Effect of amino acid supplementation in reduced crude protein diets on growth performance and gut health indices of broiler chickens exposed to sub-clinical enteric health challenges

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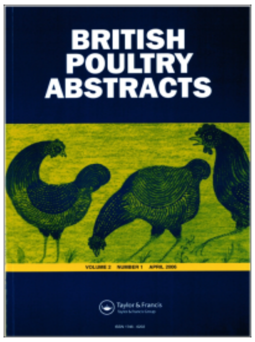
Print publication: 17/12/2021



British Poultry Abstracts

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/cbpa20

2021 Abstracts

To cite this article: (2021) 2021 Abstracts, British Poultry Abstracts, 17:1, 1-29, DOI: 10.1080/17466202.2021.1983311

To link to this article: https://doi.org/10.1080/17466202.2021.1983311

BRITISH POULTRY ABSTRACTS
2021, VOL. 17, NO. 1, 1–29
https://doi.org/10.1080/17466202.2021.1983311

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2021 Abstracts

Oral communications and invited talks accepted for presentation at the Virtual WPSA UK Branch Meeting held on the 15th and 16 April 2021. These summaries have been edited for clarity and style by the WPSA UK Programme Committee but have not been fully peer-reviewed.

Pirgozliev, V., Whiting, I. M. Rose, S. P. Lanckriet, A., and Van Der Veken, W. Dietary probiotic improves laying efficiency in Hy-line Brown hens.

Pirgozliev, V., Whiting, I. M., Rose, S. P., Lanckriet, A., and Nollet, L. Feeding a novel phytase at 250 FTU/kg to laying hens improves P digestibility.

Pirgozliev, V., Johnson, E. A. Drijfhout, F., Orczewska-Dudek, S., Whiting, I. M. Mansbridge, S. C. Rose, S. P. Mihova, T., Kljak, K., Atanasov, A. G. and Oluwatosin, O.O. Short chain fatty acids production and villus morphometry in chickens fed diets containing graded levels of supplementary sea buckthorn dried berries.

Yates, K., Burton, E. J., Scholey, D. V., Perry, C. C., and Lee, S. Evaluation of broiler response to silicon rate of dietary inclusion and particle size.

Afrin, S., Burton, E. J., Scholey, D. V. and Brinch-Pedersen, H.. Amino acid digestibility is improved in broiler diets containing high-phytase HIGHPHY wheat.

Afrin, S., Burton, E. J., Scholey, D. V., and Lee, S. A. Evaluation of phytase super-dosing on broiler intestinal development.

Riva, F., Burchmore, R., Mckeegan, D., Mrljak, V., Horvatić, A., Rubić, I., Peinado-Izaguerri, J., Bruggeman, G., Hermans, D., Mclaughlin, M., Eckersall, D., And Bain, M. Untargeted metabolomics on plasma and liver samples: effects of novel plant extracts in broilers pre and post LPS challenge.

Arthur, C. P., Mansbridge, S. C., Rose, S. P., Brearley, C. A., Whitfield, H, Kühn, I. and Pirgozliev, V. *myo*-Inositol concentration in chicken tissues is influenced by dietary myo-inositol and phytase.

Alkhtib, A., Sanni, C., Burton, E. and Scholey, D. What is 'normal'? Morphology and mineralisation of tibias from healthy, on farm broilers.

Dunn I. C., McCormack H. M., Fleming R. H., Wilson P.W., Andersson B. and Schmutz M. B. Lack of genetic correlation between laying hen bone quality and egg production suggests egg number may not explain bone quality.

Introduction of slower growing broilers in the market in the Netherlands

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In the 2000s, the idea of a so-called middle-segment arose in North-West Europe to address the criticism on intensive broiler production systems. Middle-segment systems being an intermediate segment between conventional and organic broiler production. The production system is based on indoor housing of slower-growing broiler strains at a lower stocking. Between 2014 and 2017 there was a complete change in the Dutch market of fresh meat for broilers leading to a new standard called the New Dutch Retail Standard (NDRS). In 2018, all the fresh meat from broilers sold in Dutch supermarkets originated from different NDRS concepts. The conventional broiler was banned by all supermarkets in The Netherlands. In this process action groups played an important role with a fierce media campaign against fast growing broilers. By attacking two large retailers an upward pressure between retailers resulted in a rapid overall transition. In 2019 approximately 30% to 35% of the broilers are kept at NDRS standard and the remaining is still kept under the conventional conditions and are for food service and export markets. An important side-effect of this partial transition towards more robust animals is, that the use of anti-microbials was further reduced. It's clear that the NDRS broilers are more robust, hence less susceptible for diseases and therefore require less anti-microbials. Based on extensive research by Wageningen University it was concluded that NDRS broilers provide a considerable increase in animal welfare at a relatively small increase in production costs and therefore offer good prospects for a cost-efficient improvement of broiler welfare.

Opportunities and challenges for added-value chicken meat

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Throughout the history, the poultry industry has undergone profound modifications mainly driven by the global population growth as well as by the changes in consumers' lifestyle and needs. The same driving forces will be responsible for defining the role of the poultry production in feeding the world in the coming years. In the future, everybody involved in the poultry meat production chain will face different challenges including those related to the changes in consumers' perception in relation to the sustainability of the production chain, to food safety and animal welfare concerns.

The concept of 'meat quality' is extremely complex and is commonly thought to be the result of genetic, nutrition and environmental conditions. Indeed, meat quality includes different aspects related to the intrinsic traits of the meat itself (such as its sensorial, sanitary and nutritional traits as well as the genetic background of the animals) but it also embraces extrinsic factors including those related to the consumers' perception of the meat itself primarily related to the production system from which it belongs to, which is currently gaining more and more importance.

Nowadays, poultry meat is considered as a commodity and, as this term implies, aside from the place and from the producer it belongs to, all its traits are highly standardized and uniform all over the world. This is a direct consequence of the poultry meat production system which is currently based on the intensive farming of few commercial hybrids selected for meat production purposes. Indeed, being selected for their fast growth rate, the commercial hybrids intended for meat production are slaughtered at young age (around 35 days-old) and, therefore, the sensory profile of the resulting meat is extremely mild. This peculiar trait of chicken meat makes it particularly suitable for further processing (as any flavor enhancer/spice can be easily added) but may also represent a constraint when differentiation needs to be achieved.

Undeniably, the selection practices carried out in the past fifty years permitted to improve the production performances and allowed to reduce the price of chicken meat which is now cheap and affordable for people having different income levels. However, from the other side, the intensive farming of few commercial hybrids is resulting in the loss of most of the autochthons and local breeds that can be found worldwide. Also, the increasing incidence of myopathies that are currently affecting the pectoral muscles of the fast-growing genotype should be kept in mind. Thus, different aspects should be considered when thinking about the opportunities and challenges related to the production of high-values meats. Indeed, differentiation can be achieved throughout different strategies.

As previously mentioned, meat quality is the result of genetic, nutrition and environmental conditions. Thus, it might be easily understood that the first strategy to achieve differentiation is through the choice of the genotype to be reared according to the production system (intensive vs. extensive) that will be implemented. In this specific framework, rearing medium- or slow-growing genotype instead of the commercial fast-growing and high breast-yield hybrids, would result in meat having a different nutritional profile and stronger flavor, which can partly be ascribed to the slaughter age of the birds and to their feeding. However, it should be mentioned that this choice is associated to longer production cycles and to an overall reduction in the sustainability of the production chain.

Aside from changing the genetic of the birds, nutritional strategies can be developed with the aim to differentiate the quality of the resulting meat. Indeed, as chickens have a monogastric digestive system, the lipid composition of their feed can strongly affect the fatty acid profile of the meat. Aside from this well-known aspect, nowadays, researchers coming from different countries in the World are investigating the impact of including alternative protein sources (including green peas, seaweeds and insects) in feed formulation on the sensorial traits and technological properties of meat. Thus, promoting the use of protein sources that commonly do not find any application and that are not intended for human consumption, this strategy would allow to confer to the meat peculiar traits that can be related to the protein source included in the feed as well as to improve the sustainability of the production chain. In addition, also the inclusion of molecules with potential beneficial effects on the productive performances as well as on meat quality traits and on its stability during storage (i.e. antioxidants, minerals, etc.) might represent a profitable differentiation strategy, especially in the case in which the added compounds can be mentioned as a claim on the product's label.

Aside from the genetic and nutritional aspects, the implementation of processing procedures aiming at producing high-value products (i.e. ready to cook and ready to eat) might be considered effective strategies to differentiate chicken meat. Indeed, the current consumers' lifestyle results in an increasing demand of meat products requiring only minimum efforts for their preparation. Thus, the implementation of processing steps, such as among the others the precutting of the fillets and the addition of marinade solutions might represent effective strategies that the companies can carry out with the aim to differentiate and produce an added-value product from chicken meat.

However, to be perceived and have positive implications on consumers' preferences and attitude to buy, all these genetic, nutritional and processing strategies need to be supported by proper information and transmitted knowledge to the consumers. It should be mentioned that, in general, consumers are getting more sensitive about food quality and animal welfare concerns and they tend to prefer products associated to the concept of 'naturality' and belonging to a non-industrial system as well. Food products containing additives are perceived as not natural and will not be longer desired. For this reason, the terms 'natural' frequently mentioned on chicken meat's label, all the claims related to its organic or extensive production system as well as the development of clean labels can be considered as useful tools to differentiate these productions from the conventional one.

Slow(er) growing breeds and the future of the poultry industry

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Conventional broiler performance improvements have been a major industry success, evolving vastly over the past decades. This is a direct result of selective breeding focused on key performance and welfare traits such as growth rate, FCR, leg health and metabolic disorders. This focus reflects the needs of a growing world population, increasingly seeking out ever cheaper sources of meat derived protein. As markets mature and birds of conventional genetics are harvested at earlier ages, retailers and integrations in developed countries have started to target the changing market needs as reaction to increased societal pressure through the production and sales of ever more diverse products.

Food safety, animal welfare, meat quality and human health are some of the considerations which provide points of difference for producers, retailers and consumers to contemplate. Most of these options inspire attention to products with different genetic characteristics, most notably in terms of growth rate and physical appearance. Alternative 'Premium' broilers can meet a large range of growth rate, FCR, yield and appearance expectations. The average daily weight gain can range from 30 to 50 g/d, depending on specific customer needs or the requirements of legislation or individual codes of practice.

Setting aside the traditional coloured bird markets across regions such as Asia and Latin America and the Label Rouge market in France, most recent developments have taken place in the mature markets of Western Europe and are under consideration in North America. In Europe, specific marketing terms set out in EU Directive (2000/13/EC) dictate that products sold as Free Range or Extensive Indoor must be a minimum of 56 days of age with Free Range having access to the range for at least 50% of their lives. Traditional or Total Freedom Free Range must be a minimum of 81 days of age before slaughter. To meet market weights this invariably means that genetically slower growing chickens are required to be used. In addition, there is a growing number of concepts evolving across parts of Europe to fit more specific market demands. The Netherlands is a unique example where in 2013 all retailers committed to all fresh broiler meat sold within their stores by 2020 at the latest coming from slower growing chickens with a maximum ADG of 50 g/d. This objective was achieved in 2017 and in total this equates to about one third of all Dutch chicken production.

The Better Chicken Commitment in Europe and the United States, initiated in 2017 by a coalition of animal welfare associations and to be implemented at the latest by 2026, requires the use of breeds of birds scientifically proven to have markedly improved welfare outcomes, a maximum stocking density of 30 kg/m^2, provision of natural light (windows) and a minimum light intensity of 50 lux, plus indoor enrichment for the birds. Several UK and French retailers and large (global) food service companies have signed up to this commitment. In the United States already more than 160 companies have committed to Global Animal Partnership (GAP) standards by 2026.

The costs of production mainly depend on the growth rate and FCR of the selected type of premium chicken, but it is inevitably higher than the cost of producing conventional broilers. This cost partly can be balanced with positive attributes such as reduced use of antibiotics, better gait, lower daily mortality and good meat quality. Within the supply chain the debate continues about premium chickens versus conventional broilers. The increased use of resources required to grow premium chickens on one side are countered by the improved social acceptability and acceptance can be further improved by using alternative and/or more local raw materials and a better utilisation of the whole carcass. It is evident from the market that there is a growing interest and there is clearly a part of the consumer base in many countries that want to purchase products from slower growing chickens. With an increasing number of suppliers stepping into the segment, customer demand will surely decide how far these new products grow in the market. Choice is not just about price; it is also about the added value and perception by consumers.

Opportunities for higher value meats, specifically chicken

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The meat sector is not spared the change in consumption trend. The 'one-size-fits-all' offer has to evolve towards a massive range of alternatives to satisfy growing individual(istic) expectations. The number of labels on meat packaging claiming better welfare, better taste, more natural or more social attributes and so on is increasing. Yet, most consumers are lacking the knowledge to grasp the true meaning of these claims. Furthermore, consumers' perception of chicken is different from that of beef, leading to different expectations. Looking at successful meat labels, recent labelling changes and peer-reviewed research, we will draw conclusions on key factors for success and future opportunities for higher value meat, especially chicken.

Does scratch mat design influence hen behaviour in enriched cages?

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Application

Scratch mat sizes and materials in enriched cages are not regulated, and thus the subsequent variety in their design may result in variation in hen behaviour. This work could help elucidate optimal design(s).

Introduction

The EU Directive on laying hens (European Commission, 1999) says 'laying hens must have: (c) litter such that pecking and scratching are possible'. In enriched cages, litter is typically provided as layer's mash ('scratch feed') on

Table 1. Mat types and their size (length x width), total area, and area per hen

Mat type	Length x width (cm)	Mat area (cm^2)	Mat area/hen (cm^2)
BD	35.0 x 26.5	927.5	15.5
K	30.5 x 19.0	579.5	9.7
V	44.8 x 18.0	806.4	13.4
Z	64.0 x 31.5	2016.0	33.6

a plastic mat, but there are no requirements for mat size, location or makeup. Commercial enriched cages offer various scratch mat materials and sizes, which may influence behaviour. This study compared hen behaviour on four mat designs.

