate. All experiments were performed in 5 replicates.

The tryptic activity in serum in treatments 2 and 3 was significantly higher in compare to control by 84.3 and 87.6%, respectively (P

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ID : 104

THE ADAPTATION OF EXOCRINE PANCREATIC FUNCTION IN LAYING HENS TO DIETARY LIPID INGREDIENTS

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The use of different vegetable oils in poultry nutrition is still an urgent problem. The published data of the research on broiler chicks evidence that different oils can affect the metabolism and growth efficiency. However, the mechanism of the influence of different vegetable oils on the exocrine pancreatic function is still understudied. The physiological study presented was performed on three Hisex White laying hens (*Gallus gallus* L.) with chronic fistulae of main pancreatic duct inserted using the method of Batoev (1970). The method of periods (7-10 days) was used. In the control period the hens were fed standard layer diet supplemented with sunflower oil; in experimental period 1 the sunflower oil was substituted by soybean oil; in period 2 – by rapeseed oil; in period 3 – by flaxseed oil. The amount of pancreatic juice released during the collection period and enzymatic activities within the juice were determined.

It was found that lipase activity is adapting to the oil used: the highest lipase activity was found in control (with unrefined sunflower oil); the increase in lipase activity was accompanied by the increase in the amylase activity. The postprandial dynamics of activity of the pancreatic lipase evidenced that sunflower and flaxseed oils featured better palatability for the hens: during the first 60 min after the feeding (the period corresponding to complex-reflex phase of regulation of the exocrine pancreatic secretion) the enzymatic activity grew 1.9-2.5-fold with these two oils while two other oils (soybean and rapeseed) produced the relatively substantial increases in lipase activity during the neurohumoral phase of the regulation evidencing the better nutritive value of these oils. The correlation between the activities of proteases and alkaline phosphatase in the pancreatic juice was found; a phosphatase-protease index can be therefore proposed for the assessment of response of the digestion to alterations in diet composition. The conclusion was made that the changes in the exocrine pancreatic function in hens fed diets with different vegetable oils can be the basis for further research of the digestion in poultry to identify optimal dietary ingredients.

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ID : 105

BACILLI CAN CREATE A PROTECTIVE BIOFILM ON THE EPITHELIUM

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Bacillus-based probiotic products containing bacterial spores seem to be particularly well-suited for use in broiler feeds, as they are metabolically dormant and resilient to environmental stresses, including pelleting. There have been years of debate on the mode of action of probiotics in chickens and more broadly in poultry. One of the cornerstones of the debate was the ability of spores to germinate and become viable organisms in the intestine due to the rapid transit time in poultry. These aspects were clarified in 2008 by Cartman et al. The research has shown that orally-administered *Bacillus subtilis* spores germinate in the chicken's gastro-intestinal tract (GIT) (Cartman et al. 2008). Continuous administration of an effective *Bacillus subtilis* probiotic is advisable to achieve consistent benefits (Latorre et al. 2014). However, another point of discussion was to know whether spore-forming Bacilli were transient organisms in the gut or if they could attach somehow to the intestinal epithelium. This led to two schools of opinion in the scientific community.

The study was conducted to investigate the effects of commercially-available spores of Chr. Hansen *Bacillus subtilis* spore-based probiotic in diets at 1.6×10^6 cfu/gram of feed on performance and microbiota activity in broilers. Fluorescence in situ hybridization (FISH) was performed to investigate the spatial organization and the formation of *Bacillus subtilis* biofilms in intestinal samples from various GIT locations in 6 broiler chickens. Tissue sections from each chicken were analyzed in duplicate and visualized by fluorescence microscopy with a 40x objective, using a fluorescence microscope.

Indeed, a first picture describes very well how *Bacillus* colonize the intestinal epithelium in the intestine. It can be seen very clearly the red fluorescence on the surface of the villi of the intestine. In a second picture with a different fluorescence, we can also see some luminescence inside the lumen of the intestine, which clearly shows that transient *Bacillus* are in the intestine, live and multiplying into the lumen of the gut content. This most recent research helps to further understand an important part of the mode of action of probiotics, such as Bacilli. Bacilli can germinate in the gut, being an active part of the bacteria microbiome in poultry, they can be transient, live organisms in the flow of the intestinal content, and can colonize the surface of the intestinal villi.

ID : 155

EFFECT OF GANODERMA LUCIDUM EXTRACT ON THE FECAL MICROBIOTA AND BURSAL TRANSCRIPTOME OF BROILERS

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This study investigated the effects of Ganoderma lucidum extract on the fecal microbiome and bursal transcriptome of broilers. A total of 60 one-day-old male broiler chicks (Ross 308) were randomly assigned into 2 dietary treatments, with 5 replicate cages per treatment and 6 birds per cage. The dietary treatments consisted of a basal diet as control and a diet of control plus 1 g/L G. lucidum extract in drinking water. The average body weight, average daily gain, average daily feed intake, and feed conversion ratio were calculated from days 1 to 35. On day 35, feces and bursa of Fabricius from broilers were freshly collected and used for fecal microbiota and transcriptome analysis, respectively. Student's t test was used for intergroup comparison, and a P value less than 0.05 was considered significant. No significant difference was observed in growth performance in broilers after feeding with G. lucidum extract. However, the microbial richness and evenness was reduced in the fecal content of G. lucidum extract-treated group. Principal coordinate analysis demonstrated good clustering of the pair of samples collected from the fecal content of the control group and G. lucidum extract-treated group. The abundance of genus Lactobacillus in feces increased in broilers fed G. lucidum extract, whereas the abundance of genus Ruminococcus torques group in feces was decreased in response to G. lucidum extract treatment. Transcriptomic analysis demonstrated that 2085 genes were differentially expressed in the bursa of Fabricius and that 1114 genes were upregulated and 974 genes downregulated in response to G. lucidum extract stimulation. Gene ontology biological processes related to immune system process, regulation of immune system process, cell activation, and organelle assembly were downregulated in response to G. lucidum extract treatment. A comparative functional analysis results demonstrate that G. lucidum extracts positively regulated T cell receptor signaling pathway, glycerophospholipid metabolism, and N-glycan biosynthesis in the bursa of Fabricius. These results demonstrate that G. lucidum extract can regulate gut microbiota and immune response in the bursa of Fabricius in broilers.

ID : 169

THE DUODENAL ACTIVITIES OF THE DIGESTIVE ENZYMES IN CHICKEN FED DIFFERENT LIPID DIETARY INGREDIENTS

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The most intensive hydrolysis of dietary nutrients occurs in the duodenum. Since the digestion can adjust to the diet composition different vegetable oils with different fatty acid profiles can produce different responses. The aim of our study was the investigation of duodenal activities of the digestive enzymes in chicken fed different oils. Three Hisex White laying hens with chronic duodenal fistulae and method of periods (7-10 days each) were used. The hens were fed a standard diet for layers. In control period the diet was supplemented with sunflower oil (SFO); in subsequent periods the latter was substituted by soybean (SBO), rapeseed (RSO), and flaxseed oils (FSO). The samples of duodenal digesta were taken in 1 postprandial hour. Activity of amylase was determined using Smith-Roy method modified for high activity; activity of proteases by hydrolysis of Hammersten's casein with colorimetric control (450 nm); activities of lipase and alkaline phosphatase (AF) and concentration of calcium were determined on semi-automatic biochemical analyzer BS-3000P (Sinnova, China) with a reagent kit by DIAKON-VET (Russia).

It was found that the activities of lipase and AF and Ca concentration are the responsive to the shifts in dietary oil ingredients. The highest lipase activity was found in the period with SFO. Substitution of the latter by SBO significantly decreased activity of lipase by 35.6%, for RSO by 37.6%, for FSO by 35.9% ($P<0.05$). The highest Ca concentration was found with FSO, higher by 12.5% ($P<0.05$) in compare to SFO; the increases in Ca concentration with SBO and RSO were 10.4 and 12.5% ($P<0.05$). The AF activity also significantly increased with these substitutions: by 92.6% for SBO, by 29.0% for RSO, and by 46.0% for FSO ($P<0.05$). The most conclusive parameter characterizing the adjustment of the digestion to diet composition is a phosphatase-protease index calculated as the ratio of the activities of AF and proteases (u/L). Optimal values of this index were found for SFO and RSO; these data are in accordance with our previous research on the pancreatic secretion rates for these oils. The indices for SBO and FSO were 2.1-fold and 1.8-fold higher in compare to the control SFO period.

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ID : 304

INFLUENCE OF DIFFERENT FIBER SOURCES ON THE ILEAL MORPHOLOGY IN BROILER CHICKENS

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The beneficial effects of fibre are well described in the feeding of monogastric animals. Recently the use of fibre in the diet of broiler chicken is a topical issue. Depending on fibre properties, the inclusion of moderate levels of fibrous material can positively influence the development and functionality of the gastrointestinal tract. In this respect, also its epithelial mucosa can be affected. This may increase the digestibility of nutrients leading to an enhanced performance of the chicken (Jimenez et al. 2013). The current study examines the effect of various dietary fibre sources on morphometric parameters of the ileum in broiler chickens.

A total of 6,300 day-old broiler chicken (Ross 308) were reared to 36 days of age on a 3 phase feeding program. The animals were randomly allotted to one of four iso-caloric and iso-nitrogenous treatments: Treatment 1 was a commercial diet for broilers consisting of corn, wheat and soybean meal without a supplemented fibre source, treatment 2 included lignocellulose I, treatment 3 included lignocellulose II and treatment 4 soybean hulls.

After removing the ileal segment, tissue samples from 2 cm to the ileocecal junction were taken and prepared for paraffin embedding. After sectioning and staining with alcian blue and periodic acid–Schiff six villi, crypts and muscular layers of each sampled animal (n=72) were examined with computerized light microscopy.

Statistical analysis of the gathered data was conducted using Statistical Analysis Software (SAS®, 9.4.). The results show that adding fibre to the diet significantly influences the morphometry of the ileum. The control treatment without any fibre supplementation showed significant shorter villi, lower crypt depth as well as a tendency towards thinner mucosal layer. An increased villus and crypt surface area is important for an improved nutrient absorption surface. Furthermore, the least amounts of goblet cells were detected in the control treatment. An increased amount of mucus building goblet cells can be evaluated positively, as the mucus layer acts cytoprotective and influences the nutrient transport mechanism. This research shows that the fibre supplementation to the diets of broiler chicken positively affects the intestinal morphology in the ileal section.

Jimenez, E.; Frikha, M.; Coca, A.; García, J.; Mateos, G (2013): Oat hulls and sugar beet pulp in diets for broilers-Effects on growth performance and nutrient digestibility. *Anim Feed Sci Tech*,182:33-43.

ID : 319

IS SELECTION FOR IMPROVED PERFORMANCE LIMITING THE GASTROINTESTINAL CAPACITY OF MODERN BROILERS?

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Genetic selection for improved carcass yield may limit the capacity of the gastrointestinal tract (GIT) of modern broilers to deal with bulky feeds. Interest has grown around the use of alternative feed ingredients in broiler diets, which are often bulky materials. Reductions in the capacity of the GIT will lead to a limitation in feed intake and consequently performance on such diets. We investigated the capacity of male Ross 308 broilers to deal with increasing levels of dietary bulk and aimed to identify a feed bulk dimension related to limited feed intake (FI). Two bulky ingredients were selected: oat hulls (O), and sugar beet pulp (S), due to the differences in their physicochemical properties. 480 day-old broilers were allocated in 48 pens and offered a common starter until d8 and 1 of 7 feeds from d8-d36 of age; a control feed (C), which was diluted to three levels (15%, 30% or 45%) with either O or S. FI was measured daily and two birds per pen were dissected for organ measurements and empty carcass weight (ECW) at d36. Cumulative FI (CFI) was calculated over the experiment and scaled relative to ECW at d36. It was hypothesised that bulky feeds would increase the visceral weight of the birds, therefore ECW was used as a scalar for CFI (g/ kg CW). Results were analysed with GLM using the nlme package in R.

Broilers were able to accommodate a greater increase in CFI on the O rather than S feeds ($P < 0.001$). CFI (g) of the birds offered C, O15, O30, O45, S15, S30 and S45 were 2683, 3678, 3977, 3795, 3521, 3041, 2808 (± 178) g. ECW was reduced by the inclusion of S, whereas the inclusion of O was limiting only at the 45% level ($P < 0.001$): 2486, 2610, 2498, 2211, 2262, 1876, 1301 (± 35.1) for birds offered C, O15, O30, O45, S15, S30, and S45 feeds respectively. Scaled CFI were 1084, 1444, 1593, 1814, 1702, 1816, 1956 (± 64) g/ kg ECW, respectively. Scaled CFI was increased to a greater extent in the S and O45 birds than the O15, O30 and C birds ($P < 0.001$). The reductions in performance of the S and O45 birds were consistent with the limitations in FI, as they are unable to increase FI to the extent that they meet the nutrient requirements for optimum growth. Nonetheless, our data show that modern broilers can substantially increase FI in response to bulky feeds, almost double their FI on feeds high in S. However, we were unable to identify the property of bulky feeds which was responsible for limiting FI and consequently performance.

ID : 378

IMPROVEMENT OF BROILER PERFORMANCES BY PROBIOTIC IN DIET IS LINKED TO EARLY LIFE
INTESTINAL TRACT DYNAMIC CHANGES

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Probiotics are currently proposed as an alternative solution to antibiotics used in poultry farming. Probiotic strains are described to improve zootechnical performances and are known as healthy beneficial microorganisms by correcting gut microbiota imbalance and gut barrier defect through mucus production stimulation. This study was aimed to determine the incidence of *Bacillus pumilus* in diet during the rearing period of broiler i) on the zootechnical performances and ii) according to the age, on intestinal tract dynamic changes.

204 broilers Ross PM3 (8 or 9 animals/cage) were used from day (d)0 to d35 and received diet enriched in *Bacillus pumilus* (Bp group: 1×10^9 CFU/kg feed) or the respective diet alone (control group) (12 cages/treatment). To determine the average daily gain and the feed intake, animals and feed were weighted for 4 periods: d0 to d10 (starter), d10 to d25 (grower), d25 to d35 (finisher), and d0 to d35 (total period). Histological analysis (muscular and mucus layer thickness and goblet cells number) were performed on the ileum at d10 and d35. For statistical analysis, a mixed model was used, treatment was considered as a fixed effect and cage as a random effect.

Compared to control group, no impact of Bp was measured on body weight and average daily gain. From d10 to d25, daily feed intake tended to decrease ($p=0.07$) with Bp. Feed conversion ratio was significantly reduced by Bp for the 2 periods d10-d25 ($p=0.039$) and d0-d35 ($p=0.015$). At d10, in ileum of Bp animals, a thicker gut muscle layer ($p=0.07$) as well as increased intraluminal mucus production ($p=0.004$) were measured, compared to control group. At d35 the mucus production in ileum was decreased in Bp group compared to control group ($p = 0.004$). At d10 and d35, no incidence of Bp was noticed on goblet cells number.

Bacillus pumilus supplemented diet contributes to improving broilers zootechnical performances: FCR improvement was mainly driven by a decrease of feed intake. This beneficial economic aspect observed with Bp diet is associated with a clear impact of Bp during early life period. These data are associated to a more important thickness of intestinal muscular layer in favor of better contact between diet and digestive enzymes and to a reinforcement of the gut barrier illustrated by the higher production of mucus by goblet cells. Finally, *Bacillus pumilus* seems to be a good candidate to counteract intestinal disorders like malabsorption syndrome in chicken.

ID : 386

INVESTIGATING THE COMBINED EFFECT OF MEDIUM CHAIN FATTY ACIDS AND PHYTOGENIC MOLECULES ON BROILER DIGESTIBILITY

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Investigating the combined effect of medium chain fatty acids and phytogenic molecules on broiler digestibility

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Dietary medium chain fatty acids (MCFAs) have strong antibacterial activities and a positive impact on intestinal morphology in broilers, while phytogenic components have antioxidant, anti-inflammatory and enzyme stimulating properties. Improving gut health results in optimizing the nutrient absorption capacity and the efficiency of the bird. Here, we evaluated the effect of a MCFAs and phytogenic mixture (M-prove[®], Agrimprove) on broilers' digestibility and its in vitro antibacterial effect. A digestibility study was performed at ILVO. 108 male day-old broiler chicks (Ross 308) were set in deep litter pens and fed a standard wheat-soy based mash diet ad libitum from day 1 to 9. On day 9, the number was reduced to 96 birds, randomly transferred to digestibility units and fed nearly ad libitum (12 replicates per diet, 4 birds per unit): one control diet and one topped with 1.2 kg/ton of the mixture. Between day 14 and 18, total feed intake and total excreta were determined. Body weight gain was recorded during the collection period. Digestibility was calculated on the basis of the total excreta/feed ratio. Crude protein (CP), crude fat (CF), gross energy (GE) and dry matter digestibility as well as energy contents (metabolizable energy corrected for nitrogen retention; MEn) were determined. Digestibility parameters were analysed with a linear mixed model with diet treatment as a fixed factor, followed by a Tukey post-hoc comparison of means. The mixture tended to slightly increase broilers' feed intake ($P=0.064$) and significantly increased daily weight gain by 2.5g ($P0.1$). An in vitro model mimicking poultry gizzard was used (pH=3, 40 min incubation time, co-culture of three bacteria and 3 replicates) and the mixture was added at 0.4 kg/ml medium (= equivalent in vivo dose taking into account a water/feed intake ratio of 2/1). The data in the model showed a decrease of the relative ratio of pathogenic to beneficial bacteria as enumerated via plate counting. Indeed, Enterococcus displayed over 1 log reduction, while Lactobacillus remained unchallenged and an intermediate effect was seen on E. coli. It is concluded that the impact of the combination of MCFAs and phytogenic molecules on digestibility and microbiota profile should be further investigated.

Key words: broiler, fatty acids, phytogenic, digestibility, antibacterial

ID : 390

HYDROXY-METHIONINE OLIGOMERS ARE EFFICIENTLY USED BY BROILERS TO SUSTAIN GROWTH PERFORMANCE.

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Synthetic methionine (Met) sources are currently added in broilers' feed to fulfill sulfur amino acids (SAA) requirements. Despite the wide use of both DL-Methionine (DL-Met) and Hydroxy-Methionine (HMTBA), there is still a debate on their relative bio-efficacy. Liquid HMTBA product presents a natural equilibrium between monomeric and oligomeric forms. The latter are claimed to be less absorbed by birds that would partly explain the difference of efficacy between DL-Met and HMTBA. The aim of this study was to compare birds' growth performance when fed with increments of HMTBA oligomers from 23 to 84%. Birds were fed from 0 to 8 days a commercial starter diet. From 8 to 21 days, 112 Ross 308 male birds were randomly allocated to 6 treatments consisting of a negative control diet, without Met supplementation (dSAA/Lys = 0.54), a positive control supplemented with DL-Met to reach SAA requirement (dSAA/Lys = 0.78) and 4 treatments supplemented on iso-molecular basis with: Liquid HMTBA (23% oligomers), and oligomers enriched products containing respectively 50, 75 and 84% oligomers to reach the same SAA as the positive control diet. The oligomers enriched products were obtained by vacuum water elimination to shift the chemical equilibrium to oligomeric forms. The total HMTBA and oligomeric contents were measured by HPLC. Birds were reared in cages of 2 birds. Animals and feed were weighted at 14 and 21 days. Data were statistically analyzed using a two-way ANOVA with the treatment and the block as main variables. Treatment means were compared using the Fisher test. Globally, feed intake was lower in the negative control ($P < 0.05$) and similar between other treatments. Body Weight Gain and Feed Conversion Ratio were significantly impaired ($P > 0.10$) of body weight, weight gain and feed conversion ratio were observed between the positive control and other treatments. DL-Met containing no oligomers was similar in performance to all treatments containing up to 84% oligomers ($P > 0.10$). A linear regression applied to only HMTBA treatments also indicated no significant effect of the oligomers level. These results highlight that modern broilers can hydrolyze, absorb and utilize HMTBA oligomers even at high level. According to these results, oligomers level in commercial HMTBA cannot be considered as limiting and reinforce the 100% relative efficacy of Hydroxy-methionine.

ID : 469

CO-EXPOSURE TO DEOXYNIVALENOL (DON) AND CAMPYLOBACTER JEJUNI INCREASES INTESTINAL PERMEABILITY AND BACTERIAL TRANSLOCATION IN BROILER CHICKENS

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The mycotoxin deoxynivalenol (DON) is a great health concern in poultry production. DON affects the epithelial cells of the gastrointestinal tract and contributes to the loss of the epithelial barrier function. Similarly, it is debated that *C. jejuni* negatively affects the integrity of the intestinal epithelium and promotes the translocation of bacteria from the gut to inner organs. So far, no data are available in regard to the co-exposure to DON and *C. jejuni* in broilers, two widespread foodborne zoonotic agents. Therefore, the aim of the present study was to investigate whether DON interacts with *C. jejuni* colonization/translocation.

To this end, a total of 120 commercial broiler chickens (Ross 308) were housed in floor pens on wood shavings with feed and water being provided ad libitum. Birds were allocated to four groups (n = 30 with 5 replicates/group) and fed for 5 weeks with either a contaminated diet (5 mg DON/kg feed) or a basal diet (control). Following oral infection of two groups at 14-days of age with 1×10^8 CFU of *C. jejuni* NCTC 12744, birds were euthanized and tissue samples were collected in weekly intervals until 3 weeks post infection. We investigated the effect of DON and *C. jejuni* on the epithelial paracellular permeability of duodenum, jejunum and cecum by measuring the mucosal to serosal flux of ¹⁴C-mannitol (a paracellular marker) in an Ussing Chamber. Furthermore, the translocation of *C. jejuni* and *E. coli* was investigated by determining CFU counts from duodenum, jejunum, cecum, liver and spleen. It was found that the co-exposure of broilers to DON and *C. jejuni* increased the paracellular permeability of duodenum, jejunum and cecum compared to the individual treatments. In agreement with this, this co-exposure also promoted the translocation of *C. jejuni* and *E. coli* to inner organs with a significantly higher bacterial load in liver and spleen. Finally, a reduced growth performance of birds was detected underlining the negative impact.

ID : 583

DISTRIBUTION OF NEUROPEPTIDE Y NEURONS IN THE MALE NATIVE THAI CHICKEN

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Neuropeptide Y (NPY), a 36 amino acid neurotransmitter, plays various physiological functions involving food intake in birds. NPY is associated with the regulation of the reproductive system in the female native Thai chickens. To date, there are no data described the functional aspects of NPY in the male birds. Thus, the objective of this study was to elucidate the distributions of NPY-immunoreactive (-ir) neurons and fibers in the male brain, enabling further studies of neuroendocrinology related to feeding behavior of the male native Thai chicken. Six male native Thai chickens, Pradoohangdum breed, were used and the distributions of NPY-ir neurons and fibers were detected by immunohistochemistry technique. The results revealed that the distributions of NPY-ir neurons and fibers were located throughout the brain, especially in the hypothalamus. The greatest density of NPY-ir neurons was found within the nucleus septalis lateralis and nucleus paraventricularis magnocellularis (PVN). A few of NPY-ir neurons was observed within the nucleus inferioris hypothalami (IH), and the nucleus infundibuli hypothalami (IN). The distribution of NPY-ir fibers was found in abundance within the nucleus supraopticus, pars ventralis, nucleus suprachiasmaticus, pars medialis, IH-IN, PVN, and the external layer of eminentia mediana (median eminence). A few of NPY-ir fibers was observed within the nucleus commissurae pallii, nucleus periventricularis hypothalamic, commissura pallii, regio lateralis hypophalami, and nucleus rotundus. These results indicated the distributions of NPY-ir neurons and fibers in the male native Thai chicken were markedly observed in the PVN which might be related to the regulation of feeding behavior in this equatorial species.

ID : 681

GROWTH PERFORMANCE, HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS CHANGES IN BROILERS CHICKENS FED VARYING LEVELS OF VERNONIA AMYGDALINA LEAF MEAL

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The growing concerns on the use of antibiotics as growth promoters in animal diets and its health implication on consumers of poultry products has necessitated a search for suitable alternative growth promoting agents especially medicinal plants. The aim of this study was to determinate the effects of dietary supplementation of Vernonia amygdalina leaf meal (VALM) on the haematology, biochemical and growth performance of broilers chickens. Four hundred and eighty day-old cobb chicks were randomly allocated to four dietary treatment viz.: a basal diet (V0) (control group with 0% of VALM); basal diets with 1 (V1), 2 (V2) and 3% VALM (V3) having 4 replicates of 30 birds each in Completely Randomized Design. Birds were managed conventionally on a deep litter system for a period of 6 weeks. Data were collected on body weights, feed intake, feed conversion ratio and blood parameters. Results showed that feed intake of the birds in V2 and V3 VALM was significantly lower (P0.05) but significantly higher than those of V2 and V3. Final body weights and weight gain were higher significantly (PVernonia amygdalina leaf supplementation improved growth performance, haematological and biochemical parameters indices of broiler chickens.

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ID : 715

EFFECT OF SUPPLEMENTATION OF LYSOLECITHIN ON PRODUCTION PERFORMANCE AND INTESTINAL MORPHOLOGY OF CHICKEN

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Lysolecithins are produced by an enzymatic conversion of lecithin. The addition of lysolecithin to diet decreased the size of fat globules and increased the active surface of fats for enzymatic digestion. Hence, the present research was conducted to study the effect of lysolecithin on production performance and intestinal morphology of Nandanam broiler 3 at Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. A total of nine hundred day old, unsexed, Nandanam broiler 3 chickens were wing banded, weighed and randomly allotted into three treatment groups and reared under deep litter system of management. All the treatment had three replicates and each consisted of 100 chicks. Birds were reared upto eight weeks of age. The experimental birds fed with diets containing lysolecithin 0 g (T1-control), 2.5 g (T2) and 5 g (T3). All the diets were iso-caloric and iso-nitrogenous. Birds were fed with pre-starter (0–14 days), starter (15–28 days) and finisher (29–56 days), respectively. The body weight, body weight gain, feed consumption, feed efficiency, livability, NewYork dressed weight, eviscerated carcass weight, ready - to - cook yield and economics were analyzed. The data collected on various parameters were grouped and subjected to statistical analysis of one way ANOVA as per the procedure of statistical analysis system (SPSS, version 20.0 for windows). Dietary incorporation of 5 g of lysolecithin (T3) recorded significantly ($P \leq 0.01$) higher body weight followed by 2.5 g of lysolecithin (T2) and control (T1) group at 8 weeks of age. The highly significant ($P < 0.01$) difference in body weight gain of Nandanam broiler-3 was observed in T3 group from second weeks to entire experimental period. Feed consumption revealed significant ($P < 0.05$) difference between treatment groups throughout the experiment period. Birds received 5 g of lysolecithin (T3) had better ($P < 0.01$) feed efficiency. Statistical analysis revealed a significant ($P < 0.05$) difference in New-York dressed weight, eviscerated carcass weight, ready - to - cook yield between treatment groups. Feeding of 5 g of lysolecithin to Nandanam broiler 3 significantly ($P < 0.01$) increased intestinal length when compared to other treatment groups. The cost effectiveness of T3 group showed increased net profit per kg live weight. From the experiment, it was concluded that 5 g of lysolecithin can be incorporated in the feed for better returns to the farmers.

ID : 743

EFFECT OF EARLY THERMAL CONDITIONING ON THE HEPATIC METABOLISM IN BROILER CHICKS

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【Objectives】 The metabolic heat production is one of the main heat production systems in chickens, and the liver is an important heat producing tissue in the system of metabolic heat production. Besides, it is well known that thermal conditioning on early age improves post-growth thermotolerance and alters heat production systems in chickens. The purpose of this study is to clarify the effect of thermal conditioning at early age on metabolism of liver in broiler chicks. **【Materials & Methods】** Three-day-old chicks were exposed at 40°C for 12 hours (thermal conditioning) and control chicks were kept at 30°C. At one week old, the both groups were exposed 40°C for 15 minutes (heat challenge), or kept at 30°C. In this study, there is 4 groups depending on whether there is thermal conditioning and whether there is heat challenge. After heat challenge, the all groups of liver tissues were collected for metabolomics analysis. **【Results】** Results of metabolomics analysis, the level of isoflavones such as daidzein, genistein and glycitein were low by thermal conditioning. In addition, the effect of heat challenge was observed in the cholesterol-related metabolites. **【Conclusion】** In conclusion, thermal conditioning at early age alters the part of metabolism of liver in broiler chicks.

ID : 758

IMPACT OF REPLACING CHOLINE CHLORIDE WITH A PHYTOBIOTIC ON THE HISTOMORPHOMETRIC CHANGES IN THE LIVER OF LAYING HENS

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The use of phytobiotics as an alternative to products of chemical synthesis in animal production is increasing due to environmental and consumer demands. A phytobiotic-based product, Biocholine® (Nuproxa, Switzerland Ltd.; Indian Herbs Specialities PVT. Ltd.) composed of the medicinal plants *Andrographis paniculata*, *Azadirachta indica*, *Trachyspermum ammi* and *Achyranthes aspera*, with proven lipotropic activity, has been successfully used to replace choline chloride (CC) in broiler feeds. The objective of this study was to compare the dietary supplementation of CC vs Biocholine® on the histomorphometric changes in the liver of layers during production. The experiment consisted of two dietary treatments; a corn and soybean meal-based diet supplemented with CC 60% (800 g/ton) (T1) and T1 but supplemented with 200 g/ton of Biocholine® in replacement of CC (T2). A total of 53.677 layers (Shaver Brown) were distributed in 4 houses with floor production, automatic chain feeders and bell drinkers. The layers allocated in houses 1 and 2 (n = 26.928) were fed T1 and the ones allocated in houses 3 and 4 (n = 26.749) were fed T2. The experimental period was 20 weeks (34 to 54 wk of age). The study was conducted in a layer farm, located at the Santander Department, Colombia, at an altitude of 1.055 m over sea level. At 38, 42, 46, 50 and 54 wk of age, 5 layers from each house and dietary treatment were randomly sampled for liver samples (n = 60 in each treatment). Liver samples were submitted to histomorphometric analysis. Although not relevant for the objectives of the study, egg production was recorded. Data across ages were submitted to an analysis of means and variances for parametric values using the Snedecor F distribution for comparing the variances as reported by Gibson-Corley et al. (2013). Results showed that CC induced a more extensive and severe cellular degeneration in liver (p 0,05) were observed in the severity or extension of inflammatory-related damage in liver tissue. Egg production and laying persistency were improved (p vs CC. In conclusion, the use of a phytobiotic product based on medicinal herbs proved to be an alternative to substitute CC in the feed of laying hens.

ID : 884

EFFECT OF FERMENTED RAPESEED CAKE ON PHOSPHORUS DIGESTIBILITY IN LAYING HENS

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Fermentation is considered to be an effective method for improving the nutritional value of diets for farm animals. Fermentation increases the bioavailability of selected nutrients and decreases the content of undesirable compounds in many feedstuffs. The aim of this study was to determine the effect of fermentation on the phytate-phosphorus content of rapeseed cake as well as ileal and total tract phosphorus (P) digestibility in laying hens. A total of 216 Hy-Line Brown layers at 36 weeks of age, kept in battery cages, were divided into 3 dietary treatment groups (36 replicates of 2 birds each). For 12 weeks, the birds were fed isonitrogenous and isocaloric diets. In the control group, the main dietary protein source was soybean meal (C), and the diets in two experimental groups were supplemented with 20% of raw rapeseed cake (RRC) or fermented rapeseed cake (FRC). During the experiment, excreta were collected for 5 days to determine total tract P digestibility. On the last day of the experiment, 10 hens from each group were sacrificed and the small intestinal digesta was collected to evaluate ileal P digestibility. TiO₂ was used as an indigestible dietary marker. The differences between group means were determined by ANOVA and Tukey's test with the use of Statistica 13.1 software. It was found that fermentation led to a nearly two-fold decrease in the phytate-phosphorus content of rapeseed cake (7.88 g/kg vs. 14.28 g/kg). Ileal P digestibility was significantly higher in hens fed FRC than in those receiving RRC, and comparable values were noted in FRC and control groups (FRC - 63.9%, RRC - 50.9%, C - 62.1%, $P \leq 0.001$). Fermentation had no positive effect on total tract P digestibility ($P > 0.05$). The coefficients of total tract P digestibility were considerably lower in all groups ($\leq 30\%$), probably because the absorbed P was re-directed into the excreta via the urine. It can be concluded that fermentation decreases the phytate-phosphorus content of rapeseed cake and exerts a beneficial influence on ileal P digestibility.

ID : 890

BRAIN VITAMIN D3 SIGNALING REGULATES CHICK ENERGY METABOLISM

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Introduction: Vitamin D3 (D3) is hydroxylated in the liver, circulates as 25-hydroxyvitamin D3 (25OH-D3), and then hydroxylated in the kidney is converted to an active vitamin D metabolite (1,25-dihydroxyvitamin D3). These vitamin D signals act on target organs expressing vitamin D receptor (VDR). VDR is also known to be expressed not only in peripheral organs such as liver, gastrointestinal tract, and kidney, but also in the brain.

In mammals, it has been revealed that the vitamin D signals in the brain act on the melanocortin system, which is the key regulator of energy metabolism, and control whole-body energy metabolism. However, little is known about the effect of brain vitamin D signaling on chick energy metabolism. Therefore, in this study, to clarify the function of the brain vitamin D signal in the neonatal feeding behavior and energy metabolism, we investigated 1) gene expression level of diencephalon VDR during growth and 2) effect of feeding D3 and 25OH-D3 on brain melanocortin system.

Materials and Methods: All experiments were demonstrated commercial broilers (Ross308). Experiment 1: Diencephalic VDR gene expression at 15, 18 and 21 days of embryonic stage and 3, 7, 15 and 42 days after hatching was relatively quantified by quantitative PCR (qPCR). Experiment 2: Four types of diets were mixed for D3 (3000 or 5000 IU) and 25OH-D3 (0 or 69mg), and fed for 11 days after hatching. The chick body weight, feed intake and feed efficiency were measured during the experimental period. At the end of experiment periods, the chicks were sacrificed, and diencephalon was collected. The expression level of VDR and melanocortin peptide (POMC, AgRP, NPY) genes were quantified by qPCR.

Results and Discussion: VDR gene expression in the diencephalon increased with embryonic development, and was higher in chicks immediately after hatching. Addition of D3 and 25OH-D3 to the starter diet for 11 days after hatching did not changed in the body weight, feed intake and feed efficiency. The POMC gene expression level in the D5000 treatment group was higher than that of D3000 treatment group, and these upregulation was diminished by the addition of 25OH-D3. Furthermore, positive correlation between diencephalic VDR and POMC gene expression level were observed. These results suggest that D3 and 25OH-D3 act on the melanocortin system in the chick brain and may be involved in regulating energy metabolism, particularly through the activity of POMC neurons.

ID : 936

THE EFFECT OF FREE FATTY ACIDS CONTENT AND SATURATION LEVEL ON DIETARY FAT PREFERENCE IN HENS. PRELIMINARY RESULTS

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Behavioural and genetic evidence show that avian taste is intimately related to nutrient sensing and, consequently, to poultry nutrition practices. Clarification of the mechanism of avian fat taste could provide meaningful information for improving new feedstuff in poultry nutrition. In this sense, acid oils could be economical and sustainable feedstuffs. Acid oils are fat by-products from the edible oil refining industry with similar fatty acids composition to their respective crude oils but richer in free fatty acids (FFA). A trial was conducted in order to study the effect of FFA content and ratio unsaturated:saturated (U:S) on dietary fat preference in hens. 48 Lohmann Brown laying hens were housed individually in cages. Three test diets containing Soybean acid oil (SA; high U:S, 50% FFA), Palm Oil (PO; low U:S, 5% FFA) and Palm Fatty Acid Distillate (PFAD; low U:S, 50% FFA) were offered in a series of double choice tests (after a 7 d training period) against a reference diet with Soybean Oil (SO; high U:S, 5% FFA). In each diet, fat was added to a basal diet at inclusion rate of 6%. Two equally sized containers were placed in front of the cage: one of them holding the reference diet (SO) and the other one of the three experimental diets assigned following a complete block design. A control "reference vs. reference" two-way comparison was included to detect such possible effects of position preferences and feeders' position was changed after every measurement. Feed intake was measured during 2 h twice a day after 1 h of fasting. The consumption was analyzed as a standard preference index (% of test diet intake over total intake). Preference value were compared to the random choice value of 50%. For statistical analysis, a One Sample t-Test was performed. Significant differences were considered for $P < 0.05$. Preference measured for the double reference control did not differ from 50% (51.9%), indicating that the observed preference values may be attributed to dietary changes in fat ingredients. Hens showed feed preference for PO (60.9%) and PFAD (57.0%). However, preference values less than 50% were observed for SA (37.9%). These results suggest that with high U:S, hens prefer feed with less FFA. Furthermore, saturation level plays an important role on dietary fat preference: hens prefer low U:S fats even when are rich in FFA. Acknowledgements: Financed by Generalitat Valenciana and the European Social Fund.

ID : 978

THE EFFECT OF DIFFERENT DIETARY RATIOS OF ARGININE AND METHIONINE TO LYSINE ON PROTEINS NITRATION, REDOX STATUS OF TURKEYS BLOOD AND DNA OXIDATION

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The aim of this study was to determine the effect of different ratios of arginine (Arg) and methionine (Met) in diets with high lysine (Lys) content on performance, metabolism, and oxidation processes of tissues and DNA in turkeys. The Hybrid Converter turkeys were assigned to 6 groups with 8 replicates per group and 18 birds per replicate. Six feeding programs, with 3 dietary Arg levels (90, 100 and 110%) and 2 dietary Met levels (30 and 45%) relative to dietary Lys content were compared. During each of four feeding phases (4 weeks each), birds were fed ad libitum isocaloric diets containing high level of Lys; approximately 1.83%, 1.67%, 1.49% and 1.20%, respectively.

The applied dietary treatments had no effect on daily feed intake and body weight in each of feeding phases. Comparing to the lowest dietary Arg ratio (90% Lys), the both increased ratios (100 and 110% Lys) were associated with the increased levels of nitrotyrosine-3-NT (139.6 vs. 158.8 vs. 179.1 nmol/L, $P<0.001$), malondialdehyde-MDA (2.3 vs. 3.2 vs. 3.1 $\mu\text{mol/L}$, $P=0.04$), and decreased activity of superoxide dismutase-SOD (1616.7 vs. 1272.1 vs. 780.3 U/gHb, $P<0.001$) in the turkey blood. Birds fed diet of highest Arg ratio (110% Lys) had the highest level of PC (4.0 vs. 4.2 vs. 4.7 nmol/mg, $P=0.002$) in the blood comparing to birds fed other dietary Arg ratios (100 and 90% Lys). Irrespective to the dietary Arg ratio, a increased ratio of Met in the diet (45% Lys) resulted in lower blood levels of protein carbonyl-PC (4.5 vs. 4.1 nmol/mg, $P=0.008$), 3-NT (162.7 vs. 155.7 nmol/L, $P=0.05$), and 8-hydroxydeoxyguanosine (8.5 vs. 7.9 ng/mL, $P<0.001$).

Our data indicated, that feeding turkeys diets containing 90% Arg and 45% Met in relation to Lys, could positively stimulate antioxidant defense system. The beneficial effect of such treatments was evidenced in down regulation of oxidation and nitration processes of key molecules in the biological system.

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ID : 1107

STUDIES ON DOSE AND DURATION OF FEEDING COMBINATION OF NUCLEOSIDES IMPROVED PERFORMANCE, IMMUNITY AND NUTRIENT TRANSPORT IN BROILERS THAN INDIVIDUAL COUNTERPARTS

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Genetic improvement and environmental conditions have led to a shift in the concept of nutrients essentiality, especially in broiler chickens. Nucleotides were once regarded as non-essential but could be considered as conditionally essential at early rapid growth and stress conditions. The chicks during the early rapid growth periods become dependent on preformed nucleotides than its de novo synthesis. To assess the impact of nucleotides, a series of experiments were carried out. In the first experiment, the effect of nucleosides (adenosine, guanosine, cytosine and Uridine) were assessed individually and in combination. A total of six experimental groups as control; adenosine (0.1%); Guanosine (0.1%); cytosine (0.1); Uridine (0.1) and combination of four having equal proportions (0.1%). All the groups were administered with respective nucleosides orally daily morning before feeding for the first 14 days of age. The birds fed with the combination of nucleosides resulted in better growth performance and immune response than their individual counterparts and control group. Based on this results, the second experiment was carried out to determine the dose as well as the duration of supplementation. Chicks (N=400) were divided into eight groups of five replicates as Group N1: (Control no antibiotics/nucleosides); N2 (positive control - BMD@150ppm) for 42 days. Group N3, 4 and 5: combination of nucleosides @ 0.05, 0.1 and 0.15% for the initial 14 days; Group N 6, 7 and 8: combination of nucleosides @ 0.05, 0.10 and 0.15% for the initial 21 days of age. The production performance, immunity and relative expression of nutrient transporter genes were studied at various time intervals and the data were analyzed using single factor analysis of variance. Higher body weight (P<0.05) difference among the treatment groups at 7 and 14 days. The results indicated that feeding combination of nucleosides @0.10% for initial 14 days of age improved the performance and immunity in broiler chickens.

ID : 1132

MODULATING MICROBIOME BY NUTRITIONAL STRATEGY TO OPTIMISE PERFORMANCE OF BROILER CHICKENS

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Microbiome is often seen as a second brain as in fact he is involved in many physiological pathways. Intestinal microbiome has a crucial role and is part of new emerging nutritional strategy as fibre represents main of the substrate available to ferment by the bacteria. That's why it becomes relevant to stimulate specific populations to take more benefits from the fibre fraction. The objective of this study was to investigate the impact of 2 different nutritional strategies on growth performance and how hindgut fermentation and caecal microbiome could be affected. Two dietary treatments were tested where treatment 1 (XYL+XOS) was a diet supplemented with a combination of xylanase and a specific xylo-oligosaccharide to stimulate the microbiome to ferment fibre and treatment 2 (XYL+BUT) was the same diet supplemented by a xylanase and micro-encapsulated butyrate offering substrate directly to the microbiome. Treatments were fed to day old male Ross 308 placed in floor pens with 65 birds/pen in 4 feeding phase program (0-10d, 11 to 21d, 22 to 32d, >33d) with 9 replicates pens per treatment. Growth performance (body weight, feed intake per pen) were measured, FCR and EPEF calculated. Caecal samples were collected on 9 birds per treatment at 42 days and analysed for their VFA content and %G+C profile. Data were analysed by a one-way ANOVA. Differences in terms of growth performance appeared at the last growing phase with XYL+XOS, improving BW ($p=0.032$) and FCR ($p=0.041$) by 67 g/bird and 12 points respectively that is translated to an overall improvement of the bwcFCR by 5 points ($p=0.087$) and of the EPEF by 46 points ($p=0.068$). Along to the performance, a reduction of BCFAs production was also noticed ($p=0.12$) without any changes regarding all the others SCFA analysed. The %G+C profile show differences where XYL+XOS increases the butyrate producers' population ($p<0.05$). These data suggest that the product from xylanase hydrolysis in treatment 1 combined with the specific XOS had positive influence on caecal bacterial population that may have allowed to extract more energy from the fibre arriving in the hind gut that is translating in better growth performance. This beneficial effect is not only related to butyrate production or the concentration may have been too low. Caecal SCFA analysis may also not be the most relevant criteria as it's a point in time measurement of a dynamic flow of production/absorption so microbiome analysis could be more meaningful.

ID : 1155

URIC ACID IS SECRETED BY ENTEROCYTE AS AN ANTIOXIDANT BUT NOT A WASTE TO PROTECT AGAINST OXIDATIVE STRESS

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Abstract

Objective Uric acid has been found an important antioxidant in avian and human. Gut is considered as an organ to excrete uric acid (UA) except of kidney. The gastrointestinal (GI) tract has the most extensive surface to be constantly exposed to a wide variety of potentially harmful substances. This study re-evaluated the role of UA secretion in the intestines to test the hypothesis that enterocytes can synthesize and secrete UA into intestinal lumen to protect from oxidative stress. Synthesis, secretion, and antioxidant effects of UA were investigated in the intestinal tract and enterocytes of chickens.

Design UA and allantoin concentrations in chyme were measured in various segments of the small intestine. mRNA expression of genes related to UA synthesis and transport was determined. Synthesis, secretion, and antioxidant effects of UA were evaluated in enterocytes and everted duodenum sacs in the presence of inosine, which is a precursor of UA. In order to test the hypothesis that enterocyte can synthesize UA to protect against oxidative damage, the in vitro cultured enterocytes was exposed to H₂O₂ challenge and the mRNA was extracted and reverted for transcriptome sequencing analysis. We further confirmed the effect of mild oxidative stress on synthesis and secretion of UA in enterocytes and cultured everted gut sacs.

Results Along the GI tract, UA and allantoin were present in the chyme and the duodenum had the highest ones, which was in line with the mRNA levels of the key enzymes in the purine nucleotide salvage-catabolism pathways. In enterocytes and everted gut sacs, inosine treatment increased UA synthesis and secretion. Low concentration of H₂O₂ stimulated UA synthesis and secretion by enterocytes. Transcriptomic study identified 206 differentially expressed genes (DEGs) with significantly higher expression level in H₂O₂ treatment, and 174 DEGs with low expression level, compared with control. In particular, the phosphodiesterase 1A (PDE1A) involved in guanine metabolism and XDH, ABCC4, ABCG2 mRNA levels increased significantly in H₂O₂ treatment. In enterocytes, UA attenuated H₂O₂-induced oxidative damage by Nrf2-ARE pathway.

Conclusions UA is synthesized by enterocytes and secreted into the intestinal lumen, where it elicits antioxidant effects on the intestinal epithelium. The result suggests that UA secretion into intestinal lumen is a protective mechanism of intestinal tract in facing to oxidative stress, rather than to excrete waste.

ID : 1239

EFFECT OF IN OVO ADMINISTRATION OF SELENIUM ON EGG TYPE CHICKEN HATCHABILITY

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Health status and stress impact poultry production. To alleviate the effects of those factors, many approaches are developed like control housing and use of drugs. But substances like Selenium can be used to reduce the negative effect. Selenium is an antioxidant that protect the organism against oxidative stress and health status of animals. The aim of this study was to evaluate the effects of in ovo Selenium administration on layer type chicks' performance. A total of 600 eggs were numbered, weighed and incubated. At 18 days of incubation, the eggs were candled and those with evidence of living eggs were divided into five groups of 120 eggs each. These groups were eggs in T1 that were not injected, T2 received NaCl 90/00 injection as positive control, T3 received 10 µg (Se), T4 received 20 µg (Se) and T5 received 30 µg (Se). The eggs were injected at the air chamber site. A hole was created at the injection site with a syringe needle. The solution was administered using an automatic syringe. The dosage of injection is 0.1 ml per egg. After the injection the sites were well sealed with a scotch tape before transferring the eggs to the hatcher. Embryonic parameters such as pipping time, incubation durations, hatchability, chick's quality using tona score, organs and chicken weight at hatch were evaluated. Result showed that birds that were not injected had the shortest hatching duration compared to others. The control group, the negative and the positive controls groups showed the lowest hatchability while groups of eggs that were injected with 20 µg (Se) and 30 µg (Se) had the highest rate. At hatch, chicks of the highest selenium dosage (30 µg (Se)) injection were the highest in term of chick's quality compared to others and the quality increased as the concentration of Se increases in the solution injected. No significant ($p>0.05$) difference was observed for chicks and organs weight after hatch. In conclusion the injection of Selenium in the fertile eggs improve the performance of the chicks after hatch. But the Selenium administrated at high level have a negative effect on the birds because of it's proven toxicity.

Key words: Selenium, In ovo administration, Hatchability, Chick's quality.

ID : 1244

CIRCADIAN RHYTHMS OF CALCIUM AND PHOSPHORUS DIGESTIBILITY IN COMMERCIAL LAYING HENS

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The objectives of this study were to investigate the circadian rhythms of digestible calcium (Ca) and phosphorus (P) in extended production cycle laying hens, with the intention to improve the efficacy of Ca and P utilization. The experimental design was a 2x2 factorial of 2 particle sizes of limestone (LM), grit (1.5mm geometric mean diameter (GMD)) or fine (0.2mm GMD) and two levels of added phytase from *Buttiauxella* sp. (0 or 600 FTU/kg). A corn-soy diet was mixed to contain 3.6% Ca from each LM and 0.11% Ca from the basal diet. Cr₂O₃ was included at 0.4% as a marker. The ileal digestibility of Ca and P were tested, at 3 hours post oviposition (POP) and at 11 hours POP. At 3 hours POP Ca and P are actively being added to the medullary bone matrix (Wilson & Duff, 1990) and at 11 hours POP the eggshell is undergoing calcification (Van De Velde et al., 1984). Amberlink hens at 33 wks of age were used and digestibility measurements were taken over four days at each time period (3;11 hours POP). Each hen served as a replicate (n = 1 hen) and 20 hens (5 per treatment) were sampled per time period per day. Distal ileal digesta was taken, lyophilized, ground and analysed for Ca and P and Cr. Data were analysed using SAS MIXED model with day of sampling as a random effect. Time POP affected Ca and P digestibility (P<0.01). Ca digestibility was higher at 11 hours POP than at 3 hours POP (P<0.05). Phytase improved P digestibility with the greatest improvement noted at 3 hours POP (P<0.05). The results from this study suggests that hens are able to increase the capacity to absorb nutrients according to physiological demands for Ca and P differently during medullary bone mineralization vs eggshell calcification and, that phytase improves P digestibility in laying hens. These time-dependent changes in the efficacy of Ca and P utilization should be considered when formulating diets for laying hens using a split feeding approach.

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ID : 1254

BLOOD PH AND BLOOD GASSES OF BROILERS AT DAY 7 AND 21 AS AFFECTED BY THE ACID BINDING CAPACITY OF THE DIET, WATER ALKALINITY, AND WATER ACIDIFICATION.

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The objectives of this study were to determine if blood pH, bicarbonate and blood gasses [partial pressure of CO₂ (PCO₂), partial pressure of O₂ (PO₂), total CO₂ (TCO₂), O₂ saturation percentage (sO₂), bicarbonate (HCO₃⁻), blood sodium (Na) and blood base excess (BE_{ecf})] were affected by the acid-binding capacity (ABC) of the feed, alkalinity of water, and water acidification. The experimental design was a 2x2x2 factorial of 2 dietary treatments (High ABC and Low ABC), 2 water alkalinities (84 ppm CaCO₃ and 416 ppm CaCO₃) with or without the addition of a water acidifier that reduced water pH to 4.0. Male Ross 308 birds were placed in 96 cages with 12 replicates per treatment. Two starter (0-10d) and finisher (11-21d) diets were formulated with a low and high ABC. The water with high (416 ppm CaCO₃) and low (84 ppm CaCO₃) alkalinity values was provided ad lib. At 7 and 21d birds were fasted for 6 hours to ensure the gastrointestinal tract was empty and birds were then provided feed for 12 minutes prior to drawing blood from the brachial vein. All blood parameters were measured using an i-STAT point-of-care laboratory system (Abbott Point of Care, East Windsor, NJ). The effects of dietary ABC, water alkalinity, acidification and their interactions on the various blood parameters were tested by SAS MIXED model using repeated measures (SAS, 9.4). Age had a significant effect on all the measured parameters (P<0.01) apart from PO₂ and sO₂. Blood pH increased significantly (P<0.0001) from 7.32 to 7.39 at 7d and 21d, respectively. There was a significant (P<0.05) interaction between water alkalinity and acidification on blood pH. There was a significant (P<0.001) interaction water alkalinity, acidification and day of sampling for TCO₂ and Base excess. Blood bicarbonate was significantly affected by day of sampling (P<0.0001) and a 3-way interaction between water alkalinity, acidification and day of age (P<0.005). The bicarbonate in the blood increased from day 7 (22.32) to day 21 (26.11). There was a correlation between blood pH and HCO₃ (R=0.46) illustrating alkaline tide. The results of this study demonstrated that certain blood gasses varied significantly when the bird's stomach was buffered through feed or water. The consequences of this on nutrient digestion and live-performance will be evaluated in future studies.

ID : 1344

A NOVEL APPROACH FOR THE MEASUREMENT OF BROILER CHICKEN ALBUMIN SYNTHESIS RATE

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INTRODUCTION: Protein Fractional Synthesis Rate (FSR) can be used as an early biomarker in health and disease. This work aims to facilitate the introduction of proteomics platforms to estimate FSR of multiple proteins by measuring tracer incorporation at the peptide level. This novel approach for FSR measurement involves the use of heavy water (D₂O), which labels free amino acids during de novo synthesis and transamination. The objective of the current study is to validate a 'dynamic proteomics' approach through isolation and conventional analysis of a single protein. Albumin is the ideal candidate due to its accessibility, high concentration in plasma and its importance as a marker of nutritional status. We applied a single D₂O bolus to calculate the FSR of multiple proteins in broiler chickens by dynamic proteomics using a non-linear model of tracer incorporation.

METHODOLOGY: 21 day old male broiler chickens of around 1kg body weight were administered an oral bolus of 10 g D₂O/kg body weight. Chickens were injected with 2mg/kg E.Coli lipopolysaccharide (LPS) or saline solution intravenously one hour after D₂O administration. One chicken per pen was blood sampled and culled at 4h, 24h, 48h and 96h after D₂O dosage. Blood was collected in 2ml tubes coated with EDTA and plasma was obtained after centrifugation. Deuterium enrichment in body water was quantified by FTIR analysis of plasma. Free amino acids were isolated by cation exchange columns and their deuterium enrichment was analysed by GC-MS. Albumin was isolated from plasma by differential solubility, acid hydrolysed and the deuterium enrichment of bound amino acids was analysed by GC-MS.

RESULTS: The elimination of a D₂O bolus follows a single exponential. Elimination half time was 2.34 days for chickens injected with LPS and 2.10 days for control birds. Mean albumin FSR of chickens injected with LPS was 78.2 % day⁻¹ calculated from body water enrichment and 77.1 % day⁻¹ calculated from free alanine incorporation. Mean albumin FSR of control chickens was 100.6 % day⁻¹ calculated from body water enrichment and 97.7 % day⁻¹ calculated from free alanine incorporation.

CONCLUSION: New data on male broiler chicken albumin FSR has been obtained. This data will be compared with FSR measured at peptide level by proteomic platforms and will contribute to unravel the effect of E.Coli LPS on protein dynamics in broiler chicken.

ID : 1387

DEVELOPMENTAL PROGRAMMING OF FAT ACCRETION IN BROILER CHICKS THROUGH LONG CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS IN THE HEN DIET

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Developmental programming can produce lasting effects on growth and metabolism in poultry (Lesuisse et al., Poult. Sci., 2018). Programming occurs in part through epigenetic mechanisms that persist across generations, yielding an efficient tool with which to improve performance. We previously demonstrated that developmental programming through long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) in the broiler-breeder hen diet reduced fat accretion in chicks after hatch (Beckford et al., Sci. Rep, 2017). The objectives of this study are to determine if LC n-3 PUFA influence embryonic development of chick adipose tissue and to identify mechanisms for programming of body composition. Broiler-breeder hens (Cobb 500) were fed corn-soybean diets supplemented with fat (2.3%) from fish oil (FO; LC n-3 PUFA) or soybean oil (SO; n-6 PUFA control) for 4-7 weeks (n>20 hens/diet). Fertilized eggs were incubated to days E12, E14, E16, E18, and E20. Maternal diet effects at each age were compared using T-test ($\alpha=0.05$). Embryo weights were significantly lower in FO vs. SO at E12 and E14, but not at later ages. Absolute and relative subcutaneous adipose weights were not affected by diet at any age. However, histological analyses revealed that maternal FO significantly reduced adipocyte size and increased adipocyte number, suggesting an effect of hen diet on adipogenesis. Breast muscle (p. major) weight was lower in FO embryos at E12 and E14, but by E18 was significantly greater than in SO controls. In mammals, maternal LC n-3 PUFA have been shown to reduce fat accretion and increase lean mass through effects on mitochondrial metabolism and fatty acid oxidation (Rudolph et al., Diabetes, 2018). To test this possibility, primary preadipocytes and satellite cells were isolated from SO and FO chicks two days after hatch and induced to adipogenic and myogenic differentiation. Oxygen consumption rates (Seahorse XF, Agilent.com) revealed that hen FO significantly altered mitochondrial respiration and enhanced fatty acid utilization in both cell types. Collectively, these results indicate that dietary programming by LC n-3 PUFA may reduce fat accretion after hatch through effects on adipogenesis that begin in the embryo. In addition, maternal FO enhances metabolic flexibility in both adipose and muscle, which may improve energy utilization by the developing chick. Efforts to identify the underlying molecular basis for metabolic programming by LC n-3 PUFA are underway.

ID : 93

SELECTED EXCRETA BIOMARKERS OF INTESTINAL BARRIER FUNCTION IN BROILER CHICKENS
SUBJECTED TO TWO GUT LEAKAGE MODELS WITH OR WITHOUT PROBIOTIC SUPPLEMENTATION

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Perturbation of the intestinal barrier can lead to increased intestinal permeability (IP), elevated inflammation, high risk of enteric diseases and compromised performance in poultry. Common assessments of intestinal barrier function have been mainly through invasive methods involving differentially sized sugar tests, tissue sampling, or blood collection. Such methods are often complex, time-consuming and less field relevant. The present study aimed to identify selected biomarkers in excreta of broilers to facilitate the non-invasive assessment of gut barrier function. A 3 × 2 factorial arrangement of treatments was used with the main factors being gut barrier dysfunction models (control, rye-based diet, and dexamethasone – DEX) with and without probiotic supplementation (a three-strain *Bacillus*). Seventy-two male Ross 308 day-old chickens were kept in two groups given the same diets with or without probiotic supplementation. From days 13 to 21 of age, birds were individually housed and subjected to experimental treatments. Each of the 6 experimental treatments was replicated 12 times. On d 14, 16, 18 and 20, birds in the DEX group (n=24) were injected with DEX (0.5 mg/kg BW). Fluorescein isothiocyanate dextran (FITC-d) uptake into serum was used to test IP on d 21. Fresh excreta samples were collected on d 20. The excreta concentrations of Alpha 1 Antitrypsin (A1AT), Intestinal Fatty acid Binding Protein (IFABP-2), Fibronectin (FN) and Intestinal Alkaline Phosphatase (IAP) were measured using chicken specific ELISA assays. Data were subjected to two-way ANOVA to assess main effects and interaction. Treatment means were separated by Fisher's LSD test ($P < 0.05$). DEX and rye-based diet depressed feed intake, weight gain and increased feed conversion ratio compared with control birds. Only DEX increased FITC-d passage to the blood, indicating a greater IP. The excreta concentration of A1AT and IFABP-2 were unaltered by the experimental treatments. DEX increased ($P < 0.05$) FN concentration in excreta compared with control birds. Conversely, inclusion of rye in the diet reduced ($P < 0.05$) FN. Independently, DEX decreased IAP ($P < 0.05$) in excreta compared with control and rye-fed birds. There was no demonstrable effect of probiotic addition on any of the studied parameters. Subject to further validation studies the results reveal that FN and IAP, determined by ELISA, show promise as excreta biomarkers for rapid assessment of gut barrier function in poultry.

ID : 1324

SUN DRIED AZOLLA ALEAF MEAL AFFECTS GUT MICROBIOME AND INTESTINAL DEVELOPMENT THROUGH REGULATION OF P70S6 KINASE IN BROILER CHICKEN

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The inclusion of soybean in other industrial branches like biodiesel will reduce its availability for poultry feeding. Therefore, other feed alternatives are crucial to sustain poultry farming. Azolla leaf meal (ALM) was used as a novel alternative protein source in broiler chicken diet (Basak et al., 2002). However, the effect of ALM on gut health, intestinal development, and major bacterial population in broiler cecum is not known.

We hypothesized that inclusion of ALM in broiler chicken diet will affect growth performance and gut health of broiler chicken.

A total of 135 male Ross 308 broiler chicks were randomly allocated into 3 dietary treatments (3 replicate/treatment; 15 birds /replicate). The groups were CON; fed on diet contain 0% ALM, AZ5; fed on diet contain 5% ALM, AZ10; Fed on diet contain 10% ALM for 37 days, diets were formulated to be iso-nitrogenous and iso-caloric.

Feed intake and body weight were recorded at the end of each feeding stage (starter, grower, and finisher). Nine birds from each group were euthanized and used for sampling of cecal contents for microbiota study, and a segment from each intestinal part was collected in formalin for pathological evaluation with routine H&E staining using microarray technique. The abundance of total and activated P70S6 Kinase protein, the main activator of mTOR pathway that regulates cell growth, was measured by immunohistochemistry using microarray technique. Gene expression of selected bacterial genera was performed using qPCR, through isolation of 16S rRNA.

Data were analyzed via one-way ANOVA using the General Linear Models (GLM) procedure of SAS 9.4. Significance was set at $P \leq 0.05$ and tendency when $P > 0.05$ but $P \leq 0.10$.

Feed intake and body weight gain were increased in both ALM fed groups ($P = 0.02$), body weight tended to increase ($P = 0.10$), however FCR was similar in all groups ($P = 0.15$).

Class Bacilli expression was downregulated in AZ10% ($L = 0.03$). Family Enterobacteriaceae was markedly downregulated in both ALM fed groups ($L = 0.01$).

The noted effect of ALM on P70S6 Kinase was in duodenum and jejunum. The ratio of activated: total Kinase was increased in duodenum of ALM fed groups, while, it was only increased in jejunum of AZ5 group (P

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ID : 1203

THE POTENTIAL OF CANNABIDIOL AND NANOSIZED SELENIUM IN MODULATING IMMUNE RESPONSE IN CLOSTRIDIUM PERFRINGENS CHALLENGED CHICKENS

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The process of inflammation resulting from specific challenging conditions can be controlled via preventive veterinary. Nutrients with bioactive properties are the potential mediators that present biological activities in different levels. This study investigated actions of Cannabis sativa-derived cannabidiol (CBD) and nanosized selenium (nano-Se) on the expression of genes encodes cannabinoid receptors (CBD1 and CBD2) and peroxisome proliferator-activated receptor gamma (PPAR- γ) in brain and spleen of broilers not challenged or challenged with necrotic enteritis (NE).

A total of 216 male broiler Ross 308 were assigned to 3 groups with 8 replicates per group and 9 birds per replicate. From days 8 to 35 birds were feed: (i) control diet (C), (ii) diet supplemented with CBD and nano-Se, and (iii) diet as (ii) but birds were subjected to NE. At days 15, 16, 17 and 18 of life birds were challenged with *C. perfringens* in a subclinical dose. At day 23 of life, samples of brain and spleen were collected from 8 birds of each treatment. Differences between groups were determined using one-way ANOVA with a least significant difference test.

The body weight gain (BWG) for the period between days 9-23 was similar in C and diet with CBD and nano-Se, but was lower in challenged birds ($P=0.018$). BWG calculated for the whole period was similar in all groups ($P>0.05$). The feed conversion ratio (FCR) in the period of 9-23 days did not differ in C and treatment with CBD and nano-Se, but was worsened in challenged birds comparing to C ($P=0.005$). FCR for the whole period of the experiment did not differ between treatments ($P>0.05$). The expression level of CBD1 and CBD2 in birds brain and spleen was similar in all treatments ($P>0.05$). Expression level of PPAR- γ in brain did not differ between treatments however, in spleen it was significantly higher in challenged birds comparing to C and that fed CBD and nano-Se ($P=0.003$).

Our data indicated, that feeding chickens diets supplemented with CBD and nano-Se was not associated with the compromised performance, and it did not evoke higher expression of cannabinoid receptors genes. The results also suggest that feeding diets with CBD and nano-Se could positively stimulate immune system in challenged birds. The beneficial effect of such treatments was evidenced in up regulation of expression of gene determining immune function in birds.

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INGESTION AND DIGESTION

ID : 213

INFLUENCE OF CALCIUM LEVEL, CALCIUM SOURCE, AND PHYTASE ON PRECAECAL AMINO ACID DIGESTIBILITY AND INTESTINAL MICROBIOTA OF BROILER CHICKENS

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Reports on effects of phytase supplementation on precaecal digestibility (pcd) of amino acids (AA) are inconsistent. Phytase supplementation increased pcd of AA in some but not all studies. The Ca level of the diet previously has been shown to influence gastrointestinal phytate degradation. The Ca source might also be a relevant factor involved in phytate degradation. Therefore, we investigated whether pcd of AA is influenced by Ca level and Ca source and whether any effect is related to changes in microbiota composition in ileal digesta.

Six diets without mineral phosphorus were formulated with 5.8 or 8.2 g Ca/kg dry matter by inclusion of CaCO₃, CaCO₃+formic acid (FA), or Ca-formate (Ca-F). Each diet was used without or with phytase (1500 FTU Natuphos E/kg). Diets were tested in 6 pens with 15 broiler chickens each. Experimental diets were provided from day 16 post-hatch. Digesta from the terminal small intestine was collected and pooled on a pen-basis on day 21 and 22. Statistical models comprised Ca source, Ca level, phytase supplementation, and interactions between these factors as fixed effects, and a random block effect. Effects were considered to be significant if $P < 0.05$.

The Ca level \times phytase interaction was significant for pcd of all AA. Other interactions were not significant. Without supplemented phytase, pcd of all AA was lower at the high than at the low Ca level, with an average difference of 2.7 percentage points (pp) between Ca levels. No difference between Ca levels was determined when phytase was supplemented. The effect of Ca source was significant for all AA except for Cys. Adding FA to CaCO₃ increased pcd of all AA except Cys by an average of 1.1 pp. Exchange of CaCO₃+FA with Ca-F increased pcd of all AA except for Asx, Cys, and His significantly, on average by 1.1 pp. Main effects, but not interactions were significant when microbiota abundance in ileal digesta was analysed using permanova. Similar to pcd of AA, permanova showed a significant difference in microbiota composition of CaCO₃ compared to CaCO₃+FA and Ca-F. Correlations of relative abundance and pcd of most AA were significantly positive for *Lactobacillus johnsonii* and negative for *Gallibacterium* sp. and *Streptococcus alactolyticus*.

The results suggest that dietary Ca levels, but not Ca sources contribute to inconsistency of phytase effects on pcd of AA found in the literature. Changes in pcd of AA may be connected to accompanying changes in microbiota composition.

ID : 255

EFFECTS OF NUTRITIONAL SUPPLEMENTATIONS ON GROWTH AND SELECTED BLOOD BIOMARKERS IN BROILER CHICKENS VACCINATED AGAINST COCCIDIOSIS

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With the increasing concerns of drug resistance, vaccination against coccidiosis has become an important tool to control the disease. However, in practice vaccinated birds are usually observed with a deterioration of body weight gain. This loss could possibly due to the nutrients spared for immune systems, inflammatory and oxidative stress, as well as the damaged gut integrity. This study aimed to identify nutritional supplementations which compensate this loss. 960 Ross 308 males, including 240 non-vaccinated chicks (NV) and 720 hatchery-vaccinated (V) were weighted and divided into 64 pens with 8 treatments and 8 replications. Experimental treatments were as following: NVC: NV with coccidiostats, NVS: NV with standard diet, VS: V with standard diet, VArg: V with higher level of Arginine, VThr: V with higher level of threonine, Valgae: V and sulfated polysaccharides from algae, VEp: V and Echinacea purpurea, VPE: V with a blend of essential oils. The feed program included 3 phases: starter (0-10d), grower (11-20d) and finisher (21-35d). Chicken weight and feed consumption were measured at D10, D20 and D35. Blood samples were taken at D0, D20 and D35 to measure malondialdehyde (MDA) and glutathion peroxydase activity (GPx), as oxidative stress biomarkers. At D20, 2 chickens per pen were randomly selected to carry out the coccidiosis lesion score. At birth, V chicks were 1.5% lower than NV chicks ($p<0.001$). During starter period, no effect of treatment was observed on zootechnical performance. During grower period, NVC and NVS had significantly greater average daily gain (ADG; 63.7 g/d) than VPE (58.8 g/d) whereas the other treatments were intermediate ($p=0.01$). No effect was recorded on feed conversion ratio (FCR). During finisher period, no effect was seen on ADG but the FCR was improved for VArg and VThr (1.55) compared to NVS (1.59) ($p=0.015$). On the whole period, no significant difference was highlighted, but all nutritional strategies permitted a numerically improvement of growth (2 to 3%) in comparison with VS group. Concerning the total mean lesion score, it was low for all treatments, with the highest score in VPE (1.31) and the lowest in NVS (0.5) ($p=0.019$), the other treatments were intermediate. No significant effect of treatments was observed on GPx and MDA, but GPx of VArg and VThr tended to be higher than GPx of VS. To conclude, this work needs further investigations but these nutritional strategies seem to be promising.

ID : 343

INFLUENCE OF FEED FORM ON STANDARDISED ILEAL AMINO ACID DIGESTIBILITY OF PROTEIN SOURCES IN BROILER CHICKENS

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Knowledge on the digestibility of amino acids (AA) in raw materials is critical for precise feed formulation and, for more efficient and sustainable use of feed resources. Published data on digestible AA values for broilers are based on mash feed, because of its simplicity. Broilers, however, are fed pelleted diets and the applicability of data generated with mash diets to pelleted diets could be questioned. Based on the above, it was hypothesised that broilers fed the same ingredient but in different feed form (FF) may show different AA digestibility values. Therefore, 336, 19-day-old male Ross 308 broilers were used to determine the influence of feed form (FF) on standardised ileal digestibility (SID) of nitrogen (N) and AA in different protein sources (PS). A completely randomised design was used to develop six experimental diets in a 3 × 2 factorial arrangement including three PS (i.e. meat and bone meal [MBM], soybean meal [SBM], and canola meal [CM]) in mash and pelleted forms. The inclusion rate of PS in three assay diets, were 383, 413 and 553 g/kg diet, respectively, and each assay diet contained about 18% protein. The basal endogenous N and AA losses were determined by offering a N-free diet (NFD) to broilers. The assay diets and NFD were fed to birds from day 19 to 23. The ileal digesta was collected from all the birds on day 23. The SID of N was higher ($P < 0.05$) in SBM followed by MBM and CM. The average SID values for indispensable AA (IAA) were similar in SBM and MBM and greater ($P < 0.05$) than CM. Average SID values for dispensable AA (DAA) was significantly ($P < 0.05$) higher in SBM than MBM and CM. Histidine was the only indispensable AA influenced by FF, and its SID was reduced ($P < 0.05$) by pelleting. Pelleting, however, resulted in significant ($P < 0.001-0.05$) reduction in the SID of all DAA and average of all AA. Pelleting decreased ($P < 0.05$) the standardised digestible protein and average of all AA contents. Overall, present data demonstrates that FF can influence N and AA digestibility of PS, in particular digestibility of DAA. Therefore, FF should be taken into account in AA evaluation assays.

ID : 368

IMPROVING FEED EFFICIENCY BY BOOSTING DIGESTIVE AND EXOGENOUS ENZYMES THANKS TO CLAY AND ALGAE

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Several types of feed additives are available to improve feed digestibility, including exogenous enzymes which target specific nutrients that are not digested by endogenous enzymes. Recently, an innovative algo-clay complex (ACC) developed by Olmix Group (France) has shown interest in increasing the activity of endogenous digestive enzymes and feed efficiency. The present work aims at reviewing the effect of this ACC on broilers performance depending on the use of exogenous enzymes in the diet. Three different studies were implemented in the Poultry Research Center of the University of Viçosa (Brazil). All studies used Cobb 500 male broilers fed corn-soybean diets. Animals were placed in pens of 20 (Study 1) or 22 (Studies 2 and 3) chicks, with 10 replicates per treatment. In each study, the treatments were control (no ACC supplementation) and test (with 0.1% ACC in all feeding phases). The diets in the 3 studies differed by their supplementation in exogenous enzymes: no exogenous enzymes were used in Study 1, phytase was used in all groups in Study 2 and phytase and carbohydrases (NSP enzymes) were used in all groups in Study 3. Results of the Study 1 showed a decrease in feed conversion ratio (FCR) when using the ACC in grower phase (day 22-35; -4%, $P<0.05$) and total cycle (day 0-35, -3%, $P<0.05$). Growth rate tended to be higher in the test group than control in the grower phase (+4%, $P<0.10$) with a final weight (at 35 days) of 2.449 kg in the test group and 2.392 kg in the control group ($P=0.127$). In the Study 2, the ACC supplementation lead to a decreased FCR of 2% in the total period (day 0-42, $P<0.05$) with the highest effect in the grower phase (-4%, $P<0.05$). The final weight (42 days) was increased by 78g in the test group compared to control (respectively 3.337 kg vs 3.259 kg, $P=0.163$). In the Study 3, the FCR (day 0-42) was decreased by 3% in the test group compared to control ($P<0.05$) with the highest effect in the finisher phase (-8%, $P<0.05$). Growth rate followed the same trend with an increase in average daily gain (ADG) of 4% ($P<0.01$), resulting in a 134g higher final weight (42 days). The highest effect was observed in the finisher phase with a 12% increase in ADG ($P<0.01$). All in all, these studies highlight the capacity of the ACC to improve feed efficiency and growth of broilers independently of the use of exogenous enzymes. The demonstration of a synergistic effect between the ACC and exogenous enzymes requires further investigation.

ID : 387

EXOGENOUS XYLANASE IMPROVES BROILER GROWTH PERFORMANCE BY INCREASING THE RATE OF DIGESTION

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The effect of dietary supplementation with the feed-enzyme RONOZYME® WX (DSM Nutritional Products Switzerland), a mono-component xylanase, on broiler performance and digestibility kinetics was investigated in Ross 308 chickens. We hypothesized that the xylanase would improve growth performance through an increase in the intestinal rate of digestion. The study included 2850 1-day old male broilers, divided into 5 treatments using 19 pens per treatment and 30 birds per pen. The positive control (PC) diet was formulated for optimal growth and based on wheat, soya bean meal and corn. Corn was substituted with increasing amounts of wheat-by-products and rye in the negative control and negative control high viscosity diets with and without RONOZYME® WX. The negative control diets were optimized using the Ross 308 Nutrition Specifications 2014 except for a reduction in apparent metabolizable energy (1 MJ/kg) and digestible amino acid content. Data were analyzed by a mixed model with age as repeated measures, showing a positive effect on final body weight at d35 and increased average feed intake compared to PC birds. The xylanase supplementation resulted in a 3% reduction in the feed conversion ratio ($P<0.001$) and the European feed efficiency of the enzyme treatments were no different from PC, but higher than unsupplemented diets ($P=0.02$). The enzymatic activity of the xylanase on cereal cell wall components was illustrated by microscopy in several parts of the digestive tract including crop, duodenum, jejunum and ileum. This was confirmed by improved digestibility of organic matter and starch ($P<0.001$) in the first half of the small intestine in xylanase supplemented birds, using TiO₂ as a digestibility marker. The improvement was expressed less in the lower parts of the small intestine. This may indicate that the improved growth performance originated from a change in the intestinal site of digestion. It is speculated that the absorbed nutrients available for growth are greater with the dietary supplementation of xylanase, because of a more rapid digestion in the proximal part of the intestine. This may decrease the microbial uptake of nutrients in the distal part of the small intestine, thus ensuring an overall greater uptake of nutrients by the bird. In conclusion RONOZYME® WX improves the growth performance in broiler chickens and promotes the proximal digestion of organic matter and starch, giving a higher final bodyweight at d35 than PC fed birds.

ID : 446

THE DIGESTIBILITY OF SOLUBLE NON-STARCH POLYSACCHARIDES AND FREE OLIGOSACCHARIDES IN BROILER CHICKENS OFFERED CORN- OR WHEAT-BASED DIET

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Poultry lack endogenous enzymes for efficient utilisation of non-starch polysaccharides (NSP), and therefore digestion of NSP is achieved by bacterial fermentation in the absence of exogenous enzymes. The efficiency of NSP digestion varies depending on animal age and the physical/chemical properties, feed processing and net concentration of NSP in the diet. This study aimed to investigate the digestibility of soluble NSP and free oligosaccharides in corn- or wheat-based diets. A total of 144 Cobb 500 mixed sex day-old broiler chickens were offered either wheat- or corn-based diets in pelleted form, with 12 replicates of 6 birds per treatment. Titanium dioxide was added at 5 g/kg to all diets as an inert marker and quantified by UV-spectroscopy to calculate the apparent digestibility. On 35 days of age, excreta was collected and pooled by pen and digesta was collected from the jejunum and ileum of four randomly selected birds per pen. Soluble NSP and free oligosaccharides in the diet, digesta and excreta samples were isolated by an enzymatic-chemical method and released constituent sugars were determined by gas chromatography. After testing for normality, an independent-sample t-test was used to compare the two treatments. The wheat-based diet was determined to contain greater soluble NSP content compared to the corn-based diet (15.9 vs. 6.2 g/kg DM), but free oligosaccharide concentrations were similar in both diets (36.9 vs. 37.2 g/kg DM). Apparent digestibility of soluble NSP in the jejunum and ileum was not affected by dietary treatment; but total tract digestibility was higher ($P<0.001$) in birds offered the wheat-based diet compared to the corn-based diet (44.8 vs. 23.2%). This suggests hindgut microbiota may prefer to utilise soluble NSP from wheat rather than corn. Apparent jejunal and ileal digestibility of free oligosaccharides was greater in birds offered the corn-based diet than those fed the wheat-based diet ($P=0.006$ and $P<0.001$, respectively). Total tract free oligosaccharide digestibility was higher in birds offered the corn-based diet compared to the wheat-based diet (82.3 vs. 75.83%, $P=0.034$). Thus, it appears free oligosaccharides from corn are more digestible throughout the small and large intestine compared to those derived from wheat. In conclusion, the digestive dynamics of soluble NSP and free oligosaccharides present in corn and wheat differ owing to the net quantities and source as well as intestinal environmental conditions.

ID : 517

COMPARING THE EFFECT OF TWO COMMERCIALY AVAILABLE COCCIDIA VACCINE CHALLENGE ON APPARENT ILEAL NUTRIENT DIGESTIBILITY AND INTESTINAL MORPHOLOGY IN 21 DAY-OLD BROILER CHICKENS FED A CORN-SOYBEAN MEAL-PORCINE MEAT AND BONE MEAL-BASED DIET

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The effect of two commercially available coccidia vaccine challenge (CVC) on the ileal amino acid, energy, calcium, and phosphorus digestibility and intestinal morphology was evaluated in 21 day-old broiler chickens. On day 14, one hundred and sixty-eight day-old broiler chicks were allotted to three treatments in a completely randomized design with seven replicate cages/treatment and eight birds/replicate cage. The treatments were the control, and control challenged with either Coccivac® B52 or D2 (Merck Animal Health). The Coccivac®-B2 contained live oocysts of *Eimeria acervulina*, *E. maxima*, *E. mivati*, and *E. tenella* while Coccivac®-D2 contained live oocysts of *E. brunetti* and *E. necatrix* in addition to those found in B52. All birds were fed a standard corn-soybean meal-porcine meat and bone meal-based diet from day 0 to 21. On day 14, the control birds were orally gavaged with 0.6 mL of distilled water, while the CVC birds were orally gavaged with 0.6 mL of distilled water containing 20x (approximately 2x of the recommended dose for day-old chick, by body weight) of B52 and D2 vaccine, respectively. Birds and feed were weighed on day 14 and 21. On day 21, birds were euthanized followed by ileal digesta collection. The mid-section of duodenum, jejunum, and ileum were removed for histology. Liver and spleen weights were also determined. Data were analyzed using the GLM procedure of SAS with mean separation using Tukey's test, where necessary. Specific contrast was used to compare the effect of B52 and D2. Bodyweight gain was highest ($P < 0.05$) in the control birds and lowest ($P < 0.05$) in the D2-challenged birds. Feed efficiency was lower ($P < 0.05$) in the D2-challenged birds compared to the control birds. The weight of the spleen relative to bodyweight for the D2-challenged birds was lower ($P < 0.05$) compared to the B52-challenged birds. Bone breaking strength (kgF) was lower ($P < 0.05$) in the B52-challenged birds compared to the control birds. Apparent ileal digestibility of dry matter, N, energy, Ca, amino acids, and digestible energy was lower ($P < 0.05$) in the CVC birds compared to the control birds. Duodenal and jejunal crypt depth, and villus height:crypt depth ratio was lower ($P < 0.05$) in the CVC birds compared to the control birds. However, ileum crypt depth was deeper ($P < 0.05$) in the CVC birds. Data from this study showed that both coccidia vaccine type reduced apparent ileal nutrient and energy digestibility compared to the control.

ID : 527

NEW DIETARY β -MANNANASE IMPROVES BROILER PERFORMANCE AND NUTRIENT DIGESTIBILITY

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Soybean meal is one of the main feed ingredients in poultry nutrition due to its high protein level and favourable amino acid composition. However, soybean meal contains non-starch polysaccharides such as β -mannans that are in general considered for being so called antinutritive factors. These factors can potentially decrease nutrient utilization and thereby impair performance of animals. Therefore, we evaluated the efficiency of a supplemented fungal β -mannanase in broiler on nutrient digestibility and growth. A total of 960 Ross-308 1 d-old male broilers were distributed to 24 pens with 40 animals each. The study was set up as a randomized complete block design, with two treatments replicated 12 times each. Dietary treatments were: a control diet based on corn-soybean meal without any additional enzyme supplementation, and the control diet supplemented with mannanase at 800 Thermostable Mannanase Units (TMU) per kg (BASF SE, Germany). Diets were fed in 2 phases (starter: 1-21d, finisher: 22-35 d). Performance from 1 to 35 d of age and apparent ileal digestibility (AID) of dry matter, energy, fat, nitrogen and amino acids at 36 d of age were measured. Mannanase increased daily gain and feed to gain ratio from 22-35 d ($p<0.05$), and daily gain from 1-35 d and final live weight at 35 d ($p<0.05$) (58.0 g/d vs. 60.4 g/d and 2071 g vs. 2156 g; respectively). Mannanase tended to improve overall feed to gain ration, from 1.474 to 1.459 ($p=0.084$). Moreover, the European Production Efficiency Factor was increased by mannanase from 384 to 411 ($p<0.05$). Mannanase increased the AID of dry matter, energy and fat ($p<0.05$), and nitrogen ($p<0.001$). In addition, mannanase increased the AID of all amino acids ($p<0.05$; $p<0.01$; $p<0.001$), on average by +2.4 percentage points, except the AID of methionine. In conclusion, dietary mannanase increased the digestibility of the nutrients in the diet and thereby improved the performance of broiler chickens.

ID : 537

CHESTNUT TANNINS IN BROILER DIETS AND THEIR EFFECT ON NUTRIENT DIGESTIBILITY

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The working mechanism of chestnut tannins and their effect on broiler metabolism are not yet fully elucidated. These tannins can be used as an additive to improve gut health and performance. The goal of this study was to determine the effect of chestnut tannins on nutrient digestibility of broilers fed maize-soy diets or wheat-rapeseed-palm oil diets. This trial is part of a larger project focused on unravelling the working mechanism of chestnut tannins.

Two contrasting basal diets, a corn-soy based and a wheat-rapeseed-palm oil based challenge diet were used to assess the interaction of chestnut tannins with dietary nutrients. These diets were formulated to similar nutritional value and chestnut tannins (Tanno-SAN®, Sanluc International, Gent, Belgium) were added to these diets (0 mg/kg, 500 mg/kg or 2000 mg/kg) resulting in 6 different treatments. 216 broiler chicks (Ross 308) of 21 days of age were allocated in 72 digestibility units (3 chicks per cage and 12 replicates per treatment) for a balance period of 5 consecutive days after which blood and faeces were collected to determine gross energy, crude protein, crude fat and uric acid. From these results apparent digestibility coefficients for gross energy, crude fat, metabolizable energy corrected for N, and crude protein corrected for uric acid were calculated. On blood samples non-esterified fatty acids (NEFA), triglycerides and glucose were determined. Results were analyzed with R Studio using linear models with dose of tannins and diet as factor.

Despite comparable nutritional values, there were significant differences between the two basal diets. Corn based diets resulted in better digestibility coefficients than wheat based diets. In contrast, addition of tannins showed no differences in all digestibility coefficients. There was no interaction between diet and tannin dose. Regarding blood values, corn based diets resulted in significantly higher NEFA and triglycerides values. Glucose levels were not different between diets. There was no effect of tannins on blood parameters nor significant interactions between diet and tannins.

From these results, it seems that chestnut tannins do not affect broiler digestibility. However, from other trials, an effect of chestnut tannins was observed on performance. We are currently looking in more detail to other parameters to help explain these apparently contradictory effects, but these results are not yet available at time of submission of this abstract.

ID : 585

ILEAL DIGESTA FLOW AND BASAL ENDOGENOUS LOSS OF PHOSPHORUS RESPONSE TO A PHOSPHORUS-FREE DIET WITH DIFFERENT FEEDING DURATION FOR BROILER CHICKENS

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The standardized ileal digestibility (SID) of phosphorus (P) is known to be more additive rather than apparent ileal digestibility for feed formulation and are calculated by correcting basal endogenous losses (BEL) which is commonly determined by feeding P-free diet (PFD). However, the impact of feeding length of PFD on BEL of P is not fully understood. Therefore, the objective of the present study was to determine the proper feeding duration of PFD to estimate the BEL of P for broiler chickens. A total of 384 male broilers (Ross 308) were fed a commercial starter diet for 14 days. On d 15, the birds were weighed and allocated to three treatment groups in a randomized complete block design with 8 replicate cages per treatment and 16 birds per cage. Birds in each of the treatment groups were fed the PFD for 2, 3, or 4 days, respectively. Chromic oxide was used as an index for determining the BEL of P. After feeding of PFD for 2, 3, or 4 days, the birds were asphyxiated with CO₂ and ileal digesta and ileum samples were collected. Growth performance and weight of ileal digesta and ileum were measured for each sampling day. The birds fed the PFD for 2 days had lower ($P < 0.05$) final BW compared to the birds fed diet for 3 or 4 days. The relative dried weight of the ileum (mg/100 g of BW) was greater ($P < 0.05$) in birds fed the PFD for 2 days compared with the birds fed diet for 3 or 4 days. However, the amount of the dried ileal digesta (g/bird) was not affected by the duration of PFD feeding. The BEL of P was calculated to be 103.2, 102.1, and 110.6 mg/DMI in birds fed the PFD for 2, 3, and 4, respectively, but there was no significant difference among the treatments. The collection of ileal digesta from broilers fed PFD for 2, 3, or 4 days might not give different amount of ileal digesta as well as different values of BEL of P.

Keywords: feeding duration, phosphorus free diet, endogenous loss, phosphorus, broiler

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ID : 680

THE SUPPLEMENTATION OF BROILER FEEDS WITH CAPSICUM BASED ADDITIVE IMPROVES ANIMAL PERFORMANCE. THE RESULTS OF A META-ANALYSIS.

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Capsaicin is the main active compound in capsicum oleoresin derived from red pepper (*Capsicum* spp.). Studies have demonstrated that the inclusion of plant extracts high in capsaicin can improve the productivity of broiler chickens. The meta-analysis is a useful tool to perform a joint analysis of different studies as it increases the statistical power of the test when comparing the mean of a control diet and a treatment (i.e. a feed additive). In this study, we used the technique of meta-analysis to address if the phytogetic additive based on capsaicin LUCTAROM® Convert (LOM) improves broiler chicken performance. A total of 8 in-vivo nutritional trials run by the company LUCTA S.A. between 2003 and 2019 were analysed. They were performed in different experimental facilities testing the effect of the additive LOM on average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio FCR and mortality in broilers fed from 1 to 21 and from 22 to 35-42 days of age. The data were analysed using a mixed model with LOM supplementation, sex, dietary fat type and their interactions as fixed effects and the trial as a random effect. Dietary fat level and age at the end of the growth period were included as lineal covariates. The supplementation with LOM during the first period (1-21 days of age) significantly improved FCR 1.431 vs. 1.418 g/g for control and treatment respectively ($P = 0.0062$). Also, the additive inclusion tended to increase ADG ($P = 0.052$) with no effects on ADFI ($P = 0.80$) and mortality ($P = 0.25$). The positive effect of the additive on FCR tended to be higher ($P = 0.060$ vs. 0.198) in feeds with animal source as added fat rather than with vegetal. Similar findings were observed for the second period from 22 to 35-42 days of age. The supplementation with LOM in grower feeds improved ADG from 95.1 in control to 96.3 g/d in treatment groups ($P = 0.028$) and tended to improve FCR from 1,759 to 1,744 g/g ($P = 0.065$). Again the positive effect of the additive on FCR tended to be higher ($P = 0.10$) in feeds with animal source as added fat than with a vegetal fat source ($P = 0.22$). The meta-analysis of 8 trials with LOM shows a significant improvement of this additive based on capsaicin on broiler performance.

ID : 808

THE IMPACT OF XYLANASE ON THE FATE OF NUTRIENTS AND NON-STARCH POLYSACCHARIDES ACROSS THE GASTROINTESTINAL TRACT OF BROILERS FED WHEAT DIETS

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Feed supplementation with enzymes active on non-starch polysaccharides (NSP) is widely practiced to improve animal performance. Enzyme inclusion has been reported to decrease the digesta viscosity facilitating feed digestion¹. Xylanase addition could also lead to the formation of arabinoxylan oligosaccharides (AXOS), compounds exhibiting prebiotic potential². The aim of this research is to determine the impact of xylanase on wheat NSP structure and passage through the gastrointestinal tract (GIT) and evaluate possible implications on animal performance.

During this research, 48 male broilers were assigned to two treatments following a randomized block design in 6 pens per treatment. The diets consisted of a wheat-soy control diet (WC) and a wheat-soy diet supplemented with commercial xylanase and cellulase (WE). Acid insoluble ash (AIA) was used as digestibility marker. After 28 days, the birds were euthanized and samples from gizzard, ileum and caeca were collected. Excreta samples were collected between day 24 and 28. Samples were freeze-dried and analyzed for dry matter, marker, starch, protein and total sugar composition.

Enzyme inclusion in the diet (WE) increased body weight and lowered feed conversion ratio compared to the control diet (WC). Additionally, enzyme supplementation resulted in increased apparent ileal digestibility of organic matter and total glucose, while the effect on individual feed components (protein, starch) is currently under investigation. The relative ratio of NSP xylose and arabinose to AIA was similar in the feed and ileum digesta for both WC and WE. These ratios decreased significantly in the excreta samples, suggesting carbohydrate fermentation in the hindgut. In excreta, the Xyl:AIA ratio did not differ between WC and WE but suggested a trend ($0.1 > p > 0.05$), indicating that xylanase improved xylose utilization in the hindgut. To further determine the impact of xylanase supplementation on NSP, ileal samples were subjected to Mass Spectrometry and the presence of AXOs was shown in WE, but not in WC. In both WE and WC, hexose oligomers (maltodextrins) were present as well.

Xylanase activity did not affect NSP passage in the GIT, but altered their structure, forming potentially prebiotic AXOs and improving glucose digestibility, leading to improved animal performance.

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ID : 875

A NEW FUNGAL B-MANNANASE IMPROVES BROILER PERFORMANCE IN A XYLANASE AND GLUCANASE CONTAINING DIET

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β -Mannans belong to the NSP fraction in feed. They are found in e.g. soybean meal, soybean hulls, and guar meal. They are considered anti-nutritional factors, potentially decreasing nutrient digestibility. Therefore, enzymes that break down β -mannans, i.e. β -mannanases, can possibly improve performance. Commercial broiler diets usually contain NSP enzymes such as xylanase and glucanase. Although its substrate is different, it is unclear whether β -mannanase adds value to diets already containing xylanase and glucanase. Thus, a broiler performance trial was carried out to study the effect of β -mannanase at two dose levels in the presence of xylanase and glucanase.

The trial had a randomized complete block design with 4 treatments and 12 replicates. Each replicate was a floor pen with 20 Ross 308 male broilers. Treatment 1 was a Control without NSP enzymes. Treatments 2-4 contained xylanase and glucanase (Natugrain® TS, BASF SE, Germany) added to the Control (= XG). Treatments 3 and 4 were additionally supplemented with β -mannanase (BASF SE, Germany) at 400 and 800 Thermostable Mannanase Units (TMU) per kg (= XGM400 and XGM800). All diets contained 500 FTU phytase (Natuphos E 5000G, BASF SE, Germany) per kg. Birds were fed with a 3-phase feeding regime: starter (0-14 d), grower (14-28 d) and finisher (28-35 d). The pelleted diets were based on wheat-maize-SBM and contained also barley, rye and soybean hulls. Drinking water and feed was available ad libitum during the whole trial. The data per phase and overall period were analyzed with one-way ANOVA.

Results: Broilers fed the XG diets showed a significantly improved FCR compared to broilers fed the Control diet in the starter phase, with no additional improvement due to mannanase. In the finisher phase, XG diets had a numerically lower FCR than the Control diet, which became significantly lower when β -mannanase was supplemented (XGM400 and XGM800). The overall experimental period showed a clear distinction between the different treatments. Adding xylanase and glucanase to the Control diet significantly improved FCR. Moreover, supplementation with β -mannanase resulted in an even further significantly improved FCR.

In conclusion, mannanase at 400 and 800 TMU/kg in NSP-rich diets had positive effects on FCR in broilers, even on top of xylanase and glucanase. The significant effect was found for both mannanase dose levels.

ID : 977

THE EFFECT OF UNHEATED, LOW-TRYPSIN INHIBITOR SOYBEANS ON THE DIGESTIBILITY AND GROWTH PERFORMANCE OF TOM TURKEYS REARED TO 14 AND 28 DAYS OF AGE

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Two trials (T) were conducted to evaluate the effect of poult age on the optimal inclusion rate of a novel low trypsin inhibitor soybean in turkey starter diets. Poults were either raised in groups of 6 from hatch to 2 wk (T1) or groups of 5 from 2 to 4 wk (T2). Male Hybrid Converter turkey poults (528) were randomly assigned to 48 battery cages. Poults were provided ad libitum access to water and feed. All finished feeds were iso-caloric and iso-nitrogenous with treatments defined by the inclusion of solvent extracted soybean meal (SBM), the unheated low trypsin inhibitor (LTI) soybean at 20, 30, or 40%, or the conventional unheated soybean (CB) at 20 or 40%. Poults and feeders were weighed weekly to determine body weight (BW) and feed intake (FI) to calculate feed conversion ratio (FCR). At d 14 and 28 (T1 & T2) excreta were collected and pooled by pen for the determination of apparent metabolizable energy (AMEn) and apparent lipid digestibility (ALD). All poults were euthanized via cervical dislocation and pancreas excised. Pancreas weights were expressed relative to poult BW (PRW). Overall, increased inclusion of the LTI bean from 0 to 40% linearly decreased BW (384, 350, 360, and 329 g, ± 14.2) and FI (415, 390, 375, and 370 g, ± 15.4) from 0 to 14 d, while increasing FCR (0.97, 1.11, 1.05, and 1.16, ± 0.05) and PRW (0.37, 0.45, 0.50, and 0.52%, ± 0.01) when compared to SBM. Based on performance and PRW of the 14 d poults, the TI was still present at levels sufficient to affect the protein digestibility. Performance was improved in T2 with older poults. At d 28, diets containing increasing LTI from 0 to 40% remained comparable to SBM in BW (1599, 1570, 1590, and 1545 g, ± 32.3), FI (1841, 1818, 1772, and 1731 g, ± 51.2) and FCR (1.75, 1.78, 1.70, and 1.74, ± 0.04), though PRW (0.28, 0.34, 0.37, and 0.41%, ± 0.01) remained greater in relation to increasing LTI inclusion. Feeding CB diets typically suppressed performance compared to SBM and LTI. At 14 d, feeding 20 or 40% CB reduced BW (312 and 240 g, ± 14.2) and FI (348 and 315 g, ± 15.4) and increased FCR (1.15 and 1.45, ± 0.05) and PRW (0.71 and 0.89%, ± 0.01). At 28 d, CB again reduced BW (1510 and 1409 g, ± 32.3) and FI (1694 and 1550 g, ± 51.2) with increased PRW (0.51 and 0.68%, ± 0.01), though FCR (1.77 and 1.80, ± 0.04) remained similar. Therefore, the more mature poult was better able to utilize the nutrients in the LTI diets.

ID : 981

DIFFERENCES IN ORIGIN AND DOSE OF DIETARY ENDOXYLANASES RESULT IN DISTINCT ARABINOXYLAN DEGRADATION PATTERNS IN THE GASTROINTESTINAL TRACT OF YOUNG BROILERS FED WHEAT-BASED DIETS

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Despite their general use in poultry feed, the predominant mode of action of endoxylanases in improving broiler performance is not fully understood. The abundance of different endoxylanases used in the field and the variable response to their application often complicates a clear understanding of the functionality of endoxylanase in vivo. In this study, we hypothesized that arabinoxylan (AX) hydrolysis and microbial fermentation in broilers fed a wheat-based diet is different for endoxylanases of different origin and at different dosages. To investigate this hypothesis, a digestibility trial was designed to provide further insights into the contribution of dose (10, 100, 1000 ppm) and origin (*B. subtilis* vs. *N. flexuosa*) of endoxylanases on the structure-function relationship of AX products formed in vivo in the hindgut of young (d 11) and old (d 36) broilers (Ross 308). The residual AX population, the AX digestibility coefficients and the metabolites produced during microbial AX fermentation were analyzed at the level of the ileum, caeca and feces. Data were statistically analyzed using a three-way ANOVA with enzyme, dose and age and their second order interactions as model effects. Endoxylanase supplementation resulted in a more extensive degradation of wheat AX and reduction in intestinal viscosity compared to the control ($P < 0.05$). In spite of their difference in substrate preference, solubilizing the wheat AX was clearly the main activity of both endoxylanases. Endoxylanases produced from *B. subtilis* stimulated precaecal digestion of dietary AX by creating a large pool of solubles, thereby increasing ileal viscosity compared to broilers fed an endoxylanase from *N. flexuosa* ($P < 0.05$). This latter endoxylanase mainly triggered caecal AX fermentation in the young broiler by delivering easily fermentable AX substrates having a low polymerization degree ($P = 0.03$). At least 100 ppm for either endoxylanase was required to obtain improved hydrolysis of the dietary AX along the GIT against the control ($P < 0.05$). The effects observed were particularly present in young broilers as they are more prone to anti-nutritional factors and dependent on the fiber degrading capacity of the young intestinal microbiome. From this study, it is clear that the origin and dose of endoxylanases added to a wheat-based diet determine the nature of the heterogeneous pool of AX substrates that is formed, which in turn affects intestinal viscosity and AX digestion at young ages.

ID : 1016

EVOLUTION OF LIPID CLASSES ALONG THE GASTROINTESTINAL TRACT IN BROILER STARTER CHICKENS:
EFFECT OF SOYBEAN ACID OIL COMBINED WITH PALM OIL

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Acid oils are by-products from the refining process of edible oils, which have a similar fatty acids (FA) profile than their corresponding crude oils, but higher proportions of free FA (FFA: 40-90%). Their use in poultry nutrition could contribute to improving the sustainability of the food chain. It has been suggested that the absorption of fats is more important as a limiting step comparing with their hydrolysis. Therefore, it has been hypothesized that the study of the evolution of lipid classes (TAG: triacylglycerols; DAG: monoacylglycerols; MAG: monoacylglycerols, and FFA) during the absorption process will allow for a better understanding the differences observed in the digestibility of FA. The objective of the present study is to evaluate the effect of the combination of soybean acid oil (SA) with palm oil (P) on the lipid-class composition along the gastrointestinal tract (GIT) and excreta in starter broiler chickens. A total of 480 birds (16 per cage; 6 replicas per treatment) were assigned to 5 dietary treatments from 0 to 11d. A basal diet was supplemented at 6% with: P (TAG: 78%; FFA: 9%), S (TAG: 72%, FFA: 14%), SA (TAG: 28%, FFA: 56%), or the combination of 33%SA-66%P, and 66%SA-33%P. At 11d, content from upper and lower jejunum, upper and lower ileum, and excreta was collected for the determination of lipid-class content (mg/g) /Ti (mg/g). Data was statistically analysed by one-way ANOVA using the SAS statistical package. In general, TAG, DAG and FFA decreased along the GIT studied, but an increase of these lipid classes was observed in excreta. The highest disappearance of the main final lipolytic product (FFA) occurs from the upper to lower jejunum, which is associated with the highest FA digestibility values obtained in this segment. The replacement of SA by P caused lower levels of MAG and higher content of FFA in all GIT and excreta samples studied (P<0.05), in agreement, these fats followed similar pattern in the digestibility of FA along the GIT. The results suggest that the study of the lipid classes can be used to understand the dynamics of fatty acids absorption. The inclusion of palm oil is not recommended in young broiler diets. Instead, soybean acid oil could be adequate in starter broiler chickens, obtaining similar FA absorption than soybean oil.

ID : 1190

EFFECT OF FEEDING PROBIOTIC, ACIDIFIER OR NUCLEOTIDE ON THE PERFORMANCE OF BROILER CHICKENS

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EFFECT OF FEEDING PROBIOTIC, ACIDIFIER OR NUCLEOTIDE ON THE PERFORMANCE OF BROILER CHICKEN

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Substantial growth in the poultry industry has been achieved in the recent past mainly due to exploitation of various growth-promoting agents. Probiotics, acidifiers and nucleotide are the front runners among them. The present experiment has been planned to study the growth performance of broiler chickens by feeding probiotics (*Bacillus subtilis*, 1.6×10^{10} cfu/g), acidifier (a mixture of acetic, propionic and sorbic acids) or nucleotide in the ration. Day-old commercial Cobb-100 chicks (n=140) were randomly distributed into four equal groups, viz., T0, T1, T2 and T3 containing 35 chicks, which were further divided into 5 replicates of 7 each. The experiment was conducted under the randomized block design. Four dietary treatments were formulated as per BIS (2007) and were assigned to the above group as T0 (no growth promoters in feed, i.e., control), T1 (T0 with probiotic @ 50g/ton of feed), T2 (T0 with acidifier, @ 2 kg/ton of feed) and T3 (T0 with nucleotide, @1 kg/ton of feed). Feeding was accomplished for 6 weeks under the battery cage system of management. The experimental data were subjected to statistical analysis (Snedecor and Cochran, 1994).

From among all groups the probiotic fed showed significantly (Pth week this group achieved 1.62% higher body weight as compared to the control group. Parallely, the retentions of dry matter, crude protein, ether extract, calcium and phosphorus were improved to the extent of 2.95%, 4.61%, 4.41%, 4.08% and 4.74%, respectively. The probiotics containing *Bacillus subtilis* organism might have helped in synthesizing more body proteins, lipids and minerals resulting in higher body growth. The broiler performance efficiency factor was highest in the probiotic fed group (98.30) followed by nucleotide (94.90), acidifier (93.33) and the control diet-fed group (91.82). Feeding of growth promoters reduced the intestinal pH whereas, significantly increased the carcass qualities. The additive fed groups showed significantly reduced blood cholesterol level, the result was more prominent in the probiotic fed group. The probiotic bacteria also improved the zootechnical parameters, gut health influencing overall performance. From among all, the probiotic fed group showed the lowest cost of feeding followed by the acidifier, nucleotide and control groups. It could be concluded that the use of probiotic fed group showed the best result followed by acidifier and nucleotide fed groups.

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ID : 1259

A SPECIFIC BLEND OF ESSENTIAL OILS AND OLEORESINS OF SPICES IMPROVES FEED EFFICIENCY AND REDUCE NITROGEN AND PHOSPHORUS LOSSES

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One new challenge for animal production is to reduce environmental impact like nitrogen (N) and phosphorus (P) losses. Considering the simple reduction in P and N recommendations might have a negative economic impact, search for alternative additives has been incentivised. The main goal of this experiment was to evaluate the effects of a blend of essential oils and oleoresins of spices on performance of broiler between 7 to 42 days compared to usual conditions of production.

A total of 1,200 newly hatched chicks (Ross-308) were randomly distributed (30/pen) into 3 groups: Negative Control (NC), Positive Control (PC) and Treatment (OLEO) respectively with 12, 14 and 14 replicates each. All broilers received from d 0 to d 7 the same non-medicated starter. At day 4 they all receive a previous dropping samples sprayed on the litter to apply a sanitary challenge. Thereafter, Negative Control group received only non-medicated feed (starter from day 0 to day 14, grower from day 15 to day 28 and finisher from 29 to 42). Birds from Positive Control group received 55 ppm of Bacitracin Methylene Disalicylate (BMD®) from day 8 to day 28 shuttled to 22 ppm of Stafac® from 29 to 42 days. In the tested group (OLEO), birds were received a phytogenic feed additive (Oleobiotec, Laboratoires PHODE - France) at 100 g/MT. Statistical analysis: A completely randomized design was used ($P \leq 0.05$), and means were separated by LSD ($P = 0.05$). Statistical analysis was carried out using the General Linear Model procedure of SPSS®, Analytical Software, IBM USA.

The Control group presented significantly lower performances from 0-42 d compared to Positive Control or OLEO group (1.906 vs. 2.111 kg BW; 1.933 vs. 1.765 feed conversion ratio or FCR), confirming the negative effect of the challenge. Both groups (OLEO vs PC) presented statistically equivalent performance on mortality rate (5.48% vs. 6.19%), growth performance (2.061 vs. 2.111 kg live weight) and feed efficiency (1.775 vs. 1.765 FCR).

N and P losses are reduced in the OLEO group vs. NC (28% vs. 32% for N and 41% vs. 44% for P). These results could be explained by FCR and Live weight improvement in OLEO group.

Oleobiotec was an effective feed additive to improve feed efficiency and reduce Nitrogen and Phosphorus losses. Further studies need to be done to investigate whether this product improves digestibility of energy and nitrogen to help explain those results.

ID : 1287

EFFECT OF A SPECIFIC BLEND OF ESSENTIAL OILS AND OLEORESINS OF SPICES ON GROWTH PERFORMANCE OF BROILERS IS INFLUENCED BY CHALLENGE ACUITY

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The consistence of plant extract efficacy is often discussed. A part of the explanation of variable result could be in the level of challenge animals have to face. The main goal of this study was to evaluate the effects of a blend of essential oils and oleoresins of spices, Oleo, with a specific focus on founding modulating factor of their response on growth performances of broilers from a meta-analysis approach. An exhaustive database including 25 trials performed from 2001 to 2018 with Oleo was built to quantify its impact on average daily gain (ADG) and feed conversion ratio (FCR) of broilers. Dietary treatments were either a positive control (C+) with antibiotic growth promoter, a negative control (C-) without antibiotic or a treatment consisting in an addition of Oleo at 100 ppm. To test the hypothesis that Oleo response was influence by the challenge the bird experienced, when C+ and C- were both present in the same trial (n=9), the relative difference between them (C+/C-, %) was also calculated as Challenge Acuity Index and used as an X variable. Metabolizable energy and crude protein has been recalculated based on diet composition using table of feedstuff composition and expressed based on requirement of the genetic line studied. Regarding the range of trial durations and slaughter ages of broilers, the Y variables were converted as relative difference in FCR and ADG expressed in percent between Oleo and C+ (ADG_Oleo/C+, FCR_Oleo/C+) and Oleo and C- (ADG_Oleo/C-, FCR_Oleo/C-). The mixed procedure of Minitab (Version 18.0) was used with the effect of the trial as random effect and the dose of Oleo and C+/C- as X variables. When comparing Oleo and C- treatments, results showed $2.8 \pm 0.70\%$ ($P < 0.001$; $R^2 = 65\%$) reduction of FCR and $3.6 \pm 1.2\%$ ($P = 0.006$; $R^2 = 56\%$) improvement of ADG with 100 ppm of Oleo. However, when comparing Oleo with C+ treatment there was no significant effect on FCR or ADG, indicating that Oleo and C+ are equal. The FCR_Oleo/C- was negatively correlated (Linear, $P = 0.03$; Quadratic, $P = 0.02$; $R^2 = 85\%$) with the Challenge Acuity Index (C+/C-) indicating that in challenge condition (when C+ performed better than C-), so in challenge condition, the reduction of FCR with Oleo addition was higher. This work help quantifying the average effect of Oleo and leads to better understand the variations observed between studies for the same alternative to antibiotics.

ID : 353

THE DOSE RESPONSE OF A PHYTASE ON THE DIGESTIBILITY AND ON THE BONE ASH CONTENT IN MALE BROILERS

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The objective of this trial was to investigate the effect of different doses of a phytase (OptiPhos) on the ileal digestibility and tibia quality of male Ross308 broilers. During the starter period, the diet was the same for all broiler chicks. From day 9 on, broilers were divided into eight treatment groups (pelleted grower): a positive control group (PC; corn-soybean meal based diet) and a negative control group (NC, -1.5 g/kg available phosphorus (aP) and calcium), and six different doses of the phytase added to the NC: 250, 500, 750, 1000, 1250, and 1500 FTU/kg (T3 to T8). On day 17, broilers were transferred to digestibility units (six replicates of four or five broilers per treatment) and on day 27 the ileal digesta and the right tibia were collected and pooled per digestibility unit. No significant differences were found in crude protein and crude ash digestibility. Adding the phytase (except for T5), significantly increased the total P digestibility (range 72.5-74.8%) compared to the PC (55.8%). Concerning the phytate P digestibility, all groups had a significantly higher digestibility (range 57.6-81.1%) compared to the PC (41.1%). Compared to the NC (57.6%) only the two highest doses (T7 and T8; 74.3% and 81.1%, respectively) were significantly higher. The crude ash concentration of the tibia was significantly higher for all treatments (range 39.5-45.4%) compared to the NC (36.3%), where the addition of the two highest phytase doses (T7 and T8; 43.6% and 43.9%, respectively) no longer significantly differed from the PC (45.4%). Based on these results, it can be concluded that adding the phytase at 1250 and 1500 FTU/kg could compensate for the decrease in available phosphorus.

FEEDING IN ALTERNATIVE PRODUCTION SYSTEMS

ID : 58

GROWTH PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF BROILER CHICKENS REARED ON DIFFERENT LEGUME PASTURES DURING DRY SEASON

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There has been a growing interest in pastured poultry production as a result of improved welfare and meat quality. The objective of this study was to evaluate the comparative benefits of different legumes pastures on the performance and physiological response of broiler chickens reared during the dry season in a tropical environment. The study involved the use of a total of 200 day-old unsexed Marshall Broiler chickens were used to investigate the growth performance and physiological responses of broiler chickens reared on different legume pastures. The chicks were weighed and assigned randomly into five experimental treatments namely; deep litter without access to run (DL), deep litter with access to *Stylosanthes hamata* (SH), deep litter with access to *Stylosanthes guianensis* (SG), and deep litter with access to *Mucuna pruriens* (MP) and deep litter with access to free run (FR) during dry season for 5 weeks. Each treatment had 4 replicates consisting of 10 birds each. Data were collected on growth performance, gait score, haematological parameters, serum biochemical indices, tonic immobility and plasma triiodothyronine (T3) hormone. Data obtained were subjected to One-Way Analysis of Variance in a Completely Randomized Design using SAS (2008). Results showed that the weight gain and final weights of the birds reared on SH were higher ($P < 0.05$) than those of the other treatment groups. Generally, the feed conversion ratio of the birds on pastures was better than those without pasture. Throughout the experimental period, the different legume pastures had no significant ($P > 0.05$) effect on plasma triiodothyronine (T3) concentration. The gait score of the birds on different legume pasture were superior ($P < 0.05$) to those in the deep litter and free runs without pastures. The total protein of the birds in SH, SG, MP was similar but higher than those of DL and FR. Also, globulin of SH was comparable to those of SG and MP but higher ($P < 0.05$) than those of FR and DL while MP, SG, FR and DL were similar ($P > 0.05$). It was concluded that access to legume pastures; *Stylosanthes hamata*, *Stylosanthes guianensis* and *Mucuna pruriens* improved performance and gait score without adverse effect during dry season in the tropical environment. Access to *Stylosanthes hamata* appeared better than the other legumes.

ID : 141

EFFECTS OF ALTERNATIVE PROTEINS TO SOYBEANS AND LIPID SOURCES ON THE GROWING PERFORMANCE AND CARCASS AND MEAT QUALITY OF EXPORT CHICKEN

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There is an increasing demand in the world for healthy food. In many countries, the place of chicken meat in diets is more and more important. Regarding nutritive value of chicken meat, the more variable component is lipid quantity and quality. The aim of the present study was to increase the amount of n-3 fatty acids (FA) in the edible part of broiler grillers dedicated to export market by testing different levels of extruded linseeds (LS) and rapeseed oil in growing and finishing diets. Another challenge was to replace a part of soybeans by faba beans. For that purpose, 3168 chickens from JV15 line were distributed into six groups. Each group comprised eight pens with 33 males and 33 females per pen. The groups were R1=control diet, R2=3 then 5% LS, R3=4 then 6% LS, R4=5 then 7% LS, R5=3 then 5% LS and low amount of soybean meal in the finishing diet, R6 = 3 then 5% LS and no soybean meal in the finishing diet. For each feeding period, all diets were isocaloric and isonitrogenous. The growing performance and mortality rate were recorded. At 31 days of age, all chickens were slaughtered in a commercial slaughter plant at an average weight of 1322 g. Thirty carcasses per group were cut to determine cut yields. The edible part of half carcass was removed to determine the chemical composition and fatty acid composition of meat with skin. The sensorial quality of fillet was evaluated on 20 carcasses per group after roasting. Data were tested with variance analysis. The effect of sex and group and their interaction were analysed by comparing means with a t-test and P value vs. 0.3 g in control group) without modifying growing performance, carcass composition and sensorial quality of meat. The environmental impact was favourable for the climate change but unfavourable for the land occupation. The extra cost of feed, in our experimental conditions, represented +3 to +8% for groups R2 to R6 compared to group control R1.

ID : 416

VARIETAL SELECTION AND TECHNOLOGICAL PROCESSES MAKE PULSES AN ALTERNATIVE OF SOYBEAN MEAL BY IMPROVING THEIR NUTRITIONAL VALUES

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A major challenge for poultry nutrition is to greatly reduce the use of synthetic inputs and to rely on more ecological processes, and consequently reduce soya bean meal in the diet. Faba beans may represent an alternative to soya but they are still poorly valued by animals; thus, improve nutritional values by genetics and processing may increase their interest in poultry. In this project, specific varieties and adapted technological processes (dehulling, extrusion) were used. Balance study was conducted on broilers, Ross strain male, between 21 and 25 days of age, with total and individual excreta collection for 3 days. Seven raw materials were compared simultaneously: faba bean alone, dehulled faba bean, faba bean 80% + full-fat soybean 20% extruded, faba bean 80% + linseed 20% extruded, faba bean variety with low vicin and convicin 80% + linseed 20% extruded, dehulled faba bean 80% + linseed 20% extruded, dehulled faba bean 80% + dehulled linseed 20%. Diets consisted in a basal part for 60 % and the tested product for 40%. 8 groups of 10 birds were randomly allocated to one of the experimental diets, one group was used to determine the digestibility of basal diet. Dry matter, gross energy and nitrogen were analysed on tested products, diets and excreta, and uric acid on excreta. AMEn (apparent metabolisable energy) and apparent digestibility of nitrogen were calculated for each diet and tested product. For raw faba bean alone, dehulling resulted in an improvement of protein content (+10.6%), digestibility of energy (+12.7%) and nitrogen (+3.7%). When extruded and associated with faba bean, replacement of full-fat soybean by linseed, improved digestibility of energy (+25.3%) but reduce nitrogen digestibility (-3.1%). Compared to entire seed, dehulling of linseed improved protein content (+1.7%), digestibility of energy (+22.4%) and nitrogen (+8.5%). Selection of faba and linseed with low antinutritional factors improved digestibility of energy (+7.0%) and nitrogen (+4.7%). These results suggested that an appropriate selection of seeds, associated with an adapted technological treatment, such as dehulling and thermomechanical processing give opportunities for an improvement in nutritional value leading to a higher inclusion of local sources of protein for broilers.

ID : 483

USE OF ALTERNATIVE PROTEINS TO SOYBEANS IN BROILERS: EFFECT ON GROWTH PERFORMANCE AND ENVIRONMENT

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A major challenge for poultry nutrition is to greatly reduce the import of soybean meal and the use of synthetic inputs favouring the use of more ecological processes and the use of local alternative protein's sources. The objective of this trial was to evaluate the effect of different alternative strategies to soya bean in broiler on zootechnical, environmental and economic point of view. For this, 1080 broilers, Ross strain males, were randomly distributed into 24 floor pen at D1 (1st day of life, day 1), and all fed a standard starter diet till D12, and then experimental diets : grower from D12 to D22 and finisher from D22 to D33. From D12 to D33, each pen was allocated to one the four experimental groups: R1 control group with soybean meal as main protein source, R2, with sunflower, rapeseed and corn gluten as protein sources, R3 and R4 with soya bean drastically reduced, but 15% and 20 % of crude or thermomechanical processed faba bean with low content in antinutrients in grower and finisher, respectively. Diets were calculated to provide the same nutritional values for AME and digestible amino-acids. At D22, there were no significant differences between the groups for growth performance. At D33, there were no significant differences between groups for liveweight (1792 g). Feed to gain ratios from D12 to D33 were 1.563, 1.618, 1.552 and 1.542 for R1, R2, R3 and R4, respectively, and were only significantly different for R2 and R4. No differences were observed between the groups for podermatitis and breast muscle yield. Soya bean meal consumption per animal between D12 and D33 was 576 g for control group, 195 g for low soya group (R2), 40 and 37 g for R3 and R4, respectively. The environmental impact is favourable to local pulses, and in particular for processed seed, for fossil fuel consumption (-15%, -16%, -21%) and in particular for climate change (-20%, -28%, -41%) but is unfavourable on the land occupation (-14%, +19, +14%) for R2, R3 and R4, respectively. Finally, the economic impact is neutral since it represents a saving for the average French consumption of 0.13 € / consumers / year for chicken consumption for R4. To conclude, alternative protein's sources and particularly processed faba bean, can contribute to reduce imported and GM soya bean meal in poultry diets, to maintain growth and feed to gain ratio, to be benefit for the environmental point of view and neutral for the economic point of view.

ID : 569

INTERACTIVE EFFECTS OF DIETARY LEVELS OF FIBER, PROTEIN AND ENZYMES SUPPLEMENTATION ON GROWTH PERFORMANCE AND SHORT-CHAIN FATTY ACID PROFILE IN BROILERS

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A total of 384 Cobb 500 male broiler chicks were used in a 21-day experiment to examine the influence of xylanase and protease supplemented individually or in combination on growth performance and short-chain fatty acid (SCFA) profile in broiler diets with different levels of dietary fiber and protein. Birds were allocated to 16 treatments in a randomized complete block design. Each treatment had 6 replicates with 4 birds per replicate cage. The 16 treatments were corn-soybean meal basal diets with different low (L) or high (H) levels of protein (P) and fiber (F) to give LFHP, LFLP, HFHP and HFLP. Wheat bran was used to increase the dietary fiber content. The HF diets also had lower ME content. All the diets were supplemented with phytase at 500 FTU/kg). Each of the basal diets were divided into four: non-enzyme supplemented or supplemented with xylanase, protease, xylanase plus protease. Birds and feed were weighed on days 0 and 21 and ceaca content of birds were collected on day 21. Correlation analysis was done to relate growth performance and caecal short-chain fatty acids (SCFA) data in addition to ANOVA. There was no significant 3-way interaction for any of the responses except for FCR. FCR was lower in birds fed with individual xylanase and protease in LP, but not HP diets. Birds fed HF diets had greater (P

ID : 1062

FEEDING BEHAVIOR OF LOCAL CHICKENS FED WITH DRIED LARVAE FROM MUSCA DOMESTICA, CRACKED MAIZE, AND A LAYER DIET, IN CAFETERIA FEEDING SYSTEM.

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The aim of this study was to evaluate fly larvae as protein source in traditional poultry feeding. Specifically, the objective was to assess the feeding behavior of 18-month-old local chickens, choice-fed with three types of food: a balanced laying diet, crushed maize, and dried domestic fly larvae. Fifty-four chickens (54) were divided into three (3) batches representing three (3) periods of the day during which the cafeteria test was conducted: in the morning at 8am; noon at 12 pm and in the afternoon at 3 pm. In each batch, the chickens were randomly divided into 4 groups representing replications with 4 birds in two replications and 5 birds in the other 2. The cafeteria test was conducted for 6 weeks. Observations on the behavior of the chickens from the recording of filmed sequences showed that, at the beginning of the test, the chickens were attracted to the feeders containing the crushed maize. From the fourth week, the chickens were distributed homogeneously around the 3 feeders. At the beginning of the 5th to the 6th week all the chickens went to the feeders where the larvae were. The results showed average consumption of 6.7 ± 2.6 g; 7.2 ± 3.3 g and 4.8 ± 2.2 g per chicken respectively for dried larvae, crushed maize and the layer diet ($P \leq 0.05$). According to the time of the day, the morning cafeteria showed consumption of 5.0 ± 0.1 g; 5.5 ± 0.2 g and 3.7 ± 0.1 g respectively for larvae, crushed maize and layer diet ($P \leq 0.05$). The corresponding values for the noon test were 8.1 ± 0.2 g; 8.41 ± 0.2 g and 5.6 ± 0.2 g respectively for larvae, crushed maize and the balanced diet ($P \leq 0.05$). The afternoon test showed 6.9 ± 0.2 g; 7.8 ± 0.2 g and 4.94 ± 0.1 g respectively for larvae, maize and the balanced feed ($P \leq 0.05$). The noon larval consumption (8.1 ± 0.2 g) was significantly higher ($P \leq 0.05$) compared to the morning (5.0 ± 0.1 g) and the afternoon (6.9 ± 0.2 g). The daily intake of the layer diet after the 30 min test was also higher ($P \leq 0.05$) in the noon group (55.0 ± 1.00 g) compared to the morning (48.7 ± 0.8 g), and the afternoon (53.9 ± 1 g). In addition, Total daily intake of dietary supplement (maggot + maize + layer feed) tested was also higher ($P \leq 0.05$) at noon (77.2 ± 1.5 g) compared to the morning (62.9 ± 1.0 g) and the afternoon groups (73.6 ± 1.4 g). It was concluded that maggots are appreciated by chickens and are more consumed at noon.

ID : 1114

TRIPHALA: JOURNEY FROM TRADITIONAL MEDICINE TO GROWTH PROMOTER AND IMMUNE-MODULATOR IN BROILER CHICKENS

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Triphala is called in different names at various parts especially Asia and Africa is an agglomeration three fruits with various biological activities. They are being used in the traditional human medicine for treatment gastric disturbances and also as a digestive stimulant. Known for its digestive stimulation and immune-modulation property, the present study was carried out in broiler chickens to explore its suitability for antibiotic-free meat production. The experiment was carried out with 300 one-day-old male broiler chicks grouped into six treatments having five replicates each. The chicks were reared from day-old to thirty-five days of age in deep litter system in a gable-roofed, open-sided poultry house. The birds were fed with either one of the following six diets: basal diet without supplementation (T1) or with supplementation of oxytetracycline at 0.004 per cent (T2), or with supplementation of Triphala at 0.025 per cent (T3), 0.05 per cent (T4), 0.075 per cent (T5) or 0.10 per cent (T6). The data were subjected to analysis of variance single factor to interpret the observations. The experiment revealed that the birds fed with T2 had significantly ($P<0.01$) higher body weight and gain than control, which recorded the lowest values. The Triphala fed groups (T3, T4, T5 and T6) not differ significantly from the antibiotic fed group in growth performance. The group T6 has the highest body weight and body weight gain among the Triphala fed groups. The feed conversion ratio of broilers was significantly ($P<0.05$) improved in T2 and T6 followed by T5, T3 and T4 groups than the control group. There was no difference in livability among treatment groups as there were non-specific mortality among the groups were observed. The immune-modulatory property was assessed in birds against the Newcastle disease virus vaccine. The titre values in the group T5 was significantly ($P<0.05$) with T5. Based on results from the experiment, it could be concluded that traditional medicine Triphala could be used as an additive @ 0.10% for improving the production performance and immune response in broiler chickens for antibiotic-free production.

ID : 1171

INCLUSION OF SILKWORM PUPA AS PROTEIN SUPPLEMENT IN BROILERS RATION

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Inclusion levels of silkworm pupa (SWP) and noug seed cake for soybean meal (SBM) as a protein supplement in broiler starter and finisher rations were evaluated at Debre-Zeit Agricultural Research Centre. The objectives of the study were to assess the performance of broilers on the basis of dry matter and nutrient intake, growth, feed conversion efficiency, survivability, carcass characteristics, as well as the economics of production. Two hundred twenty-five day-old Hubbard Classic unsexed chicks with uniform average group weight (42.32 ± 0.297 g (\pm SEM)) were randomly allotted to five dietary treatment groups in a completely randomized design (CRD) each with three replicates of fifteen chicks per replicate. The treatments were T1 that contains no SWP (0% SWP+30% SBM (the recommended level of SBM)), T2 (1.25% SWP+22.5% SBM), T3 (2.5% SWP+15% SBM), T4 (3.75% SWP+7.5% SBM) and T5 (5% SWP (the recommended level of SWP) +0% SBM) of the total rations. The treatment is based on substitution of SBM by SWP and NSC hence, the formulation also requires NSC to vary, therefore NSC increases as SBM is replaced by SWP. So as the level of SWP inclusion increased the noug seed cake was also increased. Every 1.25 % SWP and (5% and 4% NSC for starter and finisher ration, respectively) inclusion, replaced 7.5% SBM in the total ration. The crude protein content of SWP and noug seed cake was 61.18 and 36.79%, respectively. During the 49 days of the experiment, dry matter and nutrient intake, body weight change, dry matter, and nutrient conversion ratio and efficiency, mortality, carcass characteristics, organ weight, and percent, and economic responses were assessed. The results revealed that daily DM ($59.2-63.9$ g/bird/day/ ($SEM = \pm 1.65$)), CP ($12.4-13.4$ g/bird/day ($SEM = \pm 0.37$)) and ME ($404.8-430.2$ Kcal/bird/day ($SEM = \pm 10.8$)) intake, and body weight gain ($19.4-23.1$ g/bird/day ($SEM = \pm 0.87$)) during the entire experimental period were not significantly ($P > 0.05$) affected by the dietary treatments. The amount of dry matter consumed per unit of weight gain or dry matter conversion ratio (DMCR) (g/g) was higher (P

METABOLISM

ID : 445

IN VIVO ASSESSMENT OF THE EFFECT OF STARCH DAMAGE LEVEL IN SOFT AND HARD WHEAT CULTIVARS CAUSED BY PROCESSING AND ITS IMPACT ON AMEN AND STARCH DIGESTION RATE IN BROILER CHICKENS

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Hardness of wheat grains may affect starch digestibility due to physical starch damage during feed processing. The aim of this study was to evaluate the level of starch damage (SD) in different wheat cultivars as affected by feed processing and its impact on starch digestibility and apparent metabolizable energy corrected to nitrogen (AMEn) of broiler chickens. The experiment was a $2 \times 4 \times 2$ factorial RCBD design with two types of wheat (soft and hard), four cultivars of each and two feed processing methods (mash vs pellet). A total of 540 one-day old broiler chicks (Ross 708) were randomly distributed to 16 treatment diets consisting of 6 replicates (cage) with 5 birds per cage and fed treatment diets from day 7 to 22. Statistical significance was considered at ($P \leq 0.05$). SD as a % of total starch was significantly lower ($P < 0.05$) in mash soft wheat (6.62%) compared to hard wheat (9.76%). Pelleting increased SD % in both wheat types, 10.53 vs 12.28%, in soft and hard wheat, respectively. Body weight gain and feed intake were increased by pelleting ($P < 0.05$). Feed conversion efficiency was improved by pelleting especially in hard wheat cultivars, 1.35, 1.28, 1.25 and 1.20 for soft wheat mash, soft wheat pellet, hard mash and hard wheat pellet, respectively ($P = 0.001$). There was no effect on empty proventriculus weight (g/kg body weight), but relative empty gizzard weight was greater in mash diets as compared to pelleted diets ($P < 0.0001$). In mash diets, AMEn of hard wheat cultivars were higher than soft wheat cultivars (3157 vs 3386 kcal/kg DM) soft and hard wheat, respectively except paramount (soft wheat cultivar) which had a similar AMEn (3352) to the hard wheat cultivars. Pelleting increased the AMEn of both soft and hard wheats, but hard wheats had higher AMEn ($P = 0.0001$; 3267 vs 3396 kcal/kg DM for soft and hard wheat, respectively). Starch digestion at proximal jejunum and distal ileum in mash diets were significantly higher in hard wheat cultivars ($P < 0.0001$) compared so soft wheat cultivars. Pelleting increased starch digestion at proximal jejunum and at distal ileum of soft wheat cultivars ($P < 0.0001$; 60.85 vs 64.34% proximal jejunum) in soft and hard wheats, respectively, and (96.42 vs 98.61% distal ileum) soft and hard wheats, respectively.). These results indicated that pelleting increases starch damage in both soft and hard wheat and improves starch digestibility and AMEn.

Keywords: AMEn, starch damage, wheat cultivars, broiler.

ID : 541

A SYNERGISTIC BLEND OF GUT HEALTH ENHANCING FEED ADDITIVES IMPROVES PERFORMANCE OF BROILERS RAISED WITHOUT ANTIBIOTICS

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With the reduction of antibiotics in their production strategies, the main concern of broiler meat producers relate to potential decreases in animal productivity. The objective of this study was to test different dietary solutions on their efficacy to increase growth performance in broilers raised without antibiotics (RWA = no in-feed antibiotic or coccidiostat use during production phase; coccidiosis vaccination at hatchery).

A total of 2,420 one-day-old, male Ross 708 broiler chickens were allocated to each of 44 pens with 55 birds in a randomized complete block design. Dietary treatments included negative control (T1), synergistic blend of organic acids and medium chain fatty acids including target release butyrates and a phenolic compound (SBSMBP; T2), T2 + copper hydroxychloride (T3), T3 + dietary structure in the form of oat hulls (T4). Treatments were all-vegetable based diets and were fed based on a 3-phase feeding program. Treatments were all-vegetable based diets and were fed based on a 3-phase feeding program. The animals were monitored for body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) was calculated. Differences in least square means were analysed through SAS.

Analysis of overall cumulative data (day 0 – 36) showed a significantly higher ADG (+1.9 g/d) in broilers fed T3 and T4 relative to those fed the control diet ($p = 0.067$) compared to T1. Cumulative FCR was analysed with the exclusion of mortality weights to reflect local commercial FCR calculations. Broilers fed T3 exhibited a significantly lower FCR relative to broilers in T1 (-2.11%) and T4 (-2.70%) ($p = 0.36$) and within typical commercial practice levels.

The present study demonstrates the efficacy of SBSMBP to significantly improve FCR and numerically increase market weights under RWA production conditions. The addition of copper hydroxychloride to the above mentioned treatment further improves RWA program performance, resulting in a significant improvement in both, both, market weights and lower FCR. Data suggests no added benefit of inclusion of dietary structure in RWA programs.

ID : 669

KEY METABOLITES PRODUCED BY THE PROBIOTIC BACILLUS SUBTILIS 29784 STRENGTHEN INTESTINAL BARRIER AND DAMPEN INFLAMMATORY RESPONSE

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The probiotic strain *Bacillus subtilis* 29784 (Bs29784) produces a large number of metabolites. Niacin (NA), hypoxanthine (HPX) and pantothenate (PTH), are produced in high quantity in in vitro cultures. These key metabolites have been also shown to be produced in vivo, in the digestive tract of poultry, mainly in the upper part. The purpose of the present study was to determine the effects of those key metabolites on the development of the intestinal epithelium, the enhancement of the intestinal barrier function, and the resilience to inflammation using Caco-2 cell models.

Caco-2 cells were subjected to different treatments through incubation with NA, HPX, or PTH at a concentration close to what is measured in the gastrointestinal tract of broilers and were compared to non-treated conditions. Differentiation was assessed by measuring alkaline phosphatase activity after 72h treatment once the cells had reached confluence (on day 5). Resilience to inflammation was evaluated by measuring TEER (transepithelial electrical resistance) and cytokine release after 2h incubation with the treatment followed by 4h incubation with Cytomix (TNF/INF/IL1). For each condition, two biological replicates, with at least 4 repeats each, were carried out and data were subjected to the ANOVA procedure of XLSTAT.

None of the metabolites tested had an impact on cell differentiation. The drop of TEER observed following Cytomix stimulation was less pronounced with the addition of both PTH and HPX. HPX treatment also led to a decrease of IL6 secretion by 47.3% compared to the control treatment group ($p < 0.0001$). The results of this in vitro study suggest that the key metabolites produced by Bs29784 have a direct effect on the host and could directly support the metabolic activity of the host epithelial cells, and therefore could reinforce the intestinal barrier at the level of the small intestine. In a next step, those observations should be evaluated in vivo in order to correlate the mode of action of Bs29784 and its effect on animal response.

ID : 768

EFFECTS OF CHRONIC INTESTINAL INFLAMMATION CAUSED BY DEXTRAN SODIUM SULFATE ON FUNCTION OF EGG PRODUCTION IN LAYING HENS

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Intestinal health is important for sustaining normal meat and egg production in chickens. It is considered that gut environment such as microbiota, mucosal barrier function and cytokine production affects egg production in laying hens. Intestinal inflammation is known to change gut environment. Previously, we reported that oral administration of dextran sodium sulfate (DSS: 0.9 g/kg BW) for 5 days caused severe intestinal inflammation and reduction of egg laying in the laying hens. However, there is no long-term investigation for mild inflammation on egg production in laying hens with DSS. Thus, the goal of the study was to determine the effects of long-term intestinal inflammation caused by low dose of DSS on egg production in laying hens. White Leghorn hens (330-day-old) were orally administered with or without 0.225 g DSS/kg BW in drinking water for 28 days (n = 8, each). Laid eggs and blood plasma were collected once every week. Cecal contents, intestinal tissues and liver were collected 1 day after the final treatment. These samples were used for the analysis of egg quality, intestinal environment and egg yolk precursor production. Operational taxonomic unit was decreased and beta-diversity was changed in cecal contents in DSS group compared with control group. The ratio of villus height/crypt depth was lower in the cecum and colon in DSS group. Gene expression of IL-6 in cecum was higher, and of TGF- β 4 in colon was lower in DSS than control group. These results suggested that the oral administration of DSS caused intestinal environment such as change of microbiota in cecal contents and enhancement of intestinal inflammation in lower segment. Egg yolk weight was 15% decreased during 1-3 weeks of experiment in DSS group. While the gene expression of yolk precursor production related molecules in the liver was not changed by DSS treatment in liver, and plasma VLDL concentration was higher in DSS group. In conclusion, disruption of intestinal environment caused by DSS could reduce egg yolk size for long term by disorder of normal follicular growth. The dysfunction of follicular growth may be caused by disorder of egg yolk precursor uptake in ovarian follicles, but not disorder on supply of yolk precursor.

ID : 1058

CLONING OF CHICKEN GLUT13 AND ITS EXPRESSION FEATURE

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INTRODUCTION

In mammals, facilitated transport of glucose into cells is mediated by a family of facilitative glucose transporter (GLUT) proteins[1]. As the member of GLUT family, GLUT13 (HMIT or SLC2A13) exhibits transport activity for myo-inositol, but not for glucose in mammals [2]. SLC2A13 can be a potential markers for CSC in various tumors[3]. The aim of this study was to clone the transcriptional sequence of chicken GLUT13 , and to detect the tissue expression of GLUT13 .

MATERIALS AND METHODS

The coding region of chicken GLUT13 was cloned by RT-PCR technique, and the biological characteristics of its encoded protein were analyzed by bioinformatics technology. The expression of GLUT13 in different tissues and time after insulin treatment was detected by qPCR.

RESULTS

Chicken GLUT13 was successfully cloned based on predicted sequence (XM_001232939.5) . It is predicted to encode a 459 AA protein. The CDS is 561 bp short than that in XM_001232939.5 . Nucleotide and amino acid sequence alignment showed that chicken GLUT13 was highly homologous among species, and collinear analysis showed that the region of chromosome 1 where GLUT13 was located was also highly conserved among species. The real-time fluorescence quantitative PCR showed that the expression of GLUT13 gene was the highest in chicken brain and kidney, which is similar to the expression pattern of human GLUT13. Exogenous insulin significantly inhibited the expression of GLUT13 gene at 240 min in brain and kidney.

DISCUSSION AND CONCLUSION

The predicted chicken GLUT13 was highly similar to known human GLUT13, While we gained the CDS region of chicken GLUT13 561bp short than the predicted sequence in NCBI, Which results to chicken GLUT13 lack of an MFS_HMIT_like domain which may result to the function change in chicken. Preliminary analysis showed that chicken GLUT13 may be related with glucose intake under insulin stimulation.

Key words: GLUT13; gene cloning; bioinformatics analysis; tissue expression

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ID : 1063

LOWER AND HIGHER CONCENTRATIONS OF BUTYRATE REGULATE FAT ACCUMULATION IN CHICKEN ADIPOCYTES VIA DIFFERENT MECHANISMS

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Background: Short-chain fatty acids (SCFAs), mainly include acetate, propionate and butyrate, are the fermentation products produced by microorganisms using dietary fibers in the cecum and colon. The beneficial role of butyrate in health, such as combating obesity and type 2 diabetes mellitus, has been widely studied in mammals and humans. The role of butyrate in fat metabolism has been demonstrated to be dose dependent. Besides, lipid metabolism in chickens is different from that in mammals, of which liver is the primary site for fat synthesis, and the adipose tissue is mainly for fat deposition. Therefore, we speculated that there might exist differences in the regulation of butyrate on fat metabolism between chickens and that in mammals. In the present study, we determined the effects of serial concentrations of sodium butyrate (SB) on fat accumulation in chicken adipocytes.

Experimental design: Firstly, in vitro experiments were performed to detect the effects of serial concentrations of SB (0.01 to 2 mM) on fat accumulation in chicken adipocytes, by using Oil-red-O staining, Bodipy-staining, TG content determination, qRT-PCR, and western blot analysis. Secondly, the role of SB in cell proliferation was examined via EdU and CCK-8 assays in preadipocytes. Subsequently, the involvement of free fatty acid receptors (FFARs), extracellular regulated protein kinase (ERK) signaling, AMP-activated protein kinase (AMPK) signaling, and inhibition of HDAC in the role of low and high concentrations of SB was elucidated, respectively. Lastly, animal experiment was carried out to determine the influence of low dose butyrate (basal diets supplementing with 0.1% SB coated with polyacrylic resin Π) on fat deposition of broiler chickens.

Results: Both Oil-red-O and Bodipy staining showed that the 0.01 and 0.1 mM butyrate groups had fewer stained lipid droplets than the control and that droplet sizes did not change significantly. However, SB at or higher than 0.5 mM resulted in larger lipid droplets and reduced cell numbers, especially at the 1 mM and 2 mM concentrations. SB activated FFAR2 and FFAR3 mRNA expression, with 2 mM showing the highest expression compared to other groups. The 0.01 mM SB treated group, had the PPARG (peroxisome proliferators-activated receptor γ), FABP4 (fatty acid binding protein 4), FAS (fatty acid synthase) and LPL (lipoprotein lipase) mRNA levels statistically lower than those in the controls. Contrarily, SB-treated groups higher than 0.5 mM showed marked elevated expressions of all adipogenic genes tested. Western blotting revealed that adipocytes treated with 1 mM or 2 mM butyrate had upregulated protein expression of PPARG and FABP4 compared with untreated cells. These findings indicate that although both lower and higher concentrations of SB reduced adipocytic fat accumulation, they exhibited different cell morphology and distinct lipogenic genes expression.

Since butyrate at all concentrations increased FFAR2 and FFAR3 mRNA expression, siRNAs specific to the two receptors were used to down-regulate their expression. We found siRNA-FFARs reversed the lessened fat droplets under 0.01 mM SB treatment. Results indicated that neither the p-ERK/ERK nor the p-AMPK/AMPK ratio was markedly altered by 0.1 or 1 mM SB. Strikingly, 1 mM SB significantly inhibited HDAC activity. Therefore, TSA, which is a highly specific inhibitor of HDACs, was used to mimic the effect of SB. The acetyl-histone H3 protein expression level was elevated by the 1 mM SB and 10 nM TSA treatments, respectively. Oil red O staining and TG content determination showed that both 1 mM SB and 10 nM TSA groups accumulated more lipids than the control group. Similar to the effects of 1 mM SB, 10 nM TSA also vastly elevated FFAR expression, and moderately increased the FAS and LPL mRNA levels. These findings demonstrate that inhibition of HDAC activity is at least partially involved in the role of 1 mM SB in fat accumulation.

Animal experiments indicated that dietary supplementation of low dose coated SB (0.1%) inhibited fat deposition in both livers and abdominal fat tissues of broilers. Liver index was reduced in SB-treated animals at d 21. H&E staining showed that fat deposition in the livers was decreased with SB supplementation. In accordance, the TG content in SB-treated livers was significantly lower than that in control livers. In contrast to the controls, SB supplementation substantially although not significantly reduced the abdominal fat ratio at both d 21 and d 42, which was decreased by 16.5% and 17.6%, respectively. The transcription of some adipogenic genes (Srebp-1c and ACC in liver; PPARG in adipose tissue) was inhibited in SB-treated livers and adipose tissues. The serum TG level from SB-treated broilers was significantly decreased compared to that from the control animals. However, the growth of the broilers and the feed to gain ratio were not changed by SB administration. These findings indicate that fat deposition in broilers was reduced by dietary supplementation of low dose SB.

Conclusion: This study showed a novel understanding on the differential between lower and higher concentrations of butyrate on fat accumulation in chicken adipocytes, and the suppression of low dose SB on fat deposition ensured a promising application of using SB as feed additive in chicken breeding.

ID : 1222

LEVEL AND SOURCE OF DIETARY METHIONINE: THEIR RESPECTIVE EFFECTS ON GROWTH AND PROTEIN METABOLISM IN GROWING BROILERS

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Like other amino acids (AA), sulfur AA affect protein metabolism. Protein synthesis and deposition are reduced when an insufficient level of sulfur AA is provided. This response may originate from the dietary AA deficiency and/or excessive AA utilization for other purposes such as the synthesis of acute-phase proteins during catabolic stress. Although dietary sulfur AA have been extensively studied, little is known about how different supplies and sources can affect protein metabolism. The aim of this study was to investigate growth performance and protein turnover of different tissues (liver and 2 types of muscles) in broilers receiving a diet either deficient or sufficient in sulfur AA and supplemented with either DL-Methionine (DL-Met) or DL-OH-Met (methionine analog) during 3 weeks. For the two Met sources, the supply of Met and sulfur AA in the deficient diets was respectively 43% and 30% below those of the sufficient diets. The birds were pair-fed to ensure the same feed intake and housed individually in wire-floored cages. Forty birds (10 birds per treatment) were used to determine protein synthesis (using the flooding dose procedure, Tesseraud et al., 1996), proteolytic enzyme activities, and expression of genes related to proteolysis. The deficient Met supply significantly reduced performance. In Met-deficient compared to Met-sufficient birds, the weight and protein mass of pectoralis major (PM) muscles were also lower ($P < 0.001$), but not those of the liver and thigh muscles. Similarly, for both Met sources, Met deficiency decreased the amount of proteins synthesized per day in PM, but not in the liver and thigh muscles. Irrespective of the tissue studied, the proteolytic activity of proteasome was greater ($P < 0.01$) in the Met-deficient groups compared with the Met-sufficient groups. Results on proteolysis-related gene expression measured in PM agreed with these findings. There were no differences between dietary DL-Met and DL-OH-Met sources on all these parameters denoting in particular that DL-OH-Met supports as DL-Met protein synthesis in PM when adequately supplied. In conclusion, a Met deficiency greatly alters chicken growth, PM development, and tissue protein turnover, whereas different Met sources do not.

Tesseraud S, Peresson R, Lopes J and Chagneau AM (1996) Br J Nutr 75: 853-65.

ID : 1390

EFFECTS OF EXOGENOUS MULTI-ENZYME CARBOHYDRASE AND CORN DERIVED PHYTASE ON MALE BROILER PERFORMANCE FED A REDUCED ENERGY DIET

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The objective of the current study was to evaluate the effects of multi-carbohydrase enzyme (MCE) containing alpha galactosidase, xylanase, and B-mannanase enzyme compared to a single xylanase enzyme and the effect of corn derived phytase over microbial derived phytase. A total of 3250, one day old Ross 708 broilers were randomly assigned to 65 replicate pens with 50 birds per pen. Pens were randomly allocated in complete randomized block design to one of the five treatments; 1) a positive control (PC) with 1500 FTU/kg of microbial fermentation derived phytase, 2) negative control (NC) with a reduction of 99 kcal/kg ME compared to the PC and phytase similar to PC, 3) the NC + xylanase, 4) the NC MCE, 5) the NC + multi-carbohydrase enzyme with phytase replaced by 1500 FTU/kg of corn derived Phytase. 0.15% ca and 0.15%P credit was provided to the diet for both source of phytase used in the experiment. Feed and water was provided ad libitum in a 4 phase feeding program for up to 48 days. Feed intake, body weight gain, and FCR was measured at d 14, 28, 42, and 48. Data was analyzed by one-way ANOVA using SAS 9.2 and tukey HSD test was used as post hoc comparison with significant difference considered at $P \leq 0.05$. Reduction of dietary energy in NC diet reduced ($P \leq 0.05$) the weight gain and FCR of broilers compared to PC diet in all phase of the experiment. Inclusion of both xylanase and MCE increased ($P \leq 0.05$) the weight of birds compared to NC diet in all phase of the experiment. Broilers fed MCE in the diets had higher ($P \leq 0.05$) body weight gain compared to birds fed xylanase enzyme on day 48. Replacing fermentation based phytase with corn derived phytase increased ($P \leq 0.05$) body weight gain at all phase of the study. The result of the study indicates the use of exogenous enzyme can improve BWG and feed efficiency in broilers by sparing more energy to the diet. Use of multi carbohydrase enzyme had benefit over the single xylanase enzyme by acting on different substrates of the diet. Inclusion of corn derived phytase improved the yield in broilers compared to the fermentation derived phytase while used at similar FTU and Ca and P matrix.

ID : 1433

INTERACTION BETWEEN NEUROPEPTIDE Y AND FEEDING REGIMENS IN AMINO ACID METABOLISM OF THE CHICK BRAIN

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Recently, we identified that feeding behavior of neonatal chicks was characterized by short resting periods between brief times spent on food intake and reported the involvement of free amino acids in the brain as innate signals (Tran et al., 2015, 2016). We hypothesized that appetite-related neuropeptides and amino acids collaborate to centrally regulate feeding behavior in neonatal chicks. This study aimed to investigate the relationship between orexigenic effect of neuropeptide Y (NPY) and brain amino acid metabolism in neonatal chicks under different feeding regimens. In Experiment 1, 5-days-old chicks (n=7–10) were intracerebroventricularly (ICV) injected with saline or NPY (375 pmol/10 µl/chick) and then subjected to fasting and ad libitum feeding condition for 30 min. Brain samples (diencephalon) and plasma were collected after experiment to analyze free amino acid concentrations. In Experiment 2, we further investigated the influence of central administration of NPY on L-tyrosine catabolism in the diencephalon due to its significant decline by NPY injection under feeding condition in Experiment 1. Chicks 5-days-old (n=9) were ICV injected with saline or NPY (375 pmol/10 µl/chick) and then subjected to ad libitum feeding condition for 30 min. The diencephalons were collected to examine the mRNA expression of tyrosine hydroxylase. Statistical analysis was conducted by the Stat View after the elimination of outliers by Thompson rejection. The stimulation of food intake by central NPY was confirmed. The results showed that central NPY significantly decreased diencephalic L-phenylalanine and L-isoleucine levels in both feeding and fasting condition (PPP

EF3

GENETICS

QUANTITATIVE GENETICS

MOLECULAR GENETICS

NEW TRAITS FOR POULTRY BREEDING

QUANTITATIVE GENETICS

ID : 299

GENOMIC CHARACTERIZATION OF EXTREME FEATHER PECKING IN LAYING HENS

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Feather pecking is a behavior frequently occurring in commercial layer flocks. It often leads to skin injuries and cannibalism. Besides economic losses, severe animal welfare problems can also not be ignored. Breeding against feather pecking is possible, but phenotyping the hens in a commercial environment is at present economically not viable due to a lack of appropriate techniques. The distribution of feather pecking is not homogenous. Several studies reported the presence of a subgroup of extreme feather peckers within groups of laying hens. It is assumed that the extreme feather peckers play a leading role in the development of feather pecking in poultry flocks. The genomic characterization of extreme feather pecking might help to select against this damaging behavior. We developed a statistical method to detect extreme feather peckers. A mixture of two negative binomial distributions was fitted to two feather pecking datasets. The first dataset consisted of about 900 hens of a large F2 cross of the 11th generation of two laying hen lines selected divergently for severe feather pecking. The second dataset consisted of about 500 hens of the 15th generation of these two lines. For phenotyping, the hens were kept in groups of about 40 animals and observed for in total 420 min over several consecutive days. The hens were genotyped with the Illumina 60K chicken Infinium iSelect chip. After data filtering, about 29,000 SNPs remained in the datasets. In both studies, the proportion of extreme feather peckers was about 35%. Hens in this subgroup showed on average over both studies 2.8 series of severe feather pecking and pecked feathers on average 8.53 times within the series. In contrast, hens in the remaining subgroup showed on average 1.63 series of feather pecking and pecked feathers only 1.65 times within the series. In order to identify the extreme feather peckers individually, the posterior probability of each hen belonging to the extreme feather pecking subgroup was calculated and used as a novel trait for genetic analyzes. Heritabilities for this novel trait were 0.35 in the F2 cross and 0.26 in the two divergently selected lines. A genomewide association study revealed a QTL region on chromosome 1. The putative candidate genes found in this QTL region are part of the GABAergic system, which has already been linked to feather pecking.

ID : 357

AN EQTL IN THE CYSTATHIONINE BETA SYNTHASE GENE IS LINKED TO OSTEOPOROSIS IN LAYING HENS

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High incidences of bone fractures and other forms of skeletal damage are being reported in egg-laying birds across a wide range of modern husbandry systems. The physiological adaptations for egg laying make laying hens susceptible to osteoporosis, resulting in increased bone fragility and susceptibility to fracture. We have previously shown that genetic factors underlie the variation in the susceptibility of individual birds to osteoporosis and bone fracture and have demonstrated that genetic factors explain approximately 40% of variation in end-of-lay bone quality (1). A quantitative trait locus (QTL) of large effect, located on Chromosome 1, was characterised in a F2 population of hens divergently selected for bone strength (2).

The aim of this study was to confirm and fine-map the QTL in progressive populations of the white leghorn strain used as the foundation of the selection lines (1). A number of SNP markers were identified which had highly significant associations with tibial breaking strength (BS), the most significant of which had BS values of 200.4 vs 218.1 Newton ($p < 0.002$) for the two genotypes.

The transcriptome of the tibia for each genotype indicated four differentially expressed genes at the locus, with one gene, cystathionine beta synthase (CBS), having a nine-fold greater expression associated with the allele for low bone quality. CBS catalyses the conversion of homocysteine to cystathionine in the transsulphuration pathway. The genotype associated with higher expression of the CBS gene and poorer bone quality showed a small but significantly increased concentration of homocysteine (19.13 vs 17.18 mM/L; $p = 0.016$).

We have defined and validated markers predicting bone strength that can be used in selective breeding. The results suggest that differences at the locus surrounding the CBS gene are involved in phenotypic effects of the QTL on bone quality. The pivotal role of CBS in mammalian sulphur metabolism, and in particular the methionine cycle, may give us additional routes to manage bone health in addition to genetic selection.

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ID : 394

TOWARDS A CANALISATION OF EGG WEIGHT. AN ILLUSTRATION IN DUCKS.

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Canalization can be defined as the action of selecting to maintain a constant phenotype in fluctuating environments or to decrease the sensitivity to environmental variations. Here, canalization parameters were addressed by a heteroscedastic model involving genetic values for the mean and the variability (log variance) associated with a single phenotypic value: the egg weight of Muscovy female ducks from an experimental population.

Two successive generations (68 females in generation 1 and 83 females in generation 2) laid 6859 individually weighed eggs (mean egg weight $\mu=80.3\pm 8.3$ grams). Across females, the number of weighed eggs ranged from 20 to 87 while the standard deviation of egg weight ranged from 2.8 to 8.2. The estimation of genetic parameters through a homoscedastic model provided additive variance for the egg weight: $\sigma_u^2=18.77\pm 5.64$ and residual variance: $\sigma_e^2=20.38\pm 0.41$. Consequently, heritability of egg weight was $h^2=0.42\pm 0.10$ and repeatability was $r=0.54\pm 0.03$, in accordance with literature. A Double Hierarchical Generalized Linear Model (DHGLM) was used to estimate the genetic and non-genetic effects affecting the mean (location parameters) and the residual variance (dispersion parameters). For the dispersion, the fixed effects of generation and age of layer were then retained beside the random genetic effect v which led to an estimation of $\sigma_v^2=0.19\pm 0.03$. The magnitude of σ_v^2 , accounted for a ratio of 2.4 for egg weight standard deviation, between extreme females exhibiting a low or high sensibility to environmental variation for egg weight. A genetic correlation of $\rho_{uv}=-0.11\pm 0.11$ was finally estimated between genetic effects for location and dispersion of egg weight. This nearly null estimated correlation is favorable to implement a selection to reduce individual variability of egg weight. However, an improvement of the confidence interval for this correlation is needed. That will be obtained with data from future generations.

Such methodology could profitably be implemented in laying breeding companies in order to improve homogeneity of products, which could easily be attained without impairing the selection goal on mean values when the correlation between location and dispersion parameters is negative or close to zero.

ID : 455

ESTIMATION OF VARIANCE COMPONENTS AND HERITABILITY OF BODY WEIGHT GAIN IN IRANIAN NATIVE CHICKENS

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Recently, much attention has been paid to native chickens due to increasing consumption demand and its resistance to environmental conditions. Investigation of chicken growth over time is critical for the genetic improvement of meat production. Twenty one generations of Iranian (Mazandaran) native chickens were used to estimate the variance components and heritability of body weight gain in different interval of ages. Body weight gains were recorded from hatching to 8 weeks of age (bwg0-8), from hatching to 12 weeks of age (bwg0-12), from hatching to sex maturity (bwg0-SM), from 8 weeks of age to 12 weeks of age (bwg8-12), from 8 weeks of age to sex maturity (bwg8-SM) and from 12 weeks of age to sex maturity (bwg12-SM). Variance components were obtained by the AI-REML algorithm under six different animal models by implementing or ignoring maternal effects and the covariance between direct and maternal genetic effects. Significant fixed effects (generation, hatch and sex) were detected using the GLM procedure of the SAS software. Also, for sex maturation related traits, Age at sex maturation was considered as covariate. The most appropriate model for each trait was determined by the Akaike Information Criterion (AIC). For all traits measured, maternal permanent environmental effect was significant. Estimates of direct heritabilities (h^2) were, respectively, 0.17, 0.21 and 0.26, 0.07, 0.31 and 0.26 for bwg0-8, bwg0-12, bwg0-SM, bwg8-12, bwg8-SM and bwg12-SM. Ratios of maternal permanent environmental effect were, respectively 0.06, 0.05, 0.05, 0.02, 0.04 and 0.02 for bwg0-8, bwg0-12, bwg0-SM, bwg8-12, bwg8-SM and bwg12-SM. Significant maternal permanent environmental effect on traits studied highlight the importance of considering this effect in genetic evaluation of birds for body weight gain. Also, Estimates of heritability show that improvement through selection is possible.

ID : 514

MULTIPLE ANCESTRAL HAPLOTYPES HARBORING REGULATORY CAUSATIVE MUTATIONS CUMULATIVELY CONTRIBUTE TO A MAJOR QTL AFFECTING DIFFERENT CHICKEN GROWTH TRAITS

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Introduction

In depth studies of quantitative trait loci (QTL) can provide insights to the genetic architectures of complex traits. A most investigated major QTL on chicken chromosome 1 has been associated with growth traits in multiple populations, but there is still no conclusive consensus of causal mechanisms in the last two decades.

Materials and Methods

Here we fine-mapped this QTL in a fifteen-generation chicken advanced intercross population including 1,119 birds and explored in further detail using 222 sequenced genomes from 10 high/low body weight chicken stocks. We developed a low-pass sequencing (0.5× coverage) method to obtain millions of SNP markers in thousands of individuals with extremely low cost. We combined linkage analysis, association analysis, haplotype association, identical-by-descent analysis, transcriptome sequencing, ATAC-seq and dual-luciferase reporter gene assay to explore this QTL interval.

Results

The strongest association in F2 and F9 generation was detected to a mosaic haplotype model inside the QTL. We traced the history of chromosomal transmission and demonstrated polymorphisms at multiple loci in the region cumulatively contributed 14.4% of the genetic variance for growth. Further, nine mosaic precise intervals (Kb level) which contain ancestral regulatory variants were fine-mapped in other 10 breeds/lines and gene expression analysis also indicated that multiple genes within these 9 haplotypes might be involved in the body growth.

We used ATAC-seq to identify chromatin accessibility near these intervals and chose one of them to demonstrate the key regulatory role in the duodenum. CAB39L gene was the candidate genes and two regulatory SNPs were the causative mutations. Besides, combining the QTL information and gene expression of gizzard, we discovered 2 hub genes, MLNR and HTR2A, and a core small effect gene list which are related to food intake.

Conclusions

This is the first study to break down the detail genetic architectures for the well-known QTL in chicken. It demonstrates the interaction mode of major-minor genes in biological pathway and provides novel insights into the likely role of minor genes in complex trait development. This is a good example of the fine-mapping of various of complex quantitative traits in any species and illustrates the common genomic complexity of complex traits.

ID : 809

GENETIC CHARACTERIZATION OF INDIGENOUS CHICKEN POPULATIONS IN CANARY ISLANDS (SPAIN)

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Since their conquest, the Canary Islands became an important maritime trading station through which different chicken breeds were introduced. Nevertheless, over the centuries, the Canarian chickens have evolved as a breed, including a set of variables that distinguishes them for their rusticity and good performance for egg and meat production, which makes them potentially suitable for dual-purpose production. However, there are no studies about their genetic characterization. This study aimed at investigating the genetic diversity, relationship and population structure of 161 Canarian chickens derived from five colour varieties: Black Barred (46), Red Barred (41), Black (38), Columbian (37) and Partridge (36), using 30 microsatellite markers and then compared to a reference set of 18 breeds taken from BIOCHICKEN project. Number of alleles, expected and observed heterozygosity values and polymorphic information content (PIC) were calculated using MS[®]Excel Microsatellite Toolkit. The Genetix software v4.04 was used to calculate the global inbreeding of individuals within breed (FIS), population differentiation index (FST) and global heterozygosity deficit (FIT). Deviations from Hardy-Weinberg equilibrium using Bonferroni correction on loci was determined by using CERVUS software v3.03. All microsatellites were polymorphic, with a mean of 7.2 alleles per locus. The observed and expected heterozygosity ranged from 0.101 to 0.802 and from 0.339 to 0.891 for the all colour varieties, respectively. Thus, the means of the observed (0.618) and expected (0.667) heterozygosity were higher than those found in other Spanish and European chicken breeds using the same set of studied markers. The PIC values were highly informative for 24 markers (>0.50), and for the rest were fairly informative (0.25–0.50). Moreover, only 3 markers (MCW014, MCW067 and MCW034) showed significant deviations from Hardy-Weinberg expectations. Across the five colour varieties, the FIS, FST and FIT were 0.039, 0.044 and 0.081, respectively. The low FST value indicated a scarce genetic differentiation among them. Finally, Canarian chickens did not show genetic proximity with other Spanish breeds or with the commercial strains included in the study. The results of the current study can be used as baseline genetic information for conservation programs, which will allow the recovery and official recognition of the Canarian chickens as an autochthonous breed.

ID : 867

MORPHO-BIOMETRIC CHARACTERIZATION OF INDIGENOUS GUINEA FOWL (NUMIDA MELEAGRIS) POPULATIONS IN NORTHERN TOGO

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The study which had for objective the morphobiometric characterization of indigenous guinea fowls populations was conducted between March and July 2018 in two agroecological zones in northern Togo. The study was carried out on 738 adult guinea fowl. Each guinea fowl was described by direct observation, and weight and body measurements were recorded. The results showed twelve (12) colours of the plumage with a dominance of Bonaparte (39.0%) and Pearl grey (27.1%) colours and a rarity of Cinnamon pied (0.7%), Isabelle (0.3%) and Blue coral (0.1%) colours. The plumage was mostly smooth (90.5%). The eyes were mostly brown (57.0%), but Albino guinea fowls predominantly had white colour (85.2%) for the eye. Regardless of the phenotype, the helmet was curved (73.2%) and mumps white-bluish colour (85.5%). In Atakora, the beak was brown (81.3%), the wattles were red-white (49.8%), the shanks were black-red (32.6%) and the toes were red (47.6%) while in the Dry Savannah the beak was red (52.9%), the wattles were red-bluish (38.4%), the shanks were black-orange (12.3%) and the toes were grey (35.2%). For body measurements such as beak length, drumstick length, body length and live weight, Dry Savannah guinea fowl showed significantly ($p < 0.05$) higher values than those of Atakora guinea fowl. Moreover, guinea fowl with Pearl grey phenotype (1.36 ± 0.28 kg) were heavier ($p < 0.05$) than the other phenotypes and appeared to be better for improving growth performance. However, further studies are needed to establish positive relationship between phenotype and biometric characteristics.

Key words : Guinea fowl, characterization, morphobiometric, diversity, Togo

ID : 1112

GENETIC ANALYSIS OF EGG PRODUCTION AND EGG QUALITY TRAITS IN THREE VARIETIES OF DOMESTICATED JAPANESE QUAILS HAVING DIFFERENT PLUMAGE

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The ultimate goal of a poultry breeder is to improve the overall genetic economic worth of the bird through multi-trait selection. So, study of heritability and correlations between productive traits is of great importance in the development of poultry breeding programs. In this perspective, the study had been planned to evaluate genetic parameters for developing white egg shell variety, for commercial exploitation. Three varieties of J. quail CARI- Ujjawal, CARI- Brown and CARI- Suneheri maintained as a closed flock at CARI were used. The single pair mating was carried out in individual pedigree laying cages by using 160 sires of each variety with an equal number of dams. Pedigreed and fertile eggs were collected, incubated and hatched out. Five female layers of 40 dam-sire families from each variety was transferred to individual laying cages after 5th week of age for recording of egg production traits. Part-time egg production of each bird was recorded biweekly at 8th, 10th, 12th, 14th, 16th and 18th week of age. The genetic analysis of the data sets were done with Wombat - (Full sib analysis) mixed model analysis by REML. There were significant differences observed in the ASM and egg production at 8th week was influenced by varieties. Egg quality traits also significantly influenced by varieties. Heritability estimates of biweekly egg production of all ranged from 0.041 to 0.583. The h^2 estimate for ASM ranged as low heritable with coefficients 0.041 to 0.166, for all the three varieties. Egg weight was moderately heritable. The heritability estimates of egg quality traits range from high to low. The ASM was negatively correlated with the numbers of egg produced for all. But the correlation components with biweekly egg production were positive up to 18th week in all the three varieties. High positive correlations were obtained between the Haugh unit and albumen height and between yolk weight and yolk diameter. Egg shell color observed in different varieties shows that CARI-Suneheri, produces more white-shelled eggs. The heritability estimates of Egg production traits in CARI Suneheri were higher. Moderate to high heritability estimates reported in the present study may leads to Mass selection for rapid improvement in growth and production traits in CARI-Suneheri because growth and production traits are positively correlated and hence whenever one goes for improvement in body weight there would be simultaneous improvement on the egg production also.

ID : 1385

PHENOTYPIC RELATIONSHIPS BETWEEN HOST RESPONSE TO LENTOGENIC AND VELOGENIC NEWCASTLE DISEASE VIRUS IN LOCAL GHANAIAN CHICKENS

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Newcastle disease (ND) is a highly contagious viral disease that constantly threatens poultry production. The velogenic (highly virulent) form of ND inflicts the most damage and can lead to 100% mortality in unvaccinated village chicken flocks while the lentogenic (non-virulent) form causes mild infections and is used in vaccine development. This study sought to identify phenotypic relationships between the response of local chickens in Ghana to lentogenic and velogenic NDV. Blood samples were collected from 1440 half-sib chicks and genotyped on a 600K Affymetrix chip in order to assign dams. At four weeks old, chicks were challenged with lentogenic ND virus (NDV) and then blood and tear samples collected for antibody detection and viral load estimation, respectively. Body weights were recorded weekly from hatch. Traits measured were pre- and post- infection growth rates (GR), antibody levels at 10 days post infection (dpi), viral load at 2 and 6 dpi, and viral clearance. The birds were then transferred to another facility and naturally exposed to velogenic NDV after anti-NDV antibody titres had reduced to 1:1000. Body weights, blood and tear samples were collected for analysis. Finally, the birds were euthanised and lesion scores (LS) recorded. Post-exposure GR, antibody levels at 21 and 34 days post-exposure (dpe), LS for trachea, proventriculus, intestines, cecal tonsils and average LS across tissues, were measured. Variance components and heritabilities were estimated for all traits using univariate animal models in ASREML with ecotype as a covariate. Pre- and post-infection GR means were 6.26 and 7.93, respectively, but mean post-exposure GR was -1.96. Heritability estimates for the traits in the lentogenic trial ranged from moderate (0.23) to high (0.55) while those for the traits in the velogenic trial were very low, ranging from 0.00 (average lesion scores) to 0.08 (antibody levels at 21 and 34 dpe). There were some correlations within traits measured in the velogenic trial and between traits measured in both trials. Lesion score means ranged between 0.52 (trachea) and 1.33 (intestine), with males having significantly higher ($p < 0.05$) lesion scores compared to females. These results suggest that selection for the traits measured in the lentogenic trial may be more feasible compared to the velogenic trial, although there was some evidence of phenotypic correlations between the two trials. Keywords: local chicken, Newcastle disease, correlations

ID : 1388

DETECTION OF QTL WHICH AFFECT EGG QUALITY TRAITS IN PUREBRED AND CROSSBRED LAYERS

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Genomic evaluation of egg quality traits was firstly carried out using ssGBLUP methodology in a purebred Rhode Island line and SNP effects were post estimated to detect QTLs. Secondly, performances from crossbred individuals issued from the same purebred line were added and new QTL locations were obtained. Egg quality traits were measured once a week on 7 982 purebred hens, from 60 weeks of age to 80 weeks of age. In addition, the performances of 37 232 crossbred hens were available. Each purebred male had 45 crossbred daughters in expectation, with unknown dams of different genetic type. Egg quality traits were measured once they reached 70 weeks of age. Purebred birds, i.e. 1 214 males and 1 148 females, were genotyped for 302 102 SNPs. Five egg quality traits were studied: egg weight (EW), eggshell color (ESC), eggshell strength (ESS), albumen height (AH) and egg shell shape (ESshape). Among the 26 QTLs detected using purebred performances, 20 were confirmed when crossbred hens performances were added and 10 new QTL locations were detected. Four to 8 QTL were detected per trait. All QTL influencing EW and ESshape detected in purebred were confirmed during the second analysis and 2 new QTL were detected for EW. Conversely, 2 or 3 QTL were not confirmed during the second analysis for ESC, ESS and AH.

ID : 1427

GENETIC ANALYSIS OF FEED EFFICIENCY TRAITS IN BROWN-EGG LAYER CHICKEN

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Feed consumption represents a large percentage of production costs in the poultry industry. Genetic selection plays a predominant role in improving feed efficiency. This study aimed to estimate genetic parameters of feed efficiency and relevant traits in late laying periods of chickens. Feed consumption, body weight and egg mass were measured from 69 to 72 weeks of age in a pure line of 725 Rhode Island Red hens from a commercial breeding company. Feed and water were provided ad libitum for all birds. Metabolic body weight (MBW), daily body weight gain (BWG), daily egg mass (EM), daily feed intake (FI), feed conversion ratio (FCR) and residual feed intake (RFI) were calculated to evaluate phenotypic and genetic parameters. The phenotypes that did not follow a normal distribution were transformed for following genetic analysis in DMU packages by using AI-REML with animal model. Mean values of FI, FCR, RFI, MBW, BWG and EM were 119.42g/d, 2.42g/g, 0, 312.86g, 1.11g/d and 52.79g/d, respectively. The coefficients of variation (CVs) of FI and MBW were less than 10%, while CVs of FCR, BWG and EM were greater than 15%. Heritabilities for FI, FCR, RFI, MBW, BWG and EM were 0.38 ± 0.10 , 0.30 ± 0.09 , 0.51 ± 0.10 , 0.56 ± 0.10 , 0.04 ± 0.05 and 0.32 ± 0.09 , respectively. Phenotypic and genetic correlations among FI, FCR and RFI were high and positive, ranging from 0.31 to 0.86. Genetically, FCR was highly correlated with MBW (0.47), whereas that with EM was negative (-0.58). RFI was correlated with EM (0.20), whereas that of RFI with MBW was low (0.04). Notably, the standard error of estimates among FCR, RFI and relevant traits ranged from 0.06 to 0.84, suggested that genetic correlation should be evaluated using larger population in breeding practice. In addition, the phenotypic correlations between RFI and its component traits were near 0. Phenotypic correlations between FCR and relevant traits ranged from 0.21 to 0.31, except that with EM (-0.68). In conclusion, the high heritability estimates for both RFI and FCR suggested that feed efficiency can be directly improved by proper selection programs. Selection for low RFI could reduce FI without significant changes in EM, while selection on FCR will increase EM. The findings would help better understand the genetic background of feed efficiency and contribute to chicken breeding and further genomic studies. Keywords: feed efficiency, heritability, genetic parameters, chicken

ID : 712

QUANTIFYING TEMPORAL GENOMIC EROSION IN LOCAL CHICKEN BREEDS

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Livestock biodiversity is declining globally at rates unprecedented in human history. Of the avian species, local chicken breeds are the most affected, as their small population size makes them more susceptible to demographic stochasticity and genetic drift [1, 2, 3]. The maintenance of genetic diversity and control over genetic drift by breeding programs is well understood, but often overlooked [2]. In this study, we used temporal whole-genome sequencing data to assess the consequences of starting a breeding program on the genetic diversity, deleterious variation, and inbreeding of two local French chicken breeds, the Gasconne and Barbezieux.

Although genetic diversity declined over time in the two populations, the reduction was more pronounced in the Gasconne ($\Delta\pi$: -0.0005) than Barbezieux ($\Delta\pi$: -0.0001). The reduction in diversity is the result of an increased number of medium and long ROHs, which cover up to 29% of the Gasconne genome (ΔFROH : 0.0776) and 13% of the Barbezieux genome (ΔFROH : 0.0051). In the Gasconne we also observed a higher number of damaging mutations (ΔL : 0.0029) than in the Barbezieux (ΔL : 0.00016). Damaging variants were mostly found in medium (0.83, p-value: <2.2-16) and long (0.95, p-value: 2.2-16) ROHs, suggesting that recent inbreeding enables rare damaging mutations to exist in homozygous state [4].

Our study shows that the size of the founding population is an important factor that can overrule genetic drift. We also show that the structure of the breeding program is key to preserve genetic diversity and reduce inbreeding. We recommend screening of individuals for damaging mutations to become common practice in near future.

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MOLECULAR GENETICS

ID : 16

PROFILING OF MICROSATELLITE LOCI AND THEIR ASSOCIATION WITH LAYER ECONOMIC TRAITS IN RHODE ISLAND RED CHICKEN

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The research work was carried out in Rhode Island Red (RIR) chicken to analyze the effects of various genetic and non-genetic factors on layer traits along with determination of allelic polymorphism at egg production-associated microsatellite (MS) loci and the influence genotypes on economic traits. Hundred and eleven pullets belonging to five hatches were used in the investigation which was maintained at institute poultry farm. The location of studied 10 MS loci were chromosome Z (ADL0273 and MCW0258), 1 (MCW0145), 2 (ADL0176 and MCW0044), 3 (MCW0103), 5 (ADL0023), 26 (MCW0069), E29 (ADL0158) and E48 (MCW0110). Data on body weight at 20 weeks of age (BW20) and layer traits viz. age at sexual maturity (ASM), egg weight at 28 weeks of age (EW28), egg weight at 40 weeks of age (EW40) and egg production up to 40 weeks of age (EP40) were analyzed by least squares analysis of variance taking sire as random and hatch as fixed effects. Least squares means of ASM, BW20, EW28, EW40 and EP40 were 135.19 ± 1.15 days, 1347.13 ± 15.28 g, 42.49 ± 0.27 g, 48.19 ± 0.42 g and 124.55 ± 1.94 eggs, respectively. Hatch effect was significant (P

ID : 162

COMPARISON OF TRANSCRIPTOMIC PROFILE OF PRIMORDIAL GERM CELLS ISOLATED FROM WHITE LEGHORN AND GREEN-LEGGED PARTRIDGELIKE CHICKEN EMBRYOS

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The Green-legged Partridgelike (GP), a native, dual-purpose Polish chicken, became recognized as a breed at the end of nineteenth century. Presently, the GP chicken is protected from extinction by means of in situ preservation and is kept in a flock without subjecting to any selection process for more than 60 generations. The White Leghorn chicken (WL) is intensively selected for several dozen years mainly to improve reproductive traits. This breed has been used to create highly productive egg-laying hybrids. The germline chimeras have been successfully produced by the transplantation fresh or frozen/thawed donor primordial germ cells (PGCs) collected from GP into recipient WL embryos. The aim of the current study was transcriptome comparison of the WL and GP PGCs at three developmental stages: 4, 8 and 12 day of embryo development. PGCs were collected from 6 embryos at each embryo development stage for each breed and RNA was isolated with commercial kit for single cells application (GeneElute Single Cell RNA Purification Kit, Sigma Aldrich). The quality and quantity of RNA was controlled by 2100 Bioanalyzer instrument (Agilent). High quality and purity RNA was intended for microarray analysis (One-color microarray-based gene expression analysis, Agilent). Microarray analysis was carried out according to the manufacturer's protocol. After scanning and feature extraction, obtained data were analyzed by GeneSpring GX software (Agilent).

Statistically significant differences (p2) was demonstrated in the case of PGCs originating from GP in comparison to WL. Up – regulated genes are mostly involved in the pathways related to regulation of biological processes (578 genes) and cellular processes (548 genes), developmental process (362 genes) and anatomical structure development (348 genes).

The obtained results might suggest a faster development of GP embryos comparing to WL embryos. The research was supported by the National Science Centre, grant no. UMO-2017/27/B/NZ9/01510.

ID : 176

ANALYSIS OF THE REGULATORY MECHANISM OF THE CHICKEN VANIN1 GENE: ROLES OF PPARALPHA AND MIRNA-181A-5P

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Introduction: Vanin1 (VNN1) is a pantetheinase that can catalyze the hydrolysis of pantetheine to produce pantothenic acid and cysteamine. Our previous studies showed that VNN1 is specifically expressed in chicken liver and negatively regulated by microRNA-122, a class of lipid metabolism-related noncoding RNAs. However, the upstream transcriptional regulation mechanisms of VNN1 gene have not been fully elucidated in the chicken liver. Here, we aimed to investigate the roles of PPAR α and miRNA-181a-5p in regulating chicken VNN1 gene.

Methods: 5'-RACE was performed to identify the transcription start site of chicken VNN1. JASPAR, TFSEARCH and miRanda were used to analyze the potential transcription factor binding sites in the 5'-regulatory region of VNN1 gene and miRNA binding sites in chicken VNN1 3'UTR. We used a knock-down function strategy to manipulate PPAR α (or miRNA-181a-5p) expression levels in vitro to further investigate its effect on VNN1 gene. Mechanistically, luciferase reporter assays were used to explore the specific mechanisms by which PPAR α (or miRNA-181a-5p) regulates VNN1 gene. These experiments were performed in triplicate wells and repeated for at least three independent trials.

Results: Several transcription factor-binding sites were recognized via sequence analysis of the 5'-regulatory regions, including HNF1 α , PPAR α and CEBP α . GW7647 (a specific agonist of PPAR α) increased the expression level of VNN1 mRNA in chicken primary hepatocytes, whereas the knockdown of PPAR α with siRNA was the opposite. Moreover, the predicted PPAR α -binding site is indeed functional and plays a crucial role in the regulation of the VNN1 gene. In addition, VNN1 3'UTR contains a sequence that is completely complementary to nucleotides 1 to 7 of miRNA-181a-5p. Overexpression of miR-181a-5p significantly decreased the expression of VNN1 mRNA, probably by directly combining with the predicted target site of the VNN1 3'UTR.

Conclusion: We provide experimental evidence that PPAR α plays an important role in the transcriptional activation of the VNN1 gene and that miRNA-181a-5p acts as a negative regulator of VNN1 gene expression in chicken hepatocytes.

ID : 332

SUPPLEMENTARY DIETARY BETAINES MODULATES PERFORMANCE, OXIDATIVE STATUS AND MRNA EXPRESSION OF HEAT SHOCK FACTORS AND HEAT SHOCK PROTEINS IN MEAT-TYPE CHICKENS UNDER HEAT STRESS

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Heat stress (HS) negatively impacts poultry performances and distorts molecular and cellular functions. Betaine supplementation is associated with the regulation biological processes associated with heat stress. To better understand the molecular mechanism of dietary betaine supplementation in mitigation HS, 3-week old Abor Acres chicks were assigned to four treatments: two diets (19%CP + 0%betaine and 19%CP + 1%betaine) and two temperature levels (24°C and 40°C) and subjected to either acute heat stress-AHS (40C for 6 hours) or chronic heat stress-CHS (40C for 7days). Five birds per treatment were euthanized and the Pectoralis(P.) major, ileum and liver were sampled for mRNA expression of heat shock factors (HSFs) and heat shock protein (HSPs) genes. Data were analyzed using ANOVA at $\alpha 0.05$, relative expression of genes was calculated using the $2^{-\Delta\Delta CT}$ method and beta-actin was used as endogenous control. Elevated temperature reduced growth and feed intake under acute and chronic HS compared to their respective control counterparts. Betaine supplementation decreased corticosterone from 2.20 ± 0.67 to 1.96 ± 0.60 and improved FCR from 1.55 ± 0.15 to 1.20 ± 0.08 , and decreased GSH:GSSG from 3.17 ± 0.58 to 2.81 ± 0.22 in AHS birds. Betaine supplementation differentially affected mRNA expression and was tissue specific. mRNA expression of HSFs and HSPs in the ileum under AHS were upregulated but downregulated in the liver and P. major respectively compared to the control. However, across the tissues, HSF2, HSF3, HSP60, HSP70 and HSP90 genes were all downregulated in the P. major except for HSF2 in the ileum and HSP90 in the liver under AHS with betaine supplementation compared to the control, while under CHS, betaine supplementation improved feed conversion ratio 1.56 ± 0.07 to 1.41 ± 0.18 , decreased corticosterone from 2.25 ± 0.72 to 2.08 ± 0.58 and GSH:GSSG from 3.08 ± 1.12 to 2.53 ± 0.5 . The mRNA expressions of HSF3, HSP60 and HSP90 were upregulated in the P. major; HSF3 and HSP60 were upregulated in the ileum and only HSF3 was upregulated in the liver. Across treatments in the liver HSF2, HSP60, HSP70, HSP90 except HSF3 were all downregulated. Betaine supplementation enhanced performance through improved feed efficiency, corticosterone and oxidation status under AHS. The changes in the heat shock factor- and heat shock protein- genes show tissue-specific regulation and may explain the efficacy of dietary betaine supplementation in mitigating heat stress in meat-type chickens.

ID : 344

IDENTIFICATION OF CHICKEN MT3 GENE PROMOTER FUNCTIONAL FRAGMENTS

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Abstract: Metallothionein 3 (MT3), is one isoform of the MTs that are low molecular weight proteins with rich-cysteine, which have high affinity to heavy metals. Our previous studies showed that the mRNA expression level of Mt3 in the chicken liver was raised highly after fasting. However, much less is known about the regulations of Mt3 gene in the chicken. In current study, the results of the quantitative PCR showed that the mRNA expressions of Mt3 in kidney and adipose tissues was higher than that of other tissues. Its level of expression in the liver was the next highest. In order to identify the transcription initiation site, the 5'UTR sequence of Mt3 with 72 bp was obtained using the 5'RACE (rapid-amplification of cDNA ends) method from chicken liver. The promoter deletion experiment was performed to find the functional segments to regulate Mt3 transcription and the potential binding transcription factors. The sequence from -1000 to +72 of Mt3 gene was analyzed and seven segments from the +72 site to different upstream sites of -57, -122, -220, -427, -556, -727 and -928 were amplified by PCR from chicken genome and were inserted into the reporter vector psiCHECK2. The relative activities of Renilla luciferase were measured after the reporter vectors being transfected to LMH cells for 48 hours, respectively. The results showed that the segment from -122 to +72 raised the activity of Renilla luciferase higher than the segment from -57 to +72. It indicated there were some cis-elements could upregulate Mt3 transcriptional level in the segment from -122 to -57. The analysis showed there were 4 clusters (-115 to -103, -106 to -95, -95 to -79 and -78 to -66) in this segment, containing 10 different potential transcription factor binding sites. After some oligonucleotides mutated in the segment from -79 to -66, the relative activity of Renilla luciferase was significantly decreased by the mutation segment from -122 to +72 to the wild segment. The 3 potential transcription factors of E2F transcription factor 1(E2F1), transcription factor AP-2 alpha (TFAP 2 α) and TFAP 2 β might upregulate Mt3 transcription through binding to the segment from -79 to -66. And the inferences would be verified through the co-transfection experiment of the reporter vectors and the overexpression vectors of these transcription factors, and ChIP in the future works.

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DEVELOPMENT OF PARENTAGE ASSIGNMENT SNP PANELS IN PHEASANT (PHASIANUS COLCHICUS) AND GOOSE (ANSER ANSER)

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In avian selection programs pedigree recording are generally dependent on the use of individual caging and individual artificial insemination. In other production such as aquaculture, parentage assignment methods based on the use of genomic markers are widely used to establish pedigree (Vandeputte and Haffray, 2014). In pheasant and goose, parentage assignment may contribute to welfare improvement of reproducers and allow selection on new traits. In goose, an existing microsatellite panel reaches poor assignment rates (70%), and no tool was available for pheasant. The objective of FaisSigne and SNPois programs were to develop assignment panels for both species. RAD-seq was performed to identify SNP (Single Nucleotide Polymorphism) on blood samples from commercial lines. DNA was pooled, (one pool/line and 15 individuals/pool). Each pool was sequenced with the Illumina HiSeq 3000 technology. In pheasant, 991K RAD loci were obtained, and 1.787 K in goose. To ensure identification of high quality SNP, several filters were used on sequencing depth, number of lines where a loci was sequenced, number of lines where a SNP was observed, number of observation of minor alleles, missing data, and minimum distance between SNP. After quality controls, 4.7 K SNPs were available for pheasant, and 3 K for goose. For each species, 288 SNP were genotyped on sire-dam-descendant trios with Kasper technology. Genotypes were filtered on missing data, mendelian compatibility and Hardy-Weinberg equilibrium. In goose, 231 SNPs were kept. Minimum Allele Frequency (MAF) was estimated within line and the 96 SNP with the highest mean MAF were kept for the panel design. Parentage assignment with this panel was performed in a routine context on commercial goose lines using APIS R package (Griot et al., 2019). The high assignment rate (94% in one line for example) was in accordance with previous work on ducks (Teissier et al., 2019). The same developments are currently being conducted in pheasant to design a specific assignment panel.

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ID : 449

MICROEVOLUTIONARY DYNAMICS OF CHICKEN GENOMES UNDER DIVERGENT SELECTION FOR ADIPOSITY

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Introduction: Decades of artificial selection have remarkably improved the performance and efficiency of animal production systems. However, little is known about the dynamics of genome microevolution during the intensive breeding process. Tackling the genome dynamics within the microevolution framework could improve our understanding of the genetic basis of complex trait. **Material and methods:** In this study, we sequenced 60 pooled-DNA samples in a time-series manner for birds used as parents from 15 generations (G4-G18) of chicken lines divergently selected for abdominal fat content for 19 generations. We explored how the chicken genomes changed dynamically by examining the genome heterozygosity levels and identified genome regions under positive selection using identity score (IS) and fixation index (FST). Furthermore, important genes for abdominal fat content were identified from time-series sequencing data coupled with a sequencing-based GWAS and multi-omics analyses. **Results:** Between our two chicken lines, continuous and directional selection on adiposity has caused striking phenotypic differences. We explored how the chicken genomes changed dynamically by examining the genome heterozygosity levels, which decreased significantly from 0.6% of G4 to 0.5% of G18. Analysis of allele frequency difference showed that the number of SNPs subjected to positive selection increased sharply from G9, which correlated closely with the pattern of selection responses, indicating that selection could recruit genomic variants to drive the phenotypic improvement. Between lines, FST increased but IS decreased from G4 to G18, whereas the opposite trend was found for within-line comparison. Divergent selection thus increased between-line but reduced within-line genomic differences. We reveal that artificial selection tend to recruit the preexisting variations of genes that related to adipose tissue growth. In addition, novel mutations contribute to the divergence of phenotype under selection, but less significantly than preexisting genomic variants. Integrating time-series genome sequencing, GWAS and multi-omics data further identified that genes involved in signaling pathways important to adipogenesis, such as autophagy and lysosome (URI1, MBL2), neural system (CHAT), and endocrine (PCSK1) pathways, were under strong selection. **Conclusions:** Our study provides insights into the microevolutionary dynamics of domestic animal genomes under artificial selection.