Material and methods

A commercial egg shed with 60-bird Big Dutchman (BD) enriched cages was used. Cages were arranged over six banks and nine tiers. Here, 24 cages in banks 2-5, tier 5 (6 cages/bank) were used. Cages contained two scratch mats each. Prior to flock arrival, some BD mats were replaced with other mat types in a balanced design, so that each mat was equally represented across banks, cage locations, and cage sides. All pairs of mats in a cage were of two different mat types. Mat types were BD, Kovobel (K), Valli (V), Zucami (Z) designs, and varied in size (Table 1), design, and colour.

Hen behaviour at the mats was recorded at three observation points relative to scratch feed application at 30, 50 and 79 weeks of age. Observations were 1st (1 h 40 min-4 h 45min since last scratch feed), 2nd (during and immediately after scratch feed), 3rd (40 min-1 h since last scratch feed). At the 2nd observation, only half the cages were observed (balanced for mat types) to capture behaviour when scratch feed was most likely to be present. Behaviour proportions were analysed in Genstat 18 using Generalised Linear Mixed models (GLMMs). Where data was sparse and thus GLMMs could not estimate effects in some models, Linear Mixed models (LMMs) were used as a weaker alternative on angular transformed data. Fixed effects were age, observation, mat type and their interactions, random effects were bank, cage, cage.age, cage.age.observation and cage side within cage. Analyses shows results on transformed scale and back transformed to proportions. This study was ethically approved by SRUC's AWERB.

Results

A total of 805 hens were observed on the mats over 360 observations (mean 2.2 hens/mat/observation) thus observed mat usage was low. Mat type significantly affected the proportion of birds observed on the mats, with a greater proportion of hens seen on the Z mats (−3.256) than any other type (BD −3.732, K −3.799, V −3.788) (mean SE 0.102, P<0.001 by GLMM, $Wald_2$= 44.01, back transformed proportions BD 0.023, K 0.022, V 0.022, Z 0.037), however Z mats are larger, and the difference in bird proportions is in reality small (3.7% of hens in a cage on Z mats, versus 2.2-2.3% of hens in a cage on other mats). Proportions of behaviours observed were stand (0.72), sit (0.25) or forage (0.02), with preen and walk behaviours making up <0.01. As forage was the behaviour of interest, only this is reported further although proportions were low. There was a significant effect of observation on the proportion of foraging observed (P<0.001), with most at the 2nd (1st 0.557, 2nd 7.046, 3rd −0.001, mean SE 1.122, by LMM, $F_{2,133}$=11.49, back transformed proportions 1st 0.000, 2nd 0.015, 3rd 0.000). There was a significant age x observation interaction also (P=0.002, mean SE 1.808, by LMM, $F_{2,146}$=4.51), where the proportion of birds foraging was similarly low across ages at both the 1st and 3rd observation, but at the 2nd observation, birds seen foraging increased with bird age (data not shown).

Conclusion

Mat types had little influence on foraging behaviour observed here, although behaviour may have been inadvertently disturbed by observer presence. Foraging behaviour was highest when scratch feed was present, but the amount of time observed foraging overall was small, and there was no evidence to suggest that mat design significantly influenced foraging behaviour.

Acknowledgments

Our grateful thanks to the commercial farm for access. This work was funded by Scottish Government (RESAS programme 2016-2022).

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The correlation between *myo*-inositol (Ins) concentration in the jejunum digesta of broiler chickens and the Ins concentrations of key tissues associated with the uptake and regulation of Ins

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Application

A relevant part of the increase of Ins in jejunum and key tissues is likely to be from dietary sources.

Introduction

myo-Inositol is a cyclohexitol sugar that is released by the complete dephosphorylation of phytate by phytase and phosphatases. The absorption of free Ins from the gastrointestinal

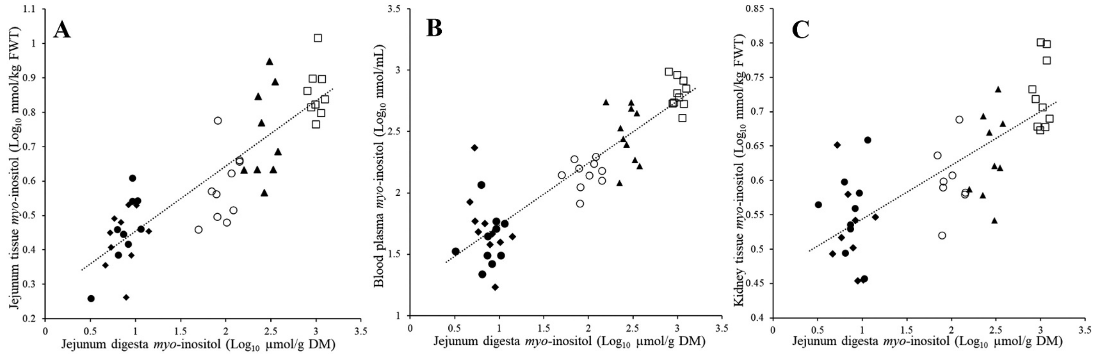

Figure 1. The correlation of jejunum digesta *myo*-inositol (Ins) (Log_{10} µmol/g DM) between jejunum tissue Ins (Log_{10} mmol/kg FWT) (**A**) (P<0.001, R^2=0.875), blood plasma Ins (Log_{10} nmol/mL) (**B**) (P<0.001, R^2=0.886) and kidney tissue Ins (Log_{10} mmol/kg FWT) (**C**) (P<0.001, R^2=0.779). Control (●), control + 4500 FTU/kg (○), control + 4.5 g/kg Ins (▲), control + 13.5 g/kg Ins (□) and control + 4.5 g/kg glucose (♦).

tract (GIT) is very efficient, 99.8% is absorbed in humans. Cells can obtain Ins from a number of sources; from the *de novo* biosynthesis of D-glucose, breakdown of phosphatidylinositol or via specific Ins transporter proteins actively transporting Ins from the extracellular fluid. Walk, Bedford, and Olukosi (2018) demonstrated that the expression of Ins transporters in the jejunum were boosted with increasing phytase dosage. The only known organ associated with the catabolism of Ins is the kidney. The interaction between free Ins in the GIT digesta and Ins in these key tissues involved in absorption (jejunum), circulation (blood plasma) and catabolism (kidney) is not fully understood.

Material and methods

A control basal diet was split into five diets with one part supplemented with one of two levels of Ins, at 4.5 g/kg or 13.5 g/kg, one diet with 6-phytase (Quantum Blue™ AB Vista, UK) at 4500 FTU/kg, one diet with glucose at 4.5 g/kg and one part was left un-supplemented. Ethics for this study was granted by Harper Adams University Research Ethics Committee. Diets were cold pelleted. Each diet was fed to 10 pens from 7-21 days, with two birds per pen resulting in a total of 100 male Ross 308 chickens being used. At day 21, one bird per a pen was killed and blood collected, tissue (jejunum and kidney) and digesta (jejunum) samples were taken and immediately frozen at −80°C. Tissue, blood plasma and digesta were extracted according to Greene, Flees, Dadgar, Mallmann, and Orlowski et al. (2019b) for Ins. Ins was determined by HPLC. Data was transformed using Log10 and analysed in GenStat® (18th edition) for Persons correlation coefficients.

Results

The data demonstrates that an increase of Ins in the jejunum digesta (either by Ins liberated by phytase or Ins dietary supplementation) has a strong positive correlation with jejunum tissue Ins (P<0.001, R^2=0.875), blood plasma Ins (P<0.001, R^2=0.886) and kidney tissue Ins (P<0.001, R^2=0.779).

Conclusion

Free Ins in the jejunum digesta is rapidly absorbed and distributed into the blood plasma, kidney and jejunum tissue. The increase of Ins in these tissues and blood plasma is likely to be from dietary sources rather than from de novo synthesis due to the significant positive correlation (P<0.001) (Figure 1), however C^{14} Ins is required to fully validate this.

Acknowledgments

The authors would gratefully acknowledge doctoral sponsorship from ABVista Germany and the technicians (Rich James and Ros Crocker) at NIPH, Harper Adams University, UK.

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Effect of rapeseed cultivar on the nutritional value of rapeseed meal in broilers

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Application

When a standardised processing method is used during the production of single-cultivar rapeseed meals, the range of variation in their metabolisable energy contents and nitrogen digestibilities for broilers is low.

Introduction

Over the past decade the use of rapeseed meal (RSM) in broiler rations has become commonplace. Despite this there is scant recent data on the variability that exists in the nutritional value of RSMs produced from modern cultivars

Table 1. Effect of rapeseed cultivar on nitrogen corrected apparent metabolisable energy[1] (MJ/kg DM) and coefficients of nitrogen digestibility[2] in broilers

Cultivar	Bar	Cha	Com	Cab	Cam	Haw	Inc	Pic	Pop	Tro	SEM	P value
AMEn	9.79	9.78	9.8	9.89	9.88	9.86	9.79	9.69	9.91	9.72	0.141	0.981
N-DBTY	0.86	0.84	0.83	0.85	0.84	0.83	0.86	0.84	0.84	0.87	0.017	0.824

Bar-Barbados; Cha-Charger; Com-Compass; Cab-Cabernet: Cam- Camelot; Haw-Hawai; Pic-Picto; Tro-Troy
AMEn - nitrogen corrected apparent metabolisable energy
N-DBTY- coefficients of nitrogen digestibility

leaving historical data as a key source of information. Much of this has been compiled over time and generated from multiple locations outside of the UK. However, after years of successful rapeseed breeding programs, the accuracy of these values remains unclear (Houdijk et al., 2017). Achieving precision during feed formulation requires knowledge on the variations that exist within feedstuffs (Rezvani, Kluth, Bulang, & Rodehutscord, 2012). With this in mind the current experiment was designed to investigate the influence of rapeseed cultivar on the range of variation in the feed value of RSMs for broilers.

Materials and methods

Ten modern cultivars of double zero, UK-grown rapeseed was processed under strict control into RSM using a cold-pressed, solvent-extraction method. To avoid potentially overriding the effect of cultivar, air-desolventisation followed by micronizing was used as opposed to industrial-desolventisation /toasting which entails prolonged exposure of RSM to extreme temperatures which can cause substantial heat damage. Ten diets containing 250g/kg RSM and 750g/kg basal feed containing titanium dioxide were blended in mash form. To enable subsequent calculations a basal control diet was also fed giving a total of 11 diets. Experimental procedures were approved by Harper Adams Ethics committee. Each diet was randomly allocated to 6 raised-floor pens (5 birds per pen/13 d age). Total excreta were collected from 17 to 21 d age and feed intake was recorded. On the final day birds were humanely slaughtered and ileal digesta were collected for analysis. A randomized complete block ANOVA was performed to investigate the effect of cultivar on the nitrogen corrected apparent metabolisable energy content (AMEn) (MJ/kg DM) and prececal nitrogen digestibility (N-DBTY) in broilers fed single-cultivar RSMs ($P < 0.05$).

Results

There was no significant effect of rapeseed cultivar on either AMEn contents (P=0.981) of RSMs or coefficients of N-DBTY (P=0.824). Amongst the ten single-cultivar RSMs AMEn content (MJ/kg DM) ranged from 9.69 (MJ/kg DM) to 9.91(MJ/kg DM) whilst the coefficients of N-DBTY ranged from 0.83 to 0.87 (Table 1).

Conclusion

When strict processing control was applied during the production of single-cultivar RSMs, cultivar had no effect on the nutritional value of RSM in broilers.

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Lacto-fermented rapeseed meal additive: a nutritional intervention to reduce *Campylobacter jejuni* colonisation and improve performance in broilers

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Application

Lacto-fermented rapeseed meal (LFRM) additive can help to reduce *Campylobacter* load in poultry and consequently can help to combat campylobacteriosis worldwide.

Introduction

New nutritional interventions to decrease *Campylobacter* spp. load in the poultry gut are urgently required. LFRM additive (EP100i: Fermentationexperts A/S) is a novel product which is produced from rapeseed fermented with lactic acid bacteria and the final product contains *Enterococcus faecium* (a probioticum), multi-enzymes (xylanase, β-glucanase and phytase), *Lactobacillus plantarum* and two *Pediococcus* strains i.e. *P. pentosaceus* and *P. acidilactici*. This study investigated the efficacy of LFRM on growth performance and *C. jejuni* carriage in broiler exposed to experimentally seeded litter with *Campylobacter spp*.

Material and methods

A total of 144 day-old male broilers (Ross 308; 42 ±1g) were allocated to two dietary treatments in a randomised block design, each having six replicate pens of 12 birds per pen. Wheat soybean meal starter (d0 to d13), grower (d13 to d20) and finisher (d20 to d35) control diets (T1) were supplemented with 3% and 4% LFRM in grower and finisher phase, respectively. Birds had *ad libitum* access to feed and water. Cloacal swabs from one bird per pen were taken at d0 to confirm the absence of *C. jejuni* at trial start. At d20, all birds were

Table 1. Effect of LFRM on growth performance and caecal *C. jejuni* counts of broilers challenged with *Campylobacter* spp

	Average body weight (ABW) (kg)					ADFI (kg/d)		*C. jejuni* (log_{10} cfu/ml)
Treatment	d0	d13	d20	d35	ADWG (kg/d)	d0-35	FCR (ADFI/ ADWG)	d35
T1: Control	0.042	0.308	0.680	2.113	0.059	0.091	1.537	7.675
T2: LFRM†	0.042	0.319	0.738	2.220	0.061	0.092	1.494	7.185
SED	0.000	0.016	0.020	0.052	0.001	0.004	0.046	0.1346
P-Value*	0.940	0.519	0.014	0.066	0.154	0.741	0.537	<0.001

ADWG = Average daily weight gain per bird; ADFI = Average daily feed intake per bird; † * P- values = Significantly different (P≤0.05) or trending (0.05<P≤0.10). †LFRM at 3% and 4% in diets during d13 to d20, and d20 to d35, respectively.

challenged with *C. jejuni* through the seeded litter tray procedure described previously (Khattak et al., 2018). At d35, caeca pooled from four sets of two birds per pen were used for *C. jejuni* enumeration, resulting in a total of 24 caecal samples per treatment. *C. jejuni* colony-forming unit (cfu) concentration (cfu/ml) was calculated from serial dilutions plated on Charcoal Cefoperozone Deoxycholate agar (CCDA) and colonies enumerated. Growth performance and log_{10} transformed *C. jejuni* concentrations were evaluated by analysis of variance (Genstat v19) with a P<0.05 level of significance. This study was approved by SRUC's Animal Welfare and Ethical Review Body and carried out under Home Office authorisation.