ID : 580

EXPRESSION LEVEL OF W-LINKED GENES IS UNLIKELY TO BE ASSOCIATED TO THE REPRODUCTION TRAITS IN DUCKS

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Birds have a ZZ/ZW system and are characterized by female heterogamety (ZW), and this sex determination mechanism different from mammals means that the W chromosome follows female-limit inheritance pattern and therefore the expression of W-linked genes may be related to female fitness traits. Recent reports in chickens have shown that the female-selected chicken breeds have higher gene expression levels compared to Red Jungle Fowl and male-biased selected breeds like fighting chickens. Reproduction traits as the important "female fitness traits" are generally measured in poultry by egg production performance. To determine whether the expression of W-linked gene in duck is related to reproduction traits, we have performed the following experiment. DNA samples were extracted from 3 ducks (1 Pekin duck, 1 Shaoxing duck, and 1 Mallard) were used for SMRT sequencing. Jinding duck (JD), Pekin duck (PK) and Mallard (MD) represent high, medium and low fertility respectively according to their egg production levels. The left gonad of 30 one-day-old ducks (5 males and 5 females per breed) was collected to perform transcriptome sequencing. SMRT reads data of three ducks were used for genome assembly at contigs level, all contigs blast with 12 known W-linked gametologs and reference genome to identify candidate W-linked genes in duck. Transcriptome data and PCR validation experiments were used to filter true W-linked genes. Finally, we obtained 31 W-linked genes (containing known W-linked genes), and some genes are present in multiple copy numbers. Analysis of RNA-Seq normalized reads counts found that there was no significant difference in the expression of W-linked gene among ducks (JD, PK, and MD) representing different reproductive capacity (paired t-test, $p>0.05$). By comparing the single gene expression of JD (high reproductive capacity) and MD (low reproductive capacity), there were only 4 W-linked genes have significantly high expression in JD (independent-samples t-test, $p<0.05$), is inconsistent with similar research on chickens. Taken together, our results indicate that W-linked gene expression level was not significantly correlated with reproductive capacity in ducks, and there is no evidence that short-term positive selection of reproductive traits on ducks leads to high expression of the W-linked genes.

Keywords: W-linked gene, reproduction traits, duck, RNA-Seq

ID : 581

GENOME-WIDE ANALYSIS OF THE GENETIC ARCHITECTURE FOR COMMERCIAL, LOCAL, FIGHT AND WILD CHICKEN POPULATIONS BY 600K SNP MICROARRAY DATA

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Abstract: After chickens were domesticated, diversified chicken populations were formed by both natural and artificial selection, which help chicken enrich abundant genetic and phenotypic variation and could be used as an ideal genetic research model. To better understand genetic architecture of chicken populations under different selection pressures, we genotyped various chicken populations with specific selection targets, including local, commercial, fighting and wild ancestral chicken breeds, by using the 600K SNP array. We analyzed the population structure, genetic relationship, inbreeding index and effective population number (N_e) and some other genetic parameters.

The wild ancestral population, red junglefowl (RJF), possessed the richest diversity with comparison to all other domesticated populations, indicated by LD, N_e and ROH results, etc. And the fight chicken breeds, which were subject to stronger male-biased selection on fighting related traits, also present comparatively higher variation than in commercial and local breeds. After being intensely selected for reproductive and productive traits, the commercial lines showed poorest diversity. We also found the European local chicken conferred lower genetic variation than in Chinese local breeds, which could be attribute to relative shorter history of the European breeds. We also estimated the effective sex-ratio of these breeds to show the change ratio of both sexes. We found commercial chickens made more sex imbalance between female and male and the commercial lines showed highest values for the ratios of female to male. Interestingly, the red jungle fowl population present more males than females.

Our results provide the population genetics information of chickens being undergone different selection pressures and could help for better conservation strategies of different chicken breeds.

Keywords: Chicken, Genetic architecture, Genetic diversity, Linkage disequilibrium, Effective population size

ID : 591

THE COMMON PHEASANT GENOME PROVIDE UNIQUE INSIGHTS INTO ITS ADAPTATION AND EVOLUTION

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The common pheasant (*Phasianus colchicus*), also called ring-necked pheasant, is a widely introduced game bird that originates from Asia. It has been the most consumed special poultry since it was reintroduced into Chinese market in 1980s. The pheasant is characterized by strong sexual dimorphism, thus an ideal model for sexual selection study. Here we present the de novo assembly of the common pheasant using single-molecule real-time sequencing, next-generation sequencing and high-throughput chromosome conformation capture approaches (Hi-C). Single-molecule sequencing polished with next-generation mapping generated an assembly with a contig N50 size of 1.33 Mb. Subsequent scaffolding by using Hi-C data generated 589 sequences with N50 size of 59.46 Mb, resolving 11 macro-chromosomes into single scaffolds. We annotated the genome using its transcript data, the method of ab initio gene prediction and homolog-based protein evidence, thus identified 23,058 genes. Quality assessment of the final assembly found 94% of the ring-necked pheasant canonical transcripts nearly complete, comparable to other published high-quality reference genome assemblies. Comparative analysis of 13 species identified 5,526 single copy orthologous genes of the common pheasant, based on which, a fully resolved phylogenetic tree was reconstructed with 100% bootstrap supports. The alignment between the common pheasant and the chicken chromosomes reveal high consistence. However, two inversions were found in the regions corresponding to chicken's MHC class I and class II genes, respectively. With the presence of reference, we performed whole genome re-sequencing to 15 individuals from 5 breeds. A total of 633 Gb of raw reads were mapped against our reference assembly, generating 12,696,645 variants across the whole genome. Selective sweep analysis was performed followed the work on Tibetan pigs. Some of the genes that showed artificial selection signatures regulate chicken's growth and productive traits, though further validation should be done for confirmation. Overall, we achieved a high-resolution reference genome assembly for the common pheasant by integrated 2nd and 3rd generation sequencing data. Having characterized the genome, we undertook detailed analyses of artificial selected genes and gene families to gain knowledge of this bird's macro- and micro-evolution.

Key words: common pheasant, de novo, MHC, comparative analysis, selective sweep

ID : 602

REFERENCE-GRADE GENOME ASSEMBLY OF WILD MALLARD (ANAS PLATYRHYNCHOS)

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Mallard duck has been long worldwide as a well model organism and considered ancestral to domestic ducks. The draft genome sequences are available but they are fragmented and incomplete, and built from domestic duck. In this study, we report high-quality genome assembly and annotation for wild mallard (CAU_wild1.0) by combining Pacbio single-molecular real-time sequencing, BioNano optical mapping and high-throughput chromosome conformation capture techniques. We generated 112 Gb of genome sequences, with an estimated depth of coverage of 100x. After assembling and polishing, a total length of 1.2 Gb of sequences were produced with scaffold N50 of 77.62Mb. The final assembly includes 1665 scaffolds, of which the largest 41 super-scaffolds representing the chromosomes occupied 95.20% of all sequences. In this assembly, we predicted 17,953 genes with 28,562 annotated transcripts. Compared with previously published draft genome (CAU1.0), new genome assembly represents the significant improvement in contiguity (The scaffold N50 7-fold against CAU1.0). Another improvements of new assembly are achieved in genome gap-filing and accuracy assembly of highly complex regions. The number of gaps of new assembly is only 309 (about 1/37 of gap number of CAU1.0). Moreover, 502 avian "missing" genes are identified for the first time, which were considered in the complex regions. Our findings will accelerate evolutionary and functional genomics studies in birds and inform future breeding programs for economic traits of domestic ducks.

ID : 647

A HIGH-QUALITY PEKIN DUCK GENOME ASSEMBLY PROVIDES NEW INSIGHTS INTO EVOLUTION DURING DUCK DOMESTICATION

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Abstract

Pekin duck is one of the major global breeds of meat-type duck and account for an increasing market share. Although the previous version(CAU_1.0) of the genome is available, the short read length of the second-generation sequencing technology leads to poor assembly performance in the de novo assembly process for regions with high repeat structure and high heterozygosity in the genome, making it difficult to obtain longer fragment. In this study, we report high-quality genome assembly and annotation for pekin duck using Pacbio single-molecular real-time sequencing (depth coverage: 92.6x), BioNano optical mapping (depth coverage: 141.07x) and high-throughput chromosome conformation capture techniques (depth coverage: 101.92x). After assembling and polishing, a total length of 1.2 Gb of sequences were produced with contig N50 of 5.46Mb and scaffold N50 of 76.28Mb. Hic data was used to assemble scaffold into 40 chromosome-level sequences (39 autosomes and Z chromosomes), with an average chromosome loading rate of 95%. Compared with previously published Peking duck genome (CAU1.0), the N50 length of new assembled genome has increase of at least 7 times at the scaffold level, and the number of GAPS is 1/37(559/20,679) of the latter. The results of the BUSCO showed an improvement of at least 3.3% in genomic completeness. In this assembly, 18507 protein coding genes were predicted using full-length transcripts. OC-17 plays an important role in the calcification of eggshells in poultry and our assembled pekin genome was the first poultry genome to obtain the complete genetic structure of OC17. Our study provides new insights into evolution during duck domestication and constitutes a promising resource on economically important genes in fowl.

ID : 738

A NOVEL LONG NONCODING RNA LNCPRDM16 IDENTIFIED TO BE RELATED TO FAT DEPOSITION IN BROILERS

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Introduction

For over half a century, commercial broiler has been selected intensively for growth rate and feed efficiency. However, intensive selection on fast growth rate brings along adverse outcomes, such as obesity and related metabolic syndromes. Currently, to reduce fat deposition is still a main objective of commercial broiler selection and breeding program. In recent years, noncoding RNAs, including microRNAs and long non-coding RNAs (lncRNAs), that regulate the proliferation and differentiation of adipocytes have been successively reported. A large number of lncRNAs have been identified to be of complicated functions in a wide variety of physiological and biological processes. However, research on the regulatory function of lncRNA on chicken adipose tissue development is still in its infancy.

Materials and methods

The experimental animals were derived from the 23th generation of Northeast Agricultural University broiler lines selected divergently for abdominal fat content (NEAUHLF). Total RNAs were isolated from 19 tissues using Trizol reagent (Invitrogen). A cDNA synthesis kit from Takara (Dalian, China) was used for the synthesis of cDNA. The full lengths of the lncRNA were obtained by the FirstChoice RLM-RACE Kti (Thermo). The quantitative real-time PCR was performed to detect the expression patterns of LncPrdm16. Data were analyzed by the t-test.

Results

We obtained the transcript sequences of LncPrdm16 from an unpublished RNA-seq experiments on abdominal fat tissues from NEAUHLF. The expression levels of LncPrdm16 were examined in various tissues from 7-wk-old fat and lean broiler lines. In fat and lean birds, LncPrdm16 was highly expressed in pancreas, moderately expressed in gizzard fat, crop fat, cerebrum, proventriculus, kidney and gizzard fat, and relatively low in heart, duodenum, pectoralis muscle and leg muscle. RACE analysis found that the full-length of LncPrdm16 was 996 bp using abdominal fat cDNA as template. In addition, LncPrdm16 was mainly expressed in mature adipocytes as compared to that of preadipocytes (p

Conclusion

Our results provide a foundation for future studies on the function of LncPrdm16 to adipose tissue growth and development in chickens.

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ID : 832

A HIGH PERFORMING BLUE EGG LAYER LINE DERIVED THROUGH MARKER-ASSISTED INTROGRESSION INTO WHITE LEGHORN

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The objective of the present study is to demonstrate an efficient way to transfer a specific monogenic trait maintained in a gene bank, here blue egg shell color from the Araucana breed, into a contemporary high performing White Leghorn line (WL) by marker-assisted introgression. This project is part of the EU project IMAGE (Innovative Management of Genetic Resources). Blue eggshell color is inherited in a dominant way and is caused by a large insertion on chromosome 1 upstream of *SLCO1B3*. Since 2016, an initial F1 generation, two marker-assisted backcross generations (BC1 and BC2) and a final intercross generation (IC) were generated, aiming at a high performing homozygous blue layer line. Breed/line specific SNP markers surrounding the introgression locus were genotyped for the detection of recombinant animals using Merlin. Furthermore, genotypes from a 52K SNP array were used for the selection of animals with the highest proportion of the recipient genome and the highest degree of diversity. Selection was based on analyses with MoBPS. Through marker assisted selection, the WL genome content increased up to 91.9 % in the BC2 animals, which is 4.4 % more than the expected 87.5 % in a BC2 generation, and the proportion of the Araucana genome in the BC2 thus could be reduced by about 35% (8.1% instead of 12.5%). In 2019, an intercross population with a total of 751 animals hatched (424 females, 327 males), of which 188 animals (116 females, 72 males) were homozygous carriers of the introgressed gene, 367 were heterozygous and 196 were non-carriers. Currently, analyses of genotyping results of the intercross generation are underway. Performance tests for the IC has not been finished yet, but will be reported. Preliminary analyses of performance data of the IC generation encompassing data until week of life 39 in comparison with commercial WL yielded promising results. The laying rate was quite similar between intercross and White Leghorn hens. The peak laying rate of IC animals reached 91.7 % in homozygous carriers, 91.1 % in heterozygous carriers, 94.0 % in non-carriers and 94.8 % in the WL control group. The eggshell strength increased from BC1 to BC2 up to 45 N on average, and was significantly lower than in WL control line (~50 N). The mean egg weight of 61.5 g in BC2 hens was about 2 g lower compared to the control group. Performance tests of the IC and genotyping results will enable to evaluate the success of the marker-assisted introgression.

ID : 836

HOST TRANSCRIPTOME AND METABOLOMIC RESPONSES TO EIMERIA SPP INFECTION IN MEAT-TYPE CHICKENS

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A study was conducted to identify transcriptome differences between two chicken genotypes infected with *Eimeria* (E.) *acervulina* and to understand the underlying gene expression changes and further delineate genotype-specific effects. Fourteen day old chicks of an unimproved (ACRB) and improved (COBB 500) genotype were orally infected with 2.5×10^5 sporulated *E. acervulina* oocysts. At 6 day-post infection, 5 birds were bled for serum and the jejunum from each genotype and their controls were used for global transcriptome analysis. The serum was used to determine the global metabolomic profiles using ultra performance liquid chromatography/tandem mass spectrometry. There were 5,262 differentially expressed genes (DEGs) between the ACRB infected and controls, and 2,884 DEGs between COBB infected and controls. There were common pathways between ACRB and COBB genotypes in response to *E. acervulina* infection. Among the common pathways were actin cytoskeleton, MAPK signaling, focal adhesion and cell adhesion molecules. However, there were also genotype-specific pathways in response to the infection. Whereas Toll-like receptor and retinol pathways were specific to the COBB genotype, oxidative phosphorylation and calcium signaling pathways were specific to the ACRB genotype. Both ACRB and COB genotypes have shared pathways in responding to *Eimeria* spp infection, however, each genotype also show distinct transcriptome signature. From the metabolomic study, unique metabolic signatures were identified in fatty acid metabolism, beta-oxidation, oxidative stress and inflammation. There were also microbiome metabolites that were putatively associated with malabsorption during infection.

ID : 851

COMBINED ATAC-SEQ AND TRANSCRIPTOME ANALYSIS TO IDENTIFY GENE REGULATION NETWORK AFFECTING CHICKEN GROWTH IN DUODENUM

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Background

Duodenum is important in food digestion and absorption and then provide nutrition for animal growth. The intestine length and body weight have the same QTL position[1], it's of great value to study chicken growth at the point of duodenum.

Materials and Methods

Here we used Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq) and RNA sequencing to profile the open chromatin regions and transcriptome in high and low body weight chicken duodenum.

Results

According to the ATAC-seq analysis, peaks at open chromatin mapped to transcription start sites (TSS) regions, as well as intergenic and intron regions. The differentially expressed peaks are associated with signal transduction and transmembrane transport, which are the important parts of duodenum function. GO analysis showed that many of the differentially expressed genes were involved in the regulation of biological processes key to intestinal function, notably the neuroactive ligand-receptor interaction pathway has important role on chicken appetite and regulate growth[2]. We also found that 263 down-regulated genes with down-regulated peaks in high body weight chickens were significantly enriched in ion transport, amino acid transport, transmembrane transport and hormone metabolic process, we considered these genes as important candidates for further exploration to find genes affecting chicken growth.

Transcriptome and ATAC-seq data revealed that in most region (including TSS, exon, intron and transcriptional termination site), the chromatin accessibility increased when the gene expression increased, while in intergenic regions the chromatin accessibility decreased when the gene expression increased suggesting that there may be function elements inhibiting gene's expression in the intergenic regions.

Conclusion

In conclusion, we found several pathway and biological processes regulating chicken growth together and the intergenic regions are associated with the gene expression inhibition.

Reference

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ID : 861

POPULATION HISTORY OF DOMESTIC JAPANESE QUAIL INFERRED FROM MITOCHONDRIAL GENOME SEQUENCES AND GENOME-WIDE SNP DATA

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To understand the genetic diversity of domestic Japanese quail, we previously examined mitochondrial D-loop sequences and microsatellite (MS) markers for 19 Japanese quail populations (Nunome et al., 2017). The populations included nine laboratory lines established in Japan, six meat-type lines reimported from Western countries, one commercial population in Japan, and three wild quail populations collected from three Asian areas. The phylogenetic tree of D-loop sequences showed two distinct haplotype groups, MT-G1 and MT-G2. MT-G1 included a dominant haplotype, which represented most of the domestic populations and wild quail. MT-G2 was composed of minor haplotypes found for six laboratory lines, two meat-type lines, and a few individuals of commercial and wild quail. Genetic clustering analyses with microsatellite markers revealed two genetic groups, MS-G1 and MS-G2. MS-G1 was found for six laboratory lines. MS-G2 was seen in the other three laboratory lines, all meat-type lines, and commercial and wild quail populations. We have recently conducted a whole mitochondrial genome (mt-genome) sequencing and a genome-wide SNP genotyping: Mt-genome sequencing was performed using the Oxford Nanopore MinION platform. The mt-genome sequences of 22 individuals from six laboratories, two meat-type, and one wild quail populations have been determined. Genome-wide SNP data for one individual each from four laboratories and two meat-type lines were obtained with new genotyping technology, GRAS-Di (Enoki, 2019; Enoki & Takeuchi, 2018). Tentative phylogenetic study for each of mt-genome sequences and genome-wide SNP data supported the two genetically different groups of D-loop sequences and microsatellite markers, respectively. Thus, we assumed two genetic sources of domestic Japanese quail populations with different genetic backgrounds. One was represented by MT-G2 (D-loop) and MS-G1 (microsatellite markers), which may originate in the population that was exported to the United States from Japan before World War II. The other was represented by MT-G1 and MS-G2 and was probably derived from limited founder populations re-established in Japan after the war. Now we are carrying out detailed phylogenetic and population genetic analyses using mt-genome sequences and genome-wide SNP data for more individuals from various quail populations to infer the population history of quail populations from genetic data.

ID : 863

COMPARATIVE MICROSATELLITE ANALYSIS OF SIX HUNGARIAN NATIVE CHICKEN BREEDS

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Genetic diversity investigations of endangered breeds of farm animals have been going on for several years. The aim of this study was to investigate the change of genetic diversity of six Hungarian indigenous chicken breeds in 15 years.

In total 180 individuals were genotyped at 29 microsatellite loci, with 4-6 markers at the same time as a multiplex. Mean number of alleles, expected and observed heterozygosity, inbreeding coefficient and effective population sizes were calculated within populations, between populations Wright's fixation indices and pairwise F_{ST} , furthermore a cluster analysis was performed with Bayesian approach (STRUCTURE). Results obtained from populations sampled in 2002 (old populations) were compared to the stocks of the same breeds sampled in 2017 (young populations).

The range of basic diversity measures was generally more narrow and lower in the young stocks than in the old populations, consequently the inbreeding coefficient was higher. While the old populations were close to the Hardy-Weinberg equilibrium state, the expected and observed heterozygosity of the young stocks differed significantly. The effective population size increased during 15 years for all breeds, but the values of populations sampled in different periods showed quite low estimates indicating lower genetic variability of the breeds. The pairwise F_{ST} estimates were considerable higher in the young populations suggesting larger differentiation between them, likely due to genetic drift. STRUCTURE results showed a clear clustering of the Hungarian populations. Based on Evanno's method, the most likely clustering was $K=6$, assorting the old and young populations of the same breed as one group. At higher level of K values the old and young flocks of the Black and Speckled Transylvanian Naked Neck chickens split from each other respectively, suggesting larger distinction between the old and young populations of those breeds.

There was no mentionable allele loss found in the Hungarian native chicken breeds investigated 15 years later; however one allele (frequency of 30%) seems to be missing from the Black Transylvanian Naked Neck breed, may allude to a selection effect. The inbreeding level was generally higher in the young populations which can be reduced using random sire rotation. With some improvements the breed management of the Hungarian local chicken breeds can be an appropriate solution to conserve the genetic variance.

ID : 864

CLONING AND EXPRESSION ANALYSIS OF THE CHICKEN NEUREGULIN 4

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Introduction

Neuregulin 4 (NRG4), a member of the epidermal growth factor (EGF) family of extracellular ligands, is highly expressed in adipose tissues. The role of NRG4 in liver has been extensively studied in mammals and demonstrated that NRG4 can improve diet-induced metabolic disorders, including insulin resistance and hepatic steatosis. To date, the role of NRG4 in adipose tissue development and adipogenesis has not been fully explored. In the present study, we cloned chicken NRG4 gene, investigated its tissue expression profile and expression difference in abdominal adipose tissue between fat and lean chicken lines.

Materials and methods

Twenty different tissues and organs were collected from the 7-week-old broiler chickens of the Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF). Total tissue RNA was extracted using Trizol reagent. Reverse transcription was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser. Based on chicken NRG4 mRNA sequence, Primer 5.0 software was used to design the primers for gene expression analysis and the coding sequence cloning of chicken NRG4 gene. Quantitative real-time RT-PCR analysis was performed using the SYBR Premix Ex Taq and ABI Prism 7500 systems. Statistical differences were evaluated using two-tailed unpaired Student's t-test.

Results

The full-length coding sequence (CDS) of chicken NRG4 gene was amplified by RT-PCR, cloned and sequenced. The sequence analysis showed that the full-length CDS of chicken NRG4 gene was 339 bp in length, encoding 112 amino acids. Nucleic acid sequence analysis showed that the NRG4 shared 24.9% and 40.65% sequence identity with human and mouse NRG4, respectively. Protein sequence analysis showed that chicken NRG4 shared 57.76% and 52.59% amino acid sequence identity with human and mouse NRG4, respectively. Real-time quantitative RT-PCR analysis showed that chicken NRG4 gene was expressed in all 20 tested tissues and organs, with the highest expression level in the kidney and the lowest expression level in the spleen. Unlike mammalian NRG4 gene, chicken NRG4 gene was moderately expressed in chicken adipose tissue. Further gene expression analysis showed that adipose NRG4 expression was significantly higher in the fat chicken line than in lean chicken line ($p < 0.05$).

Conclusion

Understanding the role and regulation of NRG4 in adipose tissue will provide insight into chicken adipose tissue growth and development.

ID : 866

PREDICTING THE FUNCTIONAL CONSEQUENCES OF NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS IN LEPR GENE IN CHICKENS

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Introduction

LEPR, a high-affinity receptor for leptin, is the key to the role and function of leptin. LEPR participates in many processes such as fat metabolism and appetite regulation in chickens. The objective of the current study is to identify the functional consequences of non-synonymous single nucleotide polymorphisms (nsSNPs) of LEPR gene in chickens based on bioinformatics tools and association analysis.

Materials and methods

In this study, the 19th generation broilers from the Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF) were used as experimental materials. The SNPs on the exon of LEPR gene were screened by whole-genome re-sequencing. A variety of online bioinformatics tools (SIFT, Polyphen-2, PhD-SNP, SNPs & GO, SNAP) were used to predict the functional SNPs. The conservation of mutant amino acids was analyzed by the online multi-sequence alignment tool Clustal-Omega and ConSurf. The stability was analyzed by I-Mutant3.0 and Mupro. The secondary and tertiary structure changes of the LEPR protein were modeled by SOPMA and Modeller 9.23 software, respectively. Finally, we used a mixed linear model to analyze the association between SNPs and phenotypic traits (abdominal fat weight and abdominal fat percentage).

Results

This study indicated that two possible functional nsSNPs (Exon19-N867I, Exon20-C1002R) maybe lead to significant changes in the structure and function of the LEPR protein. Association analysis showed that Exon20-C1002R was significantly correlated with abdominal fat weight and abdominal fat percentage. This significant mutation site is the first to be identified and further laboratory validation is required.

Conclusions

Based on bioinformatic prediction and association analysis, it is concluded that Exon20-C1002R is a functional SNP that could change in the physical and chemical properties of LEPR protein. The findings will provide a new insight into lipid formation and help decrease abdominal fat in broiler using marker-assisted selection (MAS) in further broiler breeding program.

ID : 908

GENETIC DIVERSITY AND EVOLUTIONARY HISTORY OF THAI NATIVE CHICKENS AND WILD RED JUNGLEFOWL INFERRED FROM MITOCHONDRIAL SEQUENCES

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Molecular phylogenetic studies of domestic chickens have been performed for a wide variety of chicken breeds throughout the world to understand their geographical origins and genetic diversities (Liu et al., 2006; Miao et al., 2013). To understand the evolutionary history of domestic chicken populations, genetic characterization of their wild progenitor, the red junglefowl (*Gallus gallus*, RJF), is prerequisite; however, their population genetic surveys are still limited (Akishinonomiya et al., 1994; 1996).

In this study, a part of the mitochondrial DNA D-loop region (780 bp) were sequenced for 298 RJFs (*G. gallus* and *G. g. spadiceus*) from 12 populations in Thailand and 138 chickens of 10 native chicken breeds from Thailand (Betong, Black meat chicken with white/black feather, Luang Hang Khaw, Chee, Pradu Hang Dum, Kheaw Palee, Decoys, fighting-cock) and Vietnam (Dong Tao). Phylogenetic trees were constructed using D-loop sequences obtained in our study and more than 5,000 sequences retrieved from Genbank. Demographic histories of haplogroups of D-loop sequences (e.g. Haplogroup A–I) were inferred by Bayesian skyline plot analysis. Twenty-nine out of 44 haplotypes found in our study were newly discovered haplotypes, and 15 of them were only found in RJFs. In addition, a rare haplogroup-like lineage, which may have separated from other haplotypes around 2,000 years ago, was constructed by one new haplotype found in three RJFs. These results suggest that some haplotypes of RJFs were never involved during the process of chicken domestication. Bayesian skyline plot analyses indicated that four major haplogroups (A, B, CD and E) exponentially increased their lineages from 250 to 500 years ago, and particularly, two waves of lineage diversification occurred in Haplogroup A. Thai fighting-cock had the Haplogroup H, which is also found in Japanese fighting cooks (Shamo) in Okinawa (Komiya et al., 2003), suggesting the possibility that Shamo chicken in Okinawa originated from Thailand.

It is well known that the distribution of domestic chickens spread throughout the world by human migration, culture contact, and trade after chicken domestication. Our present results reconfirmed the a long history of strong relationship between chickens and humans. We are now conducting whole mitochondrial genome sequencing for RJFs to perform highly resolved demographic analysis with about 150 mitochondrial genome sequences of chickens deposited on Genbank.

ID : 1031

INVESTIGATING ALLELE SPECIFIC EXPRESSION IN CHICKEN SPLEEN TRANSCRIPTOME RESPONSES TO AVIAN PATHOGENIC ESCHERICHIA COLI INFECTION

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Colibacillosis, caused by extraintestinal infections with avian pathogenic *Escherichia coli* (APEC), negatively impacts poultry health, welfare, and performance. In chickens, exposure to APEC can reduce growth and egg production, and virulent strains, such as APEC O1:K1:H7, can even cause high mortality. Our long-term goal is to reduce the negative impacts of APEC infections through novel strategies for veterinary control or selective animal breeding. This study aims to improve our understanding of host functional responses to APEC by identifying allele specific expression (ASE), or allelic imbalance, in the chicken spleen transcriptome that occurs in the early stages of infection. APEC-associated ASE SNPs act as markers for cis-acting regulatory elements that affect the host response to APEC, which could be potential selection targets to improve resistance to APEC. For pathogen challenge, F1 progeny from reciprocal crosses between Fayoumi (disease-resistant) and broiler (disease-susceptible) chicken lines were inoculated at 14 days of age with APEC O1:K1:H7 or sterile PBS via intra-air sac injection. Samples of spleen tissue were collected 1 or 2 days post infection (DPI) and used for RNA-sequencing (RNA-seq) on the Illumina HiSeq 3000 (n = 5-6 samples/F1 cross/inoculation type/DPI). Resulting reads were mapped to the chicken genome (Galgal6a) and used to identify SNPs and to detect ASE at these loci (with the GATK4 pipeline). Significant ASE was detected within individual samples for 8,832 SNPs, of which 3,054 (almost 35%) were unique to APEC infected samples and were confirmed to be significant across biological replicates (at the group level). The Fayoumi sire x Broiler dam cross had a greater number of ASE SNPs at 1 DPI, while the Broiler sire x Fayoumi dam cross had more at 2 DPI. Many of these APEC-specific ASE loci were identified within genes that also had significant differential expression under infection, including *SERPING1* and *MGST1*. These loci were also used for functional analysis in Ingenuity Pathway Analysis (IPA) to understand pathways where APEC induces cis-regulation of gene expression. Splenic ASE revealed the importance of cis-regulation in transcriptome responses to APEC and provided targets to investigate for markers of resistance to colibacillosis. Support: This work was funded by USDA-NIFA-AFRI #2015-67015-23093 as part of the joint NIFA-BBSRC Animal Health and Disease program and Hatch project #5424 and #5458.

ID : 1038

THE FUNCTIONAL ROLE OF CELLULAR SENSOR IN THE RECOGNITION OF RNA LIGAND IN CHICKEN

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Sensing and recognition of pathogens is the first line of defense in host innate immunity. The host pattern recognition receptors (PPRs) recognizes the pathogen-associated molecular patterns (PAMPs) and interact with adapter proteins to form complexes that subsequently triggers an antiviral state via the interferon-mediated signaling pathway. In mammals, RIG-1 has been identified as a major PPRs for RNA viruses [1]. However, in avian species such as chicken, the RIG-1 is lacking implying that chicken could be more susceptible to RNA virus mediated infections. Since RIG-1 has been lacking in chicken, we investigated to identify which PPRs could compensate for the function of RIG-1.

In order to investigate which PPR is critical for recognition of RNA ligands, we first assessed mRNA expression of cellular sensors in DF-1 cells in response to poly I:C or influenza virus and then conducted knockdown studies to assess the functional effect on IFN- β activity. Thereafter, we established knockout cell lines to identify which PPR plays a pivotal role in the establishment of antiviral innate immune response of IFN- β activity. Significant differences between mock and poly I:C-treated or AIV groups were determined by one-way ANOVA with Turkey's multiple comparison, and differences of relative mRNA levels between groups with different treatments were conducted by the Student t test. A value of $P < 0.05$ indicated statistical significance.

In our results, in response to poly I:C or influenza infection, the mRNA of PPRs related genes in DF-1 cells were significantly upregulated due to IFN-mediated positive feedback. Then our knockdown revealed that two of the PPRs are involved in the induction of interferon-mediated innate immune response in response to RNA ligand. By using CRISPR/Cas9 gene knockout, we found that, of the two identified cellular, one cellular receptor had the immune response impaired while the other could still stimulate immune response. In double knockout studies, we identified that of the two sensors, one could abolish the recognition of RNA ligands thus been the major receptor while the other sensor was secondary.

In conclusion, we demonstrated that even in the absence of RIG-1, chicken has a potent sensor of RNA ligands that regulate IFN- β pathway.

Reference

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ID : 1102

INTEGRATED GENOME-WIDE MRNA AND PROTEIN EXPRESSION FOLLOWING SALMONELLA ENTERICA SEROVAR ENTERITIDIS INOCULATION IN CHICKEN CECUM

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Salmonella enterica serovar Enteritidis (S. Enteritidis) can infect a wide range of hosts and cause food-borne disease in the world. Poultry is considered as a primary source of Salmonella Enteritidis infection. The correlation between mRNA and protein responding to S. Enteritidis infection remains unclear.

One hundred twenty two-day S. Enteritidis free Jiningbairi chickens were divided into two groups. Chickens in inoculated group were orally inoculated with S. Enteritidis. Chickens in non-inoculated group were mock inoculated with PBS. The cecum were collected at 3 days post inoculation for RNA and protein isolation. The genome-wide transcriptome and proteome in cecum were analyzed through bioinformatic tool.

There were 365 significantly differentially expressed genes including 200 up-regulated and 165 down-regulated genes ($P < 0.05$, fold-change > 2.0). The up-regulated genes were mainly associated with TLR6 signaling pathway, detection of diacyl bacterial lipopeptide, cellular response to interferon-gamma, regulation of inflammatory response, cellular response to tumor necrosis factor, immune response, inflammatory response, innate immune response, T cell receptor signaling pathway. Down-regulated genes were related with multicellular organismal water homeostasis, sodium ion homeostasis and glutathione metabolic process. There were 563 differentially expressed proteins consisting of 225 up-regulated and 338 down-regulated ones. Enriched immune-related GO-BP terms associated with differentially expressed proteins were defense response to bacterium, acute-phase response and positive regulation of protein secretion. There were 74 differentially expressed mRNA and 57 proteins matched. Related mRNA-proteins with same regulation direction were associated with enriched in GO terms of oxidation-reduction process, JAK-STAT cascade, retina homeostasis, endodermal cell differentiation and immune response. Related mRNA-proteins with reverse regulation direction were associated with enriched GO terms of substrate adhesion-dependent cell spreading, extracellular exosome, plasma membrane, adherens junction and brush border.

Transcriptome and proteome play different roles in the response to S. Enteritidis inoculation. The immune-related function was mainly associated with transcriptome, however, metabolism mainly associated with proteome.

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ID : 1103

GENOME-WIDE VARIATION FACILITATES RESPONSE TO BIDIRECTIONAL SELECTION FOR SHEEP RED BLOOD CELL ANTIBODY RESPONSE IN CHICKENS

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Objective:

Disease resistance is an important economic trait in the production of broilers. Long-term selection experiments provide a powerful approach to obtain empirical information on adaptation, allowing researchers to discover the objectives of the selection and infer their contributions to the mode and pace of adaptation. Here we implement a 55K SNP chip approach to investigate the consequences of 10 generations of bidirectional selection in White Leghorn chickens for a humoral immune trait: antibody response to sheep red blood cells.

Material and methods :

Three groups were included in the study (up selection line, down selection line and the control group). At 56d of age, 30 birds from each group were intramuscularly injected with 1ml of 25% SRBC diluted in PBS. The peripheral blood samples were collected from all the chickens from the wing vein of each bird at 6d after challenged (on day 56). Antibody titer to sheep red blood cells (SRBC) was determined. Genomic DNA was isolated from peripheral blood and hybridized to a chicken 55k DNA chip.

Results:

We observed the broad variation in the chicken genome in response to this selection regimen. Many genomic regions were highly differentiated as a result of this experimental selection regimen, although genetic drift has certainly contributed to this. Pathway enrichment analysis showed immunoglobulin, immune response, deubiquitination superoxide-generating NADPH oxidase activity, toll-like receptor signaling pathway have been significantly enriched in the candidate genes. We also identified five strong candidate genes associated with response to SRBC, including NFKB1, NOX1, TLR3, TLR15, and USP1.

Conclusions:

Adaptation to bidirectional selection for antibody response in this experimental chicken population has been facilitated by revealing genetic variation across many regions of the genome, functional studies highlight four particularly interesting candidate sweeps and underlying candidate genes involved in immune response.

ID : 1118

THE ANALYSIS OF SPLEEN TRANSCRIPTOME RESPOND TO INFECTIOUS BRONCHITIS VACCINATION IN TAIWAN COUNTRY CHICKEN

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Vaccination is the first line to protect chicken from severity of diseases, then rapid and high antibody level responses to vaccine are achieving results after vaccination. Local chicken breeds usually have better disease resistance. Our previous studies have shown that Taiwan Country chicken can induce high antibody level response to Marek's and Newcastle disease vaccines in a short period. In addition, Taiwan Country chicken has more resistance to coccidiosis and Leucocytozoonosis compared with White Leghorn chicken. To find out the genetic effects on immune responses of Taiwan Country chicken compared to White Leghorn chicken. B strain, Taiwan Country chicken which selected for body weight at 8 weeks of age over 30 generations were used in this experiment. B strain and Single Comb White Leghorn were vaccinated with live attenuated infectious bronchitis virus (IBV) vaccine (2(3.6log₁₀EID₅₀)) via a nasal route at 3 weeks of age. Spleens from control and vaccinated groups from both breeds were collected at day 7 post-vaccination then analyzed with RNA-sequencing. The results indicated that after comparing between B strain and Single Comb White Leghorn, In vaccinated group, B strain chicken show higher expression in C-type lectin domain family 2 member L-like and IgG Fc-binding protein-like responses to vaccination. While in White Leghorn chicken, T cell-related genes like T-cell surface glycoprotein CD8 alpha chain-like and T-cell surface glycoprotein CD8 alpha chain-like were highly expressed. In unvaccinated group, B strain was greatly expressed in immune related-genes including, interleukin 4 induced 1, interleukin 6, interleukin 22, glutamate decarboxylase2. In another way, White Leghorn chicken highly expressed genes were interleukin 1 receptor-like 2, mitogen-activated protein kinase 10, and T -cell-interacting, activating receptor on myeloid cells protein 1-like responses to IBV vaccination. In conclusion, differences in gene expression will affect the pattern of immune responses and this study provides the basis for the future study about immune-related genes responses to IBV vaccination in Taiwan Country chicken.

ID : 1227

THE INTEGRATION OF MULTI-OMIC DATA IN MULTI-TISSUES FROM LAYERS DIVERGENT FOR FEED EFFICIENCY PROVIDES SOME INSIGHTS INTO THE MOLECULAR MECHANISMS ASSOCIATED WITH THIS COMPLEX TRAIT.

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Improving feed efficiency remains a challenge to meet the needs of a growing human population and to contribute to the sustainability of the livestock sector. Improving feed efficiency also contributes to improve production efficiency considering that feed represents up to 60% of the production cost in monogastrics, and to reduce the competition between livestock feed and human food for the use of cereals.

In this work, we aimed at better understanding the pathways involved in feed efficiency in layer chickens. We used two experimental lines divergent in feed efficiency thanks to 40 years of selection that led to efficient birds, the R- and inefficient ones, the R+. First, we conjointly analyzed their transcriptomes in the adipose tissue, blood, hypothalamus and liver in order to determine the contribution of each tissue in the lines differentiation, and to identify genes and pathways shared between tissues. To this end, we realized a Multiple Factor Analysis (MFA) that provided a good separation between lines at the first factorial plan.

We identified approximately 7000 genes contributing to this axis with roughly 1000 shared by at least two tissues and less than 100 shared by the four tissues. Using regularized Canonical Correlation Analysis or co-Inertia Analysis, we produced correlation-based networks that we are currently studying to find potential hub- and regulator- genes. Moreover, using a recently produced (Jehl et al, Sci Rep., submitted) annotation of the chicken genome enriched in long non-coding RNA genes (LNC) known to be regulatory gene, we are analyzing the line difference in LNC. For the liver, transcriptomic data were enriched with lipidomic and metabolomic data and for the adipose tissue with lipidomic data. We conjointly analyzed these data within each tissue, selected between 1000 and 2000 variables found by MFA to separate the two lines, and observed interesting patterns of connectivity between genes and molecules in the resulting networks. In particular, we found genes highly correlated with different lipids and metabolites, some of which being known regulators of fatty acids metabolism. Overall, this work provides interesting insights in the mechanisms at work at the molecular level in different tissues in relation with contrasted feed efficiencies in layer chickens. This work was supported by the French National Research Agency (Chickstress project - ANR-13-ADAP) and Europe (Feed-a-Gene project - grant agreement no. 633531).

ID : 1236

GENOMIC SNP DETECTION BY RNA-SEQ: LESSONS FROM MULTI-TISSUE & MULTI-POPULATION DATA ANALYSIS IN CHICKENS

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The RNA-seq, a technology that gives access to tissue transcriptomes, is mainly used for gene expression analysis to address a variety of scientific questions in a given species. However, since this technology involves a sequencing step, it can also be used to detect DNA variants in the expressed regions of the genome, thus allowing to take advantage of the accumulation of this type of data in multiple tissues and populations.

The identification of a large number of variants in the genome is crucial to unravel the relationship between genotype and phenotype, to analyze the genetic diversity between populations or to detect variants with deleterious effect in protein-coding regions.

However, the transcriptome is much more complex than the genome, thus requiring specific bioinformatic tools for sequence data analysis: it is characterized by mature transcripts corresponding to spliced genomic regions that can have highly variable expression levels, leading to depths of a few reads to tens of thousands of reads from one genomic position to another. Nevertheless, few pipelines have been proposed since 2013 for detecting reliable SNPs in RNA-seq data, but no benchmarking has been undertaken. Here we used the reference tools for RNA-seq sequence alignment (STAR-2-pass) and for variant calling (PicardTools and GATK pipeline). We analyzed 700 RNA-seq samples from more than 300 chickens belonging to 8 experimental and commercial layer and broiler populations with more than 16 individual per population.

We first compare SNPs detected from RNA-seq data with those detected from DNA-seq data in two populations for which both data types, on the same individuals and tissue, were available and show the reliability of the SNP detection procedure with RNA-seq data and commented the filters proposed by GATK. We then provide a description of the SNPs detected from RNA-seq data for the eight populations, obtaining an overview of the SNP distribution at the population scale, with several millions of reliably detected SNPs. We refine this analysis by analyzing these SNPs at the individual scale, selecting those with reliable genotypes resulting in hundreds of thousands of SNPs with a call rate > 95% for which we provide their frequencies in each population. Finally, we describe some interesting variations across populations.

This study was supported by INRA (Elastic project), the French Research National Agency (FatInteger and ChickStress projects) and Europe (Feed-a-Gene).

ID : 1265

COMPLETE TRANSCRIPTIONAL INSIGHTS INTO KEY GENES AND PATHWAYS INFLUENCING EGG YIELD IN NANDANYAO CHICKENS

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INTRODUCTION: Nandan-yao chicken is an excellent local breed in Guangxi, which has been included in ANIMAL GENETIC RESOURCES IN CHINA POULTRY. Egg production is a complex economic trait controlled by multiple genes with low heritability. Therefore, this study intends to use RNA-seq to analyze the full transcriptional state of the ovary and uterine tissues of Nandan-yao chicken with high and low egg production, and to screen out important candidate genes and related regulatory pathways related to egg production.

MATERIALS AND METHODS: 300 Nandan-yao chickens, raised to 60 weeks old, and recorded their egg production. The three chickens with the highest egg production were selected as the high-yielding group (185 eggs), and the three with the lowest egg production were selected as the low-yielding group (82 eggs). Uterine and ovarian tissues from high - and low-yielding egg groups were collected for transcriptome sequencing. FASTQC, HISAT2, htseq-count, DESeq2, cluster profiler and other software were used to process data. The differentially expressed genes were screened by the general criteria ($\log_2|\text{Fold Change}| > 1$ & FDR

RESULTS: In the ovary, the numbers of differentially expressed mRNA, lncRNA and circRNA, respectively, were found to be 95, 558, 5, respectively. GO and KEGG analysis revealed their target genes were enriched in extracellular matrix regions for regulation, intracellular parts, protein binding, and immune system processes, etc. In addition, 16 differential miRNAs were screened (p, the numbers of differentially expressed mRNA, lncRNA, miRNAs, circRNA were found to be 402, 1421, 97, 45 respectively. GO and KEGG analysis revealed their target genes were enriched in ECM receptor interactions, oxidative phosphorylation, cell cycle, DNA metabolism, DNA methylation, cell cycle, oocyte meiotic division, cell metabolism, metabolic process of organic matter, etc. Among them, the ECM receptor interaction pathway is significantly enriched in both uterine and ovarian tissues, and the genes mainly enriched in this pathway include COL1A2 COL4A4, ITGB6, ITGA8, COL3A1 and VTN, which are most likely related to the regulation of egg-producing traits.

CONCLUSION: At present, we are still carrying out in-depth data mining and follow-up verification, which will be of great significance to the improvement and development of genetic resources of local chicken breeds in Guangxi.

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ID : 6

ONE THOUSAND CHICKEN GENOMES

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The 1000 Gallus genomes project aims at gathering whole genome sequence data of chickens, produced by research teams as well as breeding companies, on the model of the 1000 Bull genomes project. The main interest is to increase the power of analyses by increasing the number of genomes. Applications are to improve the knowledge of the chicken genome, to characterize with a high accuracy the genetic diversity at the species level, to facilitate the identification of causal mutations and to provide support to genomic selection by facilitating imputation of sequence data from SNP data. A pilot project has started in France with the collection of 207 individual genome sequences produced by 9 publicly funded projects on a range of chicken populations (fast-growing and slow-growing broilers, layers, local breeds, wild ancestors). Technical metadata include sequencing method, depth and technology, fragment length, individual or pooled sequencing, date of data production. Animal metadata include population type, breed or line name, sex, pedigree information. No phenotypic information is required. All sequences have been aligned on GalGal5 reference genome and SNP calling was performed with a common pipeline. The organization of the project makes possible to identify the analysis of common interest to be performed on the whole data set, whereas the leader of a given project can use the entire set of SNPs to address a specific question, while recognizing the origin of the whole data set. The number of SNPs varied from 5 to 10 millions per project whereas the number of private alleles varied from 1000 to 10000 according to the project. A Structure analysis led to group the individuals in 7 genetic clusters. Among them, recently selected broilers were well separated from old broiler lines, showing the impact of selection within broilers, whereas a variable pattern of diversity was observed in local breeds with no real clustering between them. As expected, brown-egg layers were clearly separated from white-egg layers. Considering that these two types of lines exhibit very contrasted egg shell color, we developed a case study on 7 candidate genes involved in the metabolism of protoporphyrin IX which determines egg shell color. We search for SNPs exhibiting contrasted allele frequencies between White Leghorns and Rhode Island Red layer lines: 57 SNPs were identified in 5 of the 7 genes, most of them involved FECH and ABCB6 genes. Regarding ABCB6, 13 variants showing opposed frequencies (0/1) covered a segment of 6920 bases, including one non-synonymous variant, which suggest a potential selection signature. Functional consequences of this SNP are being explored.

ID : 1466

IDENTIFYING SIGNATURES OF SELECTION RELATED TO COMB DEVELOPMENT

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Abstract : The aim of this study was to identify the genes involved in the comb development and to provide insights into molecular mechanism of comb formation in chickens. Fixation index (F_{ST}) and average number of base differences ($\theta\pi$) of two groups of males with large and small combs were calculated based on whole-genome resequencing data. All males used in the present study were sourced from a Partridge Shank chicken breeding line with single comb. To obtain males with different comb sizes at 9 weeks of age for sequencing, comb areas and heights of 200 males determined from the descendants of 6 families of Partridge Shank chicken line were measured. Based on those results, 22 males with bigger combs (BC) and 19 males with smaller combs (SC) were selected for further analysis. DNAs in each population (BC, SC) were pooled in each tube. Two pooled samples (BC and SC) were separately sequenced using next-generation sequencing technique on the Illumina sequencing platform (HiSeq 2000). The chromosome regions with larger F_{ST} value and smaller $\theta\pi$ were considered as candidate regions of selection. Through further annotation of gene functions and pathways, we sought to screen possible selected genes associated with comb development. By screening whole genome resequencing data, the F_{ST} and $\theta\pi$ were calculated using a 40Kb sliding window strategy. Eight regions were identified. QTLs (FOX1 gene) related to comb length were found on chromosome 1. QTLs (GLP1R, BTBD9, MIR6633, MDGA1 genes) related to comb weight were found on chromosome 3. QTLs (ALDH1A1, TMC1, ANXA1 genes) associated with comb area were found on the Z chromosome. Nineteen genes, Wnt signaling pathway and neuroactive ligand-receptor interaction signaling pathway directly or indirectly related to comb growth and development were found through functional annotation and GO analysis. Among the selected genes LYN, GLP1R, FOX1, TBK1, STRAP, ST6GALNAC and Wnt signaling pathways were related to immunity. MDGA1, BTBD9, MTSS1, SrGAPs and neuroactive ligand receptor interaction signaling pathways related to neural function were screened. ALDH1A1, ANXA1, THBS, HIF-1 α , ACTN1 genes were related to heat dissipation. Among the selected genes FOX1, MDGA1 and ANXA1 associated with immunity, neurological function, and heat dissipation function coincided with genes affecting the length, weight, and area of the comb. Comprehensive analysis suggested that comb development was due to multiple genes and signaling pathways.

Key words: comb; whole-genome resequencing; selection signatures; candidate genes; genetic differentiation

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NEW TRAITS FOR POULTRY BREEDING

ID : 360

ASSOCIATIONS OF FEATHER PHENOTYPES WITH BODY AND FEATHER GROWTH PERFORMANCES IN SLOW OR RAPID FEATHERED QINGYUAN PARTRIDGE CHICKENS

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Introduction: In China, quality chickens are sold as whole carcass with skin. Immature plumage may result in black spots in the feather follicles of skin after feathers removal, which may therefore severely reduce the product quality and value, especially for breeds with dark plumage. This study was conducted to evaluate associations of feather phenotypes with body and feather growth performances.

Materials&Methods: A total of 320 day-old Qingyuan partridge chicks from the slow feathering strain (80 males and 80 females) and the rapid feathering strain (80 males and 80 females) were randomly selected. The two strains have been developed by phenotypic selection of five generations. Feathering parameters including primary feather length (PFL), primary-covert feather length (PCFL), average feather length (AFL), and feather length difference (FLD) were measured as hatched. Body weight (BW), comb height (CH), the percentage of matured feathers of dorsum (MFD), and the number of matured feathers of wing (MFW) were measured at 110-day-old, a typical marketing age of quality chickens in China. Effects of feather phenotypes and sex on growth traits, and correlations between feathering parameters and growth traits were estimated by GLM and CORR with SAS9.2.

Results: The MFW for rapid feathering strain was higher than slow feathering strain ($PP_r = -0.44$, $P = 0.002$) in rapid-feathering males, and between MFW and BW ($r = -0.31$, $P = 0.04$) in slow-feathering males. For rapid-feathering males, there were positive correlations between PFL and MFW ($r = 0.33$, $P = 0.02$), PCFL and MFW ($r = 0.43$, $P = 0.002$), AFL and MFW ($r = 0.38$, $P = 0.007$); for slow-feathering males, there were negative correlations between PCFL and MFW ($r = -0.36$, $P = 0.02$), AFL and MFW ($r = -0.33$, $P = 0.03$); for rapid-feathering females, there was a positive correlation between FLD and MFD ($r = 0.33$, $P = 0.03$); for slow-feathering females, there was a negative correlation between FLD and CH ($r = -0.32$, $P = 0.02$).

Conclusion: Influences of feathering parameters on growth traits of quality chicken breeds were proved first time by our results, providing scientific basis for the breeding of local chicken breeds. Further genetic correlation analysis is needed to confirm the possibility of improving feather maturity of Qingyuan partridge chickens by positive selection of PCFL and AFL for rapid-feathering males, and by negative selection of PCFL and AFL for slow-feathering males.

ID : 535

INDIVIDUAL FEED INTAKE AND FEEDING BEHAVIOUR COMPARISON BETWEEN PEKIN AND MUSCOVY DUCKS BASED ON HIGH THROUGHPUT PHENOTYPING

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The production of duck meat is covered by different species including the Pekin and the Muscovy ducks. Generally, Pekin ducks are commonly used in the Asian market and Muscovy ducks are mainly used in European market. Most of the studies published present result on feed efficiency on a small number of animals (Bley et al, 2008, Howie et al, 2010). The aim of the study is to compare those two genetic on the individual feed intake and feeding behaviour.

Animals of 2 Pekin lines (C, D) and 2 Muscovy lines (A, B) of Orvia hatched in 2016 were kept on floor to express their natural feeding behaviour as they do in normal production environment. Large group were studied and compared using high throughput phenotyping. Data have been collected using automatic feed station with ducks equipped of a radio frequency identification. A total of 12 068 ducks have been measured. Ducks were housed in large pen of around 1 500 animals. The ducks were feed ad libitum with a pelleted grower. Measurement have been done from week 3 to week 7 for the Pekin lines and from week 3 to week 12 for the Muscovy lines. The number of meals per duck has been record, meal size, duration of meal and the frequency of meals, feed intake, duration of feed intake per day. A total of 20 430 239 records have been studied.

R software has been used to remove abnormal data and to carry out the statistical treatments. Anova and Duncan tests have been performed. A focus from 3 to 7 weeks have been done. All differences between lines are significant. Muscovy meal duration per day is 1539s vs 826s for Pekin. Feed intake per meal is respectively 9.8g and 9.0g for Muscovy and Pekin. During the day Pekin are coming more often to the feeder 20.8 vs 13.9 for the Muscovy. Feed consumption per day is higher for the Pekin (186g) than Muscovy (136g). A clear variability within lines and between products have been identified. Although Pekin and Muscovy duck are on the meat market, these results highlight the fact that they have different feeding behaviour. This implies different managements. Future analyses will look the difference in term of genetic component of those traits within different Pekin lines and Muscovy lines.

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ID : 653

GENOME-WIDE ASSOCIATION STUDY FOR THE DYNAMIC CHANGE OF EGGSHELL GLOSS ACROSS THE WHOLE LAYING PERIOD IN CHICKEN

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The appearance of eggshell can influence the purchasing behavior of consumers. Eggs with a shiny appearance are more attractive than others. Previous studies found that the eggshell gloss was related to the texture of the surface. However, the genetic architecture underlying the dynamic change of eggshell gloss remain elusive. Herein, we measured the eggshell gloss at 6 age points (36, 56, 60, 72, 80 and 90 wk) and conducted comprehensive genome-wide association studies (GWAS) with 50 K density SNP array in a pure line of 706 Rhode Island Red hens. The results showed that the average value of gloss ranged from 2.21 GU to 3.15 GU with a variation of 15.09% to 23.73%. The eggshell gloss of 36 wk old hens was higher than that at other ages. Also, significant positive correlations were found between the eggshell gloss of hens with adjacent ages ($R=0.51, 0.53, 0.51, 0.52$ and 0.59). Subsequently, 6 genome-wide significant SNPs were identified at 60 wk. According to these candidate loci, six genes (ACADSB, IKZF5, GPR26, SLC2A11L3 and SLC2A11L2) were considered to play an important role in eggshell gloss. This study will greatly advance the understanding of the genetic basis and has the practical significance in breeding program for the improvement of eggshell gloss.

Keywords: chicken, eggshell gloss, GWAS, 50 K SNP array

ID : 1205

THE DIFFERENCE IN SOME PHYSICAL EGG QUALITY AND PRODUCTIVE TRAITS BETWEEN PARENTAL LINES OF WL AND RIR AND THEIR RECIPROCAL HYBRIDS OF DOMINANT TINTED D 723 AND D 732

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The creation of new hybrid combinations in poultry husbandry is connected with practical testation of selected and predicted lines and theirs crosses. The objective of the present work was to evaluate the effect of the particular genotype, age of layers and their interactions on egg quality traits in laying type hens Dominant. The following genotypes were examined in the experiment: as parental lines White Leghorn, slow- and fast-feathering (next WL FF, WL SF) and Rhode Island Red, slow- and fast-feathering (next RIR FF, RIR SF) and the crossbreeds of the above mentioned strains in the F1 generation (Dominant TINTED D 723 = LW FF x RIR SF and Dominant TINTED D 732 = RIR FF x WL SF). There was used feather sexing method. The layers were fed by feed mixture N1 (16.64% of CP) from the 20th week of age and by feed mixture N2 (15.02% of CP) from the 40th week. Husbandry conditions met the regular requirements for laying type hens. Egg quality traits were examined at the layers' age of 28, 36 and 59 weeks. For analysis, 300 eggs were collected from each genotype per time. The eggs were weighed using an electronic scale Ohaus. Eggshell strength was determined by the analyzer Instron. Eggshell thickness was measured by a digital micrometer. The proportions of yolk, albumen and eggshell were calculated in relation to egg weight and expressed as percentages. The egg shape, yolk and albumen index, yolk to albumen ratio and Haugh unit score were computed as well. The statistical analysis was processed by the computer application SAS. All monitored traits of physical egg quality were affected by genotype ($P \leq 0.05$) and age of hens ($P \leq 0.05$). There were detected significantly ($P \leq 0.05$) mutual interactions between genotype and age of hens in yolk weight, yolk and albumen proportion and their index, yolk to albumen ratio, Haugh unit score and eggshell thickness. Eggs from crossbreeds (D 723 and D 732) had better quality than those from parent lines (e.g. egg weight, eggshell thickness, albumen weight, yolk weight and proportion). The results of this study helped to select and start practical use of the new laying programme DOMINANT TINTED D 723, which produced egg production per hen day 338 eggs in age of 518 days in XIV. International performance test of commercial layers – alternative system (described in The final report 2015 – 2016) and started to be popular among programmes of DOMINANT CZ company. This research was funded by an "S" grant of the MEYS of the CR.

EF4

EGG QUALITY

CONSTRUCTION OF EGG QUALITY

QUALITY OF EGG PRODUCTS

CONSTRUCTION OF EGG QUALITY

ID : 59

AGING-ASSOCIATED CHANGES IN THE ISTHMIC AND UTERINE MUCOSAE LOWERING THE EGGSHELL QUALITY

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Understanding why aged hens produce inferior quality eggs may help to produce long-life layers. Thus, the aim of this study was to determine the aging-associated molecular changes in the isthmic and uterine mucosae causing an inferior quality eggs produced by aged hens. White Leghorn hens were used in this study to compare the expression of proteins related to eggshell membranes formation in the isthmus and eggshell biomineralization in the uterus of young (35 weeks old) and aged (130 weeks old) hens at 16 h following oviposition. The results showed that collagen V protein density was lower in the isthmic mucosa and eggshell membranes of aged hens than in young hens. The mRNA expression of calbindin, osteopontin, and ovocalyxin-36, and the densities of calbindin and carbonic anhydrase-2 were lower in the uterine mucosa of aged hens than in young hens. There was no observable histological difference in the isthmus or uterus between young and aged hens. We conclude that the eggshell quality is decreased in aged hens compared to young hens due to a molecular disorder to produce structural and functional proteins that share in the eggshell membranes formation and eggshell biomineralization process.

ID : 139

MICROSTRUCTURE OF THERMALLY INDUCED EGG YOLK GEL

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Egg is a kind of animal food with balanced nutrition. Gel properties are one of the most important processing characteristics of eggs in food processing. The thermal gelation process of yolk is a complex process, which determines the rheological properties and gel properties of egg yolk gel products. Through a lot of research and breeding work on local chicken breeds in China, it is found that some local chicken breeds in China have the characteristics of producing yolk. Therefore, it is necessary to find out the causes of egg yolk non blocking at the molecular level

The experiment was divided into four parts: (1) pigeon eggs, common eggs and oil eggs were steamed for 10 minutes, 20 minutes and 30 minutes respectively. (2) cook the common eggs at the corresponding time, including duck eggs, eggs, goose eggs, quail eggs and pigeon eggs (3) cook 8 big black eggs and 8 Luodao red eggs at the same time, and send the above three groups of cooked eggs to the company for scanning electron microscopy observation. (4) lipoproteins were extracted from 20 large black eggs, 20 Luodao red eggs and 20 pigeon eggs, and the content was calculated.

The results showed that: (1) the yolk particles of egg yolk and pigeon egg were smaller than those of common eggs. (2) cooking time had no significant effect on the size of yolk particles. (3) the size of yolk granules of five kinds of bird eggs is very different, but they are basically granular. Under 500 nm scanning electron microscope, the structure of goose egg and duck egg is significantly different from that of other birds. (4) in the determination of lipoproteins, the content of lipoproteins in big black chicken was the highest, while that in Luodao red egg was the lowest.

The conclusions are as follows: (1) the reason for the good oil property of egg yolk and non choking may be that the egg yolk particles are small. (2) due to the existence of oil, duck eggs and goose eggs are closely arranged at 500 nm, and their oil shape is larger. (2) the content of lipoproteins may be related to the particle size, but it needs to be further explored.

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ID : 259

THE EFFECTS OF THE “SUBJECTIVE DAY” LENGTH ON DAILY OVIPOSITION RHYTHM AND PRODUCTIVE PERFORMANCE IN LAYING HENS

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The effects of the “subjective day” length and intermittent lighting regimes on daily oviposition rhythm and productive performance in laying hens (cross SP-789). Four treatments of hens (100 birds per treatment) were kept from 124 to 290 days of age in cage batteries (5 birds per cage). The lighting regimes for treatments 1-4 were 1L:6D:4L:2D:3L:8D; 1L:5D:4L:2D:3L:9D; 1L:4D:4L:2D:3L:10D and 1L:4D:4L:2D:3L:10D; the “subjective day” length was 16, 15, 14 and 13 hours, respectively. First switch-on was at 2 am for all the regimes; light intensity was 10 Lx. The oviposition time was determined during 5-day periods in the beginning of the trial, in the middle and in the end of the trial using a camera recorder on a 24-hour basis. It was found that the decrease in the “subjective day” length resulted in earlier onset and earlier termination of the oviposition: in treatments 1-4 the oviposition started at 4, 3, 2, and 1 am and ended at 6, 4, 4, and 3 pm, respectively. The percentage of eggs laid between 2 and 8 am in treatments 1-4 was 52.3; 70.8; 88.0 and 89.9%; between 2 and 10 am 61.0; 76.9; 95.2 and 91.9%; between 2 am and 1 pm 92.9; 90.8; 98.8 and 98.0%, respectively. The average oviposition time in these treatments was 8 hrs 40 min; 7 hrs 22 min; 5 hrs 22 min and 5 hrs 18 min (time of the day), or 14 hrs 40 min; 14 hrs 22 min; 13 hrs 22 min and 14 hrs 16 min after the onset of the longest dark phase of the respective regimes (the difference between treatments 1 and 2 was significant at $P<0.05$; between treatments 3, 4 and 1, 2 at $P<0.001$). Average live bodyweight in treatments 1-4 at 290 days of age was 1664, 1660, 1714, 1681 g (the difference between treatments 2 and 3 was significant at $P<0.05$); mortality levels were 2.0; 3.0; 1.0 and 1.0%; egg production per initial hen 123.9; 124.1; 130.3 and 126.1 eggs (the difference between treatments 3 and 1, 2 was significant at $P<0.001$; between treatments 3 and 4 at $P<0.05$); average egg weight 60.0; 59.9; 60.4 and 59.7 g (the difference between treatments 3 and 4 was significant at $P<0.05$); feed conversion ratio per 10 eggs laid 1.37; 1.36; 1.32 and 1.36 kg, per 1 kg of eggs laid 2.28; 2.26; 2.18 and 2.26 kg, respectively. The conclusion was made that manipulations with the “subjective day” length can regulate daily oviposition time in correct direction; the best parameters of productivity and feed conversion were found at the 14-hr length (treatment 3).

ID : 287

GUINEA FOWL EGG SHELL STRUCTURAL ORGANIZATION AND PARTICULAR ORGANIC MATRIX PROTEIN PATTERNS TO DECIPHER ITS EXCEPTIONAL BIOMECHANICAL PROPERTIES

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The Guinea fowl (*Numidameleagris*) presents a highly resistant eggshell compared to the other birds. We will described in this study the particular ultrastructure of the Guinea fowl shell that confers exceptional mechanical properties and how changes in organic matrix components controls the development of this structure. The inner part of the shell is similar to other birds, but an additional change in the size and orientation of crystals (switch) is observed at about 1/3 of the calcified layer. Large columnar calcite units break into smaller crystal units with varying crystallographic orientations forming a microstructure with an intricate interlacing of calcite crystals.

We recently reported the Guinea fowl shell structural organization from the micro to the Angstrom, which underlined that this particular shell is a bilayer structure. Organic matrix is suspected to firstly induce the initial microstructure shift and then the secondary nucleation events resulting in smaller crystals with increasing misorientations. Consequently, the change of the intra-crystalline organic matter level during the crystal switch was also investigated. A proteomic survey allowed us to identify and characterize 149 proteins in Guinea fowl shell. These proteins were quantified at five calcification stages corresponding to the first events of mineral deposition, the growth of calcite units just prior the shift of crystal orientation, then to the period of the deposition of newly formed crystalline shape and to later stage when the growth of the newly formed crystals is stabilized after the microstructure shift and the secondary nucleation events. We have observed 61 matrix proteins only present in the shift period and potentially responsible of the change of mineral in the shell. Amongst them are calcium binding proteins (NPNT-X1, CALBP1, Protein S100-A6, ANXA1 and 2, CDH2...), core proteins of proteoglycans (TSKU, GPC4...), and other proteins regulating the activity of proteins driving the mineralization (SSP1, OC-116GDF6...).

These proteins interact with mineral to produce changes in crystal size and orientation and consequently the new shell structure and its resulting mechanical properties. Data obtained will allow the determination of biological markers that will be used for the genomic selection of chicken layers with improved mechanical shell mechanical properties. Additionally, they provided a list of organic products that will be tested as additives for material and ceramics.

ID : 339

COMPARATIVE STUDY OF PERCENTAGE OF EGG LAYING AND EGG QUALITY TRAITS IN ITALIAN SLOW-GROWING CHICKEN BREEDS

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Introduction. Bionda Piemontese (BP) and Bianca di Saluzzo (BS) were the most widespread local chicken breeds of Piedmont region and currently are reared in small-scale farms for eggs and meat production. The aim of this study was to characterize the percentage of egg laying, as well its physical and shell parameters of these two Italian chicken breeds.

Material and methods. A total of 180 laying hens of both BP and BS (23 wk of age) were homogenously distributed in 12 pens with outdoor access (15 hens/pen/breed); all the birds were fed the same commercial feed (ME 11.8 MJ/kg; CP 14.2%) for 36 wk. Percentage of egg laying and mean egg weight were recorded per pen throughout the laying hen cycle. From 37 to 61 wk of age, egg samples were collected every 6 wk, (5 eggs/pen/breed) to determine both egg and egg shell characteristics. Data were analysed by IBM SPSS Statistics V25.0.0 software. Percentage of egg laying and mean egg weight of the two breeds were analysed using Student's t-tests for independent samples. General Linear Model (GLM) procedures have been performed for all egg quality parameters, using breed and age as principal effects. Tukey multiple comparisons have been performed after the GLM procedure for slaughtering time. Significant differences are considered at $P \leq 0.05$.

Results. No significant difference was observed for the percentage of egg laying (BP $54.6 \pm 2.7\%$; BS $53.3 \pm 3.0\%$; mean \pm SEM) and for mean egg weight (BP 58.6 ± 0.8 g; BS 56.9 ± 0.6 g; mean \pm SEM) among breeds. Physical egg parameters (egg weight (BP 62.4g, BS 59.8g), width (BP 43.3mm, BS 43.0mm); length (BP 59.7, BS 58.1 mm), yolk weight (BP 18.8g, BS 17.7g), albumen weight (BP 37.4g, BS 36.5g) yolk/egg percentage (BP 30.1%, BS 29.6%) and total egg surface (BP 73.6 cm², BS 71.5 cm²) resulted higher in BP hens ($P \leq 0.05$). Egg shell parameters did not differ among breeds and age except for colour indices. Age influenced all the egg quality parameters with linear trend over the age ($P \leq 0.05$). Interaction between breed and age of hens were observed for yolk weight, yolk/egg ratio and for colour indices of yolk and egg shell ($P \leq 0.05$).

Conclusion. Promising results were obtained in both production and physical quality parameters of eggs between BP and BS hens. This yielded evidence for several valuable egg characteristics that might be exploited for the improvement of these Italian pure breeds.

ID : 430

DIETARY SUPPLEMENTATION OF LINOLEIC ACID FOR INCREASING THE PULLET EGG SIZE

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Dietary supplementation of linoleic acid for increasing the pullet egg size

Linoleic acid (LN) is beneficial to human health for its multiple properties of antiobesity, antidiabetic, anticancer, antiatherogenic, and immunity improvement. The use of LN in laying hen diets to produce eggs enriched with essential nutrients has become a common practice in the table egg industry and linoleic acid levels in egg yolks increase in a dose-dependent manner. An experiment was conducted to investigate the effect of LN of the diet on egg weight, egg production and egg quality characteristics on NovoGen brown commercial layers of 20 to 40 weeks of age. There were three dietary treatments of four replications per treatment maintaining four birds per replication. All the diets were iso-caloric and iso-nitrogenous except for the doses of LN. The diets contained T1 (control diet with no LN), T2 (control diet + 1.5% LN) and T3 (control diet + 2.5% LN). In this experiment, we supplied soybean oil as the potential source of LN, as because soybean oil contain about 53% LN, soybean oil frequently use as the laying birds ration formulation and we had try to avoid the use of synthetic LN for the safer egg production. We had measured the boiled yolk weight, because the yolk weight directly reflects the egg weight. The results showed the egg weight, yolk weight, and egg quality parameters were better in the diet contained higher in linoleic acid. The yolk weight was significantly improved in the diet containing 2% soybean oil (2.5% LN) compared to 1% soybean oil (2.5% LN) and control (0% LN) groups. Additionally, both the internal and external egg quality parameters, especially shell thickness, Haugh Unit (HU), yolk index were improved in the dietary group treated with both 1% and 2% soybean oil (both 1.5 % and 2.5% LN). Taken together, it may be concluded that LN has a positive effect in not only the increase in egg size but also in the egg quality characteristics and thus improve the overall health benefit for human consumption.

Key words: Linoleic acid, pullet, egg size, diet, supplementation.

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ID : 474

LAYING HENS PRODUCTION EFFECTIVENESS INCREASING AND QUALITY IMPROVING BY INCLUDING TO THEIR DIET SUSTAINABLE PLANTS (HELIANTHUS TUBEROSUS L.)

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Nutritionally enhanced eggs could play a larger role in promoting the nutritive value of people's diets (Shinn et al. 2018). The factors that affect egg shell quality and egg internal quality are housing systems, management, storage period and environmental temperature, diseases, age of hen and nutrition (Tamiru et al., 2019). To improve egg quality different feed additives are tested, as well as *Helianthus tuberosus* L. tubers, as an inulin source, can be very promising from this point of view. In in study was hypothesized that feeding with *H. tuberosus* L. tubers (HTT) powder, rich of inulin, might improve laying hens' egg quality characteristics. A total of forty 30-week-old laying hens we distributed into two groups and for 8 weeks were fed by diets were supplemented with nothing (control) and HTT powder at the level of 2g/kg of feed. The fatty acids (FAs) profile in egg yolk by gas chromatography, yolk cholesterol by HPLC and egg, yolk, albumen and egg shell weight, eggshell breaking strength, albumen height and Haugh unit as well as body weight (BW), feed conversion ratio (FCR), laying rate were evaluated. In statistical analysis, data were subjected to univariate analysis of variance (ANOVA) and the Tukey HSD test was applied. Results were considered statistically significant at $P \leq 0.05$. In the present study, the treatment with HTT powder did not significantly influence the total sum of yolk SFAs, MUFAs, PUFAs and omega-6/omega-3 FAs ratio. The total cholesterol content in egg yolk was significantly lower in treatment group by 15.95% compared with the control. There were no significant effects of HTT on BW, FCR, but the laying rate of hens increased by 4%, compared with the control group ($P \leq 0.05$). A significant effect of HTT on shell breaking strength by 2% and Haugh unit by 5% was found, compared with the control ($P \leq 0.05$). The results of the study indicate that *Helianthus tuberosus* L. tubers powder has a positive effect on eggshell quality, Haugh unit, and it reduces yolk cholesterol level. Finally, laying hens productivity effectiveness and quality of egg can be improved by including to their diet sustainable plants.

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ID : 547

IDENTIFICATION OF POLYMORPHISMS IN PROTEIN CODING GENES WHICH AFFECT EGGSHELL QUALITY TRAITS IN LAYERS

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Chicken egg is a high quality food at a low cost to the consumer but must be healthy and free from pathogens. Consumer demand for the development of alternative farming systems to cages is growing (barns, free-range, etc.). However, these systems present an increased risk of microbial contamination of eggs, which reinforces the importance given by breeders to the eggshell quality. A large number of QTLs influencing production and egg quality traits have been detected. However, the number of known causal polymorphisms remains low. In the context of genomic selection, knowledge of these polymorphisms is a key step to increase the evaluation accuracy. In the context of scientific knowledge, identifying genes and polymorphisms involved in the variability of traits is also important.

To address these two contexts, we have selected potential causal polymorphisms in QTL and candidate genes. Polymorphisms in DNA from laying hens were first selected in QTL regions according to a strong contribution to additive genetic variance of a trait. Then, they were selected according to their position in candidate genes in QTL regions. We have defined candidate genes as genes known to play a role in eggshell quality, differentially expressed in uterus or coding proteins with different levels in uterine fluid during eggshell formation. Finally, deleterious polymorphisms localized in coding regions (creation or loss of start or stop codon, reading frame shift or modification of splicing site) were identified. This strategy for identification of putative candidate genes and causal polymorphisms susceptible to affect protein abundance, structure and function is generic and can be applied to other species and traits. In our study, we have identified 4849 deleterious loci as putative causal mutations, localized in 418 candidate genes for eggshell formation.

Finally, we have selected 255 deleterious polymorphisms which have been genotyped on 480 laying hens from 8 laying breeds. Association studies analysis will be conducted to validate the effect of these polymorphisms on eggshell quality.

Polymorphisms in coding regions are a small proportion of all polymorphisms identified in QTL regions. We don't suggest that polymorphisms outside coding regions are not causal mutations. Conversely, when experimental validations are needed it's more obvious to analyze the effect of these polymorphisms on the function of proteins and define a link between polymorphism and protein function and trait.

ID : 617

EFFECTS OF ADDING THE FUNCTIONAL CO-EXTRUDATES AND NATURAL PIGMENTS IN THE DIET OF LAYING HENS ON EGG QUALITY

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Eggs are cheap and nutritionally valuable products of animal origin. Using specially designed mixtures for the production of table eggs, it is possible to produce eggs enriched in ω -3 fatty acids, as well as in natural pigments and vitamins. Thus, this study aimed to investigate the effects of inclusion of co-extrudate based on flaxseed and camelina seed in combination with natural pigments in hen's diet on egg quality parameters.

One hundred and eighty Lohmann Brown laying hens divided into six groups (two controls (C1 and C2) and four experimental (E1-E4)) were fed for four weeks. The laying hens were fed with corn-soybean meal based diet (C1 and C2). Flax-corn meal co-extrudate was added in amount of 13.5% (E1) and 22.5% (E2) while camelina-corn meal co-extrudate was added in amount of 16.6% (E3) and 27.6% (E4) in based diet. C1 contained up to 3% fat without added pigments, while C2 contained up to 5% fat and synthetic pigments. The same amount of natural pigments (1% carrot and 0.5% paprika) was added in all experimental treatments. Content of fat in E1 and E3 was 3%, while in E2 and E4 was 5%.

No significant changes ($p>0.05$) on the internal egg quality characteristics were observed for eggs from the experimental treatments when compared with eggs from control treatments. All experimental treatments achieved desirable color demanded by consumers, from 12.67 to 13.28 RYCF value (Roche Yolk Color Fan). The content of PUFAs in eggs from all experimental treatments was significantly higher ($p<0.001$) than in eggs originated from hens fed with control diets. The ω -6/ ω -3 ratios of 1.43, 1.01, 1.74 and 1.73 in treatments E1, E2, E3 and E4, respectively, were significantly lower ($p<0.001$) than in treatments C1 and C2 (9.40 and 8.88, respectively). The addition of co-extruded flaxseed in treatments E1 and E2 had a negative effect ($p0.05$).

Based on obtained results it can be concluded that with the addition of natural pigment, as well as selected co-extrudates, it is possible to design functional eggs that will have good egg quality parameters, optimal yolk color, increased content of ω -3 fatty acids, better ω -6/ ω -3 fatty acids ratio, as well as good sensory profile of the eggs.

Keywords: functional eggs, flaxseed, camelina seed, co-extrudates, natural pigment

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ID : 674

GENETIC VARIATIONS FOR THE EGGSHELL CRYSTAL STRUCTURES REVEALED BY GENOME-WIDE ASSOCIATION STUDY IN CHICKEN

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Avian eggshell is a complex bio-ceramic characterized by layer structure. Eggshell forms an embryonic chamber for the developing chick by providing a stable internal environment. For the egg industry worldwide, the integrity of eggshells is critical to the economic viability of the poultry production. However, the genetic architecture underlying the eggshell crystal polymorphism remains to be elucidated. Herein, we measured the eggshell crystal structures including integral intensity of the first nine major diffraction peaks, total integral intensity (TA) and eggshell crystal orientation degree (OD), then the genome-wide association studies in 927 F2 hens was conducted. The results showed that TA had a positive correlation with the ultrastructure and common quality traits of eggshells. There was a negative correlation between TA and OD, indicating that the larger the crystals, the lower the degree of orientation, the more prone to random distribution. The SNP-based heritabilities of TA and OD were 0.23 and 0.06. Moreover, 621 SNPs located in 55.7-69.3 Mb in GGA1 were significantly associated with TA, and the most significant SNP accounted for 4.87% of the phenotypic variance. Based on mRNA and protein expression in uterine tissue, we suggested that DERA gene may play an important role in the regulation of eggshell crystal growth. The findings greatly advance our understanding of the genetic basis underlying the crystal ultrastructure of eggshell quality and has the practical significance in breeding program for the improvement the eggshell quality.

Keywords: chicken, eggshell, crystal structure, X-ray diffraction, GWAS

ID : 810

EVALUATION OF EGG QUALITY TRAITS OF FOUR VARIETIES OF THE CANARIAN HEN IN FREE-RANGE CONDITIONS

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Despite the economic and cultural importance of poultry sector in the Canary Islands throughout their history, there is no information to date on the main productive performance and egg quality characteristics of Canarian hens. The objective of this study was to determine the quality of the egg produced by four colour varieties of Canarian hen (Black Barred, Red Barred, Black, and Partridge) reared under free-range conditions and comparing with commercial barn eggs from Isa Brown hens. All hens were fed with the same standard layer feed during the experimental period. A total of 180 eggs were collected at 32, 36 and 40 weeks of age, and were used to evaluate external and internal parameters. For the statistical analysis, the genotype effect was determined using an ANOVA followed by the post hoc Tukey's method. Statistical differences were considered significant at Pvs. 75.07 (0.30), respectively. There were not differences in shell and albumen weights, but yolk weight was higher in Canarian than in commercial eggs: 18.12g (0.17) vs. 16.19g (0.24), respectively. Moreover, eggshell colour parameters did not vary among Canarian varieties. However, significant differences were reported for the three coordinates defined by the International Commission on Illumination: lightness (81.48 vs. 62.34, SEM 0.687), redness index (5.64 vs. 19.72, SEM 0.459) and yellowness index (17.88 vs. 30.94, SEM 0.505) for local and commercial eggs, respectively, showing the Canarian eggs are less brown than the commercial ones. No differences were found for shell hardness, yolk index and pH of the yolk and the albumen, and ranged between 31.87–37.84N, 46.85–50.59%, 6.09–6.20, and 8.99–9.16, respectively. Finally, there were not differences for yolk colour parameters among Canarian varieties, but the yolk from local hens had a higher yellowness index than the yolk from commercial laying hens: 54.10 (0.43) vs. 48.53 (0.57), respectively. In conclusion, the results obtained confirm there is no division among the four Canarian varieties in terms of differences in egg quality traits. Nevertheless, the local hens presented some differentiating attributes, as shell and yolk colours, which may contribute to consumers' initial selection.

ID : 929

STUDY OF MEASUREMENT METHODS ON PHENOTYPE OF TRANSLUCENT EGGS

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Scoring is a commonly used method to evaluate eggshell translucency, which mainly depends on the area size and density of translucent spots on eggshells. However, a lack of common scoring criteria and quantitative measurements of spots on eggshells does not allow for effective comparisons between research papers and greatly hinders the progress of research on translucent eggshell formation. To make measurement of translucent eggshells more objective, we optimized the scoring method and explored two new methods: the grayscale recognition and colorimeter methods, and further compared these three methods. Briefly, a total of 354 eggs from 600 395-d-old dwarf brown laying hens were collected and classified into 4 score levels subjectively according to their translucent degrees. This process was repeated five times. Then we captured profile side of each egg using a camera and measured spot characteristics by grayscale recognition, including quantities of spots (QS), diameter of each spot (DS), average area of each spot (AAES), sum of spot areas (SUSA), sum of shell area (SUSHA), and ratio of SUSA to SUSHA (RSS) on an eggshell. Furthermore, L, A, and B values of each egg on sharp, middle, and blunt ends were separately measured using a colorimeter. As results, average values of 31.31%, 29.78%, 29.81%, and 9.08% of all eggs were divided into score levels 1, 2, 3, and 4 (from opaque to translucent), which corresponds with RSS values of 1.34%, 3.23%, 6.21%, and 11.89%, respectively. By grayscale recognition, QS, DS, AAES, SUSA, SUSHA, and RSS all increased along with increased translucency scores ($P < 0.05$). Using scoring, an egg with specific RSS value was more easily divided into specific score level (50%) and adjacent score levels (50%). The L, A, and B values of eggshells that fall into score level one were significantly different from those of eggshells scored as levels 3 or 4 ; however, the adjacent score levels were not. In summary, the present study explored objective reference metrics for measurements of eggshell translucency.

ID : 795

MITOCHONDRIAL TRANSCRIPTION FACTOR A-INDUCED DECLINE IN MITOCHONDRIAL BIOGENESIS IN RELATION WITH THE DEPIGMENTATION OF BROWN EGGSHELL

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Eggshell color is an important characteristic of poultry eggs and has a positive effect on consumer preference. It would be favorable for table eggs with dark brown color. However, poor shell color of aged hens results in unacceptable shell color. The objective of this study was to examine what factors were related with the occurrence of brown eggshell depigmentation in aged laying hens. There eggshell color was determined by brightness value (L^*). We selected 24 chickens laying stable dark ($46 \leq L^* \leq 53$) and light-eggshell color ($59 \leq L^* \leq 65$) respectively from 300 62-wk-old Hy-Line brown-egg laying hens reared in individual cages. Oviposition time was recorded after collecting the successive 7 eggs per hen. A total of 8 hens per group were slaughtered at 15 h after the oviposition and were collected the eggshell gland region of the oviduct. All analyses were performed using Student's t-test with SAS9.1. Results showed that egg weight ($P < 0.05$), eggshell weight ($P < 0.01$), protoporphyrin IX (Pp IX) content of the eggshell and the shell gland ($P < 0.001$), and biliverdin content of the shell gland ($P < 0.001$) were significantly declined in the light-eggshell group. The mRNA relative expression of δ -aminolevulinic acid synthase 1 ($P < 0.05$), coproporphyrinogen oxidase ($P < 0.01$), ATP-binding cassette transporter ABCG2 ($P < 0.01$) and mitochondrial transcription factor A (TFAM) ($P < 0.01$) was downregulated in hens laying lighter brown eggs. There was significant decrease in mitochondrial DNA (mtDNA) copy numbers ($P < 0.01$), mitochondrial ND4 ($P < 0.05$), ATP8 ($P < 0.05$) and COX1 ($P < 0.01$) relative expression in the shell gland of the light-eggshell group. In addition, NAD⁺ contents of the shell glands were increased in the dark-shell group ($P < 0.01$). In conclusion, the remarkable traits of the depigmentation are showed to reduce Pp IX content in the eggshell and shell gland; the downregulation of ALAS1 mRNA relative expression make Pp IX biosynthesis declined; downregulated TFAM mRNA relative expression induce the decline in mitochondrial biosynthesis independent of peroxisome proliferator-activated receptor- γ coactivators β pathway, and it is associated with the depigmentation by lessening the synthesis of ALAS.

QUALITY OF EGG PRODUCTS

ID : 23

EVALUATION OF PHYSIOCHEMICAL CHANGES IN BOILED EGGS STORED AT DIFFERENT TEMPERATURE

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Hard boiled eggs are used widely in the world but once these eggs are stored under refrigerated conditions lead to customers' rejections due to its rubbery texture. Therefore, objective was to evaluate the effect of storing temperature on structural changes in hard boiled eggs. Newly laid, medium sized eggs were purchased from a commercial farm and boiled at 95 °C for 15 minutes and stored at 27 °C, 4 °C and -18 °C for 0, 6, 12, 18, 24 and 48 hours as different time intervals. 18 eggs per each experimental group were studied and subjected to analyze the texture and Fourier Transform Infrared (FTIR) spectroscopy. The color was measured as lightness (L^*), redness (a^*) and yellowness (b^*). Egg white surface was observed under gemological microscope for changes in structure. Sensory evaluation done by 7-point hedonic scale with 30 untrained panelists [Age 21-24 years, both male and females]. Salmonella was checked in boiled eggs stored at 27 °C. Texture and color data analysis were performed by one way ANOVA model and sensory data were analyzed with Friedman test in MINITAB 17.1. According to the results, frozen eggs showed the highest hardness due to formation of the ice crystals by freezing of water in the elastic gel of cooked egg white (denatured protein). The starting lightness (0 hours) of boiled egg white was 97.69 ± 1.15 and gradually decreased after 48 hours in 27°C, 4 °C and -18 °C as 93.84 ± 1.27 , 96.36 ± 1.60 , and 95.10 ± 0.84 respectively while changing the color from white to greenish white due to microbial and chemical activities. Boiled eggs stored for 48 hours at 27 °C were positive for Salmonella may be due to removal of barriers in egg and destroying of proteins which act as anti-microbial agents. Frozen eggs showed a significantly lower acceptance than those kept refrigerated or at 27°C (pOC could not use for the sensory analysis due to spoilage. Texture hardness of the frozen eggs (0 hours) was 272.00 ± 28.3 and it has increased up to 1060.75 ± 595 after 48 hours while chewiness (8.15 ± 0.0707 , to 14.966 ± 12.80), gumminess (227.50 ± 2.12 to 619.00 ± 380) springiness (3.623 ± 0.0802 to 3.40 ± 0.157) and cohesiveness (0.825 ± 0.0778 to 0.55 ± 0.1365) decreased. This texture variation has clearly explained by the reflection of light changes in microscopic images. FTIR data explained the textural changes in bonds of Amide A (3271 cm^{-1}), Amide I (1626.2 cm^{-1}), Amide II (1539.0 cm^{-1}), C=O stretch of COO- (1397 cm^{-1}), asymmetric PO₂- stretch (1240 cm^{-1}). Concluding, refrigeration condition (4 °C) for 24 hours was the more suitable way to store the boiled eggs to avoid consumer rejection when consider with texture profile and egg white lightness.

ID : 24

EVALUATION OF PHYSIOCHEMICAL CHANGES OF RAW CHICKEN EGGS STORED AT DIFFERENT TEMPERATURES

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Eggs are great and cheap source of high-quality protein which are widely used in the food processing industry. However, eggs are undergone deterioration with the time due to its' high perishability and affects to economic losses. Refrigeration is very effective in preserving egg quality. The study was carried out to evaluate the physiochemical changes of raw chicken eggs (RCE) stored at room temperature (28-31°C) and refrigeration temperature (4°C). A total of 111 medium sized white shelled RCE obtained from 61-week old Hyline. 6 eggs per each experimental group were studied and subjected to analyze weight loss (%), yolk color, Haugh unit, USDA grade, yolk and white pH and Fourier transform infrared (FTIR) spectrums at both room temperature at 4°C for 0, 1, 4, 8, 12, 16, 20, 24, and 29 days. Salmonella examined was done to RCE in room temperature at day 0. The data were analyzed by one-way ANOVA model in MINITAB 17.1. According to the result the eggs were not contaminated by Salmonella. USDA grade has decreased in both temperature from AA to B as at room temperature after 8 days and after 16 days at (4°C). Yolk color in room storage RCE increased significantly than those kept at refrigeration temperature (4°C) (p3 Stretching (2925 cm⁻¹) and Symmetric CH₂ Stretching (3304 cm⁻¹) in room storage condition. No chemical changes observed in egg albumin of refrigerated RCE. Moreover, secondary structural changes were detected in protein in RCE albumin during the storage. In conclusion, RCE stored at refrigerated storage showed less physiochemical changes in weight loss, Haugh unit, albumin pH, yolk pH and chemical structures during the storage period up to 29 days than room temperature.

ID : 185

EFFECTS OF GENOTYPE ON THE EGG QUALITY CHARACTERISTICS IN BROWN-EGG LAYING HENS

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Egg quality characteristics are a very important factor both for the consumers, as well as for the egg producers. The main factors that affect egg quality characteristics are hens strain, hens age, storage temperature and time, humidity, laying season, and nutrition. On the market there is a number of different hybrids, and egg producers are often in doubt as to which hybrid to choose.

This study aimed to determine the influence of hybrid on egg quality parameters (external and internal) of commercial laying hens. 60 eggs of Hy line silver and Hy line brown were examined from the hens of the same age (50 weeks). All eggs were collected on the same day. The hens were reared on same farm, under same conditions, and fed with same diets.

Eggs were analysed in the laboratory for determining poultry meat and egg quality on the Faculty of Agriculture in Novi Sad, Department of Animal Science. The determinants of external and internal egg quality traits were: egg weight, shape index, shell cleanness, shell colour, shell breaking force, shell thickness, shell weight, yolk colour, albumen height, yolk height, Haugh unit (HU), yolk width, yolk index, USDA, blood / meat spots, and albumen and yolk pH.

Results obtained showed that both strains produced eggs which have good external and internal egg characteristics. Excluding egg shell breaking force, albumen and yolk height, yolk pH and index, all other egg characteristics studied were not significantly ($p \leq 0.05$) affected by the strain.

The eggs of Hy line brown were superior in terms of internal parameters (albumen and yolk height, yolk index, and pH), while the hybrid Hy line silver was better in shell strength. The albumen and yolk height, yolk index, and pH significantly differed ($p \leq 0.05$) between Hy line brown (7.37mm; 19.51mm; 48.82; 6.42), and Hy line silver (6.55mm; 18.82mm; 46.28; 6.25). There was statistically significant difference ($p \leq 0.01$) between shell strength of observed hybrids. In Hy line silver eggs shell strength was 4.38, while in Hy line brown it was 3.45. A slight difference was found in HU, 83.92 for Brown vs. 80.48 HU for Silver.

The egg weight, shape index, shell cleanness, shell colour, shell thickness, shell weight, yolk colour, HU, yolk width, USDA, blood / meat spots, albumen pH were not significantly affected by the genotype.

The results obtained within this research suggest that different strains may possess different quality. This has a large influence on the specific egg use. Concerning the egg breaking industry, the eggs from Brown hens showed superior egg quality, due to their higher internal quality. On the other hand, for the egg producers, the Silver hens are more suitable for rearing, owing to their strong shell.

ID : 633

EVALUATION OF CHEMICAL EGGS COMPONENTS IN LOCAL POULTRY BREEDS AND DEVELOPMENT OF NIRS SPECTROSCOPIC TECHNIQUE FOR THE PREDICTION OF THE BIOCHEMICAL COMPOSITION.

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The present study aims to analyse quality eggs traits and develop infrared prediction models of Italian local chickens breeds.

The chemical analyses (proteins, moisture, lipids and ashes in yolk and white) were determined using reference methods on 25 eggs belonging to 8 genotypes: 4 breeds with white shell eggs (Padovana Camosciata, Padovana Dorata, Polverara Nera and Polverara Bianca) and 4 breeds with red shell eggs (Pépoi, Ermellinata di Rovigo, Robusta Maculata and Robusta Lionata), reared under the same farming conditions from a single farm. Spectra information were collected on visible and near-infrared part (from 400 to 2500 nm) using DS2500 (Foss, Hillerød, Denmark). Prediction models were carried out using a cross-validation approach and the accuracies of calibration equation were evaluated through the coefficient of determination (R^2), error standard of prediction (SE_{cv}), and the ratio of performance to deviation (RPD) in cross-validation. Red shell eggs show a lower yolk-to-egg white ratio compared to white shell eggs. The yolks of white shell eggs are richer in protein and moisture but lower in lipids compared to the red shell eggs. In detail, Padovana breed differs from the others for the greatest content of moisture and the lowest amount of lipids in the yolk; whereas in the albumen, Padovana differs just to Polverara showing a higher level of proteins and a lower amount of ashes. In the group of the red shell breeding hens, Pepoi differs significantly to the other breeds, both in yolk and albumen, for a higher amount of proteins and moisture, and lower quantities for the other considered chemical traits. The accuracy of the predictions were higher for the freeze-dried compared to the fresh egg yolk matrix, as expected. The fitting statistics of prediction models for eggs quality traits allowed a quite satisfactory prediction with a R^2 range from 0.234 (ash) to 0.662 (moisture) in fresh eggs and from 0.486 (ash) to 0.951 (moisture) in the freeze-dried. Taken together, these results suggest an innovative approach to predict the chemical composition of eggs from local hens, that could be used as a discriminatory analysis.

ID : 747

NON-DESTRUCTIVE PREDICTION OF SHELL EGG QUALITY BY NEAR-INFRARED SPECTROSCOPY

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Freshness and quality characteristics play a pivotal role in defining the acceptability of eggs by the consumers. Different approaches for their assessment are available, but they are mostly destructive, while food industries call for rapid, easy and non-destructive methods, able to grade shell eggs along the entire production and distribution chain. In this context, the aim of this work was to evaluate the possibility of using near-infrared (NIR) spectroscopy as a non-targeted technique for non-destructive egg quality assessment.

Eighty-four shell eggs from organic and barn farming were analysed at different times and storage conditions for weight, albumen and yolk ratios, Haugh Unit (HU), air cell height, yolk coefficient, and shell strength and thickness. Diffuse reflectance spectra (12,500 to 4,500 cm⁻¹, FT-NIR, MPA, Bruker Optics, Milan, Italy) were collected by a fibre optic probe at four sampling points: two in the equatorial region and two at the poles. Before spectral acquisition, the effectiveness of beam penetration was assessed by comparing the spectra obtained by substituting the egg content with water and ethanol with those of pure water and ethanol.

The spectral information was reduced (7,300-4,200 cm⁻¹) and pre-treated with SNV, smoothing and first derivative, alone or in combination, to be used for the development of prediction models based on the Partial Least Squares regression algorithm. The calibrated models were evaluated for internal (cross-validation) and external prediction (test set with 29% of the total samples). The best models were obtained for HU (range = 5.93 – 87.16; standard deviation = 19.95) and air cell height (range = 4 - 13 mm; standard deviation = 2.4 mm). The calculated Root Mean Square Error (RMSE) values for the prediction were similar to those previously reported in studies on egg freshness (Giunchi et al., 2008; Kemps et al., 2006). However, the models here developed can be considered more robust as the reliability of beam penetration was assessed and they were built considering eggs from different rearing methods.

Thus, the presented results do the groundwork for a non-destructive tool to assess egg freshness and quality at industrial level.

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ID : 897

EFFECT OF DIFFERENT COMBINATIONS OF NATURAL ZEAXANTHIN (YELLOW) PIGMENTS, AND NATURAL CAPSANTHIN AND SYNTHETIC CANTHAXANTHIN (RED) PIGMENTS ON COMMERCIAL LAYER HEN-EGG-YOLK COLOUR

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Addition of pigments in the diet is a common practice in the laying hen industry, responding to the perception of the consumers about the correlation between yolk colour and egg quality, and their direct benefit to ocular and skin health. Recently, there has also been an increased preference for the use of natural pigments over their more commonly used synthetic variants. Carotenoids are responsible for colouring egg yolks and chickens cannot synthesise them de novo so they need to be provided in the diet. In Australia, ration raw materials that contain natural pigments, such as maize, are not typical ingredients in layer diets because of limited availability. The objective of the trial was to compare the effect on the yolks colour of different combinations of natural yellow pigments derived from marigold flowers (Lutein; Zeaxanthin), with natural red pigments from paprika *Capsicum annuum* (Capsanthin) and synthetic red pigments (Canthaxanthin).

Hy-Line Brown laying hens (111) were divided into 6 groups (n = 24, 17, 18, 17, 18 and 17 from Groups 1 to 6), and fed without the addition of pigments. At 10 days before yolk colour scoring, designated groups received diets with different combinations of natural Zeaxanthin pigments (yellow), two concentrated (Leader Lutein Ester 10% and Colortek 10%) and one of typical source (Leader Yellow 2%). They also received red pigments, two from a natural origin derived from paprika (Leader Red Natural 1% and Xarocol 2%) and one synthetic (Leader red 10%, Canthaxanthin). Groups 1 and 2 received only concentrated yellow pigments from different sources, while groups 3 to 6 received different combinations of natural yellow and natural and synthetic red pigments. Yolk colours were measured every week for three weeks.

Median yolk colour scores did not differ between Groups receiving only concentrated yellow natural pigments, being 6 (4-7) for Leader Lutein Ester and 6 (4-8) for Colortek. Median scores in Groups 3 to 6, 12 (10-13), 12 (9-13), 12 (10-12) and 13 (11-14), were significantly higher. Inclusion rate for the non-concentrated Leader Yellow was 2.35 times higher than the concentrated alternatives and inclusion rate of natural reds was 3.50 and 3.52 times higher than that of synthetic reds.

Concentrated yellow pigments have proved to be as effective in adding colour to yolks as the typical non-concentrated pigments and with a lower inclusion rate. Natural red and synthetic red pigments bioequivalence was calculated as 3.5:1.

ID : 1094

YOLK INDEX (YI) AS A FRESHNESS INDICATOR OF HEN EGGS ALSO RELATE TO SALMONELLA GROWTH INSIDE SHELL EGG.

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[Introduction] It has been known that hens infected with *Salmonella enterica* Enteritidis (SE) lay eggs with SE-contaminated on yolk membranes inside their shell eggs. Since Japanese eat raw eggs, it is necessary to ensure the freshness and safety of shell eggs. The expiry date for hen eggs is calculated by the relationship (Humphrey's equation) between the storage temperature and SE growth at the surface of yolk membrane inside shell eggs. $D=86.939-4.109T+0.048T^2$ (D: days of SE rapid growth T: stored temperature)

Iron and yolk components are required for the growth of SE, and when the yolk membrane is weakened during egg storage, SE growth is accelerated due to the iron and yolk components leaked from the yolk.

[Experimental design] Fresh eggs were stored at 30°C with 75% humidity for 0-15 days, and at 25°C for 0-4 weeks. Then, the egg freshness was measured during the storage time. For the HU and YI, digital egg tester (DET6000: Navel Co., Ltd.) was used. Yolk membrane strength was measured as a breaking load (gf/cm²). Egg yolk of shell eggs stored at 30°C with 75% humidity for 0, 3, 6, 9, 12 and 15 days and stored at 25 °C for 0.1, 2, 3, and 4 weeks were aseptically separated. Then, each separated yolk was individually immersed in 10-100 CFU/ml SE bacterial solution, and after 24 hours incubation, the amount of the bacteria was individually measured using an ATP luminescence measurement method (BackTiter-Glo: Promega). Statistical analysis was performed using ANOVA.

[Results] The strength of yolk membrane decreased in the eggs stored at 30 °C and 25 °C in correlation to decreases of both HU and YI. The SE bacterial count with being soaked egg yolk significantly increased when the yolk of the eggs whose expiration date have passed (P

[Conclusions] In this study, the validity of the expiration date, which indicates the shelf life of eggs in Japan, was confirmed. Furthermore, using "YI" as a new freshness index for hen eggs, we were able to propose, "YI = 0.3 or more" as a cut-off value for judging that raw eggs can be eaten even in egg SE-contaminated.

EF5

MEAT QUALITY

CONSTRUCTION OF MEAT QUALITY

MEAT PROCESSING

CONSTRUCTION OF MEAT QUALITY

ID : 42

POLYPHENOLS AS EFFECTIVE SOLUTION TO IMPROVE MEAT QUALITY AND ANTIOXIDANT STATUS OF BROILERS

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Intense farming contributes to increased oxidative stress, with harmful implications for health, productivity, carcass dressing and meat quality. The aim of this study was to determine the effect of different doses of a polyphenol blend (ELIFE®) on the antioxidant potential and meat quality of broiler chickens. The experimental design comprised 1440 Cobb® male day-old chicks (4T*9R*40) assigned to 4 dosages: T1 – negative control, T2 – supplementation of 250 mg kg⁻¹, T3- supplementation of 500 mg kg⁻¹, and T4 – supplementation of 1000 mg kg⁻¹ in finisher phase only. A 3-phase standard corn soybean meal diet (60 ppm vit E) was fed. At the end of the experiment, breast muscle and legs were removed from the carcass and weighed to calculate carcass yield (n=9). Meat quality was determined by the evaluation of thiobarbituric acid reactive substances (TBARS) on breast muscle and oxidative stress was determined by the analysis of glutathione (GSH), glutathione transferase (GST), and lipid peroxidation (LPO) on the liver samples from these same birds. Although not significant, a dose response was found for proportional breast muscle weight. A one percent increase was also found for proportional drumstick weight for the highest inclusion compared to the control. TBARS are formed as a byproduct of lipid peroxidation and indicate damage produced by oxidative stress. TBARS were significantly (p<0.0001) lower for all supplemented groups compared to control. LPO in the liver was also reduced with the inclusion of the polyphenol blend compared to the control, although not significant. GSH content in the liver significantly (p=0.0066) increased with supplementation, indicating less oxidation. GST is an enzyme that interferes in the conversion of reduced glutathione (GSH) into its oxidized form (GSSG). High intracellular concentrations indicate injury to those cells. Its concentration in the liver decreased significantly (p=0.019) with supplementation above 500 mg kg⁻¹ compared to control. In conclusion, supplying polyphenols to fast growing broilers protects them against oxidative stress, with decreased lipid peroxidation and improved meat quality as a consequence.

ID : 158

DIETARY ANTIOXIDANT SUPPLEMENTATION IMPROVES MEAT QUALITY OF FEMALE KORAT CHICKEN.

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We investigated the effects of a dietary antioxidant supplementation (ID PHYT BIOLOX, ID4FEED SAS, France) composed of natural or nature-identical ingredients (containing 7% carvacrol, 6% thymol, 4% 1-8 cineol, 7% alpha-pinene, 0.03% carnolic acid, 3% Capsicum powder) when given to Korat slow – growing female chicken (KR). Chicken were fed practical corn-soybean meal diets with a single feed the first 3 weeks of age. After 3 weeks, female chickens were randomly allocated to 2 treatments, CT = control and ANT = control + 0.07% of antioxidant preparation. The performance, body weight, body weight gain, feed intake and feed conversion ratio were measured at the starter period (3-6 week) and finishing period (6-10 weeks). At the 10 weeks of age, 5 chickens per replicate were slaughtered and breast meat were collected to measure the drip loss, muscle color (L^* , a^* , b^*), pH values at 48 min and 24 hour, cooking loss and TBARS values. The results showed that the growth performance, muscle color and pH values were not affected in ANT birds ($P>0.05$) but cooking loss, TBARS and drip loss were significantly decreased (respectively -4.8%, -25.1% and -13.0% ; $P<0.05$) in ANT treatment. These results suggest that plant antioxidants may reduce meat lipid oxidation and retain the properties of meat proteins during processing. The reducing of water loss is the parameter to indicate the meat juiciness in which has been affecting to consumer acceptance.

Key words: antioxidant, plant extract, chicken, meat quality

ID : 183

UNDERSTANDING THE ROLE OF HISTIDINE DIPEPTIDES ON POST-MORTEM PH DECLINE IN BROILER BREAST AND LEG MUSCLES

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The rate and the extent of muscular post-mortem acidification influence several qualitative and technological properties of meat. Poultry muscles are highly abundant in histidine dipeptides (HD) such as anserine and carnosine, that possess a great buffering ability in vivo and thus might influence muscular acidification during early post-mortem, where muscle pH is still close to their action range. The aim of the study was exploring the role of HD on post-mortem metabolism in 3 chicken muscles having different energy metabolism and belonging to the main cut-ups (breast, thigh and drumstick), previously selected based on their ultimate pH and carnosine content as indicators of muscle glycolytic activity. For this purpose, 8 carcasses of broiler chickens (Ross 308 strain, females, 49 days of age, 2.8 kg body weight) were obtained from a commercial producer and samples from bone-in Pectoralis major (PM; breast), Extensor iliotibialis lateralis (EIL; thigh) and Gastrocnemius pars interna (GI; drumstick) muscles were collected at 15, 60, 120 and 1,440 min post-mortem, instantly frozen in liquid nitrogen and used to evaluate pH and glycolytic metabolites. At 1,440 min, buffering capacity and HD (anserine and carnosine) concentration were also assessed. Data were analyzed using One-Way ANOVA to test the effects of both sampling time and muscle type. According to their metabolic characteristics, selected muscles showed different acidification kinetics and carbohydrate metabolism. Indeed, if post-mortem glycolysis terminated at 120 min in both EIL and GI due to their lower glycolytic potential ($P < 0.05$), it continued in PM allowing the achievement of a significantly lower ultimate pH ($P < 0.05$) measured at 1,440 min. However, considering both its lactate production pattern and the existence of residual glycogen at 24 h post-mortem, PM should have shown a faster/greater pH decline. We suggest that the remarkably higher content of HD detected in breast muscle ($P < 0.001$) exerted an effect on post-mortem metabolism by reducing the rate and the extent of muscle acidification. Indeed, if compared to leg muscles, PM showed significantly higher buffering capacity in the pH range 6.0-7.0 ($P < 0.001$). In light of these outcomes, dietary strategies aimed at modulating the muscular content of histidine dipeptides could regulate post-mortem acidification kinetics, thus improving poultry meat quality traits (i.e. limit the incidence of PSE-like meats and improve microbial stability).

ID : 221

CAN ONE WEEK'S USE OF NETTLE IN BROILER FEED AFFECT THE CHEMICAL QUALITY OF MEAT?

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This experiment aimed to evaluate the effects of adding fresh or dry nettle (*Urtica dioica* L.) to broiler diets during the last week of fattening on proximate composition, fatty acid and mineral content of meat. The trial was performed on a total of 600 ROSS-308 unsexed day-old chickens, divided into 3 groups (C - Control group; FN - Fresh Nettle group; DN - Dry Nettle group). All broilers were bred and fed equally, with standard diets from age 1-42 days, except for the last seven days of fattening when in FN group was added daily to the feeders (on top) 20g/chicken freshly collected, blanched and chopped nettle, or in case of DN group of broilers, 10g/kg finely ground dry nettle mixed into a finisher. On day 42, random sample of 6 male and 6 female birds were taken from each group and slaughtered. Meat samples (breasts and drumsticks) were collected and the proximate composition analysis was conducted on each portion of each chicken (total of 72 samples). The fatty acid composition (Shimadzu GC-2014 with FID detector) and mineral analysis (Na, Mg, K, Ca, Mn, Fe, Cu, Zn and Se content measured by iCap Q) was conducted on each portion (breast and drumstick) of 6 chicken per group (3 male and 3 female). An analysis of variance and t-test (IBM SPSS Statistics 20 software) was performed. Meat fat content was significantly lower ($P=0.028$) in broilers fed with the addition of dry nettle compared with other two groups, which could have an impact on quality characteristics of meat, such as flavor and tenderness. The addition of fresh or dry nettle influenced meat ash content as well, which was significantly larger compared with C group ($P<0.001$). Total MUFA was highest in DN group (31.20%), while there were no significant differences between other two groups ($P<0.001$), mainly due to higher share of palmitoleic and oleic acid found in meat from DN group. The lowest amount of total PUFA (35.08%) and n-6 fatty acids (32.19%) was found in DN group ($P<0.001$), specifically significantly lower content of linoleic acid. Also, DN group have better n-6/n-3 ratio (11.83) compared to control (13.46, $P=0.036$), while there was no effect when fresh nettle was used. Analysis of minerals in meat showed significantly higher level of Na and Se in DN group, and Fe and Zn in FN group. Generally, research shows that extra addition of 1% dry nettle in broiler feed during the last seven days of fattening can result in different chemical, fatty acid and mineral composition of meat.

ID : 310

INTERACTION BETWEEN CHICKEN GENOTYPE AND FEEDING REGIMEN FOR THE CONTROL OF MEAT QUALITY TRAITS

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In poultry production is great interest to provide consumers with high quality and safe meat. Meat quality has been affected by variable factors of which genotype and feeding regime play an important role. There is a question if both factors interact in meat quality traits, therefore, the aim of the study was to evaluate interaction between chickens genotypes of variable growth intensity and feeding regime in physical and chemical meat parameters. In the study, meat quality was analysed in 120 chickens; fast- growing Ross 308, medium- growing JA 575 and slow- growing ISA Dual chickens. In each genotype, a half of chickens were fed ad libitum (AL) and the second half was restricted (R) from 14 to 21 days of age. During restriction chickens received 70 % ad libitum feeding. The meat quality was assessed in breasts of chicken`s slaughtered at live weight 2 kg, which Ross chickens reached at the age of 32 days, JA 757 45 days and ISA Dual 80 days. Results were processed by two-way analysis of variance with interaction of genotype and feeding regime. Meat pH, colour and cooking loss were significantly affected only by chicken genotype. Interaction of genotype and feeding regime was detected in texture ($P \leq 0.05$). The lowest texture was in restricted JA 757 (8.4 N), in Ross (AL 15.2 N vs. R 11.7 N) and JA 757 (AL 9.1 N vs. R 8.4 N) texture was significantly lower in in restricted groups, whereas, in ISA Dual in the ad libitum fed group (AL 9.8 N vs. R 11.3 N). In meat chemical composition, interactions were observed in dry matter ($P \leq 0.05$), ether extract ($P \leq 0.001$) and hydroxyproline ($P \leq 0.001$). On the other hand, crude protein was the highest ($P \leq 0.001$) in ISA Dual (AL 23.7 % and R 23.9 %). Based on the data of the present study we can assume that interactions of genotype and feeding regime are more important in meat nutritional value than in physical meat traits, and genotype more affected meat quality traits than feeding regime.

ID : 313

THE MEAT QUALITY OF MALES AND FEMALES OF FAST-, MEDIUM- AND SLOW-GROWING CHICKEN GENOTYPES

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The aim of the study was to evaluate the meat quality parameters of males and females of fast- (Ross 308), medium- (JA 757) and slow-growing (ISA Dual) chickens slaughtered at 2 kg of weight. Males and females of each genotype were fattened under identical conditions. At 2 kg live weight, 20 males and 20 females were slaughtered. For meat quality analysis, the samples of Pectoralis major (PM) were taken 24 hours post mortem. The fast-growing chickens reached 2 kg slaughter weight at 32 days of age, medium- at 35 days of age and slow-growing at 74 days of age. No effect of sex or interactions of genotype and sex were detected in monitored traits except of meat tenderness. The dressing out percentage and breast percentage were lower ($P \leq 0.001$) in ISA Dual compared to medium and fast growing chickens. From physical meat characteristics, the pH value was affected by genotype ($P \leq 0.001$) with lower values in JA 757 and ISA Dual compared to the Ross. All of meat colour characteristics (L^* , a^* and b^*) were influenced by genotype. The ISA Dual chickens had lighter ($P \leq 0.001$) and less intensive colour parameter a^* ($P \leq 0.001$), but more intensive colour parameter b^* ($P = 0.004$) in PM than both genotypes Ross and JA 757. The drip losses were not affected by any of tested parameter. However, cooking losses were higher ($P \leq 0.001$) in JA 757 than in other genotypes. The toughest meat was observed in cockerels of ISA Dual ($P \leq 0.001$) and the most tender in Ross of both sexes. It can be concluded that slow-growing chickens had lower dressing out percentage and breast yield and less intensive meat colour than fast- or medium-growing chickens.

ID : 573

DIETARY INCLUSION OF HEMP (CANNABIS SATIVA L) AND DILL SEED (ANETHUM GRAVEOLENS)
IMPROVES SHELF LIFE AND QUALITY OF BROILER MEAT

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A study was conducted with dietary inclusion of hemp (*Cannabis sativa* L.) and dill (*Anethum graveolens*) seed in varying combinations against antibiotic growth promoter in broiler diet with an objective to study their possible synergistic effect on meat quality of broiler chickens. 192 CARIBRO-VISHAL broiler chickens were grouped under completely randomized design into six dietary treatments for 42 days comprising varying combinations of hemp and dill seed in addition to the basal diet. Each treatment had four replicates with eight birds per replicate, viz. Diet T1 (Basal Diet), T2 (BD+0.2% HS), T3 (BD+0.2% HS+0.3 DS), T4 (BD+0.3% HS), T5 (BD+0.3% HS+0.3 DS) and T6 (BD+0.025% Bacitracin Methylene Disalicylate). At 42 days post-hatch, eight birds per treatment group were sacrificed to collect breast and thigh meat samples to study the quality of fresh and stored meat (30 days). The muscle lipid, cholesterol content, DPPH and ABTS+ activity, TBARS value and Free fatty acid was determined by using methods of AOAC (1990); Wybenge et al. (1970); Kato et al. (1988); Shirwaikar et al. (2006); Witte et al. (1970) and Koniecko (1979) respectively. Statistical significance was declared at $P < 0.05$ using the Tukey's multiple comparison test. Lipid content of thigh and cholesterol of both thigh and breast meat remained unchanged due to seed supplementation. Free fatty acid content in breast meat did not differ significantly, however, increased significantly at low inclusion levels (T3 & T2). TBARS value was significantly higher ($P < 0.001$) in both pre and post storage breast and thigh meat of control birds. ABTS+ ($P < 0.001$) radical scavenging activity was reduced significantly in fresh and stored meat. Lowest inhibition was witnessed in control (T1) under pre and post storage phases. DPPH activity increased significantly ($P < 0.05$) in fresh meat, however, remained comparable in all groups in post-storage conditions except for antibiotic control (T6). It is concluded here that, the dietary hemp and dill seed, especially in combination improved shelf life of broiler meat under cold storage without compromising the meat quality.

Keywords: Hemp, Dill, meat quality, shelf life, Broiler

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ID : 575

SYNTHETIC VITAMIN E AND A NATURAL ALTERNATIVE : COMPARISON ON BROILER ZOOTECHNICAL PERFORMANCE AND MEAT QUALITY

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Vitamin E (VE), found in most of raw materials used in poultry feed, is a biological antioxidant beneficial for farming under stressful environment such as high stocking densities, high temperature and immune suppression. It is also known for its interest in meat quality improvement. Synthetic VE is a mix of eight isomers of α -tocopheryl acetate with variable bioavailability. Natural VE is more complex and is composed of tocopherols, tocotrienols under 4 forms, α , β , γ and δ . Natural alternatives such as Herbal E, a polyherbal mixture composed of *Ocimum Sanctum*, *Phyllanthus emblica*, *Emblica officinalis* are interesting because of their biological antioxidant activity. The purpose of this experiment was to compare the impact of synthetic VE and Herbal E on broiler zootechnical performance and meat quality.

The experiment was conducted with 400 broilers divided in 4 groups of 10 repeats of 10 Ross 308 males from day 21 to 42. The treatments were: T1: Negative Control; T2: 30 ppm of synthetic vitamin E; T3: 30 ppm of Herbal E; T4: 15ppm of synthetic vitamin E and 15ppm of Herbal E. Mortality and individual weight were measured at days 21, 32, 42. Feed consumption and feed conversion ratio (FCR) were measured for periods 21-32 and 32-42 days. Meat quality, pH, colour and drip loss, were evaluated at day 42. Fixed effects were tested using ANOVA, SAS software.

There was no effect ($p > 0.05$) of treatment on average body weight at day 32 and 42. During period 21-32 days, FCR was higher ($p < 0.001$) for T1 and T2 (1.58 and 1.59, respectively) vs T3 and T4 (1.53 and 1.55, respectively). This difference was not significant ($p > 0.05$) for the period 32-42 days but remained significant ($p < 0.001$) during the whole period 21-42 days for T1 and T2 group (1.72 and 1.74, respectively) vs T3 and T4 groups (1.69 for both). There were not differences ($p > 0.05$) in carcass and breast yield, meat pH and colour between treatments. There was a reduction ($p < 0.001$) of drip loss in filets of groups T3 and T4 (1.07 and 1.12%, respectively) vs T1 and T2 (1.41 and 1.29%, respectively).

Vitamin E had no effect on zootechnical performance and meat quality under the conditions of this experiment but broilers fed diets supplemented with the natural alternative improved both FCR and filet drip loss. Polyherbal mixture of plants known for their biological antioxidant activity can be an interesting alternative to VE for finishing broilers.

ID : 596

MANIPULATION OF LIGHT INTENSITY IMPACT ON GROWTH, MEAT QUALITY, BLOOD PARAMETER AND BEHAVIOR OF BROILERS

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The aim of this study was to investigate the influence of different light intensities on the growth performance, meat quality, blood parameters and behavior of broilers raised to 42 days of age. A total of 1280 one-day-old broiler chicks were randomly assigned to eight treatment groups based on light intensity in 32-floor pens of 2.86 m² per pen. Each light intensity treatment had four replicates of forty chicks each. The light intensity treatments were as follows: 30-30-30; 30-30-20; 30-20-20; 30-20-10; 20-20-20; 20-20-10; 20-10-10; and 20-10-5 lx for the pre-starter (1 to 11 d), starter (12 to 25 d) and grower (26 to 42 d) period, respectively. Experimental birds were fed ad-libitum commercial mash diets during respective rearing period and given free access water. Data were subjected to statistical analysis using the GLM procedure of SAS software (SAS 9.1, 2009) in a completely randomized design. The results of the study showed that weight gain was differed by the light intensity treatments over the experimental period from 1 to 42 days of age and higher was in 20-10-10 lx intensity group (3050.71 g; p

ID : 672

SKIN COLOUR PREDICTION IN BROILER ACCORDING TO THE SEX AND CAROTENOID IN THE DIET

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A total of 1080 one-day-old males and 1080 one-day-old females, both ROSS 308, were randomly divided into 3 groups with 6 replicates (cages) of 60 animals each from the same sex. All were fed with 34 ppm of natural xanthophylls (mainly lutein + zeaxanthin) in the starter phase (1 to 14d). In the grower phase (15 to 41 d), all were fed with 5 ppm of canthaxanthine, but three different pigments were added: 68 ppm of lutein (natural pigment, group A), 34 ppm of apo-carotene-ester (synthetic pigment, group B) and 68 ppm of natural xanthophylls (group C). At days 14 and 28, both at the farm, and at day 41 at the abattoir, the colour of the skin was measured with a Minolta CM 600d in the CIEL*a*b* space in 5 chickens per cage, in two regions: underneath the wing and at the tibiotarsal joint in the thigh. Analyses of variance were used through a GLM model with sex and diet as fixed effects, and regression equations were calculated per each treatment. At 14 days, no differences were found due to the diet, but females showed higher redness in both measured areas. Since the amount of xanthophylls was identical for each gender, we can assume that differences in fatness in subcutaneous fat were responsible for colour differences at this early age. At 28 days of age, no differences were found at the tibiotarsal joint, but underneath the wing females showed higher yellowness (13.02 vs 11.29, $P<0.01$) with higher saturation (13.34 vs 11.62, $P<0.01$) than males. Also group A and C diets showed higher yellowness and saturation underneath the wing than group B diet ($P<0.01$). At the end of the trial, females had the skin underneath the wing more yellow (28.74 vs 25.31, $P=0.001$) and with higher saturation than males, whereas males showed lighter skin (69.20 vs 70.39, $P=0.001$) and slightly redder ($P<0.05$) than females at the tibiotarsal joint. The lower amount of carotenoid in group B showed lower colour indexes in both measuring areas. However, although with similar amount of carotenoid in the diet, group C showed similar colour indexes than group A underneath the wing, but higher redness ($P<0.01$), yellowness and saturation ($P=0.60$) than underneath the wing ($R^2<0.35$), better in females than in males. Quadratic predictions of yellowness are slightly better than linear predictions, very similar in both sexes and measuring areas in diets A ($R^2=0.83$) and C ($R^2=0.86$), and more variable in diet B ($R^2=0.64-0.79$).

ID : 673

BROILER BREAST COLOUR SHELF LIFE ACCORDING TO THE SEX AND CAROTENOID IN THE DIET

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A total of 1080 one-day-old males and 1080 one-day-old females, both ROSS 308, were randomly divided into 3 groups with 6 replicates (cages) of 60 animals each from the same sex. All were fed with 34 ppm of natural xanthophylls (lutein + zeaxanthin) in the starter phase (1 to 14d). In the grower phase (15 to 41 d) all were fed with 5 ppm of canthaxanthine but three different pigments were added: 68 ppm of lutein (natural pigment from Marigold, group A), 34 ppm of apo-carotene-ester (synthetic pigment, group B) and 68 ppm of natural xanthophylls (lutein + zeaxanthin, group C). At 24h after slaughter, and 2d and 6d after display with O₂ permeable film at 4°C in the dark, colour was measured in the external surface and sliced breast, with a Minolta CM 600d in the CIEL*a*b* space in 15 chickens per treatment. Analyses of variance were used through a GLM model with diet and display as fixed effects within sex.

Diet highly influenced colour variables in females, but did not affect lightness in males. In the sliced breast, group C showed higher yellowness (25.55, 25.90) and saturation (21.97, 21.79) in both males and females respectively, but lower in the intact breast in males (21.66, 19.44, respectively), than in groups A and B. Group B had the lowest values of yellowness, especially in females in the sliced breast (18.70). Display had a large effect in males, but did not affect redness in females. Throughout display, meat was darker, with less yellowness, Chroma and Hue. The decrease was especially evident in males, which started with higher values than females in yellowness (26.90 vs 23.40) and Chroma (27.21 vs 23.79), but after 2 days of display colour was already less intense, whereas females did not show a significant decrease between 24h after slaughter and 2 days of display in the sliced breast, keeping better the colour. After 6 days of display, the lost in yellowness and Chroma was very intense, especially in males (21.56, 10.62, respectively). The studied pigments at the used doses behave differently according to the sex, although group C with natural xanthophylls shows more intense colour than the rest of groups. Both intact and sliced breast lose colour intensity throughout display, more evident in males than in females.

ID : 737

THE USE OF TURMERIC (*CURCUMA DOMESTICA*) AND PAPAYA LEAVES (*CARICA PAPAYA* L.) POWDER MEAL AS FEED ADDITIVE IN THE BASAL DIETS OF BROILER CHICKENS TO IMPROVE THEIR MEAT QUALITY

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An experiment was conducted to determine the use of turmeric (*Curcuma domestica*) and papaya leaves (*Carica papaya* L.) powder meal as feed additive in the basal diets of broiler chickens to improve their meat quality. A total of 100 day old chicks were divided into four groups, then sub-divided into five replicates of five birds/treatment. The turmeric powder and papaya leaves powder were not added into the dietary treatment groups as T0 (only basal diet as control), T1(basal diet+0.5% turmeric powder), T2 (basal diet+2.5% papaya leaves powder), and T3 (basal diet+0.5% turmeric powder+2.5% papaya leaves powder). The meat quality traits of broiler chickens measured were water content, protein content, fat content, tenderness and cooking loss of broiler meat. Data on broiler chicken moisture content were 76 to 78% within the range of normal poultry meat of 60 to 80% water (Florkowski et al., 2002). The results of protein content within the range of normal poultry meat of 15 to 25% protein (Florkowski et al., 2002). The fat content is higher than 1.5 to 5.3% lipids reported by Florkowski et al. (2002). The tenderness of broiler meat were extremely tender of (<3.3 kg/cm²; Suryati et al., 2008). The cooking loss of meat were 30.34 to 34.01% within the range of normal cooking loss of broiler meat of 15.55 to 36.0% (Rehman et al., 2012). In conclusion, the addition of turmeric and papaya leaf powder as feed additives in the basal diets of broiler chickens affected the content of protein, fat, and meat tenderness. The addition of papaya leaves powder alone can increase their protein content, water and tenderness while reduce their fat content and produced the lowest water content in broiler chicken meat.

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ID : 835

USE OF ALLYL-ISOTHIOCYANATE AND CARVACROL TO PRESERVE FRESH CHICKEN MEAT DURING CHILLING STORAGE

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The effect of active compounds (ACs); Allyl-isothiocyanate (AITC) and carvacrol (CARV), as natural additives on the quality of fresh chicken meat was evaluated. The meat was treated with 500, 1000-ppm of ACs vacuum packaged and stored at 4 °C up to 8 days. Samples were taken at different time intervals for different analyses on day 1, 3, 6 and 8. Physicochemical characteristics: pH, colour values (L^* , a^* and b^* , Chroma and hue angle), lipid oxidation (thiobarbituric acid reactive substances-TBARS), microbiological evaluation: in vitro three Gram-positive (*Listeria monocytogenes* CCM, *Staphylococcus aureus* ATCC and *Bacillus cereus*) and three Gram-negative (*Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Pseudomonas lundensis*) were used as target bacteria, in meat model aerobic mesophilic counts-AMCs were tested, sensorial properties (Electronic-nose based) were examined. AITC, particularly 1000-ppm, showed greater activity than CARV and resulted in cumulative colour changes higher and increasing L^* , b^* and h^* , decreasing a^* values, accumulative odour production. Besides AITC triggered reduction in the growth of *Pseudomonas lundensis*, *Staphylococcus aureus*, and *Bacillus cereus* and 3 log₁₀ CFU/g reduction in AMCs. However, CARV was more active in increasing chroma properties and reducing the growth of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium. Concomitantly, 500-ppm CARV showed greater activity than AITC in controlling lipid oxidation, producing higher level of water holding capacity and protecting the colour changes. The knowledge of properties and mode of inhibition of AITC and CARV, may contribute the great potential of these natural preservatives to preserve the quality characteristics and extend the shelf life of meat and meat products.

ID : 906

INVESTIGATION OF THE RELATIONSHIP BETWEEN PH AND COLOUR OF CHICKEN BREAST MEAT AND LIPID PEROXIDATION PARAMETERS BY FEEDING COCONUT, PALM AND SUNFLOWER OIL

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Our aim was to measure the effect of coconut, palm and sunflower oils on lipid peroxidation and meat quality parameters and to investigate the correlation between these values.

Cobb500 cocks (n=245) were divided into five experimental groups: C (control), CO (5% coconut oil), PO (5% palm oil), COPO (2.5-2.5% coconut+palm oil), SO (5% sunflower oil). After the fattening period (42 days), the pH of the breast meat was measured 45 minutes (pH45) and 24 hours (pH24) after slaughter. The colour (CIELab L*a*b*) was detected on fresh surface (n=40). Lipid peroxidation parameters (reduced glutathione-GSH and malondialdehyde-MDA content, and glutathione peroxidase activity-GPx) were determined in blood plasma, red blood cell hemolysate and liver. Significant correlations were found in plasma: a*-MDA (r=0.3306; p=0.0372); b*-MDA (r=-0.4019; p=0.0102) and in liver: b*-GSH (r=0.3575; p=0.0235); pH45-GSH (r=0.3216; p=0.043); pH45-GPx (r=0.4043; p=0.0097); b*-MDA (r=0.3295; p=0.0379) according to the complete dataset. The correlations were also performed separately to analyse the correlation differences of the four experimental groups.

In the liver samples of SO group, pH45-GSH (r=-0.2443; p=0.5598) and pH45-GPx (r=-0.1891; p=0.6538) parameters were contradictory, suggesting that the addition of sunflower oil may have influenced the metabolism in the liver compared to other oils containing moderately lower level of long-chain unsaturated fatty acids.

In plasma, a* value was positively correlated with MDA in each group except the control, which is believed to be influenced by the additional oil. The b* value was also associated with MDA negatively in plasma and with GSH and MDA positively in liver which was reinforced by the addition of coconut oil.

Further studies are needed to clarify the knowledge about these relationships.

ID : 1123

THE COMPONENTS OF VOLATILE FLAVOR COMPOUNDS IN CHICKEN BREAST MEAT AND THEIR RELATED GENES

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Background

The quality of food flavor will affect the choice of consumers. In different foods, the source of flavor is also different. The phenotype of flavor character is composed of different volatile compounds. The formation of flavor substances in meat products mainly depends on Maillard reaction, lipid oxidation and degradation and their interaction. The volatile components degraded by esters include aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids and esters. Therefore, in order to determine the main volatile compounds that affect the flavor of chicken breast meat, the relative content of flavor compounds was determined by GC-MS. Through genome-wide association analysis (GWAS), the possible genes related to flavor were screened to provide molecular reference for the analysis of complex flavor characters.

Materials

Population: Jingxing (DW, n = 520) population, Qingyuan Ma (n = 400) population and Wenchang (n = 53) population

Determination of flavor substances: GC-MS

GWAS model:

α : PCA and batch effect (fixed effect)

β : marker effect (fixed effect)

ξ : polygene effect (random effect)

The likelihood ratio test (LRT) was used to test the significance of each point effect.

Results

Hexanal and 1-octen-3-ol are the main volatile components in chicken breasts through Principal component analysis (PCA). Correlation analysis results show that hexanal and 1-octen-3-ol have a significant negative correlation. The relative contents of hexanal and 1-octene-3-ol were used as phenotypic data, and the genotype of DW population was used for GWAS analysis. In the significant association region located by hexanal and 1-octen-3-ol, the predicted candidate genes are LPP and BCL6 genes.

Discussion

Reports show that 1-octen-3-ol and hexanal can change the taste of dry-cured ham, which means that 1-octen-3-ol and hexanal are the main volatile components, and their contribution to the flavor cannot be ignored. As their candidate genes, LPP and BCL6 genes provide a possible range for the study of the genetic mechanism of flavor.

Keywords: Chicken; Volatile flavor compounds; GC-MS; Metabolomics

ID : 1154

IDENTIFICATION OF MAJOR GENES FOR INTRAMUSCULAR FAT BY A GENOME-WIDE ASSOCIATION STUDY IN CHICKEN

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Introduction: Chicken, known for the exquisite and delicious meat quality, is the important animal protein to the human as the second largest meat product after pork. Intramuscular fat is a quantitative trait collectively controlled by minor-major genes and has a great contribution to meat color, tenderness and flavor meat quality. To date, the genetic mechanism of IMF metabolism and many functional genes had been reported. However, the systematic screening of major gene on IMF deposition of chicken is yet not clear.

Experimental design and methods: In this study, the excellent Jingxing-yellow (JX-Y) chicken population with 16 generations selection of IMF content, and the unselected natural population as the control, were used to systematically elucidate the genetic basis of IMF metabolism by a genome-wide association study (n = 516).

Results: IMF was a complex of lipids, we found that triglycerides (TG) was the main component of IMF with the high positive genetic and phenotypic correlations, so TG could replace IMF for follow-up analysis. As expected, we excavated 131 SNPs associated with TG, and these 131 SNPs were located in the inside of LIN52 (Chr 5) and in the upstream of SLC16A7 (Chr 1). Excitingly, the results by XP-EHH analysis revealed that LIN52 was selected in used two population. And then, a big data of functional validation and the clarification of regulatory mechanism of SNP had supported that SLC16A7 was the major gene on TG biosynthesis in pectoralis of chicken. Meanwhile, our results by XP-EHH analysis revealed that the SNPs in the upstream of SLC16A7 (Chr 1) also were selected in used two population. The further structural analysis of variable region and functional verification of SLC16A7 confirmed that SLC16A was another major gene on TG biosynthesis in pectoralis of chicken.

Conclusions: In summary, we identify two major genes (LIN52 and SLC16A7) on TG biosynthesis in pectoralis of chicken, and our findings will help to understanding and perfect the underlying molecular regulatory mechanisms of IMF deposition in chickens.

ID : 1216

EFFECT OF DIFFERENT SOURCES AND LEVELS OF SELENIUM ON SLAUGHTER ANALYSIS AND MEAT QUALITY OF BROILER CHICKENS

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Selenium (Se) is an essential mineral that has antioxidant properties in poultry. The supplementation diets with Se is required because of the wide variation of Se concentration in feedstuff. The objective of this study was to evaluate the effects of dietary levels of inorganic and organic Se, salinized yeast and nano form of selenium (nano-Se) on slaughter analysis and meat physicochemical characteristics of broilers chickens.

A total of 300 d-old male broilers Ross 308 were divided into 4 experimental groups in 5 replication of 75 birds each. Birds were fed following treatments: (i) standard commercial diet containing inorganic Se at the level of 0.3 mg/kg diet (C), (ii) diet with the increased level of inorganic Se (0.5 mg/kg diet), (iii) as in treatment (ii) but with salinized yeast as Se source, and (iv) as (ii) but nano-Se was used as a Se source. At 42 day of life, 10 birds were selected from each group, and dissected to achieve breast muscle samples for analyses. The pH₂₄ value and water holding capacity (WHC) were replicated three times, and the mean value was reported. Color parameters (a^* , b^* , L^*) were analyzed in minced meat while, the drip loss was computed as the percentage ratio of meat weight after and before storage. The analysis of variance (ANOVA) was applied to determine the effect of diet supplementation with the selenium on slaughter analysis and broiler meat quality.

The dietary treatments significantly affected slaughter yield of birds. Both, the highest slaughter yield and the highest yield of breast muscle was found in birds fed diet with nano-Se while, the lower yields of these cuts was in birds fed diet with increased level of inorganic Se ($P < 0.01$). Legs meat share was the highest in groups fed diet with salinized yeast whereas, the lower one was found in treatment with increased level of inorganic Se ($P < 0.01$). This group was also characterized by the highest relative weight of heart, liver and gizzard but the lowest weight of adipose fat comparing to other groups ($P < 0.05$). Feeding diets of increased level of inorganic selenium affected color of the breast meat (the highest L^* value) and WHC of the breast meat (the lowest level) comparing to remaining groups ($P < 0.05$).

Our data indicated that feeding birds diet containing nano-Se at the level of 0.5 mg/diet throughout the 42-days feeding period did not compromised quality indices of the meat.

This work was supported by the National Science Centre, Grant No. 2018/29/B/NZ9/01351

ID : 1280

EFFECT OF AFLATOXIN ON PERFORMANCE AND CERTAIN MEAT QUALITY PARAMETERS IN BROILER CHICKEN

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Mycotoxins can cause production losses and health risks both for livestock and human. Aflatoxin is considered to be the most hazardous one as it has carcinogenic property and it might be excreted in animal products (i.e. milk and egg). Due to climate change, nowadays, aflatoxin producing *Aspergilli* intriguing troubles for poultry farmers even in the temperate regions.

In our study effects of aflatoxin contaminated diet on performance and certain physico-chemical quality parameters of meat were investigated.

Three groups of Cobb 500 cockerels (n=600) were created (5 replicates each). Control birds (T1) were fed with three-phase commercial broiler diets. Birds in the treatment groups were fed with control diets contaminated with 0,1 mg AFB1/kg (quintuple of limit value 0.02 mg/kg (574/2011 EC)) between 1-21 or 21-42 days of age in Group T2 and T3, respectively. Live weight, feed consumption and feed efficiency were analysed in each group. At Day 42 birds were culled and meat productivity was evaluated and breast fillets were taken from 30 birds/group for further analysis. Colour, drip loss, texture, kitchen loss were analysed in breast samples.

Feed intake in Group T2 was significantly reduced on the first week of aflatoxin load, but from Day 21 birds started to compensate the initial low consumption. As a result, live weight in Group T2 was significantly lower than in the control, but became similar by culling. Feed refusal caused by aflatoxin was confirmed also in Group T3 as from the start of aflatoxin load feed intake dropped markedly. However, weight of birds in Group 3 was notably different from the control only on Day 35. Altogether, feed efficiency was debased in both treatment groups.

Proportion of primary cuts was reduced mainly in Group T2. Considering meat quality the most remarkable changes were found in the colour as the breast samples in Group T3 were lighter and yellower, which resulted in visible difference, as well. However, statistically significant difference was revealed also in water holding capacity, as drip loss was notably higher of the breast samples in Group T2 than in the control (T1).

Altogether, aflatoxin has harmful effect both on performance and on certain quality traits of meat (water holding capacity), and these are more prevalent, when contamination occurs in the initial period of fattening.

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ID : 1309

PHYSICOCHEMICAL TRAITS AND FATTY ACID PROFILE IN BREAST MUSCLES OF CHICKENS KEPT IN A SMALL-SCALE FARM, FED WITH FABA BEAN AND RAPESEED MEAL

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The possibility of using other protein feeds than soybean meal raises the importance of conducting research and seeking solutions to produce good quality broiler meat in small farms in EU based on own feed. The aim of the study was to compare the fatty acid profile in the breast muscles of chickens fed with feed with faba bean and rapeseed meal as a high-protein replacement for soybean meal. ROSS 308 chickens were divided into 2 groups (170 birds/group). In the control group (I), nutrition was based on soybean meal. Experimental group (II) was fed with faba bean and rapeseed meal. After 6 weeks of rearing, 10 randomly selected chickens from each group were slaughtered. The breast muscles (BM) were collected, weighed, and the color of the meat was assessed with a colorimeter (Konica Minolta) in the CIE system (1986) (L^* , a^* , b^*), water-holding capacity (Grau and Hamm, 1952) and the fatty acid profile was determined. Lipid extraction from BM was performed (Folch et al., 1957). Fatty acids (FA) were quantified as methyl esters (FAME) using a GC Trace 2000 gas chromatograph (ThermoQuest EC). Results are expressed as a percentage of total FAs identified. To assess the nutritional implications, the ratio of n-6 to n-3 FA ($n-6 / n-3$) and the ratio PUFA to SFA (P/S) were calculated. Atherosclerotic index (AI) and thrombogenic index (TI) were calculated (Ulbricht and Southgate, 1991). Numerical data were analyzed in the Statistica 10.0 (2011). Body weight before slaughter and carcass weight were significantly higher in group I ($p < 0.05$). A significantly higher proportion of C16: 0 (palmitic), C22: 4 n-6 (docosatetraenoic) acids was found in group II ($P < 0.05$). In group II, a significant increase in the ratio of n-6 to n-3 acids ($p < 0.05$) was found. The results indicate no negative impact of the use of feed with faba bean and rapeseed meal in chicken feed, except for an increase in C16:0 content and a positive increase in C22:4n6, which belongs to EFA, which allows to state that the use of replacement soybean meal may be recommended for rearing broiler chickens.

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MEAT PROCESSING

ID : 630

SENSORY SCORES AND MICROBIAL COUNTS OF KILISHI-LIKE CHICKEN JERKY MADE WITH THREE SLURRY TYPES

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Introduction

Production of kilishi-like product from chicken in this region increase demand for chicken and help to stabilize income for poultry farmers who are often compelled to sell their produce at competitively low prices during periods of glut. The quality acceptability of Kilishi is affected by the nature of slurry used (Ogbonna and Linus, 2009; Mgbemere et al., 2011). This study assessed the effect three slurry types; groundnut paste, melon paste and soya bean residue (Okara) on sensory qualities and microbial status of kilishi-like jerk from chicken after 12 weeks of storage.

Materials and Methods

Thinly sliced chicken meat from breast and thigh muscles were broiled for 2 hours at 60 °C, and then infused for 15 min in a slurry mixture containing either groundnut cake, melon cake or okara (280g) blended with water (1 L), onion (100g), garlic (25g), ginger (50g), ground pepper (20g), 2 Maggi® cubes, granulated sugar (20g), Yaji (kilishi pepper). The samples were then oven dried.. The products were cooled to room temperature and hygienically packed in airtight polyethylene bags and stored on the shelf. After 12 weeks, they were assessed by a trained sensory panel using a 9 point category hedonic scale. 1 g of jerky from slurry type was dropped in a test tube containing 9 ml of distilled water, shaken vigorously and then allowed to settle for 5 min. The resulting solution was used to inoculate six media namely; Mackonkey, CLED, SSA, Sabourand desxtrose, chocolate blood and blood agar and the set up were incubated for 24 hours. The data were subjected to analysis of variance in a completely randomized design. Significantly different means were separated using LSD.

Results and Conclusion

No significant differences ($p < 0.05$) were found for all the sensory parameters evaluated. The overall hedonic rating for the product types were very high (7.44 to 8.44) and no significant microbial growth was observed in all the cultures. It is therefore concluded it is feasible to produce Kilishi-like jerky from chicken using defatted groundnut paste, melon or okara as slurry materials and the product is safe to consume and will be stable after 12 weeks of storage under ambient conditions.

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ID : 687

COMPARISON OF THREE DIFFERENT METHODS USED FOR ESTIMATING COOK LOSS IN BROILER BREAST MEAT

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The objective of this study was to evaluate the different methods used for estimating moisture loss in cooked broiler breast meat (pectoralis major). Two experiments were conducted. In the first experiment, both intact breast meat and cored meat samples were used to compare three different methods (5 min in room temperature, 5-min; cooling down to ambient temperature RM; or chilled down to room temperature in ice water, IW) for estimation of cook loss in broiler breast meat. In the second experiment, cut meat samples were used to compare the effect of the endpoint temperature (23, 40, 53, 57, 68, 75, or 90 °C) on the cool loss estimation using the 5-min and RT methods. Chicken breast meat were collected from local chicken processing plants and trimmed, cored, or cut into similar size/weight prior to cooking. Moisture loss after cooling, moisture retention, and total moisture loss after 24 h in cooked broiler breast meat were measured for comparison. Data showed that there were no significant differences ($P > 0.05$) between 5-min and RT methods in the cook loss (19-21%) and total loss (21-25%) regardless of coring. However, the cook loss ($< 17\%$) and total loss (19.3%) of the IW method was significantly lower ($P < 0.05$) than those of the other two methods in whole breast meat samples but there were no significant difference in the cored samples (23.2%). When the endpoint temperature was $\leq 40^{\circ}\text{C}$ or $\geq 75^{\circ}\text{C}$, no moisture accumulation was observed in packages between 5-min and RT method after 24 h. However, when the temperatures were ≥ 53 and $< 75^{\circ}\text{C}$, cook loss estimations were significantly different ($P < 0.05$) between these two methods (more than 4%), with reducing the endpoint temperature resulting in increased differences (from less than 5% to more than 9%). These results demonstrate that the cook-loss estimating methods may affect moisture loss results in cooked broiler breast meat and the differences in cook loss results between estimating methods could be affected by the final endpoint temperature.

ID : 722

SENSORY CHARACTERISTICS, PROXIMATE AND MICROBIAL COUNTS OF KILISHI LIKE JERKY FROM CHICKEN

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Introduction and Objective

In many sub-Saharan African countries, the demand for Kilishi – a shelf stable, intermediate moisture product that is prepared from thin flat beef slices infused in slurry made from a mixture of defatted groundnut paste and spices, is on the increase (Mgbemere et al., 2011). Successful production of kilishi-like product from chicken will help to stabilize farm income for poultry farmers who are often forced to sell at abysmally low prices due to poor marketing arrangements and in seasons of glut. This study evaluated the feasibility of producing a kilishi-like jerky (meat crackers) from chicken using oven drying method.

Material and Methods

Meat crackers were produced from chicken and beef following the usual procedures for Kilishi production from beef (Ogbonna and Linus, 2009; Igene et al., 1990) except that charcoal oven was used in place of sun drying. The samples were subjected to sensory evaluation on day of production and after 7 days of storage. Proximate analysis and total plate count were also conducted on 7 day. The data generated was subjected to t-test at 0.05 and 0.01 significance levels.

Results

The panellists preferred meat jerky from chicken to beef on the day of production, but no significant differences ($p < 0.05$) were found in the degree of likeness, flavour, texture and off-flavour intensity after 7 days of storage. Meat jerky from chicken had significantly higher moisture (34.21%) and Nitrogen Free Extract content (17.73%) but lower protein (42.65%) and fat (2.30) contents when compared to those from beef (21.05, 13.03, 51.02 and 10.19 respectively). The mean bacterial load of the chicken jerky was 3.501×10^5 (E. coli/ml) was higher ($p < 0.05$) than those jerky from for the beef jerky samples (1.159×10^5 E. CFU/ml).

Conclusion

It is feasible to produce a kilishi-like product from broiler chicken meat using the oven drying method. The product was well accepted to consumers, had sensory scores similar to traditional kilishi from beef and the microbial load are within the recommended safety levels for human food.

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ID : 872

ASSESSMENT OF MICROBIOLOGICAL QUALITY OF GRILLED CHICKEN MEAT AND SPICES ACCORDING TO PROCESSING METHOD IN SOUTH OF BENIN REPUBLIC

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Grilled chicken meat are well appreciated by consumers. However, grilling and sale conditions may affect quality grilled chicken meat (Djoulde et al., 2015; Alonge et al., 2017; Somda et al., 2018). This study aimed to assess microbiological quality of grilled chicken meat sold in Benin. A total of 65 samples of grilled chicken meat from different grilling processes at Cotonou and Abomey-Calavi (the two biggest cities) in Benin were collected respectively after grilling and 2h after exposure for selling. A total of 35 spices used for grilled chicken meat consumption were also collected. They were analyzed for microbiological quality according to ISO standards methods. Data were analyzed by student T test and ANOVA one-way followed by tukey post test using GraphPad 5. Results showed that total plate count of all chicken grilled kinds exceeded maximum limits of 3×10^6 cfu/g fixed by EU regulation (n°2073/2005) except gaz-grilled chicken meat ($1.47 \times 10^6 \pm 6.26 \times 10^5$ cfu/g). Total coliforms, E. coli, ASR, yeast and mold respected regulation's limits. As well, fecal coliforms exceeded limits in some grilled chicken meat. Microbial loads did not change significantly within 2 hours of exposure during selling. Spices collected from barrel and charcoal cabinet kiln grilling processors were higher in total plate count exceeding limits, contrary to gaz-cabinet and local chicken grilling processors. Spices collected for gaz-grilled chicken meat consumption contained lowest load of total ($7.7 \times 10^1 \pm 3 \times 10^1$ cfu/g) and fecal ($7.1 \times 10^1 \pm 6.8 \times 10^1$ cfu/g) coliforms, and spices used for local chicken grilling recorded highest loads. E.coli, ASR, mold and yeast loads were under maximum limit. Staphylococcus aureus and salmonella were not found in all samples of grilled chicken meat and spices analyzed. In conclusion, this study suggested that efforts should be made by processors for grilling and selling chicken meat in hygienic conditions.

ID : 873

DIFFERENCES IN TEXTURE ANALYSES OF CHICKEN BREAST FILLETS AFFECTED BY SEVERE WOODEN BREAST AND SPAGHETTI MEAT MYOPATHIES

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Texture of broiler meat is one of the most important attributes for consumers, and it can be affected by breast meat myopathies, such as wooden breast (WB) and spaghetti meat (SM). However, differences in textural measurements between samples can vary by the test applied, meat preparation (raw vs. cooked), and severity of myopathy. Thus, this study employed three tests: compression, Meullenet-Owens razor (MORS), and Allo-Kramer (AK) - to compare normal, WB, and SM fillets. A total of 209 fillets (68 normal, 71 WB, 70 SM) were selected from 3 flocks, at a large commercial processing plant, 3 h after slaughter. The fillets were analyzed for dry matter, crude protein, and fat contents (9 per meat type) as well as pH plus color, and then frozen for later texture analyses (181 fillets; 59 normal, 61 WB, 61 SM). Thawed fillets were submitted to: compression test (raw and cooked), and later cooked samples to the MORS test (blade: 0.5 mm thick, 8.9 mm wide, and the AK test (5 blades, 2 mm blade thickness). Data were analyzed by a mixed model with myopathy and flock as the main effects (SAS, 2013). Normal meat had lower fat content than WB meat (0.91% vs. 1.54%; $P<0.05$) with intermediate values for SM (1.25%), whereas protein content was higher in normal meat (23.9% vs. 22.3% and 22.7% in WB and SM; $P<0.01$); similar to previous results. Normal breasts were lighter (188 vs. 230 and 262 g in WB and SM; $P<0.01$), had lower L^* and a^* values than abnormal meat, and lower cooking losses (22.4% vs. 27.8% and 26.9% in WB and SM; $P<0.001$). Raw, normal and SM meat showed lower compression force (5.61 and 4.69 vs. 9.52 N), work (25 and 22 vs. 45 N x mm) and Young's modulus (2.71 and 2.11 vs. 4.29 N/sec) than WB ($P<0.001$); the same pattern was observed in cooked meat. For the MORS test, SM showed lower shear force (12.8 vs. 14.7 N), work (249 vs. 288 N x mm) and fewer peaks (5.39 vs. 7.57) than normal meat ($P<0.01$), whereas WB had intermediate values. For the AK test, SM showed lower force (10.5 vs. 14.5 N) and Young's modulus (31.0 vs. 46.0 N/sec) than WB ($P<0.01$), whereas normal meat had intermediate values. Overall, results revealed that texture tests show different sensitivity with respect to meat preparation and type. The compression test was useful to identify WB even in raw meat as well as in cooked meat. In cooked samples, MORS distinguished SM from normal fillets, whereas the AK test identified SM as different from WB.

ID : 942

BACTERIAL MICROFLORA IN POULTRY GROUND MEAT TREATED WITH CARNOSINE AND COLD PLASMA WERE ASSESSED BY SINGLE CARBON UTILIZATION

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Introduction: Poultry meat contains abundant nutrients for human health and also harbors many potential spoilage and foodborne microorganisms. To provide safer food for human consumption, many reagents and technologies have been applied to reduce and/or eliminate these microorganisms in food matrices. However, bacterial communities in food before and after treatments with cold plasma, carnosine or both have not been extensively investigated. In this communication, the bacterial communities in poultry meat samples after various treatments were assessed with EcoPlates™.

Experiment Design: After treatment with cold plasma and carnosine, the ground meats were diluted in PBS, and the supernatants were dispensed into 96-well EcoPlates™. The plates were incubated at 25 °C for seven days in an OmniLog® chamber. Bacterial growth in the plates was monitored on formazan production every 30 minutes at OD590 nm. The OD readings of the chemicals were adjusted by the subtraction from the average of the OD values of the water blank in the plates. Means of the resulting OD values for each chemical compound were calculated. The SAS software package (version 9.4) was used to determine the bacterial growth curves based on three-parameter Gompertz growth algorithm. The PAST software package (version 3.22) was used to calculate principal coordinates analysis based on the variance-covariance matrix. GraphPad Prism® 8 (GraphPad Software) was used for plotting the bacterial growth curves.

Results: The kinetics of bacterial growth patterns were well fitted a sigmoidal Gompertz curve with the lag, log and stationary phases, but with different inflection times and asymptotes at the log phase and the stationary phase, respectively. The cold plasma technology and carnosine could inactivate bacterial growth in treated meat samples. Among 31 chemicals tested, seven chemicals could not be used by bacteria in meat samples as the carbon source: phenylethylamine, alpha-D-lactose, D,L-alpha-glycerol phosphate, 2-hydroxybenzoic acid, gamma-hydroxybutyric acid, alpha-ketobutyric acid and D-malic acid.

Conclusions: Whether the chemicals that inhibit growth of bacteria from the samples can be used as food preservatives is required for further investigations. Further, whether the profiling on bacterial communities be as an indicator for effectiveness of meat sample treatments is needed. To address what the bacterial compositions are in the communities of the meat samples is also needed.

ID : 972

IMPACT OF NATURAL PRESERVATIVE FORTIFICATION ON OXIDATIVE STABILITY OF CHICKEN SEEKH KEBAB PREPARED BY USING SPENT HEN MEAT

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Present study was carried out with in vitro inclusion of natural preservatives viz., curcumin, α -tocopherol acetate and eugenol @ 0.025, 0.04, and 0.02% respectively to observe their effect on oxidative stability of chicken seekh kebab (CSK). Spent hens were taken as meat source following standard slaughter technique at division of Post-Harvest Technology ICAR-CARI, Izatnagar. Formulation was selected on the basis of earlier studies carried out in the Post-Harvest Technology division, CARI, Izatnagar. Pre-standardized processing and cooking techniques were followed for CSK preparation. For comparison a control devoid of natural preservatives and treatment sample containing a mixture of curcumin 0.025%+ α -tocopherol acetate 0.04%+eugenol 0.02% were prepared. These samples were then packed in self-sealing low density polyethylene bags aerobically and stored at 4 ± 1 °C till insipient spoilage occurs. At an interval of 5 days different physicochemical and sensory quality parameters viz., TBARS value (Witte et al., 1970), free fatty acid, peroxide value (Koniecko, 1979), protein oxidation (Metmyoglobin formation %) (Krzywicki, 1979) and antioxidant activity (ABTS+ and DPPH activity) (Biswas et al., 2015) were evaluated. Duncan's multiple range test was used to test the statistical significance. During early storage period, differences amongst various physicochemical and sensory parameters varied numerically but as storage time progressed it varied significantly. TBARS value FFA, and PV lowered significantly (P

Keywords: Natural preservatives, oxidative stability, curcumin, eugenol.

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EF6

REPRODUCTION

REPRODUCTIVE PHYSIOLOGY

REPRODUCTIVE BIOTECHNOLOGIES

REPRODUCTIVE PHYSIOLOGY

ID : 62

GENE EXPRESSION OF GONADAL GERM CELLS (GGCS) IN DOMESTIC CHICKEN

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Study on the understanding of mechanism of sexual differentiation is crucial for the poultry industry, since the commercial use of poultry is heavily skewed in favor of either one of the sex. Accordingly, it is evident that the development of the technology to produce newly hatched chick of desired phenotypical sex regardless of the genetic sex benefit the poultry industry. At present, however, studies on the sexual dimorphism of chick embryos has been primarily focused on the gonadal sex differentiation, whereas limited information is available on the sexual differentiation of gonadal germ cells(GGCs). The present study was conducted, therefore, to examine the genetic factors that regulate the sexual differentiation of GGCS in chick embryonic gonads.

Fertilized eggs were incubated in an air forced incubator for 5 to 9 days and developing gonads were dissected carefully using a sharp edge of 30 gauge needle under a dissection microscope. GGCs were isolated from isolated gonads by using PBS(-) method as described by Nakajima et al. (2011). Briefly, embryonic gonads were incubated for 1 hour in the phosphate buffered saline without containing Ca and Mg ions [PBS(-)]. After incubation, GGCs dispersed in the PBS(-) from embryonic gonads were manually recovered using a fine glass pipette under dissection microscope. RNA were recovered from GGCs and subjected to gene expression analysis by RNAseq. Quantitative gene expression data were obtained by annotating the outcome of RNAseq data using chicken genome database.

Results showed that by 9th day of incubation, down regulation of genes characteristics to undifferentiated germ cells such Nanog and Prdm14 were observed, whereas upregulation of genes characteristics for sexual differentiated cells such as Dnd1 in male and Stra8 in female were observed. The results from present study strongly indicate that sexual differentiation of GGCs takes places in concert with gonadal sex differentiation. Future study should be directed toward identifying the initial factor that regulate the cascade of germ cell sexual differentiation in the poultry.

ID : 276

SPERM WITH A LONGER FLAGELLUM EXHIBIT BETTER FERTILIZATION SUCCESS BY FACILITATING SPERM ENTRANCE INTO THE SPERM STORAGE TUBULES IN JAPANESE QUAIL (COTURNIX JAPONICA)

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During passage through the female reproductive tract, the ejaculated spermatozoa massively decrease in number, and a small number of sperm are able to reach the site of fertilization in animal reproduction to fuse with ova. In birds, vagina and sperm storage tubules (SSTs) located in the utero-vaginal junction are thought to be the site of sperm selection because less than 1% of sperm inseminated are successfully stored in the SSTs. However, the exact mechanism of sperm selection by which sperm enter the SSTs is poorly understood. Here, we investigated sperm entry into the SSTs and subsequent fertilization success under a competitive situation created by artificial insemination of a sperm mixture obtained from two males. We employed two quail strains, a wild type and a dominant black (DB) type, that allow easy assessment of any offspring's paternity by feather coloration. We found paternity of embryos was significantly biased towards DB males when mixed sperm were artificially inseminated into females, despite the two strains having similar sperm motility in normal medium assessed by computer-assisted sperm motility analysis. Our novel sperm staining method with two different fluorescent dyes, Hoechst33342 and pHrodo-red-AM clearly demonstrated that the DB-biased fertilization is due to the better ability of DB sperm to enter the SSTs. Moreover, we found that DB sperm had longer flagella solely coupled with a longer midpiece and they swam fast in with high viscosity medium created by the addition of methyl cellulose that may be a similar environment to the lumen of the female reproductive tract. Our results indicated that sperm competition occurs to win a place in the SSTs, and that filling the SSTs with their own spermatozoa is a critical step to achieve better fertilization success for the male Japanese quail. Thus, males produce the sperm with longer flagellum may have advantage for fertilization in birds.

ID : 374

EXPANDING DUPLICATION OF A TESTIS GENE FAMILY IN THE CHICKEN GENOME

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Background: At the cellular and genetic levels, spermatogenesis represents a relatively well-conserved process event among phylogenetically distant animal species. In a previous study [1], we pointed out a substantial list of uncharacterised genes highly conserved in non-vertebrate and vertebrate species and exhibiting a high expression in chicken testis. Among these genes, we have studied two families of genes that exhibit numerous duplications in the chicken genome.

Material&Methods: Gene copy numbers investigated by PCR (genomic DNA from Leghorn adult roosters), gene conversion, and genomic localization by FISH were previously described in the lab [2][3]. Bioinformatics data were retrieved from Ensembl database (<http://www.ensembl.org/index.html>).

Results: We investigated the copy number of genes in two gene families, named “novel genes” in Ensembl database: 1) the chicken co-orthologs of the mouse PHD finger protein (7) gene and 2) the chicken co-orthologs of the Sun3-5/Spag4 genes. These genes are involved in the determination and/or differentiation of germ cells in vertebrates and we showed that they are highly expressed in the rooster testis. The gene dosage allows to confirm the high duplication of these genes in the chicken genome (around 60 copies for the first family and 20 for the second). The gene clusters of chicken PHD finger protein were localized by FISH on 8 loci on chromosomes 1, 2, 12, 19 and Z, as predicted by Ensembl.

Conclusion: We showed that two gene families of testis genes conserved across evolution and highly expressed in the gonad, are highly duplicated in the chicken genome. The identification of reliable fertility markers in farm chickens is critical in the context of the assessment of decline of reproductive performance (reduction in the longevity of male reproduction and deterioration in sperm quality). The characterization of such testis-specific (enriched) genes in the chicken might be of agronomic interest regarding the need for fertility markers in avian species.

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ID : 391

PROTEOME AND BIOLOGICAL FUNCTIONS OF INNER AND OUTER PERIVITELLINE LAYERS IN THE CHICKEN EGG

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The chicken egg perivitelline layer (PL), also known as the vitelline membrane, is a proteinaceous structure surrounding the egg yolk, in contact with the albumen. In avian reproduction, PL is involved in the acrosome reaction, in the early embryonic development and in the physical and antimicrobial protection of the embryo. It thus contributes to the quality of both hatching and table eggs. It consists of two sublayers: the inner PL (IPL) and the outer PL (OPL), that are in contact with the yolk content and the egg white, respectively. About 140 different proteins were identified in the PL to date; however, the distribution of most of these proteins between IPL and OPL remains to be elucidated. The specific sites of synthesis, namely the ovary for the IPL and the oviduct for the OPL, suggest that these two sublayers possess different biological functions. The present study aims at characterizing the proteome composing IPL and OPL, and identifying the intrinsic putative functions of each perivitelline sublayer. For this purpose, PLs were harvested from ISA-Brown eggs collected on the day of lay and carefully washed to remove traces of yolk and egg albumen. IPL and OPL were carefully manually separated from isolated PLs and proteins composing these two layers were independently solubilized, fractionated by SDS-PAGE and analyzed by tandem mass spectrometry coupled to nanoliquid chromatography (GeLC-MS/MS). The proteomic analysis revealed more than 400 proteins in the whole PL including around 310 and 230 proteins that were identified in IPL and OPL, respectively. Among these, 141 were common to both sublayers and encompass the most abundant proteins of the PL. The tissue origin of some of these shared proteins was investigated by the analysis of gene expression (RT-qPCR) in different reproductive tissues assumed to be involved in the formation of IPL (liver, theca, granulosa) and OPL (oviduct segments). The functional annotation (gene ontology/bibliography), carried out on the entire set of identified proteins, revealed the presence of numerous proteins associated with fertilization and antimicrobial defense, but also with many other biological functions such as cell adhesion and migration, metabolism, morphogenesis, etc. Altogether, the data generated from this study give new insights into the structure and composition of the chicken egg PL and provide an integrative overview of the respective physiological functions of IPL and OPL.

ID : 606

MIR-26A-5P REGULATES THE EXPRESSION OF VEGFA BY TARGETING NLK IN CHICKEN FOLLICULAR GRANULOSA CELLS

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Introduction

The miRNAs are a class of small non-coding RNA molecules of about 22 nucleotides in length, which are involved in ovarian follicle function. Our previous study have found that miR-26a-5p facilitates theca cell proliferation in chicken ovarian follicles. However, the regulatory role of miR-26a-5p in chicken hierarchal follicular granulosa cells remains unclear. In this study, we found that miR-26a-5p regulates the expression of VEGF-A by targeting NLK in chicken follicular granulosa cells.

Materials and methods

The follicular granulosa cells were obtained from Hy-line brown hens. NLK 3' UTR target site wild and mutant type luciferase reporter gene vectors as well as siNLK vector were constructed. The dual-luciferase reporter assay was used to detect the regulation relationship between miR-26a-5p and NLK. After overexpression, inhibition of miR-26a-5p and interference with NLK in chicken granulosa cells, RNA and protein were extracted for qRT-PCR and Western Blot, and the cell culture medium was used for ELISA test of VEGF-A. DNAMAN 6.0 and SAS 9.2 were used to design primers and analyze data. Differences were considered significant at $p < 0.05$.

Results

(1) miR-26a-5p targets NLK to down-regulate its expression

The luciferase results showed that the relative luciferase activity with miR-26a-5p mimic and the mutant-NLK luciferase reporter vector showed significantly higher than that of the wild type, indicating that miR-26a-5p could target and negatively regulate the expression of NLK gene.

(2) miR-26a-5p targets NLK to down-regulate the expression of VEGFA mRNA

After overexpression of miR-26a-5p in follicular granulosa cells, qRT-PCR showed that the levels of NLK and VEGF-A mRNA were significantly decreased; after inhibition of miR-26a-5p, the levels of NLK and VEGF-A mRNA were significantly increased. Furthermore, after interference with siNLK, the levels of VEGFA mRNA were also significantly decreased.

(3) miR-26a-5p targets NLK to down-regulate the expression of VEGFA protein

After overexpression of miR-26a-5p, as well as interference with siNLK in granulosa cells, The Western Blot and ELISA results showed that the protein levels of NLK and VEGFA were significantly downregulated. After inhibiting miR-26a-5p, NLK and VEGF-A protein levels were significantly upregulated.

Conclusions

(1) NLK is a direct target gene of gga-miR-26a-5p. (2) gga-miR-26a-5p regulates the expression of VEGFA by targeting NLK gene in chicken granulosa cells.

ID : 610

ROLE OF TTR, PrRP AND GHRELIN AND CRH SYSTEMS ON APPETITE REGULATION IN INCUBATING SILKIE.

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We previously identified that mRNA expression of the transthyretin (TTR) and prolactin-releasing peptide (PrRP) are changed significantly in the hypothalamus of incubating Silkie hens. However, it is unclear whether the observed changes are specifically associated with broodiness induced anorexia or not. In addition, Ghrelin and CRH suppress food intake in chicken, and these expressions are known to be regulated by thyroid hormone and PrRP, respectively. From these points, we investigated mRNA expression of TTR, PrRP, and ghrelin and CRH systems among different feeding states in order to clarify the regulatory mechanism of food intake in incubating hens.

Egg laying and behavior of matured female Silkies (36-week-old) were monitored daily, and divided into three groups (n=5); 1) incubating hens that sat in the nest with characteristic behaviors for at least 1 week, 2) laying hens that lay eggs regularly for at least 1 week, and 3) pair-feeding hens that were given feed equivalent to the level of incubating birds for 2 weeks. Finally, hens were weighed, sacrificed by decapitation, and the hypothalamus was collected. Hypothalamic TTR, PrRP, Ghrelin, Ghrelin receptor (GHS-R1alpha), CRH and CRH receptors (CRH1R, CRH2R) were quantitatively analyzed by RT-qPCR. The Smirnov-Grubbs test was applied to exclude outliers, and difference in mRNA expression was determined by the Tukey-Kramer test. P-values less than 0.05 were considered significant. All statistical analyses were performed by R statistical package (www.r-project.org).

TTR mRNA expression was significantly increased in pair-feeding compared to the other two groups. PrRP mRNA expression was significantly decreased in incubating and pair-feeding than in laying. Ghrelin mRNA expression tended to decrease in pair-feeding compared to laying and incubating. GHS-R1alpha mRNA expression in incubating showed an increasing trend compared to laying and pair-feeding. CRH mRNA expression was significantly reduced in incubating and pair-feeding compared to laying, and that was similar to the PrRP. CRH1R mRNA expression was not different among the groups, and CRH2R mRNA expression in pair-feeding was significantly reduced than in laying and incubating. In conclusion, TTR, PrRP and CRH systems are possible to respond generally to chronic under nutrition. Contrary, the ghrelin system may involve specifically regulating natural anorexia during incubation behavior in Silkies.

ID : 638

EFFECTS OF SEMINAL PLASMA ON THE SPERM REPLACEMENT IN CHICKEN SPERM-STORAGE TUBULES

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In birds, the ejaculated sperm does not directly travel to the site of fertilization, but they initially entered and stored in the sperm storage tubules (SSTs) located in the utero vaginal junction (UVJ) of the oviduct. After storage, sperm are gradually released from the SSTs and achieve fertilization in successive days (several days to months depending on the species). In domestic chicken, approximately 14 eggs are fertilized after single insemination by natural mating or artificial insemination (AI). Previously, in the chicken, it is reported that when consecutive AI of semen obtained from different males were performed, paternity of the chick significantly biased to the male which inseminated in the last. This phenomenon is referred to as last male sperm precedence (LMSP) and is also known to operate in the insect fertilization such as fruit fly. In this study, we investigated whether LMSP in the chicken is related to the replacement of the resident sperm in the SST after additional insemination. The ejaculated sperm of White Leghorn (WL) were fluorescently stained with pHrodo-Red and inseminated into WL female vagina, sperm filling rate which calculated as sperm filled SSTs/total SSTs decreased in a time-related manner, but approximately 50% of SSTs still contained the sperm after 5 days. When we performed another insemination of the sperm which fluorescently labelled with Hoechst33342 after 3 days of initial AI, we observed most of the resident sperm were labelled with H33342 dye under fluorescent microscope. This result suggests that LMSP in the chicken is occurred by the replacement of resident sperm with recently inseminated sperm. To examine the effects of seminal plasma on the sperm release from the SST, we injected seminal plasma of WL intravaginally for three consecutive days after AI, and the sperm filling rate was compared with the control birds (PBS injection). As the results, sperm filling rate significantly decreased in the seminal plasma-injected birds compared to the control. Moreover, when the seminal plasma of the second AI was removed, sperm replacement decreased, and this decrement recovered by the addition of seminal plasma. These results suggested that LMSP in the chicken operates at the stage of the sperm entrance into the SSTs, and seminal plasma components may play important roles in the process of sperm replacement.

ID : 639

TWO ISOFORMS OF DDX4 AND THEIR DIFFERENTIAL EXPRESSION DURING EARLY EMBRYO DEVELOPMENT OF JAPANESE QUAIL

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DEAD (Asp-Glu-Ala-Asp) box polypeptide 4 (DDX4) encoding RNA helicase plays essential roles for proper germ cell formation in several animal species. In mice, it is reported that DDX4 is essential for spermatogenesis, whereas in chicken, DDX4 are necessary for maintenance of germ cells in both sexes, and subsequent oogenesis, not spermatogenesis. The difference in the role of DDX4 between mice and chicken is suggested to be due to the different mechanisms in germ cell specification in each species, however, authentic RNA targets of DDX4 remain unknown. In the present study, we investigated the expression profile of DDX4 during embryogenesis of Japanese quail. In addition, we attempted the screening of target RNA of DDX4 capable of interacting with DDX4 protein by in vitro binding assay. Western blot analysis using specific antibody revealed the presence of two DDX4 isoforms in the gonad of quail embryo. cDNA cloning and the sequencing analyses demonstrated the presence of short isoform which is the truncated version of DDX4 that lacks a part of the N-terminal region (DDX4-S), in addition to full length DDX4 (DDX4-L). Immunohistochemical and western blot analyses showed that DDX4-S isoform was expressed in male blastoderm and the gonad throughout the embryogenesis after the migration of primordial germ cells (PGCs) into their gonads. Interestingly, DDX4-S signals did not detect in the female blastoderm. On the other hand, DDX4-L was expressed in ovulated egg and was detectable through blastoderm and embryonic development, irrespective of genotypic sex. In addition, the number of genotypic male and female PGCs was apparently decreased in homozygous mutant DDX4 blastoderms generated by microinjecting CRISPR/Cas9 sgRNA into the egg at pronuclear stage, suggesting that DDX4 is an important germ cell determinant and/or regulates initial proliferation of male and female PGCs in Japanese quail. Furthermore, in vitro binding assays of DDX4 and blastodermal and gonadal mRNAs revealed that DDX4-S strongly binds to several male-specific mRNAs. Our results suggested that DDX4-L is essential for germ cell survival and proliferation in both sexes and DDX4-S may participate in male germ cell differentiation in Japanese quail.

ID : 1179

TRANSCRIPTOME SEQUENCING REVEALS CANDIDATE GENES FOR EGG RATE DURING PEAK LAYING PERIOD AND END-OF-LAY HENS

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Introduction

Laying hens have a sharp drop in laying rate when ovarian follicles are exhausted. However, low laying rate in aging laying hen is a result of a dynamic interaction between neuroendocrine changes that take place in the brain with the reproductive endocrine axis governing the function of ovaries.

Material and Methods

To elucidate the key regulatory genes involved in Dawu Jinfen chicken with high and low rates of egg production, RNA from hypothalamus tissue during 35-week-old high laying rate 100%,100%,100% (Group YH:YH1, YH2 and YH3), 70-week-old high laying rate 100%,98%,100% (Group OH: OH1, OH2 and OH3) and 70-week-old low laying rate 55%,45%,30% (Group OL:OL1, OL2 and OL3) were sequenced using the Illumina HiSeq 2500 sequencing platform.

Results:

A total of 391.59 million clean reads with an average of 43.51 million reads per sample (range: 40.04 to 45.71 million) were acquired after removing the low-quality and adaptor sequences. A comparison between Groups OH and OL revealed 32 differentially expressed genes, with 15 displaying up, and 17 displaying down, A comparison between Groups OH and YH revealed 56 differentially expressed genes, with 30 displaying up, and 26 displaying down, A comparison between Groups OL and YH revealed 127 differentially expressed genes, with 30 displaying up, and 97 displaying down. Ten genes that are mainly involved in pathways for reproduction regulation, such as PRLH, ESR2, CGA, CRH, CYP19A1, CYP26B1, PGR, OXT. All of the predicted genes of them were mapped to terms in the GO and KEGG databases. In total, in OH-VS-OL groups, 2 GO terms and 55 KEGG pathways in hypothalamic tissues were significantly enriched.

Discussion and conclusion

The hypothalamus is a key component of the HPG system. As the tip of the HPG axis, it plays an important role in hormone secretion and regulation of reproductive performance in laying hens. As we expected, our results aggregated many reproductive-related signaling pathways, such as the GnRH signaling pathway, the estrogen signaling pathway, and the prolactin signaling pathway. The obtained RNA sequencing data from the hypothalamus could be a valuable resource for genomic studies and provide new insights into the biological processes that regulate chicken reproduction. These data provide comprehensive gene expression information at the transcriptional level that might increase our understanding of the laying hens' reproductive biology.

ID : 1204

EFFECT OF LEPTIN ON THE OVARIAN DEVELOPMENT AND FUNCTION IN JUVENILE CHICKS

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Leptin advances the onset of laying and suggested to be involved in the regulation of reproduction. Leptin receptor (LEPR) appears in early embryonic days and continuously expresses in chicken ovary. However, the role of leptin in juvenile chicks' ovary is still not fully understood. Furthermore, formation of primordial follicles and its transition to primary follicles occurs around day 7 and day 28 after hatching in chicken. Therefore, the mRNA expression of LEPR, FSHR and CYP19A1 were measured in the ovary from day 1 to day 28 at 7 days interval. In addition, changes of the same gene expression after leptin administration was measured in the ovary in 7-and 28-days-old chicks.

Newly hatched White Leghorn female chicks were used in the present experiments. All birds were maintained at 24h light at 30°C for the first two weeks and 23h light at 29°C thereafter. All birds freely accessed to feed and water. In the first experiment, at the age of days 1, 7, 14, 21, and 28 (n = 5) were killed by decapitating and ovary was collected. In the second experiment, chicks received intraperitoneally recombinant mouse leptin at a dose of 25 and 250 µg/kg body weight (BW) at 7- and 28-days old. After 24h of administration, all birds were sacrificed by decapitation after taking blood and ovary was collected. mRNA expression of LEPR, FSHR and CYP19A1 was analyzed by RT-qPCR and serum estrogen was measured by ELISA in the second experiment.

In the first experiment, ovarian LEPR, FSHR, and CYP19A1 expressions showed an increasing trend by age progress and peaked at day 28. In the second experiment, leptin showed specific effects on ovarian gene expression, but the effect was differed by age. In 7-days-old chicks, both doses of leptin significantly increased LEPR and CYP19A1 mRNA expressions, and FSHR mRNA expression was dose-dependently increased by leptin. In the ovary of 28-days-old chicks, leptin dose dependently decreased LEPR mRNA. FSHR mRNA expression was elevated by lower and declined by higher doses of leptin. CYP19A1 mRNA remained unchanged by leptin treatment. Leptin increased significantly serum estradiol in the 7 days old chicks. Besides, high dose of leptin administration significantly decreased the serum estradiol in 28 days old birds.

In conclusion, leptin may have age specific effect on ovarian development in early juvenile chicks and that supports previous findings that leptin administration at early age advances puberty in birds.

ID : 1223

THE MOLECULAR MECHANISM STUDY OF SPERM STORAGE ABILITY BASED ON RNA-SEQ

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Sperm storage tubules is a special tubular gland that exists in the hens' utero-vaginal junction, allowing sperm to survive in the fallopian tube and maintain fertility for a long time. The objective of this study was to identify the molecular regulation of sperm storage ability based on RNA-seq. A total of 158 White Leghorn laying hens and 28 rosters of 27 weeks of age were used in this study. After the artificial insemination with mixed semen for two consecutive days, the eggs were collected for each individual laying hen starting from the third day for 21 days and incubated. The fertility was calculated for each laying hens, based on which, the top four chickens with the highest fertility and the four with the lowest fertility were selected and named high and low sperm storage ability group, respectively. The uterus and vaginal junction area of these eight laying hens was dissected and divided into two parts in the longitudinal direction. One was made into paraffin sections and HE stained for the morphology evaluation including the number and cross section area of the sperm storage tubules, and the other one was used for transcriptome sequencing. There was no difference in sperm storage tubules numbers between high and low sperm storage ability groups ($P > 0.05$). The average cross section area of sperm storage tubules of high sperm storage ability group was higher than that of the low group ($P < 0.05$). Analysis of transcriptome data shows that the gene of high abundance in the utero-vaginal junction mainly includes the biological process of protein synthesis, genes related to sperm plasma membrane protein interaction and sperm state. A total of 555 mRNA were differentially expressed between groups, of which, 118 mRNA were up-regulated and 437 mRNA were down-regulated in the low sperm storage ability group. GO annotation analysis revealed that these genes were involved in muscle contraction, membrane receptors, signal transduction, and lipid synthesis and so on. The present study indicates that the cross section area of sperm storage tubules may be related to the sperm storage ability of laying hens. Based on the RNA-seq data, we get important clue that smooth muscle contraction and mucous membrane secretion-related lipids may also be involved in sperm release and ascending fertilization. The molecular mechanism involved in the sperm storage and release regulation needs further digging of the RNA-seq data.

ID : 586

EFFECTS OF PHOTOPERIOD UPON THE REPRODUCTIVE SYSTEM AND REPRODUCTIVE CYCLE IN THE FEMALE NATIVE THAI CHICKEN: ROLES OF STEROID HORMONES

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In birds, besides the neuroendocrine factors, the environmental factors may influence the reproductive cycle. This study was aimed to elucidate the roles of steroid hormones in the regulation of the reproductive cycle and its relationship with photoperiod. Female native Thai chickens, Pradoohangdum breed, were used. Blood samples were collected according to their reproductive stages (n=6); non-laying, laying, and incubating eggs. Plasma samples were analyzed for estrogen, progesterone, and testosterone by enzyme-linked immunosorbent assay (ELISA). The results showed that plasma estrogen and testosterone levels were significantly ($p<0.05$) higher in laying than that of non-laying hens, however; plasma levels were not different when compared with incubation hens. In contrast with estrogen and testosterone, plasma progesterone levels were not changed during the reproductive cycle. To investigate the role of photoperiod in relationship with steroid hormones, chickens were divided into 4 groups (n=6). They were reared in different photoperiodic treatments as long day (16 hours of light and 8 hours of darkness; 16L:8D), short day (8L:16D), normal day (12L:12D), and control group (giving natural light photoperiod). Blood samples were collected from each chicken 2 times a week for 4 weeks and then analyzed for estrogen, progesterone, and testosterone levels by ELISA. The results indicated that plasma estrogen and progesterone of chickens reared in long day, short day, and normal day were not different. In contrast, plasma estrogen and progesterone levels were higher in the control group when compared with other groups. Plasma testosterone levels were as the same among 4 groups. These results support the pivotal role of steroid hormones in associated with the reproductive cycle of this equatorial gallinaceous species. In addition, photoperiod might have some effects on plasma estrogen and progesterone levels, but not on testosterone levels. These data presume to suggest that photoperiod may, in parts, have an effect on the reproduction of the native Thai chicken.

ID : 1228

EFFECT OF NANOSELENIUM ON THE IMPROVEMENT OF CRYOPRESERVATION OF DOMESTIC FOWL SEMEN

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During semen cryopreservation, the spermatozoa undergo notable stress effect. The avian spermatozoa are extremely sensitive to the harmful effects caused by the formation of free radicals. Under natural conditions various types of antioxidant systems in spermatozoa and seminal fluid are able to bind to free radicals. Selenium with vitamin E is essential component of the antioxidant system in a multi-step biochemical process. In several animal species, antioxidants added to the semen diluent have been shown to improve the efficiency of semen freezing. Recent researches have shown that nanoparticles could be more efficiently utilized due to their increased surface area and reactivity. In the present work we investigated the effect of vitamin E and nanoselenium in various concentrations in semen diluent on the changes of qualities of rooster spermatozoa after freezing/thawing.

Semen of 20 individually placed Hungarian yellow males was collected twice weekly. The pooled semen was divided into four equal parts and diluted with Lake's diluent in 1:2 ratios. 5 µg/ml α-tocopherol was added to the diluted semen samples with the exception of control group, as well as 50, 100 and 200 µg/ml of nanoselenium solution were put to the three experimental samples, respectively. After 20 min of equilibration at 5 °C, all samples were further diluted in 1:0.5 ratios with DMA containing diluent to give a final concentration of 6% DMA. The samples were frozen 1 cm above the surface of liquid nitrogen in 0.25 ml French straws for 20 minutes and then placed in liquid nitrogen. The samples were thawed at 5°C. From the spermatological parameters motility was examined using SCA® CASA. The live/dead cell ratio and the ratio of morphological abnormalities were determined by aniline blue-eosin staining.

After thawing, the proportion of motile cells in the samples with nanoselenium at different concentrations did not differ significantly from the control group ((34.6%; 26.4%; 34.5% and 29.2%, respectively). There were no differences in the survival rates of the cells (20.2%; 19.2%; 17.2%; 22.4%), and the morphological abnormalities, either. Based on in vitro studies, the use of nanoselenium and α-tocopherol in the given concentrations did not improve spermatological parameters after freezing/thawing. Despite the in vitro results further studies are planned with artificial insemination to investigate the effect of the above additives on the fertilization capacity of frozen/thawed sperm.

ID : 1330

EXTRACELLULAR VESICLES ISOLATED FROM SEMINAL PLASMA OF CHICKENS WITH CONTRASTED FERTILITY SHOW DIFFERENTIAL CHARACTERISTICS

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Extracellular vesicles (EVs) are present in seminal plasma of different mammalian species but their existence in chickens remains controversial. In addition, the relationship of these structures with male fertility is still unclear. The aim of the present work was first to isolate the EVs from chicken seminal plasma, then to characterize their morphology and protein content and finally to investigate their interactions with sperm in both fertile and subfertile roosters. For this purpose, in vivo fertility was determined by artificial insemination for 40 French SASSO T44 roosters and animals were classified as fertile (F+, >90%) or subfertile (F-,

REPRODUCTIVE BIOTECHNOLOGIES

ID : 222

HOW SINGLE LAYER COLLOID CENTRIFUGATION CONTRIBUTES TO CHICKEN SPERM CONSERVATION IN VITRO

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Conservation of animal genetic resources is a crucial issue for the future. In chicken, sperm cryopreservation is an important method of reproductive cell conservation to restore genetic diversity (Blesbois et al 2008). Glycerol is a highly efficient cryoprotectant for chicken sperm freezing but it must be removed before artificial insemination due to vaginal contraceptive effect. Stepwise dilutions followed by centrifugation is a general method used to remove glycerol from frozen-thawed semen. However, it is time-consuming, must be processed at 4°C, and could decrease fertilizing potential due to added sperm damages (Purdy et al 2009). Percoll™ is a colloid used for sperm preparation. This technique has recently been successful in chicken sperm selection (Lin et al 2019). Therefore, the purpose of this present study was to determine whether Percoll™ centrifugation could be a workable method to remove glycerol from cryopreserved semen. Twenty slow-growing, broiler type of T44 line roosters were used in this study. Semen was collected by dorso-abdominal massage, pooled, diluted 1:1 in Lake pre-cryopreservation (LPC) diluent and kept 10 min at 4°C under gentle shaking. LPC containing 22% glycerol was then added 1:1 (11% glycerol final concentration) for 10 min at 4°C. Percoll treatment: 1 ml of glycerolized semen was then gently placed on top of 2 ml Percoll™ 60%, 70% or 80% colloid or PBS in a 15 ml conical plastic tube and centrifuged at 800g for 20 min. Sperm pellets were resuspended in 100 µl Lake 7.1 (L7.1). Sperm motility was analysed by CASA over 3.5 hr. The results indicated that motile sperm (%) and progressive sperm (%) of 11% glycerolized chicken sperm treated with Percoll™ centrifugation were significantly higher than without. Furthermore, levels of motile sperm (%) and progressive sperm (%) stayed almost stable after Percoll™ centrifugation until 3.5 hr by opposite to 11% glycerolized sperm untreated with Percoll that lost 2/3 motile sperm in the first hour. Thus, Percoll™ centrifugation reduce the sperm motility alterations due to glycerol. This is an encouraging alternative method to remove glycerol in order to optimize the fertility obtained with cryopreserved semen in the chicken.

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ID : 595

IMPROVED SERUM- AND FEEDER-FREE CULTIVATION OF CHICKEN PRIMORDIAL GERM CELLS

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Primordial germ cells (PGCs) are the embryonic progenitor cells for gametes. In chicken, a technique was developed in which intravascularly transplanted PGCs undergo germline transmission (Tajima et al. 1993). Recently, a serum- and feeder-free culture system for chicken PGCs was established (Whyte et al. 2015). However, the growth of PGCs was significantly slower than that of PGCs in serum-containing medium. Therefore, a need existed to improve a serum- and feeder-free culture for chicken PGCs. KnockOut Serum Replacement (KSR), a defined, serum-free formulation, could improve the growth of various types of stem cells in serum- and feeder-free culture (Garcia-Gonzalo et al. 2008; Kanatsu-Shinohara et al. 2014). Here, we demonstrate the impact of KSR on derivation and growth of chicken PGCs in serum- and feeder-free cultures. FAot medium (Whyte et al. 2015) was used as a basal medium for chicken PGC culture. We used male PGC-line (F5) of White Leghorn MK line that was derived under serum-free conditions. To investigate the effect of KSR on growth of PGCs, 1,000 PGCs were placed and cultured in FAot medium with different concentrations of KSR for 10 days. As a result, the addition of KSR to FAot medium could promote PGC propagation in vitro. FAot medium supplemented with 1% KSR was optimal for proliferation of PGCs. To investigate whether KSR could improve the derivation of PGCs in primary culture, blood sample (1 μ L) containing PGCs obtained from 2-day-old embryos of MK line were cultured in FAot medium with 1% KSR for 4 weeks. Resulted cultures that contained more than 50,000 PGCs were determined as derivation. In all, we demonstrated the addition of KSR accelerated the derivation of chicken PGCs in primary culture. In the presence of KSR, PGCs could propagate for more than 6 months and maintained normal morphology and karyotype. Immunocytochemical analysis showed that all PGC lines derived in a medium containing KSR were positive for Vasa and Dazl, germ cell markers, as well as SSEA-1, an undifferentiated cell marker. Germ cell transplantation showed that PGCs derived in KSR-containing medium could migrate toward and settle to the gonads of recipient embryos. In conclusion, the present study showed that serum- and feeder-free medium supplemented with KSR improves the derivation and propagation of chicken PGCs in culture.

ID : 891

DISTINCT BASE EXCISION REPAIR CHARACTERISTICS IN CHICKEN PRIMORDIAL GERM CELLS INDUCES VARIOUS GENOTYPES BY BASE EDITING

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Recently, base editing technology provides the opportunity to generate precisely genome-modified animal model (1). In addition, genome-modified chicken can be produced through genome-modified primordial germ cells (PGCs), but germ cells have a sophisticated DNA repair system. Therefore, we want to understand the influence of base editing in repair pathways of PGCs and to identify relationship between germ cell specific characteristics and the consequences of base editing.