Results

Table 1 shows that LFRM significantly affected body weight and *C. jejuni* colonisation. All cloacal swabs taken at the start of the study were negative for *C. jejuni*. Supplementation of grower and finisher diets with LFRM resulted in 8.5% and 5.0% heavier birds (P < 0.05) at d20 and d35, respectively. The overall weight gain, feed intake and FCR did not show significant improvement. The seeded litter challenge reliably colonised broilers with *Campylobacter* spp. as indicated by the presence of *C. jejuni* in all pens at d35. Supplementation of diets with LFRM significantly reduced log caecal *C. jejuni* counts at d35. Resulting back-transformed means with 95% confidence interval showed a 66% reduction, i.e. from 50 (41-61) to 17 (13-23) million cfu for T1 and T2, respectively. Mortality was low (0.69%) and did not differ between treatments.

Conclusion

This is the first study to demonstrate that LFRM supplementation result in heavier birds and reduced gut colonisation of *C. jejuni* in broilers exposed to *C. jejuni*. These positive effects are likely to be attributed to fermentation metabolites within LFRM, which is known to improve nutrient digestibility, increase the lactic acid level, and modulate the immune response by preventing colonisation of enteropathogens through competitive exclusion. The study supports the view that dietary LFRM has the potential to produce poultry with a lower public health risk of campylobacteriosis.

Acknowledgments

The authors acknowledge the funding provided by Fermentationexperts A/S, Denmark. SRUC is financially supported by the Scottish Government. The authors also acknowledge the husbandry and technical support by Abid Ahmed and Fraser Whyte.

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A reduced duration of the feeding period does not affect the estimates of dietary digestible energy and nutrient ileal digestibility in broiler chickens

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Application

Shorter feeding periods may be used for estimates of dietary digestible energy and nutrient digestibility coefficients.

Introduction

Measuring digestible energy (DE) is becoming a valuable alternative to evaluating energy availability of poultry feeds and feed ingredients. There are reliable techniques to determine DE using indigestible markers, avoiding the complications of dealing with urine mixed in the droppings. Most DE protocols use at least an eight-day feeding period before the digesta are sampled (McDonald et al., 2011). There are number of situations where the amount of experimental material is limited and so a shorter feeding period is needed. However, information on the effect of the duration of the feeding period on DE is lacking. The reported study evaluated the effect of four feeding periods, 6, 8, 10 and 12 days on DE, dry matter (DMD), nitrogen (ND) and fat (FD) digestibility coefficients of three *ad libitum* fed mash diets that were designed to have different energy densities. The impact of energy density on these digestibility coefficients was also studied.

Material and methods

Harper Adams University Research Ethics Committee approved the experiment. A basal diet containing 499.5 g/kg of wheat, 235.0 g/kg soybean and 100.0 g/kg of maize, as major ingredients, was mixed. The basal diet was then split into 3 batches and one of them was used as a control (Diet 1), AMEn was 11.68 MJ/kg and CP was 220.1 g/kg, a second lot had 100 g/kg of vegetable oil added (Diet 2), AMEn was 10.512 MJ/kg, CP was 198.09 g/kg. The third lot had 100 g/

Table 1. The effects of the pre-feeding period on digestible energy (DE), dry matter (DMD), nitrogen (ND) and fat (FD) digestibility coefficients in broiler chickens

Treatment factor		DE (MJ/kg DM)	DMD	ND	FD
Diet	1	12.29^a	0.641^a	0.759^a	0.912
	2	13.87^b	0.608^b	0.734^b	0.926
	3	10.71^c	0.555^c	0.719^b	0.921
	SEM	0.177	0.0089	0.0067	0.0105
Feeding period duration (d)	6	12.35	0.599	0.736	0.934
	8	12.31	0.606	0.737	0.899
	10	12.12	0.588	0.732	0.928
	12	12.38	0.612	0.745	0.918
	SEM	0.205	0.0103	0.0077	0.0121
P-Value					
Diet		<0.001	<0.001	<0.001	0.617
Adaptation		0.818	0.403	0.663	0.203
Diet X Adaptation		0.797	0.613	0.713	0.205

abc: means in the same column having different superscripts differ significantly (P <0.05).

kg of soy hulls added (Diet 3), AMEn was 10.812 MJ/kg and CP was 207.09 g/kg. Acid insoluble ash was used as the indigestible marker. At 19 days old, 144 birds were allocated to 48 pens (3 birds in each) and experimental diets were fed. Each pen had a solid floor covered with bedding material. At 23 days old, 144 of the remaining birds were allocated to another 48 pens and experimental diets were fed. A total of 96 pens were involved in this study, and each diet was fed to 32 pens following randomisation. Digesta collection was done at 29 and 31 d age, respectively, resulting in pre-feeding period of 6, 8, 10 and 12 days. Two birds per pen were electrically stunned and digesta from the ileum were collected, pooled per pen, freeze dried, milled, and subjected to further analysis. Data were statistically analysed by ANOVA using a 3 × 4 factorial arrangement of treatments.

Results

The determined DE of diet 2 was higher compared to the rest (Table 1). The control diet had higher DMD and ND than the rest. Diets did not affect dietary FD. Studied variables were not affected by the duration of the feeding period and there were no interactions.

Conclusion

Feeding duration does not influence the estimate of dietary digestible energy and nutrient digestibility coefficients.

Acknowledgements

I would like to thank the World Poultry Science Association, UK Branch, for kindly funding this project.

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Feeding non-starch-polysaccharidase enzymatic complex to laying hens

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Application

Feeding a novel non-starch-polysaccharidase enzymatic complex to laying hens reduces the FCR, thus results in less expensive egg production bringing more profit to the producer.

Introduction

Non-starch polysaccharides (NSP) in grains are responsible for several anti-nutritive effects in poultry. To alleviate the negative impact of NSP the inclusion of NSP degrading enzymes (NSPases) is a common practice in poultry production. For NSPases, there are three principal modes of action, including digesta viscosity reduction, grain cell wall destruction and generation of prebiotics (Yang, Iji, & Choct, 2009). Exogenous NSPases are being constantly improved and upgraded so there is a continued need to evaluate their efficacy and mode of action. Therefore, the aim of the experiment was to study the effect of a next generation of enzymatic complex (ECOM) containing different NSPases on egg production and quality variables.

Materials and methods

The experiment was conducted at the National Institute of Poultry Husbandry and approved by the Harper Adams University Research Ethics Committee. A new ECOM (CE001, Huvepharma NV) was used in the present experiment. The ECOM contains 3 enzyme activities (3000 EPU/kg endo-1,4-beta-xylanase (EC 3.2.1.8); 200 CU/kg endo-1,4-beta-glucanase (EC 3.2.1.4), aka. cellulase and 200 XGU/kg xyloglucanase (EC 3.2.1.155)) derived from a single fermentation process induced for over-production of the referred activities. An enzyme free pre-lay diet was fed to all birds from 17 to 22 weeks of age. Two experimental wheat-based diets were prepared for the study. A wheat-based control mash diet (520 g/kg wheat, 200 g/kg soyabean meal, 50 g/kg full fat soya) was formulated to contain 181 g/kg crude protein, and 11.59 MJ/kg ME. The second diet was made by adding CE001 at 500g/tonne to the control diet. One hundred and sixty 22 week old Hy-Line hybrid pullets were individually weighed and randomly allocated to 40 enriched coups, giving four birds per coup. Each diet was fed to 20 coups in an environmentally controlled house following randomisation. The experimental diets were fed *ad libitum* from 22 to 34 weeks of age. Variables measured included feed intake per egg (FI), average egg weight (EW), feed conversion ratio for egg production (FCR), % egg production, shell thickness (ST), shell weight (SW) and Haugh units (HU) when fed to Hy-Line laying hens. Data was statistically analysed by ANOVA using Genstat 18th edition.

Table 1. The effect of dietary non-starch-polysaccharidase enzymatic complex (ECOM) on feed intake per egg (FI), average egg weight (EW), feed conversion ratio for egg production (FCR), % egg production (EP), shell thickness (ST), shell weight (SW) and Haugh units (HU) when fed to Hy-Line layers from 22 to 34 weeks of age

Diets	FI (g)	EW (g)	FCR (g:g)	EP (%)	ST (mm)	SW (g)	HU
Control	131	58.9	2.221	91.4	0.354	5.59	92.0
Control + ECOM	125	59.3	2.113	94.6	0.366	5.72	90.6
SEM	1.4	0.43	0.0270	0.84	0.0082	0.148	1.15
P	< 0.05	NS	< 0.05	<0.05	NS	NS	NS

Results

Overall, birds fed CE001 had 4.3% lower feed intake for egg produced ($P < 0.05$) and 4.9% lower (better) FCR compared to the birds fed the control diet ($P < 0.05$) (Table 1). Birds fed ECOM for the period produced 3.4% more eggs ($P < 0.05$) compared to the birds fed the control diet. There were no other statistical differences ($P > 0.05$) between the studied variables.

Conclusion

Feeding non-starch-polysaccharidase enzymatic complex to laying hens from 22 to 34 weeks of age improved overall egg production and FCR under the conditions of this trial. The size of the improvements in rate of egg production and FCR are commercially important.

Acknowledgements

The authors acknowledge the help of Richard James and Ros Crocker of The National Institute of Poultry Husbandry (Edgmond, UK) for their help in taking care of birds used for this study.

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Effect of soyameal replacement by Black Soldier Fly (*Hermetia illucens*) meal in broiler diets on productive performance

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Application

The inclusion of 15% defatted Black Soldier Fly meal in poultry diets as a replacement of soya may reduce the pressure on deforestation in South America and increase the feed security in the UK.

Introduction

The use of soyabean meal as the major protein source in animal feeds raises concerns due to the increased pressure on the food-feed-fuel competition and deforestation. Black Soldier Fly (BSF) (*Hermetia illucens*) represents the most promising insect species produced in a large-scale that can be included in broiler diets as replacement of soyabean meal; however, little information is available on their nutrient value. The current study aimed to demonstrate that BSF meal can partially replace soymeal as a protein source in broiler diets during an entire productive cycle of 35 days without detriment on bird performance.

Material and methods

The study was performed in the poultry facility of the Veterinary Science Division of AFBI NI (Belfast, UK). The trial was conducted under the Animals (Scientific Procedures) Act 1986 and procedures were approved by the Animal Welfare and Ethical Review Body. A total of three experimental diets were formulated to meet the nutrient requirements of broilers according to ROSS 308 Nutrition Specifications (2019) and be as isoenergetic and isoproteinaceous as possible. The diets were prepared to include, increasing levels of BSF defatted larva meal (0%, 7.5% and 15%) in substitution of 0, 21%, 42%, 0, 28%, 56% and 0, 33%, 66% for the starter, grower and finisher stage, respectively. Mash feed and water were provided ad libitum. Starter, grower and finisher diets were offered from 1-10d (Starter;S), 10-21d (Grower;G) and 21-35d (Finisher; F). A total of 508 'as hatched' broiler chickens (Ross 308) were reared for 35 days and assigned to the 3 dietary treatments (8 pens/treatment and 21 birds/pen). Chickens were weighed at the beginning of the trial and after every diet change (LW). The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for each stage. Data were analysed by

Table 1. Effects of dietary BSF larva meal inclusion on the growth performance parameters of broilers at different productive stages

Treatment	Level	Diet C	BSFL	BSFH	SEM	P-value
Live Weight (g)	0d	41.7	41.6	41.9	0.11	0.388
	10d	253.4[c]	269.3 [b]	298.3 [a]	4.57	<0.001
	21d	870.6[b]	893.5[b]	976.5[a]	8.51	<0.001
	35d	2298[b]	2332[b]	2476[a]	20.46	0.013
Average daily gain (ADG) (g)	Starter	21.2[c]	22.8[b]	25.6[a]	0.45	<0.001
	Grower	56.1[b]	56.7 [b]	61.7[a]	0.64	<0.001
	Finisher	102[b]	103[ab]	107[a]	0.82	0.014
	1-35d	64.5[b]	65.4[b]	69.6[a]	0.58	<0.001
DFI (g)	Starter	30.2	32.7	32.5	0.67	0.237
	Grower	74.8[b]	80.5[a]	81.0[a]	0.91	0.003
	Finisher	160.3	162.7	162.2	3.41	0.871
	1-35d	92.8	96.3	96.3	0.92	0.221
FCR (g/g)	Starter	1.43	1.44	1.27	0.035	0.069
	Grower	1.33[b]	1.42[a]	1.31[b]	0.016	0.013
	Finisher	1.57	1.58	1.52	0.021	0.358
	1-35d	1.44[ab]	1.47[a]	1.38[b]	0.015	0.053

SEM: standard error of the mean; Contrasts: 1 – C vs BSF7.5; 2 – C vs BSF15; 3 – BSF7.5 vs BSF15; 4 – C vs BSF diets. d – days. [a,b,c] Superscripts within the same line indicate differences between dietary treatments.

a one-way ANOVA with the diet as the main treatment and the pen (n=8) as experimental unit employing SPSS 25th edition. Significant means were further compared using Tukey HSD test.

Results

The inclusion of 15% defatted BSF meal improved (P<0.05) LW and ADG in every productive stage, whereas the LW and ADG were only improved for birds offered the 7.5% inclusion in the starter stage (Table 1). However, the higher ADG in birds offered diets containing BSF did not translate to improved FCR as ADFI was numerically higher in birds offered both BSF diets (significantly higher during the grower stage P<0.05). Consequently, the birds offered the diet containing 7.5% were less efficient (P<0.05) in terms of FCR during the grower stage than birds offered the diet containing 0% and 15%. Considering the entire cycle, BSF diets improved ADG but not FCR, whereas birds fed the BSF7.5 diet were less efficient in terms of FCR than (P<0.05) than birds offered diets containing BSF15.

Conclusion

The inclusion of 15% defatted larva meal as partial replacement of soymeal revealed no detrimental effect on broilers' productive performance during the 35-day cycle. Results tended to suggest an improvement in performance indicating that BSF is of a higher nutritive value than assigned in formulation, thus confirming the need for research to accurately determine its nutritive value. BSF larvae meal can be considered as a potential sustainable source of protein in broiler nutrition.