First, we constructed all-in-one base editing plasmids. Then we applied the base editing plasmids into chicken DF-1 cells and PGCs, and genome modification was confirmed by T7E1 assay and sequencing analysis. Furthermore, qRT-PCR for BER pathway related genes and functional studies for BER gene on the effect of base editing purity was conducted. As a result, significant differences of gene expression and editing purity between cell types were evaluated by student t-test. Then, base edited WL PGCs were microinjected into the Ogye recipient embryo to generate genome-modified chickens.

First, we introduced base editing plasmids into two chicken cell lines. Specifically, in vitro genome modification showed that the base editing purity in DF-1 cells and PGCs were different as base editing purity was lower in PGCs in two economic related genes. Then, we examined the expression levels of several BER pathway related genes by qRT-PCR. As a result, most of the DNA glycosylases and BER complex genes are upregulated in PGCs. Subsequently, functional studies for upstream gene of the BER pathway demonstrated that regulation of BER gene affects base editing purity. By transplanting the base edited PGCs into recipient embryos, we produced more than 100 genome-modified chickens, which had nucleotide substitutions as well as indel mutation in the targeted locus.

Our results suggest that the distinct repair property of chicken PGCs causes low editing purity during genome-modification, but modulation of BER functions could enhance the production of genome-modified organisms harboring desired genotypes

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ID : 1153

SUCCESSFUL CRYOBANKING OF SEVEN INDIGENOUS CHICKEN BREEDS WITH PRIMORDIAL GERM CELL LINES

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The economically important or indigenous chicken breeds are held in in situ populations worldwide, thus they are exposed to various risks. Therefore, it is increasingly important to protect the genetic information these breeds represent. From the available methods, cryopreservation of primordial germ cells (PGCs) is the most promising one.

The goal of the present study was to establish a cryobank based on PGCs for seven indigenous Hungarian chicken breeds and to test the viability of the cryopreserved cells by creating germline chimaeras with one of them; the Partridge colour Hungarian. The germline chimaeras were then back-crossed to recover the donor breed.

We collected blood from each embryo individually, then the isolated blood samples, containing the PGCs, were cultured in a medium which was selective for the PGCs. Later, samples from the cell lines were collected for DNA, RNA isolation and immunohistochemistry to characterize the quality of the cells and to perform microsatellite analysis to evaluate the representation of the genetic variability of the original population. As a next step, parallel vials were frozen from each PGC line. To evaluate the freezing process and to prove the functional integrity and migrating ability of PGCs, some of the vials were thawed and the cells were injected into recipient embryos. First, the cells were labelled with an in vivo fluorescent dye, thus the migration of the injected cells was followed toward the developing gonads, and the ratio of the colonization was analyzed. As a second experiment, after injection with the frozen/thawed PG cells, the eggs were left to incubate until hatching. The hatched chicks were then kept until maturation and are going to be cross-tested with animals from the donor genotype to examine the germline transmission.

During the study, 21 PGC lines from Partridge colour Hungarian chicken were established with a derivation rate of 31.1%. Based on the general diversification indexes which were used for the microsatellite analysis the cell lines represent the genetic variability of the original population. The PGC lines were frozen and then successfully thawed with a cell viability of 50%. The preserved cells were capable of colonizing the gonads of the recipient embryos; furthermore, we have 24 adults (13 roosters and 11 hens) which presumably contain the donor PGCs.

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ID : 1316

FRENCH AVIAN CRYOBANK: DEVELOPMENT OF REPRODUCTIVE BIOTECHNOLOGIES BASED ON PRIMORDIAL GERM CELLS (PGCS) AND INVESTIGATION OF THE IMPACT OF IN VITRO STEPS ON PGCS INTEGRITY AND REPRODUCTIVE CAPACITY.

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Introduction. Cryopreserved primordial germ cells (PGCs) derived in vitro present a tool complementary to frozen sperm for the conservation of avian genetic resources. Recently, we have developed in France reproductive biotechnologies based on PGCs and enriched the national avian cryobank by PGCs collections of a local breed La Poule Noire du Berry (NB), which have benefited from a national program of conservation for endangered breeds. However, little is known on the impact of in vitro steps on molecular integrity and reproductive capacity of PGCs. In this study we investigated the effect of culture duration and cryopreservation on DNA methylation, gene expression and germline transmission rate of NB PGCs.

Materials and methods. Male and female PGCs isolated from the embryos were derived in in vitro culture according to (Whyte et al., 2015). PGCs transcriptome and methylome were evaluated using RNAseq and RRBS analyses respectively after short term and long term cultures and after cryopreservation. To evaluate germ line transmission rate of in vitro derived cryopreserved NB PGCs, germline chimeras were created by transplanting PGCs after thawing and reamplification in vitro in Rhode Island embryos, and then crossing the obtained chimeras with Rhode Island chickens and obtaining their progeny.

Results and discussion. RNAseq study revealed important differences in transcript abundance between male and female PGCs that suggests their early sexual identity. Moreover, RRBS analyses showed that culture duration and cryopreservation significantly affected the epigenetic landscape of PGCs, and this effect was stronger in male PGCs than in those of females. The gender differential effect of culture duration was also observed on the PGCs transcriptome. In contrast, cryopreservation had little effect on gene expression in both sexes. The germline transmission rate for female and male NB PGCs was up to 60.6% and up to 42.4% respectively, and tended to vary according to the culture duration in a gender-specific manner.

Conclusions. This study shows that in vitro environment may affect PGCs at different molecular levels with consequences for their reproductive potential in gender dependent manner. This knowledge will be useful to better adapt in vitro steps to PGCs physiology and improve PGCs quality.

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ID : 1378

ANALYSIS OF REPRODUCTIVE QUALITY OF DUCK GERMLINE CHIMERAS

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Avian species play a vital role in livelihood of human beings throughout the world, especially in developing countries. Duck is an under-researched scientific (breeding) object, but one of the most economically promising species of farm poultry. In Ukraine and in the world there is a problem of reduction and irreversible loss of genetic resources of birds, including local breeds of ducks. During the past several years, the production of transgenic animals has been a prominent theme in animal biology. The study was carried out to estimate the quality characteristics (external and internal) of germline chimeras, which was created by blastodermal cells method, Shan partridge and Shaoxing duck eggs through analyzing different physical properties of eggs. The research was conducted on a duck farm of the company Zhejiang Generation Biological Science and Technology Co., Ltd and at the Zhejiang Academy of Agricultural Sciences (Zhejiang Province, China) bird genetics laboratory. Recipients were sterilized using ultraviolet light irradiation. The isolated blastodermal donor cells were transfected with the DNA-construction (CRISPR/Cas9-mediated gene insertion into the duck genome) with Lipofectamine (liposome) which were inoculated under embryo of the (blastodisc) of the recipient eggs.

After hatching, eleven female and eight male alive birds (3 % of the manipulated embryos) were obtained. We formed 3 groups (n=6), according to the results of their sperm productivity of group of chimeras was higher compared to Shan partridge group ($P > 0,01$) and group of Shaoxing ($P > 0,05$) by collection sperm 6 times a week and higher when sperm collection was performed every other day compared to group 1 ($P 0,05$) in reproductive season. In total, we used 35 drakes to check the quality of the daughters. We conducted a comparative analysis and showed that for all productive traits, the chimera group had average indicators between the Shan partridge group and Shaoxing groups. We checked 10 loci of 5 genes and found that only a few of them are correlated with productive traits such as PRL, GH, DRD1, OIH. We found both positive correlations and negative correlations. It turned out that according to some signs, these mutational events are undesirable, such offspring should be discarded, and on the contrary, on the contrary, populations with such mutations should be maintained that give a good correlation with egg production and body weight. The prospect of further research is to evaluate the breeding qualities of the chimeric bird of the Shaoxing-Shan partridge breeds and to create new high-performance duck lines to produce products with optimal technological properties.

ID : 225

IMPLEMENTATION OF NEW METHODS IN THE HUNGARIAN IN VITRO POULTRY GENE BANK

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The part of protection of biodiversity is the preservation of those farm animal species and breeds, which were excluded from public farming due to the use of intensive, high yielding lines and hybrids. The advantage of frozen storage of the reproductive material over in vivo preservation is that it has no animal health risk and does not involve cost of the housing and feeding. While the cryopreservation of gametes and embryos is a routine procedure in many species of mammals, in birds only the sperm freezing is still the most reliable preservation practice. The establishment of in vitro gene bank of the Hungarian poultry was started in 2012 - among the first ones in Europe - by NBGK-HGI. The work started with the frozen storage of spermatozoa according to the FAO protocol which gives recommendations only for domestic fowl, therefore the sperm cryopreservation of other poultry species is still under continuous development in our institute. In avian species, the females are heterogametic (ZW) and the males are homogametic (ZZ); the ovum carrying the W sex chromosome and the embryo per se cannot be frozen. Therefore, there is a need to develop methods, which allow the female genome to be involved in the in vitro preservation. Transplantation of the ovarian tissue of day-old chicks on one hand and the primordial germ cells (PGC) on the other may be the solution. Both materials can be frozen, by which gonadal chimeras are formed and after sexual maturation donor-derived ovum can be obtained. In usage of PGCs the challenge is to establish stable cell lines moreover improve hatchability after injection of donor cells into recipient embryos. According to our previous studies on tissue grafting not all breeds are suitable as recipients, thus one of the most difficult tasks is to form appropriate donor/recipient combinations. Although the optimization of these methods is still ongoing, reconstruction of the donor genotype has been already successful, so storage of PGCs and gonadal tissues from 7 native hens has begun. Currently, there are 1034 PGC samples stored from which 517 are core, while 517 working collections. In case of ovarian tissue of the day old chicks, 239 working collections are placed in the bank. The sperm bank currently has 250-300 straws/breed - altogether 4000 straws as core collection and 200 samples as working collection. This work was supported by Horizon 2020 "IMAGE" n°677353 and GÉNNET_21 - VEKOP-2.3.2-16-2016-00012.

ID : 306

A NEW MODEL FOR PGCS REINTRODUCTION INTO HOST ANIMAL USING INFERTILE INTERSPECIFIC HYBRIDS

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Related to the avian gene preservation activities, the aim of our research was to produce interspecific hybrids, to investigate their ability hosting the primordial germ cells from native poultry breed (donor) and according to our expectations, this donor genotype may appear in the offspring of hybrids.

To achieve our goals, Hungarian guinea fowl x Hungarian yellow chicken hybrids were produced, the crossing was repeated inversely and the hatching time, the hatching rate, the sex ratio, the presence of own germ cells, the fertility and the phenotype of viable hybrids and the incidence of chromosomal abnormalities of dead hybrid embryos were described.

Crossing of Guinea fowl hens with domestic fowl males resulted 6.65% viable offspring but the reverse crossing was unsuccessful due to the high rate of infertile eggs (0.14% viable offspring). During the investigation of chromosomes 2.7% of the samples showed abnormalities (mosaicism) or aneuploidy, but this ratio is average compared to previous literary data. The hybrids hatched between the 21st and 27th day of incubation and raised until maturity. Three kinds of phenotype (light brown, dark brown and white spotted) were observed on the hatched hybrids (6.65%) and none of the hybrids had helmet, crest or facial wattles.

Between the 16th and 30th week of growth the gonads of hybrids weren't symmetrically positioned, their shape were bent and their size didn't increase really with the age. Histological analysis shows that the germinal epithelium is not active, but the normal tubular structure suggests that the gonads of the hybrids may be suitable for hosting of donor PG cells and to produce gametes.

Based on the investigations, the hybrids from the successful crossing were sterile male hybrids. 3 days old hybrid embryos were tested by injecting fluorescently labeled chicken PGCs. The integration rate of labelled PGCs was measured in 7.5-day, 14.5-day and 18.5-day old embryonic gonads. 13.6% of the injected hybrid embryos survived and 85% of the examined gonads contained fluorescent labelled donor PGCs.

This research work shows that the sterility of hybrids can be used in gene conservation to be a universal host for PGCs of different avian species.

The research leading to these results was founded by the European Union's Horizon 2020 Research and Innovation Programme n°677353 IMAGE, by the VEKOP-2.3.2-16-2016-00012 and by the EFOP-3.6.3-VEKOP-16-2017-00008 grant, co-financed by the EU and the European Social Fund.

ID : 61

EFFECTS OF CALCIUM LACTATE ON VIABILITY AND BLOOD COMPONENTS OF CHICK EMBRYOS UNDER THE SHELL LESS CULTURE SYSTEM.

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The complete shell-less culture system in chicken (cSLC) was developed by Tahara and Obara (2014). During the course of developing cSLC system, however, a supply of calcium lactate together with distilled water into the culture vessel were obligatory for the embryos to hatch. The aim of the present study, therefore, was to clarify effects of calcium lactate on the development of chick embryos under cSLC system.

Fertilized White Leghorn eggs (MB line, NLBS, Okazaki, Japan) were allocated to one of the following four treatments: Treatment 1. cSLC system supplemented with both calcium lactate together with distilled water; Treatment 2. cSLC system without calcium lactate but only with distilled water; Treatment 3. cSLC system without both calcium lactate and distilled water, Treatment 4. Intact fertilized eggs for control. At 17th day of embryo culture, blood was collected from the embryos and plasma lactate dehydrogenase (LDH), calcium (Ca), inorganic phosphorus (iP) were measured. The tibia length was also measured.

The viability of embryo in Treatment 1, 2, 3 and 4 were 24.1%, 75.0%, 100% and 100%, respectively. In Treatment 1, a significant bimodal decreases of the embryo viability were observed between 5th and 8th day and between 15th and 17th day of the culture ($P<0.05$). Plasma LDH concentration in Treatment 1 was significantly higher than in Treatment 3 ($P<0.05$). Blood Ca concentrations were significantly higher in Treatment 1 compared with other 3 treatments ($P<0.05$). On the other hand, no significant differences in iP concentration were observed among treatments. The tibia length was significantly shorter under cSLC systems (Treatment 1, 2, and 3) compared with Treatment 4 ($P<0.05$). In conclusion, even though calcium lactate supplementation is necessary for hatching of chick embryos under cSLC system, adverse effects on the viability of embryos was observed. Future study should be directed toward decreasing the embryo mortality while supplying Ca source under cSLC system.

WELFARE

WELFARE AND BEHAVIOUR

MANAGING POULTRY WELFARE

WELFARE AND BEHAVIOUR

ID : 17

CARCASS WEIGHT UNIFORMITY IN BROILER FLOCKS IS ASSOCIATED WITH PRODUCTION MEASURES ON FARM AND AT SLAUGHTER

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The flock weight uniformity in a poultry flock can be defined as the percent individuals within 10 % of the mean body weight (BW) and the variability of this uniformity can be expressed as the CV (coefficient of variance) of BW. Flock weight uniformity is a standardized and objective measure, and could potentially be used as a welfare indicator. Poor flock uniformity (i.e. a high CV) may indicate reduced animal welfare, due to either general housing or management problems, or bird health problems. However, little is known about the relationship between flock uniformity and other production measures on-farm or at slaughter. Therefore, the aim of this study was to investigate associations between carcass weight uniformity (CV of BW) and production measures on-farm and at slaughter in Norwegian commercial broiler flocks. A total of 45 randomly selected mixed-sex Ross 308 broiler flocks were visited prior to slaughter at 28 – 30 days of age (average slaughter age 30.6 days). All flocks were raised under similar farm management systems. The Welfare Quality® protocol for broilers was used to assess different animal welfare indicators in each flock. For each flock, production data from the slaughterhouse were collected, including carcass weight uniformity (%), mortality (%), growth rate (g), feed conversion ratio (FCR), and rejected birds (%) in different rejection categories. Flock uniformity varied from 11 % to 18 % between flocks within the same hybrid, similar management standards and similar slaughter age (day 29 – 32). Poorer uniformity (i.e. higher CV) was associated with increased first week mortality ($P < 0.004$, $r = 1.48$), increased total mortality ($P < 0.013$, $r = 0.01$), increased FCR (i.e. less efficient growth) ($P < 0.024$, $r = 0.06$), reduced growth rate ($P < 0.0012$, $r = -0.01$), and a reduced rejection rate at slaughter ($P < 0.006$, $r = -0.01$). The results show that flock uniformity varies across broiler flocks, and is associated with several production measures.

ID : 125

PILING BEHAVIOUR AND STIMULUS ATTRACTION IN LAYING HENS

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Piling behaviour (PB), the tight agglomeration of several laying hens, often leads to suffocation and is therefore considered a major welfare problem in the loose-house egg industry. Results of an observational study showed that PB is mostly triggered by specific stimuli (e.g. light spots and novel objects) and occurred when hens were attracted towards these stimuli (Winter et al. 2019). Since these observations lack experimental validity, we experimentally tested two hypotheses: one, that specific stimuli attract laying hens and, two that this attraction leads to PB. We conducted this study within 8 identical experimental pens, each holding 55 Lohmann Selected Leghorn hens between 21 - 27 weeks of age. Each pen consisted of two locations where stimuli were applied with opposite locations serving as controls, i.e., four locations/pen. We observed the animal responses towards three stimuli: a light spot (flashlight, presented for 10min), a glittering tape (for 35min), and increased local temperature (heating plate, mean: $T \sim 44^{\circ}\text{C}$, $\sim 20\text{-}24^{\circ}\text{C}$ above environmental temperature, for 35 min) at the stimulus locations. Each pen received each stimulus once as well as a control with no presented stimulus stratified over a 7-week period. To test hypothesis one, we compared the number of hens at the stimulus and the control location at specific times where stimuli were presented: light spots: at 0, 5, 10min, glittering tape and increased local temperature at 0, 5, 10, 17.5, 25, 30, 35min. We assessed the influence of the interaction between stimuli and time on the number of attracted laying hens by applying generalised linear mixed-effects models. To test hypothesis two, we compared the number of PB events at the stimulus and the control locations within each pen. Our results showed that only the light spot affected the number of attracted hens ($p < 0.0017$) with more hens being present in the stimulus location when this stimulus was presented compared to the other stimuli. In addition, the attraction to light spots occasionally led to PB (3 PB at the stimulus vs. 0 PB at the control side, $n=16$). Our findings suggest that specific stimuli attract laying hens, but that stimulus attraction does not always lead to PB. Further studies should investigate which other factors (e.g. social mechanisms) lead to PB.

Winter J, Stratmann A, Toscano M. 2019. Piling Behaviour of Laying Hens in Switzerland: Origin and Contributing Factors. 53. ISAE Congress Proceedings, Bergen, Norway

ID : 195

UNDERSTANDING BEAK BLUNTING AND ITS POTENTIAL TO BE USED AS AN ALTERNATIVE TO CURRENT BEAK TREATMENT METHODS

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There is increasing pressure to ban the practice of beak treatment in the commercial egg industry and with this comes the need to find viable alternatives. Natural beak blunting by the inclusion of an abrasive material in the feeder has been suggested as an alternative but its effectiveness or impacts on pullets are not yet fully understood. The objective of this study was to examine the effects of beak blunting on the beak characteristics, production, and welfare of Lohmann Brown-Lite (LB) and Lohmann LSL-Lite (LW) pullets. Newly hatched chicks (n=900 per strain) were sorted into 3 beak treatments: sham untreated control (C), infrared beak treated (IR), or provided with a specialized beak blunting feeder (B), which had an abrasive inner feed pan. Pullets were housed in 18 floor pens (n=100 per pen; 900 cm² per pullet) from 0 to 16 wk of age. Feed and water were provided ad libitum. Body weight, beak length, beak histology, and heterophil/lymphocyte (H/L) ratios were measured at 0, 2, 4, 10, and 16 wk. Mortality was recorded throughout the 16 wk. The study, a 3x2 factorial arrangement of beak treatment and strain, was performed in a completely randomized design. Data were analysed using PROC MIXED (SAS 9.4) with Tukey's test to separate means. Differences were significant when $P \leq 0.05$. Body weight did not differ between treatments except at 4 wk when C pullets were heavier than B pullets (290.8 vs. 284.3 g, respectively). Over the 16 wk, IR pullets had shorter top and bottom beaks compared to C and B pullets. No differences in beak length were found between C and B pullets. At all ages, B beaks showed no histological differences from C beaks. At 4 wk, all IR beaks except 1 had healed. H/L ratios did not differ between treatments at any age. Mortality was highest in the C pullets compared to B pullets over the 16 wk (7.7 vs. 3.6 %, respectively), although the primary cause of mortality was yolk sac infection and unrelated to beak treatment. LB pullets were lighter than LW from 0 to 4 wk but heavier at 10 and 16 wk. LB pullets also had higher H/L ratios compared to LW at 2, 4, and 10 wk. Mortality was higher in the LW compared to LB (8.0 vs. 3.0 %, respectively). Overall, the results suggest that while the specialized feeder did not negatively impact pullet body weight or welfare, it was not effective at blunting the beak, which poses welfare risks for outbreaks of severe feather pecking as the pullets age and enter lay.

ID : 282

TOE PECKING – AN EMERGING BEHAVIOR PROBLEM IN SWISS LAYING HENS

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During the past 3 years, an increasing number of Swiss egg producers have reported mortality due to toe pecking. Studies on toe pecking in laying hens are rare. Therefore, we conducted a survey among Swiss egg producers, examined histological changes in affected toes, and scored toe damage after an experimentally altered feeding schedule. An online questionnaire was sent out to 490 Swiss egg producers. We included 96 complete questionnaires from 48 farms with and 48 farms without toe pecking. Results were analyzed using Fisher's Exact Tests and generalized linear models in univariate and multivariate analyses. Tissues of affected and intact toes of six laying hens were stained with HE and PAS for histology. Additionally, in an experimental barn, 4 of 8 pens missed the last feeding of the day. Toes were scored before and after 7 weeks of this change of feeding schedule at 39 weeks of age. Toe pecking at the own and other birds' toes and pecks to the comb were scored from videos. Data were analyzed with generalized mixed models. Pen was taken as a random variable and for the number of pecking events a Poisson distribution was chosen. Toe pecking was almost only reported in white hybrids (Fisher's Exact Test $P = 0.01$, $N = 79$). Infections with *E. coli* during the last 5 years ($F_{1,50} = 5.69$, $P < 0.02$), metal instead of plastic slats ($F_{1,50} = 5.61$, $P < 0.02$), and direct sunlight in the barn ($F_{1,50} = 7.52$, $P < 0.008$) increased the probability for toe pecking ($N = 54$). The presence of high frequency lights also increased the risk of toe pecking ($\chi^2_{21} = 4.73$, $P = 0.03$, $N = 45$). Tissues affected by toe pecking showed ulcerating dermatitis in the deeper skin layers and the adjacent bone tissue. Treatment affected the frequency of toe pecking directed at other birds and was less frequent in the pens that missed the last feeding (treatment: $F_{1,14} = 7.58$, $P = 0.02$). The severity of toe lesions was positively associated with the frequency of toe pecking ($F_{1,14} = 5.17$, $P = 0.04$) and pecking at the comb (treatment: $F_{1,14} = 10.59$, $P = 0.006$; lesions: $F_{1,14} = 12.27$, $P = 0.004$; interaction: $F_{1,14} = 4.09$, $P = 0.06$). The severity of lesions of the toes and comb were correlated within hen ($r_s = 0.54$, $P = 0.007$, $N = 23$). Although this study suggested that toe pecking, like feather pecking, was a multifactorial problem, genetics seemed to be a major factor as only white hybrids were affected. The histological findings indicate that toe pecking is of high relevance to bird welfare.

ID : 301

A PRACTICAL TOOL TO IMPROVE BROILER WELFARE AND TO DISCUSS ANIMAL WELFARE

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Animal welfare is a shared responsibility of all the actors involved in raising animals and farmers have to meet the European citizens' demand for high broiler welfare levels. The observation of footpad dermatitis (FPD) is a common measure used in Europe to assess broiler welfare. It provides information on the welfare status of broilers but it does not cover all dimensions of animal welfare. The self-welfare assessment method EBENE® was developed in France to provide a complete overview on the welfare status of broilers through 12 scores. Mainly animal-based indicators (behaviour and health) are observed with no need to handle animals and a mobile app is available. The aim of this article is to present farmer's perception of welfare and on the EBENE® method.

The welfare perception of broiler farmers in Italy (n=15) and in France (n=11) was assessed between April and November, 2019. Open questions, multiple-choice questions and Likert-scale questions from 0 to 5 were asked. French farmers also gave their opinion on the EBENE® app.

Among 6 proposals, to improve animal welfare is the 3rd priority for farmers. To assess animal welfare (open question), only half of them rely on animal-based indicators (eg behaviours) while the other half use only resource-based indicators (eg litter quality). When asked to choose among several proposals on how they assess animal welfare, almost 60% of the farmers answered behaviours. Not all the farmers consider behaviours as part of animal welfare assessment. Half of them declare that it is not easy to observe behaviours according to the EBENE® method because they are not used to it, which may explain why only few farmers observe behaviours. However, the behavioural observation is interesting for all of them, probably because it is different from their usual observations and that it gives them positive indicators to communicate on. Farmers are more used to negative animal-based indicators to assess welfare such as lameness, injuries or FPD, probably because these measures are easier to observe. Indeed, for around 80% of the farmers questioned, health indicators are easy to observe according to the EBENE® method.

There is a need for practical tools to help farmers talk about animal welfare. Welfare assessment tools such as EBENE® might help farmers to assess all the dimensions of animal welfare, and to be able to talk about positive animal-based indicators. It is also a practical tool to improve broiler welfare.

ID : 375

DISTURBANCE OF RESTING BEHAVIOUR OF BROILERS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Sleep is essential for several important functions in the body and sleep deprivation can lead to health problems and stress. In broiler production, the chickens are generally kept in large flocks in a barren environment. In this production situation, chickens are exposed to physical disturbances by other individuals, forcing individuals to become active when they are highly motivated to rest.

Therefore, this study investigated separate resting places in the form of elevated platforms. By providing such platforms we hypothesized that the broilers would get more undisturbed rest compared to groups without elevated platforms. Three treatments were applied; treatment EP providing elevated platforms 30 cm above the litter (stocking density at 40 kg/m²), treatment SD with a lower stocking density at 34 kg/m² and a control group C (no platforms and a stocking density at 40 kg/m²). Behaviour of the chickens was video recorded in the pen, during both night and day, and recordings at days 20 and 34 of age were used for further analysis. Analysis was made in R (version 3.6.1) using an ANOVA test to compare the duration of resting bouts between treatment groups and post hoc comparisons of significant factors were made using Tukey's HSD test. Results presented as mean \pm SE.

The results show that a separate resting place increased the duration of resting bouts and reduced the number of physical disturbances between chicks. A treatment effect of the duration of resting bouts (df=2, F=8.499, p

Our results show that elevated platforms provide an area where broiler chickens can rest with longer resting bouts and with less risk of being physically disturbed by other individuals. Also, reducing the stocking density increases the duration of resting bouts. In addition, the study shows that even with elevated platforms, disturbance of sleep occurs frequently.

ID : 393

ANIMAL WELFARE INDICATORS IN BROILER FARMS ARE NOT IMPAIRED BY COCCIDIOSIS VACCINATION COMPARED TO COCCIDIOSTATS - FIELD STUDY.

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Coccidia vaccination is often associated with the reduction of antibiotic use in broiler production. Societal and regulatory expectations also include animal welfare compliance. Tarsal and breast burns, scratches and infected wounds, and rejects are indicators measured at the slaughterhouse. Mortality is the fourth criterion studied.

From March 2018 to May 2019 all chickens fed by one feed plant (plant B) were vaccinated at the hatchery with Paracox®-5 (approximately 16 million chickens). Results are compared with chicken batches fed a coccidiostat feed supplied by the same plant B over the previous period (February 2017 to March 2018) and with chicken batches fed a coccidiostat feed manufactured by another plant (plant A) from February 2017 to March 2019.

In order to take grower effect into account, only farms for which results from more than 6 batches were available were retained for the statistical analysis with the software R. The analysis of 379 batches from 32 farms is presented here.

For the mortality criterion, there was a (non-significant) trend towards a slight increase in total mortality (+0.28%) and mortality at 10 days (+0.18%) for the vaccinated batches. Since the use of TMP sulfa on vaccinated flocks is limited due to its negative effect on vaccinal coccidia recycling, it may have resulted in a few colibacillosis outbreaks with higher mortality.

It is interesting to note that while the rate of tarsal and breast burns evolves seasonally, with an increase on winter flocks, vaccination has no negative or positive impact on this criterion.

Similarly, the switch to vaccination has no impact on the rate of infected scratches and wounds.

Finally, the rate of rejects evolves in the same way over time for batches of broilers fed by plant A or plant B, before or after the introduction of vaccination.

This study therefore shows that under real field conditions, Paracox®-5 vaccination of broilers is compatible not only with the antibiotic reduction approach, but also with animal welfare regulations, the indicators on vaccinated batches being at the same level as on batches with coccidiostat.

ID : 472

INFLUENCE OF CATCHING METHOD ON ANIMAL HEALTH AND BEHAVIOUR OF BROILERS AT MANUAL LOADING

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Two manual loading techniques (1LCM = one leg catching method; 2LCM = two leg catching method) of broilers were compared concerning animal health and animal behavior. For this purpose broilers of one container of each method were evaluated within 12 loadings. Assessments of animal health prior and after loading and detailed analyses of the behavior were performed. A logistic regression model was used for statistical analysis.

By the use of the 2LCM (8048 broilers/hr) the time of loading was doubled compared to the 1LCM (3408 broilers/hr) and deviations from the target stocking density were observed with a higher variance. The risk for wing fractures was raised by the use of the 1LCM (1.12% in 1LCM, 0.42% in 2LCM). An increase of the catching duration and the height of the drawer in the container influenced the risk for wing fractures. Animals of low drawers were at higher risk for fractures, than broilers of middle or high drawers. Wing flapping was observed in 74.5% of the broilers of the 2LCM and in 83.6% of the 1LCM. A lower risk of wing flapping was achieved if the broilers were carried with a neighboring animal or grasped proximal to the body in the area of the tarsal joint. Whether the broilers are settled, dropped or thrown in the drawer significantly influences the behavior of the broilers in the drawer. The catching duration, the height of the drawer and the weight of the broilers influenced the method of settlement.

The design of the study needs to be considered as a limitation and accounted for when interpreting the results. The assessment of the broilers during loading was assessed on the basis of one container of each method within 12 loadings. In a previously performed study, we compared manual (2LCM) with mechanical loading of entire barns and observed wing fractures in 1.02% of the broilers of the 2LCM. Thus, it seems unlikely that the advantage of the 1LCM method observed in this study concerning wing fractures could be transferred to the loading of entire barns. An exhaustion of the loading staff when loading an entire barn with the 2LCM is expected and would lead to a higher risk for lack of concentration and careless or hectic performance. A deliberate and respectful handling of the broilers is essential for an animal friendly loading. By the consideration and implementation of the stated risk factors the risk for wing flapping and wing fractures can be lowered, irrespective of the number of grasped legs.

ID : 484

GENERAL PERFORMANCE AND EGG QUALITY OF LAYING HENS UNDER DIFFERENT REARING SYSTEMS

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Considering the issue of animal welfare which European Union (EU) and other nations are clamouring for, there is a need to consider such in sub-Saharan Africa. This study seeks to evaluate the general performance, production performance and egg quality of Isa-Brown laying hens reared under different systems. Ninety (90) Isa-Brown hens were used for this study and the study lasted for eighty-four days (84). The hens were randomly distributed to three (3) treatments (battery cage, deep litter and outdoor systems) of three (3) replicates and ten (10) hens per replicate in a Completely Randomized Design. Each hen was given 115g of feed per day, while fresh clean water was provided throughout the experimental period. Results show that highest final weight (1586.7g), highest percentage hen-day production (74%), highest egg weight (63.2g) and best feed conversion ratio (2.45) were observed in hens reared on battery cage system, while lowest percentage hen-day production (68.3%), lowest egg weight (56.7g) and worst feed conversion ratio (2.70) were observed in hens reared on the outdoor system. Meanwhile, highest total feed intake (9660g) was observed in hen reared on battery cage and deep litter systems, while the lowest total feed intake (8710g) was observed in hen reared on the outdoor system. Highest dressed weight (1446.6 ± 112.6 g) was observed in bird reared under the deep litter system, while lowest dressed weight (1383.3 ± 63.6 g) was observed in bird reared under the outdoor system. Highest gizzard weight (29.7 ± 1.68 g/kg body weight) was recorded in bird reared under the conventional battery cage system, while lowest gizzard weight (26.9 ± 0.46 g/kg body weight) was recorded in bird reared under the deep litter system. Hen on battery cage had the highest gross returns on the egg (N1555), while hen on the outdoor system had the lowest gross returns on the egg (N1475). In all, the highest profit (N309) was recorded in hen on the outdoor system, while the lowest profit (N284) was recorded in hen on deep litter system. In conclusion, outdoor could be considered as an alternative to battery cage system for hens considering the highest profit recorded and going by the European (EU) campaign on animal welfare.

ID : 494

LIVE INSECT LARVAE FOR LAYING HENS: EFFECTS ON PLUMAGE STATUS AND ANIMAL BEHAVIOR

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Introduction. Feather pecking and cannibalism are two phenomena widespread in intensive farming of laying hens that have negative impact on egg production. The aim of the study is to evaluate the effects of black soldier fly (*Hermetia illucens*) live larvae as environmental enrichment for laying hens on plumage status and animal behavior.

Material and methods. A total of 169 adult laying hens and 24 males of Bionda Piemontese (BP) and Bianca di Saluzzo (BS) breeds (308 days of age) were allotted to 12 pens. Each pen was assigned to one of two dietary treatments (6 replicates/treatment, from 12 to 19 birds/replicate) as follows: i) control diet (C): commercial feed and ii) HI: C + *H. illucens* live larvae. The commercial diet and water were distributed ad libitum and the amount of live larvae was distributed based on 6% of the expected daily feed intake (120 g/bird). To evaluate the plumage status, all the birds were given feathering scores for neck, breast, cloaca/vent, back, wings, tail and hump foot lesions. The behavioral observations were carried out using video recordings. Each pen was filmed for 5 minutes before larvae administration in 4 different periods. The analysed behaviors were divided into 2 different categories: duration (walking, ground pecking, standing still, resting) and frequencies (scratching, grooming, allogrooming, trotting, severe feather pecking, stretching, chasing, sand bath, feather shake, wing flapping). Data were analysed by General Linear Mixed Model (IBM SPSS Statistics V20.0.0, P value < 0.05).

Results. No significant differences were observed for both the mean and the total feathering scores between the C and the HI hens independently of the breed (P<0.05). On the contrary, the HI BP animals showed higher (better) mean and total feathering scores when compared to the C group (P<0.05). The HI birds spent more time walking than the C group (P<0.05). Differently, the other duration behaviors were not affected by the treatment (P<0.05). Considering the frequencies, the HI hens displayed more grooming, allogrooming and trotting than the C birds. On the contrary, the C group performed more severe feather pecking than the HI hens.

Conclusions. The use of live larvae as environmental enrichment positively affects the plumage status in the BP breed. Furthermore, the addition of live larvae to the commercial diet leads to a decrease in severe feather pecking and an increase in some comfort behaviors.

ID : 498

IMPACT OF PHOTOPERIOD ON THE WELFARE OF BROILERS RAISED WITHOUT ANTIBIOTICS

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The North American broiler industry is facing increasing pressure to eliminate use of antibiotics which may impact bird welfare. Therefore, it is important to assess how management factors can ensure optimal welfare in flocks raised without antibiotics (RWA). The objective of this study was to investigate the impact of photoperiod on stress, gait and footpad scores, and behaviour of RWA broilers reared to 36d. Two repeated trials were conducted with a total of 8,064 mixed run Ross 308 broilers over both trials. Birds were housed in 8 environmentally controlled rooms (12 pens of 42 broilers per room). Lighting treatments were initiated at 6d and included 14L:10D, 17L:7D, 20L:4D, and 23L:1D. Light was provided by white LEDs with an intensity of 10 lux (d6-36). At 25d, blood was collected from the brachial vein of 96 birds per treatment to assess H/L ratios. At 29d, gait (trial 2 only) and footpad scores were assessed on 40 and 80 birds per treatment, respectively. At 30d video cameras recorded behaviour in 4 pens per treatment for 24h; data were analyzed with scan samples every 15 minutes. Data were analyzed with regression analyses using Proc Reg and RSReg of SAS 9.4. Prior to analyses behaviour data were log +1 transformed. Significance was declared when $P \leq 0.05$ and a trend noted when $P \leq 0.10$. A quadratic response was observed for H/L ratio ($P < 0.01$), with highest values, indicating increased stress, found in birds reared on 23L. Mobility, assessed via gait score, showed a linear tendency for poorer mobility under longer photoperiods ($P < 0.08$). A linear increase in more severe footpad scores with increasing photoperiod ($P < 0.01$) was observed. A linear increase in time performing nutritive behaviours was observed under longer photoperiods ($P < 0.01$). Time spent standing responded quadratically ($P < 0.01$) with the 14 and 23L treatments being highest. Preening responded in a linear fashion ($P < 0.01$) with birds reared on 17 and 20L spending numerically more time preening. Birds raised on 20L spent more time environmental pecking (quadratic response; $P = 0.01$). Object pecking occurred more under 14L (linear response; $P = 0.01$). These results indicate that rearing RWA broilers on longer photoperiods has negative effects on bird welfare, as indicated by poorer mobility, chronic stress, more severe footpad lesions, and changes in behaviour.

ID : 530

THE EFFECT OF A BACTERIAL LITTER PRODUCT APPLICATION ON LITTER QUALITY AND GROWTH PERFORMANCES OF BROILERS

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The objective of this trial was to examine the effect of the application of a dry litter product (formula with a complex of microorganisms and enzymes) on the physicochemical characteristics of the litter as well as the performances of broilers. For this 194 male broilers (Ross 308) were equally divided in 2 groups of 8 pens. Bedding material was sawdust. The litter of the treatment pens (T) was seeded with the dry litter product at the rate of 30g/m² (2 applications) at trial set up (below and above the sawdust). For the remainder of the trial application of 30g/m² was repeated weekly. The control pens (C) did not receive any litter treatment. Litter quality (humidity, temperature, litter scoring and pH) was monitored regularly from start (day 0) until end of the trial (day 35). Individual body weight (BW) was registered at day 0 and day 35. Footpad dermatitis (FPD) has been assessed at day 35. Litter crust height was measured as a visible indicator of litter quality at the end of the trial. Data were analyzed according to General Linear Model with Treatment as fixed effect (SPSS v24). Results indicate a significant difference between the control and the treatment group for humidity (C=64.6% vs T=56.8%; P<0.05), temperature (C=26.4°C vs 23.4°C; P<0.05) and litter score (C= 4.7 vs T=3.4; P<0.05) indicating an improved litter quality for the treatment group. Moreover, at the end of the trial, a strong tendency of improved growth was observed for the treatment group compared to the control (BW at day 35: C: 2432.3g vs. T: 2348.6g, P=0.06). There have been a significant reduction of number of broilers suffering from FPD (C=83% vs T=31%; P<0.05) and in the severity of these FPD (Average FPD-score: C=1.41 vs T=0.54; P<0.01). A lower height of the litter crust was also recorded (C=5.7 cm vs T=4.2 cm), indicating this could be a good visible sign of litter quality. These results indicate that the application of a bacterial dry litter product can improve litter quality and therefore help improve the environment of livestock barns. A better litter quality also reduces the occurrence of FPD, which could positively impact animal welfare.

ID : 542

OPTIMIZING SPACE AND ITS IMPACT ON WELFARE BEHAVIOUR AND PRODUCTION OF COMMERCIAL LAYING HENS IN CONVENTIONAL CALIFORNIA CAGE SYSTEM

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In Asian countries commercial laying hens are mostly kept in confined conventional cages with automatic feeding, controlled lighting and ventilation to optimize production performance. Recently, in India cage rearing system was argued by animal welfare activists complaining that the welfare of the laying hens is not maintained ensuring reasonable space for standing up, lying down, flapping wings, turning around and access to feed and water. Scanty information are available in context to Asian condition to understand the welfare, behavior and stress-free production performance in conventional California cage rearing system. A 20-weeks experiment was conducted in 28 weeks old commercial white leghorn layers to investigate the required conventional cage floor space allowance per bird to optimize the welfare, behaviour and egg production. The four different experimental groups were assigned with different space allowance of 387, 484, 548 and 645cm² per bird with 18 replicates in each. Laying birds were fed commercial diet (110 g/bird) and maintained under standard management practice. Data were analyzed using SPSS software package .Higher hen day egg production (89.71 and 90.92 %, $P<0.001$), best feed conversion ratio per dozen eggs (1.47 and 1.46, $P<0.001$) and best welfare parameter (better feather score, $P<0.05$) were recorded in the birds reared at 548 and 645 cm² per bird (which were non-significant among themselves), respectively, compared to the 387 and 484 cm² space provided birds. For H/L ratio and serum corticosterone measured at the start and end of the experimental period no significant difference was detected between treatments. Behaviors of the laying birds were observed through installed video recording cameras and different activities were calculated by distributing the state and event activities of 24 hour period into time frame of one minute. Significant different behavioral activities were observed for standing, sitting, walking, investigating, drinking and feather pecking among the different treatment groups ($P<0.05$). The birds reared at 548 cm² per bird had highest activity of sleeping, feather-pecking ($P<0.001$), walking, sitting ($P<0.05$) as compared to others space allocated birds, indicating a more comfort zone. Hence it could be summarized that cage floor allowance of 548 cm² per bird provides optimum space without compromising welfare behaviour and production of commercial laying hens in conventional California cage system.

ID : 699

EFFECT OF INCLUSION OF SUN-DRIED MANGO KERNEL MEAL ON HEMATOLOGICAL PARAMETERS OF JAPANESE QUAILS

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The Increasing cost of conventional feed ingredients has compelled most poultry farmers to compound their feed. Alternate use of non-conventional feed stuff can reduce production cost. This study was to determine the effect of inclusion of sun-dried mango kernel meal (SMKM) on hematological parameters of quails.

The study was conducted at the University of Agriculture, Makurdi, Nigeria. Experimental design used was complete randomized design. 144 unsexed Japanese quails of 2 weeks old were purchased from Veterinary research center, Vom, Nigeria. The Quails were fed the control diet (Maize= 55.2%, SMKM 0%, Full-fat soybean=26.67%, fish meal=5.2%, wheat offal=6.93%, bone ash=7%, salt=0.5%, vitamins/mineral premix= 0.5%) for one week prior to the commencement of the experiment. They were randomly allotted to three dietary treatments where SMKM replaced maize at 0%, 25% and 50%. Each diet was replicated thrice with 16 quails per replicate. 3 wooden cages with wire mesh floors, roofings and side covers were used for the experiment. Each cage had 3 layers to represent the 3 treatment replicates. Feed and water were given adlibitum for 28 days and no vaccines administered. Coccidiostat and antibiotic were administered at 6 weeks of age to control coccidiosis and bacterial infections. At the end of the trials, blood samples from 10 quails were collected from each replicate by cutting through the jugular vein using a sharp knife into bottles containing ethylene diamine tetracetic acid as anti-coagulant for hematological analysis. The hematological values were compared with standard reference values.

Results showed that, White blood cells ($11.0-13.1 \times 10^9/L$) and Lymphocytes (63-74%) values increased above normal reference ranges across treatments. The values for Red blood cells ($4.2-4.7 \times 10^{12}/L$), hemoglobin (10.3-11.3 g/dL), packed cell volume (31-34%), Mean Corpuscular Volume (78-79 fL), Mean Corpuscular Hemoglobin (22-26 pg), Mean corpuscular Hemoglobin Concentration (33.1-33.2g/dL), basophil (0) and eosinophil (0) values were within the normal reference ranges. Monocytes (0-1%) and Neutrophils (25-37%) values were slightly below the reference ranges across treatments. Abnormalities observed in the hematological parameters could have been influenced by the antibiotics given to the quails. Since the abnormalities were not only limited to quails fed SMKM, it confirms that SMKM was not responsible for the abnormalities. Therefore, SMKM can replace maize for up to 50% in quail diets without deleterious effects on hematological parameters.

ID : 700

AN ADAPTABILITY INDEX FOR POULTRY GENOTYPES ORGANICALLY REARED

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In recent years, the interest towards friendly-farm systems increased. Especially in poultry, alternative rearing systems (free-range, organic, low input) spread. The EU reg. 889/2008, establishes that: "...the choice of breeds should take into account of their capacity to adapt to local conditions...". Moreover, the EU Reg. states that each Member State has to determine criteria for the definition of Slow-Growing (SG) genotypes evaluation of their adaptability to the organic system. In most EU countries, the definition of SG genotypes is based on the Daily Weight Gain (DWG); however, the relationship between genotype, growth rate and adaptability seems more complex. The present study develops an index to evaluate the adaptability of different poultry strains taking into account: explorative aptitude (calculated by time need for get out from the shelter), kinetic activity, foraging behaviour, plumage and body lesions. Accordingly, the adaptability of one hundred chicks males of six genotypes (Ranger Classic (R1), Ranger Gold (R2), Rowan Ranger (R3), Hubbard RedJA (A), CYgen5xJA87 (CY), M22xJA87(M)), were compared in organic condition (4 m² outdoor run, organic diets, slaughtering at 81 days of age). Principal Component Analysis extracted a component including behavioural and body condition variables called Adaptability index (AI). Regression models were used to evaluate its relationships with genotype and DWG. ROC analysis was also performed to find the cut-off of DWG which discriminates high and low AI scores. The AI was positively related with grass intake, walking, drinking, and plumage score while negative loadings were found for explorative aptitude, static behaviours, and body lesions. The genotype strongly influenced the AI ($P < 0.001$): R3 and A exhibited the highest adaptability score, R1 and R2 showed an intermediate value, whereas M and CY had the lowest scores. The optimal cut-off for DWG predicts the low and high adaptability of animals with a sensitivity of 87% and a specificity of 73%. Animals with $DWG \geq 38.6$ g/d showed a low adaptability whereas for values < 38.6 the DWG did not affect the AI. In conclusion, even if all the genotypes tested are "theoretically" SG, they did not show the same adaptation. Moreover, a cut off for DWG within these strains can be identified. In particular, M and CY seemed not adapted to organic system and a $DWG \geq 38.6$ g/d is associated with poor adaptability.

ID : 716

COMPARATIVE DIFFERENCES OF 6-WEEK OLD BROILERS AND 65-WEEK OLD BROILER BREEDERS DURING VENTILATION SHUT DOWN WITH HEAT

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The US Poultry Industry weathered one of the worst highly pathogenic avian influenza (HPAI) disease outbreaks in history, with 48 million turkeys, ducks and chickens depopulated in 2015. This HPAI outbreak made its mark by illuminating a need for control measures pertaining to timely depopulation methods during an epidemic of pathogenic disease. Studies have been conducted to determine the effectiveness and physiological response of the procedure on different birds using Ventilation Shut Down Plus (VSD+). The addition of heat has been found to decrease the time to death and lower stress physiology markers of broilers. In 2019, the American Veterinary Medical Association included VSD+, in the depopulation decision tree as a method dependent upon resources and timeliness. Using data from the VSD+ studies with the addition of heat (VSDH), physiology parameters of 6-week old broilers versus 69-week old broiler breeders was examined. The simulation chambers allowed for 3.4 ft³ that represents the space volume/hen under industry depopulation conditions. Brain activity was monitored by affixing electroencephalogram electrodes to non-anesthetized birds and used as a component to determine time of death (TOD). VSDH was repeated four times with each bird type. Cloaca temperatures were also taken before and after the procedure. Blood was drawn pre- and post-VSDH to determine corticosterone (CORT), and blood chemistry parameters. Upon death, brain and liver tissue were collected and preserved in RNALater® to determine heat shock protein 70 mRNA expression. The analysis of the treatment differences was accomplished using one-way ANOVA through JMP Pro 14, alpha was 0.05. Despite a significant differences in body weight between the broiler breeders and broilers ($P=0.0086$), no differences in CORT levels or HSP70 in the brain were observed. However, HSP70 in the liver has a trend towards significance ($P=0.051$) with broiler breeders having 0.611 pg/ml and broilers having 0.492 pg/ml. TOD tended to be quicker for the broilers at 75 minutes and broiler breeders at 113.5 minutes ($P=0.091$). Broiler breeders have a greater expression of HSP70 in the liver compared to broilers while also having less post-CORT. Bird type/age appears to play a more important role in CORT values and HSP70 relationships and thereby heat tolerance more so than body weight. Further studies are needed to observe this relationship in successive time points to verify points of fluctuations.

ID : 726

FORMATION OF DOMINANCE HIERARCHY BY FIGHTING AMONG GUINEA FOWL (NUMIDA MELEAGRIS)

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Guinea fowl live in flocks over a wide area in Africa. But they are bred by artificial insemination in commercial production since they have a monogamous mating system. In order to efficiently breed naturally, it is necessary to know the process of forming relationships between individuals within a group. However, there are no studies on guinea fowl that have investigated the formation of hierarchical relationships between individuals in the flock by fighting. In this study, we examined the relationship between individuals in a group by focusing on the fighting behavior and squeal patterns, and aimed to enrich our knowledge base for better floor housing of guinea fowls. We reared guinea fowl in floor pens and observed their behavior via video footage, in particular, their fighting behavior and squeal patterns. In addition, squeals were analyzed by sound spectrogram analysis. We designed three experimental groups as follows: 1st group) both male and female (two individuals each), 2nd group) only male (four individuals), and 3rd group) only female (four individuals). All three groups were built three teams (total nine teams) and observed every two weeks each. We randomly picked the birds who make up all the groups and exchanged them for new individuals. As a result, in the mixed and only-male groups, male-to-male pecking and chasing were observed. In contrast, there was almost no female-to-female fighting. Peck order was determined in approximately 1–7 hours based on the result of the fighting between male-to-male fowls. The dominant individual ate a major portion of the food, whereas the subordinate individual ate little food and crouched in the corner of the pen. All females ate sufficient food. The lighter-built male tended to be pecked by dominant individuals. Further, the pecking behavior tended to occur between near-weight individuals. We found two new squeal patterns that are associated with fighting. These new squeals were produced by subordinate males when they crouched in the corner of the pen. These results suggest that attention must be paid while group rearing male individuals, but female-to-female and female and male groupings did not need such attention as there was no or little fighting in these groups. If there is more than one male being housed in the same area, preparing a larger area with sufficient number of feeders creates a better environment for subordinate male individuals and provides a better chance for their welfare.

ID : 729

ABOLISHMENT OF BEAK TRIMMING IN LAYING HENS: FIRST RESULTS FROM NON-CAGE HOUSING SYSTEMS UNDER FIELD CONDITIONS IN GERMANY

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In order to reduce the impacts of feather pecking and cannibalism e.g. on mortality, hens kept for egg production are routinely beak trimmed in most countries. In Germany, beak trimming in hens was abolished in 2017, which is in accordance with the German Animal Welfare Act. From 2017 to 2019 a total of 92 flocks ($34,972 \pm 18,676$ hens per flock) of commercial layer breeds with untrimmed beaks were monitored under commercial housing conditions in floor, free-range and organic production systems. Mortality and laying performance were recorded daily. Additionally, the integument of the birds was visually scored in irregular intervals to derive indirect information about behavioural disorders. Deviating from former production conditions in Germany (production period until 65 – 75 weeks of age), the flocks in the current study were kept until 85.3 ± 4.5 weeks of age in white and 79.8 ± 8.4 weeks of age in brown genotypes, respectively. The cumulative mortality rate per flock and laying period ranged between 1.4 % and 29.8 %. Throughout the entire laying periods, mortality rate increased to $\geq 0.2\%$ per week at an age of around 62 weeks in brown and 87 weeks of age in white genotypes. It could be carried out, that refraining from beak trimming requires specific management strategies. Influencing factors seem to be lighting (no flicker, no light-spots), feed quality (amount of aminoacids, sodium, crude fibre), feeding frequency (more than 3 times a day), litter management (manipulable and dry litter, usable for dust bathing) and environmental enrichment by offering additional materials such as alfalfa hay, straw or pecking blocks. Flocks using one or more of these strategies had less problems with behavioural disorders. Feather pecking occurred in all flocks over the laying period, but at different weeks of age. The occurrence of feather pecking did not differ between the different housing conditions. In case of the presence of severe behavioural disorders, reducing the light intensity below 20 lux was observed to be the first countermeasure in 76 of 92 flocks. Considering animal welfare aspects, early warning systems need to be established on-farm, accompanied by indicator based management strategies in order to reduce the risk for behavioural disorders corresponding with high mortality.

Keywords:

Abolishment of beak trimming, Layers, Hens, Non-cage-housing, Mortality, Behavioural disorder

ID : 732

EXPLORATION AND NEOPHOBIA IN DOMESTIC FOWL

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Fear leads to distress which affects animal welfare and productivity in layers and broilers. Adaptation and coping strategies are favoured that enable the animal to deal with a changing environment. In local breeds, not only genetic resources might have preserved but also favourable behaviours. These behaviours include exploration behaviour, e.g. of unknown objects, which is negatively correlated with fear behaviour, e.g. neophobia. The objective of our study was to observe local breeds in different standardized tests concerning exploratory and fear behaviour directed towards objects and humans. For this reason, the dual-purpose breeds Bresse Gauloise (n = 24) and Malines (n = 25) were tested in the Novel Object test (NOT), Touch test (TT) and Avoidance Distance test (ADT) according to the Welfare Quality® assessment protocol for poultry (broilers, laying hens). For the NOT, the number of animals which undercut the distance of one animal's length to a novel object is recorded. For the TT, the number of animals which can be touched by a stationary experimenter is counted. For the ADT, the number of animals which undercut a one arm length distance to the stationary experimenter is counted. Every test was carried out at four different points in the pen on different days and a weekly repetition. Differences were found between breeds in each test, the NOT, TT and ADT (all $P < 0.001$). In each test, Bresse Gauloise have been found to show stronger fear reactions than Malines. According to the negative correlation between fear and exploration, Malines were found to exhibit a stronger exploration behaviour than Bresse Gauloise. The results of this study show that exploration and fear can be quantitatively measured in standardized tests. To favour better adapted animals, it is necessary to find a balanced relationship between these two behaviours. Local breeds like Malines and Bresse Gauloise show different adaptive values and might, therefore, be appropriate models to understand behavioural diversity and to unlock opportunities in modern breeding towards animal welfare.

ID : 761

PECKING BEHAVIOUR OF NON-BEAKTRIMMED LAYING HENS IN AVIARIES ON COMMERCIAL FARMS IN GERMANY

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Severe feather pecking (SFP) impairs animal welfare due to pain and injuries of affected hens. The aim of the project was to investigate both SFP in different functional areas of commercially used aviaries and identify influencing factors.

Behavioural observations were conducted in commercial laying hen farms in Germany. 4 out of 8 farms used Lohmann Brown (LB)-, 3 mixed LB- and Lohmann Selected Leghorn (LSL)- and 1 farm Bovans Brown- and Dekalb White-flocks. Additionally to these non-beaktrimmed flocks, 4 farms kept beaktrimmed flocks under equal Management conditions (Multi-tier-aviaries, feeding and light regime according to the breeding companys management guidelines). Video cameras were installed at different functional areas (litter, aviary platforms, perches). Pecking behaviour was analysed using "continuous recording" for 5 minutes/hour during light day. For the distribution of the flocks in different functional areas "scan sampling" was used.

To analyse effects (partial Eta-Square) and significant effects of single independent factors an univariate multifactorial analysis of variance (UNIANOVA) with the area under the curve (AUC) derived from data of the functional area usage, was calculated. The calculated average of the AUC-data for AP and SFP were tested on normal distribution using a Shapiro-Wilk-Test. As data were not normally distributed, a Mann-Whitney-U-Test was used.

The functional areas were used in a circadian rhythm. While perches were not frequently visited during day, usage at night was as high as expected. However, in some cases aviary platforms were used for roosting instead. Usage of the litter area (25%) correlated with data found in literature. A consistently high number of hens were seen on aviary platforms. The functional area had a significant effect on SFP (p one laying hybrid only) revealed a significant effect on SFP (p flocks with white and brown layers) flocks. But in these cases the factor "management" had an influence on the result. LSL-hens were responsible for the majority of SFP (59%). Beaktrimming neither had an effect on the occurrence of aggressive pecking nor on SFP.

We found a high usage of aviary platforms and the highest rates of SFP in this area. Therefore trying to increase the space of this area and/or the use of other areas (eg. litter) could help to prevent SFP. But as SFP is a multifactorial problem, an individual risk analysis is recommended.

ID : 914

CORTICOSTERONE IN FEATHERS (CORTF) AS AN APPROACH TO QUANTIFY ANIMAL WELFARE: A PROMISING TOOL FOR LAYERS?

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CORTf has become a promising tool to monitor wild birds coping with environmental situations over a longterm period. The stress-related hormone is deposited into the feathers during growth, when they are supplied with blood. In layers, CORTf may be used to assess birds' ability to cope with rearing conditions and thus, to evaluate their susceptibility of developing behavioral disorders, such as feather pecking. It may also be suitable to perform selection decision to provide stress-resilient animals. The objective of the current study was to adapt and validate a protocol to measure CORTf in layers.

Ten tail and 80 interscapular feathers of three adult laying hens, Lohman Brown, were pooled and crushed. Tail and interscapular feathers were processed and analyzed separately. Three aliquots of 50mg each were created to examine precision and specificity of the assay. 40 aliquots of 10mg each were created and five aliquots were assigned to one assessment group. CORTf extraction was done using a modified protocol of Bortolotti et al. (2008), followed by an ELISA with four repetitions each sample (n=188). For precision, intra-assay CV within repetitions and inter-assay CV amongst two assays was calculated. Specificity was tested by examining the linearity of a serial dilution via linear regression. Aliquots of one assessment group went through the same treatment to examine the extraction efficiency using five different methanol volumes, two different methods of crushing and the effect of feather types. Group effects were calculated using Kruskal-Wallis or Mann-Whitney-U test.

Intra-assay CV was 7.5% (median over all repetitions), inter-assay CV 6.4%. Linearity (R^2) was 99.7%. No significant effect was detected between the five different methanol groups ($p>0.05$) and the two crushing methods ($p>0.05$). CORTf values of interscapular feathers were significantly higher than values of tail feathers ($p<0.05$).

It can be concluded that the validated protocol is suitable to analyze CORTf in layers. To choose feathers carefully is important, as our results indicate different values in different feather types. Subsequent studies should focus on individual feathers of single hens and identifying stressful conditions during feather growth and the related responses of individuals in terms of CORTf deposition.

Bortolotti, G.R., T.A. Marchant, J. Blas, T. German. 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Funct. Ecol.* 22:494-500

ID : 919

A PARTIALLY PERFORATED FLOORING SYSTEM AND ITS EFFECT ON ANIMAL WELFARE IN BROILER HOUSING

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In practice, broilers often spend the fattening period in contact with litter with an increasing moisture and excrement content over time. Contact to those litter conditions is known to reduce animal welfare. Totally (100%) perforated flooring systems separate the animals from the litter and showed positive effects on animal health. However, this system leads to a restriction in the natural behavior of the animals due to the missing bedding material. Furthermore, totally perforated flooring systems resulted in an increased ammonia concentration, if manure underneath the perforated floor was not removed between the fattening periods. The objective of our case-control study was to evaluate the effect of a partially (50%) perforated flooring system on health indicators and ammonia concentrations compared to a conventional deep-litter system. The experimental barn was partially (50%) equipped with a perforated floor directly underneath the feeders and water lines accessible by perforated ramps. Wood shavings were used in the remaining area (50%) and in the control barn (100%). Two fattening periods (repetitions) with 31 to 32 days were carried out with 500 Ross 308 broilers per barn (39 kg/m²). Foot pad dermatitis, hock burn, and plumage cleanliness were assessed weekly for 50 randomized selected birds per barn by Welfare Quality® (2009). Ammonia concentrations were measured continuously via photoacoustic infrared spectroscopy (LumaSense Technologies SA, Ballerup, Denmark). Because there was no homogeneity of variance, a Mann-Whitney-U-test was used to analyze the health indicators and Welch's t-test for the ammonia concentrations. Results for the whole fattening period showed a positive effect of the partially perforated flooring system on foot pad dermatitis ($U(999) = 93,420.0$, $Z = -7.47$, $P(999) = 111,189.0$, $Z = -3.89$, $P(999) = 116,878.5$, $Z = -1.88$, $P = 0.061$). The ammonia concentration was higher in the barn with the partially perforated flooring system compared to litter flooring ($T(2620.35) = -9.02$, P

ID : 1142

QUANTIFICATION OF THE BENEFITS OF LOW PROTEIN DIETS ON FOOT PAD DERMATITIS INCIDENCE AND SEVERITY IN BROILER CHICKENS

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Foot pad dermatitis (FPD) is recognized as one of the main indicators of welfare in broilers. Specific nutritional strategies, especially low protein diets, are recognized as effective tools to reduce this condition. The effect of dietary protein content on dietary potassium, water intake, litter moisture and litter nitrogen content has already been quantified, allowing to better understand the mode of action of low protein diets on FPD. However, the quantification of the effects of low protein diets on FPD incidence and severity still needs further investigation.

All published and internal trials from 2007 which measured and reported both FPD incidence and severity were collected, leading to a database of 10 trials from 8 publications and 27 treatments. As birds were fed various levels of protein in different grow-out segments, weighted average crude protein content (WACP) was calculated to standardize protein supply. The different FPD scoring systems were harmonized: 0, 1 and 2 for birds with respectively no, moderate and severe FPD. The final score (foot pad score, FPS) was then recalculated according to the official method as $100 \times ((0.5 \times \text{birds scored } 1) + (2 \times \text{birds scored } 2)) / \text{scored birds}$, resulting in a number from 0 and 200, 200 being the worst score. Mixed effects model of Minitab 19 was used to quantify the impact of dietary WACP on FPS and foot pad incidence (FPI). Experiment was taken as a random factor, and the level of FPS (cFPS) or FPI (cFPI) of the control treatment was also included to test for the interaction between cFPS or cFPI and WACP.

In the database, WACP, FPS and FPI averaged $19.0 \% \pm 0.17$, 44.8 ± 6.5 and $51.8 \% \pm 4.5$, respectively. Experiment effect explained only 6% of the variation ($P=0.06$; $R^2=97$ vs 91%), making the model able to predict many situations. Dietary WACP content significantly and linearly reduced FPS and FPI (P). In conclusion, this work shows that low protein diets are an efficient strategy to control FPD by quantifying their beneficial effects on the FPD incidence and severity.

ID : 1241

IMPACT OF HOUSING SYSTEMS ON BONE HEALTH AND WELFARE ISSUES IN JAPANESE QUAIL

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Impact of housing systems on bone health and welfare issues in Japanese quail

Quails can be used to obtain meat within a short period of 4-5 weeks. The high performance in quails depends on special care and management. Bone abnormalities like low bone mass, bone deformities, and bone fractures increased the risk of osteoporosis in quails. This leads to high mortality and reduce feed consumption, weight gain and feed efficiency. The present study was conducted to address leg problems and welfare concerns under two quail housing systems used in the country. A total of 500 quail one-day old chicks were maintained in into two experimental housing systems viz., battery cage system and deep litter system. Each housing system had five replicates and each replicate consisted of 50 quails. The experimental design consisted of Completely Randomized Design and analyzed by using General Linear Model techniques with the assistance of Statistical Analysis System. The results indicated non-significant ($p>0.05$) effect of two housing systems on feed intake, mean body weight gain and feed conversion ratio up to 6 week of quail age. The mortality rate was significantly higher ($p<0.05$) in both treatment groups. Likewise, the carcass weight and the European broiler index as growth indicator was also non-significant ($p>0.05$) in both housing systems. Bone health parameters like fumer length, tibia weight, tibia length and tibia seeder index were significantly (p

Key words: welfare, quail, feed intake, weight gain, cage system, deep litter system

ID : 1300

COST ACTION 15224 'KEELBONEDAMAGE': BOOSTING RESEARCH, INNOVATION AND CAREERS IN GREECE AND SERBIA

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The aim of this paper is to present and discuss the networking opportunities and scientific outcomes of Short-Term Scientific Missions (STSMs) with exchange visits between researchers involved in CA15224 'Keel Bone Damage' in Greece and Serbia. Within the 1st STSM entitled 'KBD occurrence in different production systems in Greece', Prof Đukić Stojčić from the University of Novi Sad was hosted by the Veterinary Research Institute of the Hellenic Agricultural Organization in Greece and visited three laying hen farms with different production systems and made welfare measurements according to the Welfare Quality® Assessment Protocol for Poultry. Palpation of laying hens' keel bone and breast was made to assess both the fractures and deviations and feather plumage was scored. Egg samples were taken from each farm, in order to investigate their internal and external quality. Within the 2nd STSM entitled 'Effect of perch arrangements in furnished cages on welfare parameters, behavior of laying hens, egg quality and KBD', Dr Dedousi from the Veterinary Research Institute in Thessaloniki was hosted by the University of Novi Sad. and has visited three laying hen farms producing in different housing systems to measure welfare and behavior parameters and palpated one hundred (100) hens from each housing system. 1st STSM (Greece): The first investigation showed that KBD were present in all three observed production systems in Greece. The average range of keel damage observed in laying hens kept in enriched cages was 23.8%. Similar results were found in layers kept in free range system in which plumage score was on average 3.23. However, the incidence of KBD was higher in this system. In floor system, laying hens were 5.5 months old, with perfect feather coverage, and without other welfare problems. KBD was not recorded. Furthermore, the results showed a significant ($P \leq 0.05$) effect of housing system on the internal and external egg quality. 2nd STSM (Serbia): KBD was present only in two of the observed production systems, enriched cages and aviary system. In the aviary system, 56.4% of the birds had completely straight and flat keel, without deformation or fracture. However, KBD was recorded in 43.6% of the layers. In this production system 27.3% of the hens had deformation, 10.9% had fractures and 5.5% had both deformation and fractures.

ID : 1305

EFFECTS OF ADDITIONAL LIGHT SOURCES ON TRAINING PERIOD OF LAYING HENS AND LAYING PERFORMANCE IN AN AVIARY HOUSING SYSTEM

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Recent studies provided important insights into the requirements of light sources for poultry housing systems and their impact on performance, behavior and welfare of laying hens. This study investigated the effect of additional light sources in an aviary housing system on the percentage of mislaid eggs in the bedding area. In addition, the effect of a training light above the perch area on the perching behavior of the hens in the evening was investigated. Hens were kept in a two-story aviary housing facility, matching conventional husbandry requirements and equipped with modern lighting solutions designed for poultry on the ceiling above the litter area and below the aviary (light period 10:14 after moving, extended by 1h/week to 16:8, 20 min. dim phase). The barn was subdivided into six compartments, each with 183 either brown (Lohmann Brown Classic, LB) or white (Lohmann Selected Leghorn Classic, LSL) layers. Compartments 1 (LB) and 2 (LSL) had a tubular LED light (2.3 W, BUBO, HATO, Netherlands) above the perching area, which was switched off with a 30 min. delay to the other lights in the evening to attract the hens to the perches. The number of hens remaining on the floor was counted 15 min. after lights off on the first 14 days. Compartments 3 (LB) and 4 (LSL) served as controls with no modifications. Compartments 5 (LB) and 6 (LSL) had additional tubular LED lightings below the aviary to investigate the effect on mislaid floor eggs (22 – 45 weeks of life). Statistical analysis was run using a Welch-ANOVA on all data sets, followed by Games-Howell Post-Hoc tests for the analysis of mislaid eggs and contrasts for the effect of the training light. The additional lights below the aviary reduced the percentage of mislaid eggs in the bedding area significantly in LB ($M \pm SE$; C3control $1.10\% \pm 0.08$; C5mod $0.25\% \pm 0.04$; Welch's $F(5, 427.887) = 32.695$, $P < 0.001$), in LSL number of floor eggs was constantly low (all $M \leq 0.09\%$). Furthermore, lighting above the perching area reduced the numbers of LB hens sleeping on the floor significantly (Welch's $F(5, 38.010) = 2.859$, $P = 0.027$). These modifications might reduce the workload for farmers and increase sales of shell eggs. The study emphasizes that functional lighting has a positive effect on the hens' behavior and performance by guiding the hens to the perches in the evening and decreasing the numbers of mislaid eggs on the floor. In conclusion, functional lighting supports animal welfare in layers.

ID : 1306

INFLUENCE OF DIFFERENT FLOOR TYPES ON THE BEHAVIOR OF CHICKS

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Although broilers have special demands on their housing system, they are often kept on litter with access to feed and water. In addition to produce meat food, the housing system should support the natural behavior of the chicks. Perforated plastic floors are discussed to improve animal welfare by separating the animals from their excrements. The disadvantage of those floor systems is the lack of bedding material to show natural behavior. In this study, perforated and plane littered areas were used to observe possible behavioral preferences. Furthermore, wood shavings, lignocellulose, maize spindle granulate, maize silage, straw pellets, spelt pellets, wood pellets, loose Miscanthus chaff, and different Miscanthus briquettes (shredded or 5 mm long fragments pressed at different pressure levels) were tested. A sieve tower analysis was used to determine which bedding materials are suitable for the two floor types. Miscanthus briquettes were most suitable for the perforated plastic floor and the other materials for the plane littered area. Chicks of the dual-purpose breed Bresse Gauloise were kept in rearing boxes and observed from 2nd to 6th day of life. For the observation, chicks were filmed continuously, and videos were analyzed using the scan sampling method with a 15-minute interval. All behaviors shown were analyzed based on an ethogram and compared to a baseline trial without bedding material. Data revealed that the chicks preened significantly more frequently (all $p < .001$) and showed more locomotion behavior (all $p < .001$) in the littered area. Compared to all other bedding materials in the plane littered area, chicks showed active behavior more often on maize silage (all $p < .001$), whereby comfort behavior was carried out most frequently on Miscanthus chaff (all $p < .001$). In the area of the perforated floor, active behavior was observed more frequently (all $p < .01$) in contact with the Miscanthus briquettes (shredded; 120 and 150 bar) in comparison to finer briquettes (5 mm; 80 bar). Furthermore, the latter was used more often to show comfort behavior (all $p < .05$). Quantitative analysis revealed that behavioral categories were exhibit on the littered area more often, although resting and comfort behavior was also observed on the perforated floor. In general, different floor types and litter materials could support the functional adaptation of the housing system towards the chick's behavioral needs, especially in terms of animal welfare.

ID : 295

HOW TO DEAL WITH INTACT-BEAK LAYING HENS IN PRACTICE?

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Feather pecking is an important welfare and economic problem in laying hens. Because of national legislation or market demands, more and more hens in Europe are non-beak trimmed. It has been shown that the consequences of injurious pecking in non-beak trimmed hens (NBT) can be much larger compared with beak trimmed hens (BT) resulting in a serious decrease of animal welfare, health and flock performance. Field studies show different outcomes of NBT flocks and conclusions are often contradictory. The first experiences of the Experimental Poultry Centre (EPC) with NBT hens showed a very high mortality (25%) and a significant lower laying % (-6% per hen present) at 77 weeks of age. A second flock was started where 5300 BT hens were compared with 5300 NBT hens in a controlled set-up. All hens (Isa Brown) were from the same breeder flock, were born on the same day, were reared in identical conditions in the same rearing house, were transported in the same way to the layer house and were placed in 4 identical aviaries (2 with NBT hens, 2 with BT hens). Compared to the previous flock, adaptations to feed and light management were implemented. All groups received pecking blocks and roughage as distraction. Production data were recorded, a flock behavior checklist was performed and plumage condition was assessed every 4 weeks. Differences between the groups were ascertained with MANOVA's. The aim of this study was to evaluate the onset and evolution of injurious pecking in the groups.

Until 71 weeks of age, all hens performed above breed standards. No differences were seen in mortality and laying percentage. However NBT hens showed higher feed intake and higher feed conversion ratio. In addition, plumage condition started to deteriorate at 60 weeks of age, especially at the lower back region, and more pecking behavior was observed in the NBT hens. Further follow-up of the flock until 85 weeks of age will give more information on the progression of the parameters assessed of the current flock.

In conclusion, the current groups of NBT hens show remarkable better results compared to the first flock. However, at 60 weeks of age a turning point seems to be reached and close observation until the end of lay is mandatory.

ID : 1145

EFFECT OF A DYNAMIC LIGHT SPECTRUM ON WELFARE AND PRODUCTION IN LAYING HENS

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Prolonging the production cycle in laying hens can only be profitable when a flock shows optimal production, good welfare and health. Farmers are looking for strategies and tools to meet this ambition. Over the last few years, the importance of light in managing laying hens has been reconsidered. The evolution in lighting technology makes it possible to adapt light spectra and light intensity during the day or the production cycle.

At the Experimental Poultry Centre, a trial was conducted with 8 groups of 960 brown laying hens in commercial conditions. Four groups had a standard LED light program with a daily fixed spectrum. The other four groups received a dynamic light spectrum with longer wavelengths in the morning and evening and shorter wavelengths during the day. All the hens received the same management, feed and distraction materials. Production and welfare parameters were evaluated from 20 to 60 weeks of age. Light intensity (in lux) was measured at week 20, 40 and 60. Feather scoring and keel bone evaluation was conducted every 4 weeks.

At 60 weeks of age, there were no significant differences between the two treatments in mortality, laying percentage, daily feed consumption, feed conversion, percentage of second choice eggs and the amount of produced eggs per hen. Feather and keel bone evaluation showed no significant effect of a changing light spectrum. Although the trial could not demonstrate an effect on selected parameters, we can still conclude an important difference. Light intensity was changed during the production cycle based on flock behavior, farmer experience and group evaluation. The groups who received a dynamic light schedule could still be kept at an average of 20 lux at week 60 while the groups with a fixed program had to be kept at an average of 14.5 lux. We conclude that these dynamic light techniques can ensure longer a higher light intensity when keeping brown layers.

MANAGING POULTRY WELFARE

ID : 292

PLANT EXTRACTS TO STRENGTHEN THE NATURAL DEFENSES OF POULTRY: DEVELOPMENT OF A SELECTION TOOL

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In order to reduce antibiotics use, plant extracts use to strengthen the animal natural defences is becoming increasingly important in poultry production. However, their development as ingredients is hampered, in part, by the lack of robust and related repeatable data. Our aim was to design and test a method to assist in selection, from literature, plant extracts potentially interesting to strengthen natural defences of poultry. We were inspired by Anses methodology that aims to evaluate scientific publications and results (Saisine 2013-SA-0122 – Fév. 2018). First step consisted in establishing, with help of scientists and practitioners, two reading grids. The first allows level of reliability of bibliographic resources to be noted by verifying 1. that studied extract is correctly characterized and 2. that experimental design and results analysis are relevant to conclude about effect of tested extract. The second grid evaluates effects of studied extract on indicators of immune, inflammatory and antioxidant status of poultry. For each article, reliability score of resource is cross-referenced with effect score of extract, thus highlighting the most effective extracts. Our method has been proven. A fairly extensive bibliographic research phase on plant extracts affecting poultry natural defences, led to the selection of 917 articles, representing 48 plants. 8 of them were selected because 1. mentioned in book Bruneton "Pharmacognosie-Phytochimie-Plantes médicinales " as having effects on immunity, inflammation and oxidative stress, 2. mentioned in at least 5 articles 3. cultivable in France. Evaluation grid and methodological considerations were allowed to select astragalus, echinacea, ginseng, nigella as interesting plant extracts to strengthen poultry natural defences.

ID : 354

CONSEQUENCES OF A LOW INFESTATION BY *DERMANYSSUS GALLINAE* ON LAYER HEN PERFORMANCE

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The consequences of a high infestation of chickens by *Dermanyssus gallinae* are well documented. They include drop in lay, increase in B-grade eggs, anemia, pecking and mortality. The overall rate of flock contamination with poultry mites in Europe is estimated as being greater than 80%. However, low poultry mite contaminations were so far not considered as detrimental to the egg production industry. This study provides documentation of the consequences of low level poultry mite infestation. A study done on six layer farms, with a history of repeated, low level infestation by *Dermanyssus gallinae* investigated specifically the economic effect of low poultry mite infestations. The flocks encompassed several chicken breeds in various housing systems such as enriched cages, closed aviary or free-range aviary. The number of hens ranged from 21,500 to 190,000 birds per farm, with a total of 700,000 layer hens.

Mite infestation in these flocks for the 3 previous production cycles were controlled by spray treatment when deemed necessary, and mite infestation was considered to be low or reasonably under control. These three production cycles were evaluated as the controls in this study.

Chickens in the study production cycle were treated with Fluralaner (0.5mg/kg BW twice at a 7-day interval, via drinking water) as soon as an active mite population was found, and before flocks reached 35 weeks of age. The average observation period following treatment was 36 weeks. The aim of the study was to treat at the very early stage of mite infestation, based on visual observation during a visit, or presence of blood-fed mites in traps. The visual score (Cox et al) was below 2 in average before treatment, then the mite load was reduced to undetectable.

The study showed that even low poultry mite infestation of layer hens induces a drop in performance when not treated. Based on deviation from the breed performance objectives, the results show an average improvement of 0,6% in egg production for fluralaner treated flocks, compared to the average deviation from the breed performance objectives of the control flocks for each farm. This improvement can be explained by less disturbance of hens by mites in the nests, leading to less floor eggs in aviaries, or by better persistency in lay. The average mortality rate was equal to that of the previous production cycles, as expected with low red mite infestation.

For 50% of the farms, A and B-grade egg number and feed intake were also reported. As no standard is available for these two criteria, we compared the trial flock to the control flocks. The average improvement in A-grade egg number was 2%, and the average decrease in feed intake was 3 grams per hen per day. It has already been described that anemia and stress due to red mites may result in higher FCR.

This study demonstrates that even when *Dermanyssus gallinae* infestation is low and kept under reasonable control by spray, economic losses due to reductions in production performance, A-grade eggs and feed efficiency can be eroding profits. More effective and earlier treatment, further to animal welfare improvement and the beneficial effect on farmer's health and comfort, can improve the economic performance of layer flocks.

ID : 1083

ASYMMETRIC LENGTH OF BILATERAL MANDIBLE RAMUS CAUSES CROSSED BEAKS IN BEIJING-YOU CHICKENS

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The crossed beak is a deformity featured by the misalignment of upper and lower beaks. The occurrence of the crossed beaks is from 0.8% to 7.0% in different chicken breeds and severely impaired welfare and production. The objective of this study was to identify the occurrence regularity and morphology characteristics of the crossed beaks based on Beijing-You chickens.

A total of 9,900 female one-day-old Beijing-You chicks without beak-trimming were observed until 70 d of age for the occurrences of beak deformity. According to the bent direction of the lower beak, the crossed beaks were classified into two categories: 1) the lower beak is left-bent (LEFT) and 2) the lower beak is right-bent (RIGHT). Four chicks of each category were randomly selected at 14 and 70 d respectively to sacrifice for the characterization of the lower beak, including the mandible weight, and length, thickness, and width of bilateral mandible ramus (MR). The normal chicks (NORMAL) were used as controls. Paraffin section and HE staining was made for the bilateral mandible of a crossed and a normal beak, respectively at 7 d for histomorphology analysis.

A total of 71 out of the 9900 chickens (0.72%) showed crossed beaks. This trait occurred between 0 to 56 d of age. The frequency of LEFT did not differ from RIGHT ($\chi^2 = 0.03$, $P > 0.05$). The LEFT had shorter and thicker left MR length than those of NORMAL and RIGHT ($P < 0.05$). The RIGHT had shorter and thicker right MR length than those of NORMAL and LEFT ($P < 0.05$). The left MR length was shorter and thicker than that of the right-side in LEFT, and the right MR length was shorter and thicker than that of the left-side in RIGHT ($P < 0.05$). There was no difference in mandible weight among LEFT, RIGHT, and NORMAL groups ($P > 0.05$). No obvious histomorphological difference was observed between the bilateral mandible of normal and crossed beaks. The chicks with crossed beaks also have their upper beak often bent horizontally at the base along, and skull asymmetry (mainly nasals and orbits).

The present study indicates that the asymmetric length of the bilateral MR causing the lower beak deformity and leads to crossed beak in the Beijing-You chickens. The branches of the mandible on the short side crossed with the zygomatic arch and prevented normal occlusion, causing the curve of the zygomatic arch. The curved zygomatic arch and the muscle attached to it may have tension on the maxilla, lead the upper beak bend horizontally.

ID : 1441

RISK FACTOR IDENTIFICATION OF KEEL BONE FRACTURES IN DANISH LAYING HENS.

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Keel bone fractures (KBF) are considered the most important welfare problem for layer hens, with potential economic losses to the industry. The reported prevalences are alarmingly high in both caged and non-caged production systems, with up to 97% in non-caged systems. Identification of risk factors is necessary to understand KBF. Some efforts to identify risk factors have been made, however there has been some uncertainties in the definition of KBF, thus risk factor identification has shown to be not as conclusive as intended. The objective of the presented work was to identify risk factors of importance on the occurrence of KBF.

The current study presents the results from a prevalence study in Denmark including all production systems, including enriched cages, indoor non-caged flocks, outdoor non-caged flocks, and parent stock (60 weeks old). Forty flocks (120 birds/flock) were investigated at end-of-lay (>75 weeks old). Following euthanasia, the birds underwent inspection, palpation and necropsy. All observations and production data were analyzed (SAS statistical software).

The overall prevalence of KBF was 86%. In non-caged flocks, the KBF prevalence were 53-100%, whereas flocks from enriched cages ranged 50-98%. Furthermore, multiple KBF (≥ 4) in individual birds were often observed. Around 50% of the birds from non-caged flocks had ≥ 4 KBF, whereas 96% of KBF involved the distal end of the keel bone). Macroscopically KBF ranged from almost absence of callus, most frequent in caged birds, to large callus formations in and around the fracture lines, a frequent and typical finding in non-caged birds and birds with ≥ 4 KBF.

Parent birds had fewer KBF per bird, which is in contrast to the other birds from cage-free systems ($P=0.01$). Heavy birds from outdoor non-caged range flocks or parents stock had significantly lower KBF prevalence ($P=0.009$). Heavy birds had also fewer fractures than lightweight birds, regardless of production system. The odds ratio of age at onset of lay (+1 week) is 0.87; for every week onset of lay is increased, the risk of having fractures at the end-of-lay is reduced by 12%. Similarly, a 100 g increase in body weight at slaughter reduces the prevalence of KBF in end-of-lay hens by 3%. Other identified factors like housing, hen hybrid, and egg production are discussed.

EF10

TURKEYS

ID : 108

EFFECT OF BACILLUS SUBTILIS AND BACILLUS LICHENIFORMIS SUPPLEMENTATION IN TURKEY DIETS ON GROWTH PERFORMANCES

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The objective of the study was to evaluate the influence of a probiotic preparation containing spores of *Bacillus subtilis* (DSM 5750) and *Bacillus licheniformis* (DSM 5749), added to feed on growth performances of female turkeys reared until 84 days of age. A total of 300 day old Hybrid converter female turkeys were randomly assigned to 20 pens (15 birds/pen) allocated to 2 treatments groups T1 (basal diet without probiotics supplementation) and T2 (basal diet -T1 with probiotic preparation supplemented at 1,28.106 CFU/g of feed). All diets were offered in crumbled/pelleted form, drinking water was supplied ad libitum. Body weight (BW) and body weight gain (BWG) were determined after individual weighing in each pen and recorded on a pen basis at 0, 28, 56 and 84 days of age, feed intake (FI), feed conversion ratio (FCR) for all experimental periods and European Productivity Index (EPI) for the entire experiment. Statistical analysis was performed by a one-way analysis of variance (ANOVA) and significant differences between treatments were determined by Duncan's multiple range test, $P \leq 0.05$ were recognized as statistically significant. Mean concentrations *Bacillus subtilis* and *Bacillus licheniformis* in different phases were satisfactory T2 diet samples and corresponded to target values. The probiotic strains were not detectable in control diets. During the second experimental period (days 29-56), BW and BWG were significantly increased for turkeys fed with probiotic preparation (T2) in comparison to the control group (T1) (+4.9% on BWG, $P=0.003$). At 84 days of age, over the entire period, turkey fed with the probiotic diet (T2) had a significantly higher BW than the control group (T1) (+4.02%, 7.659 vs 7.363 kg). In this study, no significant difference on FCR in between T1 and T2 was obtained on all the feeding phases but an overall trend of -1.8% in favor of turkeys fed with probiotic preparation (T2) for the entire experimental period. In term of productivity performances, female turkeys from group T2, fed-supplemented diets, achieved higher significant EPI values (481 vs 453, $P=0.028$). For birds which received the probiotic preparation, significantly higher final BW, BWG and EPI were achieved. The results of this study indicate that a preparation of *Bacillus subtilis* and *Bacillus licheniformis* positively impacted the zootechnical performances of female turkeys.

Keywords: *Bacillus subtilis*, *Bacillus licheniformis*, turkeys, zootechnical performances

ID : 324

EFFECT OF DIETARY SUPPLEMENTATION WITH VITAMIN E AND ORGANIC SELENIUM ON TURKEY MEAT QUALITY DURING STORAGE

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Turkey meats have relatively high PUFA and heme pigment contents, which make them prone to oxidative reactions that can affect their storage stability. This study evaluated the effect of vitamin E level (vitE; dl-tocopheryl acetate at 70 [Basal-B] vs. 300 ppm [High-H]) and selenium (Se) sources (inorganic [I] vs. organic zinc-L-selenomethionine [O], both at 0.3 ppm) on quality traits and oxidative stability of turkey breast and desinewed minced thigh meat. A total of 1,064 one-day-old female turkey poults were randomly divided into 4 experimental groups: BI, BO, HI, and HO. At processing (100 d, avg live weight 9.7 kg), 200 Pectoralis major (PM; 50/group) muscles were randomly collected to evaluate quality and technological properties of fresh meat, as well as oxidative stability after 3, 7, 10, and 12 days of storage at 4°C in modified atmosphere packaging (30% CO₂, 70% O₂). The same parameters were assessed on desinewed minced thigh meat after 3 and 90 days of frozen storage at -20°C. Data were analyzed as a 2x2 factorial design (ANOVA) to investigate the main effects of vitE and Se source, as well as their interaction at P < 0.05. Supplementation with O-Se resulted in remarkably higher muscle deposition of this nutrient, as well as lower cooking losses at 3 d. In detail, HO exhibited the highest Se content (0.035 mg/100 g meat), suggesting that adding H-vitE may enhance muscular deposition of Se. Regardless of Se source, supplementing turkeys with H-vitE resulted in darker meat at 3 d and increased oxidative stability of the lipid fraction (up to 10 d). At this time point, a significant (P = 0.001) interaction was found for TBARS: BO and HO exhibited the greatest and lowest values, respectively, whereas BI and HI did not differ from each other. On the other hand, aside from the vitE level, a significant (P < 0.01) antioxidant effect (reduced TBARS) of O-Se was observed when performing the analyses at 12 d. With regard to desinewed minced thigh meat, H-vitE significantly (P < 0.05) increased the lipid fraction stability after 3 d of frozen storage. This can be ascribed to the ability of vitE to act as a scavenger against lipid oxidation. However, a time-dependent effect can be hypothesized, as this effect was not observed at 90 d. In conclusion, because of their time-dependent antioxidant effect, the supplementation with H-vitE and O-Se may be considered as a promising feeding strategy to control oxidative reaction in turkey meat during storage.

ID : 418

EVALUATION OF ALTERNATIVE ON-FARM EUTHANASIA METHODS FOR BROAD BREASTED WHITE TURKEYS

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Euthanasia of market weight turkeys using manual cervical dislocation is not feasible due to their size and the strength required by the operator, which has led to the use of blunt force head trauma. The objective of this study was to evaluate euthanasia application efficacy of four veterinary approved alternative methods: carbon dioxide (CO₂), Turkey Euthanasia Device (TED), Koechner Euthanizing Device (KED), and electrical euthanasia in achieving rapid unconsciousness and death in broad breasted white turkeys. Euthanasia methods were applied to males and female turkeys (18.8, 12.8 kg) prior to exsanguination for processing. To improve safety, ease of use for a single operator, and permit timed observations, CO₂, TED, and KED methods were performed with birds placed in a mobile bird euthanasia apparatus. For CO₂ euthanasia the head was enclosed in a single-bird portable device for 4 min (n=13). TED was applied at the crest of the head (n=13). KED was placed at the base of the skull, perpendicular to the neck, and the handles pressed together (n=12). Electrocutation was applied at 110 V AC for 15 s to induce cardiac fibrillation (n=16). Effectiveness was assessed based on kill success after one attempt, broken skin (presence/absence), external blood loss (presence/absence), and durations of persisting nictitating membrane reflex, gaping (jaws opening/closing), and musculoskeletal movements. Nonparametric data were analyzed using Chi-square and duration data using the GLM procedure of SAS with means separated by Tukey's HSD test. There were no differences between methods for kill success; CO₂ (13/13), KED (12/12), TED (12/13), and electrical (15/16). Broken skin (BS) and blood loss (BL) were more frequent in TED (13/13 BS, BL) and KED (10/12 BS, BL) than CO₂ (0/13 BS, BL) and electrocution (0/16 BS, BL, P<0.0001). The induced nictitating membrane reflex duration for KED (174 s) was longer than TED, CO₂, and electrocution (0, 0, 0 s, P<0.0001). Gaping duration after KED application persisted longer (145 s) than TED, CO₂, and electrocution (0, 0, 0 s, P<0.0001). KED resulted in significantly longer duration of muscle movement (184 s) than TED (144 s) which was longer than both CO₂ and electrocution (0, 0 s, P<0.0001). All methods were determined to be successful with CO₂ and electrical euthanasia resulting in minimal skin damage or blood loss. Electrical euthanasia and CO₂ resulted in the shortest duration of reflex and muscle movement, followed by TED and KED.

ID : 489

EFFICACY OF A NICARBAZIN/MONENSIN, A NOVEL COCCIDIOSTAT COMBINATION PRODUCT, FOR THE CONTROL OF COCCIDIOSIS IN TURKEYS

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The efficacy of Monimax® (nicarbazin/monensin) at a concentration of 40mg/kg (40ppm) nicarbazin and 40mg/kg (40ppm) monensin, administered in feed was assessed in turkeys after an experimental coccidiosis challenge under floor pen conditions.

Birds were reared without coccidiostats till the age of 13 days when they were allocated to the different treatment groups. Monimax® treated birds were compared to an infected untreated control (IUC) and an uninfected untreated control (UUC) group. Each group consisted of 6 replicate pens (6 BIG 9 males/pen). Daily weight gain (DWG), feed conversion ratio (FCR), intestinal lesion scoring (ILS) for the pathogenic *E. meleagrimitis* and *E. adenoeides* species and oocyst shedding were compared after an experimental challenge with coccidiosis. Statistical analysis methodology was in accordance with those outlined in the WAAVP guidelines for evaluating anticoccidial drugs in chickens and turkeys (Holdsworth et al., 2004). All tests were 2-sided and the level of significance was set at 5%.

At 16 days of age all birds in the IUC and Monimax® groups were inoculated with a mixture of *Eimeria meleagrimitis* and *E. adenoeides* (European origin). Birds from the UUC were sham-inoculated.

The challenge was successful as proven by the significantly lower DWG, increased FCR, higher average lesion scores and oocyst production in the IUC compared to the UUC.

Administration of Monimax® was able to significantly improve performance (DWG and FCR). In the acute (D16-23) and subacute phase (D23-D28) after challenge no significant differences were noted between the Monimax® supplemented birds and the UUC. ILS at D23 for *E. meleagrimitis* and *E. adenoeides* as well oocyst counts at D23 and D28 were significantly suppressed by Monimax® supplementation in comparison to the IUC.

The results from this trial demonstrate the efficacy of Monimax® (nicarbazin/monensin) for prevention of coccidiosis in turkeys. Under present study conditions, we can conclude, based on significant lower oocyst shedding, lower coccidiosis lesions and significant better performance, that the administration of Monimax® proved efficacious against coccidiosis infection in turkeys.

Holdsworth PA, Conway DP, McKenzie ME, Dayton AD, Chapman HD, Mathis GF, Skinner JT, Mundt HC, Williams RB; World Association for the Advancement of Veterinary Parasitology. WAAVP guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. (2004), Vet. Parasit. 121: 189-212.

ID : 665

DIETARY NITROGEN REDUCTION STRATEGIES IN TURKEY FEEDS

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Introduction and Objective

Minimising nitrogen emissions from turkey production systems is a major priority for both the industry and turkey breeding sector. An evaluation was conducted applying various strategies to reduce dietary Nitrogen in commercial turkey diets.

Materials and Method

A total of 1,968 male Premium male turkey poults were allotted in groups of 80 to 24 pens, stocking density was 3.2 birds/m². Treatment 1 (TRT1), was formulated to typical commercial diet crude protein (CP) levels. TRT2 CP was reduced by 1% in absolute terms while in TRT3 CP was reduced by 2%, in both these treatments Lys, Met+Cys and Thr specifications were the same as in TRT1. TRT 4 CP was reduced by 1% with amino acids L-Ile, L-Arg and L-Val added to balance these amino acids from P1 to P7 as per TRT1. TRT5 CP was reduced by 2% with amino acids L-Ile, L-Arg and L-Val added to balance these amino acids as per TRT1. TRT6 CP was the same as TRT1 for P1 to P4 diets while P5 to P7 diets were the same as per TRT3 P5 to P7 diets.

Results and Discussion

There was no significant difference in bodyweight between TRT1 (control) and the other treatments however bodyweight was significantly higher ($P<0.05$) in TRT4 and TRT5 relative to TRT2 suggesting a bodyweight response to correcting amino acid balance of lower crude protein density diets. TRT4 and TRT5 showed a numeric improvement in FCR relative to all other treatments. There was no difference in breast meat yield (BMV) between TRT 1 and the other treatments, however BMV in TRT4 and TRT5 was significantly higher than in TRT2 and TRT3 ($P<0.05$) suggesting that readdressing the amino acid balance of the lower crude protein density diets recovered BMV to the level of the control. Estimated nitrogen intake was significantly lower ($P<0.05$) in TRT 3 relative to the control while all other treatments trended toward lower intake relative to the control. Litter nitrogen was analysed in pens fed treatments TRT 1, 3, 5 and 6 and followed a similar trend to nitrogen intake, lower levels in TRT 3, 5 and 6 relative to the control.

Conclusion

The results of the trial demonstrate that significant reduction of dietary protein, through all phases of the growing period, is possible without compromising performance. However the entire profile of essential amino acids also needs to be considered. Reduction in dietary CP levels reduced nitrogen intake which had a positive impact on litter nitrogen levels.

ID : 701

LED LIGHTING COMBINATION EFFECTS THYROID HORMONES IN TURKEYS

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Lighting consists of three main components: daylength, intensity, and chromaticity with each potentially impacting bird well-being, including plumage. Triiodothyronine (T3) is the bioactive form of thyroid hormone facilitating feather growth and linked to photorefractoriness in turkeys. To quantify the effects of lighting regimens on turkey hen feather coverage, the objective of these two studies were to evaluate the effects of lighting combinations on T3 hormone levels in turkey hens. The first study consisted of long and short (18h & 12h) daylengths (DL) in combination with 5000K (5K) LED and 5K LED + RED LED in environmental controlled rooms (ECR); natural decreasing DL and 5K LED blocked for 18h in natural, curtain sided facility for a total of 6 treatments. The second study consisted of high and low intensity (10 footcandles (FC) & 2FC) in combination with 5K LED and 2700K LED in ECR; natural lighting conditions supplemented with 14h of 75 Watt incandescent bulbs to match DL but naturally fluctuating intensity for a total of 5 treatments. In both cases, blood was collected from the brachial vein and serum was analyzed using a commercially available RIA kit for T3. Data were analyzed using JMP14 as a one-way ANOVA and significance obtained using Tukeys HSD procedure at P0.05). Based on the first study, there was a significant difference in feather coverage recorded by infrared imaging at 9 WOA ($P<0.0001$). However, this same effect was not recorded in the second study which focused on light intensity ($P=0.56$). At 9 WOA, birds exposed to an 18h DL with 5K LED in an ECR had significantly higher T3 ($P=0.001$) than bird exposed to natural conditions, natural conditions + 18h DL 5K LED, and 12h DL of 5K LED in ECR (5.29, 3.98, 3.90, and 3.86 ng/mL, respectfully). Birds exposed to RED LED, both 18h and 12h DL in ECR, had an intermediate response (4.65 and 4.37 ng/mL, respectfully). Based on previous results using infrared imaging, these results in hormone levels do not directly correlate with the recorded feather coverage ($R^2=0.688$). Therefore, actual feather coverage may be further affected by behavioral responses of birds under various lighting regimens which will have to be quantified further to analyze feather pecking and other detrimental behaviors to feather coverage.

ID : 802

EFFECTS OF DIETARY SUPPLEMENTATION OF ORGANIC, INORGANIC AND NANO PARTICLES OF CHROMIUM ON IMMUNITY, ANTIOXIDANT PARAMETERS AND NUTRIENT COMPOSITION OF BREAST AND THIGH MUSCLES OF TURKEY POULTS.

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Trace minerals are routinely added to diets, involved in numerous biochemical functions, making them essential for optimal bird growth and health. Chromium (Cr) is essential for poultry. Chromium in poultry feed is basically supplemented in three forms i.e. organic chromium, inorganic chromium and nano particles of chromium picolinate. There are very few research findings showing comparative effects of dietary supplementation of different forms of chromium as feed additive in poultry including turkeys. Hence, the present study was designed to study the effect of dietary supplementation of organic, inorganic and nano chromium particles on the immunity, antioxidant parameters and nutrient composition of breast and thigh muscles of turkey poults. One-hundred-and-twenty-day old straight run turkey poults were divided into four treatment groups comprising of three replicates and ten birds in each replicate. T1 birds were fed basal turkey diet, T2 were fed basal diet + inorganic chromium@ 0.5 mg/ kg diet, T3 were fed basal diet + organic chromium@ 0.5 mg/ kg diet, T4 were fed basal diet + nano chromium@ 0.5 mg/ kg diet. The general innate immune-competence status of turkey poults were assayed by measuring two important immunocompetence traits, as antibody response to SRBC and cell mediated immune response to PHA-P after 8 weeks of age (Corrier and De Loach, 1990). Concentration of IgG, IgM and Cortisol was determined in the serum of experimental birds by ELISA kits. Superoxide dismutase (SOD) activity in serum was measured using the method as described by Madesh and Balasubramanian (1998). The MDA assay was done by the TBARS (Thio Barbituric Acid Reactive Substance) method of Ohkawa et al. (1979). Samples of the experimental feed, breast (pectoralis major) and thigh (ilio tibialis) muscles were processed and analyzed for dry matter (DM), crude protein (CP), ether extract (EE) and total ash (AOAC, 1990). Chromium content of the experimental feed, breast and thigh meat was determined by Atomic Absorption Spectroscopy. The data were analyzed statistically as per the standard procedure (Snedecor and Cochran, 1989) and difference between the treatment means were obtained by using Duncan multiple range test (Duncan, 1955). There was no significance difference observed in humoral immune response to 1% SRBC and cell mediated immune response to PHA-P among the treatment groups. There was no significant difference observed in serum concentration of cortisol, IgG and IgM. Serum SOD values were significantly higher (Ppectoralis major) and thigh (ilio tibialis) muscle among the treatment groups. Chromium content was significantly higher (P

ID : 960

VALUE OF AN AUTOGENOUS VACCINE AGAINST ORNITHOBACTERIUM RHINOTRACHEALE IN TURKEY BROILERS ON TECHNICAL PERFORMANCES AND ANTIBIOTIC REDUCTION

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Nowadays, more than ever, commercial turkey producers need more alternatives to antibiotics and medication.

In the EcoAntibio 2012, a French national plan aiming to reduce the risks of antimicrobial resistance in veterinary medicine, it was stipulated that autogenous vaccines are an attractive alternative for minor species including turkeys.

Statistical results of RESALAB laboratories of the « Cristal Group », shown that *Ornithobacterium rhinotracheale* (ORT) is the second cause of pathology diagnosed in commercial turkey production after colibacillosis requiring antibiotic treatments.

According to the French network of animal diseases, 92% of ORT isolates are from commercial turkey production in 2018. This bacteria is mostly isolated of pneumonia and arthritis (49,5% and 29,8% of isolates respectively). In case of septicaemia, ORT infections are third after *E. coli* and *Enterococcus*.

With this in mind and in the logic to promote ORT autogenous vaccines in commercial turkey production, a group of veterinarians RESEAU CRISTAL conducted a comparative study based on economic and antibiotic exposure indicators of two groups of turkey flocks, without autogenous vaccines (N=20) and with autogenous vaccines (N=20).

Currently in France, there are not a commercial vaccines for ORT and the only effective solution offered to turkey producers are the autogenous vaccines. But this solution is still for the moment not systematically used for various reasons and obstacles that we want to overcome through this study.

We collected the antibiotic prescription data for each flock in order to calculate indicators of exposure to antibiotics, farm and slaughter data for one economic approach.

The results of this study and the market expectations tip the balance of the comparison in favor of the group of flocks having received the autogenous vaccines. For example, for the number of treatments, we reduce from 1,3 for flocks without autogenous vaccines to 0,9 for flocks with autogenous vaccines, and the ALEA from 2,05 to 0,8.

This allowed us to build relevant communication tools in favor of this alternative solution as a means of essential medication for the meat turkey sector.

For further, we carried out a serological follow-up study after autogenous vaccines to assess the success of the vaccination. The results demonstrate the benefit of serology to evaluate good practices of autogenous vaccines activity.

ID : 1116

ASSOCIATIONS BETWEEN WELFARE MEASURES ON FARM AND SLAUGHTERHOUSE DATA IN FLOCKS OF TURKEY HENS (MELEAGRIS GALLOPAVO)

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There is a demand for documentation of animal welfare status in meat production industry. Work on turkey welfare has focused on toms at the end of production cycle, since this period is considered most challenging. Information on the relationship between welfare on farm and slaughter house recordings is currently lacking in flocks of meat turkey hens. The aim of this study is to investigate the relationship between routinely collected registrations at the slaughter house from turkey hens and their welfare measures on-farm by means of the transect walks. The current study was conducted between November 2017 and March 2018 in 20 commercial turkey flocks on farms in Norway. On each farm, one barn, divided into tom and hen area, was evaluated using the transect walk method when the birds were 11 weeks old. An observer walked the transects in random order and recorded the total number of birds per transect that were: immobile, lame, with visible head-, tail- or wing- wounds, small, featherless, dirty, sick, terminal, or dead. Routinely collected data from the slaughter house was provided for each flock. We calculated Spearman correlations using the PROC CORR in SAS 9.4. The prevalence of birds identified at the slaughterplant with old wing fractures was positively correlated ($r=0.52$) with the percentage of birds with wing wounds on-farm. This suggest that old wing fractures noted at the slaughterplant can be a potential indicator of the birds' condition during the production cycle. The results also showed that a higher prevalence of wing wounds on the farms was associated with more airsacculitis identified at the slaughterplant ($r=0.47$). The wing fractures observed at the slaughterplant may result from the final period of birds' life on-farm, when the disease could be intensifying and when birds are suspected to flap their wings to support breathing. We found that featherless birds had a higher rejection rate due to odor at slaughter ($r=0.45$). Featherless areas may reflect feather pecking occurring on farm, which is a well-known issue in turkeys, since olfaction may play role in regulating bird behavior, and has been suggested to be involved in feather-pecking. On farm welfare of the hen turkeys can be retrospectively suspected based on parameters obtained from the slaughterplant, mainly related to wing fractures. Some like rejections based on the odour may be promising on-farm welfare indicators collected at the slaughter, however need further investigations.

ID : 42

TURKEY PRODUCTION AND HEALTH : CURRENT CHALLENGES

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In the future, several challenges and problems, in addition to already existing, will face turkey production worldwide. These include strong global competition between countries and other food producing animals. Furthermore, the continuous changes of consumer perceptions with regard to food safety, animal welfare and environmental protection.

The loss of consumer confidence and trust in the quality and safety of poultry meat and poultry products will remain a continuous major challenge. Several foodborne bacterial infections such as Salmonella and Campylobacter are linked to poultry and poultry products. Control and/or elimination of these organisms present a great challenge. Furthermore, the spread of antibiotic resistant bacteria such as Vancomycin-resistant Enterococci (VRE) and Methicillin-resistant Staphylococcus aureus (MRSA) will also be a continuous public health hazard.

The future concept of animal health will cover not only the absence of disease in birds, but also the relationship between the health of animals and their welfare. It will also take into account social, economic and ethical considerations, as well as support the achievement of a high level of environmental protection.

Emergence and re-emergence of infectious turkey diseases will remain an important never ending challenge. Currently and in the future, only a few authorised pharmaceutical veterinary products will be available for the treatment of turkeys as food producing animals. The development of efficient vaccines against bacterial infections will lead to a reduction of the use of antibiotics and subsequently will reduce the development of resistant bacteria.

Continuous improvement of rearing technology, management and nutrition to maintain bird comfort and healthy are essential.

Finally, farmers, veterinarians, stockholders and all other partners involved in the production chain need to share more responsibilities.

ID : 1035

THE PREDICTION POWER OF BODY WEIGHT WITH MACHINE LEARNING AND DATA MINING OF MICROBIOME AND PERFORMANCE DATA OF MALE TURKEYS FED FORMIC ACID.

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Formic acid has been used in poultry feed to reduce the microorganism load and for acidification purposes. It affects the microbiome and ultimately the body weight (BW). The objective of this effort was to apply Machine Learning (ML) and Data Mining (DM) algorithms to predict with high confidence the BW at 19 weeks of age and to data-mine the most important attributes (variables) for the model. Nicolas Select male poult (1056) were randomly assigned to 48 concrete-floor pens. The experimental design was a completely randomized block design with a one-factor arrangement of 4 levels of formic acid at 0 lb/ton, 4lb/ton, 8lb/ton, and 12lb/ton (Amasil Na[®], BASF SE, Ludwigshafen, Germany). BW at 19 weeks of age was analyzed in SAS 9.4 in a mixed model. Birds' BW, weekly body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), gut pH, and microbiome data were used as variables for the ML and DM analysis. A total of 352 variables were used in ML and DM in both continuous (regression) and categorical (classification) analyses. BW at 19 weeks of age was classified as low and objective based on a 10% for BW margin of the Aviagen male objectives at 19 weeks of age. Support vector machine, neural networks, linear models, decision trees, Bayes, and k-nearest neighbors algorithm were used for ML with a 10-fold cross-validation system using WEKA 3.8. As a categorical analysis, the Support vector machine (SMO) yields an average of 91 % correctly classified BW at 19 weeks of age with an optimal of 7 variables. In comparison, decision trees (M5P) created a model with an average correlation coefficient of 0.93 to predict BW at 19 weeks with 3 variables. Different levels of formic acid were not statistically significant or were part of both classification and regression models to predict BW at 19 weeks of age. Both models decrease in efficiency when the attributes for modeling used are closer to the placement. In conclusion, Formic acid and its potential change in the microbiome were not deemed as important by ML and DM algorithms in both regression and a classification models. The predictive and attributes model is different depending on the question asked and the age of the attributes when the prediction is done. For this effort predicting with a regression model would serve as the most practical for application in the field. These ML and DM tools could be applied to turkey research and production systems for prediction and attribute selection.

ID : 1290

EFFECT OF THE DIETARY ADMINISTRATION OF A YEAST FRACTION RICH IN MANNAN-OLIGOSACCHARIDES AND B-GLUCANS (1,3 AND 1,6) ON FECAL MICROBIOTA AND PRODUCTIVE PERFORMANCE OF TURKEYS

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The dietary administration of prebiotic compounds is thought to positively affect productive efficiency and health of poultry mainly by modulating the gut microbiota composition. The aim of this study was to investigate the effects of the dietary administration of a yeast fraction rich in mannan-oligosaccharides and β -glucans (1,3 and 1,6) on fecal microbiota and productive performance of turkeys. A total of 1,620 one-d-old female poults (BUT Big 6) were randomly divided in 2 experimental groups (9 replicates of 90 birds/each): CON, fed a commercial basal diet, and TRT receiving the CON diet supplemented with the feed additive (500 g/ton). Productive traits were determined on a pen basis at the end of each feeding phase (16, 37, 56, 77, 91, and 106 d). Fecal samples were collected at 21 and 85 d (n=18 samples/group/sampling time) and microbiota analyzed using a 16S rRNA-seq-based approach. Turkey-Kramer t-test was applied to compare the means of the relative frequency of abundance of specific taxonomic groups identified in CON and TRT whereas productive performance data were analyzed through Student-T test. Microbiota analysis revealed interesting effects ascribed to the treatment. Feces from TRT turkeys were characterized by a lower abundance of Gammaproteobacteria (mainly Enterobacteriaceae including Klebsiella, Shigella, Escherichia, Enterobacter, Erwinia and Serratia) at 21 d (0.45 vs. 20.6%, for TRT and CON group, respectively; $P<0.001$) associated with a significantly greater amount of health promoting microorganisms at 85 d (i.e. Lactobacillus spp., Clostridium bifermentas and Pediococcus acidilactici). Growth performances of CON and TRT turkeys were similar till 91 d. However, TRT group reached a lower body weight at slaughter (9,508 vs. 9,658 g; $P<0.05$) due to worsen productive performance during the last feeding phase (92-106 d) which might be possibly ascribed to an overdosage of the feed additive for this particular period of growth. Taken together, these results indicate that the dietary supplementation of the yeast fraction determines positive effects on fecal microbiota composition of turkeys promoting healthy gut conditions; however, the dietary inclusion level requires additional insights and fine tuning for the last period of life.

EF11

EDUCATION

ID : 1024

VICKY THE VIRTUAL CHICKEN

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As part of Poultry Hub Australia's training initiative, we have developed a virtual chicken experience. 'Vicky' the virtual chicken allows users to experience all parts of the chicken from inside out. This project aims to provide not only an interactive learning tool but also an engagement tool that will facilitate a greater understanding and interest in the Poultry Industry.

The experience is run through virtual headsets and responds directly to the user ensuring each experience is unique. Both meat and egg chickens can be explored separately allowing the user to experience what each bird looks like inside. It is possible to explore different layers of the chicken such as organs, skeletal, muscle, separately just as you would if doing a live dissection. The meat chicken focuses on the different cuts of meat and the laying hen highlights the reproductive system allowing users to explore where an egg is made. There is also a testing function that asks the user to select a specified part of the chicken with tweezers and place on the tray, providing direct feedback to the user on a screen that indicates if they selected the correct part. The testing function can be played in a challenge mode providing an output score and printable certificate. The testing function collects metrics that may be used by an assessor to evaluate a user's competency.

This interactive tool has applications across all levels of education. We have already received fantastic feedback from users within Australia and we look forward to bringing this resource to the international poultry industry and public.

ID : 1151

QUALIFICATION AND CONTINUING EDUCATION FOR PEOPLE WORKING IN THE POULTRY SECTOR

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The framework of the poultry production is constantly changing. New production systems and techniques, an increasing importance of animal welfare as well as management tools and the current level of scientific knowledge are challenging the competences of people who work with poultry at different stages of production. Furthermore qualification and continuing education in order to approve, expand or renew knowledge becomes more and more important in quality assurance schemes (e.g. supplier contracts) and is even required e.g. in the German Animal Welfare Act. Therefore, on the one hand people are 'required' to participate in further training and on the other hand people participate voluntarily in order to improve their competences. Thus, continuing education – and the certificates to validate that it has happened – is therefore mandatory for those involved in the poultry industry. This emerging demand opens the discussion for a closer integration of vocational training, continuing education and advanced further education. Presently, the "in" catchwords are "Dual Course Studies" and "Open Universities". Within a project, funded by the German Federal Ministry for Education and Research (BMBF), the University of Applied Sciences Osnabrück founded the "Osnabrücker Poultry Academy" (OPA) which worked out a concept that may meet all demands and requirements and may be best explained using the example of the certificate course "Poultry Professional" (PP). The PP is an on-the-job certificate course targeting people working in the poultry sector who want or need to approve, renew or expand their knowledge and skills. The course, which comprises 300 hours workload and covers level 5-6 of European Qualification Framework (EQF), is designed as a blended learning education program combining online digital media with traditional classroom methods. The gained certification "Poultry Professional" and acquired knowledge must be maintained by collecting 20 continuing education credits (CEPS) within 2 years. Therefore the "Poultry Professionals" have state-of-the-art knowledge. However, the same program can also be completed on EQF level 6-7 which includes further examinations and workload (600 hours). During the project, which started 2017 and ended in 2020, we were able to train 50 persons who now hold a PP certificate and are now obligated to maintain their certificate through continuous training. The PP course is offered twice a year in two different forms: block course (11 days) or 11 single courses (one course per month). The different types of offerings adress the heterogenic target group and their respective possibility to take the course part-time. Generally, the demand for the PP course is high and cannot sufficiently be matched at the moment. Thus, the OPA will expand their offerings in width and depth as continuing education offers quality assurance in the whole production chain and addresses inter alia the sensitive issues of food safety and animal welfare and thus, may promote the public image of the poultry industry.

ID : 372

INVESTIGATION OF RISK TOLERANCE AND RISK ATTRIBUTES OF POULTRY FARMERS IN TAIWAN

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The perceptions of risk and risk management of farmers have considerable influence on their management and implementation of preventive strategy. The study investigated the agricultural background, financial background, and risk self-assessment of poultry farmers in Taiwan, to provide the references of management strategy and self-risk checking for poultry industry. There were 81.3% male respondents in 107 valid questionnaires (missing value under 30%), 81.7% were full-time farmers and 82.2% was the breadwinner of a family. 35.5% loaned from the farmers' association and 46.7% were own funds. There was a significant relationship between the source of funding and the full-time / part-time ($P<0.01$) and working capital ($P<0.05$). Most poultry farmers did not have a relevant academic background but hold actual experiences. 57.0% of respondents were second generation of farmer, 74.0% had more than 5-year farming experiences, and 84.9% had at least one agriculture-related job before thought only 9.3% had agricultural academic background. The average of self-assessment risk tolerance level of poultry farmers were 6.3, high risk tolerance group seemed higher than other groups divided by age-adjusted risk interval. We investigated the selection of various risk sources of poultry farmers including human, production, financial, sale and organizational risks, each question in each risk section were given a certain related risk tolerance score. All respondents were divided into three group, which were conservative group, steady group and active group, according to the total risk scores. The results showed that the risk tolerance scores in agricultural related experiences, breeding methods, expenditure sustain, acceptance of price fluctuations, acceptance of investment loss ratio, level of financial loss impact, stop loss disposal, increasing investment, joining the organization and information sharing were different among groups by ANOVA test. Conservative group had lowest risk tolerance scores in agricultural related experiences, breeding methods, acceptance of price fluctuations, level of financial loss impact, stop loss disposal, increasing investment, joining the organization and information sharing. The scores of expenditure sustain and acceptance of investment loss ratio were highest in active group. In general, poultry industry farmers in Taiwan were steady on the risk attributes of investment.

ID : 1350

GAUGING USER ENGAGEMENT OF BACKYARD POULTRY VIDEOS ON YOUTUBE FOR TARGETED OUTREACH

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Introduction. Backyard poultry (BYP) ownership is increasing in the United States (NAHMS, 2013). However, there are limited resources available to educate BYP owners. On top of this, survey and anecdotal observations indicate that BYP owners have poor biosecurity practices (USDA, 2005). This combination of poor biosecurity practices (e.g. exposure to wild birds) and unregulated bird movement can facilitate the spread of disease among poultry flocks. Consequently, the need for a robust outreach program is essential with respect to public health, food safety, animal health and the environment.

Objective. Gauge user engagement of BYP videos on YouTube to develop online outreach programs and to characterize online information using network analysis.

Methods. YouTube videos from searching “biosecurity for backyard chickens” were webscraped using Google Chrome’s extension, web scraper io, and called “featured videos.” Videos that were suggested as videos to watch next were also scraped and called “recommended videos.” Web scraped videos were classified as “excellent”, “adequate” or “poor” based on their biosecurity recommendations. Number of views as reported by YouTube was used to gauge user consumption. Number of likes and dislikes were used to gauge user participation. An edge list was uploaded to Gephi, a network analysis software.

Results and Conclusion. The resulting network consisted of 282 nodes representing featured and recommended videos and 249 directed links. Overall, the network seems decentralized with a network density of 0.004. In terms of social media engagement, it seems that users are not consuming content related to backyard poultry as much as they could. The number of views of featured videos with excellent content ranged from 8 to 3,345 views. Furthermore, it seems that participation or interaction with videos is as very low with only a few people liking (less than 30) or disliking (1) a video. From an outreach perspective, it seems that public engagement with YouTube videos can be improved and studying videos with the most views and likes could help understand user preference.

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EF12

PHYSIOLOGY

GENERAL PHYSIOLOGY

INCUBATION PHYSIOLOGY

GENERAL PHYSIOLOGY

ID : 112

INFLUENCES OF DIETARY AMINO ACIDS SUPPLEMENTATION ON L CELLS IN THE CHICKEN SMALL INTESTINE.

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Glucagon-like peptide (GLP)-2 derived from proglucagon (PG) is secreted from intestinal L cells and affects the growth of intestinal epithelium. Dietary supplementation of amino acids, methionine (Met) and lysine (Lys), had influences on the secretion of GLP-1 coexisting with GLP-2 in the same cells (Nishimura et al., 2015). This finding indicates the possibility that GLP-2 secretion is controlled by the dietary amino acids. The present study aims at clarifying the influences of the dietary supplementation of Met and Lys on PG mRNA-expressing and GLP-2-immunoreactive cells.

Male White Leghorn chicks of 7-days-old (average BW 80 g) were divided into one control and three experimental groups; crude protein (CP) 0%, CP 0%+Met and CP 0%+Lys groups. Chicks were forcedly fed with the control or experimental diet three times a day for two days at 8 hour intervals. And then they were sacrificed by decapitation under anesthesia. The middle part of ileum was dissected out from each bird as tissue sample. Tissue samples were fixed with Bouin's fluid and embedded in paraffin wax. Paraffin sections were used for in situ hybridization (ISH) and immunohistochemistry (IHC). Oligonucleotide probe based on the PG sequence was synthesized for ISH. Rabbit anti-GLP-2 serum was used as primary antibody for IHC. Frequencies of the occurrence of PG mRNA- expressing and GLP-2-immunoreactive cells were calculated. Crypt depth (CD) and the number of cells in mitosis per a crypt (NoMC) were detected in each group. All parameters were compared by Scheffe's F test for multiple comparisons.

The frequency of PG mRNA-expressing cells was significantly higher in the control group than three experimental groups (p

It is concluded that dietary amino acids supplementation doesn't influence PG mRNA expression and GLP-2 secretion of the chicken ileal L cells.

Nishimura K et al., J. Poult. Sci., 2015, 52(3):221-226.

ID : 168

EFFECT OF FEED PROTEASE SUPPLEMENTATION ON PERFORMANCE AND DUODENDAL ENZYMES ACTIVITY OF BROILERS FED DIFFERENT TYPES OF WHEAT-SOY AND PEAS-CONTAINED DIETS

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The aim of the study was to evaluate effect of two doses of the feed protease added to wheat-soy, 5 and 10% peas inclusion broiler diets. Two hundred twenty-five 1-day old Cobb 500 broilers fed commercial diets for 35 days. Birds were randomly allocated by 25 birds each group: C1–wheat-soy diet, C2–wheat-soy diet, 5% of peas, C3–wheat-soy diet, 10% of peas. C2 and C3 fed with calculated amino acids reduction. Body weight (BW) and FCR recorded at 10,21,35 d. Six experimental groups with feed protease: E1–C1+50 mg/kg, E2–C1+100 mg/kg, E3–C2+50 mg/kg, E4–C2+100 mg/kg, E5–C3+50 mg/kg, E6–C3+100 mg/kg. For physiological study, totally 27 broilers age of Cobb 500 fed C1 diets, on 21d surgically fitted with a duodenum T-cannula, divided according experimental design above. Duodenum chyme collected for 6 days, analyzed amylase, proteolytic, lipase activity. BW was significantly decreased by 10 % of peas inclusion on 3% ($p<0.05$). Protease inclusion significantly improved BW on 10,21,35 day in all types of diets ($p<0.05$). Observed no statistical differences between BW and doses of 50,100 mg/kg of feed protease. Dose of 100 mg/kg protease with soy-wheat diet significantly inhibited proteolytic activity to $18,44\pm1,27$ ($p<0.05$). Whereas 5%, 10% of peas diets and 100 mg/kg of protease significantly ($p<0.05$) increased proteolytic activity to $52,56\pm2,66$ and $46,5\pm1,89$ respectively. Broilers fed 5%, 10% peas and 50 mg/kg of feed protease had significantly higher proteolytic activity than control in both diets ($p<0.05$). Only 5 % of peas inclusion significantly decreased amylase activity in E2 diet ($p<0.05$). Diet with 10% of peas and 100 mg/kg protease significantly reduced amylase activity ($p<0.05$), comparing to 5% peas. Lipase activity was significantly affected by 5 and 10% of peas without protease. We suggest that feed protease significantly affected proteolytic activity in wheat-soy and peas-contained diets, improve BW, FCR. However, dose of 100 mg/kg protease and soy-wheat diet inhibited proteolytic, amylase activity we observed improvement in BW and FCR. Physiological adaptive and compensative response can have a place with high doses of feed protease.

ID : 417

AGE AND ROUTE OF INFECTION WITH STAPHYLOCOCCUS AUREUS ELICIT DIFFERENTIAL RESPONSES IN LAYING HEN IMMUNE CELL PROFILES MEASURED BY FLOW CYTOMETRY

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In 2019, instances of increased mortality in older caged laying hens in the United States were linked to *Staphylococcus aureus* infection. While this pathogen is commonly isolated from *Staphylococcus* infections in poultry, specific host responses and outcomes from varying infection routes remain unclear. The study objective was to examine systemic immune responses to an emerging disease in young and old laying hens infected with *S. aureus* by varying administration methods. 22-week and 96-week-old laying hens (100 birds/age) were randomly assigned to 1 of 4 treatment groups consisting of uninfected controls and birds inoculated with *S. aureus* isolated from affected laying colonies by oral gavage, subcutaneous (SQ), or intravenous (IV) injection. Prior to inoculation, 6 birds (3/age) were euthanized for baseline tissue sampling, with subsequent samples taken at 3 and 8d post-inoculation (pi). Body temperatures from 10 hens/ treatment were taken daily and blood was sampled at 6h, 1d, 3d, and 7d post-infection (dpi). Innate immune cell profiles in the spleen and peripheral blood were measured using multicolor flow cytometry. Results were analyzed by PROC MIXED (SAS 9.4) as a 2×4 factorial with fixed effects of age, infection route, and age×infection route (significant: $P \leq 0.05$). Prior to inoculation, 96-week-old hens had 34.9% more splenic CD1.1+ lipid antigen-presenting cells ($P = 0.05$). At 3dpi, 96-week-old hens infected with *S. aureus* by SQ and IV injection showed significant reductions in splenic CD1.1+ cells compared to the corresponding control group ($P < 0.0001$). At 3dpi, 22-week-old birds infected by IV injection displayed 24.2% reductions in T-cells compared to the corresponding control ($P < 0.0001$), indicating recruitment to peripheral infection sites; whereas older birds infected in the same manner did not display reductions in T-cells until 8dpi ($P < 0.0001$). T-cell subpopulations indicated that 22-week-old birds recruited CD3+CD8α+ cytotoxic T-cells at 3dpi ($P = 0.0002$), while older birds did not mobilize any of the examined T-cell populations. These results give insight into variations in the age-based immune response to *S. aureus* that may be underlying the increased mortality seen in older birds.

ID : 470

THE EFFECT OF SEX REVERSAL ON SEXUALLY DIMORPHIC OREXIGENIC AGRP EXPRESSION.

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Sexual dimorphism for growth exists across the animal kingdom and it is well known that male chicks grow faster than females. In chickens, as in mammals, genes expressed in orexigenic neurones in the hypothalamus control feed intake and these appear to have been affected through selection for growth [1]. In previous studies we have demonstrated that there is a highly significant sexual dimorphism in hypothalamic gene expression of the orexigenic agouti related protein (AGRP) with a higher level in the faster growing male than the female [2]. The relationship between genetic loci that determine growth and body weight with the expression of AGRP have led us to the hypothesis that orexigenic AGRP expression may be important in determining body weight.

To specifically test the hypothesis in the case of sex, a sex reversal experiment was carried out to find out if AGRP expression sexual dimorphism is related to the genetic sex of the chicken or alternatively its steroid milieu.

Broiler breeder (BB) eggs were incubated at 37°C for 72h. At 72h 100ul of PBS (pH 7.2) or Fadrozole (10mg/ml PBS), an aromatase inhibitor which prevents ovary differentiation, was injected into the albumen and the eggs incubated until hatch. Chicks were raised in a single room on a standard BB programme in a 2x2 factorial design [male v female, fadrozole (FAD) v PBS (C)], repeated 3 times. Birds were sacrificed at 10 and 26 weeks, and samples taken including hypothalamus for expression analysis by qPCR.

Fadrozole treatment resulted in a clear sex reversal of females, which had bilateral testes-like gonads and plasma oestradiol (ng/ml) at 26 weeks of age of 0.01 ± 0.002 v 0.44 ± 0.13 in C females. AGRP expression (normalized units) in the hypothalamus was significantly different ($p < 0.001$) between sexes, but not between treatments in either sex: Female C 0.166 ± 0.043 ; Female FAD 0.141 ± 0.044 ; Male C 0.479 ± 0.079 ; Male FAD 0.346 ± 0.152 .

The clear effect of Fadrozole on the sexual phenotype of the hens and the lack of effect of Fadrozole on AGRP expression suggests that the sex differences in AGRP expression may be genetically determined. This supports the role of AGRP expression in determining genetic differences in growth and body weight and is further evidence of the effects of genetic sex [3] rather than steroid dependent phenotypes in chickens.

[1] Am. J. Physiol. Endocrinol. Metabol. 2013 304 E909-E921.

[2] Biology of Sex Differences. 2018; 9.

[3] Nature. 2010; 464:237-U115.

ID : 568

VARIATION IN MUSCLE ENERGY STORES: IMPACT ON REPRODUCTION AND EGG CHARACTERISTICS IN MEAT-TYPE CHICKEN STRAINS

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The selection of meat-type chicken lines for increased growth and muscle development was paralleled with physiological changes. While considerable gains have been made in production performance, limits have appeared in terms of product quality and reproduction, questioning the sustainability of meat production. The genetic antagonism between production and reproduction remains a major challenge in maintaining the sustainability of selection on meat-type lines. In heavy strains, there is a decrease in muscle energy reserves, as assessed through the Glycolytic Potential. Divergent selection on breast meat ultimate pH from a commercial grandparental female broiler line has originated in two experimental lines: pHu+, presenting the lowest muscle energy status, and pHu-, exhibiting the highest energy status. These two lines constitute valuable genetic models to address the link between the energy status of the animal, reproduction, egg content, embryo development and ultimately chick quality at hatch.

The present study focused on the eggs of the breeding hens from the 11th generation of selection, where a difference of 0.6 pH units was observed for the selection criterion (pHu measured in chicken Pectoralis major muscle at 6 weeks of age). Data collected between 23 and 39 weeks of age showed a delayed first egg laid, lower egg laying rate, as well as a higher percentage of broken eggs in the pHu+ line compared to the pHu- line. Characterization of the resulting eggs revealed differences between the two lines, with heavier and more rounded eggs in the pHu+ line. Divergent selection also impacted animal fertility (lower in pHu+), embryonic mortality (higher in pHu-), and post-hatching mortality (higher between D0 and D7 in pHu+).

In conclusion, these results suggest an alteration in the reproductive performance and changes in egg characteristics when selecting for decreased muscle energy reserves. These preliminary observations pave the way for future genetic and physiological studies to assess the contribution of muscle energy status in reproductive and chick robustness traits, whose impairment penalizes meat-type broiler production. In an original way, they also suggest the possibility of identifying new indicators or biomarkers of energy status by measuring reproduction performance and egg characteristics.

This project (CHICK'TIP) received financial support from French Agriculture Ministry (CASDAR)

ID : 579

EFFECTS OF DIETARY CARBOHYDRATE LEVEL ON GLUCAGON-LIKE PEPTIDE-IMMUNOREACTIVE CELLS IN THE CHICKEN SMALL INTESTINE.

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Glucagon-like peptide (GLP)-1 and GLP-2 derived from proglucagon (PG) are secreted from intestinal L cells in response to nutrient ingestion and accomplish different physiological functions such as the stimulation of insulin secretion and the growth of intestinal epithelium. Dietary protein level affects the frequency of GLP-1-immunoreactive cells in the chicken ileum (Monir et al., 2014). But the effect of dietary carbohydrate (CHO) level on GLP-immunoreactive cells is not clear. The purpose of the study was to investigate effects of dietary CHO levels on GLP-1 and GLP-2-immunoreactive cells in the chicken small intestine.

White Leghorn male chicks of 6-week-old were divided into one control and two experimental groups; low CHO (12.5% of the control content) and CHO-free groups. After habituation for three days with the control feed chicks were fed with the control or experimental diets for 7 days. Proximal and distal parts of jejunum and ileum were removed from each chick as tissue samples under anesthesia. Tissue samples were fixed with Bouin's fluid and embedded in paraffin wax. Paraffin sections at 5 µm thickness were made from each sample. GLP-1 and GLP-2 were detected by streptavidin-biotin method. Frequencies of the occurrence of GLP-1- and GLP-2-immunoreactive cells were calculated with image analyzer and were statistically analyzed by one way ANOVA manner.

Endocrine cells showing immunoreactivity for GLP-1 and GLP-2 were observed in all intestinal parts of each chick. They were located in crypts and villous epithelium. No obvious differences were found in the distributional pattern of immunoreactive cells among three groups. Frequencies of GLP-1-immunoreactive cells of the control group were significantly higher than those of all intestinal parts in the CHO-free group and those of ileum and proximal jejunum in the low CHO group. No significant differences were recognized in frequencies of GLP-1-immunoreactive cells in ileum and distal jejunum between low CHO and CHO-free groups. Frequencies of GLP-2-immunoreactive cells were also significantly higher in all intestinal parts of the control group than the CHO-free group. No significant differences were recognized in frequencies of GLP-2-immunoreactive cells in ileum and distal jejunum between low CHO and CHO-free groups. It is concluded that the dietary CHO level affects GLP-1- and GLP-2-immunoreactive cells in the chicken small intestine.

Monir et al., Anim. Sci. J., 2014, 85: 581-587

ID : 677

PROTEOMIC ANALYSIS OF TWO WEIGHT CLASSES OF MULE DUCK "FOIE GRAS" AT THE END OF OVERFEEDING PERIOD

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INTRODUCTION:

Foie gras is the result of the accumulation of lipids in hepatocytes following a short period of gavage. Despite identical rearing conditions, different liver weights are always observed at the end of the overfeeding period. Thus, the aim of this study is to try to better understand what explain the difference in liver weights by comparing proteomic profiles of two groups of fatty livers differing in final weights: LWL =550-599g and HWL =700g.

MATERIAL ET METHODS:

A flock of male mule ducks was normally reared (11weeks) and finally overfed twice a day (10 days) according to a standard overfeeding program. After slaughtering, 24 livers (2x12) were randomly sampled to create 2 experimental groups (LWL and HWL). In addition to the gross biochemical contents of those samples, a proteomic analysis was performed according to a bottom-up approach (Chait, 2006) using NanoLC MS/MS. The raw data were obtained after searching in the local bank (Canard_Anas_Cairina 190524) and a label free protein quantification was also performed. To facilitate comparisons, statistical analyses were carried out on abundance values (peptide signal intensity in MS). Filters were applied to identify protein variants according to 3 conditions:

- The proteins must be detected in all the samples of one of the two conditions. If missing values are present in the other condition, they are replaced by an intensity value corresponding at 5% of the noise.
- The significance threshold of the Student test is <0.01 .
- The expression ratio is $1,5 < < 0.66$.

RESULTS:

In accordance to previous observations (Rémignon et al., 2018), proteins content decreases significantly (-18%) in HWL ($p < 0.001$). However, no significant differences ($p > 0, 05$) were observed for dry matter and total lipid contents between the two studied groups.

Finally, 1444 quantified proteins were identified among which 78 respect the validation parameters described above. When comparing the LWL to the HWL group, 42 and 36 proteins were under- and overexpressed respectively.

CONCLUSION:

The results showed that LWL are characterized by greater exports and beta-oxidation of lipids which indicate a more efficient storage of triglycerides in HWL. In HWL, this more massive storage of lipids reduced the amount of proteins accompanying cellular responses to hypoxia and apoptosis.

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ID : 740

MSTN GENE KNOCKOUT CHANGES THE TRANSCRIPTOME PROFILE IN CHICK LEG MUSCLE

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Postnatal muscle growth is an important aspect of animal production. However, limited knowledge is available for molecular mechanisms underlying muscle growth. CRISPR has been used for postnatal gene editing to study gene function in mice, but less has been done in chicks. To verify whether postnatal gene editing could be achieved in chicks, we knocked out Myostatin (MSTN), an important negative regulator of muscle growth, in chicks and performed transcriptome analysis on knock-out (KO) muscles and wild-type (WT) muscles to study the molecular mechanism of muscle growth. A pair of sgRNAs targeting MSTN exons 1 and 3 were packaged with Cas9 and adenoviruses (AdV) and delivered to the leg muscles of six newborn chicks. We collected muscles and detected the large fragment deletion by RT-PCR in 3-day-old (3d) and 14-day-old (14d). Transcriptome sequencing was used to determine the transcriptome profiles of 3d KO muscles, 3d WT muscles, 14d KO muscles and 14d WT muscles. We analyzed differentially expressed genes (DEGs) using the DESeq2 package and used the DAVID along with IPA software for enrichment analysis. RT-PCR and sequencing results indicated that large fragment deletions were achieved in the genome of all 3d KO muscles and 14d KO muscles. Compared to the WT group, there were 1339 DEGs at 3 days and 597 DEGs at 14 days. Several DEGs were associated with lipid metabolism (ARL4C), immune response (CCL19) and skeletal muscle growth (Ex-FABP). Gene Ontology analysis and KEGG pathway analysis results showed that many DEGs were involved in muscle growth, immune response, signal transduction and metabolism processes. IPA analysis revealed that two important classical pathways (PDGF signaling pathway and STAT3 signaling pathway) were associated with muscle growth. Our results confirm the feasibility of postnatal gene editing in chicks and provide valuable resources for studying the molecular mechanisms of muscle growth in chicks.

ID : 754

EFFECT OF EGG STORAGE TIME ON HEAT PRODUCTION AND HAEMATOLOGICAL PARAMETERS OF EMBRYONIC AND POST-HATCH DEVELOPMENT OF GUINEA FOWL BROILERS

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It is well known that egg storage affects egg quality (Kirk et al., 1991) embryo survival and post hatch growth (Tona et al., 2003), gas exchanges and the change in embryonic CO₂ metabolism (Fasenko, 2007). There is however dearth of information on the mechanisms involved. This study evaluated the effect of egg storage duration on heat production and haematological parameters on embryonic and post-hatch growth of guinea fowl broilers. A total of 800 guinea fowl hatching eggs were weighed and assigned to two treatment groups of storage duration: 5d and 10d having 4 replicates of 100 eggs each. Prior to setting, egg Haugh units were determined (Tona et al. 2002) then the eggs were incubated at 37.7°C of temperature; 55% of relative humidity. At 23 d of incubation and at 12 weeks post-hatch, 60 eggs with evidence of living embryo and 2 birds respectively per storage treatment were transferred to respiratory chambers to determine CO₂ production and O₂ consumption. CO₂ and O₂ were recorded and used to calculate heat production by the formula of Brouwer (1965) at internal pipping, hatch and at 12 weeks posthatch. They were reared for 12 weeks including starter, grower and finisher phases. Feed intake and body weight were recorded and used to determine feed conversion ratio. Also Blood samples were collected at hatch and 12 week post-hatch from 24 guinea fowls per treatment for determination of haematological parameters. The guinea fowl were later slaughtered and carcass characteristics, organs and intestinal morphometric were determined. The data were analyzed with the Graph pad Prism 5 statistical software package. T test was used for analyse. The results showed that average Haugh unit of 5d stored eggs was higher ($P < 0.05$) than of 10d stored eggs. Similarly heat production of embryo and 12 wk-old guinea fowl from eggs stored for 5d was higher ($p < 0.05$) compared to those from 10d. Also, basophil, eosinophil and lymphocyte concentration in guinea fowl from egg stored for 10d was higher ($p < 0.05$) than 5d. Body weights in guinea fowl for 5d were higher ($p < 0.05$) than those 10d. Feed conversion ratio for 5d was higher ($p < 0.05$) than those 10d. Also, weight and length duodenum were higher ($p < 0.05$) for 5d than those 10d. It was concluded that egg storage affects the metabolism of embryos through heat production, immune system during embryonic and post-hatch growth.

Key words: Egg storage, egg quality, guinea fowls, heat production, haematological parameters.

ID : 823

RESPONSES OF EXOTIC TURKEYS FED SPIRULINA (ARTHROSPIRA PLATENSIS) WITH OR WITHOUT VITAMIN B2 AND B6 DURING DRY SEASON

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Thermal stress is a challenging environmental stressor in poultry production especially in the tropics, resulting in poor growth rate, reduced productivity and immunosuppression. There is a dearth of information on nutritional intervention to ameliorate the effect of thermal stress on exotic turkeys during hot season in the tropics. This experiment was carried out to investigate the responses of exotic turkeys fed spirulina (*Arthrospira platensis*) with vitamin B6 or vitamin B2 during hot-dry season. Ninety unsexed exotic poults were allotted to 5 dietary treatments in a completely randomized design. The treatments were: corn-soybean meal basal diet (CT); basal diet supplement with 100mg/kg of spirulina (SP), 100mg/kg spirulina + 7mg/kg vitamin B6 (SP6); 100mg/kg of spirulina + 8mg/kg of vitamin B2 (SP2); 100mg/kg of spirulina + 7mg/kg of vitamin B6 + 8mg/kg of vitamin B2 (SPC). The experimental diets were fed at the pre-starter phase, starter, grower and finisher phase. The birds were raised using a conventional turkey management method. Blood samples were collected from two birds in each replicate at each phase of growth for the determination of plasma corticosterone, Superoxide dismutase (SOD) and malondaldehyde. The results showed that at the grower phase, birds in SP2 and SPC had similarly low plasma corticosterone (P

ID : 848

RNA EXPRESSION DIFFERENCES IN THERMAL CONDITIONING AND THERMONEUTRAL BROILER CHICKENS FED DIETARY METHIONINE FROM DIFFERENT SOURCES AND LEVELS AND SUBJECTED TO HEAT STRESS

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Heat stress (HS) negatively affects the performance and metabolism of chickens. Several cellular mechanisms are elicited to mitigate the negative effects of heat stress. Methionine is a precursor to glutathione, a major antioxidant in the body. It is therefore thought that methionine could mitigate some of the negative effects of HS. Thermal conditioning (TC) has also been a strategy used to mitigate HS. Our goal was to examine differential gene expression of heat stressed broiler chickens versus thermally conditioned broiler chickens as well as the effect of methionine source. For this study, Cobb500 broiler chickens were subjected to thermo-neutral temperature (TN) (25°C) or HS (35°C) from day 7 to 20. The two groups were all subjected to HS from day 21 to 35. Additionally, birds in both treatments were fed recommended diets supplemented with either DL-Methionine (DL-Met) or Methionine Hydroxy Analogue (MHA) at either 100% or 140% of requirement. RNA sequencing analysis was done on Pectoralis major samples (5 birds per treatment group) at day 10, 21 and 35. Only differentially expressed genes with a $p < 0.05$ and log2 fold change either greater than 1 or less than -1 were considered significant. KEGG analysis revealed oxidative phosphorylation and phagosome pathways, among others, to be significantly downregulated in birds TC birds and fed either 100% DL-Met or MHA in relation to the TN birds at were heat stressed from day 21 to 35. Analysis shows a reduction in significantly differentially expressed genes in TC birds when supplemented with either source of methionine at 140% of the requirement. Interestingly, FOXO signaling pathway and the cell cycle was upregulated in TC birds fed 140% DL-Met and downregulated in TC birds fed 100% DL-Met, with similar genes being either up- or downregulated in those pathways. The regulation of significant genes of pathways in relation to heat stress, thermal conditioning, and dietary methionine elucidate the biological mechanisms that underlie thermal conditioning.

ID : 853

POSSIBLE EFFECTS OF LIMINAL MICROBIOTA AND PROBIOTICS ON THE INNATE IMMUNO-DEFENSE SYSTEM IN THE CHICK INTESTINE

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[Introduction] Innate immunity plays a crucial role in preventing infection in the chick intestinal mucosa before the maturation of the lymphoid system. In the process of innate immune response, Toll-like receptors (TLRs) recognize microbe-associated molecular patterns, leading to the synthesis of cytokines and anti-microbial peptides (AMPs) including avian β -defensins (AvBDs) and cathelicidins (CATHs). The mechanism by which the expression of innate immune factors is regulated in the chick intestine remains to be established. We recently published the reports on the effects of microbiota regulation by antibiotics and probiotics treatments on the chick innate immune system. The present paper reviews those findings to discuss the effects microbiota and probiotics on the chick intestinal innate immune functions. [Experiment 1] We examined whether the expression of innate immune molecules, including that of TLRs, AMPs, and cytokines in the intestine was altered by antibiotics treatment. Broiler chicks were administered antibiotics (penicillin and streptomycin) daily for 7 or 14 days. It caused an increase in the relative frequency of the Enterobacteriaceae family and a decrease in some gram-negative and -positive bacteria families. The gene expression levels of some TLRs, cytokines, and AMPs showed a tendency to decrease by antibiotic treatment at day 7. [Experiment 2] We examined the effects of live probiotic *Clostridium butyricum* (CB) on the expression of innate immune molecules. Broiler chicks were administered 500 μ l water with or without CB daily for 6 days. The expression of TLR2 and TLR5 as well as IL-1 β and TGF β 2 in the ileum was upregulated by CB. The gene expressions of AvBD1 and CATH 3 were upregulated by CB in the cecum. [Conclusions] Our results revealed that the expression of cytokines and AMPs might be affected by luminal microbiota in the cecum because their expression was altered in association with the changes in microbiota frequencies that were regulated by antibiotics. It was also found that probiotic live CB treatment affected the expression of innate immune molecules, which may be due to the recognition of CB molecules by TLRs and/or the stimuli by organic acid released from CB. Thus, we suggest that regulation of intestinal luminal microbiota by probiotics is an effective strategy to enhance a part of the innate immuno-defense system in the intestine of chicks.

ID : 895

ALKYLATION REPAIR HOMOLOG 5(ALKBH5) KNOCKOUT PROMOTES THE PROLIFERATION OF CHICKEN PREADIPOCYTES

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Introduction

m6A RNA modification plays an important role in the differentiation of mammalian preadipocytes and hepatic lipid metabolism. ALKBH5, the m6A demethylase, is involved in the regulation of a variety of biological processes, such as spermatogenesis, carcinogenesis and development. So far, the relationship between ALKBH5 and the growth and development of adipose has not been reported. The objective of the current study was to determine the role of ALKBH5 in preadipocyte proliferation.

Material and methods

Immortalized chicken preadipocyte (ICP2) was used as the cell model. ALKBH5 knockout cell line was established by CRISPR/Cas9 system. The expression levels of genes were detected by RT-qPCR and western blot. Cell proliferation was measured by CCK-8. Cell cycle was determined by flow cytometry.

Results

Three gRNAs were ligated to the Cas9 expression vector, respectively (named by ALKBH5-g1 to ALKBH5-g3). Then the Cas9/gRNA expression plasmid was transfected into ICP2 cells. 48 hours after transfection, the gRNA targeting efficiency was detected. The results of T7E1 enzyme digestion showed that only ALKBH5-g2 had genome cutting activity, so the ALKBH5-g2 vector was selected for the following experiment. The ALKBH5-g2 vector was transfected into ICP2 cells. 24 hours after transfection, monoclonal of the GFP positive cell was selected by flow cytometry. A total of 20 cell lines were obtained (named by ALKBH5-g2-1 to ALKBH5-g2-20). The results of sequencing and western blot showed that ALKBH5-g2-11 was the ALKBH5 knockout cell line (ALKBH5^{-/-}). The results of CCK-8 showed that the proliferation of chicken preadipocytes was enhanced after the knockout of ALKBH5. The results of cell cycle showed that the proportions of cells in S-phase and G2-phase were increased significantly after the knockout of ALKBH5. The results of RT-qPCR showed that the expression levels of proliferation-related genes (Ki67 and PCNA) were increased after ALKBH5 knockout.

Conclusion

ALKBH5 knockout promotes the proliferation of immortalized chicken preadipocytes.

ID : 1022

AGE AND SEX-SPECIFIC LONG-LASTING EFFECTS OF PERINATAL TEMPERATURE MANIPULATIONS ON NEURONAL HYPOTHALAMIC MECHANISMS

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In precocial birds the hatching phase is a critical period in which perinatal temperature manipulations (PTM) induces long-lasting changes in physiological peripheral and central nervous mechanisms. This review focuses on own collaborative research projects on the influence of chronic and short-term PTM on the hypothalamic microstructure controlling body temperature, feed intake, body weight and metabolism in terms of age and sex dependence. All experiments were carried out in brain slices containing the preoptic area of the anterior hypothalamus (PO/AH) or the Nucleus infundibuli hypothalami of the ventrobasal hypothalamus (NI/VH). In Muscovy ducks aged between embryonic day (E) 28 and day (D) 10 post-hatching, the neuronal thermosensitivity of the PO/AH and its modification by chronic PTM (35°C; 38.5°C; control 37.5°C) were investigated using extracellular recording. During early ontogeny (E28-D5) the PO/AH is characterized by a high cold and low warm sensitivity (up to 30% and 5%, respectively). Between D5 and D10 a strong qualitative change occurs towards the “adult” neuronal thermosensitivity, which is characterized by high warm and low cold sensitivity (D10 15% warm and 14% cold sensitivity). PTM related modifications show also a clear age-dependent pattern starting with proximate non-adaptive changes up to a clear incubation temperature dependent and proximate adaptive modification at D10 (cold-incubation increased warm sensitivity and decreased cold sensitivity and warm-incubation induced opposite modifications, $p < 0.05$). The NI/VH contains orexigenic neurons expressing neuropeptide Y (NPY) and anorexigenic neurons expressing proopiomelanocortin (POMC), which are also key player in stress response control. In female and male broiler chickens effects of short-term PTM (+ 1°C, 2 hrs/day) on neuronal NPY expression were investigated on D35 using immunohistochemistry. Analysis of 365 slices results in a significant ($p < 0.05$) decrease in NPY expression exclusively in males. It is corresponding to changes in thyroid hormones (T3/T4) and performance. Females show no or only tendential changes in the same parameters. Studies on POMC gene expression in three weeks old normal incubated laying type chickens, showing correlation of methylation pattern and gene expression along with sex-specific differences ($p = 0.03$). In conclusion, PTM induced lasting changes in the microstructure of the hypothalamic neuronal network are sex-specific and follow ontogenetic rules.

ID : 1135

THE EFFECT OF PHOTOPERIOD AND WAVELENGTH ON BROILER PERFORMANCE

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Previous studies have found that broilers reared on short (12h) photoperiods had reduced breast meat yield (BMV) and in some cases reduced body weight (Liu et al., 2010; Mlaba et al., 2015). Previous studies have also shown that broilers reared on green wavelengths have improved muscle growth and development (Rozenboim et al., 1999; Cao et al., 2008). Thus, this study aimed to determine if the loss in BMV in broilers reared on short photoperiods could be counteracted using green light. 3200 Ross 308 day old chicks were randomly placed in one of 8 light-tight rooms (400 chicks per room). A 2x2 factorial design was used with two wavelengths (green and white) and two photoperiods (short; 12L: 12D and long; the current commercial recommendation of 23L: 1D for the first week then down to 16L: 8D). The performance parameters body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and BMV as a percent of BW (BMV%) from a sample of 8 birds/room were measured weekly until 35d of age. All results were analysed using ANOVA. Broilers that were reared under the short photoperiod had better FCR with a significantly higher BW at d 35 and had a significantly lower cumulative feed intake compared to broilers that were reared under the long photoperiod regardless of wavelength. Broilers on the white wavelength had a significantly higher BW at 21d than those on the green wavelength, but not at any other age. At 14d there was a significant interaction with BW of broilers on the long white treatment showing the highest BW. At 7d BMV% from broilers that were reared under a short green photoperiod and long white photoperiod was significantly higher compared to BMV % from the other groups. However, this interaction was not found at any other age. Mortality was not effected by photoperiod or wavelength. This study didn't find a reduced BMV from birds on short photoperiods, and regardless of wavelength there was an increase in BW on birds on short photoperiods, as well as an improved FCR, perhaps because of lower activity levels. However, the BW of all broilers was under target and therefore the results should be interpreted cautiously.

ID : 1269

TRANSCRIPTIONAL INSIGHTS INTO KEY GENES AND PATHWAYS CONTROLLING ABDOMINAL FAT METABOLISM IN BROILER CHICKENS

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INTRODUCTION: With the increase in growth rate and feed conversion efficiency, the abdominal fat rate of Chinese domestic breeding broilers has been increasing. The increase in belly fat greatly reduces the slaughter rate and disease resistance of broilers. To explore genes and regulatory pathways related to abdominal fat deposition in Chinese domestic broiler breeds, transcriptome sequencing technology was used to detect the expression levels of genes in the high abdominal fat group and the low abdominal fat group of Guangxi Sanhuang chicken.

MATERIALS AND METHODS: San Huang chicken from Guangxi province, China, Under the same feeding and management conditions, the animals were raised to the day of listing. After slaughter, the abdominal fat of each chicken was measured. The abdominal fat rate was divided into high and low abdominal fat groups, and three samples of each group were taken Liver, back fat, pectoral muscle, and leg muscle tissues. RNA was extracted and sequenced. Sequence adapters and low-quality reads were removed by Trimmomatic, and quality control was performed with FastQC. Sequencing reads were mapped to the chicken reference genome using the HISAT2. To quantify the expression of the HTseq-count. Analysis of differential expression of transcripts with DESeq2. Genes with P-value

RESULTS: A total of 2233 differentially expressed genes were identified between four groups. Among them, there were 474,459,1134,166 genes Differentially expressed in the liver, dorsal fat, pectoral muscle, and leg muscle. We conducted enrichment analysis on these differentially expressed genes, which found that these differentially expressed genes were mainly enriched in the ATP binding(MYH15, TOP2A, SYK, ASS1), extracellular region part(LPL, HBEGF, TSHB). PPAR signaling pathway(ACSL1, ACOX1, PLIN1).

CONCLUSION: This study showed that Genes related to abdominal fat deposition are mainly involved ATP binding, extracellular region part biological functions and PPAR signaling pathway, Additionally, key pathways related to lipid storage and metabolism (PPAR signaling pathway)may be the key pathways regulating differential lipid deposition.

ID : 1282

THE INFLUENCE OF DIFFERENT FRACTION OF ALUMINOSILICATE IN SLAUGHTER CHICKEN DIET ON JEJUNUM MORPHOLOGY

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In slaughter chickens production more often is paid attention on high emission of toxic gases as the ammonia, hydrogen sulfate and carbon dioxide to the atmosphere. Searching of solutions a. e.: feed supplements for decreasing toxic gases emission level by the higher absorption capacity in digestive tract. The aim of this study was compare feed supplementation by different fractions of aluminosilicate (zeolite, halloysite) and evaluation of impact on slaughter chickens Ross 308 digestive tract morphology. In this study 3 groups of chickens Ross 308 (100 bird per group) were evaluated. In all groups birds were fed with standard diet. In group I (control) fed without aluminosilicate was used. The group II was fed with 1% halloysite additive. In the group III fed with 1% zeolite additive was used. After 6 weeks slaughtered 30 chickens (10 bird per trial group). After slaughtering small intestine samples were collected (3-4 mm long) for histological analysis. In samples detected: villus height and width, which were used to villus surface area and crypt depth calculation. Statistical significance difference were found in villus height between group with 1% halloysite additive (II) and group with 1% zeolite additive (III) in fed. Similar differences were found between group I and III. No significant differences were found in the villus height between group I and II. The statistical significant difference of villus width was found between groups I and III, II and III as well. High significance differences (P

ID : 499

IMPACT OF PHOTOPERIOD ON PRODUCTIVITY OF BROILERS RAISED WITHOUT ANTIBIOTICS

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An increasing percentage of the global broiler industry is shifting towards antibiotic free production. Moving from conventional rearing to raised without antibiotics (RWA) can have detrimental effects on productivity. To minimize the negative impacts associated with RWA flocks, producers must adapt management practices. While it is known that providing a dark period improves productivity (growth rate and feed efficiency and reduced flock mortality) in conventionally reared birds, it is unclear what benefits will be realized in RWA flocks. Therefore, the objective of this research was to assess the impact of photoperiod on the productivity of RWA broilers. Two identical blocked trials were conducted, each using 4,032 Ross 308 broilers (N=8,064) reared to 36 d. Birds were housed in 8 identical environmentally controlled rooms with 12 pens per room at a stocking density of 21 kg/m² for each block. From 6 to 36 d, lighting treatments were 14L:10D, 17L:7D, 20L:4D, and 23L:1D. Each treatment was replicated in two rooms per trial resulting in 4 room replications overall. Body weight (BW), feed intake and feed efficiency were calculated at 7, 21, and 36 d. Flock uniformity was assessed at 34 d (n=336 birds per treatment per trial). Mortality was monitored daily and birds were necropsied by an independent laboratory to determine cause of mortality. Data were analyzed using regression analyses (Proc Reg and Rsreg of SAS 9.4). Differences were considered significant when $P \leq 0.05$. A quadratic relationship between BW and treatment existed at 7 ($P < 0.01$), 21 ($P < 0.01$), and 36 d ($P < 0.01$) with the highest final BW at 36 d under 20L. Feed consumption responded quadratically at all periods with birds on 20L consuming the most feed. Feed efficiency with mortality correction responded in a linear fashion from 21-36 d and 0-36 d, with longer dark periods resulting in improved feed conversion. There was no effect of photoperiod on mortality. A quadratic response was observed for the percentage of birds within 5% of the mean BW for uniformity ($P = 0.05$) with the highest percentage under 23L. A linear increase with increasing photoperiod was observed for the percentage of birds within 15% of the mean ($P = 0.04$) with higher uniformity under 23L. In conclusion, the results indicate that including a minimum 4 h of continuous darkness in the lighting programs of RWA broilers improves performance.

ID : 1395

ONTOGENY OF HORMONAL SYSTEMS REGULATING CALCIUM AND PHOSPHORUS METABOLISM IN COMMERCIAL BROILER CHICKENS

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Lameness due to pain or leg weakness is a major welfare concern in the broiler industry, with approximately 2–6% of birds manifesting skeletal problems even in the absence of nutritional deficiency, poor management, or injury. Though it is known that vitamin D3, parathyroid hormone (PTH), and calcitonin (CALC) play important roles in Ca and P homeostasis and are essential for bone strength, there remains a lack of data relating to physiological control of D3 conversion into its biologically active forms [25(OH)D3 and 1,25(OH)2D3] and hormonal regulation of Ca and P metabolism in broilers. The objective of this study was to investigate ontogenic expression of enzymes responsible for conversion of exogenous D3 to its metabolites and receptors for hormones critical in regulating Ca and P metabolism. Liver and kidney were collected from eight Cobb 500 broilers on day (D) of hatch (D0) and post-hatch D10, D20, D30, and D40. Levels of mRNA for enzymes involved in conversion of D3 and hormone receptors associated with Ca and P homeostasis were measured by RT-qPCR. Data were analyzed with one way ANOVA followed by Tukey's post hoc comparison and considered significant at $P \leq 0.05$. Levels of mRNA for CYP2R1, the enzyme responsible for converting D3 to 25(OH)D3, were highest in liver on D0 and decreased to the lowest levels on D20. In kidney, levels of CYP24A1, the enzyme responsible for deactivating 1,25(OH)2D3, were lowest on D0 and peaked by D10. Both RXRA and RXRG, transcriptional partners of vitamin D receptor, were highest on D0 and reduced to their lowest expression on D20 or D10, respectively, in liver. Levels of mRNA for both these genes remained relatively constant in kidney. In liver, mRNA for the two PTH receptors exhibited reciprocal expression patterns: PTH1R was highest at D0 and reduced steadily through D40, while PTHRL was lowest at D0 and increased steadily through D40. While PTH1R did not change in kidney, PTHRL was highest in this tissue on D0 and remained low after D20. Expression of CALC receptor was only detectable on D0 in liver; in kidney, CALC receptor levels were also highest on D0, though its expression remained detectable at all ages. These results indicate that systems involved in enzymatic conversion of vitamin D3 are not established until well after hatch and suggest that differential expression of receptors involved in hormonal control of Ca and P metabolism allows for tissue-specific regulation of this process.

INCUBATION PHYSIOLOGY

ID : 238

THE ASSIMILATION OF CARBOHYDRATES BY EMBRYOS OF BROILER AND LAYER CHICKEN

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The difference in growth rate between broiler chicken and layers can be found since the embryonic stage of development. Certain differences in the metabolic rate can also be found: broiler embryos more intensively assimilate the lipids of yolk and produce more heat since day 12 of incubation in compare to embryos of layers. The study presented was performed on the embryos of Cornish broiler chicken and "Mini-myasnye" (universal breed). At day 18 of incubation eggs with live embryos were injected with the solutions of different carbohydrates (0.5 mL per egg). Four hundred eggs for each breed were allotted to four treatments: treatment 1 (negative control) was intact; treatment 2 (positive control) was injected with normal saline; treatments 3 and 4 were injected with 10% solutions of glucose and dextrin, respectively; then the eggs were set for further incubation. Biochemical analyses of the embryos were performed in 1 hour and 1 day after the injection. It was found that concentration of glucose in embryonic blood in 1 hr after the injection was higher in the treatments injected with glucose (difference with control was significant at $P < 0.001$) and dextrin ($P < 0.05$). The assimilation of carbohydrates during days 18-19 of incubation was faster in Cornish embryos. The significant difference with control was found in the lactate concentration in embryonic blood in the glucose-injected Cornish eggs in 1 hr after the injection (2.16 vs. 4.28 mM/L in treatments 1 and 3, respectively, $P < 0.001$); this effect was not found in the dextrin-injected eggs. Similar trend was found in the glucose-injected "Mini" eggs (1.71 vs. 2.34 mM/L, $P < 0.05$). Since the increase in the lactate concentration is related to the anaerobic decomposition of glucose it can be assumed that the injection of glucose prior to hatch stimulates the glycolysis. The concentration of lactate in embryonic blood from dextrin-injected eggs in 1 hr after the injection was at the level of control evidencing slow decomposition of the starch to glucose and gradual transfer of the latter to embryonic cells. In a day after the injection the concentrations of lactate were similar in all treatments for each breed. The conclusion was made that the injections of exogenous carbohydrates into the chicken embryos at day 18 of incubation improved the assimilation of glucose and enhanced glycolysis as a source of energy in the hypoxic pre-hatch period; Cornish embryos were found to assimilate glucose faster.

ID : 239

THE BIOCHEMICAL MARKER OF GENETICALLY PRECONDITIONED MEAT PRODUCTIVITY IN POULTRY AT THE EMBRYONIC STAGE OF DEVELOPMENT

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For successful breeding to increase meat productivity it is necessary to know certain biochemical or morphological markers of the genetic predisposition to intense growth of muscle tissue. The difference in phenotypic parameters between meat and other breeds is manifested only at the postembryonic stage. But there are some biochemical factors acting at the embryonic stage and affecting the postnatal growth of muscle tissue. The synthesis of these factors, as the presence of their targets, may be predetermined genetically. Some researchers suggested that the activity of these factors is particularly related to the nitric oxide (NO) – universal cell mediator synthesized in living tissues from arginine. Using a highly sensitive and highly specific enzyme sensor that allows to assess the concentration of all nitro- and nitroso compounds in living tissues, the following patterns were established. The intensity of NO synthesis in the poultry embryos of one species is roughly the same while the rate of NO oxidation to nitrate is different in embryos of different breeds. In embryos of meat-type breeds this rate is high and about 90% of all synthesized NO undergoes oxidation to nitrate. In egg-type breeds this rate is low, and only several per cent of the synthesized NO oxidized and other NO accumulates within an embryo in content of co-called NO donor compounds – nitrosothiols, different nitrosyl-iron complexes. The difference in NO oxidation rate between the embryos of meat- and egg-type breeds is several orders of magnitude. But this difference varies by no more than 10% within a breed, line or cross. The intensity of NO oxidation in bird embryo is not depended on sex, age of the layer and feeding regime. These patterns were found in more than 50 breeds of 5 poultry species.

It was found that selection to increase meat productivity is always associated with the intensification of NO oxidation in embryo. In chickens after hatching the content of nitro- and nitroso compounds in the tissues sharply decreases, and becomes equal for all breeds of the species. Only embryonic NO oxidation is correlated with meat productivity.

We suppose that this rate of NO oxidation is predetermined by the presence or state of the NO targets. Thus, selection to increase meat productivity leads to a qualitative change in embryonic NO metabolism. Further research should reveal the mechanism of these changes.

ID : 356

COMPUTED TOMOGRAPHY (CT) AND MAGNETIC RESONANCE IMAGING (MRI) TO PHENOTYPE FERTILIZED EGGS DURING INCUBATION

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Studies of the bird embryo has led to major advances in developmental biology in the 1950s, and the Hamburger and Hamilton table related to the different stages of chicken embryonic development is still very widely used as a reference by embryologists [1]. However, because of decades of intensive selection on meat and egg production criteria (fast-growing broilers versus slow-growing broilers, laying hens), we expect that some developmental/growth features may have changed since the publication of the reference table.

To further explore this hypothesis, we decided to focus on a commercial fast-growing broiler strain. We used Computed Tomography (CT) and Magnetic Resonance Imaging (MRI), 1) to assess the evolution of egg quality during storage prior to incubation; 2) to analyze the growth and development of the embryo at various developmental stages.

Eggs from Ross 308 broiler strain (Aviagen) were stored for either 3 or 10 days (16°C, 75-85% relative humidity) and incubated for 11, 13 or 15 days prior to analysis (37.8°C, 55% relative humidity). CT on 10 eggs was performed on the day of lay, after 3 or 10 days of storage, and their global quality (egg weight, eggshell strength and thickness, internal quality of egg white and yolk) was assessed in parallel. As expected, most egg quality parameters decreased with duration of storage while the volume of the air chamber and the localization of egg yolk within the egg was also affected (CT). After 11,13 and 15 days of incubation, 10 eggs were analyzed by both CT and MRI. Embryos were removed from the egg, weighted and analyzed once again by CT followed by staining with Alcian blue and Alizarin Red for further analyses of their skeletal mineralization. The acquisition of all images is now achieved and the analyses of the results are in progress.

The final data integration will help to better appreciate internal movements of egg components during storage (which affects egg quality and potentially embryo viability) and during incubation, and to gain new insights into the development of the chicken embryo (anatomical characterization). In addition, by comparing these data with the Hamburger and Hamilton table, we will be able to determine whether some developmental features have been specifically affected by selection in this fast-growing broiler strain.

[1]. Hamburger V1, Hamilton HL. A series of normal stages in the development of the chick embryo. 1951. Dev Dyn. 1992 Dec;195(4):231-72.

ID : 622

INCUBATION TEMPERATURE AFFECT YOLK SAC DYNAMICS AND FUNCTIONALITY

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During incubation, the yolk, a main source of nutrients for the embryo, is enclosed by the yolk sac tissue (YST), a multifunctional organ responsible for nutrient absorption & digestion, for glycogen synthesis, for gluconeogenesis and for thyroid hormones (THs) regulation. Current work examined whether altered incubation temperatures (ITs) affect YST functions. Hence, 300 eggs were assigned to 3 temperature groups and incubated during E0-E18 in Control-37.8°C; Hot-39.3°C; Cold-36.3°C treatments. At E18 eggs were assigned to hatching trays and incubated in 37.8°C until hatch. At each sampling day along incubation (E5, E7, E9, E11, E15, E18, E19, E21), embryo and residual yolk (RSY) weights were recorded and YST was sampled. YST functionality was examined by regression analysis to RT-PCR expression patterns of genes encoding for lipid uptake & metabolism (LRP2, ApoA1), for oligopeptides uptake (PepT1), for gluconeogenesis (FBP1), for glycogen synthesis (GYS2) and for THs regulation (TTR, DIO1, DIO2).

Our results show that ITs significantly affect the expression of genes indicating YST functionality. In the Hot group almost all functionalities were affected while in the Cold group only few. Genes representing nutrient uptake showed a decrease in expression towards hatch, while the Control and Cold groups exhibited a linear increase. The expression of ApoA1 (indicating lipid metabolism) and DIO2 (THs activating enzyme) in Hot and Cold groups showed moderate linear expression pattern compared to Control. YST Glycogen synthesis was affected as expression of GYS2 in Hot and Cold groups had no significant change along incubation while in Control exhibited polynomial fit. The expression patterns of TTR (THs carrier) and FBP1 (gluconeogenic regulator) were not affected by ITs and showed similar expression patterns in all groups.

The effects on YST functionality probably led to the changes observed in yolk utilization, a parameter of hatchling quality. While the Control group showed optimal yolk consumption, as RSY decreased by 71.7% (E15-E21), the Cold and Hot groups exhibited poor consumption as RSY decreased by 63.8% and 37.2% respectively.

In conclusion, our findings show for the first time that IT's change the function of the YST and explains the reason for variations in RSY weight. It also highlights the importance of the YST in supporting the embryo and exhibit its functionality by fulfilling the roles of organs (intestine, liver) prior to their maturation in the embryo.

ID : 696

IN-OVO ELECTROLYTE FEEDING IMPROVES THE HATCHABILITY IN BROILER BREEDERS AND DOESN'T ALTER THE GROWTH TREND IN BROILERS

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In-ovo electrolyte feeding improves the hatchability in broiler breeders and doesn't alter the growth trend in broilers

Perinatal period is the time when post hatch chick undergoes body functioning activities shift from egg nutrients to exogenous feed. In ovo feeding via amnion fluids also enhances or facilitates healthy gut development of embryo and chicks by facilitating production of healthy microbiome. Objective of current study was to check the effect of in ovo inoculation of different electrolytes to broiler breeder eggs and their subsequent effect on hatchability and growth performance of broiler chicks. Present experiment was conducted by injecting different concentration of electrolytes solution via in-ovo technique into the amniotic sac of two hundred and forty broiler breeder hatching eggs at 18th day of incubation. The aim of the present study was to check the effect of in-ovo feeding of different concentration of electrolytes solution on hatchability in broiler breeder eggs and its subsequent impact on growth response in broiler. Eggs were arranged into four experimental groups with sixty eggs in each experimental group. Electrolytes used in study were combination of sodium chloride (3.5g), potassium chloride (1.5g), sodium citrate (2.9g) and dextrose (20g), respectively. Treatment group 1 was labelled as control group while treatment group 2, 3 and 4 were inoculated with combination of electrolytes i.e. sodium chloride, potassium chloride, sodium citrate and dextrose in 100ml, 500ml and 1000ml volume of distilled water. All experimental eggs were kept in an aseptic environment. Eggs were kept in incubator for 18 days in setter and then experimental and non-experimental eggs trays were shifted to an isolated room where fumigation is done and in-ovo inoculation was performed and afterwards, transferred into hatcher. After successful hatch out analysis, it was observed that 52 eggs were hatched in control group while 56 eggs were hatched in all other three in-ovo electrolytes fed groups. All hatched out chicks were transported to farm for evaluation of growth performance parameters. Data regarding hatchability and growth parameters i.e. feed intake, weight gain, feed conversion ratio (FCR) and mortalities was analyzed by using Completely Randomized Design (CRD). The results indicated significantly higher ($P < 0.05$) effect of in-ovo electrolytes feeding was noted on growth performance parameters viz., daily feed intake, weight gain, mortality and feed conversion ratio.

Key Words: Incubation, Concentration, FCR and Hatchability

ID : 698

EFFECT OF SUPPLEMENTATION OF FISH OIL ON HATCHABILITY PERFORMANCE IN CHICKEN

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The main energy source for the developing chick embryo is supplied by fats in the egg yolk. The alteration of the egg yolk fatty acid profile could improve the growth of the embryo during the incubation period. Hence, the present research was carried out to study the effect of fish oil on hatchability performance of native chicken namely TANUVAS Aseel and Kadaknath at Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The biological experiment was started using two hundred and seventy layers each of TANUVAS Aseel and Kadaknath (25 weeks of age) of single hatch were obtained and maintained under deep litter system of management with sex ratio of 1:6. These birds were randomly allotted into three treatment groups. All the treatment had three replicates and each consisted of 30 layers. The experimental birds fed from 25 weeks to 36 weeks with diets containing fish oil 0 % (T1-control), 1.5 % (T2) and 3 % (T3). All the diets were made iso-nitrogenous and iso-caloric by adjusting the other ingredients. A total of 1450 hatching egg were collected from 30 weeks to 36 weeks. The per cent total hatchability, fertile hatchability and total embryonic mortality were recorded and analyzed. The data collected on various parameters were grouped and subjected to statistical analysis of one way ANOVA as per the procedure of statistical analysis system (SPSS, version 20.0 for windows). TANUVAS Aseel received fish oil had numerically lesser egg weight when compared to control group, whereas numerically higher egg weight recorded in birds received fish oil. Similar trend was recorded in chick weight. Fish oil treatment groups showed significantly ($P < 0.01$) lower early and late embryonic mortalities during incubation. The statistical analysis did not show any significant variation between treatment groups on weak chicks in TANUVAS Aseel, whereas a significant ($P < 0.05$) difference was noticed on weak chicks among treatment groups in Kadaknath. The hatchability (fertile and total egg set) was significantly ($P < 0.05$) higher in birds fed with 3 per cent fish oil in TANUVAS Aseel, whereas 1.5 per cent fish oil fed group showed significantly ($P < 0.05$) higher hatchability in Kadaknath. The cock maintained on fish oil diet recorded significantly higher ($P < 0.05$) sperm motility and showed relationship between sperm motility and hatchability. This study concluded that incorporation fish oil in poultry feed will improve the hatchability in chicken.

ID : 745

USE OF MORINGA OLEIFERA LEAVES IN BROILERS PRODUCTION CHAIN: EFFECT ON HATCHABILITY OF SASSO BREEDER EGGS AND DAY-OLD CHICK SERUM LIPIDS

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This study was designed to investigate the in ovo and or dietary effects of *Moringa oleifera* leaves or a combination of both on hatchability and serum lipids of day-old chicks. Hatching eggs (n=2400) from Sasso broiler breeders fed with or without *Moringa oleifera* leaves were incubated at standard incubation conditions. At day 18, both eggs were candled and those with evidence of living embryos were divided into 2 new groups of 450 eggs each. *Moringa oleifera* extract was injected into the air chamber of one group. After extract inoculation, eggs were transferred from the turning trays to hatching baskets. Between 456 and 510 h of incubation, hatching events such as external pipping and hatch were monitored every 3 h. Hatched chicks were recorded and weighed. Eighty newly chicks from each group were used for liver, heart and yolk sac weight; and for serum lipids determination. General Linear Models procedure was used to analyze data. When means were statistically different, then they were further compared using Tukey's test based on $P < 0.05$. Results indicated that in ovo inoculation of extract reduced external pipping duration. Chick weights increased with *Moringa oleifera* leaves used only in broiler breeders diet but hatchability was greater in all groups treated with this material. Liver relative weights of day-old chick showed no remarkable changes while in ovo injection of extract reduced heart weights. Yolk sac utilization was better with *Moringa oleifera* leaves, regardless of the stage where this material was used. Serum concentration of total cholesterol and LDL cholesterol decreased with in ovo treatment while HDL cholesterol increased. These results suggest that *Moringa oleifera* leaves had positive effects on hatchability.

Keywords: *Moringa oleifera* leaves, broiler breeders, in ovo, hatchability, serum lipids.

ID : 1192

INCREASING INCUBATOR CO₂ LEVEL BY NON-VENTILATION DURING THE FIRST 10 D OF INCUBATION: EFFECT ON THE EMBRYONIC DEVELOPMENT, HATCHABILITY AND POST-HATCH GROWTH OF 2 COMMERCIAL LAYERS STRAINS

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During embryonic development, Lowman Brown (LB) embryos differs from that of Lowman White (LW) in terms of embryonic growth and hatching time (Tona et al., 2010). Therefore, these strains might adapt differently on environmental factors such as exposure to high CO₂. The aim of this study was to investigate the effect of increasing incubator CO₂ concentration by non-ventilation during the first 10 d of incubation on the embryonic development and post-hatch growth of LB and LW strains. Prior to setting for incubation, a total of 1,200 hatching eggs produced by LB and LW breeders were weighed, individually numbered and randomly assigned to 4 replicates of 75 eggs each/strain. The eggs were then divided equally into 2 incubators that were either ventilated (control group) for the entire incubation or non-ventilated for the first 10 d and subsequently ventilated. At d 18, the eggs were weighed, candled and fertile eggs were transferred from the turning trays to hatching baskets. Between 457 and 510h, hatching eggs were checked individually every 3h for hatching events. After pull out at 21.5 d, chicks were raised until d 56. Data were processed with the statistical software package graph pad prism Version 5.0. Anova 2 way and Chi square test were used for data analysis. The results showed that CO₂ level in non-ventilated group was higher (0.15%) compare to control group (0.03%). Hatchability was higher in non-ventilation incubator compare to control group in LB strain but the result was the reverse in LW strain. In both strains, non-ventilation treatment did not impact internal pipping duration, but external pipping duration was higher in LW strain compare to LB strain in both ventilated and non-ventilated group. The hatching window and the average time of hatching was shorter in non-ventilation group compare to ventilated group for both strains. Embryo weight was higher in non-ventilation treatment at ED12, ED14 and at IP compare to control group but there was no significant difference on day-old chick body weight. During post hatch, non-ventilation did not affect chick's body weight but chicks from LB strains had higher body weight compare to LW strains in both ventilated and non-ventilated group. It can be concluded that LB strain was more sensitive to incubator CO₂ level than LW strain and the two strains do not have the same developmental trajectory.

Key words: Layer strain, embryo development, non-ventilation, post-hatch growth.

ID : 1267

INTERMITTENT THERMAL MANIPULATION DURING INCUBATION: EFFECT ON EMBRYO DEVELOPMENT, HATCHING PROCESS AND POST HATCH GROWTH OF TWO BROILERS STRAIN IN TROPICAL CLIMATE.+

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Several studies have shown that thermal manipulations have effects on embryo development and post-hatch growth. It is also known that genetic background of broiler strains influences embryonic development. It was hypothesised that effect of thermal manipulation might depend of the genetic background of broilers. Therefore, the aim of this study was to investigate the effects of thermal manipulation during incubation (TMI) on embryo development and post-hatch growth of Cobb and Ross broilers under tropical climate. A total of 1500 hatching eggs obtained from Ross and Cobb broiler breeders were incubated at standard incubation conditions (37.8°C and 60% relative humidity) until day 7 and then randomly divided into four groups of five replicates: Rosscont and Cobbcont groups incubated at standard conditions and Rosstemp and Cobbtemp group subjected to TMI (39.5 °C at 65% for 12 h/d from d7 to d16). Between 456 and 504h of incubation, hatching eggs were checked for hatching events. From day15 to day42 post hatch, chicks from the four groups were reared at a natural ambient temperature (ranging from 22 to 34°C). Embryo weight, Hatchability, hatching time, chick's temperature, day-old chick's weight and 6 week-old body weight were determined. Data were analysed using Graph pad prism 5.0 in two-way ANOVA and chi square test. Results indicated high hatchability in both control group ($p<0.05$) compared to treated groups. Hatching time was shorter in Cobb strain compared to Ross strain. The body temperature was lower in TMI groups compared to control group and higher in Cobb strain compared to Ross strain. There was an interaction between incubation temperature and the strain on hatching time and chicks body temperature ($p<0.05$). Chick weight was comparable in all groups but the 42day-old body weight was higher ($p<0.05$) in Cobb strain compare to Ross strain. In conclusion, the effects of thermal manipulation are influenced by the genetic background. Further research should be carried out for the optimization of thermal manipulation during incubation for each genetic line.

Key words: Thermal manipulation, Broilers strain, Embryo development, Post-hatch growth, hot climate.

ID : 1449

CELLULAR AND MOLECULAR MECHANISMS WHICH ACCOUNT FOR EMBRYONIC SURVIVAL FOLLOWING PROLONGED STORAGE

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The chicken embryo, known as the blastoderm, consists of ~100,000 cells by the time the egg is laid. Following oviposition, avian embryos can suspend their developmental progress, at low temperature in a process known as diapause. The diapause phenomenon is exploited in the poultry industry to store freshly-laid eggs prior to incubation. In our previous studies, we defined millstone criteria to challenge and predict the effects of different storage conditions on embryonic survival, following prolonged storage. These criteria consist of accurate analysis of developmental stage, total cell count, cell viability and mitotic index.

Comparing prolonged egg storage, for up to 28 days, at 12oC and 18oC, we found embryos stored at the lower temperature exhibit better cytoarchitectural characteristics, lower apoptotic-mediated cellular death, higher mitotic index and far better embryonic survival and hatchability. Moreover, we found that in freshly laid eggs in modern commercial broiler breeds, the predominant embryonic developmental stages, are XI EG&K and XII EG&K in young and old flocks, respectively. This reflects an advanced stage compared with similar pioneering studies, decades ago, which found that the predominant stage is around X EG&K. This variance can be accounted to differences between breeds or to changes in the reproductive system and/or developmental rate, which occurred during years of broiler genetic selection.

To disclose the roots of these variances, we compared the reproductive system, developmental stages and embryonic growth rate between modern commercial broilers and commercial legacy breed which was not subjected to genetic selection since 1986. Our results show a marked increase in growth rate in modern breeds. Furthermore, using RNA seq we identified signaling pathways which are involved in entering diapause state and promoting embryonic survival during prolonged storage. Notably, we found a marked down-regulation in genes associated with metabolism, while up-regulation was found in pathways associated with maintaining pluripotency, at lower temperatures. These results were further validated by real-time PCR and RNA in-situ hybridization techniques. Our study shows that maintaining the pluripotency during diapause, even when the metabolism is drastically decreasing, can be one of the mechanisms involved in embryonic survival during diapause.

ID : 454

SCREENING OF KEY PROTEINS IN EGG YOLK AND WHITE THAT INFLUENCING CHICKEN HATCHABILITY

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During avian embryo development, the egg yolk and white provide almost all the nutritional and functional components. At present, the roles of these components in embryo development remain unknown. In this study, hens with divergent high/low hatchability (HH, LH; 100% vs. <65%) were selected from a population consisting of 88 males and 704 female Rhode Island Red chickens (σ : φ = 1: 8) to analyze the nutrients, minerals, and functional proteins in their egg yolks and whites. A tandem mass tag labeling quantitation approach was used to innovatively identify the differential proteins in (1) the egg whites/yolks of fresh eggs produced by hens with divergent high/low hatchability and (2) the egg whites of embryonated eggs with healthy and dead embryos. In egg yolks, a total of 401 proteins and 31 differential proteins were quantified. These differential proteins participate in some biological processes such as antimicrobial activity, protein processing, blood coagulation as well as differentiation and metabolism. The protein DNAJB11, which were upregulated significantly in high hatchability group and whose abundance were 270.65 times higher than that in the low hatchability group ($P < 0.05$), binds to misfolded proteins and regulates the correct folding and degradation proteins. Other proteins including WFDC2 and UMOD related to host defense contribute to embryo development when over-represented. In egg whites, a total of 378 proteins were quantified in egg white, and up to 102 differential proteins were identified. GO enrichment, pathway, and hierarchical clustering analysis revealed some of the differential proteins that are the main participants in several biological processes, including blood coagulation, intermediate filament, antibacterial activity, and neurodevelopment. A list of 11 putative protein biomarkers, such as keratin (KRT19, KRT12, KRT15, and KRT6A) and fibrinogen (fibrinogen alpha chain, fibrinogen beta chain, and fibrinogen gamma chain) were ultimately screened. The current study screened egg yolk and white proteins that can predict low hatchability and embryonic death and deciphered the role of these proteins in embryonic development, which is meaningful for the comprehensive understanding of embryonic growth.

Key words: chicken, hatchability, proteomics, egg white and yolk protein

AP1

SMALL SCALE FAMILY POULTRY FARMING

ID : 634

CLIMATE RESILIENT TANUVAS ASEEL X NANDANAM CHICKEN 4 CROSSBRED STRAIN (ANC4)

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TANUVAS Aseel is a multicoloured native chicken variety which has got attractive plumage, unique juice flavor of the meat, good disease and heat tolerance, produce 160 eggs per annum and best suited for backyard rearing. Nandanam Chicken 4 developed for backyard rearing. It has got multi colour plumage, produces brown shelled eggs and produce 220 eggs per annum. Hence, the present research work was undertaken to evaluate the production and reproduction performance of TANUVAS Aseel x Nandanam chicken 4 crossbred strain (ANC4) and to develop a synthetic strain with Aseel characters for table purpose with native favour of meat, to get more chicks per dam, owing to more egg production per dam at the Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. For development of ANC4 Chicken, individual selection for male and family selection was carried out. In each generation 1200 day-old chick were hatched out as a replacement population.

A selection pressure of 1 in 2 for females and 1 in 16 for males were applied for each generation to achieve a target. Day old fourth generation ANC4 chicken were obtained from the Poultry Research Station. The collected data were subjected to statistical analysis of one way ANOVA as per the procedure of statistical analysis system (SPSS, version 20.0 for windows). ANC4 chicken production and reproduction parameters showed significantly ($P < 0.05$) better than TANUVAS Aseel. The hatch weight of ANC4 chicken was 35.80 ± 0.93 g. Body weight was recorded to select best individual for next generation. Eight week body weight of male was 488.12 ± 5.18 g, female 425.15 ± 4.26 g and twelfth week body weight of male was 1320.22 ± 17.53 g and female $1023.45.11.01$ g. Age at sexual maturity was 150 days. The per cent hen day (HDEP) and hen housed egg production (HHEP) was 50.05 ± 1.23 and 50.27 ± 1.32 respectively. The per cent total and fertile hatchability was 72.60 ± 1.39 and 87.54 ± 1.31 respectively. The annual egg number, day old chick per dam and per cent livability 183.13, 133 95.5 were recorded from 20 to 72 weeks. Liver samples was taken from ANC4 for transcriptome analysis. A total of 25,840,415 raw reads were observed and 94.75 per cent aligned against Red Jungle fowl. Differential gene expression analysis showed 133 genes were unregulated. The results revealed that production and reproduction performance of ANC4 was better than TANUVAS Aseel in terms of egg number and chick per dam.

ID : 767

FAMILY POULTRY AND PUBLIC POLICY: LESSONS, LINKAGES AND LOOKING FORWARD

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INTRODUCTION

The number of people living with chronic hunger has risen for the third consecutive year to over 820 million in 2018 while incomes for men and women smallholder farmers are not increasing in line with SDG2 targets. Animal-source food and income from family poultry production can play a significant role in enhancing diet quality and quantity in resource-poor settings where monotonous cereal-based diets are common. However, family poultry does not always receive adequate or appropriate public policy attention which undermines farmers ability to achieve financially, environmentally and socially sustainable family poultry systems.

METHODOLOGY

As part of a wider livestock and aquaculture policy project, and with a focus on Bangladesh and Nigeria, we employed mixed methods - including stakeholder mapping, literature review, key informant interviews, online public consultation and national roundtables - to assess public policy successes, challenges and opportunities.

RESULTS

Globally a lack of inclusive policies, stakeholder consultation and implementation plans for existing smallholder livestock policies were highlighted as major challenges. Specifically, regarding family poultry, the key issues identified were: policy support for vaccination of family poultry without appropriate focus on adequate supply, cold chain and distribution contributes to ineffective disease prevention; policies banning the routine use of antibiotics as growth promotants without identification of and support for feasible transition strategies can cause additional antimicrobial resistance; inclusive policies that address adequate feed quantity and quality in relation to efficiently meeting the nutrient requirements of poultry without impacting negatively on supply of food fit for human consumption are largely lacking; introducing 'high producing' breeds without sound national breeding and indigenous breed conservation policies can lead to poor performance and impact negatively on farming household income; and focusing on increasing production without simultaneously addressing issues concerning food safety, efficient marketing and poultry product preservation can also impact negatively on farming households and consumers.

CONCLUSION

A multisectoral, inclusive approach is required to ensure that policies impacting on smallholder producers align optimally to facilitate efficient, safe, sustainable family poultry production that is available to consumers at affordable prices.

ID : 953

CAN VILLAGE POULTRY IMPROVEMENT EMPOWER WOMEN?

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Introduction

Gender analysis and gender sensitive instruments developed and used in development and research related to livestock and specifically to village poultry has evolved in the last three decades. Because poultry is the most widespread animal species in the world and because its potential for demand growth in the next decades is high it constitutes a very important animal husbandry to promote and improve. In addition, as poultry constitute one of the most common assets under the responsibility of women in the world, the motivations to use it as a leverage to increase rural women's visibility, economic benefit and empowerment is frequent.

Methodology

A dozen female key players who participated in projects and research related to village poultry in Asia (HPAI) and in Southern Africa (Newcastle disease) in the last three decades speak about women empowerment and village poultry work and comment on interventions carried out and results achieved individually and collectively. The paper also put in perspective, revises, compiles and analyses quantitative and qualitative data collected in different contexts through the prism of current knowledges and practices toward gender equality and women's empowerment.

Results

Women from different backgrounds and ages, in distinct period of their carrier and in diverse positions and roles in the several interventions under analysis experienced, exercised and valued different forms of power. By example, female students or female national researchers were given central role in the project empowering them both individually (power within), as professional (power to), as advocate for gender issues (power with). Approaches shared, methodologies applied, and experiences discussed, both in relation to research and development processes, in the search for gender equality and women's empowerment highlight the importance of placing women at the centre of the process.