Acknowledgments

Funded by Agri-Food Quest Competence Centre programme (INI Agri-Food QUEST-11-12-17-004–AFQCC) funded by Invest NI and Industry (Finnebrogue, Moypark, Cranswick).

Standardised ileal digestibility of amino acids from spent barley-bean for broilers

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Application

Dried spent barley-beans' standardised ileal amino acid digestibility indicates its possible use in poultry diets.

Introduction

Dehulled faba beans may replace 30% of malted barley as a novel brewing adjunct (Black, Tziboula-Clarke, White, Iannetta, & Walker, 2020). The spent barley-bean arising has more crude protein (CP) and amino acids (AA) than regular spent barley. We assessed standardised ileal digestibility (SID) of dried spent barley-bean and hypothesised that this would be intermediate to that of barley and dehulled faba beans.

Material and methods

A single group of 180 Ross 308 male broiler chickens were floor-reared and fed standard diets from day-old. On day 21, broilers were allocated to five feeding treatments in a randomised block design, consisting of semi-purified diets with barley, dehulled faba beans, oven-dried spent barley-bean (48 h; 60°C), soya bean meal (SBM) and faba beans as the only source of N, with 10.7, 27.4, 23.6, 46.5 and 23.1% CP, respectively. Treatments had six replicates, with six birds per replicate (HO approved wired-bottom floor cages). The experimental diets were fed for 5 days. The chickens were euthanized on day 26, and digesta from the terminal ileum were collected for dry matter (DM), titanium dioxide, CP and AA analysis. The SID of AA was calculated using basal endogenous flow previously derived (Olukosi, Walker, & Houdijk, 2019). Data was analysed through ANOVA, with Tukey-adjusted multiple comparisons to locate effects (P<0.05). SRUC Animal Welfare and Ethical Review Body approved this experiment.

Results

Table 1 shows that although spent barley-bean had a relatively low DM digestibility, which is likely a reflection of its elevated levels of fibre, SID of most eAA was intermediate to that of barley and dehulled faba beans. Whilst the SID of spent barley-bean eAA was lower than that of SBM, these differences were significant for selected

Table 1. Apparent ileal digestibility of dry matter (DM) and standardised ileal digestibility (SID) of essential amino acids (eAA) and across non-essential amino acids (non-eAA) in broilers (%)

	Test feedstuff					
Parameter	Barley	Dehulled faba beans	Spent barley-bean	Soya bean meal	Faba beans	SEM
DM	53.8[bc]	58.7[c]	48.2[b]	81.9[d]	36.7[a]	1.71
Arg	67.1[a]	94.7[c]	84.1[b]	90.9[bc]	74.9[a]	1.88
His	69.2[a]	93.8[c]	78.9[b]	88.8[c]	69.1[a]	2.00
Iso	66.7[ab]	85.5[c]	76.8[bc]	86.9[c]	63.6[a]	2.56
Leu	72.0[ab]	88.3[c]	80.3[bc]	87.1[c]	67.2[a]	2.30
Lys	61.2[a]	91.2[c]	77.4[bc]	87.6[bc]	73.7[ab]	3.44
Met	74.9[b]	75.9[b]	74.1[ab]	88.0[b]	52.5[a]	5.11
Phe	72.4[ab]	88.9[c]	79.5[bc]	87.5[c]	67.0[a]	2.40
Thr	63.2[a]	84.5[bc]	71.7[ab]	85.6[c]	63.5[a]	3.11
Trp	65.6[b]	82.4[cd]	70.8[bc]	87.6[d]	51.3[a]	3.14
Val	70.4[ab]	85.2[c]	76.2[bc]	86.7[c]	64.7[a]	2.61
non-eAA	71.3[ab]	85.4[c]	74.1[b]	85.8[c]	63.5[a]	2.35

[abcd]Means without common superscripts differ (P<0.05)

AA only. The data confirms that faba bean dehulling significantly improves AA digestibility to similar levels as observed for SBM.

Conclusion

These results support the view that SID of AA in the spent barley-bean tested was as hypothesised intermediate to barley and dehulled faba beans, though were generally smaller than those of SBM. Data obtained can inform diet formulation to assess performance response against increasing dietary levels of spent barley-bean to determine upper inclusion limits.

Acknowledgments

Horizon-2020 project TRUE (grant 727973) funded this work. SRUC and JHI are supported by Scottish Government, RESAS. We thank Abid Ahmed and Fraser White for technical input, and Barney's Beer for suppling the spent barley-bean co-product.

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The use of faba beans as an alternative protein source to soya bean meal in grower and finisher broiler rations is independent of starter feed form and in-feed coccidiostat

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Application

Faba beans can safely be included at 15% to reduce reliance on soya in nutritionally balanced broiler grower and finisher diets.

Introduction

A quadratic response to faba bean inclusion at the expense of soya bean meal in grower and finisher rations on broiler performance was previously shown, with minimum and optimal performance at 0% and 15% faba beans, respectively (Houdijk & Walker, 2021). This unexpectedly low performance in the absence of faba beans was on meal rather than crumbed starters, without the use of coccidiostats. Here, we assessed whether growth responses to faba beans or coccidiostat in grower and finisher rations were sensitive to starter feed form and coccidiostat inclusion in broilers.

Material and methods

Ross308 day-old male broilers were reared under one of nine feeding treatments (n=8 pens; 14 birds/pen; randomized block design). Three starter treatments, i.e. meal, crumbs and medicated crumbs (+Med) were factorially combined with three pelleted grower/finisher treatments, i.e. control, control with 15% faba beans and medicated controls. Rations were iso-nitrogenous and wheat- soya bean meal based, with faba beans being exchanged against soya bean meal based on digestible lysine levels, formulated to meet Ross308 requirements, using pure amino acids as required and variations in wheat and oil to maintain similar AME. Averaged bird feed intake, weight gain and their ratio as mortality corrected feed conversion ratio (FCR) were assessed over starter phase (d0 to d9), grower phase (d9 to d24) and finisher phase (d24 to d37) and overall (d0 to d37). Data were analysed via 3 × 3 ANOVA for starter treatments, grower-finisher treatments and their interaction. Since 3 × 3 interactions were not significant (P>0.15), only main effects are reported. SRUC Animal Welfare and Ethical Review Body approved this experiment.

Results

Faba bean and coccidiostat treatments resulted in similar weight gain, FCR (Table 1) and feed intake (data not shown) to their controls throughout. However, Table 1 also shows that starter meals underperformed compared to crumbs. This carried over into the grower phase, though with improved FCR (1.158 vs 1.180; s.e.d. 0.009; P=0.018), and disappeared in the finisher phase. The starter treatment tended to impact mortality during the starter phase, averaging 2.08, 1.19 and 0.30% for Meal, Crumbs and Crumbs +Med, respectively (s.e.d. 0.79%; P=0.086).

Table 1. Main effects of starter and grower-finisher treatments on averaged broiler body weight gain (WG, g/bird) and mortality corrected feed conversion ratio (FCR, g/g). +Med refers to the inclusion of coccidiostats

		Starter					Grower-finisher				
		Meal	Crumbs	Crumbs +Med	sed	P-value	Control	15% Faba beans	Control +Med	sed	P-value
WG	Starter	157[a]	223[b]	221[b]	2	0.001	200	201	200	2	0.803
	Grower	1130[a]	1183[b]	1191[b]	10	0.001	1174	1162	1169	10	0.485
	Finisher	1646	1649	1637	24	0.867	1644	1644	1644	24	1.000
	All	2934[a]	3056[b]	3049[b]	28	0.001	3017	3007	3013	28	0.941
FCR	Starter	1.253	1.125	1.133	0.037	0.002	1.150	1.171	1.190	0.037	0.572
	Grower	1.158	1.180	1.168	0.009	0.058	1.171	1.168	1.166	0.009	0.836
	Finisher	1.437	1.462	1.463	0.021	0.404	1.451	1.440	1.470	0.021	0.367
	All	1.294	1.299	1.293	0.009	0.788	1.293	1.290	1.303	0.009	0.345

[ab]Means without common superscript differ (P<0.05)

Conclusion

These data support the view that independent of starter feed form and coccidiostat inclusion, faba beans can safely be included at 15% in nutritionally balanced grower and finisher diets. Performance benefits of crumbs over course meal in starter phase remain presented throughout, suggesting that compensatory growth was limited under the current conditions.

Acknowledgments

SRUC receives support from Scottish Government (RESAS). We thank Abid Ahmed and Fraser Whyte for technical input.

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Effect of amino acid supplementation in reduced crude protein diets on growth performance and gut health indices of broiler chickens exposed to sub-clinical enteric health challenges

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Application

Ideal protein formulation remains the basis as an effective feeding strategy even during sub-clinical enteric health challenges.

Introduction

The ideal protein (IP) concept proposes setting the ratio of digestible essential amino acid (AA) to lysine in the diet as in their requirements, thus allowing for reduced crude protein (CP) rations. However, since sub-clinical challenges may reduce AA digestibility and/or increase AA demand for immune responses (Adedokun, Helmbrecht, & Applegate, 2016), the IP concept might be affected. We hypothesised that supplementation of threonine (Thr), arginine (Arg), and glutamine (Gln) to IP based reduced CP diets could improve broiler resilience to a combined reused litter and sub-clinical coccidiosis challenge.

Material and methods

A total of 1008 Ross308 day-old male broilers were placed in 72 pens (14 birds/pen). Two challenge treatments were factorially combined with four diets. Birds were either unchallenged controls (placed on fresh litter and gavage with water) or challenged (placed on reused litter and gavage with 2500 *Eimeria maxima* oocysts at day 14). Diets were high-CP (D1), reduced CP (D2), D2 with extra glycine (D3), and D3 with Thr and Arg at 25% above requirements, and 1% Gln at the expense of glycine (D4). Resulting D1, D2, D3 and D4 had 22.25, 20.25, 21.74 and 21.90% CP, respectively. Birds were fed starter mash (d0 to d10) and grower pellets (d10 to d28), with D1 and D2 formulated to IP concept. Body weight gain (BWG) and feed efficiency (FE) were assessed over starter, pre- and post *E. maxima* challenge grower phases. At day 28, two birds per pen were blood sampled for plasma uric acid and albumin, and litter samples were collected for pH and dry matter (DM). Data were analysed via ANOVA for challenge, diet and their interactive effects. Tukey-adjusted multiple comparisons located treatment effects, considered significant at $P < 0.05$. SRUC's Animal Welfare and Ethical Review Committee approved this Home Office authorised study.

Results

Diet did not affect FE and albumin but D1 birds had greater BWG and lower litter DM than D2, D3 and D4 birds (Table 1). D1 and D4 birds had similar and higher uric acid levels and litter pH than D2 and D3 birds, with D2 and D3 resulting in the lowest uric acid and litter pH, respectively. Challenge during starter phase improved BWG (+4.3%) and FE (+4.6%, $P < 0.05$). However, independent of diet (no interactions observed), subsequent *E. maxima* exposure

Table 1. Effect of high CP (D1), reduced CP (D2), D2 with Gly (D3) or Thr, Arg and Glu (D4) on body weight gain (BWG), feed efficiency (FE), plasma metabolites and litter traits of broilers unchallenged or exposed to reused litter and *E. maxima* (C)

	Control				Challenge					P values		
	D1	D2	D3	D4	D1	D2	D3	D4	s.e.d.	D	C	D × C
BWG[1] (g)	385	346	329	329	377	351	349	350	14	0.001	0.186	0.492
FE[1] (g/g)	0.91	0.94	0.87	0.87	0.89	0.91	0.94	0.90	0.04	0.629	0.514	0.388
BWG[2] (g)	1202	1033	1040	1037	928	853	852	835	45	0.001	0.001	0.471
FE[2] (g/g)	0.75	0.72	0.74	0.74	0.68	0.69	0.70	0.68	0.02	0.826	0.001	0.437
UA[3] (µmol/l)	355	304	366	395	388	305	347	350	38	0.049	0.689	0.534
Albumin (g/l)	14.54	14.14	12.91	13.06	12.44	10.87	11.72	12.59	1.13	0.478	0.003	0.335
Litter DM (%)	81.07	85.12	82.91	82.29	82.28	84.81	87.3	86.64	1.99	0.018	0.097	0.154
Litter pH	6.91	6.49	6.29	6.57	7.49	7.38	7.12	7.46	0.13	0.001	0.001	0.296

[1]0–14 days (challenge: reused litter at day 0); [2]14–28 days (challenge: additional *E. maxima* at day 14); [3]UA, uric acid

reduced BWG (−19.6%) and FE (−7%). Moreover, again independent of diet, challenge reduced plasma albumin level, increased litter pH and tended to increase litter DM.

Conclusion

These results suggest that AA supplementation to IP based reduced CP diets does not improve resilience to enteric challenge, potentially due to AA imbalances. The reduced CP ration may have underperformed due to limiting non-essential AA.

Acknowledgments

We thank Newton-Mosharafa for a scholarship to MAH, and Evonik Nutrition & Care GmbH for amino acids and feed analysis. SRUC and Roslin Institute receive support from RESAS (Scottish Government) and BBSRC (BBS/E/D30002276), respectively.

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Effect of main cereal particle size in mash feed on performance and gut organs in broilers

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Application

Understanding the increased particle size of a main cereal in mash feed affects productive performance and gut organs in broilers.

Introduction

It is important to get uniformity of particle size while manufacturing mash feed to retain the diet structure homogenously (Xu, Stark, Ferket, Williams, & Brake, 2015). Poultry birds are known to prefer bigger particle size with increasing age (Parsons, Buchanan, Blemings, Wilson, & Moritz, 2006). Previously, smaller particle size creating increased surface area for exposure to digestive enzyme activity, was considered to enhance nutrient digestibility (Preston, McGragken, & McAllister, 2000) but recently, a positive correlation between increased feed particle size and broiler growth has been reported (Aguzey, Gao, Haohao, & Guilan, 2018), which may be due to improved function or development of gut organs (Hetland, Svihus, & Olaisen, 2002). The current study was conducted to test the hypothesis, if increased particle size of the single main cereal in mash feed improves bird performance and development of gut organs in broiler chickens.

Material and methods

The study was ethically approved by the Ethical Committee, Nottingham Trent University (NTU) where a total of 240 day-old male broiler chicks (Ross 308) were individually weighed before allocating in groups of 5 chicks to 48 pens of 0.64 m^2 each at the Poultry Research Unit, NTU. Wheat-soybean based diets were formulated according to commercial standard (Ross 308 guidelines) with wheat ground to pass through screens of 2.0 mm and 5.0 mm. Dietary treatments of wheat with grinding sieve size of 2.0 mm and 5.0 mm, were given to 24 pens each, without measuring sieve profile of the final feed. Feed and water were provided ad libitum. On day 35, all birds were euthanized by cervical dislocation and data on individual bird weight, pen performance and gut organs weight was collected and analysed with independent sample t-test using IBM SPSS Statistics v25 software. Difference between treatment means were considered significant at $p < 0.05$, while p-value between 0.05 and 0.10 was considered as a trend (Ege et al., 2019).

Results

Feed intake ($p = 0.016$) and proventriculus absolute weight ($p = 0.042$) in birds with wheat particle size of 5.0 mm was significantly higher than that of the 2.0 mm group while other performance parameters were not affected significantly ($P > 0.05$). An increasing trend in intestinal absolute weight ($p = 0.083$) was also observed in the same treatment group of 5.0 mm (Table 1).

Conclusion

Increased grinding or particle size of the main cereal i.e. wheat, in mash feed significantly increased ($p < 0.05$) absolute weight of proventriculus and feed intake by 9.63% and 5.84%, respectively.

Table 1. Effect of main cereal particle size in mash feed on performance and gut organs in broilers

Parameters	2.0 mm	5.0 mm	SEM	p-value
Pen based performance (n = 16)				
Bird weight (g)	1833.926	1882.783	34.985	0.331
Body weight gain (g)	1793.696	1841.327	34.820	0.341
Feed intake (g)	2894.399	3063.439	46.680	0.016
FCR	1.615	1.673	0.028	0.158
Individual Bird weight (g) (n = 24)	1899.708	1956.271	58.562	0.498
Gut Organs (n = 24)				
Proventriculus weight (g)	7.259	7.958	0.237	0.042
Gizzard weight (g)	33.291	33.333	0.821	0.971
Small intestine weight (g)	44.620	48.500	1.549	0.083
Relative proventriculus weight (g/kg BW)	3.846	4.126	0.125	0.119
Relative gizzard weight (g/kg BW)	17.895	17.354	0.657	0.564
Relative intestine weight (g/kg BW)	23.573	25.165	0.807	0.170

BW = Bird Weight

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Impact of feeding graded levels of triticale on the productive performance of laying hens

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Application

Triticale is a suitable feed for laying hens and can be incorporated in diets at the expense of at least 20% dietary maize.

Introduction

Maize is recognised as one of the most efficient energy sources for livestock in general and particularly in the poultry industry. However, due to its high demand for both human and livestock, its price is continuously increasing (Bakhtiyary Moez, Mirzaie Goudarzi, Saki, & Ahmadi, 2020). This has highlighted to need to find an alternative ingredient that has a lower production cost but also maintains good productive performance. Triticale, a human-made crop produced from the crossing between wheat and rye distinguishes itself with having a low price and is a resistant to dry weather comparatively to maize or wheat. However, the majority of the research undertaken using triticale has been used in broilers trials and the information on the impact of triticale on the productive performance of laying hens is lacking. Thus, the aim of this study was to investigate the impact of graded levels of triticale on feed intake (FI), egg weight (EW), % egg production and feed conversion efficiency for egg production (FCR) when fed to Hy-Line laying hens from 31 to 36 weeks of age.

Table 1. The effect of dietary graded inclusion rates of triticale on daily feed intake (FI), average egg weight (EW), daily egg mass (EM), feed conversion ratio for egg production (FCR) and % egg production (EP) when fed to Hy-Line layers from 31 to 36 weeks of age

Triticale (g)	FI (g)	EW (g)	EM (g)	FCR (g:g)	EP (%)
0	103.0	55.5	52.4	1.967	94.44
50	102.3	55.6	52.3	1.956	94.11
100	104.3	55.5	52.9	1.974	95.24
150	103.9	56.2	53.2	1.954	94.74
200	103.2	55.6	52.1	1.982	93.78
SEM	0.82	0.39	0.41	0.0152	0.838
P	0.468	0.698	0.313	0.667	0.771
Linear	0.419	0.546	0.795	0.547	0.797
Quadratic	0.417	0.579	0.128	0.501	0.374
Cubic	0.270	0.422	0.133	0.712	0.480
DEV	0.319	0.374	0.876	0.253	0.555

Material and methods

The experiment was conducted at the College of Agriculture and Life Science experimental station, Jeonbuk National University, Korea. The Jeonbuk National University Research Ethics Committee approved the experiment. Five experimental isocaloric (2800 kcal/kg) and isonitrogenic (170 g/kg crude protein) diets containing 0, 50, 100, 150 or 200 g triticale per kilogram diet in replacement of maize were prepared. Three hundred and sixty 31-week-old Hy-Line hybrid hens were individually weighed and randomly allocated to 20 enriched colony cages, giving eighteen birds per cage. Diets were fed *ad libitum* from 31 to 36 weeks of age in an environmentally controlled house. The data were analysed by ANOVA and polynomial contrasts were performed to test for linear (L) and quadratic (Q) relationships between triticale inclusion rate and the variable in question.

Results

The replacement of maize with graded triticale inclusion rates did not influence ($P > 0.05$, Table 1) the layers' productive performance over the six-week experimental period.

Conclusion

In agreement with Hermes and Johnson (2004), the results in this experiment showed that triticale can replace 20% of maize in laying hens' diets without affecting the productive performance of the birds.

Acknowledgments

This study was funded by the Rural Development Administration (RDA, Republic of Korea), grant number PJ01383501

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The effect of *Agrimonia pilosa* Ledeb, *Anemone chinensis* Bunge, and *Smilax glabra* Roxb on performance, nutrient digestibility and gastrointestinal tract microbiota of broilers

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Application

S. glabra Roxb and *A. chinensis* Bunge can be used to support bird performance and gastrointestinal tract microbiota.

Introduction

There is a global effort to discover plants that can support poultry health and weight gain in the absence of antibiotics to reduce antibiotic resistance (OIE, 2018). *A. pilosa* Ledeb and *S. glabra* Roxb are used to treat infections in several parts of the world (Chinese Pharmacopoeia Commission, 2015). The antibacterial activity of these plants was identified previously *in vitro* (McMurray, Ball, Tunney, & Situ, 2020). This study determined the effects of plant dietary inclusion on production performance, nutrient digestibility, *Campylobacter* spp. and *Escherichia coli*, and Lactic acid bacteria in the chicken caecum.

Material and methods

Day-old male Ross 308 (n = 420) broiler chicks were randomly allocated to 30 pens. The trial was approved by the Animal Welfare Ethical Review Body at AFBI and conducted under the Animals Scientific Act 1986. The chickens were assigned to one of six dietary treatments (five pen replicates/treatment). Dietary treatments were: Negative control (NC) (reduced nutrient specification); positive control (PC) 1 (NC + 40 mg/kg amoxicillin), PC2 (recommended nutrient specification), NC + *A. pilosa* Ledeb (NC1), NC + *A. chinensis* Bunge (NC2), and NC + *S. glabra* Roxb (NC3). Dry plants were added at 20 g/kg. Starter (0–14d) and grower/finisher (14–35d) diets were formulated according to breed recommendations and offered *ad libitum*. Energy, protein and ideal protein were reduced by 1% in the NC diets. Birds were offered wheat/soyabean meal based diets as mash. Feed intake and weight were measured weekly. Lactic acid bacteria, *Campylobacter* spp. and *E. coli* were enumerated from the caecum of randomly selected birds (five birds/treatment) at days 7, 14, 21, and 35 using a traditional plating method. ANOVA was used with $P < 0.05$ to determine significant differences.

Results

From 0 to 35d feed intake was highest for birds receiving diets containing amoxicillin and *A. chinensis* Bunge ($P < 0.05$). Diets containing amoxicillin, *A. chinensis* Bunge, and *S. glabra* Roxb resulted in higher ($P < 0.001$) weight gains and liveweights at 35d than those observed for birds offered other diets. Feed conversion ratios were most efficient for birds offered diets containing amoxicillin, *A. pilosa* Ledeb and *S. glabra* Roxb and least efficient in birds offered the NC diet ($P < 0.001$; Table 1).

Conclusion

S. glabra Roxb and *A. chinensis* Bunge improved weight and feed conversion ratios to levels equal to the diet including the antibiotic. This could be explained by the modulation of the gastrointestinal tract microbiota.

Acknowledgments

Thanks to AFBI for their support and contribution of lab materials. Thanks to DAERA for funding this studentship.

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Table 1. The effect of plants on feed intake (g), weight gain (g), and feed conversion ratio from 0 to 35 days of age

		Treatment							
Day	Parameter	PC1	PC2	NC	NC1	NC2	NC3	SEM	P value
0–35	FI (g)	3205.9^{ab}	3072.3^{a}	3082.9^{a}	3017.5^{a}	3462.0^{b}	3197.7^{a}	86.99	<0.05
	WG (g)	2231.5^{b}	1989.6^{ab}	1887.7^{a}	2031.6^{b}	2240.6^{b}	2236.7^{b}	41.96	<0.001
	FCR	1.44^{a}	1.54^{b}	1.63 ^{c}	1.49^{ab}	1.55^{b}	1.43^{a}	0.027	<0.001
0	Live weight (g)	44.14	43.82	43.8	43.42	44.46	43.54	0.385	0.419
14		538.0^{c}	476.8^{a}	481.4^{a}	508.9^{abc}	527.5^{bc}	503.8^{ab}	10.94	<0.01
35		2275.7^{b}	2033.4^{ab}	1931.5^{a}	2075.0^{b}	2285.0 ^{c}	2280.2 ^{c}	41.95	<0.001

Differences in superscripts (ab) indicate significant differences between treatment groups (columns).
S. glabra Roxb increased lactic acid bacteria compared to the amoxicillin fed group by 35d (9.6 log^{10} CFU g^{-1}; 7.8 log^{10} CFU g^{-1}, respectively; $P < 0.01$). *S. glabra* Roxb decreased *E. coli* and *Campylobacter spp*. relative abundance from (8.2 log^{10} CFU g^{-1}; and 3.3 log^{10} CFU g^{-1}) at 14d to (7.4 log^{10} CFU $g^{-1}$and 2.2 log^{10} CFU g^{-1}) at 21d.

Xylooligosaccharide-based prebiotic improved body weights and gut health parameters in broilers raised under sub-optimal conditions

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Xylooligosaccharides (XOS) may be used to improve body weight of broilers raised under suboptimal conditions

Introduction

There are contradictory results in literature on the effects of XOS on performance of broilers, but most studies have been conducted under research conditions. Broilers in industrial production systems are often exposed to a wide range of stressors such as overcrowding, changes in temperature, poor feeding and dirty litter (Sohail et al., 2011). Therefore, the effects of a feed additive on gastrointestinal tract could be more obvious at times of stress (Baurhoo, Phillip, & Ruiz-Feria, 2007). This trial was therefore conducted to investigate whether XOS could improve performance and gut health of broilers under sub-optimal conditions.

Material and methods

Approximately 800, Ross 308 broilers were placed in one of four purpose-built pens in a farm barn with very limited temperature control and biosecurity. They received a wheat-corn-soyabean meal diet with (100 g/ tonne) or without XOS supplementation. Sixty birds per pen were individually tagged at the start of the trial (120 replicate birds per treatment blocked in two pens) to measure weekly body weights (BW). On days (d) 7, 21 and 35, ten untagged birds per treatment were euthanized and their caeca were snap frozen. Short chain fatty acids (SCFA) in caecal contents were analysed using Gas Chromatography Mass Spectrometry (GCMS). On d35 approx. 2 cm piece of the ileum was taken to study the expression of gut integrity and SCFA receptor genes using RT-PCR with GAPDH as reference. Data was statistically analysed in R version 3.6.1. Ethical approval was granted by the University Animal Sciences ethics review committee.

Table 1. Body weight and relative gene expression ratios of gut integrity and SCFA receptor genes in ileum of birds fed control (CON) and XOS diets

Parameter		CON	XOS	SEM	P-value
Bird Weight Gain (g)	day 0	37.7	37.5	0.11	0.162
	day 7	165.1	174.4	1.63	<0.01
	day 14	439	465	4.65	<0.01
	day 21	951	1005	9.86	<0.01
	day 28	1619	1689	17.05	0.004
	day 35	2439	2431	31.30	0.812
Relative gene expression ratios	MUC2	1.077	1.548	0.121	0.033
	sIgA	1.341	2.127	0.397	0.211
	CLDN1	1.163	1.063	0.243	0.78
	CLDN5	1.087	1.353	0.152	0.263
	OCLN	1.075	1.564	0.132	0.039
	IL-1β	1.182	1.068	0.278	0.782
	FFAR2	1.089	1.08	0.147	0.968

MUC2 = mucin 2; sIgA = secretory immunoglobulin A; CLDN1 = claudin-1; CLDN5 = claudin-5; OCLN = occludin, IL-1β = interleukin 1β, FFAR2 = free fatty receptor-2.

Results

XOS supplementation was shown to significantly improve BW up to 28 days of age (Table 1). On d35 acetate and propionate were significantly higher ($p = 0.021$ and 0.017 respectively) (Figure 1) and the expression of mucin glycoprotein (MUC2) and one of the tight junction proteins, occludin (OCLN) (both considered as biomarkers of gut integrity) was significantly upregulated in the XOS group compared to the control group.

Conclusion

XOS improved BW and gut health parameters of broilers raised under challenging condition.

Acknowledgments

The authors gratefully acknowledge the funding from AB Agri for the trial.

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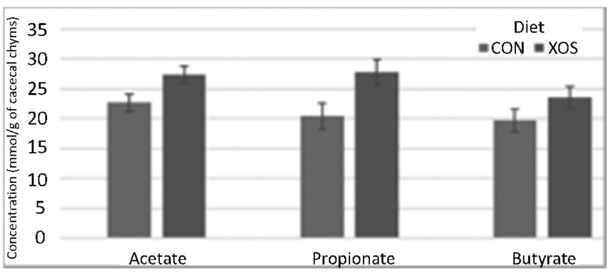

Figure 1. SCFA concentrations in caecum at day 35.