Conclusion

The diverse forms of women's empowerments experienced and achieved at different nodes of the interventions processes illustrate the multiplicity of ways by which gender sensitive interventions can transform individuals, processes as well as institutions.

ID : 998

COCCIDIOSIS IN KADAKNATH CHICKEN REARED IN MIXED FLOCKS UNDER SEMI-INTENSIVE HOUSING SYSTEM

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An investigation was taken up during our routine field visit to backyard poultry farms wherein, few Kadaknath chicken flocks were reported to have mortality and morbidity issues for 6 consecutive days. The affected backyard farm comprised about 450 birds of 8 weeks age group which included Kadaknath and Aseel ecotypes, and were being maintained under semi-intensive system. These flocks were supplied by Central Avian Research Institute which is instrumental in enhancing rural poultry production in the country. Affected birds were witnessed to show anorexia, dullness with closed eyes and bloody droppings. Necropsy examination indicated dilation of the intestinal segments through distal end of jejunum in an increasing manner and its lumen contained necro-hemorrhagic contents amidst necrotic foci on the mucosal surface. Ceca showed varying intensities of mucosal hemorrhages and contained pasty to thick fluidic contents. Microscopically, H & E stained sections of intestinal segments including cecum revealed mucosal hemorrhages, marked epithelial necrosis, denudation of superficial lining cells of intestinal villi, and presence of numerous oocysts and schizonts in the epithelial cells of villus as well as crypt glands. The stromal tissues adjacent to the infected epithelium showed mild to moderate degrees of heterophilic as well as mononuclear cell infiltration. Molecular based diagnosis (PCR) revealed positive for *Eimeria acervulina* (321 bp), *Eimeria mitis* S (193 bp) and *Eimeria tenella* (278 bp). Administration of Diaverdin and sulphaquinoxaline combination in drinking water witnessed no further mortality. Indigenous breeds are considered to be adaptable to tropical conditions and resistant to many diseases despite their frequent exposure to contaminated places where the incidence of potential pathogens is relatively high, which might be due to strong innate immune response that they elicit. Scientific evidence confirming these assumptions are lacking. Kadaknath and Aseel are most popular breeds that reflect India's heritage and are mostly preferred on various festive occasions, and therefore been used extensively for village chicken production. From this study, we understood that coccidiosis can affect diversified breeds of chicken with high disease incidence typically at the age of 8 weeks, especially in intensive housing conditions particularly during autumn and multiple strains of *Eimeria* are involved in exacerbating the intestinal pathology.

ID : 1263

RELATIONSHIPS BETWEEN RESPONSES OF TANZANIAN FREE-RANGE LOCAL CHICKEN ECOTYPES TO INFECTION WITH LENTOGENIC AND VELOGENIC STRAINS OF NEWCASTLE DISEASE VIRUS

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Village poultry in Tanzania is dominated by free-range local chickens (FRLC), they provide about 100% of meat and eggs to households. They are resilient to harsh conditions of tropical hot climate, poor nutrition, diseases, and lack of veterinary care. These conditions, affect productivity in these chickens. The increasing human population in Tanzania is driving the need to increase production of FRLCs to supply essential animal protein in human diets as well as improvement of family income. Newcastle disease is still a major cause of massive losses to the FRLCs. This study aimed to improve production of the FRLCs through control of Newcastle disease. A breeding flock of three FRLC ecotypes was prepared from randomly sampled chickens, consisting of Kuchi, Morogoro-medium, and Ching'wekwe from the Lake, Central and Coastal climatic zones of Tanzania. The chickens are not pure ecotypes, genetically they belonged to two populations; (population 1 dominated by Ching'wekwe and Morogoro-medium and population 2 dominated by Kuchi) In a two-stage study, progenies of the breeders were infected with LaSota strain of Newcastle disease virus (NDV) and later exposed to virulent field strains of NDV (vNDV). In the first stage, chicks were infected with LaSota NDV at 28 days of age. Tear samples were collected at 2 and 6 days post infection (dpi) to determine viral load by PCR and blood samples at 10 dpi to determine antibody titers by ELISA. Body weights were measured weekly from hatch to six weeks. In stage two, the chicks were kept for four additional months to wane acquired immunity and then exposed to vNDV by contact with sick chickens. Body weights were measured at two-day intervals. At mortalities, post-mortem examination and lesions scores of dead chickens were recorded for three weeks, every morning and evening. Correlation analyses were used to determine relationships between the response traits. Survival analysis was also done. Pre- and post-infection growth rates were significantly positive but moderately correlated. There were no strong correlations between traits during infection with LaSota strain and exposure to vNDV in the FRLCs, apart from positive but weak (0.12) correlation of post-infection GR with average lesion scores during exposure to vNDV. Population 2 chickens with hazard ratio of 0.52 were more likely to survive vNDV infection than population1 chickens. Males of all populations were 1.4 times more likely to die of vNDV than females.

ID : 1281

GENDER DYNAMICS AND POULTRY HEALTH ISSUES IN SEMBABULE DISTRICT , UGANDA

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Introduction

This paper presents findings of the study that was part of a broader research project entitled “Hearing their voices: Action research to support women’s agency and empowerment in livestock vaccine distribution, delivery and use in Uganda”.

Poultry in Uganda is a source of income for many communities and offer some form of employment for a sizable number of households especially women and children. In Sembabule almost all rural households keep poultry. The objectives of the research study were:

1. To assess women’s and men’s access and control over poultry Resources in Sembabule District
2. To assess gender roles, responsibilities and time use in poultry production in Sembabule District.
3. Prioritization of problems related to poultry vaccines in Sembabule District.

Research Methodology

The research was carried out in the two counties of Sembabule District, namely. Mawogola County which is predominantly agro-pastoralist and Lwemiyaga County, predominantly pastoralist. It was a cross sectional study using qualitative/participatory research methodologies, involving secondary data collection, stakeholder engagement, a rapid appraisal to the district for a scoping study and field data collection for gender analysis involving both traditional participatory methods, namely Focus Group Discussions, Key Informant Interviews and Participants observations. In addition, novel methods of collecting qualitative data, namely jar voices and focus meals were used. Domains 2 & 3 of The USAID Five Domains of Gender Analysis Framework were used to collect data. The data collected was analyzed using qualitative data analysis tools.

Results

Many women were found to have access and control over poultry resources. Men have control over land and cattle. Men are slowly encroaching on the goat resources which were previously a domain of women. It was found that more of the roles, responsibilities and time use in poultry production are done by women. The prioritized poultry health related challenges were: diseases especially New Castle Disease, lack of authentic drugs and vaccines, and lack of training.

Conclusions

In Sembabule District, Poultry is the only animal resource still accessed and controlled by women. If managed properly, poultry could be a starting point for enhancing livelihoods of women in this community. New Castle Disease is still a big challenge to poultry farming in the area, and more efforts are needed in combating this disease preferably through vaccination.

ID : 1291

THE FAO CONTRIBUTIONS TO THE SUSTAINABLE DEVELOPMENT GOALS THROUGH POULTRY PRODUCTION IN DEVELOPING COUNTRIES

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Commercial poultry (CP) production has made substantial progress during the last seventy years in developing countries of Africa, Asia, Latin America and Oceania. This type of production depends on costly imported inputs such as day-old chicks from high-performance hybrid stocks, balanced feeds, drugs, vaccines, etc. and on the presence of consumers who can afford to buy commercial poultry products.. Family poultry (FP), which make up more than 80% of poultry stocks in most developing countries, are still important. Analysing of grey literature and mid-term reports of targets and indicators towards achieving 17 Sustainable Development Goals (SDGs) by 2030 showed that FP are central to contribute to achieving directly 12 of the SDGs. The growing demand for poultry products in developing countries, driven by population growth, higher incomes, scarcity of land and urbanization, represents a huge opportunity for hundreds of millions of poor smallholder poultry farmers, processors and marketers, many of whom are women, to meet that market demand and rise out of poverty.. Improving the efficiency of poultry production in developing countries, especially the productivity per poultry bird, can triple poultry productivity while reducing by more than a third its adverse environmental impacts, including reducing emissions of greenhouse gases (GHG), in those developing countries that are less GHG emitters but highly negatively impacted by them. However, FP are facing many constraints, including high mortality (mainly due to Newcastle and other avian diseases). Significant improvements in FP production systems can be achieved through well-designed and implemented research and development programmes that endow FP actors with necessary knowledge and skills. In addition to the need for substantial improvement in human and institutional capacity building, planners and policy makers must be sensitized about the potential of poultry as a tool in poverty reduction, food security and gender equity strategies.

The Food and Agriculture Organization of the United Nations (FAO) is committed to FP production and, through the International Network for Family Poultry Development (INFPD) and other initiatives such as the Hand-in-Hand. This paper highlights the importance of poultry for poverty reduction, food security, and the promotion of gender equality in developing countries and discusses the FAO contributions towards the SDGs adopted by the world leaders of 195 nations in 2015.

ID : 1292

DIVERSIFIED POULTRY PRODUCTION IN INDIA

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Diversified poultry includes ducks, turkeys, Japanese quail, Guinea fowl, Goose, pigeons and commercial rearing of native chicken. Diversified poultry systems are now considered as viable livelihood options for small and marginal farmers in India. Ducks accounts for seven per cent of poultry population and are mostly found in coastal states of the country and the duck eggs contribute about 1.15 per cent of total egg production in the country and is preferred over chicken eggs by some consumers. Commercial Japanese quail farming in India can be a great source of handsome income and employment opportunity to small and marginal farmers. At present Japanese quail have become the third largest avian specie grown in the country. About 45 million meat type broiler quail are produced in the southern state of Tamil Nadu alone and about 30 % in other states; 30 million Japanese quail eggs produced annually. Turkey, guinea fowl and goose farming are still in their infant stage. Turkey farming is planned on a small scale and seasonal sales. Guinea fowl are reared in backyard of farm houses and a few Guinea fowl breeder farms do a lucrative business in Odisha and Tamil Nadu. Native chicken farming provides subsidiary income to the rural families. Native chicken meat and eggs attract premium price in local market. Hence, commercial intensive farming of native chicken has become popular and about 12 million native chicken are under intensive production. Whichever species is preferred based on local market preference, farmers mostly go for same species rearing and avoid multi species in the same farm. Commercial duck production for eggs and meat with improved breeds has not been attempted on a larger scale and its scope depends on change in demand for specific duck eggs and meat as chicken eggs and meat production costs are cheaper and management including health cover is easier. Change in consumption pattern with wider acceptance of frozen meat will usher in growth of turkey production in future. As the egg production performance of availability turkey germplasm does not support viable commercial production, impart of quality small variety turkey breeds as parent stock is required. Guinea fowl meat is preferred only by a few. To diversify and substitute commercial chicken production in India especially to support small and marginal farmers, Japanese quail farming and intensive rearing of native chicken offer much scope. With proper inputs, turkey farming also may do so.

ID : 1328

MONITORING OF RESPIRATORY PATHOGENS IN COMMERCIAL SPENT LAYING HENS AFTER INTRODUCTION IN BACKYARD FLOCKS

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Small scale family flocks (SSF) usually found in rural areas are getting more common in urban areas worldwide. Some health surveys showed that SSF could be infected with *Avibacterium paragallinarum* (AvP), *Mycoplasma synoviae* (MS), *Mycoplasma gallisepticum* (MG), *Ornithobacterium Rhinotracheale* (ORT), Infectious bronchitis (IB), *Chlamydia* spp. (Chl.spp), *Chlamydia gallinacea* (Chl.g), Avian metapneumovirus (aMPV) and *Pasteurella Multocida* (PM) which are pathogens of economic importance for commercial poultry industry (Madsen et al., 2013, Donati et al., 2018). Studying how SSF could be reservoirs for pathogens and understanding their transmission patterns are important issues to prevent clinical outbreaks in small or large-scale poultry farms, especially in a recent context of growing urban agriculture (Blecha et al., 2013). In France, a common practice for family flocks' owners is to get spent hens from commercial farms. In order to assess the transmission of pathogens from a "family flock" environment to commercial layers, a focus was made on AvP, MS, MG, ORT, IB, Chl.spp, Chl.g, aMPV and PM status of commercial layers. Layers from a negative free-range commercial farm for the previously cited pathogens were monitored after being introduced into 28 existing family flocks. Biosecurity practices were studied and sanitary status was assessed by qPCR at introduction and six months after. Commercial layers became respectively positive for AvP (32%), MS (39%), MG (11%), ORT (21%) after their introduction in SSF. All layers were negative for Chl.spp, Chl.g, aMPV and PM. Median flock size was of 7 hens and 25% of SSF were flocks having less than 2 years old. More than 80% of owners claimed to have layers for egg quality and recycling organic waste. They observed no specific biosecurity practices. The study showed new agricultural practices in (sub)-urban areas as well as flocks hosting pathogens. In this case, contamination from SSF to commercial farms seemed limited since direct or indirect contact between commercial farms and urban family poultry were rare (Alexander et al., 2007); (sub)-urbans family flocks could more be considered as "dead-end hosts". Nevertheless, monitoring respiratory pathogens could be of interest to limit disease spread in the SSF "compartment". Considering other pathogens such as *Salmonella* spp. and *Campylobacter* spp. would help preventing eventual food-borne outbreaks and assess public health issues.

ID : 1331

ACTION RESEARCH TO IDENTIFY CONSTRAINTS AND OPPORTUNITIES FOR INCREASED NEW CASTLE DISEASE VACCINE ADOPTION AMONG WOMEN SMALL HOLDER POULTRY FARMERS IN KENYA, RWANDA AND UGANDA.

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Objectives: In Rwanda, Kenya and Uganda, 75 to 90% of small-scale poultry farmers are women. Diseases are a major issue preventing them from optimizing production. Their flocks are frequently decimated by NCD despite availability of an effective vaccine. Packaging and reliable structures for vaccine delivery remain an obstacle to vaccine uptake and use. Women are disproportionately affected because of their reliance on poultry income. Our objectives were to: 1) identify barriers and opportunities for women's participation in NCD vaccine value chain (VVC) 2) formulate strategies that position women to effectively contribute to and benefit from poultry vaccines, 3) enhance women's participation in vaccine distribution, delivery and use, 4) provide data that impact programmatic and policy interventions.

Methodology: We used three methods for gender analysis and NCD-VVC analysis:1) The USAID Five Domains of Gender Analysis Framework administered through focus groups captured barriers, opportunities and strategies for improving women's participation in poultry ownership and VVC. 2)The Women Empowerment in Livestock Index (WELI) a standardized household quantitative survey tool captured the empowerment index of women farmers and vaccine usage. 3) Outcome Mapping systematically mapped and tracked critical stakeholders in the VVC focusing on changes in gender norms and cultural practices, organizational systems, institutional and governance policies that lead to increased vaccine adoption. We also introduced novel tools such as Vaxer Calendars, focus meals, jar voices, VacZines and PhotoVax to collect data and identify entry points for interventions.

Results: Preliminary results highlight limited vaccine availability and accessibility, as well as infrastructure challenges, lack of knowledge and information to guide poultry farmers on vaccinable diseases, women's limited decision-making agency in their households and communities, gender related technical, social, cultural, and economic barriers, lack of partnership/networking with public and private sectors as key reasons for low adoption of NCD vaccines.

Conclusion: The project will pilot interventions that target women's individual and collective decision-making control within the households and communities, resulting in a recognized productive role in poultry management, strengthened skills and knowledge on vaccines, opportunities to influence governance structures leading to increased poultry vaccine adoption.

ID : 1409

DEVELOPING SUSTAINABLE VALUE CHAINS FOR SMALL-SCALE POULTRY PRODUCERS

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In developing countries, small-scale poultry production has an important role for food security and nutrition, income generation and women's empowerment. Small-scale poultry producers face however many challenges, including growing environmental constraints, poor access to quality input and services, market linkages and limited capacities and decision making in the value chains governance, which often hamper their productivity and competitiveness vis-à-vis their larger counterparts.

The sustainable food value chain development (SFVCD) framework developed by FAO is a market led approach that integrates the multifaceted concepts of value-addition and sustainability to address the challenges small-scale producers are facing. The value-chain development is a dynamic process, which begins with the characterisation of stakeholders and their activities, the enabling environment, the behavioural change and the governance of the value chain. This allows to identify root problems, leverage opportunities to upgrade the targeted value chain, and develop a vision and a strategy supported by appropriate action plans, in accordance with the value chain stakeholders.

The interest of the SFVCD for small-scale poultry production is illustrated through the results of various projects in Asia, Africa and Europe. The specific characteristics of small-scale poultry production systems (i.e. multi-functionality, poultry as a liquid asset, input and output provision, health and quality, social equity, etc.) underline the need to analyse value chains in a holistic manner, considering not only the supply chain itself, but also its broad environment, its dynamics and its connections to other systems. When considering value chain development involving/or/focusing on small-scale poultry producers, a particular challenge relates to the interest of these producers, especially in a scavenging system, to move from a subsistence to a market oriented system.

AP2

WATERFOWL

ID : 219

THE EFFECT OF MULTI STRAINS BACILLUS AMYLOLIQUEFACIENS ON GROWTH IN MUSCOVY DUCKS

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Probiotics are known to improve gut health in poultry. *Bacillus amyloliquefaciens* have shown to decrease pathogenic *Escherichia coli*, reduce intestinal permeability, increase nutrient digestibility and performance in broilers in recent paper (a). For these reasons, *Bacillus amyloliquefaciens* was applied in the growing ducks to monitor the performance. The trial was conducted in France at a semi-commercial setup, in MG2MIX trial farm facility. Male Muscovy ducks R71M, (n = 1312) were randomly distributed in two treatments i.e. with *Bacillus amyloliquefaciens*, serving as treated or without serving as control. The treatment groups received feed with 300,000 cfu/g for starter and 150,000 cfu/g for grower. Birds were kept on a raised slotted floor with free access to feed and water with 41 ducklings per pen and 16 replicates. Ducks were fed with a starter crumbles (day 1- 21) and grower pellets (21- 49 days). For starter and grower, diets mainly consisted of wheat and corn (respectively 72% and 83% of total cereals plus cereals by products) and soybean meal. Finished feeds were analyzed for crude protein (CP 19.3%), crude fat (CF 2.1%) and crude fiber (CFb 2.9%) for starter and for grower CP 15.1%, CF 3.0% and C Fb 2.65%. Body weight gain (BWG as gram/bird/day), feed intake (FI, g/b/d) were measured and feed conversion ratio (FCR) calculated at the end of starter and grower phases (day 21 and 49). The results were analyzed using JMP pro statistical analysis program and one-way ANOVA. Average BWG in control vs treated (23.6 vs 23.0), FI (36.5 vs 36.1 g/b/d) and FCR (1.55 vs 1.57) were not statistically different in the starter phase (d 1-21). These parameters improved significantly during grower phase (day 21-49) i.e BWG (65.7 vs 70.0) and FCR (2.13 vs 2.07). However, FI was not statistically different versus control (140 vs 145). Combining overall performance from day 1-49, FCR was significantly ($P < 0.05$) improved by 2.0%. Improved gut health status and reduced immune challenge could be one explanation of increased performance, however, unfortunately these parameters were not measured in this study. In conclusion, addition of multi-strain *Bacillus amyloliquefaciens* improved BWG and reduced FCR in Muscovy ducks at 49 days of age.

a. K. Gibbs, E. White, D.J. Cadogan and S.J. Wilkinson. The effects of a multi-enzyme and bacillus probiotic combinations on calcium and phosphorus digestibility and broiler performance. 2018. Aust Poult Science Symposium 29.

ID : 1293

FATTY ACIDS PROFILE OF BREAST MUSCLES FROM DUCKS FED WITH YELLOW LUPIN

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In duck rearing in small farms in EU more often is searched of alternative vegetable protein source for soybean meal. The possibility of using the high protein content plants produced in small farms is a reason for conducting studies about quality evaluation of duck meat produced in this conditions. The aim of the study was to comparison of fatty acids profiles in breast muscles of duck fed with diets based on yellow lupine (*Lupinus luteus* L.), brewery yeast and potatoes protein as high-protein alternative for soybean meal. Chery Valley ducks were kept in small farm in pens on litter in two groups, 100 birds in each. Both groups divided into 4 replications with 25 birds per each. The control group (I) was fed with balanced feed containing soybean meal. The treatment group (II) received balanced feed containing yellow lupine. Ducks were kept for 8 weeks. After 8 weeks 10 randomly chosen ducks from each group were slaughtered. From each carcass breast muscles were collected and weighted. In all collected samples the acids profile was determinate. Lipids extraction from breast muscles was made by Folch et al. (1957) method. Fatty acids (FA) quantity level was determinate as fatty acids methyl esters (FAME) with using of gas chromatography GC Trace 2000 (ThermoQuest EC Instruments). Presented results embodied as percent complete determinate FA. Evaluation of feed implication was made by calculation of ratio between n-6 to n-3 FA (n-6/ n-3) and ratio of PUFA to SFA (P/S). Atherogenic index (AI) and thrombogenic index (TI) were calculated by Ulbricht and Southgate (1991) method. Numerical data were processed with Statistica 10.0 PL software (2011) ANOVA was processed and the significant differences was verified with Scheffe test when $P < 0.05$. In group I the % of breast muscles in carcass was bigger for 0.26. Percent of linoleic acid (LA; C18:2 n-6) was significant bigger in group II and C18:1 n-9 (oleic acid) as well ($P < 0.05$). It could be a positive effect of using the yellow lupine for profits from LA. In group II shown significant smaller quantity of C18:0, C20:4 n-6, C22:5 n-3 ($P < 0.05$). Shown bigger quantity in total of SFA in group I. Ratio P/S was significant bigger in group II ($P < 0.05$). Significant smaller factors of AI and TI were found in group II ($P < 0.05$). Results of this study shows positive influence of using feed with yellow lupin in ducks feeding, because it's improve quantity of linoleic acid and lower factors AI and TI compared soybean meal diet. [Resolution No. 222/2015; Grant No. PPI/APM/2019/1/00003].

AP3

RATITES

ID : 608

GENETIC EVALUATION OF PRODUCTION TRAITS OF OSTRICHES REARED UNDER SUB-TROPICAL FARMING CONDITION

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In India, ostrich is being promoted as an alternative poultry farming. It is not a traditional farm of India. However, it is in existence since 2000. Our experience revealed that rearing, feeding, breeding and hatching is quite different from regular chicken rearing. Experiences also revealed that phenotypic / performance selection is not sufficient to maintain a good breeding stock. With this background, a study was undertaken to estimate the genetic parameters for production traits. Data pertaining to the chick weight, periodical body weights and pedigree information were collected (period from 2001 to 2018) from the ostrich breeding unit located at Post Graduate Research Institute in Animal Science, TANUVAS, Kattupakkam. The description of production traits studied were chick weight, body weight at different ages, and average daily weight gain. The effect of non-genetic fixed factors on the production traits were studied during the period 2000 – 03, 2004 – 07, 2008 - 11, 2012 – 15 and 2016 – 18) and seasons of hatch Winter (Jan– Feb), Summer (Mar – May), SWM (June– Sep) and NEM (Oct – Dec). All the analyses were carried out using WOMBAT software (Meyer, 2007). The overall mean chick weight was 766.54 + 8.15 g. The least square mean for body weight at 2, 4, 6, 8, 10 and 12 m were 10.35 + 0.31 , 37.46 + 1.35, 52.22 + 0.74, 66.49 + 0.73, 85.04 + 1.28 and 99.78 + 1.33 kgs respectively. The least square mean ADG of 0-2, 2-4, 4-6, 6-8, 8-10 and 10-12 m were 166.07 + 5.97, 335.55 + 9.22, 346.07 + 10.64, 231.88 + 8.10, 321.3 + 11.48 and 245.28 + 12.74 g respectively. The effect of seasonal period of hatch was found significant ($P < 0.05$) for chick weight, body weight traits [except 12 m (period) and 10 m (season)], ADG [except above 6 m (period) and 6-8 m (season)], mortality [except 3-6 m (period) and 6-18 m (season)]. The heritability estimates for chick weight, 2 months body weight and ADG (0-2 m) were high (above 0.3) and for body weight at 4 and 6 m and ADG (2-4, 4-6 and 8-10 m) were moderate (0.1 to 0.3). The estimates for body weight at 8, 10 and 12 m were low (0.0 to 0.1), the heritability estimate for chick weight was 0.551 + 0.143. Higher values of genetic correlation between early and late body weight traits provided better scope for improvement through indirect selection. Negative genetic trend was observed for early body weight traits and positive genetic trend was found for body weight traits at later ages.

ID : 736

OPTIMAL SPERM DOSE AND FREQUENCY OF INSEMINATION FOR MAXIMAL FERTILISATION RATE IN OSTRICHES

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Artificial insemination (AI) may help reducing infertility, mating incompatibility while improving genetic gain in ostriches. We investigated the minimum sperm dose (Trial 1) and optimum frequency of insemination (Trial 2) that would also maintain high fertility rates over extended periods. Twenty-two South African Black ostriches (7 males and 15 females) aged between 2-10 years old were used. Semen was collected using the dummy female method and diluted 1:4 (semen:diluent) with an ostrich specific semen diluent. In Trial 1, females were inseminated three times a week on every second day with various sperm doses of fresh semen from the same male, resulting in a total sperm dose of A=7.5 billion sperm/week. Fertilization status of the germinal disc of eggs produced after the last AI was determined using the egg breakout technique. The fertile period was calculated as the number of days fertilized eggs were laid after the last AI. The rate of sperm loss on eggs produced after AI was also estimated. In Trial 2, the minimum sperm dose along with the longest fertile period obtained in Trial 1 were used for inseminations on two consecutive days, followed by a single weekly dose for 4 weeks. Fertilization status, fertile period and rate of sperm loss were also evaluated. Generalized linear mixed models in SAS were applied on the data and P<0.05). Rate of sperm loss between doses also did not differ (P>0.05). Preliminary results for Trial 2 revealed an overall fertilization rate of 47%. Hence, two consecutive AI events with sperm doses between 2.5-5 billion sperm/week followed by a single weekly dose appeared to maintain relatively good fertility rates of ostrich eggs. Additional data to expand on Trial 2 is underway to further refine the ostrich AI protocol and demonstrate its utility as a feasible reproduction option for ostriches.

ID : 1278

BRISTLE HAIRS, FILOPLUMES AND PINHOLES IN OSTRICHES

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Ostrich leather is deemed a luxury leather, with quality being of utmost importance. The widespread presence of small pinholes on ostrich skins has become increasingly problematic. Filoplumes and bristle hairs are implicated in the formation of the pinholes (Lunam & Weir, 2006; Weir & Lunam, 2011), and while both are deemed a natural phenomenon on avian skins, it is nonetheless regarded as a defect that retracts from leather quality. The presence of hair and hair follicles (pinholes) were consequently scored at different ages to determine environmental and genetic factors. It was found that the hair follicle scores on processed skins were heritable (\pm SE) at 0.40 ± 0.06 . The presence of hairs on live birds was accordingly heritable at 0.51 ± 0.11 in juvenile birds and 0.23 ± 0.09 in adult birds assessed repeatedly. The animal permanent environmental variance ratio was also significant at 0.22 ± 0.09 for adult birds. Hair follicle score was genetically correlated to hair score on live slaughter birds (0.49 ± 0.23) while the phenotypic correlation between these traits amounted to 0.36 ± 0.08 . The genetic correlation was lower than expected but it should be considered that only 476 hair scores was available on SA Blacks, of which only about 20% ($n=97$) had hair follicle scores on their skins as well. Genetic correlations of hair scores on live adult birds with other traits of economic importance were 0.64 ± 0.30 with egg production, -0.65 ± 0.35 with chick production and 0.27 ± 0.21 with adult live weight. Corresponding animal PE correlations were 0.93 ± 0.38 with egg production, 0.78 ± 0.32 with chick production and -0.10 ± 0.21 with adult live weight. Phenotypic correlations amounted to respectively 0.14 ± 0.07 , 0.15 ± 0.07 and 0.05 ± 0.05 . Hair scores on adult and juvenile birds essentially represented the same trait, as indicated by a genetic correlation of 0.93 ± 0.11 . Adult hair scores were also positively related to hair follicle scores in their slaughtered offspring, with the genetic correlation amounting to 0.61 ± 0.27 between these traits.

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WVPA

HYGIENE AND PATHOLOGY

ID: 22

THE IMPACT OF CLEANING AND DISINFECTION OF POULTRY HOUSE ON BROILER PERFORMANCE AND MICROBIAL STATUS

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The study evaluated the impact of broiler house degree of cleaning and disinfection on growth performance and microbial status. The degrees of cleaning included (dry cleaning only (T1), dry and wet cleaning with ordinary water (T2), dry and wet cleaning with detergent (T3), dry and wet cleaning with detergent and disinfection (T4), and dry and wet cleaning with detergent, disinfection and whitewashing (T5). A total of 375 Ross 308 broiler chicks were randomly allocated to each of the treatment and reared for 8 weeks. Each treatment was replicated 3 times with 25 birds each. Data recorded included feed intake, weight gain, feed conversion ratio (FCR), liveability and microbial load. Data were analyzed as one-way ANOVA using the Proc. GLM SAS® at $P \leq 0.05$. The bacterial counts were analyzed as log₁₀ CFU and ANOVA obtained my GLM. Birds from T5 had higher feed intake than birds from T1 and T2 for the period of 1-28 days but none was different from T3 and T4 ($P = 0.04$). The broiler weight gain per bird tended to be different at 49 days ($P = 0.07$) and increased with the degree of cleaning. The FCR up to 49 was lower in T4 compared to T1 ($P = 0.022$). The weekly mortality reduced with increasing degree of cleaning ($y = 1.24x^2 - 13.70x + 46.95$; $R^2 = 0.97$). Similarly, bacterial load in the litter also reduced ($y = -0.99x + 8.39$, $R^2 = 0.82$ for total viable count, and $y = -0.92x + 7.64$, $R^2 = 0.82$ for *E. coli* count). It can be concluded that complete cleaning of the pens up to the application of disinfection increased broiler performance and there may not be the need to include whitewashing of the pen walls before broiler chick placement.

ID : 35

EFFECT A NOVEL TRIPLE STRAIN PROBIOTIC BACILLUS ON THE DIGESTIVE IMMUNE SYSTEM UNDER SUB-CLINICAL NECROTIC ENTERITIS CONDITIONS

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A commonly used immune-functionality technique is the examination of levels of cytokines. Cytokines are the communication substances secreted and received by cells in the body, particularly regarding immune function regulation. Research was conducted to measure the effect a new 3-strain Bacillus-based probiotic (GALLIPRO® Fit) on the immune system, particularly investigating the impact on cytokines. In this trial, broilers were the study animal and a mild NE (Necrotic enteritis) challenge was included to better investigate cytokine levels and influence of the probiotic in the face of a pathogenic event.

36 pens were populated with 50 day-old birds each. There were 9 repetitions for each of the 4 treatment groups: negative control T1, negative control + probiotic T2, positive control, T3, positive control + probiotic T4. The NE challenge consisted of the addition of *Clostridium perfringens* at a dose of 1×10^8 CFU/ml drinking water on days 18, 19, and 20. On day 21, three randomly-obtained birds were sampled for intestinal cytokine gene expression. The pro-inflammatory cytokines IL-1b, IL-18, IL-6, and IL-12b and the regulatory cytokine IL-10 were assayed. The quantification was determined for each sample by comparing the number of cycles required to cross a certain threshold of fluorescence (Ct). The NE challenge group receiving the probiotic (T4) showed significant upregulation of IL-1b, IL-6, and IL-18. These cytokines are pro-inflammatory and anti-inflammatory. Of the three other treatment groups, only T3 (NE challenged, but not fed probiotic) showed a significant upregulation of IL-6. This is an expected result and indicates a normal, initial inflammatory response effect produced by the NE challenge. The two groups not receiving the NE challenge showed no upregulation of the cytokines. There was no expression of IL-12b and no significant upregulation of IL-10 for any of the treatment groups. These data indicate the new 3-strain Bacillus-based probiotic 'primes' the immune response. The probiotic treatment group and no NE challenge showed no up-regulation of the pro-inflammatory cytokines, thus maintaining normalcy but not stimulating the immune system. In the presence of the NE challenge, the probiotic-treated group showed significant enhancement of the intercellular cytokines.

This demonstrates the immune modulator activity of the novel probiotic to enable the triggering of these defense mechanisms in the face of a disease challenge.

ID : 118

BACILLUS AMYLOLIQUEFACIENS CECT 5940 IMPROVES THE EXPRESSION OF IMMUNITY GENES IN BROILERS UNDER A SUBCLINICAL NECROTIC ENTERITIS CHALLENGE

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The ban of in-feed antibiotics has negatively affected the poultry industry by causing necrotic enteritis (NE) to emerge in the flocks and has caused a significant economical concern for poultry farmers. *Bacillus* spp. namely, *Bacillus amyloliquefaciens* (BA), has shown to have substantial antimicrobial activities and the ability to produce several extracellular enzymes that enhance digestibility and absorption of nutrients. This strain can also produce bacteriocins, which can be effective in controlling *C. perfringens* (Bindiya et al. 2019). The study consisted of two 2 × 2 factorial arrangements where birds were subjected to NE challenge (yes or no) and probiotic treatment (yes or no). The dietary concentration of probiotic was 1x10⁶ CFU of BA CECT 5940 per gram of feed. Oral administration of *Eimeria* oocysts (d 9) followed by inoculation with 1 mL 10⁸ CFU/bird of *Clostridium perfringens* (d 14 and 15) was used to induce NE in the challenged groups. On day 24, NE challenged birds showed lower weight gain and increased feed conversion ratio (FCR) ($P < 0.001$), however the supplementation of BA improved the weight gain and FCR in all groups ($P < 0.001$ and $P < 0.05$, respectively). At d 16, jejunum tissue samples were collected for gene expression analysis. Results showed a NE challenge × BA interaction for interferon- γ (IFN- γ) where IFN- γ expression was highest in the NE challenged group with no supplementation and BA supplementation significantly reduced IFN- γ of challenged birds compared to the non-supplemented group ($P < 0.001$). Downregulation of IgA ($P < 0.001$) and IL-18 ($P < 0.001$) genes were observed in the NE challenged birds compared to the non-challenged groups. Furthermore, NE challenge × BA interaction was observed for IgG ($P < 0.01$) and IgM ($P < 0.05$), where the NE challenge with no supplementation of BA shows the lowest values, however, the supplementation of BA gave similar results as the unchallenged birds with or without supplementation. These findings suggest that supplementation of BA in broiler diets can help improve gut health by possessing positive effects on the expression of important genes related to the immunity functions in the gut.

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ID : 137

DIETARY SUPPLEMENTATION OF PLANT EXTRACT SHOWED IMPROVED FEED EFFICIENCY AND ENHANCED GUT INTEGRITY IN BROILERS CHALLENGED WITH NECROTIC ENTERITIS

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Plants extracts are being increasingly researched for their potential to improve gut health as in-feed antibiotics are phased out of the poultry industry. A feeding study was conducted to examine the efficacy of plant extract (PE), a micro-encapsulated product composed of eugenol and garlic tincture on growth performance, gut integrity and caecal microflora in broilers challenged with necrotic enteritis (NE). A total of 960 d-old Cobb 500 broiler chicks were assigned to 48-floor pens each stocked with 20 birds. A randomised complete block design was used with 6 treatments replicated 8 times and the treatments were: T1 - unchallenged group, without additives; T2 - challenged group, without additives; T3 - challenged group plus PE (100 g/t feed, Pancosma SA); T4 - challenged group plus antimicrobial (AM, 625 g/t) (Maxiban, Elanco US Inc.); T5 - challenged group plus AM with PE; T6 - challenged group plus half dose of AM (312.5 g/t) with PE in starter, grower and finisher phases, respectively. Diets were based on wheat, sorghum, soybean meal, and meat and bone meal and were supplemented with phytase. Challenged birds were given 1 mL per os field strains of *Eimeria* spp. oocysts consisting of *E. acervulina* (5000), *E. maxima* (5000) and *E. brunetti* (2500) at d 9. On d 14, challenged birds were gavaged with 1 mL per os 10⁸ CFU/mL of *Clostridium perfringens* (NE-18). NE caused mortality was determined by necropsy and mortality data were used to correct the FCR. Bird performance was measured from d 0 to 35. A leaky gut marker, serum fluorescein isothiocyanate dextran (FITC-d) and caecal microflora were measured at d 16. All data were analysed using JMP software (v.14). The treatments T1, T4, T5, and T6 had higher body weight gain (BWG), higher livability and lower FCR compared to T2 ($P < 0.05$). Birds supplemented with PE (T3) had lower FCR and feed intake compared to T2 ($P < 0.05$). A higher concentration of serum FITC-d was observed in T2 compared to unchallenged and challenged groups with additives ($P < 0.05$). Birds treated with PE (T3) had lower counts of *Lactobacillus* spp. ($P < 0.05$) in the caecal contents than T2. These findings suggest that different combinations of a PE with antimicrobials were effective in alleviating the impact of NE as indicated by improved BWG, FCR, higher livability, and enhanced gut integrity. These results also demonstrated that diet supplemented with plant extract helped to improve feed efficiency and enhance gut integrity.

ID : 194

HAEMOPROTEUS INFESTATION IN A FLOCK OF COMMERCIAL BROILERS IN BELGIUM

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Haemoproteus constitutes a genus of parasites that infest the red blood cells of amphibians, reptiles and birds. More than 120 Haemoproteus species have been reported in birds, predominantly wild waterfowl, raptors and passerines (1). To the best of our knowledge, infestations of chickens have only been described in Bangladesh, Malaysia, Kenya, Mosambique and Iraq.

Since Haemoproteus is spread through blood-sucking insects, the geographic distribution might be explained by the presence or absence of appropriate vectors. In birds, these often include Culicoides spp. and Hippoboscids flies. The vectors for Haemoproteus in chickens have not been identified.

Haemoproteus can easily be recognized microscopically, based on morphological characteristics and the localization in the cytoplasm of erythrocytes.

In June 2018, a stagnation of feed and water intake was observed in a Belgian flock of 25-day-old commercial broilers. This resulted in reduced growth. The chicks did not give the impression of being sick and mortality was not increased. At 28 days of age, 10 broilers of the flock were euthanized and necropsied. From 4 of these, trachea, lung, heart, liver, kidney and bursa were examined cytologically. For this purpose, impression smears were stained with the Diff Quik staining reagents (Medion Diagnostics) and evaluated microscopically at a magnification of 1000 times.

In all necropsied birds, the intestinal tract was poorly filled with feed. Besides paleness of the heart and a few petechiae in the bursa of one broiler, gross pathological organ lesions were not observed. Cytologically, moderate infiltrations of lymphocytes were detected in multiple organs of all birds. Examination of the heart and lungs of one specific chick revealed gametocytes of Haemoproteus in the cytoplasm of multiple erythrocytes. Mononuclear phagocytic cells were often observed in close relationship to the infested cells, indicating onset of erythrophagocytosis.

To the best of our knowledge, this is the first report on Haemoproteus in chickens in Western Europe. Since this parasite usually spreads through vectors, the present observation implies the presence of an appropriate vector or any other, unknown mode of transmission.

It is not clear to which extent the lower performance of the flock can be ascribed to the infestation with Haemoproteus.

(1) Bermudez, A.J. 2014. Haemoproteus infections. In: Diseases of Poultry, 13th ed. D.E. Swayne et al., eds. Wiley-Blackwell, Ames, IA, pp. 1189-1190.

ID : 250

ANTIMICROBIAL RESISTANCE PROFILES OF KLEBSIELLA ISOLATES FROM DIFFERENT TYPES OF CHICKEN IN IMO STATE, SOUTHEAST NIGERIA

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Extensive use of antibiotics in poultry in Nigeria has resulted in the emergence of resistant bacteria in poultry pathogens, highlighting the need for frequent monitoring of antimicrobial resistance profiles in important pathogens. This study reports the antimicrobial resistance profiles of Klebsiella species isolated from the faecal droppings of different types of chicken raised by small-scale farmer in Imo state, in the rainforest south-eastern Nigeria. Six replicate cloacal samples each, from broilers starter (BS), broiler finisher (BF), grower birds (GB), layer hens (LH) and local chicken (LC) were collected with sterile swabs, at five different locations and cultured for Klebsiella species. Serial dilutions of each sample was streaked on MacConkey agar and incubated at 37°C for 24 hours, and pink coloured colonies showing lactose fermentation sub-cultured further on eosin methylene blue agar to differentiate between Klebsiella and E. coli species. The isolates were subjected to antimicrobial resistance profiling on MacConkey agar using the disc diffusion method. Data generated were subjected to descriptive statistics such as means, percentages and histograms. An overall 43.00% Klebsiella prevalence was recorded; 60% in LC, 50.00% in GB, 40.00% in LH, 30% in BS and 35.00% in BF. Overall, the organisms exhibited very high resistance to trimethoprim (septrin), penicillin and nalidixic acid (77.42–87.1%), average resistance to streptomycin and gentamycin (51.61% each), moderate resistance to perfloxacin, ciprofloxacin and augumentin (29.00-35.48%) and low resistance to cephalixin and ofloxacin (16.13-19.35%). Isolates from GB were highly susceptible to ofloxacin, perfloxacin, ciprofloxacin, cephalixin and gentamycin, while those from LM were highly susceptibility to ciprofloxacin and cephalixin. BS isolates were also highly susceptible to the ofloxacin, perfloxacin and augumentin, while the BF isolates were susceptible to ofloxacin, augumentin, cephalixin and streptomycin. The isolates from LC were equally susceptible to ciprofloxacin, cephalixin and streptomycin. In conclusion, the floquinolones, augumentin and cephalixin could be used in the treatment of Klebsiella infection in the broiler and pullet flocks, while for the local chicken, ciprofloxacin, cephalixin, streptomycin and septrin should be used. Effective treatment of poultry infections in the study area should be based on the results of antimicrobial resistance profiling of causative pathogens.

ID : 256

THE APPLICATION OF AMALGAM BACTERICIDAL LAMPS IN POULTRY PRODUCTION

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The trial was performed to evaluate the efficiency of ultraviolet (UV) irradiation for air disinfection in populated poultry house for floor-housed broilers kept in two boxes (15 m² each) with sawdust litter. The air in the control box was not treated with UV. In the experimental box an amalgam bactericide lamp (87 Wt of UV at 253.7 nm) was set (2 m above the floor). UV irradiation of the air was performed by upper-room method in an intermittent regime: since 1 to 7 days of age – for 1 hour 6 times a day; from 8 to 28 days – for 10 min 12 times a day (each 2 hours); from 29 days of age to slaughter – for 15 min each 2 hours. The exposure to UV at the level of chicks was 11.4 mWt/m²; average exposure at the box 287.8 mWt/m².

It was found that total microbial loads in the air of the experimental box were lower in compare to the control box: at 7 days of broilers' age by 76.4%; at 14 days by 37.5%; at 21 days by 66.3%; at 28 days by 47.5%; at 35 days by 50.9%. The elimination efficacy against *E. coli* at 7, 14, and 21 days of age was 100; 90.1; and 93.1%, respectively. Since the UV doses lethal for *E. coli* are also lethal for certain viruses it could be suggested that the studied regime of air disinfection with amalgam UV lamp can substantially decrease the risks of circulation of airborne diseases.

The decrease in total microbial loads in the air of experimental box resulted in the significant increase in average live bodyweight at slaughter (35 days of age) by 4.76% (P

No detrimental effects of UV were found on the sense of vision in chicks. No erythema were found on the skin of UV-treated chicks; color of the skin in open and feathered areas was similar in control and UV-treated chicks.

UV treatment also stimulated the chicks to attend feeders and drinkers more actively. As a result the bodyweight uniformity in the treated chicks (the percentage of chicks with bodyweight falling within the range of average value $\pm 10\%$) was higher by 6.2% in compare to control treatments.

The conclusion was made that UV irradiation with new-generation ozone-free amalgam lamps in the studied intermittent regime significantly decreases the microbial load in the air of broiler poultry house and improve growth efficiency and immune status in broilers. The use of this modern UV equipment for air disinfection in populated poultry houses can substantially improve the efficiency of the prevention of hazardous infectious diseases.

ID : 268

SUCCESSFUL VACCINATION AGAINST GUMBORO DISEASE IN LABEL ROUGE SLOW GROWTH CHICKENS USING AN IMMUNE-COMPLEX VACCINE

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Label Rouge [Red Label] chicken is highly appreciated by the French consumers because of its traditional, free-range production methods using slow growing birds slaughtered at 81+ days of age. Its specifications ensure a meat with superior organoleptic properties, with a positive image in terms of animal welfare and consumers' health. Disease prevention is critical on such high-value birds with long lifetime. To preserve a good health status of their flocks, producers need an efficient, safe and convenient vaccination plan. Infectious bursal disease (IBD, Gumboro disease) is of significant economic importance, because it can induce immunosuppression, leading to morbidity, sometimes mortality, and frequent bacterial opportunistic superinfections which require using antibiotics. IBD immune-complex vaccines enable a timely immunization according to the maternal immunity status and a full protection against field viruses through bursa colonization as soon as the vaccine virus is freely released. Its technology allows the combination with vector HVT vaccines against other diseases (eg, rHVT-ND) and with Rispens vaccines. The aim of this study was to use a novel IBD immune-complex vaccine (Novamune) applied at the hatchery, in different conditions, in a large scale commercial operation of Label Rouge chicks. A total of 89 commercial flocks, representing almost 500,000 Label Rouge chickens were vaccinated with Novamune over a period of 3 months, including 11 flocks via subcutaneous route and 78 flocks via in ovo route (out of label). On the same period, a control group of 48 flocks was in-ovo vaccinated with a vector HVT-IBD vaccine. Other vaccines (including systematic Rispens vaccination) were applied as per the usual standards of the commercial operation. Post-vaccination monitoring criteria included production performances (mortality, ADG, FCR), IBD ELISA serology (D0, D28, D42 and D81), and IBDV RT-PCR (D28 and D42). The serology results confirmed the expected onset of immunity against IBD after immune-complex vaccination. This finding is confirmed by the detection of the vaccine strain (SYZA26) in the bursas. In terms of performance, no statistical difference between the two groups (immune-complex and vector rHVT IBD vaccination) was shown. The obtained results showed that this novel immune-complex vaccine is an efficient, safe and convenient option for preventing Gumboro disease in slow growing Label Rouge chickens via subcutaneous or in-ovo route.

ID : 290

APPLICATION OF INERT OILS IN THE CONTROL OF RED CHICKEN MITES DERMANYSSUS GALLINAE

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The use of inert oils in control of *Dermanyssus gallinae* (De Geer, 1778) is recommended due to: non-toxicity; effective, complex acaricidal action, which excludes the possibility of generating resistance; and multipurpose application (in prevention, curative and biosafety). The first dedicated formula of inert oils in control of red chicken mite is P 547/17. The new formula P 2020/0033, aims to increase economic accessibility to farmers and ease the conditions for more detailed application. The aim of this study is to consider the current arguments for the use of assigned inert oil formulations in the control of *D. gallinae*.

The study of 10% aqueous emulsion of formula P 2019/0033 was performed in a laboratory test, by the petri dish. By direct exposure of the adult *D. gallinae* to 1 min exposure, all of a 100% effect was found. With subsequent exposure on tin boxes with examination of prolonged action, after 1 month, we found the efficiency at an oblique position of 38%, inverted 81% and flat 98%.

Optimization of the clinical application of the formula P 547/17 was achieved by two treatments of detailed application using the spray method in an empty facility, at intervals of 5 or more days. Single application on cages and equipment with 20%, in repeated treatment at an absorbent surfaces (floor, walls) with 10% water emulsion and it proved the possibility of eradication of *D. gallinae* in parent breeding facilities (2 facilities with a capacity of 9,000 parents); in cage-laying for laying hens (8 facilities, capacity 18,000 chickens each); small farms for the exploitation of litters in a conventional cage system (capacity 2,500 and 4,000 stretchers). Project ID 1115 improves the application for large industrial cage-type facilities (conventional and enriched). In the first treatment, 20% excess water emulsion was applied. In the second treatment, a small dose of 50% water emulsion was used, which was applied only to the most important places of the cage, while for an absorbent surfaces 10% excess water emulsion was used. So far, eradication has been proved in two facilities with a capacity of 14,000 and 30,000 carriers. Eradication was observed after detailed and systematic examinations (early dust detection method, visual and tactile examinations, staff observations and surveillance of the flock's health status) for 12 months or more, and the established negative finding of *D. gallinae*.

It can be concluded that by the conditions and proper application of non-toxic formulations P 547/17 and P 2020/0033, we are able to effectively control *D. gallinae* and at the same time exclude the toxicological risk of acaricides.

ID : 297

THE CAPABILITY OF A DETOXIFYING AGENT TO PREVENT FUNCTIONAL AND MORPHOLOGICAL LESIONS CAUSED BY T-2 TOXIN IN BROILER CHICKENS

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Objective

Mycotoxins such as T-2 toxin (T-2) are responsible for severe pathological lesions and performance losses in poultry. Impaired gut morphology and liver function are corresponding consequences. The objective of this trial was to evaluate the capability of the detoxifying agent B.I.O.Tox® Farm (BTF; Biochem GmbH, Germany) to prevent the cytotoxic effects and performance losses due to T-2 toxin.

Material and methods

360 one-day-old male broiler chicks (Cobb 500) with an average initial-weight of 39.1 g were randomly divided into 6 treatment groups (n = 6 pen, 10 chicks per replicate): negative control group (NCG) with no BTF or T-2 added, a positive control group (PCG) with 2 ppm T-2 in the diet, a BTF-0 group with 0.4% BTF, a BTF-1 group with 0.1% BTF plus 2 ppm T-2, a BTF-2 group with 0.2% BTF plus 2 ppm T-2 and a BTF-4 group with 0.4% BTF plus 2 ppm T-2. All birds were given ad-libitum access to feed and water during the whole trial period (d1-21). Diets were based on corn and soybean meal meeting the nutritional recommendations according to NRC 1994. Following parameters were observed: zootechnical performance, blood serum biochemistry, and jejunal morphology.

Results

On d 21, body weight (BW), body weight gain (BWG), and feed intake (FI) were significantly decreased in the PCG compared to the NCG (9.7%, 12.0% and 6.3% respectively; $P < 0.05$), whereby all BTF-groups showed significantly higher BW, BWG and FI compared to PCG and were comparable to NCG ($P > 0.1$). Total plasma protein (TPP) and albumin (ALB), both mycotoxin-specific parameters, were significantly lower in the PCG in comparison to the NCG and to BTF-groups ($P < 0.01$). The villus:crypt ratio was significantly decreased in PCG compared to the NCG and BTF-groups ($P < 0.05$). The relative weight of liver and the feed conversion ratio did not differ between treatments.

Conclusions

This study confirms the capability of BTF to prevent the manifestation of T-2 associated cytotoxic effects. This was proven by maintained performance data, consistent serological values like TPP and ALB serum levels as well as a stabilized intestinal morphological parameter in the presence of 2 ppm T-2.

ID : 303

BACTERIOSTATIC AND BACTERICIDAL EFFICACY OF COMMERCIAL DISINFECTANTS AGAINST SALMONELLA INFANTIS VARIES CONSIDERABLY

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Salmonella (S.) Infantis is currently the most common serovar in broilers and in poultry meat in the European Union. In the field, eradication of S. Infantis in affected poultry flocks is considered extremely difficult. Despite stringent cleaning and disinfection measures between the placement of flocks, recurrent infections are often reported. Consequently, it could be hypothesized that S. Infantis has a higher tenacity compared to other Salmonella species, coinciding with a higher resistance to disinfectants. So far, no studies are available which focus on the bacteriostatic and bactericidal efficacy of disinfectants against S. Infantis. Therefore, in the present in-vitro study the efficacy of 10 disinfectants (5x Glutaraldehyde + Quaternary ammonium (QUATS), 3x Peroxygen, 1x Chlorocresol, 1x Diamidine) was tested on 7 S. Infantis field isolates. The testings were performed according to the guidelines of the German Association of Veterinary Medicine [1]. The following variables were included in the tests: 1) different concentrations of disinfectants, 2) different temperatures (10°C, 20°C) and 3) low (3 g bovine serum albumin(BSA)/l) and high protein exposure (10 g BSA/l and 10 g yeast extract/l). Also, an additional incubation step after neutralizing the disinfectants was introduced to investigate the revitalisation potential of S. Infantis. The bacteriostatic efficacy could not be assessed for 3 disinfectants due to turbidity right after compounding. For 4 disinfectants a bacteriostatic effect could only be noticed by raising concentrations above recommendation. Testing disinfectants at different temperatures had no influence. In contrary, the bactericidal effect of 9 disinfectants decreased significantly in the presence of a high concentration of proteins. Additional incubation of the product-bacteria mixture after neutralizing the active disinfectant resulted for 9 disinfectants in a revitalisation of S. Infantis.

Overall, products based on QUATS were most efficient in the present test settings followed by Peroxygen, Chlorocresol and Diamidine. The present in-vitro investigations on the bacteriostatic and bactericidal efficacy of disinfectants against several S. Infantis isolates provide important data to improve performances of cleaning and disinfection measures of contaminated premises.

[1] DVG-Prüfrichtlinie, 2017. V. Tierhaltung - Anhang: Methoden der Prüfung von chemischen Desinfektionsmitteln für die Tierhaltung

ID : 364

STABILITY OF ANTIBIOTIC DRUGS IN DRINKING WATER - IMPACT OF TREATMENT WITH BIOCIDAL PRODUCTS

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Biocidal products are often used to treat drinking water of animals, in order to improve its bacteriological quality. Antibiotic treatments are often administered to animals in drinking water, and there are no compatibility studies between medicines and biocidal products, in pharmaceuticals registration dossiers. This study aims to evaluate the impact of drinking water disinfectants on antibiotics stability in drinking water, in poultry, pig and rabbit farming.

Ten antibiotics formulations including doxycycline, amoxicillin, sulphonamides, tiamulin and colistin were tested with two biocides (H₂O₂ and sodium hypochlorite) in standard, soft and hard waters, and compared to same formulations and waters without any biocidal treatment.

First part of the study consisted in testing different situations that reproduce field conditions : the stock solution storage (with concentrated antibiotics) during 24 hours, and then, injection of the stock solution in the drinking system during 6 hours, to simulate a dosing pump administration.

The second part of the study consisted in testing amoxicillin, tiamulin and doxycycline with H₂O₂ in a field well water rich in iron and manganese.

The different antibiotic molecules were dosed by liquid chromatography coupled with UV detection. Same methodology was used for the two parts of the study (stability, dosage, data analysis). For each test (doubled), stability was stated as decreased if average concentration of the active compound was more than 10% lower to the control sample with significant difference (t-test, p<0,05).

In the first part of the study, water treatment with H₂O₂ at 50 ppm decreased concentration of only one antibiotic : amoxicillin.

Water treatment with sodium hypochlorite (0,5 ppm of active chlorine) had a negative influence on antibiotic stability of 7 antibiotic products, especially in hard water.

In the second part of the study, similar results compared to the first part were obtained for amoxicillin and tiamulin. Contradictory results were found for doxycycline, demonstrating the multifactorial and complex nature of antibiotic stability in drinking water in the presence of biocidal products.

In conclusion, practical recommendations regarding the treatment with antibiotics in drinking water when using biocidal products are provided. Some proposals could have been sent to EU to improve recommendations in guideline EMEA/CVMP/540/03-Rev1 about the evaluation of stability for antibiotics in drinking water.

ID : 365

POULTRY HELMINTHS: HOW TO SEARCH FOR AND IDENTIFY THEM MORE EFFICIENTLY?

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To meet societal expectations, poultry sectors are moving towards more outdoor area farming (meat, egg laying), with a greater risk of exposure to outdoor parasites, such as helminths. At the same time, the desire to reduce the number of veterinary treatments in livestock and to use them in a more targeted way requires a good knowledge of parasites present in field. Nematodes, also known as roundworms, are usually the most frequent and pathogenic parasites encountered, especially Ascarids, Heterakis and Capillaria. Other worms can also infest poultry, such as trematodes and cestodes (tapeworms).

The most popular method for routine poultry helminths diagnosis is coproscopic analysis, detecting parasite eggs in the animal feces. But the immatures worms do not lay eggs, and the female worms can pause laying during several days, so this method is not fully reliable to detect helminths infestation. Furthermore, cestode eggs are often layed independently from feces emission.

Direct examination of the intestine and ceca allows evidence of large roundworms like Ascaridia and large tapeworms; but smaller worms, like Heterakis, are only visible in case of heavy infestation, and tiny worms located deep into the mucosa, like Capillaria and Davainea, are not visible although they are highly pathogenic.

Subsequently, current helminth screening methods used routinely in veterinary practice laboratories on the field are not satisfactory nor reliable, especilally concerning Capillaria.

Therefore a specific method has been developed to improve helminths diagnosis, using successive sievings of the digestive content and superficial mucosa.

The content and superficial mucosa from intestines of the necropsied animals are collected and suspended in water, then sieved with a 1mm mesh strainer (that retains for example cestode heads and small Ascaridia), then with a 250 µm mesh strainer, and finally with a 100 µm mesh strainer (to withhold young Ascaridia and Capillaria detectable using a black background).

The same method is used for cecal content (using only 1mm and 250 µm mesh screen) in order to observe Heterakis worms when they are infrequent.

This method for easily identifying helminths will be deployed in poutry vet practices laboratories, in order to build an field observatory of helminths in France, as no inventory of parasitic helminth infestation is available. This field observatory will allow to use the anthelmintic treatments more efficiently, in a more targeted way.

ID : 414

A STAPHYLOCOCCUS AUREUS INFECTION HINDERED SPARE RESPIRATORY CAPACITY OF COMMERCIAL LAYING HEN IMMUNE CELLS MEASURED USING A METABOLIC ASSAY

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Staphylococcus aureus isolated from a commercial layer operation following unexplained mortality was used to inoculate 200 commercial laying hens to determine the possible route of infection and to study manifestation. Hens of 2 age groups, 22 and 96 wk, were randomly assigned to one of 4 treatments: CON (no *Staph. aureus* challenge), oral gavage (OG, intended CFU=10⁸), subcutaneous injection (SQ), or intravenous injection (IV); n=25 hens per age group/treatment. Hens were transported from a commercial facility and allowed 48 hours of acclimation pre-inoculation. Ten randomly selected hens per age group/treatment were weighed daily. Blood was drawn from 4 hens per age group/treatment pre-inoculation, 6h post-inoculation, 1-day post-inoculation (dpi), 3dpi, and 7dpi. Peripheral blood mononuclear cells isolated from whole blood collections were cultured for use in the Seahorse Cell Energy Phenotype (Agilent) metabolic assay, which measures extracellular acidification rate (ECAR, glycolytic activity) and oxygen consumption rate (OCR, mitochondrial respiration) before and after a metabolic pathway inhibitor challenge. Spare respiratory capacity was calculated by subtracting baseline OCR and ECAR from the stressed/peak values following the assay's inhibitor challenge, and data were analyzed using Proc Mixed of SAS Version 9.4. There were no differences across treatments at the baseline or 6h post inoculation. At 1dpi, a dampened immune cell ECAR spare respiratory capacity was observed in the SQ treatment group compared to the control (P=0.0293), indicating a diminished glycolytic response. At 3dpi, the SQ hens' capacity recovered to the level of the control, but the IV treatment group had an ECAR response that was significantly lower than all other groups (P=0.0023). Interestingly, by 7dpi all inoculated treatments showed an ECAR spare respiratory capacity lower than the control, (P<0.05), indicating that *Staph. aureus* may not have attained its full impact until a week post-inoculation. Age did not have an impact on ECAR response (P=0.9847). OCR spare respiratory capacity was not affected by age (P=0.5449) nor treatment (P=0.7682). Treatment and timepoint did have an impact on body weight (interaction P=0.0001), with IV hens weighing less at 3dpi (0.34 kg lower) and 7dpi (0.39 kg lower). Hence, an intravenous route of *Staph. aureus* had the greatest impact on laying hen immune cell glycolytic capacity, an outcome that was corroborated by diminished body weight.

ID : 426

EVALUATION OF PROTECTION BY ND VACCINATION PROTOCOLS AGAINST EARLY CHALLENGE WITH VELOGENIC NEWCASTLE VIRUS-VII.1

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Newcastle disease virus (NDV) is endemic in Egypt since 1960s and vaccination strategy is the main tool to control it in Egypt. Recently, NDV-VII.1 became endemic in Egypt associated with mortalities in vaccinated flocks, so the farmers moved to a combined live and inactivated vaccine strategy. The aim of this work was to evaluate effectiveness of three different vaccination protocols (G-1 double inactivated ND vaccine at 1/10 day of age, G-2 single inactivated ND vaccine at 7-day of age and G-3 single inactivated ND at one-day of age) in-combination with three-dose from live ND vaccine against challenge with velogenic NDV (vNDV). 180,000 one-day old chicks obtained from commercial hatchery were placed in three commercial broiler station "60,000 birds/station" and 40 one-day old chicks were moved to Biosafety level-3 isolators (BSL-3) at MEVAC facility to serve as control groups (non-vaccinated group challenged "G-4" and Non-vaccinated non-challenge-G-5). On a weekly basis, blood samples, cloacal swabs and oropharyngeal swabs were collected to check the development of humoral immune response and exposure to any life threatening Respiratory virus (Avian influenza" AIV", NDV and infectious Bronchitis "IB" virus). At 24-day of age, 25 birds from each station (G1-3) were moved to BSL-3 and kept under observation for 36 hours; cloacal and oropharyngeal swabs were collected and three birds from each group were euthanized and internal organs examined for three repeated times with 12 hours interval, to ensure that birds were free from any live threatening viral respiratory pathogen (AIV, NDV, IB). Birds in G1-4 were challenged with vNDV VII.1 (106 EID50) in 0.5 ml/ bird PBS via intranasal route, and birds in G-5 received 0.5 ml PBS via intranasal route. Birds in G1-5 were kept in BSL-3 for 10 days under observation and oropharyngeal swabs were collected on 3,6,9 days post challenge (dpc). Protection against mortalities after challenge with vNDV-VII.1 was 100% (12/12), 100% (12/12), 91.7% (11/12), 0% (0/12) and 100% (12/12) in groups 1-5 respectively. Virus shedding of birds in G-1 was significantly lower (amount of virus shedding and number of shedding) in comparison to G2/3. In conclusion, using NDV inactivated vaccine in two-dose protocol with live vaccines can provide protection to commercial broiler chicken against early challenge with vNDV in endemic areas as early as 26 day of age with significant lower shedding rate. The application of such protocol can be an effective tool to control NDV via a vaccination strategy.

ID : 427

EVALUATION PROTECTION OF H5 VACCINATION REGIMES AGAINST EARLY CHALLENGE WITH HPAI-H5N1

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The aim of this work was to evaluate effectiveness of inactivated H5 vaccine in different vaccination regime either as single vaccine dose at 7-day of age, two-dose regime (1/10-day of age) or using as one and half manufacture recommended dose at 7-day of age against early challenge at 26-day of age with HPAI-H5N1 clade 2.2.1.2 virus. 180,000 one-day old chicks obtained from commercial hatchery placed in three commercial broiler station "60,000 birds/station" (G-1 two-dose regime, G-2 one and half dose regime and G-3 single-dose regime) and 40 one-day old chicks moved to Biosafety level-3 isolators (BSL-3) at MEVAC facility to serve as control groups (non-vaccinated group challenged "G-4" and Non-vaccinated non challenge group "G-5"). On weekly basis blood samples, cloacal swabs and oropharyngeal swabs collected check develop of Humeral immune response and exposure to any life threatening Respiratory virus (Avian influenza "AIV", Newcastle disease "NDV" and infectious Bronchitis "IB" virus). At 24-day of age 25 birds from each station (G1-3) moved to BSL-3 and kept under observation for 36 hours; cloacal and oropharyngeal swabs collected and three birds from each group euthanized and internal organs examined for three repeated times with 12 hours interval to ensure that birds free from any live threatening viral respiratory pathogen (AIV, NDV, IB). Birds in G1-4 challenged with HPAI-H5N1 clade 2.2.1.2 (106 EID50) in 0.5 ml/ bird PSB via intranasal route and birds in G-5 received 0.5 ml PBS via intranasal route. Birds in G1-5 kept in BSL-3 for 10 days under observation and oropharyngeal swabs collected on 3,6,9 days post challenge (dpc). Regarding protection virus against mortalities following challenge with HPAI-H5; were 100% (8/8), 87.5% (7/8), 87.5% (7/8), 0% (0/8) and 100% (8/8) in groups 1-5 respectively. Regarding virus shedding birds in G-1 showed significant lower shedding virus (amount of virus shedding and number of shedding in comparison to G2/3. In conclusion, using homologous H5 inactivated vaccine in two-dose regime can provide protection to commercial broiler chicken against early challenge with HPAI-H5 virus as early as 26 day of age, with significant lower shedding rate: the application of such regime can be an effective tool to control HPAI under a vaccination strategy.

ID : 473

CONTRARY EFFECT OF DEOXYNIVALENOL (DON) AND DEEPOXY-DEOXYNIVALENOL (DOM-1) ON
CAMPYLOBACTER JEJUNI RESOLVED IN VITRO

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Deoxynivalenol (DON) is the most common trichothecene mycotoxin detected in feedstuffs globally. Similarly, *Campylobacter jejuni* is very widespread in broilers and the most common zoonotic pathogen. In a recent study, we were able to demonstrate that DON not only increased the load of *C. jejuni* in the jejunum and cecum of infected birds but also raised the counts of these bacteria in the inner organs. In contrast, the less toxic DON metabolite deepoxy-deoxynivalenol (DOM-1) reduced the *Campylobacter* load in vivo. Therefore, it can be hypothesized that DON and DOM-1 might have a direct or indirect effect on the growth of *C. jejuni*. The aim of the present study was to investigate the interaction between *C. jejuni* and DON or DOM-1 in vitro.

For this purpose, two *C. jejuni* strains were tested, one reference strain NCTC 12744 and one field strain 1303 derived from a broiler flock. Both strains (105 CFU/ml) were inoculated in 96-well plates and incubated without and with either DON or DOM-1 (5 and 20 µg/mL) under microaerophilic conditions at 41.5 °C. Colony forming units (CFU) of *C. jejuni* were determined in a time interval of 6 h (24 h – 30 h – 36 h – 42 h – 48 h). It was found that the presence of DON resulted in significantly higher growth rates of *C. jejuni* from 30 h incubation onwards confirming the in vivo data. On the contrary, the presence of DOM-1 lead to a significant decrease in the *C. jejuni* CFU counts, indicating that this DON metabolite might contribute to reduce the burden of *C. jejuni* in birds.

Furthermore, RNA sequencing of *Campylobacter jejuni* incubated with either DON or DOM-1 was performed and revealed specific effects on bacterial metabolic mechanisms.

ID : 490

PERSISTENCE OF ANTIBIOTIC RESISTANT PATHOGENS AND COMMENSAL BACTERIA IN “NO ANTIBIOTIC EVER” PRODUCTION BROILER HOUSE LITTER

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We conducted an on-farm longitudinal study to evaluate the occurrence and persistence of pathogens (Salmonella and Campylobacter) and commensal bacteria (E. coli, Staphylococcus and Enterococcus) with antibiotic resistance under a “No Antibiotic Ever (NAE)” production system. The broiler farm was comprised of four broiler houses (> 22, 000 birds/house) and used peanut hulls for bedding material. Broiler litter samples (n =288) were collected at the beginning (7 to 10 days after bird placement) and at the end (4-6 weeks after broiler placement) of each grow-out cycle (49 days), for three consecutive flocks. Pathogens and commensals were quantified, identified and characterized with VITEK® (bioMerieux), whole genome sequencing and antimicrobial susceptibility testing. Additionally, broiler house and litter physico-chemical parameters such as temperature, pH and moisture were monitored throughout the study. Overall, pathogen and E. coli populations in litter declined significantly ($p < 0.05$) from flock 1 to flock 3. Salmonella isolates (n = 47) were grouped into six O serogroups (C2, C3, DI, D2, B and E) and S. Kentucky (~ 56%) and S. Enteritidis (~ 30%) were the top serovars found in litter at the end of the 3rd flock. None of the Salmonella isolates tested carried antibiotic resistance. While Campylobacter coli isolates (~ 80%) were resistant to tetracycline, C. jejuni isolates (~ 90%) were susceptible to all drugs tested. The majority of E. coli isolates (~ 62.5%) were resistant to at least one class of antibiotics and multidrug resistant (resistance to two or more classes of antibiotics) E. coli persisted from flock to flock. The major Enterococcus spp identified were E. hirae (~ 65%) and E. faecalis (~ 15%), and these isolates harbored resistance to at least one class of antibiotics. Staphylococcus isolates (~ 45%) displayed resistance to lincomycin, and S. cohnii subsp. urealyticus (~ 27%) and S. xylosus (~15%) were the representative species present in litter from flock to flock. The temperature of the broiler house was the most important parameter that could explain the variability in our data. Our results suggest that the reuse of the same litter over the grow-out of multiple flocks under “NAE” production resulted in a reduction of Salmonella and Campylobacter population in litter, however, multidrug resistant commensal bacteria persisted from flock to flock.

ID : 501

A COMPARISON OF SALMONELLA ENTERITIDIS COLONIZATION IN BROILER CHICKENS CHALLENGED ON DAY 14 WITH EITHER AN OCULAR OR ORAL CHALLENGE

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Salmonella Enteritidis (SE) is an important food borne pathogen that is typically associated with poultry. This study was conducted to determine which organs are colonized by this bacterium when challenged with these different routes. For this study, 250 straight run broiler chicks were obtained from a commercial hatchery and were randomly placed into 10 pens (25 birds/pen). Standard management and feed practices were followed for the duration of the trial. On day 14, half of the pens were challenged orally by administering 500ul of 10x4 cfu of SE via oral gavage while the other 4 pens were challenged with 50ul of 10x4 cfu of SE into each eye.

The SE utilized in this study was nalidixic acid and novobiocin resistant. On days 35-38 a total of twenty birds were removed from each pen and necropsied. Samples collected include bursa and thymus (pooled), breast, ceca, crop, kidney, liver and spleen (pooled), skin, spinal cord, thigh, and trachea. A sterile swab was utilized to take samples from the abdominal cavity, bone marrow, cloaca and lung. After harvesting, samples were immediately placed into BPW and stored at 4C until all the birds were necropsied after which, samples were incubated at 37C for 24 hours. From each BPW sample a subsample was taken and used to inoculate TTB tubes. The TTB tubes were incubated for 24 hours at 41C. After that time samples were plated onto XLT4 agar that contained nalidixic acid and novobiocin. These plates were incubated at 37C for 48 hours after which the plates were removed, and Salmonella colonies noted. Data was analyzed using a t test at a $p > 0.05$. Results of this trial had shown at least one sample was positive for SE in all the birds. There were significant differences between the ocular (60.6%) and oral (18.4%) routes of challenge for the cloaca swab, lung swab (ocular=8.3%, oral=1.0%), pooled bursa and thymus (ocular=39.0%, oral=21.0%), ceca (ocular=61.0%, oral=28.0%), crop (ocular=59.6%, oral=20.0%), skin (ocular=21.0%, oral=6.1%). Differences in colonization may be due to the ability of this bacteria to more readily go systemic when administered via the eye compared to an oral gavage. This study shows that the route of inoculation may influence where the bacteria may colonize.

ID : 532

PROTECTION AGAINST AL-2 AND 9109-LIKE VARIANT INFECTIOUS BURSAL DISEASE VIRUSES USING DUAL RECOMBINANT HVT-ND-IBD VACCINE ALONE AND IN COMBINATION WITH TWO LIVE IBDV VACCINE

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Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus, characterized by immunosuppression and mortality generally at 3 to 6 weeks of age. IBDV is ubiquitous in commercial chicken operations. IBDV causes a prolonged B-lymphocyte immunodeficiency and increased susceptibility to various viruses and parasites. Both classic and variant strains of IBDV had been isolated in the southeastern United States. IBDV variant AL-2 (AL for Allen Laboratory) originated from Delaware and in recent years has affected broilers between the ages of 20 to 30 days or older with moderate to severe bursal atrophy. 9109 is a variant strain of IBD that has been a problem in the southern states of United States. 9109 has been characterized as a classical, very virulent, and variant strain. IBDV variant AL-2 and 9109 isolation in commercial broilers has increased in recent years in the southeastern of the United States, associated with broiler performance issues. A new first dual construct rHVT-ND-IBD vaccine (Innovax®-ND-IBD) has been launched in the United States. An evaluation of the protection against AL-2 and 9109 variants, alone or in combination with a live intermediate standard strain and 89/03 strain of IBDV vaccine, was performed. A subcutaneous injection was used for injection of the chicks for each treatment group at hatch. A minimum of 500 mls of each vaccine at 1x concentration was needed for vaccination. 300 SPF embryos were used in the study. The eggs were purchased from Charles River SPAFAS and set in a hatchery. The challenge viruses used in this study were the AL-2-like IBDV field isolate 124024 and 9109-like IBDV field isolate 127823 case submission to PDRC. The viruses were expanded in 3-week-old SPF chickens prior to the start of the study, then titrated in chicken embryos. The viruses were diluted to the target challenge dose in tryptose phosphate broth (TPB) to a target dose. Each bird in challenged treatment groups received 0.03 mls by the intraocular route of inoculation. Birds were housed in negative pressure isolation units and had unrestricted access to feed and water. Birds were fed unmedicated Southern States All Grain Start-N-Grow diet. Protection was evaluated by challenged birds at day 18. This early age was intended to mimic the age in the field when maternal antibody is expected to decline and when variant IBD challenge is commonly observed. The study was terminated on day 24 and birds and bursae weighed data for Bursa /Body Weight (Bu/BW) ratio were collected and bursas also were collected for histopathology to determine mean bursal lesion scores to determine atrophy. Protection against challenge was determined by Bu/BW ratios greater than or equal to the Bu/BW ratio of the Unvaccinated Challenge Group plus two standard deviations. The histopathology bursal lesion scoring system is based on scores of 0-4 where 0 is an absence of lesions and 4 is marked lymphoid atrophy. Statistics were performed on the Bu/BW ratios using the unpaired t-test (GraphPad Prism, v. 6). Statistics were performed on the bursal lesion scores using the unpaired t-test (Graph Pad Prism). Results showed protection in all the vaccinated groups when they were compared with the Non-vaccinated/Challenged group. The Bu/BW ratios for all vaccinated/ AL2 challenged groups were significantly higher as compared to NoVAXAL2 group, providing evidence that all vaccine combinations and the rHVT-ND-IBDV prevented significant bursal atrophy following AL-2 challenge. In addition, the Bu/BW ratios for all vaccinated/AL2 challenged groups were greater than 2 standard deviations above the mean Bu/BW of the NoVAXAL2 challenged group providing additional evidence for protection against challenge. While the Innovax vaccinated/AL2 challenged birds were protected, significant

bursal atrophy was observed in this group based on Bu/BW ratios when compared to the NoVAX and all other vaccinated/AL2 challenged groups. However, significantly less bursal atrophy was observed, based on Bu/BW, in the Innovax vaccinated/AL2 challenged group when compared to the NoVAXAL2 group. The extent of lymphocytic depletion in the bursa was most significant in the NoVAXAL2 group with a mean bursal lesion score of 4 compared to all other groups in this study. This confirms that the challenge virus and dose were valid. The bursal lesions scores for all vaccinated/AL2-challenged groups were significantly lower compared to the NoVAXAL2 group suggesting that all vaccine combinations provided protection against significant lymphocytic depletion in the bursa. In addition, the bursal lesion scores for the Innovax + 8902 vaccinated/AL2 challenged group, and Innovax+ Univax vaccinated/AL2 challenged group, were not significantly different from the NoVAX group. The bursal lesion scores for the Innovax vaccinated/AL2 challenged group were significantly higher than the NoVAX group and significantly lower than the NovaVASAL2 group. The mean lesion score for the Innovax vaccinated/AL2 challenged group was 2.4 and considered normal for broilers in the field. In fact, the mean bursal lesion score for the NoVAX group (1.1) is lower than normal birds in the field and likely due to the fact that birds were SPF and housed in HEPA filtered isolation units. Similar results were observed with the 9109 strain. The Bu/BW ratios for all vaccinated/ 9109 challenged groups were significantly higher, compared to the NoVAX9109 group, providing evidence that all vaccine combinations and the rHVT-ND-IBDV prevented significant bursal atrophy following 9109 challenge. In addition, the Bu/BW ratios for all vaccinated/9109 challenged groups were greater than 2 standard deviations above the mean Bu/BW of the NoVAX9109 challenged group providing additional evidence for protection against challenge. The bursal lesions scores for the rHVT-ND-IBDV group and the vaccine combinations were significant difference compared to the NoVAX9109 challenge group which provided evidence for protection against the 9109 challenge at 18 days of age. The rHVT-ND-IBD alone or in combination with a live IBD vaccine protected against AL-2 and 9109 like variant IBD virus when challenged at the early age of 18 days.

ID : 590

COMPARATIVE FIELD STUDY BETWEEN RHVT-IBD-ND VS RHVT-IBD VACCINE IN COMMERCIAL BROILERS

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In this study, we determined the effects on the field performance parameters and serological responses in commercial broilers induced by rHVT-ND-IBD in comparison with a recombinant rHVT-IBD vaccine. For this purpose, a total 197,000 commercial Cobb broilers were monitored from a day of old till slaughter age for 3 sequential production cycles. In each production cycle, more than 60,000 birds were obtained from the same hatchery and reared in the same farm under the same management. These birds were separated in 2 houses with the same vaccination program except one house was vaccinated subcutaneously at a day of old with rHVT-ND-IBD and second house with a recombinant rHVT-IBD vaccine. Blood samples were randomly collected at slaughter age and the serum samples were tested for specific detection of IBDV antibodies by the IBD ELISA test using two different commercial IBD ELISA kits. Field performance parameters were evaluated for the two houses for the three sequential production cycles. these parameters were included; Mortality%, Survival rate%, Average slaughter age, Average Body Weight, FCR & EPE. The results demonstrated that both rHVT-ND-IBD and rHVT-IBD vaccinated birds showed high antibodies titer against IBDV at slaughter age. The field performance parameters did not show significant differences except a significant increase in mean body weight in rHVT-ND-IBD vs rHVT-IBD vaccinated birds with an average 100 gm per bird difference in rHVT-ND-IBD vaccinated birds. This comparison revealed that rHVT-ND-IBD® vaccinated birds have higher return of investment.

ID : 593

EFFECT OF ARTEMISIA ANNUA ON CHICKEN LEUCOCYTOZONOSIS

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Poultry leucocytozoonosis is an endemic disease in East Asia. It is caused by protozoan parasites *Leucocytozoon caulleryi* (*L. caulleryi*) and decreases the laying rate of layers. Unfortunately, laying hens should not be given antibiotics during the laying period. It is important to find the natural herbs that can replace antibiotics. In this study, we used the herb which has the effect of antimalaria, *Artemisia annua* (*A. annua*), as an additive. After artificial infection, 39 of three week-old Nogoya chickens were divided into three groups, control group (CON), antibiotic group (ANT) with 40 mg/kg Sulfadimethoxine, and *A. annua* powder group (TRT) with 0.5 g/day per chick. From Day 0 to 42 post of infection, body weight (BW), body weight gain (BWG), rectal temperature (RT), rectal temperature change (RTC), mortality rate, pale cockscomb rate, green feces rate, blood smears, polymerase chain reaction (PCR) and necropsy of the sudden death chicks investigated every week. The results show that success rate of infection was 23%. TRT had lower BW and BWG than ANT ($P < 0.05$), but similar with CON. The rate of mortality, pale cockscomb and green feces of TRT and ANT were significantly lower than CON ($P < 0.05$). Some important tissues of sick chicks were scattered with multifocal petechiae and white mottling which caused by second-generation schizonts. There is no significantly difference between TRT and CON on PCR, but the percentage of *L. caulleryi* in blood of TRT was significantly lower than CON by blood smears ($P < 0.05$). In conclusion, feeding with 0.5 g of *A. annua* powder daily can effectively reduce the proportion of *L. caulleryi* and thereby reduce the mortality, pale cockscomb and green feces.

ID : 598

EFFICACY OF BIVALENT RHVT- ND-IBD VECTOR VACCINE AGAINST CHALLENGE WITH VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS IN COMMERCIAL BROILERS

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Infectious bursal disease (IBD) control has focused mainly on vaccinations, which provide protection against mortality and immunosuppression. The efficacy of commercial bivalent herpes virus of turkey –Newcastle disease and infectious bursal disease (rHVT-ND-IBD) vector vaccine against very virulent infectious bursal disease viruses (vvIBDV) Egyptian isolate (GenBank accession no.KX646373) was evaluated in commercial broiler chicks vaccinated at day-old by subcutaneous (sc) administration. Birds were challenged on 24 or 28-days of age, and the protection assessment were based on clinical signs, mortality, bursal/body weight ratios (B/BWR), gross lesions, mean severity index (MSI) of histopathological lesion scores of bursae and serological response at 7 days post-challenge (dpc). As result, the complete protection against mortality (100% and 100%) vs (100% and 96%) was adopted after challenge on days 24 and 28 in vaccinated and non-vaccinated challenged birds, respectively. In addition to partial and complete protection against bursal atrophy (B/BWR) were 0.57 and 0.42 vs 0.75 and 0.33 in vaccinated and non-vaccinated challenged birds at 24 and 28-days of age, respectively. A significant difference in MSI was shown (1.7 and 3 vs 1.5 and 3) in vaccinated and non-vaccinated birds when challenged on days 24 and 28, respectively. Our results revealed that the day-old vaccination of bivalent rHVT-ND-IBD vector vaccine is providing early onset of protection against the Egyptian vvIBDV in commercial broilers. In conclusion the pressure of vvIBDV in poultry industry, especially in IBDV-endemic countries, requires the early protection against vvIBDV. Indeed, the earliest development of immunity can be induced in chickens by day-old vaccination, moreover, day-old vaccination with rHVT-ND-IBD has been proven to be an efficient way of vaccine delivery in large scale poultry production.

ID : 628

INHIBITION OF SALMONELLA TYPHIMURIUM FECAL SHEDDING IN COBB 500 BROILERS WHEN FED ZN AND MN AMINO ACID COMPLEXES

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Food borne diseases of humans, caused by Salmonella infected products, are an insidious problem that cause significant damage to consumers, food brands, and poultry companies. Zinc (Zn) and manganese (Mn) are essential nutrients, important for immune response and intestinal health. Feeding highly available Zn and Mn sources, such as those from amino acid complexed minerals (AACM), has resulted in improved epithelial tissue structure and function, as well as immunity. A challenge-study was designed to evaluate the effect of feeding Zn or Zn and Mn AACM (Availa®Zn and Availa®Z/M, Zinpro Corporation, Eden Prairie, Minnesota, USA) on fecal and cecal recovery of Salmonella Typhimurium. A total of 100 Cobb 500-day-old chicks were tested for being Salmonella--free and assigned to 4 treatments in a randomized complete block design. Treatments were: T1) Control, with no added Zn or Mn; T2) Availa-Zn60 with 60 ppm Zn and 0 Mn; T3) Sulfates with 40 ppm Zn and 40 ppm Mn; T4) Availa-ZM, 40 ppm Zn and 40 ppm Mn. Birds were placed in plastic containers and received an oral challenge on d 3, 6, 10, 13, and 16 with Salmonella Typhimurium DT104 at 1X10⁸ CFU/bird. Dietary treatments were offered from d 22 to 42. Fecal samples were individually collected using rectal swabs on d 23, 30, 37, and 40. On d 42, birds were euthanized and cecal contents cultured. Fecal and cecal samples were diluted in Lennox L broth and cultured, then quantitated using Xylose Lysine Deoxycholate (XLD) agar (18 hr at 37°C). Bacterial counts and body weight were analyzed using Analysis of Variance with Tukey's ad hoc test for multiple comparison, and prevalence with Chi Square test (GraphPad Prism, v8). For all variables, significant differences were defined at $P \leq 0.05$. Compared to Sulfate or Control groups, birds fed Availa-Zn60 and Availa-ZM had: lower Salmonella excretion in feces (CFUs/g) at d 37 and 40 ($P \leq 0.05$), lower Salmonella prevalence in feces at d 30 ($P \leq 0.01$) and in ceca at d 42 ($P \leq 0.01$), higher BW and lower FCR at d 42 ($P \leq 0.01$). Supplementation of poultry feeds with amino acid complexed minerals, specifically Availa-Zn or Availa-Z/M, could be a useful tool in the prophylaxis of Salmonella infections.

ID : 632

DETECTION OF CHICKEN ANEMIA VIRUS (CAV) TITERS IN FIGHTING COOKS FROM PICHINCHA PROVINCE IN ECUADOR.

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The Chicken Anemia Virus is transmitted primarily through vertical transmission, which is of importance to intensive poultry populations. The age of the bird has a marked effect on the development of clinical signs, but its importance lies in the immune suppression that occurs in chickens that survive the disease. Although this has not been studied in populations of backyard chickens or fighting cooks, these are important transmitters in the spread of diseases having no vaccine immunity and the type of operation it has. The reason for this study is to determine if the virus is present in fighting cooks farms at Pichincha province detecting antibodies with ELISA test.

The study sample was randomly selected in ten farms from Pichincha province. For each farm twenty-seven birds were selected for serum sampling among ten farms kept confidentially.

From 270 serum analyzed: 226 were positives, 155 (57%) showed protective antibodies (S/N 0.6). The positive result for hens are higher (eighty-eight serums, 86%), than males (One-hundred-Twenty-nine serums, 83%). The seropositivity is common in Rural and Urban areas.

The presence of CAV titers in serum samples which don't perform any CAV vaccination is explained because this circovirus has a high contagious capacity, dissemination and transmission. In this study some challenges can be understood because most of farms are located close to roads with transit of commercial and rural birds for commercial purposes (which spread fomites and feathers). Other way of contamination can be the presence of wild birds interacting with fighting cooks when look for water sources and feed in the nature. The main risk for CAV challenge of this type of gallinaceans is when participate in championships with cooks without sanitary control, which travel from different places of the country and the continent. CAV titers detection in studied samples from fighting cooks determines a CAV exposition at some moment of their life.

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ID : 641

DIETARY SUPPLEMENTATION OF A BUFFERED FORMIC ACID AND A MIXTURE OF MONO- AND DI-GLYCERIDES OF BUTYRIC-, CAPRIC- AND CAPRYLIC ACID ARE BENEFICIAL IN GUT INTEGRITY AND MICROBIOTA OF BROILERS CHALLENGED WITH SUBCLINICAL NECROTIC ENTERITIS

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A study was conducted using 816 as-hatched 1-d-old chicks (Cobb 500) to investigate the effects of a buffered formic acid (Amasil™ NA, BASF SE, Germany) and a mixture of mono- and di-glycerides of butyric-, capric- and caprylic acids (Balangut™ LS P, BASF SE, Germany) on gut permeability and microbiota of broilers challenged with necrotic enteritis (NE). These additives were added to the wheat-soy basal diet to produce six treatments with eight replicates of 17 birds per pen: unchallenged negative control (NC) with no additive (T1); and five challenged groups orally inoculated with 1 mL *Eimeria* spp. (Bioproperties Pty Ltd., AU) on d 9 and *Clostridium perfringens* (type A strain EHE-NE18, CSIRO, AU) on d 14 with: no additives as positive control (PC, T2); buffered formic acid (0.3% throughout all the phases, T3); a mixture of mono- and di-glycerides of butyric-, capric- and caprylic acid (0.5%, 0.3%, and 0.2% in starter, grower and finisher phases respectively, T4); a combination of buffered formic acid and a mixture of mono- and di-glycerides of butyric-, capric- and caprylic acid (0.3% + 0.3%, 0.2% + 0.2%, and 0.2% + 0.15% in starter, grower and finisher phases respectively, T5); and Zn bacitracin (0.05 % throughout all the phases, T6). On d 16, four birds (two males and two females) were orally gavaged with FITC-d. Marked birds were euthanised 2h thereafter, and serum samples were collected to analyse FITC-d concentration. The ileal and caecal contents were analysed for selected microbiota counts through 16S rDNA using qPCR. The results confirmed the presence of sub-clinical NE in the challenged PC. While the birds in On d 16, NC had no lesions in the gut, those in PC had significant lesions ($P < 0.01$). During 9-21 d, challenge increased FCR by 8 points ($P < 0.01$) but T5 had similar FCR as the NC ($P > 0.05$) and 5 points less FCR than PC. The birds in T5 had decreased ($P < 0.05$) FITC-d concentration in the serum and higher counts of *Lactobacillus* spp. and *Bacillus* spp. in the ileal contents compared to PC ($P < 0.05$), while other bacterial communities were not affected. Similarly, T5 increased ($P < 0.05$) the counts of *Bifidobacterium* spp. and *Lactobacillus* spp. and decreased ($P < 0.05$) Cp counts in the caecal contents compared to PC. In conclusion, it is suggested that the dietary supplementation of buffered formic acid and a mixture of mono- and di-glycerides of butyric-, capric- and caprylic acid may benefit poultry industry by improving gut health and thus feed efficiency for broiler chickens.

ID : 662

EFFECT OF THE COMBINATION OF A MULTI-STRAIN YEASTS FRACTIONS PRODUCT AND A COCCIDIOSTAT ON THE IMPACT OF INFECTION OF BROILER CHICKENS WITH EIMERIA SPP

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The objective of this trial was to examine the combined effects of dietary supplementation of a multi-strains yeast fractions product (MSYF) and a coccidiostat on performance and health parameters of broiler chickens orally challenged with coccidian oocysts. A total of 192 male one-day-old Ross-308 chicks were randomly allocated to 4 groups with 6 replicates of 8 chicks each, housed in separate pens. All birds were offered ad libitum water and the same basal diets of meal feed. The first group (negative control, NC) was unchallenged, whereas the second group (positive control, PC) was orally challenged with *Eimeria* oocysts. The other two challenged groups had their diets further supplemented: Group SAL with a coccidiostat (salinomycin 60 mg/kg feed); Group COMB with a combination of the same dose of the coccidiostat plus the examined MSYF (0.4 g/kg feed). At 14 days of age, the three challenged groups were orally inoculated with sporulated oocysts (3.5×10^4 *Eimeria acervulina*, 7.0×10^3 *Eimeria maxima*, and 5.0×10^3 *Eimeria tenella*). A week after the inoculation (21 days of age), coccidiosis symptoms were evaluated and two birds per pen were autopsied to score coccidiosis lesions in the intestine and to collect intestinal digesta (duodenum, ileum, ceca) for coccidian enumeration by quantitative real-time PCR (qPCR). Performance parameters were recorded, along with fecal oocyst numbers and mortality. All data were subjected to one-way analysis of variance (ANOVA) or Kruskal-Wallis test, depending on their format. Based on the trial results, group COMB when compared to PC had improved (*Eimeria* species and sampling site compared to the PC, but additionally in some cases lower (*E. maxima* & *E. tenella*; ileum *E. acervulina*, *E. maxima* & *E. tenella*; ceca *E. maxima* & *E. tenella*). In conclusion, MSYF can be used in combination with salinomycin to reduce the negative effects of an *Eimeria* challenge and by consequence improve growth performance in the challenged birds.

ID : 666

A DIETARY COMBINATION OF NICARBAZIN AND NARASIN COMPARED TO OTHER ANTICOCCIDIALS SIGNIFICANTLY IMPROVES GUT HEALTH IN BROILERS: A META- ANALYSIS.

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Currently potentiated as well as single ionophores are being used in the poultry industry to control coccidiosis and improve intestinal integrity. It is hypothesized that potentiated ionophores have a bigger impact on intestinal integrity due to their unique mode of action. Gut health remains the major focus of today's poultry production. Intestinal health and feed conversion ratio (FCR) are closely linked and its association has widely been studied. Enhancing intestinal health and improving FCR not only reduces the environmental impact of poultry production but can also contribute to increased animal welfare and reduced use of antimicrobials. The importance of these topics has grown over the past two decades.

The impact of an extended use of a potentiated ionophore; nicarbazin and narasin, from 21 days up until at least 28 days was evaluated on broiler health and FCR. Similarly, a comparison between the single ionophore narasin, with salinomycin and monensin from as early as 14 days up until 40 days was analysed. Using a Health Tracking System (HTSi), a global surveillance tool for poultry intestinal health developed by Elanco, flock-level information on general and intestinal health parameters, together with the anticoccidial program was collected. Data were extracted from the global HTSi database from 2015 until 2019 including 1 million broilers. A second dataset containing information about 304 million broilers from 2011 until 2019 was used to evaluate the impact of different anticoccidial programs on FCR. A standard least squares regression model was built for each set of data to control for geography, seasonality and bird age. Results coming from the first dataset indicate that the prevalence of coccidial lesions as well as footpad lesions were significantly reduced (P

This meta-analysis clearly demonstrates that the extended use of a dietary combination of nicarbazin and narasin significantly improved intestinal health as well as FCR. Moreover, the use of narasin from day 14 was shown to ensure superior intestinal health and FCR compared to other commonly used single ionophores. This indicates that application of the appropriate anticoccidial program can reduce the impact of poultry production on global footprint.