Peracetic acid precursors for non-antibiotic modulation of gut microbiota and possible modulation of performance in broilers

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Application

In-water peracetic acid (PAA) derived from sodium percarbonate (SP) and tetraacetylethylenediamine (TAED) precursors, could impact poultry gut microbiota and possibly increase performance, functioning as a wide spectrum potential antibiotic alternative.

Introduction

Finding alternatives to antimicrobials has a critical importance due to the rise in antimicrobial resistance associated with animal and therefore poultry production (Koch, Hungate, & Price, 2017). During this study, the documented wide spectrum biocide effect of PAA (Kitis, 2004) was tested for its impact on bacterial number (BN) in different gut locations and performance in broilers.

Material and methods

A total of 96 Ross 308 male day-old broilers were used during the 14-day trial (randomised block design with 2 rooms, 12 pens per room, 4 birds per pen and 4 pens per level of inclusion), whilst PAA treatment was administered during the last week. Six different PAA levels of inclusion (i.e. 0ppm, 10ppm, 20ppm, 30ppm, 40ppm and 50ppm) were tested through in-water daily administration of SP and TAED. Possible PAA effect on body weight gain (BWG), FCR, feed/water intake and BN were assessed through fitting linear mixed model (i.e. treatment as fixed effect and hierarchy of room/pen/bird when possible as random effect). Birds were fed standard commercial diet (*ad libitum*) throughout the trial. Longitudinal data were used when repeated measures were taken. Total bacterial number in DNA extracted from crop, jejunum and caecal content was calculated through qPCR targeting 16S rRNA gene (V3 region). Study design and protocol were approved by SRUC Animal Welfare and Review Body.

Results

The results here presented strongly suggest that PAA administered in-water for one week, through SP and TAED precursors delivery, increased BWG during treatment (P = 0.05) whilst reducing BN in the crop (P = 0.02; Figure 1), especially in one of the experimental rooms (data not shown). Crop $\log_{10}$ BN was found to be significantly correlated (P = 0.006) with BWG during PAA administration. In particular, PAA administered at 40ppm had the most evident effect on BWG from D7 to D14 (P = 0.05). On the other hand, control treatment at 0ppm was associated with the lowest BWG and highest BN (Figure 1). The findings presented here could therefore indicate that increasing in-water PAA level up to 50 ppm, through precursors administration, could positively affect broiler performance through interacting with upper-gut bacterial population opening the way for further studies investigating PAA administration in-water or in-feed and correlations with changes in the level of particular microbial species. PAA administration was not correlated with bird mortality (0%) or side effect in general.

Conclusion

Whilst no adverse effects or mortality were correlated with PAA in-water administration, the concentration of total number of bacteria in crop content appeared to be reduced by higher dosage. Although qualitative composition of microbial communities was not explored, the results of this trial showed that the reduction of total bacteria in the crop could be correlated with an increased BWG. Therefore, PAA seemed to reduce crop absolute microbial number, which could have enhanced performance.

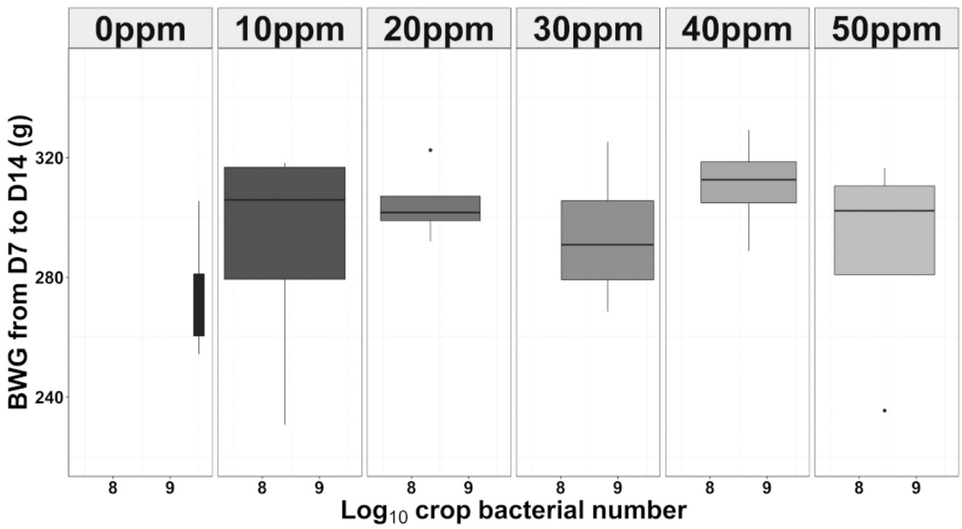

Figure 1. Observed effect of PAA on crop $\log_{10}$ BN. The latter was significantly correlated to BWG especially when PAA was administered at 40ppm. This could indicate a possible correlation between PAA and performance through interacting with upper-gut microbiota.

Acknowledgments

This work was funded through Innovate UK (Project 104990). SRUC receives support from Scottish Government (RESAS).

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The effect of oil and grape pomace on the quality of stored quail eggs over a four-week period

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Application

Dietary grape pomace, maize and linseed oils did not prevent changes in egg quality during storage of 4 weeks.

Introduction

The global demand for quail eggs is increasing (Genchev, 2012). Eggs are often stored for a prolonged period of time, which may cause quality to deteriorate. This may be alleviated by dietary inclusion of antioxidant rich compounds like grape pomace (GP). Different types of vegetable oils such as corn, soy and linseed are added to diets to increase energy content however knowledge of potential interactions with GP and effect on egg quality is limited. Thus, the aim of the current study was to evaluate the effects of feeding GP in combination with corn or linseed oils on the quality of stored quail eggs.

Material and methods

The study was approved by the Animal Experimental Committee of Wrocław University of Environmental and Life Sciences, Poland. In the experiment 4 maize-wheat-soy-based diets were offered: corn oil (CO) 40 g/kg, CO + 20 g/kg dried GP, linseed oil (LO) 20 g/kg, LO 20 g/kg + GP 20 g/kg. The trial was conducted on 120 Japanese laying quails (from 5 to 10 weeks of age) which were individually weighed and assigned to 20 cages (6 birds/cage). Each diet was fed as mash to 5 random pens. On the final day of the trial eggs were collected from each cage. Egg quality was determined at the day of collection and after 4 weeks. Variables measured included: egg weight, albumen height (AH) and pH, Haugh units (HU), yolk pH and colour. Data were statistically analysed with ANOVA using a 2 x 2 × 2 factorial arrangement of treatments.

Results

The main effects were oil, GP and storage time (Table 1). The inclusion of corn oil increased yolk colour intensity and reduced yolk pH in stored eggs only ($P < 0.001$). The GP reduced albumen pH ($P < 0.05$). Storage tended ($P = 0.082$) to reduce egg weight and reduced AH, HU and yolk colour intensity ($P < 0.05$), and increased albumen and yolk pH ($P < 0.05$). There were no further interactions observed in the study.

Table 1. The effect of dietary oil, grape pomace and storage on weight and some internal egg quality variables of quail eggs

	Egg weight	Albumen height	Albumen pH	Haugh Units	Yolk pH	Yolk colour
Corn oil	10.80	3.72	9.06	86.10	6.38	11.11
Linseed oil	10.81	3.76	8.86	86.15	6.40	10.25
Grape pomace						
No	10.76	3.78	9.06	86.30	6.40	10.57
Yes	10.85	3.70	8.85	85.96	6.38	10.79
Storage (weeks)						
0	10.93	4.39	8.80	89.81	6.19	10.98
4	10.68	3.09	9.11	82.45	6.59	10.39
SEM	0.101	0.097	0.073	0.567	0.009	0.144
Oil x Storage						
Corn x 0	10.89	4.35	8.85	89.72	6.20a	11.53
Linseed x 0	10.98	4.43	8.76	89.90	6.18a	10.43
Corn x 4	10.72	3.09	9.27	82.48	6.56b	10.70
Linseed x 4	10.65	3.08	8.96	82.41	6.63 c	10.07
SEM	0.142	0.137	0.104	0.802	0.013	0.203
P value						
Oil	0.946	0.798	0.054	0.946	0.069	<0.001
Grape pomace	0.510	0.547	0.047	0.673	0.205	0.297
Storage	0.082	<0.001	0.003	<0.001	<0.001	0.004
Oil x Storage	0.569	0.742	0.285	0.878	<0.001	0.244

Conclusion

It can be concluded that dietary grape pomace and oils did not interact with the measured variables of stored eggs in this study.

Reference

Genchev, A. (2012). *Trakia Journal of Sciences*, *10* (2), 91–101

Dietary probiotic improves laying efficiency in Hy-line Brown hens

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Application

Feeding a *Bacillus licheniformis* probiotic to laying hens reduces the FCR, resulting in less expensive egg production and more profit to the producer.

Introduction

Probiotics have been defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (Hill *et al.*, FAO/WHO,

Table 1. The effect of dietary probiotic on feed intake per egg (FI), average egg weight (EW), feed conversion ratio for egg production (FCR), % egg production (EP), shell thickness (ST), shell weight (SW) and Haugh units (HU)

Diets	FI (g)	EW (g)	FCR (g:g)	EP (%)	ST (mm)	SW (g)	HU
Control	131	59.0	2.221	91.5	0.354	5.59	92.0
Control + Probiotic	117	59.0	1.982	96.2	0.359	5.67	93.5
SEM	1.9	0.53	0.0295	1.23	0.0102	0.200	1.36
P	< 0.001	NS	< 0.001	<0.05	NS	NS	NS

2014). Probiotics do not lead to the development or spread of resistant pathogenic bacteria in animals, thus offering immense potential as an alternative to some antibiotic growth promoters in the poultry industry (Mikulski, Jankowski, Mikulska, & Demey, 2020). However, the efficacy of probiotics has been variable because of multiple factors, including differences in their microbial composition, liveability in the gastrointestinal tract, dose, way of application, diet composition, bird age and rearing conditions. Dietary probiotics are being constantly improved and upgraded so there is a continued need to evaluate their efficacy and mode of action. The mode of action of probiotics in poultry includes, but is not limited to, maintaining healthy intestinal microflora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activity, decreasing bacterial enzyme activity and ammonia production, neutralizing enterotoxins and improving feed intake and digestion (Mikulski et al., 2020). Not all mode of actions are applicable to every probiotic, and it is highly unlikely one probiotic will be able to exert all described modes of action. Therefore, the aim of the experiment was to study the effect of a novel probiotic on egg production and quality variables including feed intake per egg (FI), average egg weight (EW), feed conversion ratio for egg production (FCR), % egg production, shell thickness (ST), shell weight (SW) and Haugh units (HU) when fed to Hy-Line laying hens.

Material and methods

The experiment was conducted at the National Institute of Poultry Husbandry and approved by the Harper Adams University Research Ethics Committee, England. A current probiotic (B-Act®, Huvepharma NV) was used in the present experiment. The probiotic is a single-strain, containing spores of a unique *Bacillus licheniformis* strain (DSM 28710). An additive free diet was fed to all birds from 17 to 22 weeks of age. Two experimental wheat-based diets were prepared for the study. A wheat-based control mash diet was formulated to contain 181 g/kg crude protein, and 11.59 MJ/kg ME. The other experimental diet was the control diet supplemented with B-Act® at 500 g/ tonne giving 1.6 x 10^9 CFU Bacillus licheniformis DSM 28710/kg of feed. One hundred and sixty 22 weeks old Hy-Line hybrid pullets were individually weighed and randomly allocated to 40 enriched colony cages, four birds each. Each diet was replicated 20 times in a randomised complete block design. Diets were fed *ad libitum* from 22 to 34 weeks of age in an environmentally controlled house.

Results

Overall, birds fed the probiotic had 10.7% lower feed intake for egg produced ($P < 0.001$, Table 1) and 10.8% lower (better) FCR compared to the birds fed the control diet ($P < 0.001$). Birds fed probiotic for the study period had greater per cent lay ($P < 0.05$). There were no differences ($P > 0.05$) between the rest of the studied variables.

Conclusion

Feeding probiotic to laying hens reared in enriched cages from 22 to 34 weeks of age improved overall egg production and FCR. The size of the improvements in rate of egg production and FCR are commercially important.

Acknowledgments

The authors acknowledge the help of Richard James and Ros Crocker of The National Institute of Poultry Husbandry (Edgmond, UK) for their help in taking care of birds used for this study.

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Feeding a novel phytase at 250 FTU/kg to laying hens improves P digestibility

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Application

Feeding a novel phytase enzyme to laying hens improves dietary P availability thus resulting in less dietary P inclusion and reduced cost of production.

Introduction

Phosphorus (P) is an essential nutrient that is involved in numerous body functions for laying hens, including bone formation, energy metabolism, cellular structure, and egg formation (Seelle & Ravindran, 2007). Over two-thirds of P in cereals and legumes, the main ingredients of poultry diets, are contained in chemical structures called phytic acid or its salts, collectively known as phytates. Poultry do not produce enough intestinal phytase, thus the inclusion of exogenous phytases is a common practice in poultry production. Exogenous phytases are being constantly improved and upgraded so there is a continued need to evaluate their efficacy and mode of action. Therefore, the aim of the experiment was to study the effect of a next generation of phytase (OptiPhos® Plus, Huvepharma NV, a 6-phytase produced by a genetically modified strain of *Komagataella phaffii*) on bone ash, bone mineral contents (including P, Ca and Mg) and total tract digestibility of dietary P and Ca when fed to laying hens.

Materials and methods

The experiment was conducted at the National Institute of Poultry Husbandry and approved by the Harper Adams University Research Ethics Committee. An enzyme free diet was fed to all layers (Hy-Line) from 17 to 22 weeks of age. Three experimental maize-based diets were prepared for the study. A maize-based positive control (PC) mash diet was formulated to contain 168 g/kg crude protein, 11.61 MJ/kg ME, 3.6 g/kg available phosphorus and 39.5 g/kg calcium. Similarly, a maize-based negative control (NC) mash diet was formulated to contain 169 g/kg crude protein, 11.63 MJ/kg ME but 1.9 g/kg available phosphorus and 37.0 g/kg calcium. The third experimental diet was the NC diet supplemented with 250 FTU/kg (phytase units per kg feed). Two hundred and forty 22 weeks old Hy-Line hybrid pullets were individually weighed and randomly allocated to 60 enriched colony cages, giving four birds per pen. Each diet was replicated 20 times in a randomised complete block design. Diets were fed *ad libitum* from 22 to 34 weeks of age in an environmentally controlled house. Excreta were collected for the last three days of the study and used for determination of P and Ca digestibility coefficients. At the end of the study, two birds from each pen were culled, tibia bones collected and the pool sample analysed for ash, P, Ca and Mg.

Results

Overall, the digestibility coefficient for P was significantly improved in NC+250 FTU/kg birds compared to those fed NC diets though still lower than in birds fed PC diets (Table 1). There were no differences in Ca digestibility coefficients between the PC and phytase containing diets. The coefficients of the NC were lower ($P < 0.001$) compared to the PC. There were no differences ($P > 0.05$) on bones ash parameters.

Table 1. The effect of diets on bone ash, P, Ca and Mg contents, and total tract P and Ca digestibility coefficients

	Content in bones (% DM)				Digestibility	
Diets	Ash	P	Ca	Mg	P	Ca
PC	78.3	15.7	33.4	0.5	0.631^{a}	0.637^{a}
NC	77.6	15.8	33.4	0.5	0.279^{b}	0.536^{b}
NC + 250 FTU/kg	77.6	15.8	33.5	0.5	0.522^{c}	0.584^{ab}
SEM	0.45	0.09	0.20	0.01	0.0140	0.0176
P	NS	NS	NS	NS	< 0.001	< 0.001

^{abc}: means in the same column having different superscripts differ significantly ($P < 0.05$).

Conclusion

Feeding 250 FTU/kg phytase to laying hens from 22 to 34 weeks of age strongly improved P digestibility coefficient compared to NC and equalised with the Ca digestibility coefficient compared to the PC.

Acknowledgements

The authors acknowledge the help of Richard James and Ros Crocker of The National Institute of Poultry Husbandry (Edgmond, UK) for their help in taking care of birds used for this study.

Reference

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Short chain fatty acids production and villus morphometry in chickens fed diets containing graded levels of supplementary sea buckthorn dried berries

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Application

Incorporating dry sea buckthorn berries can negatively influence caecal fermentation and villus morphometry in broiler.

Introduction

Sea buckthorn (SB) (genus *Hippophae*) is a berry-bearing, hardy bush of the family *Elaeagnaceae* that is widely spread in Asia, Europe and recently introduced to Americas.

Table 1. Effect of the experimental diets on bird caecal production of short chain fatty acids and jejunal villus morphometry

	Sea buckthorn (g/kg)						Probability		
Treatment	0	3	6	9	12	SEM	P	L	Q
Body weight (g)	776	761	772	775	748	15.7	0.679	0.399	0.570
Acetic acid (ppm)	378	286	256	220	188	52.2	0.132	0.012	0.566
Butanoic acid (ppm)	56	57	48	40	26	9.7	0.162	0.017	0.444
Pentanoic acid (ppm)	6	6	4	3	1	1.1	0.031	0.002	0.567
Propanoic acid (ppm)	48	36	44	40	35	5.6	0.412	0.203	0.969
Villus height (µm)	1581	1599	1637	1567	1632	19.8	0.075	0.266	0.841
Crypt depth (µm)	114	115	123	119	123	2.0	0.004	0.001	0.365
Villus height: Crypt depth	13.91	13.95	13.30	13.24	13.32	0.245	0.113	0.022	0.467

Feeding berries of SB has shown some health benefits that was associated with the abundance of various antioxidants (Pengzu, Yao, & Xin, 2009). However, there is a lack of information on the impact of SB on the caecal short chain fatty acids (SCFA) fermentation and villus morphometry in broilers. Thus, the objective of the present study was to quantify the responses in caecal SCFA, jejunal villus height, crypt depth and the ratio between them resulting from feeding graded levels of SB from 7 to 21 d old broilers. The final body weight of birds was also measured.

Material and methods

The experiment was approved by the Harper Adams University Research Ethics Committee. Eighty female Ross 308 chicks were reared from 7 to 21 days of age in 40 pens (2 birds in each). Five diets in total were fed; a basal diet (Control; C) with 211 g/kg CP, 12.69 MJ/kg ME, 9.7 g/kg Ca and 4.8 g/kg available P. The C was then split into 5 parts and dried SB berries were incorporated into a diet in mash form at 0, 3, 6, 9 and 12 g/kg, resulting in five diets. The SB berries were collected from Troyan region of Balkan mountain, Bulgaria, cold pressed and the residues of berries and seeds were air dried and preserved in a freezer for about 3 months. A kilogram of air-dry SB berries contained 23.62 MJ GE, 233 g fat, 180 g CP and 151 g total non-starch polysaccharides (NSP), from which 112 g insoluble NSP. Each diet was fed to 8 pens following randomisation. At the end of the study, one birds per pen was sacrificed and caecal digesta was analysed for SCFA. About 3 cm segment of middle jejunum was also collected and morphometric measurements were determined on 20 intact well-oriented villus-crypt units for each bird. The data were analysed with ANOVA. Orthogonal polynomials were used to compare treatment differences for linear (L) and quadratic (Q) relationships with increasing SB level.

Results

Feeding SB did not change the final body weight of the birds (Table 1). Feeding graded levels of SB resulted in a linear decrease of the production of acetic, butanoic and pentanoic acids, which coincided with a linear increase in crypt depth and a decrease in the villus height to crypt depth ratio. There were no deviations from linear relationships for any studied variable.

Conclusion

Reduction in villous height to crypt depth ratio, involving shorter villi and/or deeper crypts, and the reduced SCFA fermentation are usually associated with poor gut health. It appears that the serial substitution of a balanced diet with SB has a negative effect on SCFA production and gut morphometry, suggesting that higher oil content may cause the reduced caecal fermentation.

Acknowledgments

Special thanks to Richard James and Ros Crocker of the National Institute of Poultry Husbandry (Harper Adams University, UK) for their technical support in conducting the study.

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Evaluation of broiler response to silicon rate of dietary inclusion and particle size

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Application

A granular silicon supplement fed at 750 mg Si/kg improves broiler performance results up to 21 days of age, with no deleterious effect on final growth.

Introduction

Intensive genetic selection for rapid growth and development has led to broiler birds being highly susceptible to skeletal disorders. Following extensive research at

Table 1. Main effects of diet, silicon supplement dose (mg/kg diet) and silicon supplement form on mean bodyweight, bodyweight gain, feed intake and feed conversion ratio (FCR) during two broiler growth phases (0–21d and 22–42d)

	Bodyweight (g)		Bodyweight gain (g)		Feed intake (g)		FCR	
Treatment	0–21d	22–42d	0–21d	22–42d	0–21d	22–42d	0–21d	22–42d
No silicon dose	361[a]	2202	324[a]	717	377	1208	1.38[a]	1.71
Silicon inclusion 250	385[b]	2238	347[b]	720	395	1210	1.35[ab]	1.72
Silicon inclusion 750	395[b]	2294	357 [c]	736	399	1197	1.31[bc]	1.66
Silicon inclusion 1250	398[b]	2256	360 [c]	709	395	1209	1.28 [c]	1.73
P-value	0.005	0.199	0.002	0.417	0.092	0.944	0.013	0.323
No silicon form	361[a]	2203	324[a]	717	377[a]	1208	1.38	1.71
Powder form	389[b]	2264	351[b]	724	393[b]	1206	1.32	1.70
Granular form	396[b]	2262	358[b]	719	399[b]	1204	1.30	1.71
P-value	0.015	0.325	0.004	0.900	0.024	0.986	0.093	0.949

[abc]: means in the same column having different superscripts differ significantly ($P < 0.05$).

Nottingham Trent University (NTU), a bioavailable stable silicon supplement was developed using the soluble monomer Orthosilicic acid ($Si(OH)_4$). Previous studies show that this supplement improves skeletal integrity in broiler birds (Scholey, Belton, Burton, & Perry, 2018). In order to further explore the biological influence of the supplement, this study investigated the effect of both supplement dose rate and supplement particle size on performance parameters.

Material and methods

Ethical approval for this study was granted by Nottingham Trent University's Ethics Committee and recorded as ARE636. A total of 728 male Ross 308 broiler birds were fed wheat/soybean meal-based diets designed for the age and strain of broiler bird in 2 phases: 0–21day starter diets (23% crude protein (CP) and 12.4 MJ/kg apparent metabolisable energy (AME)) and 22–42 day grower diets (21% CP and 13 MJ/kg AME). The study was designed as a randomised block-controlled diet trial with a 2*3 + 1(control) factorial design with rate of silicon inclusion (250, 750 and 1250 mg silicon per kilogram of diet) and silicon product form 'powder' (0–250 micron) or 'granular' (200–1250 micron) as factors. There were 13 replicate pens per treatment. Bird performance parameters bodyweight (BW), bodyweight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured weekly. Statistical analysis software SPSS (v25) was used to perform one-way and two-way ANOVAs.

Results

There is a significant positive effect of both silicon dose rate and form on bird bodyweight, bodyweight gain and feed conversion ratio between days 7 and 21, with these significant results being lost after this time point (Table 1).

Conclusion

Regardless of form or dose, the birds fed the silicon supplement showed significantly improved growth rate up to 21 days of age, but the silicon supplement had no effect on the final weight of the birds. Further work will be undertaken to explore the optimal time point for inclusion and the effect of this supplement on edible soft tissue.

Reference

Scholey, D. V., Belton, D. J., Burton, E. J., & Perry, C. C. (2018). *Scientific Reports*. *8*(1): 17022.

Amino acid digestibility is improved in broiler diets containing high-phytase HIGHPHY wheat

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Application

A novel variety of HIGHPHY wheat shows improved amino acid digestibility to compare a standard wheat.

Introduction

Exogenous bacterial or fungal-derived phytases are routinely added to poultry feed to hydrolyse plant phytate and make phosphorous (P) and amino acids more bioavailable in poultry and to release less P in the environment. In addition to exogenous phytase, the mature grain phytase activity (MGPA) of cereal grains also contributes to phytate hydrolysis in feed, with purple acid phosphatases (PAPhy) is considered to be responsible for bulk of MGPA. In recent years, increased MGPA were achieved in cis-genic barley holding extra copies of barley PAPhy and in the wheat HIGHPHY mutant, where MGPA was increased to ~6200FTU/kg. Previous studies showed that replacement of conventional wheat with HIGHPHY wheat significantly improved Ca & P digestibility over the diet supplemented with exogenous phytase (Scholey et al., 2017). In order to further explore the potential of this novel wheat in broilers, this study investigated the effect of graded inclusion of HIGHPHY wheat at 60, 40 & 20% level in comparison to standard wheat.

Material and methods

Ethical approval for this study was granted by Nottingham Trent University Ethics Committee and recorded as ARE460. The study was designed as a randomised block-controlled

Table 1. Pre-cecal digestibility of different amino acids from HIGHPHY wheat and standard wheat

	HIGHPHY wheat		Standard wheat		
Amino acid	Slope	Confidence interval	Slope	Confidence interval	$Z_{Critical}$
Threonine	0.710 ± 0.094[a]	0.515–0.905	0.475 ± 0.068[b]	0.334–0.615	2.026
Valine	0.859 ± 0.065[a]	0.724–0.993	0.501 ± 0.073[b]	0.349–0.652	3.663
Isoleucine	0.916 ± 0.058[a]	0.796–1.036	0.612 ± 0.075[b]	0.455–0.768	3.206
Lysine	0.887 ± 0.061[a]	0.759–1.014	0.600 ± 0.085[b]	0.424–0.775	2.743
Histidine	0.828 ± 0.057[a]	0.710–0.945	0.640 ± 0.058[b]	0.520–0.760	2.312
Leucine	0.879 ± 0.059[a]	0.757–1.000	0.710 ± 0.051[b]	0.605–0.815	2.167
Phenylalanine	0.881 ± 0.053[a]	0.771–0.991	0.742 ± 0.046[b]	0.647–0.838	1.981

[abc]: means in the same row having different superscripts differ significantly ($P < 0.05$).

diet trial with two factors: wheat type and inclusion rate of the wheats. Six test diets where diet A, B, C contained HIGHPHY wheat at an inclusion rate of 60, 40 and 20% and diet D, E, F contained Standard wheat at 60, 40 and 20% inclusion rate. All the test diets contained 0.5% Titanium dioxide as an inert marker and were fed as a mash from d15 to day 20 to 384 male Ross 308 broilers with 8 replicates per treatment and 8 broilers per replicate. Ileal digesta was also collected and analysed at for amino acid content and Ti at the end of trial. The digestibility of amino acids was calculated according to Rodehutscord, Kapocius, Timmler, and Dieckmann (2004).

Result

The pre-cecal digestibility of most of the essential amino acids were significantly higher in HIGHPHY Wheat compared to that of Standard Wheat (except Cystine and methionine) (Table 1).

Conclusion

This study indicates *in planta* wheat PAPhy has potential for improving amino acid digestibility in poultry feed. Further studies will explore the other wider implications associated with use of HIGHPHY wheat in broiler diets.

Acknowledgement

We are grateful to Plant Bioscience Limited, Norwich Research Park, Norwich for provision of materials used in the current study.

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Evaluation of phytase super-dosing on broiler intestinal development

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Application

Phytase super-dosing does not appear to alter intestinal microbiota but alters digestive tract gross development compared to a standard phytase dose.

Introduction

Phytic acid is an anti-nutritional factor, which reduces bioavailability of nutrients through complex formation in the gastro-intestinal tract. To reduce the adverse effects and to improve the utilization of plant phosphorus as well as broiler performance, lower doses of phytase supplementation is a common practice. Due to rising feed ingredient and mineral costs and lower enzyme prices, there is a growing interest for poultry producers to use higher doses of phytase beyond the standard dose, termed super-dosing. Super-dosing has shown to further increase phytate degradation resulting in generation of more soluble phytate esters and myoinositol, therefore translating to enhanced nutrient digestibility, greater bone mineralization and improved broiler performance. In addition to these benefits, the super-dosing effect of phytase on microbial ecosystem and different measurements of digestive tract are essential for better understanding of digestive and immune functions of broilers. So, the purpose of the current study was to further understand super-dosing of phytase on digestive tract measurements, bone mineralization, intestinal microbiota along with broiler performance.