ID : 811

EFFECTS OF DIETARY EUBIOTICS BASED ON ESSENTIAL OILS ON SALMONELLA GALLINARUM CONTROL IN LAYER HENS.

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The infection caused by Salmonella Gallinarum (SG) is of great importance in animal and public health due to the possibility of generating residues and antimicrobial resistance issues. It is necessary to establish alternative control methods to reduce the effects of the disease and ensure food safety. The objective of this study was to evaluate the efficacy in vivo of three eubiotic products based on essential oils EOs of Lippia origanoides and Eugenia caryophyllata (EUB1), EOs + Formic and Lactic organic acids OAs (EUB2) and EOs+OAs+ b-glucans (EUB3) on productive performance, control of infection, and SG excretion of layers experimentally challenged with a field strain of SG. A total of 40 hens at 25 weeks of age were placed in 20 cages (2 birds each) and randomly allocated to 5 treatments (4 reps each) with the same basal diet. Treatments were: Birds no challenged (Negative control; NC); birds challenged (Positive control; PC); and three groups of birds challenged and supplemented with EUB1; EUB2; and EUB3. Groups PC and EUB1, 2, and 3 received 1mL of 3.5×10^5 CFU of SG via oral gavage on week 5 of the experiment. Microbiological cultures for SG were made daily during the first week post-inoculation (wpi) and weekly subsequently from individual cloacal swabs, eggshell membranes, and yolk. At the end of 10 wk, the animals were euthanized, and samples of spleen, liver, ovarian follicles, oviduct, and bone marrow were cultured. Histological evaluation was performed, assigning a score of injury to the main organs. Samples of the jejunum and ileum intestinal mucosa were taken to evaluate the expression of genes for ZO-1 and claudin-3 tight junction proteins (TJ). Feed intake (FI), egg production (EP), and feed conversion (FCR) were analyzed. Data were analyzed by ANOVA and Kruskal-Wallis test. There were significant statistical differences (P

ID : 816

SURVEILLANCE OF AVIAN SALMONELLA: CURRENT SITUATION OF RESISTANCE TO ANTIBIOTICS

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Introduction

The number of human non-typhoid salmonella isolates multi-resistant to antibiotics has grown steadily in the past five years. Thus, we are witnessing the emergence of new serotypes, the selection of such strains is often consecutive to the use of antibiotics in animals.

Materials and methods

In Tunisia, the control of salmonella in poultry production is covered by the regulations. The control program in the poultry sector is mainly based on systematic screening for zoonotic serovars in flocks of broodstock brooders and eggs for consumption and hatcheries.

The antibiogram was performed on 776 salmonella strains, it was performed according to: CA SFM / Eucast: Standard technical recommendations for diffusion in agar medium.

Results

The results show around twenty different serovars of which S. Enteritidis, S. Kentucky and S. Zanzibar are the most frequently identified.

In the context of monitoring phenotypes of public health interest, three phenotypes are particularly identified: resistance to fluoroquinolones, resistance to 3rd generation cephalosporins (C3G), pentaresistance ACSSuT (resistances associated with ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline).

The highlights in our study are:

- 81% of the isolates are resistant to nalidixic acid. However, high-level resistance to fluoroquinolones (Enrofloxacin) was detected in 41% of the strains studied. The strains resistant to fluoroquinolones are mainly represented by the serovars Kentucky 53%, Enteritidis 20% and Zanzibar 12%.
- Detection of C3G resistant strains remains infrequent, varying between 1% and 9% depending on the antibiotic tested. 31% of Kentucky salmonella strains were resistant to both C3G and fluoroquinolones.
- 25% of the strains were multidrug-resistant to more than three families of antibiotics; and 1% of the strains tested had the ACSSuT phenotype, of which 60% belonged to the Kentucky serovar.

Conclusion

Faced with the growth of salmonella resistant to certain critical antibiotics, there is an urgent need to rationalize their use and to continue studying the relationship between this use and the development of resistance.

ID : 837

COMPARISON OF THE EFFICACY OF THREE DIFFERENT MULTIVALENT INACTIVATED RT+IB+ND+EDS VACCINES AS BOOSTER OF IB VACCINATED LAYERS AGAINST CHALLENGE WITH 5 IBV CHALLENGE STRAINS.

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Infectious bronchitis virus (IBV) affects chickens of all ages, having worldwide distribution. It is significantly important for laying hens, causing up to 70% egg production drop combined with abnormal eggs. Vaccination is a standard required control method to increase resistance of layers to IBV infections, commonly following the principle of priming with live IB vaccines and boosting with an inactivated IB vaccine. In this study the efficacy of boosting, after priming with a live attenuated Massachusetts type IB vaccine, applied at day 0, combined with a live attenuated IB serotype-4-91 vaccine applied at day 14, with the IB component of three multivalent inactivated vaccines was investigated against a M41, 4-91, D388 (QX), Q1 and Var2 challenge, on SPF pullets. The three inactivated vaccines compared were multivalent combinations protecting against Avian Rhinotracheitis, Infectious Bronchitis, Newcastle Disease and Egg Drop Syndrome, including RT+IBmulti+ND+EDS (MSD), applied at day 105. M41, 4-91 and D388(QX) challenges were performed at day 166, while Q1 and Var2 challenges at day 187. Egg production, post mortem abnormalities in the reproductive organs and abdomen, respiratory signs and serological response using the virus neutralization test (VNT) were monitored. The average egg production drop post challenge with one of the 5 serotypes, was 17.4% and 12.4% for the two competitor vaccines and 11.0% for RT+IBmulti+ND+EDS boosted birds. The latter induced an average increase of VN antibodies against D388(QX), Q1 and Var2 of 3.9, 3.6, and 3.9 log₂ respectively, versus 1.1, 1.1, and 1.3 log₂ and 2.9, 2.0, and 2.4 log₂ for competitor vaccines. The lowest number and percentage of birds with free yolk and a degenerated ovary was found in the RT+IBmulti+ND+EDS boosted birds. The average score of the clinical respiratory signs was 0.25 for the challenge groups that had been boosted with RT+IBmulti+ND+EDS, versus 0.58, 0.45 for the two competitor groups. This is well correlated with the level of VN antibodies post vaccination. There is a correlation between the level of VN antibodies induced by the vaccination program and the level of protection using different parameters. In this comparison birds primed with Massachusetts type +4/91 type IB and boosted with RT+IBmulti+ND+EDS showed the best results, making it a reasonable candidate for efficacious long-term protection against IBV.

ID : 869

MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF THE CHICKEN ANEMIA VIRUS IN SRI LANKA

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In Sri Lanka, both layer and broiler parents are vaccinated against Chicken Anaemia Virus (CAV) using live vaccines. Maintaining maternal antibodies at a protectable level in commercial flocks at day 0 has been practiced to control Chicken Infectious Anaemia (CIA). However, recently CIA outbreaks were reported in commercial poultry, causing a constant threat to the poultry industry in Sri Lanka. Therefore, this study was performed to optimize the Polymerase Chain Reaction (PCR) assay to detect CAV in poultry and to conduct genetic characterization based on the partial sequence of viral protein (VP1) of CAV strains. For this study, bone marrow and spleen samples of eleven CIA suspected layer flocks and eight CIA suspected samples from broilers flocks in Sri Lanka were used and history revealed that all those flocks were not vaccinated against CAV. DNA was extracted from collected samples and proceed with PCR using gene-specific primers. Amplified PCR products were viewed following gel electrophoresis. Sequences of positive PCR products were obtained and aligned to generate a consensus sequence using MEGA X. The consensus sequences were deposited to the GenBank at NCBI. Percentage Identity (PI) of consensus sequences obtained for positive samples was calculated using BLASTn, NCBI with published sequences. Phylogenetic tree construction and Pairwise Sequence Comparison (PASC) were performed with 45 previously published VP1 gene sequences representing different continents of the world including CAV P4 vaccine strain. Five out of nineteen samples (26.32%) showed 419 bp target PCR products namely 001/19, 002/19, 003/19 originated from broiler flocks and, 004/19, 005/19 from layer flocks. Respectively, the GenBank accession numbers of the VP1 sequences were obtained as MT178266, MT178267, MT178268, MT178269 and MT178270. BLASTn results of Sri Lankan isolates have shown more than 98% PI to previously published VP1 sequences of CAVs. Among positive samples, 001/19 and 003/19 sequences were 100% identical while 005/19 originated from layer flock and 002/19 originated from broilers showed the lowest PI of 96.77 %. Further, the highest PI of 98.94% to the CAV P4 vaccine strain was observed in 001/19 and 003/19 and 005/19 strains whereas 002/19 showed the lowest PI (96.78%). The first time in Sri Lanka, optimized PCR detected the target gene sequence of VP1 in CAV, and therefore, it can be used to diagnose CAV in non-vaccinated flocks. Further, phylogenetic and PASC analysis of the VP1 gene in CAV with the vaccine strains can be used to determine the CIA caused by field strains in vaccinated flocks and non-vaccinated flocks.

ID : 880

COMPARATIVE EFFICACY OF THREE DIFFERENT PROBIOTIC STRAINS AND THEIR COMBINATION IN THE PERFORMANCE OF CLOSTRIDIUM PERFRINGENS-INFECTED BROILER CHICKENS

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There are many poultry probiotics to reduce the antibiotic use. However, their effectiveness is not always reliable. Highly efficient probiotics are the result of in-depth research on the modes of action of single strains and their complementarity in the gut. A study was conducted to compare the effect of GalliPro®Fit (1.6E+09 *Bacillus subtilis* strain DSM32324 + 1.0E+09 *Bacillus subtilis* strain DSM32325 + 0.6E+09 *Bacillus amyloliquefaciens* strain DSM25840 CFU/g; Christian Hansen A/S, Denmark) with the effect of its constituent strains on performance of broilers challenged with *Clostridium perfringens*. 2,250 Cobb 500 male one day old chicks were randomly distributed among five experimental treatments (9 floor pens per treatment, 50 birds per pen): T1, control group without probiotics in the diet; T2, *B. subtilis* strain DSM32324 (0.8E+06 CFU/g feed); T3, *B. subtilis* strain DSM32325 (0.5E+06 CFU/g feed); T4, *B. amyloliquefaciens* strain DSM25840 (0.3E+06 CFU/g feed) and T5, GalliPro® Fit (0.8E+06 strain DSM32324 + 0.5E+06 strain DSM32325 + 0.3E+06 strain DSM25840 CFU/g feed). A starter diet from 1 to 21 days of age, grower diet from 22 to 35 days of age and finisher diet from 36 to 42 days of age were fed ad libitum. On days 19, 20, and 21 all birds were challenged with *C. perfringens* (1.0E+09 CFU/bird) by mixing an inoculum into the feed in the base of the tube feeders and necrotic enteritis lesion scores (LE) were measured at day 21. At 21, 35 and 42 days of age, body weight gain (BWG), feed intake, feed conversion (FCR) and mortality were assessed. The raw data were analyzed statistically (ANOVA) using a Random Complete Block Design. Tukey's HSD test was used to separate means when ANOVA F values were significant ($p \leq 0.05$). The supplementation of GalliPro®Fit (T5), strains DSM32324 (T2) and DSM32325 (T3) improved (p At 35 days of life, GalliPro®Fit (T5) and each single strain (T2, T3 and T4) supplementation improved FCR compared to the control group (pC. perfringens.

ID : 907

ANTIBIOTICS MAY MODIFY THE COLONIZATION AND INVASION PATTERN OF CAMPYLOBACTER JEJUNI IN CHICKENS

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Campylobacter (C.) jejuni colonization of chickens is usually asymptomatic, but occasionally it may lead to clinical symptoms and is associated with changes in the intestinal nutrient absorption. Although the use of antibiotics has been reduced during the last years in the EU broiler production, still there is the need for treatment of various bacterial diseases. Previous studies indicated that the colonization rate of C. jejuni and number of colony forming units (CFU) may be increased, if chickens were raised under germ-free conditions or the gut microflora was modified for example by changes in the diet composition. In this study we compared the C. jejuni colonization pattern in chickens, which were treated with a cocktail of antibiotics (AB), with untreated (C) birds. We speculated that AB-treatment with associated changes in the gut microflora may lead to an increase of CFU of C. jejuni in the gut and possibly enhances adhesion and invasion contributing to lesion development and clinical signs. Significantly higher numbers of CFU of C. jejuni were observed in the cecum of AB-birds, with increased evasion and higher colonization rates of the extra-intestinal tissues such as liver and spleen compared to C-birds ($p < 0.05$). AB-birds showed histopathological lesions of the cecal wall as well as distended ceca, which as not observed in the C-birds. C. jejuni adhered to and invaded the cecum mucosa in AB-chickens, as confirmed by a modified Gentamicin Protection Assay and electron microscopy. Our results clearly indicate that an antibiotic-induced modification of the gut microbiota may allow the direct adherence of C. jejuni to intestinal cells, which subsequently facilitates the invasion and disease development.

ID : 918

PHYTOGENIC FEED ADDITIVE AS A NEW CONTROL APPROACH AGAINST THE PRESENCE OF POULTRY RED MITES (*DERMANYSSUS GALLINAE*, DE GEER, 1778)

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There is a global need to find an easy, safe and effective solution to prevent the deleterious effects of poultry red mites within the egg production industry. The aim of this research was to evaluate the effectiveness of a new phytogetic feed additive (PFA) in controlling a natural infestation of laying hens with *D. gallinae* in a cage system.

The tested PFA, RIDofMITE® (PATENT CO. DOO), consisted of essential oils' blend that predominately originated from *Thymus vulgaris* and *Origanum vulgare*, was dosed at 500g/ton of feed. The trial was designed to last 44 days on a farm with 1,560 laying hens (ISA Brown line) housed from 52 to 58 weeks of age. Egg production and general health of the hens were monitored daily. Standardized AVIVET traps were used to collect the mites. The traps were left for 2 days and collected at day -12., -5. and 0. before (control period), and on days 2., 5., 8., 11., 16., 23. and 30., after (trial period) hens started to consume PFA. The weight of the *D. gallinae* mites, in traps, was determined after storing at low-temperature (-80°C) for 48 hours. Normal distribution of data was tested with the Kolmogorov–Smirnov test, while the Kruskal-Wallis analysis of variance and Mann-Whitney U test (post hoc) were used to evaluate the impact of PFA on the population of poultry red mites.

In the control period of the trial, the sample weight from traps collected on day -12. (150.90 ± 36.84 mg) was significantly less than the weight measured in traps collected on day 0. (316.10 ± 51.08 mg), $P < 0.05$. In the trial period, the trend of decrease in the weight of mites, in comparison to day 0., was observed after days 2, 5, 8, and 11 of the treatment, but these changes were not statistically significant. Statistically significant ($P < 0.001$) decrease in mites' weight in the traps, comparison to the day 0., was observed after days 16 (114.75 ± 40.43 mg), 23 (81.05 ± 26.71 mg) and 30 (49.40 ± 16.54 mg) of PFA administration. Egg production (% hen-day) in the control period was 88.9, while in the trial period was 89.5. The obtained results showed that RIDofMITE® addition in hens' feed correlates with a gradual time-dependent decrease in the weight of mites with no observed negative effects of PFA on egg production and general health of the hens.

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ID : 933

ANALYSIS OF ITS-1, 18S, AND CO1 GENES FOR IDENTIFICATION OF EIMERIA PARASITES IN POULTRY.

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Coccidiosis is a costly enteric disease of commercial poultry worldwide caused by single-celled, parasitic protozoa of the *Eimeria* genus. The parasite reproduces rapidly both sexually and asexually causing substantial damage to intestinal linings, and often mortality. Furthermore, infection with certain species of *Eimeria* predisposes the chicken gut to development of necrotic enteritis caused by *Clostridium*. Control of coccidia and necrotic enteritis infections cost the poultry industry billions annually. The most common method for the identification and differentiation of *Eimeria* of species is classic microscopy, using morphometric characteristics (size, shape) and region of the gut parasitized. While a simple technique, microscopy can be subjective and sensitivity is not ideal. As molecular tools have advanced, using PCR to differentiate species has become more common place and may afford the opportunity to further characterize *Eimeria* samples past simple species differentiation. Three different genome regions have been used to molecularly differentiate species of coccidia; Internal Transcribed Spacer-1 (ITS1), Ribosomal 18s DNA (18S), and Cytochrome Oxidase C-1 (CO1). Our hypothesis is that, when all three genes are evaluated together for a single sample, inter- and intra-species variation in these regions will allow for a molecular fingerprint of current multispecies samples. Therefore, the overall goal of our research is to produce profiles from sequence data to promptly differentiate *Eimeria* species, but also identify *Eimeria* strains within a species. To test this, DNA was extracted directly commercially available vaccines in the US. Pan-*Eimeria* PCR to amplify either the ITS1, 18S or CO1 genes was performed on the mixed species DNA samples. PCR amplicons from each gene were then sequenced using the Illumina next generation sequencing (NGS) platform and sequences were separated and compiled using Geneious software. To date, our results show that using this technique can differentiate species in a multi-species sample, eliminating the need for monoclonal samples or species-specific PCR. We can also see that individual vaccines carry an identifiable sequence combination of these three genome regions, enabling differentiation from each other. With this data, we hope to build a profile database of vaccine coccidia, which will be an extremely useful diagnostic tool in the future.

ID : 935

EFFECTS OF USING DRY HYDROGEN PEROXIDE IN A COMMERCIAL HATCHERY ON HATCHERY PERFORMANCE

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Bacteria in a commercial broiler hatchery can be detrimental to chick health and hatchery performance. Even though sanitation and disinfection occur routinely, a method of continual sanitation could be a valuable tool for commercial hatcheries. A commercially available product, gaseous dry hydrogen peroxide (DHP) from Synexis Biodefense, has been proposed for this purpose. The purpose of this study was to evaluate the effects of DHP on bacteria levels and hatchery performance in a commercial hatchery setting. For this trial, a hatchery with two identical sides was used. Half of the hatchery was treated with DHP, while the other half remained non-treated. Treated areas included an egg cooler, setter hall, hatcher hall, chick processing areas, and vaccine laboratories. The non-treated locations included an egg cooler, setter hall, and hatcher hall. Bacterial loads were measured by total ATP bioluminescence swab samples and static air plates using tryptic soy agar (TSA). Air samples and ATP swabs were collected for two weeks prior to treatment to establish baseline microbial load, then samples were taken bi-weekly from similar locations on each side of the hatchery for 27 weeks for comparison. Hatchery performance data, chick quality evaluations, and residue breakout data were also collected. Data were analyzed using Prism statistical software with student's T-tests or two-way ANOVA and Sidak's multiple comparisons test. Microbial load testing was affected by a "super clean" performed in the hatchery in week 5 of the study for an internal audit, but reductions in microbial load as compared to baseline were seen. Chick quality data showed a significant decrease ($p<.05$) in the percent of chicks with *Aspergillus* in the lungs. Early dead, bacterially contaminated, and *Aspergillus* contaminated embryos were all numerically reduced on the treated side when compared to the non-treated side in residue breakouts. The percent hatch and hatch of fertile were numerically increased (90.2% non-treated vs. 90.9% treated HOF; $p=.08$) on the treated side of the hatchery as well. Three-day mortality of chicks hatched from the treated side was also numerically reduced compared to chicks hatched from the non-treated side. Taken together, this study suggests that the DHP product was beneficial as a method of continual sanitation to reduce microbial load, which in turn improved hatchery performance and production in a commercial setting.

ID : 946

FIELD EXPERIENCE OF THE USE OF A DUAL RECOMBINANT HVT- ND- IBD VACCINE IN BROILER BREEDERS IN COLOMBIA

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Newcastle disease (ND) infects wild birds and poultry species worldwide, severely impacting the economics of the poultry industry. ND is especially problematic in Latin America (Mexico, Colombia, Venezuela, and Peru) where it is either endemic or re-emerging disease and is a present threat in the poultry industry that affects the habitability and performance of birds. The presence of ND can also limit international trade, and cause production losses in egg-producing birds, causing greater economic damage. Vaccination against ND is effective, but traditional live vaccines induce reactions to vaccination and inactivated vaccines in broilers or broilers breeders can affect performance of the birds. Outbreaks of infectious bursal disease (IBD), a significant contagious immunosuppressive disease of poultry, are still reported throughout the world despite efforts to control the disease through vaccination. IBD control has only been possible through the use of efficacious vaccines, but vaccination efforts are complicated by the fact that frequent viral genetic mutations, reassorting of genome segments, and recombination can potentially increase virulence and alter antigenicity, rendering vaccines and vaccine protocols less effective. Historically, breeder pullets were primed with strong conventional live Infectious Bursa Disease (IBD) vaccines between 5 and 6 weeks of age. That stimulated immunity to IBD virus, and the birds responded better to the killed vaccine they received between 10 and 20 weeks of age. Several recombinant turkey herpesviruses (rHVT) have been developed within the past decades, and they are now used commercially worldwide. In broilers chickens, rHVT are usually administered alone but in long living birds they are used in combination with Marek's disease (MD) vaccines of other serotypes.

The objective of this work was to replace a single rHVT-IBD vaccine and an inactivated ND-IBD vaccine in the vaccination program of a breeder pullet for a dual rHVT-ND-IBD and no inactivated vaccine without affecting the protection for ND and IBD. A new dual rHVT-ND-IBD vaccine (Innovax®-ND-IBD) has been launched worldwide. rHVT-ND-IBD is a unique dual-construct herpesvirus of turkey (HVT) based vaccine that provides comprehensive protection against infectious bursal disease (IBD), Newcastle disease (ND) and Marek's disease (MD) in a single non-reactive vaccine that significantly expand flock protection and improve efficiency. rHVT-ND-IBD is administered in ovo or subcutaneously into day-old chicks at the hatchery to ensure the viability of this cell associated vaccine.

18,379 Ross AP and Cobb 500 one-day-old birds were used to evaluate the new vaccination program dual rHVT-ND-IBD vaccine by subcutaneous route and no inactivated vaccine (Montearroyo 2 Farm) and compared to 18,379 Ross AP and Cobb 500 one day-old with the current program single rHVT-IBD vaccine by a subcutaneous route and an inactivated ND-IBD vaccine (Montearroyo 1 Farm). On days 7, 14, 21, 28 and 35 days of age 6 birds from each farm were selected to collect body weight and bursa of fabricius to determine the Bursa/Body Weight (Bu/BW) ratio and for histopathology to determine mean bursal lesion scores to determine atrophy. Additional, 20 blood samples from each farm were collected at 13, 31, 40 and 60 weeks of age to run Hemagglutination Inhibition (HI) test to assess the efficacy of ND vaccine in field. An HI titer less than 1/64 was considered negative.

Protection against IBD was determined by Bu/BW ratio statistical differences and the histopathology bursal lesion scoring system based on scores of 0-4 where 0 is an absence of lesions and 4 is marked lymphoid atrophy. Protection against ND was determined by the difference of the titers from the HI

comparing the previous vaccination program with a single rHVT-IBD vaccine and an inactivated ND-IBD vaccine and the new program with a dual rHVT-ND-IBD vaccine and no inactivated vaccine. Statistics were performed on the Bu/BW ratios using the unpaired t-test (GraphPad Prism, v. 6). Statistics were performed on the bursal lesion scores using the unpaired t-test (Graph Pad Prism, v.6). Different letters above the bars in the graphs denote groups with significant differences in bursal lesion scores.

Results of the Bu/BW ratio showed not statistical differences at day 7, 21, 28 and 35 days of age comparing the two vaccination programs, but at day 14 statistical difference of the Bu/BW ratio was observed. There was not statistical difference in the bursal lesions scores at day 7, 14, 21, 28 and 35 days of age. The mean lesion score for both vaccination program was 2 (25-50% follicular atrophy) which is considered normal for field. HI test means titers result for ND showed that at 13 and 31 weeks of age in both farms antibodies were numerical similar and prevalence of ND antibodies in this population were low. However, a different picture was observed at 40 and 60 weeks of age were different antibodies levels were present. Montearroyo 1 Farm prevalence of 90% and 95% at 40 and 60 weeks respectively were observed. Montearroyo 2 Farm prevalence of 55% and 10% at 40 and 60 weeks respectively were observed.

Based on the results of Bu/BW ratio and bursal lesion score there were no significant different between the two vaccination programs at day 7, 21, 28 and 35 day of age. At 14 day there was a significant different in Bu/BW ratio, but the bursa means lesion score showed that there was mild follicular atrophy which showed an adequate protection at that specific age for IBD. HI mean titers showed that the breeders at Montearroyo 1 and Montearroyo 2 were protected at 13 and 31 weeks of age, however at 40 and 60 weeks of age adequate protection for field ND were observed in Montearroyo 2 that received the dual rHVT-ND-IBD vaccine and no inactivated vaccine. The dual rHVT-ND-IBD vaccine showed adequate protection against IBD and ND disease in the broiler breeders vaccinated at day of age by subcutaneous route.

ID : 996

IDENTIFICATION OF EFFICIENT NATURAL COMPOUNDS THAT RESTRICT EIMERIA TENELLA INVASION, DEVELOPMENT AND SPORULATION, TO LIMIT THE INCIDENCE OF CHICKEN COCCIDIOSIS.

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Introduction and aim of the study:

Coccidiosis is one of the most common and economically important parasitic diseases of chickens worldwide. Anticoccidial chemicals, coccidiocides, coccidiostats, and ionophores, have long been used as a mainstream strategy to control avian coccidiosis in modern poultry production. The increase in resistance to all anticoccidial drugs and the expectation of consumers for residue-free meat has encouraged development of alternative control strategies. Together with IDENA society we evaluated 150 active ingredients mainly of natural origin (plant extracts or essential oils) but also synthetic aromatic compounds naturally present in the environment, for their direct cytolytic effect on the dissemination stage of the parasite (oocyst) or for their ability, alone or in combination, to limit invasion and/or development in epithelial cells.

Experimental design:

Activity against oocyst was determined by the ability of the active ingredients to limit *E. tenella* oocyst sporulation after an incubation period of 72h at 26°C. We generated transgenic *Eimeria* strains to easily monitor anticoccidial effect of the active ingredients in vitro on the invasion or development stages in epithelial cells.

Results :

Three recombinant *E. tenella* parasite strains were generated to screen the efficacy of the 150 IDENA library compounds against all stages of the parasite life cycle. Six compounds were able to restrict *E. tenella* oocyst sporulation by more than 90%. Three compounds limited invasion by at least 50% with a selectivity index (SI) up to 12. Finally, 15 compounds were capable of inhibiting parasite development in epithelial cells with an efficacy ranging from 50% to 100% and a SI that can be as high as 100. Most promising compounds have been evaluated in association on broilers and none of them presented loss of performance (DWG, DFI, FCR). For some of them, a significant increase (Anova) in animal weight was noticed. A field study with IDENA compounds revealed an improvement in weigh performance (similar to Maxiban/Monteban treated group) compared to Paracox vaccine.

Conclusion :

This study allowed to develop new tools to screen in a simple and precise way the efficacy of natural compounds and the identification of promising ingredients limiting parasite growth at several stages of development in vitro. These compounds, will be evaluated in a challenge experiment (postponed/ Covid-19) in order to have results available for WPC2021 meeting.

ID : 1027

THE USE OF MONOVALENT OR BIVALENT IBV VACCINES SIGNIFICANTLY REDUCES CLINICAL SIGNS BUT NOT VIRAL LOAD AFTER HETEROLOGOUS CHALLENGE

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Infectious bronchitis virus (IBV) is an economically significant upper respiratory tract pathogen of poultry. Infection with IBV results in a mild respiratory infection but predisposes chickens to secondary bacterial infections leading to airsacculitis and condemnation at processing. Because of the economic significance of condemnation, nearly all commercial poultry are vaccinated against IBV. Vaccines are effective in a serotype specific manner, meaning they do not induce fully neutralizing antibodies against heterologous serotypes. The major challenge to control is that current vaccines are not effective against novel IBV variants, which seem to arise every few years. Recently, producers have begun to use multiple IBV serotype vaccines given together in an effort to induce cross-protection. This strategy has been used with some success, but variant IB viruses have still been detected in flocks indicating that the vaccine combinations have not prevented infection. In this study, we seek to determine how a single versus a combination of 2 different IBV vaccine types protect against DMV/1639, a currently circulating and economically important IBV variant. For this project, we vaccinated chickens by eye-drop on day of hatch with either a commercial Massachusetts (Mass), Georgia 08 (GA08), or Arkansas (Ark) type IBV vaccine, or with a combination of 2 Mass types, a Mass and GA08, or a Mass and Ark vaccines, and challenged on day 28 with the variant DMV/1639 virus isolated from a clinical case in Georgia in 2019. Necropsy was performed 5 days post challenge. Clinical signs were significantly reduced in all vaccinated chickens at 5 days post-challenge however, the DMV/1639 variant virus was detected by RT-PCR and by isolation in embryonated eggs in a majority of vaccinated chickens. Taken together, it seems the cross-protection strategy with these vaccines will aid in reducing or eliminating clinical presentations, but does not prevent infection or replication of the virus. Additionally, it was interesting to note that all vaccines, whether alone or in combination, performed equally in this experiment. In the future, it will be necessary to follow variant IBV viruses in flocks using this cross-protection strategy to evaluate the influence of these vaccines on viral evolution and development of new variants.

ID : 1125

PATHOGENIC CHARACTERIZATION OF AVIAN REOVIRUS VARIANTS ISOLATED FROM DIFFERENT ORGANS

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Avian reoviruses (ARV) cause negative economic impacts in the poultry industry. Its double-stranded RNA genome divided into ten segments makes this virus prone to mutation and recombination events generating molecular variants (ARVv). In previous studies, we reported isolates of ARVv with homologies under than 77% based on sigma C compared to S1133 vaccine virus. Despite the increased detection of ARVv, there is a lack of understanding about the pathobiology of these different variants. Therefore, the objective of the present study was to compare the pathobiology of selected ARV variants obtained from chicken hearts (H-strain) and tendons (T-strain). Four groups of one-day-old SPF chickens were placed in BSL2 rooms. Half of the chickens in each group (n=28) were challenged with 10^5 EID50 of the H-, T-, S-strain (vaccine S1133) or PBS as a negative control group via footpad. The other half of the chickens remained as contact birds. Body weights (BW), body-weight/hock joint ratios (BW-HJ) and viral loads in heart and tendons were obtained weekly for 4 weeks. Viral loads in tendons and hearts were calculated by using real-time RT-PCR. Statistical analysis was done by ANOVA and multiple comparisons using Tukey test. At seven days post-inoculation (DPI) a high mortality was detected in the S1133 inoculated birds (28.6%) compared with H- (7.1%) and T-strain (7.1%) inoculated birds. Body weight and BW-HJ ratios in hearts and tendons were different between inoculated and exposed birds in all groups ($P < 0.05$), except in the negative controls. In terms of viral load, we detected differences between groups at different times post challenge. While higher ARV viral loads were detected in hearts of H-strain inoculated birds compared with birds inoculated with the other strains at 8 and 15 DPC ($p < 0.05$), the S strain seemed to persist better in heart of the of inoculated birds at 21 and 28 DPC. Something similar happened with T-strain exposed birds that showed higher viral load than the other inoculated birds 21 and 28 DPC. We can conclude from our results that differences in clinical presentation are mainly related with mortality. In addition, ARV's from different sources have different tissue tropism. Finally, some strains persist better in hearts or tendons of challenged SPF birds.

ID : 1159

ISOLATION, PREVALENCE AND ANTIMICROBIAL AGENTS SUSCEPTIBILITY TESTING OF BRACHYSPIRA SPECIES IN POULTRY AT YUN-CHIA-NAN AREAS OF TAIWAN

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Avian intestinal spirochaetosis (AIS) is an intestinal infection caused by anaerobic spirochaetes of the genus *Brachyspira*, colonizing in avian caecae/rectum. It is known as a cause of chronic diarrhea, delayed onset and low egg production in commercial layers and breeders. According to epidemiological surveys, *Brachyspira* spp. are more occurred in adult poultry. Since AIS has not previously been studied in Taiwan, this study was aimed at investigating the prevalence of *Brachyspira* species and determining their antimicrobial susceptibility.

The cloacal swab and caecal content were collected from 24 flocks in Yun-Chia-Nan areas of Taiwan. Furthermore, the cases of sick poultry have been collected as well in Animal Disease Diagnostic Center (ADDC) of National Chiayi University. Samples were cultured anaerobically on selective agar and using PCR to identify the *Brachyspira* isolates. The isolates were tested by agar dilution method for determining the minimum inhibitory concentration (MIC) with 9 antimicrobial agents.

In 17 layer flocks, the following species were determined by PCR: *B. intermedia* (76.7%, 222/290), *B. pilosicoli* (29.0%, 84/290), *B. innocens* (25.5%, 74/290), *B. "pulli"* (4.5%, 13/290), *B. murdochii* (0.7%, 2/290) and *B. alvinipulli* (0.7%, 2/290). In 6 native breeder and 1 layer breeder flocks only isolated *B. intermedia* (50.0%, 32/64) and *B. "pulli"* (56.3%, 36/64). In ADDC cases of poultry, founding *Brachyspira* species are most occurred in waterfowls (44.4%, 8/18), followed by layers (38.7%, 12/31) and native breeders (10.0%, 1/10). Antimicrobial susceptibility testing of 19 *Brachyspira* isolates revealed MIC₉₀ of tylosin, amoxicillin, lincospectin, cephalixin, trimethoprim/sulfamethoxazole, tiamulin, doxycycline, gentamycin and florfenicol were >256, >256, 256, 32, 32, 8, 8, 4 and 4 mg/ml, respectively. The isolates showed lower susceptibility to tylosin, amoxicillin and lincospectin, although it indicated higher susceptibility to tiamulin, doxycycline, gentamycin and florfenicol.

The demonstration indicated different *Brachyspira* species particularly *B. intermedia* and *B. pilosicoli* in layers, native breeders and waterfowls are vital. It needs further research to assess their potential role and pattern of causing AIS in poultry flocks in Taiwan.

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Medhanie GA, McEwen SA, Slavic D, Guerin MT. *Brachyspira* spp. and avian intestinal spirochaetosis: an epidemiological review. 2013.

ID : 1161

THE POTENTIAL CONTRIBUTION OF BOTANICAL HERBS (ETHNOVETERINARY MEDICINE) AGAINST POULTRY DISEASES AND AS AN ALTERNATIVE TO THE USE OF INORGANIC MINERAL SUPPLEMENTS IN BROILERS (*DOMESTICUS GALLUS*) IN GURUVE DISTRICT OF ZIMBABWE.

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An exploratory study in the area of ethnoveterinary practises as an alternative to the use of synthetic drugs (inorganic) and as a complete substitution of inorganic mineral supplements was carried out at Chifamba High School in Guruve District. A total of 90 day-old broiler chicks, Cobb 500 supplied by reputable supplier, Irvines Zimbabwe were used because its the commonly used and preferred strain in Guruve. An invivo experiment was carried out through a controlled experiment with three (3) treatments replicated three (3) times each using botanicals herbs that is sausage tree barks and moringa tree barks soaked in water for extraction. Alcohol extraction was avoided since alcohol is believed to have some pharmacological effects by some researchers. 30 birds were randomly allocated to three treatments using Complete Random Design, Treatment 1 had moringa, Treatment 2 had moringa plus sausage tree and Treatment 3 had conventional medicines and inorganic mineral supplements. This study was important so as to increase livestock production, improve the living standards of rural communities and fight poverty and hunger in the poor societies of Guruve. Most of the farmers in Guruve district do not have money for or access to chemical medicines or to other cost effective control measures. Hence there was a serious need to find alternatives to the commercially supplied drugs. The results from this research provided evidence that botanicals herbs are essentially important in comparting the effects of poultry diseases and also acting as an organic minerals supplement. The growth and health performance of broilers on botanical herbs was statistically insignificant from those on conventional medicines. The health status and performance was measured through repeated measurements and observation of growth in kilograms, feed intake, water intake and diseases in each treatment. There were no diseases recorded in treatments 1 and 2 with botanicals inclusion whereas in treatment 3 cases of coccidiosis and respiratory diseases were noted. Mortality rate was insignificant in all the treatments. Broilers on botanical herbs also reached final slaughter weight of 2kgs at day 38 faster than those on conventional treatments which reached 2kgs at day 42. The main conclusions drawn from this study were suggestions since chemical composition of water was not performed. The researcher suggested that ethnoveterinary medicine can be safely used by the poor rural societies with no access to expensive synthetic drugs. This study suggested that farmers should control and prevent poultry ailments using botanical herbs so as to increase productivity as they work comparable to other drugs. Another recommendation was that all botanicals being used by farmers must be documented well before this knowledge is lost since the indigenous knowledge system is transmitted from the elders to the younger generation. More research must be done accompanied by experiment that is invivo and invitro so as to ascertain the efficacy on the ethnopharmacological effect of such herbs on different poultry ailments.

Keywords: poultry, growth promotors, ethnoveterinary medicine, moringa tree and sausage tree.

ID : 1162

ISOLATION RATE OF GALLIBACTERIUM ANATIS IN SICK CHICKENS IN THE CENTRAL AND SOUTH TAIWAN AND THE MECHANISMS OF QUINOLONE RESISTANCE

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In order to know the influence of *Gallibacterium anatis* (*G. anatis*) on poultry health, we investigated the flock isolation rate of *G. anatis* in sick chickens of different breeds in the central and south Taiwan from September 2014 to August 2019. The previous research in Taiwan demonstrated the high resistance rate of the field strains for quinolones. To investigate the possible mechanisms responsible for quinolone resistance, 98 *G. anatis* isolates were tested in the present study.

A total of 174 layer flocks, 161 native chicken flocks, 107 breeder flocks and 70 broiler flocks were submitted for examined. Bacteriological investigations were carried out from lung, airsac, heart and liver when necropsy, and each sample was plated out directly on a blood agar for isolation of *G. anatis*. The results showed that the flock isolation rate of layers, native chickens, breeders and broilers were respectively 33.33% (58/174), 19.25% (31/161), 12.15% (13/107) and 7.14% (5/70). According to the result of chi-square statistic, we concluded that there is a relationship between isolation rate and chicken breeds ($p < 0.05$).

We assess the antimicrobial susceptibility for 5 quinolones by broth microdilution method, and found the high resistance rate. Polymerase chain reaction amplification and sequencing of *gyrA* and *parC* genes were performed to analysis the quinolone-resistance determining region (QRDR); besides, the detection of plasmid-mediated quinolone resistance (PMQR) genes including *qnrA*, *qnrB*, *qnrS*, *oqxA*, *oqxB* and *aac(6')-Ib-cr* were conducted. The QRDR mutations in *GyrA* has been observed in all of the isolates, and the most common mutations were serine-83 to phenylalanine (which were also found the asparatic acid-87 to alanine), valine or isoleucine. In *parC* genes, 97 isolates showed threonine-58 to isoleucine and 37 of the above showed glutamic acid-62 to glycine. The one-way analysis of variance (ANOVA) is used for data analysis, and the results indicated that double mutations in *GyrA* exhibited the significantly lower quinolone susceptibility than the isolates with single mutantion ($p < 0.05$), whereas in *ParC*, there was no significant difference. *oqxA* and *oqxB* genes were detected in 13.3% and 3.1% of all isolates, and the other PMQR genes were absent. However, those carrying PMQR genes didn't show a relatively low susceptibility ($p > 0.05$).

In conclusion, we found the isolation rate of *G. anatis* is associated with chicken breeds, and the data showed the highest rate in layers. On the other hand, this study indirectly elucidated the contribution of amino acid substitutions in *GyrA* to quinolone resistance.

Reference:

Singh SV, Singh BR, Sinha DK, Kumar V, Vadhana P, Bhardwaj M, Dubey S. J Veterinar Sci Techno doi: 10.4172/2157-7579.1000324, 2016.

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ID : 1170

SALMONELLA INFANTIS STRAINS VARY CONSIDERABLY IN COLONIZATION ABILITY OF BROILERS

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Food-borne infections with *Salmonella* are among the most common causes of human diseases worldwide. EFSA (2018)¹ reports a slight increase in human salmonella infections, whereby in addition to *S. Enteritidis* and *S. Typhimurium*, infections with the Serovar *S. Infantis* are becoming increasingly important. Poultry meat is considered as an important reservoir for *S. Infantis* salmonellosis in humans, being actually the most common serovar in broiler flocks in the EU. So far, different genetic clones of *S. Infantis* are reported. Therefore, the aim of the present study was to investigate the colonization ability of 2 genetically different *S. Infantis* strains in broilers. For this, 75 conventional broilers (ROSS 308) were randomly divided into 3 groups comprising 25 birds each. 15 birds each of groups 1 and 2 were infected orally on day 2 of life with 10⁸ CFU/ml *S. Infantis* MSR-16/1939 (multiresistant) and *S. Infantis* MRS-17/00712 (fully sensitive), respectively. In both groups, 10 birds were kept as sentinels. Group 3 served as negative control. Sequential killing of the birds was done weekly after challenge until the age of 35 days. Tissue samples from liver, spleen and caeca were taken for determination of *S. Infantis* by CFU counting and enrichment procedure. Furthermore, weekly cloacal swabs were investigated to determine shedding. A clear difference was found in the colonization and shedding of the 2 strains. Strain MSR-16/1939 was re-isolated from most organs of infected and sentinel birds from 5 dpi onwards until the end of the study. The bacterial load determined by CFU counting ranged for liver and spleen from 10⁰ to 10² CFU/g and for caeca from 10⁸ to 10¹⁰ CFU/g. Additionally, all cloacal swabs from infected as well as sentinel birds of this group were positive. In contrast, only caeca were found positive for strain MRS-17/00712 applying enrichment of samples. Interestingly, the majority of cloacal swabs was negative.

The present investigation demonstrates substantial differences in the potential of *S. Infantis* strains to colonize broilers. This finding harmonizes with observations in the field and contributes to a better knowledge in regard to the infection dynamic of different *S. Infantis* strains belonging to different genetic clusters.

¹ European Food Safety Authority, 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017.

ID : 1172

ESCHERICHIA COLI ISOLATED FROM ORGANIC LAYERS REVEALS HIGH LEVEL OF ANTIMICROBIAL RESISTANCE EVEN IN THE ABSENCE OF ANTIMICROBIAL TREATMENTS

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In the actual study *Escherichia coli* was isolated from organic layers at 3 different points: before onset of lay (S1), at the peak of lay (S2) and at the end of lay (S3). For this, ovary, oviduct, heart, liver and lung were sampled and single colonies were confirmed by matrix-assisted laser desorption ionisation time of flight-mass spectrometry analysis (Microflex LT instrument, Bruker Daltonic, Leipzig, Germany). A total of 209 isolates from 18 organic layer flocks were obtained and tested against 21 antimicrobial substances (ampicillin, amoxicillin, oxacillin, amoxicillin/clavulanic acid, cefazolin, ceftazidim, ceftaxime, cefotaxim, ceftazidim, imipenem, streptomycin, neomycin, gentamicin, chloramphenicol, tetracycline, tylosine, colistin, nalidixic acid, enrofloxacin, trimethoprim, trimethoprim/sulfamethoxazole) by broth microdilution method.

All isolates were resistant against oxacillin and tylosin and a high level of resistance was also noticed against sulfamethoxazole (95.22%), nalidixic acid (91.87%), ceftazidim (82.30%) and colistin (73.68%). Nearly all isolates were susceptible against amoxicillin (97.61%), gentamicin (93.78%), imipenem (90.91) and cefotaxim (90.43%). A slight increase of resistant strains was noticed in older birds most obvious for enrofloxacin and tetracycline. No difference was noticed with regard to the level of resistance between isolates obtained from systemic vs reproductive organs, different to isolates of *Gallibacterium anatis* which were processed in the course of the same longitudinal study¹.

Overall, the actual results indicate a high level of resistant *E. coli* isolates in organic layers even in flocks without antimicrobial intervention. Great heterogeneity between isolates from birds of the same farm and even within a single birds underlines the difficulties to implement targeted treatment, although much less relevant in organic flocks.

1 Hess, C et al. (2019). Antimicrobial resistance profiling of *Gallibacterium anatis* from layers reveals high number of multiresistant strains and substantial variability even between isolates from the same organ. *Microb. Drug. Resist.* doi: 10.1089/mdr.2019.0056

ID : 1175

ASSESSING COMPLIANCE WITH BIOSECURITY MEASURES IN FRENCH POULTRY FARMS USING
INSPECTIONS REPORTS FROM VETERINARY PUBLIC HEALTH AUTHORITIES

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Biosecurity is a key parameter for the prevention of poultry farm contamination by pathogens. In 2016-2017, in the context of two epidemics of Highly Pathogenic Avian Influenza (HPAI) in France with respectively 81 (serotypes H5N1, H5N3, H5N9) and 485 (serotype H5N8, clade 2.3.4.4.) reported domestic outbreaks, regulations on biosecurity practices in the poultry industry were reinforced. In order to control compliance with those regulations, a program of on-farm inspections was carried out by the veterinary public health services on the whole French territory. A thorough assessment of on-farm biosecurity measures may require time-consuming farm visits. In that context it may prove difficult to conduct a study on a large number of farms.

This study provides an assessment of biosecurity in a large number of French poultry farms, using reports from on-farm inspections carried out by veterinary public health services.

A total of 1616 commercial poultry farm inspections was analysed and encompassed various poultry species (gallinaceous poultry, waterfowl), production types (breeders, layers, meat-type) and poultry-producing areas over the French territory. Due to the nature of the data and the heterogeneity of species and production types, farm size could not be studied. The most respected biosecurity measures included having single-age flocks, a satisfying management of farm equipment, the absence of pets in production units, and appropriate food and manure storage. The least respected biosecurity practices included the redaction of a farm-adapted biosecurity plan, the definition of different zones within the farm, the presence of well-equipped anterooms, pest management and proper decontamination protocols. A higher level of compliance with biosecurity measures was found in Southwest France area and in waterfowl farms, where previous HPAI outbreaks had occurred.

Those results provide insights on how to focus efforts for maintaining and improving biosecurity in French poultry farms.

ID : 1177

IN VITRO ANTIPROTOZOAL ACTION OF 7 SPICE, MEDICINAL PLANTS AND ESSENTIAL OILS AGAINST 3 STAGES OF EIMERIA TENELLA

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Growing societal concerns to replace coccidiostats in poultry farms lead to the use of alternatives as plant bio-actives. To investigate natural molecules modes of action we designed this in vitro study to screen anti-protozoal activity of 4 spices or medicinal plants described by non-volatile active molecules (MedP) (Fabacea powder, Urticaceae extract, Punicaceae extract, Acanthaceae extract) and 3 essentials oils (EO) (Lilaceae EO, Lamiaceae EO, Lauraceae EO) against 3 different stages of *E. tenella*. First, cytotoxicity of samples was evaluated using MTS test against MDBK cells. The lowest dilution of each MedP or EO samples showing no cytotoxicity (lethal dose 10%) was used to perform 3 antiprotozoal tests. Sporozoite viability test was conducted in triplicate with Evans blue dye (incubation 41°C for 1h). Invasion inhibition test was performed in 96 microplate: 1.5×10^4 MDBK cells, infected with sporozoites (MOI = 1), were incubated with each sample (4 replicates) at 41°C. *E. tenella* strain expressed an enzymatic activity which can be quantify. After 2h, substrate was added and enzymatic reaction measured after 2h incubation at 37°C. Invasion rate was calculated as percentage of samples respective absorbance compared to absorbance of control. Finally, 5×10^5 non sporulated oocysts were incubated in triplicate with each MedP or each EO samples in solution of potassium dichromate (2%) at 26°C for 72h. Sporulation rate was defined as percentage of sporulated oocysts in tested samples compared to sporulated oocysts in control. Mann-Whitney test was used to compare each sample rate to control. The 4 MedP samples were less cytotoxic than 3 EO samples (MedP diluted at 10-2, EO diluted at 10-4-10-6). MedP samples showed antiprotozoal activity against different *E. tenella* stages: sporozoites viability rate were between 76.2% and 27% ($p = 0.05$). Urticaceae extract showed also a sporulation rate of 68.8% ($p=0.05$). Punicaceae extract exhibited invasion rate of 74% ($p<0.001$) and sporulation rate of 81.6% ($p=0.05$). EOs showed only direct anti sporozoites activity (sporozoites viability rates between 76.7% and 81.6% ($p=0.05$)). MedP group showed antiprotozoal activity against different *E. tenella* stages, on the contrary of EOs, which only targeted sporozoites. This study helps to understand how bio-actives plants could act in association to reduce *E. tenella* pathogenicity.

ID : 1279

PATHO-MORPHOLOGICAL AND GENE LEVEL CHANGES IN INDUCED AFLATOXICOSIS IN WHITE PEKIN DUCKS (ANAS PLATYRHYNCHOS DOMESTICUS)

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The present study attempts to analyze gross and microscopic changes in tissues and characterization of gene level changes in ducks induced with aflatoxin B1. The study was carried out in 120 nos. of white Pekin ducklings. Ducklings were reared under standard managemental system with ad libitum feed and water etc. for a period of 8 weeks. Aflatoxin B1 was added to the feed with different proportion at the dose rate of 6ppb, 12ppb, 24 ppb, & 48ppb through premix which were fed to the ducklings of Group 2, 3, 4, 5 respectively, after 3 days with Group 1 as control group. Grossly liver was enlarged, pale, soft and friable with marked congestion in 48 ppb AFB1. Microscopically, at 48 ppb, liver revealed vacuolar degeneration of hepatocytes, mild sinusoidal congestion and focal necrotic area. Toxicopathological effect was pronounced at 48 ppb of aflatoxin B1. This experiment registered the first confirmation study of toxicopathological changes in the host upon induced aflatoxicosis at molecular level through gene expression of two candidate genes, IGF1 and Cytochrome P450 with in-house designed primer by qRT-PCR analysis. Expression of IGF1 in kidney, liver and spleen was down regulated to a very significant level ($p < 0.005$) in case of ducks treated with 48 ppb of AFB1. CYP450 level was up regulated up to 2.14 fold in case of kidney, 1.57 fold in case of liver, and spleen 1.75 fold at 48 ppb. Expression of IGF1 & CYP450 gene in tissue can be used as a marker for evaluating toxic effect of aflatoxicosis. Toxic level at 48 ppb of Aflatoxin B1 in white pekkin duck was validated by this study.

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ID : 1289

FIRST REPORTED COINFECTION OF ESCHERICHIA COLI AND KURTHIA GIBSONII IN LAYING HENS

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E. coli is a very common and well-known pathogen in the poultry industry. Although coinfections with other microorganisms have been often reported, to our knowledge, this is the first report of a coinfection with *Kurthia gibsonii*. Five different laying hen farms have recently reported increased morbidity and mortality rates, and suspected of colibacillosis. Their daily mortalities were examined and the most common lesions were perihepatitis, fibrinous peritonitis and salpingitis. During necropsies, swabs were taken from macroscopically changed organs and bone marrow, and examined bacteriologically. Identification of *E. coli* was done based on morphological characteristics and confirmed by MALDI-TOF mass spectrometry method, while identification of *Kurthia* was done based on 16S rRNA sequencing. Both *E. coli* and *Kurthia gibsonii* were isolated from liver, peritoneum, ovaries, oviduct and bone marrow samples in high quantity. In total, nine *Kurthia* isolates were phylogenetically analysed. Phylogenetic tree was constructed by Maximum Likelihood method and Jukes-Cantor model using Molecular Evolutionary Genetics Analysis (MEGA X). The results showed high level of homogeneity between seven isolates, while two isolates from different farms were separated from the main cluster. Although *Kurthia* spp. are not very well-known species, they are widely distributed in the environment. There is no evidence of pathogenicity in poultry, but they have been isolated from healthy animals and humans, as well as causative agents of infections. Considering the origin of our isolates, we assume the infection could occur ascendingly through the oviduct. Since there has been no reports on *Kurthia* infections in poultry, it is probably an opportunistic pathogen. Further research is needed to understand the pathogenesis of infection and to determine the importance of *Kurthia gibsonii* in poultry farming.

ID : 1301

SYNERGISM OF MS-H STRAIN AND AUTOGENOUS E. COLI + G. ANATIS VACCINE IN THE PROTECTION OF BROILER BREEDER FLOCK

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Monitoring showed that more than 70% of the layer and breeder flocks in Europe are positive for *Mycoplasma synoviae*. It can cause respiratory distress, synovitis and changes on egg shells. However, it is usually manifested as mild infection causing oxidative destruction of the epithelia, thus opening entrance for other, generally opportunistic pathogens, like *E. coli* and *G. anatis*. Vaccination of MS using MS-H strain showed excellent results in the protection of layers and breeders, as well as autogenous vaccines against *E. coli* and *G. anatis*. In this study we present results of simultaneous application of MS-H and autogenous *E. coli* + *G. anatis* vaccine in broiler breeder flocks. In total 8 flocks of 31000 Ross 308 breeder hens were longitudinally monitored. Three flocks were vaccinated twice using commercial live and inactivated vaccines for *E. coli*, while two flocks were vaccinated using only autogenous *E. coli* vaccine twice. One flock was vaccinated once using MS1 strain of MS in combination with autogenous *E. coli* twice. On the other hand, two flocks were vaccinated once using MS-H vaccine at 9 weeks, and twice using autogenous vaccine, in one flock only with *E. coli* and in the second *E. coli*+ *G. anatis* vaccine at 9 and 16 weeks of age. Production parameters (egg production, total and weekly mortality) were collected in each flock during production till the 60 weeks of age and analysed statistically. Results showed statistically significant drop in mortality and increase in egg production in the flocks simultaneously vaccinated with MS-H and autogenous vaccines compared to the flocks vaccinated only with commercial vaccines, with average decrease from 19,37 to 10,3%. Also, same flocks have had lower mortality compared to flocks vaccinated only with autogenous vaccines, but not significantly, which is 14,93%. In conclusion, combination of MS-H and autogenous vaccine significantly lowers the mortality in broiler breeder flocks and improves the production.

ID : 1321

INFECTIOUS ENDOCARDITIS IN THE MULE DUCK: CLINICAL EPIDEMIOLOGY AND ETIOLOGICAL ASSOCIATION OF STREPTOCOCCUS PLURANIMALIUM

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In winter 2018, fourteen farms breeding mule duck in South West of France showed sudden mortality between 15 and 58 days of age. Major lesions were severe splenomegaly associated with vegetative endocarditis and spleen infarcts. In gallinaceous birds, bacterial septicemia leading to endocarditis was usually associated to *Streptococcus* and *Enterococcus* infections (Hedegaard et al., 2009; Bisgaard et al., 2010). However, it was never reported in ducks. Eleven farms were investigated to better assess the etiology of observed mortality. For seven clinical cases, bacterial strains were isolated from the heart by field veterinarians for further identification. For additional cases, samples were directly taken on farms in order to perform histology, bacteriology and biomolecular analysis. Fragments of abnormal tissue located on the mitral valve were removed in sterile conditions in order to perform 16S sequencing analysis and culture on specific media for MALDI-TOF identification. Additional PCR assays were also performed for the presence of other viral agents of interest (parvovirus of Derzsy's disease, circovirus, polyomavirus). Affected flocks presented a strong heterogeneity associated with brutal mortality ranging between 1.1 and 30%. Two farms presented birds having short beaks and "geese profiles" which led to suspect Derzsy's disease. Necropsy always showed splenomegaly with necrotic foci and vegetative endocarditis as main lesions. Bacteriological examinations from the mitral vegetations revealed pure cultures of bacterial colonies of small white smooth appearance having partial hemolysis. The colonies from each of the farms were identified by mass spectrometry as being *Streptococcus pluranimalium*. Additional 16S sequencing confirmed DNA sequences belonging to *Streptococcus pluranimalium*. Sequencing and assembly of 2 complete genomes were performed using Illumina NGS and confirmed the identification and some genetic diversity between these 2 strains. In human medicine, the mechanisms of infectious endocarditis have been well described and looked similar to our observations (McDonald et al., 2009). However, the origin of this severe sepsis remains to be clarified. Looking further ahead, including new field cases and possible experimental reproduction of the infection will give the opportunity to assess the pathogenesis and etiological role of *Streptococcus pluranimalium*.

ID : 1362

EFFECTS OF A BLEND OF GLYCEROL ESTERS OF FATTY ACIDS ON THE PATHOGENESIS OF EXPERIMENTAL NECROTIC ENTERITIS IN BROILER CHICKS

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The aim of the present study was to investigate the effect of a blend of glycerol esters of fatty acids on the pathogenesis of experimental necrotic enteritis in broiler chicks. One hundred and twenty 1-day old broiler chicks were randomly allocated to 4 treatment groups according to the following experimental design: group A, which served as negative control, group B, to which dietary supplementation of a blend of monoglycerides of fatty acids was applied, group C, to which a challenge of the birds with *C. perfringens* and *Eimeria* spp. was applied and group D, to which dietary supplementation and challenge was applied. The intestine, gizzard and liver were collected and scored for gross lesions from each bird, while one caecum was taken for microbiological analysis. The intestinal digesta were collected for pH and viscosity determination. The statistical analysis and evaluation of the experimental data revealed that the challenge of birds caused significantly ($P \leq 0.05$) more severe necrotic enteritis lesions, while the dietary supplementation reduced them significantly ($P \leq 0.05$). In addition, the challenge of birds, as well as its combination with a blend of glycerol esters of fatty acids, caused significantly ($P \leq 0.05$) more severe coccidiosis lesions, reduced significantly ($P \leq 0.05$) the pH and increased significantly ($P \leq 0.05$) the viscosity of ileum digesta. Finally, the dietary supplementation, the challenge as well as their combination increased significantly ($P \leq 0.05$) the *Bifidobacterium* spp. counts in the caecum. In addition, their combination increased significantly ($P \leq 0.05$) the *E. coli* counts in the caecum. The study provides evidence that the dietary supplementation of a blend of glycerol esters of fatty acids can significantly promote the performance and can reduce the severity of necrotic enteritis lesions and improve the gut health in broiler chicks.

Keywords: necrotic enteritis, glycerides, fatty acids, broiler chicks, microbiota

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ID : 1370

IN VITRO EFFICACY OF COMMERCIAL POULTRY ACIDIFIERS AGAINST POULTRY PATHOGENS

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Commercial poultry acidifiers have been widely used in poultry production. The aim of this study was the in vitro investigation of the antimicrobial activities of five commercial poultry acidifiers (A-E) against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Campylobacter* spp. and *Listeria* spp. The antibacterial activity of the products was tested by the micro-MIC method and expressed as the lower concentration of the products which inhibits bacterial growth. All the tested products exhibited antibacterial activity against the pathogens used in the study. Product A and Product B, mixtures of formic, lactic, acetic, propionic and sorbic acid, exhibited almost equal antibacterial activity. Both products were effective against the majority of the tested bacteria on MIC values of 0.14% v/v. In addition, the products exhibited high anti-*Campylobacter* activity; such were effective against *C. jejuni* and *C. coli*, in a concentration of 0.07% v/v. Product C, a mixture of propionic, benzoic and sorbic acids blended with copper-chelates, ethanol and prebiotics was effective inhibiting bacterial growth in concentrations varying from 0.02-1.14% v/v. Particularly, the product was effective against *C. jejuni* under low concentrations (0.02-0.28% v/v), in contrast to *L. innocua*, for which higher concentration was required (1.14 % v/v). Product D, a blend of organic acids, short-chain fatty acids in the form of butyric acid and prebiotics was effective against tested microorganisms in a concentration ranging from 0.04-0.57 % v/v. Remarkable was its efficacy against *C. jejuni* and *L. monocytogenes* with MIC ranging from 0.04-0.14% v/v and 0.08-0.16 % v/v, respectively. Product E, an acidifier based on hydrogen peroxide, blended with complexed silver was effective under extremely low concentrations against the tested bacteria, with MIC values lower than 0.01% v/v. The MIC tests indicated that the tested products exhibited promising antimicrobial effects in vitro against all the pathogens used in the study. Since the majority of these products are registered as feed additives and the dosage scheme has been designed for growth enhancement, further studies are needed to define the appropriate dosage protocol for pathogen control. Thus, more precisely targeted strategies for applying acidifiers as feed additives may be developed.

Keywords: poultry, acidifiers, disinfectants, organic and inorganic acids, antimicrobial activity, MIC.

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ID : 1373

IN VITRO EFFICACY OF COMMERCIAL POULTRY ACIDIFIERS AND PHYTOGENICS AGAINST POULTRY PATHOGENS

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Phytogenics and acidifiers have been used as feed additives in order to improve nutrient absorption, stimulate the secretion of digestive enzymes and promote birds' performance. The aim of the current study was to investigate the Minimum Inhibitory Concentration (MIC) of commercial phytogenics and acidifiers against common poultry pathogens. This study includes in vitro research of the antimicrobial activity of five commercial poultry products (A-E), from both categories, against *Staphylococcus* spp., *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp. and *Listeria* spp. The antibacterial activity of the products was tested by the micro-MIC method and expressed as the lower concentration of the products which inhibits bacterial growth. All the tested products exhibited antibacterial activity against the pathogens used in the study. Product A, a blend of EOs with a high concentration of cinnamon, was effective against *C. jejuni* and *C. coli* in low MICs values 0.017-0.071% v/v. Higher concentration (1.14-4.54% v/v) was needed against *S. aureus*, *Salmonella* spp., *E. coli* and *Listeria* spp. Product B, a blend of EOs with cinnamon, was also effective against all the bacteria strains with MIC values ranging from 0.14-9,10% v/v. Product C, a blend of formic and propionic acid, was effective against *Escherichia coli*, *L. monocytogenes* Scott A, *C. jejuni* and *C. coli* in a concentration of 0.57% v/v. Product D, a blend of propionic, acetic and formic acid, was remarkably effective against *E. coli*, *S. aureus* and *L. innocua* in low MICs values varying from 0.14- 0.57% v/v. Product E, a blend of lactic, citric and orthophosphoric acid, was effective against all the bacteria tested in concentration from 0.28-1.14% v/v. The results of this study indicate the antimicrobial properties of phytogenics and acidifiers against common pathogens. However, the majority of these products are registered as feed additives and the dosage scheme has been designed for growth enhancement, further studies are needed to define the appropriate dosage protocol for pathogen control.

KEYWORDS: commercial poultry products, MIC, essential oils, acidifiers, antimicrobial activity, bacteria.

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ID : 1392

BROILER HOUSE PARTICULATE MATTER, AEROBIOME, AND ANTIBIOTIC RESISTANT E. COLI UNDER “RAISED WITHOUT ANTIBIOTICS” PRODUCTION.

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Broiler houses introduce high levels of particulate matter (PM) and airborne bacteria which may lead to health concerns and pathogenic transfer in birds and handlers. The general shift towards antibiotic (AB)-free live broiler production indicates the need for active research on AB resistant commensal bacteria that may persist in broiler houses under “Raised Without AB (RWA)” production. While studies have shown compositional profiles of PM and bacteria in broiler houses, there is no data on temporal or spatial changes in broiler house aerobiome or AB resistant bacteria. The purpose of this study was to determine the relationship between PM, litter factors (moisture and E. coli abundance), aerobiome, and AB resistant E. coli. 125 Cobb-500 broiler chicks were raised in floor pens on “built-up” litter for 49 days following RWA production conditions. A DustTrak DRX (TSI Inc) was used to measure airborne PM sizes of 2.5 and 10 micrometers. Open petri dishes with ECC CHROMagar(TM) or Brain Heart Infusion agar were placed in the broiler house to quantify levels of airborne coliforms, E. coli, and total bacteria. Bacterial and PM sampling took place at heights representing bird level (height 1), human level (height 2) and upper room circulation (height 3) for 12 minutes. PM sizes consisted of 18-25% PM_{2.5} and 33-50% PM₁₀ regardless of height or day of sampling. PM changed consistently throughout the study, and there was no significant difference between heights ($P>0.05$). No airborne E. coli was detected at day 0, however, E. coli was measured on day 7 at 2.98 and 3.76 log CFU/m³ for height 1 and 3. Although airborne E. coli increased and ranged from 4.49-4.95 log CFU/m³ from day 14-28, there was no significant difference between heights ($P>0.05$). In contrast, coliform bacteria concentration decreased at all heights from day 14–28. AB susceptibility testing was done on a selected number of E. coli isolates (n=71) with 66% of isolates susceptible to 14 antibiotics tested and 23% displaying multidrug resistance to two or more classes of antibiotics. A multiple regression test revealed that airborne E. coli was positively correlated to litter E. coli and PM_{2.5} ($P<0.001$) and negatively correlated to PM₁₀ ($P<0.001$) with litter moisture having no significant effect. There was no significant correlation found between these factors and concentrations of airborne coliforms. This study shows that decreasing levels of PM_{2.5} may decrease airborne bacteria concentrations.

ID : 1402

PROTECTIVE EFFICACY OF RECOMBINANT HVT ND-IBD VACCINE AGAINST VIRULENT NEWCASTLE DISEASE VIRUS CHALLENGE IN BROILER CHICKENS

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Newcastle disease virus (NDV) is one of the most important infectious agents encountered in poultry industry worldwide due to the high mortality and production loss associated with the disease. Preventive measures are including biosecurity and vaccination. The objectives of this study were to determine the efficacy and performance of different vaccination programs of recombinant HVT (rHVT) ND-IBD (Innofusion ND-IBD, MSD) vaccine compared to rHVT ND (Vectormune HVT-ND, Ceva)+immune complex IBD (Transmune, Ceva) vaccines against challenge with virulent Newcastle disease virus (vNDV) in broiler chickens. Chickens were randomly allocated into 4 groups. Groups 1-3 received different combinations of vaccine at 1-day-old, these were: Group 1 received live combine ND+IB vaccine (Ma5/Clone 30, MSD) simultaneously vaccinated with rHVT ND-IBD vaccine. Group 2 received the same live vaccines simultaneously vaccinated with inactivated ND (iND) (ND Broiler, MSD) and rHVT ND-IBD vaccines. Group 3 received live combine ND(B1)+IB(Mass) vaccine simultaneously vaccinated with rHVT-ND vaccine. At 7-day-old, Groups 1 and 2 received live ND (Clone30, MSD) vaccine and Group 3 received live ND (LaSota) vaccine. Group 4 was the non-vaccinated control group. At 14 and 28-day-old, each chicken in Groups 1-4 received approximately 10⁵ EID₅₀ of virulent Genotype VII NDV challenged, 20 chickens of each vaccinated group and 10 chickens of the non-vaccinated group at each time point. The presence of HVT in spleen at 14 and 28-day-old, protection and performance after they received the challenge virus and antibodies against NDV were determined and compared statistically. The results revealed that the presence of HVT in spleen of the vaccinated groups 1, 2 and 3 at 14 and 28-day-old was 100, 100 and 70 percent and 70, 70 and 90 percent, respectively. Protection of chickens in Groups 1, 2, 3 and 4, that received the challenge virus at 14-day-old, was 35, 75, 35 and 0 percent, respectively. While protection of chickens which received the challenge virus at 28-day-old was 70, 70, 70 and 10 percent, respectively. Body weight gain and serological response of chickens in Group 2 were among the highest. It is concluded that chickens which received live vaccine simultaneously vaccinated with iND and rHVT ND-IBD vaccines at 1-day-old and received live vaccine at 7-day-old had the highest protection and performance against challenge with vNDV at 14 and 28-day-old.

ID : 1423

EVALUATION OF POULTRY RED MITES SUSCEPTIBILITY TO COMMERCIAL ACARICIDES

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The poultry red mite (*Dermanyssus gallinae*) is one of an economic problem in egg-laying hens today. This hematophagous ectoparasite is found up to 95 % flocks in Europe, USA, Japan and China. The cost expends on fight with it and production losses are calculated on 360 million EUR per year. Infestation by poultry red mites can lead to decrease in egg production and quality, aggressive feather-pecking among hens, cannibalistic behavior or death syndrome. Poultry red mite may be a vector of a variety of poultry pathogens including zoonoses.

The most often used chemical substances to eliminate poultry red mites are pyrethroids, derivatives of fenols (DEET), carbamates, spinosin, organofosfates, amidine. Using products based on these substances is legislative constrained or absolutely prohibited in some countries. Their advantages are simple application and lower costs, very effective on the beginning. Their disadvantages are nonavailability of new molecules, residues, toxicity, short-term effectiveness, necessary to repeat application until a week, resistance of poultry red mites to chemical substance, some products may be used only in empty poultry house. These products are still used for poultry red mites elimination.

This study evaluated susceptibility of poultry red mites to four commercially used acaricides (Milba STOP ultra, Elector, Poultry Shield, Byemite). Poultry red mites came from a farm with cages system for table eggs production in the Czech Republic in 2019. For the tests in vitro direct contact method was used; Method No. 11 from IRAC (Insecticide Resistance Action Committee) Susceptibility Test Methods Series. According to the method concentrations of acaricides were used as follow: 0% (acetone only control); 20%; 100% of the recommended field application rates. All commercial acaricides caused mortality of poultry red mites in all their stages of development. The evaluation of susceptibility and resistance of poultry red mites to commercially used acaricides by Susceptibility rating scheme showed, that poultry red mites were highly susceptible to Poultry Shield and they were resistant to acaricides Milba STOP ultra, Elector, Byemite on the farm. Poultry Shield caused 100% mortality of all poultry red mites at tested concentrations (20% and 100%). Acaricide Milba STOP ultra caused 95% mortality of poultry red mites at 20% concentration and 70% mortality at 100% concentration. Acaricide Elector caused 77% mortality of poultry red mites at 20% concentration and 87% mortality at 100% concentration. Acaricide Byemite caused 78% mortality of poultry red mites at 20% concentration and 87% mortality at 100% concentration.

ID : 1431

IMMUNE FUNCTION AND MACROPHAGE-SURVIVAL IN A STAPHYLOCOCCUS AGNETIS MODEL FOR BACTERIAL LAMENESS IN BROILERS

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Objective

Staphylococcus agnetis has been previously associated with subclinical cases of mastitis in dairy cattle. We first reported the isolation of this species from the bones and blood of lame broilers at the University of Arkansas. Since then, others have identified this same species in chickens. We have demonstrated transmission of bacterial chondronecrosis with osteomyelitis (BCO) through aerosols or in drinking water. BCO primarily affects the growth plate in the proximal femur and tibia, the fast-growing leg bones. We have identified a particular BCO isolate, *S. agnetis* 908, that can induce very high incidences of lameness and bacteremias in broilers under standard growing conditions. We have determined that certain feed supplements enhance peripheral blood monocyte activity against strain 908. We have published the complete annotated genome of strain 908 and have compared it to existing genomes for isolates from mastitis in cattle and chicken isolates in commercial operations. Phylogenomic analyses of chicken and cattle isolates show the hypervirulent chicken isolate, 908, is closely related to two cattle isolates, including strain 1379. We have found that strain 1379 is efficiently killed by immortalized chicken macrophage, while strain 908 not only survives phagocytosis, it kills the macrophage within 2 days. We have therefore employed directed genome evolution (DGE) to identify the determinants of macrophage survival and killing as a model for survival in the blood stream.

Methods

Genome analyses have identified 40 genes and 3 plasmids from strain 908 absent or poorly conserved in any of the cattle *S. agnetis* isolates. DNA from strain 908 was electroporated into strain 1379 which was then passaged through chicken macrophage to select for resistant bacteria that kill chicken macrophage. Survivor genomes for multiple independent transformants were sequenced and assembled.

Results

Survival and killing is associated with particular amino acid substitutions in either of two copies of the deoxyribose-phosphate aldolase gene.

Conclusions

The deoxyribose-phosphate aldolase gene has been previously associated with stress response in other systems. The exact mechanism for facilitating survival in *S. agnetis* is not known but it likely is critical for survival in the blood system in order to reach and colonize the proximal growth plates resulting in BCO.

ID : 100

ENHANCED BIOSECURITY THROUGH IN-HOUSE COMPOSTING OF ROUTINE MORTALITIES

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Routine mortality losses occur in poultry production systems and the carcass disposal method is an important component of the farm biosecurity program to prevent disease transmission. Several methods exist for carcass disposal – incineration, burial, composting, rendering, landfill, etc. – all are typically performed outside the production area but within or near the farm creating a biosecurity risk for spreading disease. Composting is commonly used for carcass disposal because it is economical and yields a valued soil amendment. However, management of open outdoor compost systems can be challenging especially in instances of excessive temperatures and precipitation. Additionally, the open area lends itself to invasion by insects, rodents and animals all of which increase biosecurity risks. Although large, industrial-type composters that completely contain the compost process are available, these typically require substantial infrastructure and space making them cost prohibitive for many producers. In biosecurity terms, a line of separation (LOS) is a defined line or physical barrier that separates poultry within a building from exposure to potential risk factors (i.e. pathogens) from outside the facility. A major advantage of in-house composting is confining mortalities within the facility (i.e. within the LOS) which enhances biosecurity because it decreases the potential of spreading diseases from barn-to-barn and farm-to-farm. Additionally, in-house composting provides ideal conditions for composting that facilitates rapid carcass decomposition and pathogen inactivation. The objective of this study was to determine the effectiveness and feasibility of using an inexpensive, commercially available, tumbler composter for in-house composting of poultry mortalities and evaluate its potential to transmit disease under laboratory and field conditions.

We assessed the biocontainment of the tumbler composters under experimental conditions using two viruses and a bacterium all of which are commonly found in poultry operations. We chose the B1type Lasota vaccine strain of Newcastle disease virus (NDV). NDV is an enveloped virus that is relatively labile in the environment. We also selected the Massachusetts vaccine strain of infectious bursal disease virus (IBDV). IBDV is a nonenveloped virus that is environmentally stable. As our bacterium, we selected and used *Salmonella* Kentucky that was isolated from a healthy broiler flock. Specific pathogen free chickens were used as sentinel birds that were placed in biosafety level 2 facilities along with the composter. The composter contained used poultry litter, poultry mortalities and was frequently inoculated with the viral vaccines or *Salmonella* Kentucky preparations. The sentinel birds were assessed for serologic antibodies to the viruses over a period of 6 weeks. Four separate virus trials (2 trials for each virus) were performed. The sentinel birds in the *Salmonella* Kentucky trial were tested weekly for a period of 12 weeks by swabbing their cloacas and culturing for *Salmonella* Kentucky. The results indicated that tumbler composters did not increase the spread of the viral or bacterial agents used in the trials. Additionally, we have tested the tumbler composters under field conditions with different commercial poultry operations. We conclude that in-house composting using tumbler composters is an efficient, effective and biosecure method of disposing of routine mortalities.

ID : 184

VALIDATION OF A MACROSCOPIC SCORING SYSTEM FOR FOOT PAD DERMATITIS BY USING HISTOPATHOLOGICAL FINDINGS IN BROILER CHICKEN

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Foot pad dermatitis (FPD) is an inflammatory lesion on the plantar skin on broiler chicken feet and a good animal welfare indicator. The aim of the study was to validate an appropriate macroscopic scoring system for FPD by measuring the histological depth of a lesion and evaluating the histopathological severity of the lesion (no lesion, mild lesion, ulcers).

Prior to data collection, the interobserver reliability was calculated as well as the sampling size by simulating different data sets and assuming proper conditional probabilities. The macroscopic scoring system was a modified 5-point score in accordance to Welfare Quality® (2009). In total 200 feet (40 of each score) from 39-42 days old Ross 308 broilers were visually scored, the size of the foot and a lesion, if present, manually measured and histological slides prepared. These hematoxylin-eosin stained slides were scored according to the scheme by Michel et al. (2012) and the depth of inflammation was measured. A score was assigned to each single foot, but took place without knowing the previous visual score. The statistical relations of macroscopic score, microscopic score and depth of inflammation were estimated via regression models for ordinal responses.

A one to one assignment is not possible if macroscopically findings are compared to microscopically findings, but a lower or higher macroscopic scores corresponded to lower or higher microscopic scores. Feet with no histological lesion were in majority assigned to feet without visual lesions. The presence of ulcer is first observed in feet with macroscopic score 1 and predominant at macroscopic score 2 (58%, e.g. superficial lesions with a lesion over 0.5 cm diameter) and macroscopic score 3 (92.5 %). Feet with macroscopic score 4 only contained histopathological scored ulcers (100%). Feet with histological no lesions were assigned with a sensitivity of 0.96 and specificity of 0.92 correctly. Feet with mild lesions could be predicted with a sensitivity of 0.71 and specificity of 0.73 and feet with ulcer with a sensitivity of 0.67 and specificity of 0.96. There was an increase in the measured inflammation depth in rising macroscopic scores, this was however not significant.

Based on our results, we can recommend visual scoring as a practicable tool to assess FPD. The study elaborates significant links between the examined macroscopic and microscopic scoring systems and shows high comparability with limitations of the diagnosis of mild lesions.

ID : 294

SELECTION OF BEST COMBINATIONS OF NON-CHEMICAL CONTROL OF THE POULTRY RED MITE FOR UPSCALING IN IPM PROGRAMMES

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The Poultry Red Mite (PRM) *Dermanyssus gallinae* causes serious health and welfare problems and production losses in the egg industry worldwide. Controlling this ectoparasite is very challenging because it spends life at a distance of hens, thus accumulating in the whole poultry house, and because of resistance emergence against chemical treatments. A sustainable approach is needed to decrease use of chemicals, ban illegal treatments, increase animal health and welfare, economic profitability and food safety. Non-chemical treatments emerge, though none of them seem to be sufficient as a stand-alone treatment. A promising course of action is Integrated Pest Management (IPM), where chemicals are only used as a last resort.

This study, part of MiteControl*, aims to evaluate the relevance of different combinations of 4 non-chemical treatments (predatory mites, plant-based feed additives, vaccines and electrified perches). Their compatibility and synergistic effect were investigated by 2 types of complementary experiments. The first were based on 60 experimental units that mimic small parts of a poultry house and allow a strict control of mite infestations and adequate replica's. Subsequent trials were conducted on an experimental farm under field conditions. In both trials, PRM populations were intensively monitored, and in the first, predatory mite populations were assessed as well. Non-parametric univariate and multivariate tests were conducted in the first trials and temporal changes were assessed in the field trials.

Preliminary results show no adverse effect from plant-based products and vaccines on predators. It was confirmed that in compartments with high start PRM infestation, control was quite difficult regardless of the treatments used, and the temporal dynamics of PRM populations show much faster PRM population growths than initially predicted by a mathematical model.

Our work provides not only useful information for the implementation of non-chemical strategies, but also progress in the general understanding of PRM control. This will feed the development of IPM strategies, to sustainably and efficiently control PRM in poultry farms.

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ID : 316

PROTECTION AGAINST IBV CHALLENGE DURING THE PRODUCTION PERIOD

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INTRODUCTION

IBV is considered as one of the highly contagious respiratory diseases of chickens, leads to economic losses in poultry worldwide. Despite of vaccination, it still a major cause of poor egg production in breeders and layers. In the last few years it was noticed that breeders and layers were facing a production drop problem during laying after the peak, in the Middle East area, as part of the world, the production drop reached up to 15%, in addition to egg quality, higher percentage when it is combined with other respiratory diseases. Thus, it was deemed worthwhile to investigate the causative agents behind this problem.

MATERIALS AND METHODS

Samples were tested by PCR, ELISA and HI. Molecular results showed different IBV variant strains were playing a role during the problem at different farms such as IBV: 274/ Var O2/Q1/ QX and 793, the same was confirmed by HI tests. So, a farm of 60, 000 birds was chosen. The flock monitored was given @ day 1 IBV Mass, @ day 14 CHB and @ 13 weeks IBV 793, Then it was given@ 16 weeks boosting inactivated killed vaccine of IB3 (Mass, 274 and 1466), then monitored monthly from 17 weeks until 42 weeks of age, the antibodies titer of IBV: 274/Mass and 1466 were measured monthly using the HI test of specific antigen for each subserotype. Additionally, the production curve monitored. As according to Box et al,1988, that higher level of protection against egg drop after IBV challenge is correlated with higher HI-titer (more is better).

RESULTS AND CONCLUSION

The HI results showed high titer of different IBV strains, which indicates IBV challenge, while ELISA showed a challenge in MS starting at week 22 weeks of age, keep in mind that no vaccine is available in the market against MS. However, when production drop monitored it was noticed that drop in production is less (2%) compared to one before administration of IB3 vaccine (15%), In addition to improvement in egg quality as well, while MS still positive.

In conclusion, it was noticed that induction of the IB3 vaccine:

- Increased the protection against several IBV strains even in the presence of MS challenge, which helps in reduction of MS damage compared to previous flock, before starting the treatment. Also, it provides high level of protection against egg drop.
- Boosting with inactivated IBV vaccines helps getting higher antibody titers, which is related with higher protection.
- Presence of more strains in inactivated vaccine is helpful to induce more antibodies against more strains.

Key words: IB3, layers, production period

ID : 320

TREATMENT INCIDENCE FOR COCCIDIOSIS IN BROILERS ON DIFFERENT ANTICOCCIDIAL CONTROL PROGRAMS IN FIELD CONDITIONS

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From January till July 2019, 737 Belgian broiler flocks were monitored for their coccidiosis prevention program and the coccidiosis treatment incidence.

For two different coccidiosis control programs, the number of flocks that were diagnosed with coccidiosis problems and were prescribed a coccidiosis intervention (either a veterinary product or an alternative substance) by the veterinarian, were compared. Programs compared were: 139 flocks on nicarbazin/narasin from one day old until thinning (nic/nar full) and 598 flocks on nicarbazin/narasin until 20 days of age followed by salinomycin (Sacox®) until thinning (nic/nar-salinomycin shuttle). Anticoccidial products were used at registered dosages. After thinning (± 32 days) all flocks received blank feed until slaughter (± 38 days). Diagnosis for coccidiosis was based upon clinical signs, necropsy and lesion scoring (Johnson and Reid, 1970).

In the nic/nar full group, 70 flocks out of 139 (50%) were diagnosed and treated for coccidiosis which is in steep contrast to the 22% treatment incidence for the shuttle program (132 out of 598). Furthermore, 65 (93%) of the treatments in the nic/nar full group were started with the indication of *Eimeria acervulina* coccidiosis problems. On the other hand, in the nic/nar-salinomycin shuttle program only 83 treatments (63%) were started for *E. acervulina* problems. Treatments in both programs were initiated at similar ages with most treatments started between 20 and 30 days of age. The difference in treatment results was highly significant when compared using the chi-square test ($p < 0.001$).

These results show that despite of the inclusion of anticoccidials in the feed, treatments for coccidiosis are often initiated. The main species for treatment on the both anticoccidial programs compared was *E. acervulina*. A significant difference was seen between the two evaluated coccidiosis control programs in the incidence of coccidiosis problems requiring treatment. Reduced sensitivity of *Eimeria* field strains for the products used is a possible explanation for the observed differences. These results therefore confirm the need for regular monitoring and evaluation of coccidiosis control programs in order to obtain and maintain optimal anticoccidial efficacy.

Johnson, J and Reid, WM (1970). Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. *Experimental Parasitology*, 28, 30-36.

ID : 406

PROTECTION AGAINST A RECENT NDV VELOGENIC AVIAN ORTHO-AVULAVIRUS TYPE-1 (VAOAV-1) GENOTYPE VII.2 BELGIUM 2018 STRAIN FROM BELGIUM USING A DOUBLE CONSTRUCT RHVT-ND-IBD VACCINE ALONE OR IN COMBINATION WITH LIVE ND CL30 VACCINE.

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Newcastle disease (ND) is a worldwide challenge for the poultry industry, since the 1950s birds are vaccinated to prevent ND outbreaks. New molecular techniques have given more diagnostic tools to follow the genetic evolution of ND field viruses but also gave the opportunity to develop modern vaccines that can be given from hatchery and are beneficial in increasing accuracy of vaccination and reduce vaccine reaction. We are still looking for a good balance between protection and performance. Worldwide, most ND vaccines are based on a ND genotype II strain, but new diagnostics have led to further differentiation in genotypes within the ND population and the logical question is raised; do we need a homologous genotype vaccine to control the different existing genotypes. In 2018 there was an ND isolation of a velogenic Avian Ortho-avulavirus type-1 (NDV) genotype VII.2. in Belgium. This isolate was used as a challenge strain to evaluate two vaccination programs based on the ND genotype II strain.

The objective of the study is the evaluation of day-old ND vaccinations in specific pathogenic free (SPF) birds. The study consisted of four groups: Group 1 rHVT-ND-IBD (with the ND insert based upon Clone 30 ND), Group 2 rHVT-ND-IBD + live ND Cl30, Group 3 positive control and Group 4 negative control. The protection was evaluated after challenge at 3 and 4 weeks post vaccination with velogenic Avian Ortho-avulavirus type-1 (vAOAV-1) genotype VII.2 Belgium 2018 strain. Protection was determined by monitoring clinical signs including mortality and reduction of shedding of the challenge virus. The study was performed in the facilities of the Belgium Sciensano laboratory.

The results show that both vaccinated groups using ND genotype II vaccines induce 100% clinical protection (P-value < 0.05) at 3 and 4 weeks challenge and a significant level of reduction of shedding (P-value < 0.05) for the group rHVT-ND-IBD + live ND Cl30 compared to the non-vaccinated - challenged (positive control) group.

ND genotype II vaccines are effective in controlling the clinical signs of a ND genotype VII challenge at 3 and 4 weeks post vaccination at day-old and for a severe ND challenge the vaccination advice should be combined administration rHVT-ND-IBD + live Cl30 to obtain also significant reduction of shedding.

ID : 459

EFFICACY OF A NICARBAZIN/MONENSIN, A NOVEL COCCIDIOSTAT COMBINATION PRODUCT, IN THE CONTROL OF COCCIDIOSIS IN BROILERS

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The efficacy of Monimax® (nicarbazin/monensin; Huvepharma®) at a concentration of 40mg/kg (40ppm) nicarbazin and 40mg/kg (40ppm) monensin, administered in feed was assessed in broilers after an experimental coccidiosis challenge under battery conditions.

Birds were reared without coccidiostats till the age of 14 days when they were allocated to the different groups. Monimax® treated birds were compared to an infected untreated control (IUC) and an uninfected untreated control (UUC) group. Each group consisted of 7 replicate cages (5 Ross 308 males/cage).

At the age of 17 days all birds in the IUC and Monimax® groups were inoculated with a mixture of *E. acervulina*, *E. maxima* and *E. tenella* (European origin). Birds from the UUC were sham-inoculated.

Daily weight gain (DWG), feed conversion ratio (FCR), intestinal lesion scoring (ILS) and oocyst shedding were compared. Statistical analysis methodology was in accordance with those outlined in the WAAVP guidelines for evaluating anticoccidial drugs in chickens and turkeys (Holdsworth et al., 2004). All tests were 2-sided and the level of significance was set at 5%.

The challenge was successful as proven by the significantly lower DWG, increased FCR, average lesion scores and higher oocyst production in the IUC compared to the UUC.

Administration of Monimax® was able to significantly improve performance (DWG, FCR) in the acute infection phase (D17-D23). Although not statistically significant, total ILS at D23 were lower in the Monimax® supplemented group in comparison to the IUC. At D27 OPG counts for all the examined species were (often significantly) lower in the Monimax® group compared with the IUC group.

The results from this trial demonstrate the efficacy of Monimax® (nicarbazin/monensin) for prevention of coccidiosis in broilers. Under present study conditions, we can conclude, based on significant lower oocyst shedding, lower coccidiosis lesions and significant better performance, that the administration of Monimax® proved efficacious against coccidiosis infection in broilers.

Holdsworth PA, Conway DP, McKenzie ME, Dayton AD, Chapman HD, Mathis GF, Skinner JT, Mundt HC, Williams RB; World Association for the Advancement of Veterinary Parasitology. WAAVP guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. (2004), Vet. Parasit. 121: 189-212.

ID : 460

MULTI-STUDY ANALYSIS ON THE EFFICACY OF NICARBAZIN/MONENSIN, A NOVEL COCCIDIOSTAT FOR USE IN BROILERS

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Multi-study analysis on the efficacy of nicarbazin/monensin, a novel coccidiostat for use in broilers
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The objective of this multi-study analysis was to compare the efficacy of Monimax® (nicarbazin/monensin) and nicarbazin/narasin against a variety of different Eimeria strains. Data of 27 different anticoccidial sensitivity tests (ASTs) conducted over a 7-year period (2013-2019) were included in this study. The Eimeria isolates originated from commercial broiler farms from 13 different countries worldwide applying different anticoccidial control programs.

Two research institutes, using a comparable standardized AST protocol, conducted these trials. Birds were supplemented with Monimax® or nicarbazin/narasin at the same inclusion rate (80-100 ppm) starting 2 days before oral inoculation. Between 5-7 days after inoculation lesions scores and performance (daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR)) was measured and compared to an uninfected, untreated control group (UUC) and an infected, untreated control group (IUC). A multivariate mixed general linear models, including research institute as covariate and including the trial as a random effect was used. Results were further evaluated with multivariable mixed logistic regression models.

The Monimax® group showed a significant reduction for all species (E. acervulina, E. maxima and E. tenella respectively 1.69, 0.93 and 1.38) compared to the IUC (respectively 2.02, 1.20 and 1.81). Nicarbazin/narasin did not result in a significant reduction of E. acervulina (Lesion scores: 1.98 for E. acervulina, 1.04 for E. maxima and 1.44 for E. tenella) compared to IUC. Monimax® showed significant lower E. acervulina and E. maxima scores compared to nicarbazin/narasin.

Monimax® resulted in significant improved DWG, DFI and FCR (respectively 55.0g, 90.2g and 1.72) compared to the IUC (respectively 46.0g, 87.1g and 2.07). Monimax® resulted in better numerical performance compared to the nicarbazin/narasin combination product (respectively 53.8g, 89.2g and 1.75).

From this analysis of 27 studies, it can be concluded that Monimax® resulted in significant reduction of all Eimeria species lesions and all measured performance parameters compared to the IUC. When comparing Monimax® with nicarbazin/narasin all performance results are numerically higher. Nicarbazin/ narasin did not significantly reduce lesion of E. acervulina compared to the IUC and lesions for E. acervulina and E. maxima were significantly higher compared to birds supplemented with Monimax®. The finding that nicarbazin/narasin did not result in a significant reduction of E. acervulina lesions after infection is in contrast to earlier findings of Long and colleagues (1988) where the nicarbazin/narasin combination showed efficacious control of coccidiosis. A potential explanation is that reduced sensitivity of the Eimeria field strains due to long-term use of both compounds over the years has attributed to the less positive results in comparison to the older work.

ID : 1274

EFFECT OF MOISTURE CONTENT AND HOLDING TEMPERATURE OF POULTRY LITTER ON THE VIABILITY OF EIMERIA OOCYSTS

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Coccidiosis, caused by the genus *Eimeria*, is an economically important parasitic disease in chickens and is prevalent in poultry houses from flock to flock. Poultry litter may be composted between flocks to reduce the risk of litter serving as the vector for bacterial, viral, and protozoal pathogens, however, temperatures required to assure proper destruction of *Eimeria* oocysts during composting have not been established. The objective of the study was to evaluate the effect of litter moisture and holding temperature on the viability of *Eimeria* oocysts in model poultry litter.

Pine sawdust was sieved (2.5 mm) and moisture content was adjusted to 20, 30 and 40% using deionized water (DIW). Sawdust (5.0 g) was transferred into a sterile centrifuge tube. Commercial anti-coccidial vaccine (COCCIVAC®-B52; 250 µL) containing a mixture of *Eimeria* species (*acervulina*, *mivati*, *tenella*, *maxima*) was added to the sawdust and mixed manually. The inoculated sawdust was transferred after 24, 48 and 72 h to a sterile container, and DIW (100 mL) was added and held at 4°C for 24 h. The sawdust was filtered through cheesecloth and the intact oocysts were enumerated using a microscope. A 3 (temperatures, 43.3, 54.4 and 65.6°C) x 3 (holding times, 24, 48 and 72 h) experimental design was used and three independent replications were performed.

Inoculation of the sawdust achieved 8.0×10^4 oocysts/g. Lower moisture content and higher holding temperatures resulted in greater destruction of the *Eimeria* oocysts. Holding inoculated saw dust (20% moisture) at 43.3°C resulted in *Eimeria* oocyst population of 8.0×10^4 , 4.6×10^3 , 2.9×10^3 and 4.4×10^2 oocysts/g subsequent to 0, 24, 48 and 72 h, respectively. Increasing the holding temperature to 65.6°C resulted in elimination of the oocysts in the sawdust after 48 h of holding. Increasing the moisture content of the sawdust to 40% resulted in recovery of *Eimeria* oocysts at 65.6°C, with 8.0×10^4 , 7.9×10^3 , 6.4×10^3 and 8.1×10^3 oocysts/g subsequent to holding of the sawdust for 0, 24, 48 and 72 h, respectively. Maintaining sawdust temperature at 65.6°C for 48 h (20% moisture) and 72 h (30% moisture) achieved $>8.0 \times 10^4$ oocysts/g destruction in *Eimeria* oocysts.

Composting temperatures of $\geq 65.5^\circ\text{C}$ are required to achieve destruction of *Eimeria* oocysts in the poultry litter ($\leq 30\%$ moisture). Higher composting temperatures may be required for poultry litter with $>30\%$ moisture content to eliminate *Eimeria* oocyst survival.

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