Material and methods

Ethical approval was granted by the University's Ethics Committee as ARE488. Five hundred (250 male and 250 female) Ross 308 day-old broilers were divided into four dietary groups: group 1 was positive control (PC 0.48 and 0.34% non-phytate phosphorous-nPP in starter and grower diet), group 2 was a negative control (0.35% and 0.21% nPP in starter and grower), groups 3 and 4 were fed the negative control (NC) plus standard 500 FTU/kg and superdose 3000 FTU/kg of phytase (Quantum Blue, 6-phytase derived from *Trichoderma reesei*. AB Vista, UK) respectively. Starter diets were fed from d 0 to 21 and grower diets were fed from d 21 to 35. Each diet had 12 replicate pens (6 male and 6 female) with 10 broilers per replicate. Treatment effects on performance, bone mineralization and gastrointestinal tract (GIT) weight were evaluated at both phases. Birds were euthanised at d 35 and caecal samples were collected and analysed by 16S rRNA gene sequencing to evaluate the effect of phytase on the caecal microbiome.

Table 1. Effect of diet on alpha diversity of broiler microbiome, intestinal development and bone mineralisation

Diversity index	PC	NC	NC + 500 FTU/kg	NC+ 3000 FTU/kg	P-value
Shannon	4.06	4.04	4.06	4.03	0.630
Observed OTUs	120.58	119.27	121.41	119.25	0.980
Chao1	127.44	126.53	128.08	125.75	0.980
Feed conversion ratio (0–35d)	1.48	1.50	1.50	1.48	0.475
D21 Tibia ash %	40.02[a]	37.79[b]	39.66[ab]	40.07[a]	0.015
D28 Tibia ash %	36.32[a]	33.17[b]	34.36[ab]	36.45[a]	0.045
D21 GIT wt (g/kg BW)	69.59[ab]	71.13[a]	69.79[ab]	63.78[b]	0.019
D28 GIT wt (g/kg BW)	58.67[ab]	60.00[ab]	60.61[a]	55.38[b]	0.036

[abc]: means in the same row having different superscripts differ significantly ($P < 0.05$).
PC: positive control; NC: negative control.

Results

Body weight gain, feed intake and feed conversion ratio were statistically similar among the experimental treatments though feed conversion ratio was numerically improved with addition of phytase compared to NC diet in male broilers (Table 1). At days 21 and 28, tibia ash content was lower ($P < 0.05$) in birds fed the NC diet compared to the PC. Supplementing 3000 FTU/kg phytase to the NC diet increased ($P < 0.05$) ash percentage to a comparable level to the PC, with 500 FTU/kg phytase giving an intermediate response. Caeca and digesta weights were unaffected by treatment, although at days 21 and 28 birds fed 3000 FTU/kg phytase had lower GIT weights compared to negative control and NC+ 500 FTU/kg diet respectively. Dietary treatments had no effect on species richness and relative abundance of microbial communities at phylum, family or genus level.

Conclusion

The results from this experiment suggest that diets have no effect on broiler performance, and caecal microbiome but significant effect was observed in bone ash content and GI tract weight when diets were super-dosing with Phytase.

Untargeted metabolomics on plasma and liver samples: effects of novel plant extracts in broilers pre and post LPS challenge

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Application

Metabolomics offers a new tool to investigate the role of novel plant extracts in broilers' diets.

Introduction

Novel plant extracts, citrus and cucumber, were identified as possible alternatives to antibiotic growth promoters in broiler diets (Savoia, 2012). The presence of active compounds in citrus and cucumber make them potential candidates for modulation of the immune system and metabolic processes (Akyildiz & Denli, 2016). Metabolomics can be used to explore the interaction among these active compounds and metabolites. In this study, untargeted Metabolomics studies were performed to test the hypothesis that citrus and cucumber dietary supplementation can modulate the immune system and metabolome of broiler chickens.

Materials and methods

All protocols were approved by the Home Office, Animals in Science Regulation Unit (ASRU), Glasgow. A total of 144 one-day-old male broiler chickens (Ross 308) were raised for 15 days at Cochno farm & Research Centre, Glasgow. Chickens (4 replicate pens/each diet; 12 birds/pen) were randomly allotted to one of the three diets: 1) CTL: commercial diet, 2) CMB: diet 1 supplemented with cucumber extract 3) CTS: diet 1 supplemented with citrus extract. At 15 days old, chickens were challenged with *E.Coli* lipopolysaccharide (LPS). Plasma and liver samples, collected pre- and post-challenge at 4 time points (T0,12,24,48 h) from 12 birds per diet, were assessed by Untargeted metabolomics (MS-LC based). Data obtained were analysed using Polyomics integrated Metabolomics Pipeline (Pimp) and Metaboanalyst software. Multivariate analysis (PCA and PLSDA) was used to invistagate significant metabolites modulated by the experimental diets (CMB and CTS) pre and post challenge.

Results

PCA and and PLSDA of plasma and liver samples showed clustering in relation to the LPS challenge, and the CTS diet. In both studies, entire pathways (e.g. Citrate cycle, Alanine, Aspartate, Glutamate) were found to be modulated by LPS challenge at all time points (T0,12,24,48 h) with a decrease of the metabolite intermediate's at T12h. Among the metabolites modulated by CTS, adenosine was identified as a primary candidate for biomarker investigation. Adenosine is a signalling molecule produced in response to stress. Adenosine was lower in the CTS versus CTL dietary treatment pre and 12 h post LPS challenge (Figure 1).

Conclusion

The CTS dietary supplement significantly modulated the overall metabolome of broilers pre and post LPS challenge while CMB had little or no effect compared to the CTL diet (only few metabolites varied). Pectin, limonene and flavonoids are all associated with CTS and could be the active molecules that interact with the specific metabolites identified in this study, including adenosine, and will form the basis of further investigations.

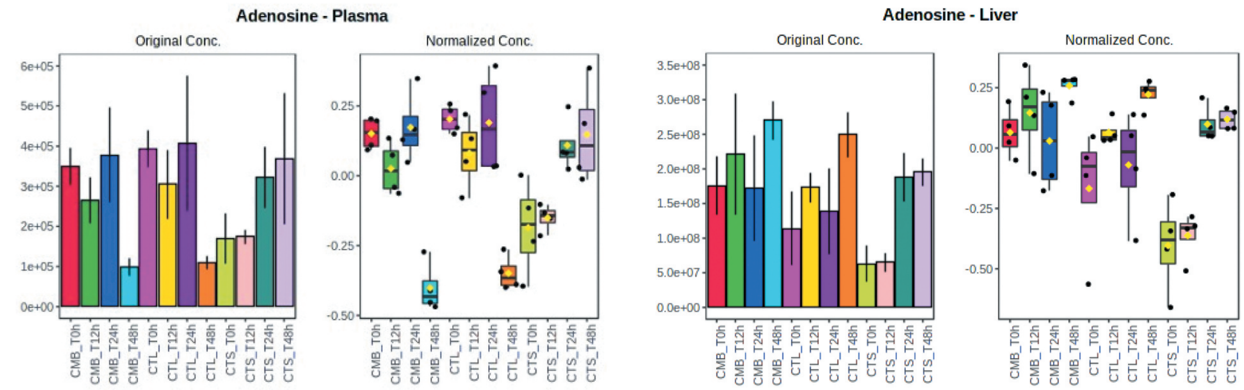

Figure 1. Adenosine levels in plasma and liver: the bar plots show the original concetrations of adenosine (mean ± SD). The whisker plots show the normalized values obtained using the Metabolanalyst software. In both studies, the adenosine level is lower in CTS vs CTL diet at T0, T12h ($P < 0.01$) .

Acknowledgments

This project is funded by the European Union's Horizon 2020 research and innovation programme H2020-MSCA-ITN-2017- EJD: MANNA and the British Poultry Society Ltd small project grant 2019.

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myo-Inositol concentration in chicken tissues is influenced by dietary myo-inositol and phytase

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Application

myo-Inositol (Ins) can be synthesised endogenously from D-glucose or absorbed from the diet via transporter proteins. It is important to assess if Ins concentration in tissues can be manipulated by either the addition of Ins or phytase in poultry diets, in order to understand its role in poultry production and performance.

Introduction

Super-dosing dietary phytase results in increased dephosphorylation of phytate which elevates the concentration of free Ins in the gastrointestinal tract of broiler chickens. This free Ins is then rapidly absorbed (Arthur et al., 2019). myo-Inositol has a number of important roles including its use a signalling molecule, component of cell membrane phospholipids and, it was regarded as a B-vitamin. Based on previous studies it is possible to hypothesise that if chicken blood plasma Ins concentration increases, then this increase of Ins should also be found in the tissues (proportionally). There is little research determining the distribution of Ins in chicken tissues and whether this can be influenced by dietary phytase or supplementation of Ins.

Material and methods

The study was approved by Harper Adams University Research Ethics Committee. One hundred and ninety-two male Ross 308 chicks were reared to 21 days in 24 raised floor pens with eight birds in each. Boilers were fed one of six mash diets: one part left un-supplemented as a control (0 g/kg Ins), the next two parts were supplemented either with two levels of phytase (1,500 or 4,500 FTU/kg; Quantum™ Blue, ABVista, UK) and the final parts with three levels of Ins at 1.5 g/kg, 3 g/kg or 30 g/kg. Each diet was fed to four pens following randomisation, to give four replicates per treatment diet. At day 21 the birds were killed, and tissue samples (breast, leg muscle, kidney and brain) were collected from one random bird per pen and immediately frozen at −80°C. Tissue (100 mg frozen weight) was homogenised in 1 ml of 5% w/v (0.83 N) perchloric acid, 20 mM EDTA, Na_2 on ice and Ins extracted according to Greene et al. (2019b). Inositol was determined by HPLC. Data was analysed using a one-way analysis of variance in GenStat® (18th edition) with a protected Fisher's least significant difference test to separate means.

Result

The addition of either Ins or phytase into broiler chicken diets increased Ins concentrations of breast, leg and kidney tissues at day 21 compared to the un-supplemented diet. High inclusion of Ins at 30 g/kg resulted in a significant increase ($P < 0.05$) in Ins content of breast, leg and kidney tissues compared to the un-supplemented diet (Table 1). Ins in brain tissue did not significantly (Table 2) correlate with Ins in either breast, leg, kidney tissue or blood plasma.

Table 1. The effect of dietary *myo*-inositol (Ins) and phytase supplementation on the concentration of Ins (mmol/kg FWT) in breast, leg, kidney and brain tissue of broilers at day 21 of age

Treatment factor		Breast	Leg	Kidney	Brain
Ins (g/kg)	0	0.452[a]	1.62[a]	4.39[a]	12.3
	1.5	0.756[ab]	1.48[a]	5.90[ab]	17.9
	3	0.638[ab]	1.49[a]	5.17[ab]	21.5
	30	1.31 [c]	2.71[b]	10.2 [c]	19.3
Phytase (FTU/kg)	1500	0.624[ab]	1.60[a]	7.65[bc]	14.7
	4500	0.925[bc]	1.65[a]	7.03[ab]	18.4
SEM (df = 23)		0.1368	0.2407	0.984	3.03
P-value		0.006	0.015	0.007	0.354
CV%		34.9	27.4	29.2	34.9

[a,b,c]superscript letters denote differences in columns (P < 0.05), N = 4.

Table 2. Pearson correlations of *myo*-inositol concentrations between plasma and tissues samples from broilers (age 21d)

Variables	Plasma	Breast	Leg	Kidney
Plasma	-	-	-	-
Breast	0.4107*	-	-	-
Leg	0.4649*	0.7582*	-	-
Kidney	0.5139*	0.5318*	0.5508*	-
Brain	0.1388	0.177	0.1379	0.2433

* denote differences (P < 0.05), N = 28.

Conclusion

This study demonstrates that dietary Ins or Ins liberated by phytase increased Ins in some tissues of broilers compared to those fed an un-supplemented diet. The distribution of Ins differs across the different tissues sampled in this study, and the uptake of Ins into the blood plasma (Arthur et al., 2019a) correlates (P < 0.05) with Ins in the breast, leg and kidney. Results are in line with Greene et al. (2019b) who also found increased Ins levels in muscle tissues in broilers fed Ins at 3 g/kg or phytase beyond a level of 1000 FTU/kg feed. This suggests that Ins increases in the tissue of a broiler by feeding free Ins or high phytase levels, however, isotope labelling of Ins is required to fully validate this.

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What is 'normal'? Morphology and mineralisation of tibias from healthy, on farm broilers

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Application

Morphological data and mineral composition of broiler tibia presented in the current study could be used by poultry practitioners to identify tibia anomalies.

Introduction

Bone anomalies and lameness due to many genetic and non-genetic factors result in reduction in performance, increase in culling rate and negatively impact animal welfare (Applegate *et al.*, 2014). A key focus of vets supporting broiler producers is to diagnose the cause of any lameness and suggest an appropriate intervention. However, there is limited information available on birds reared on a farm rather than a research setting. Thus, exploring tibia morphology and mineral composition of healthy broilers would provide guidelines for poultry practitioners to assay tibia anomality. The aim of the current study is to characterise morphological traits and mineral composition of healthy broilers tibia.

Material and methods

Morphological characters (weight, length, width and strength) and mineral composition (ash, Calcium (Ca) and Phosphorus (P)) of tibia of 144 healthy Ross 308 broilers (72 of each sex) were collected from 6 farms in Nottinghamshire, UK. Each farm obtained its birds from 10 different hatcheries. Birds in all farms received the same standard broiler growing diet. Morphological traits were measured directly for the right and the left tibia of every bird, normalised for one kg bird live weight then averaged. Tibia bones ash was determined by burning at 650°C for 24 hours and Ca and P was determined using an inductively coupled plasma optical emission spectrometry. General linear model was used to analyse the effect of farm, hatchery, season, sex, age, farm×age, hatchery×age, season×farm and sex×age interaction on morphological traits, ash, Ca and P content of tibia. Bird live weight was added to the model as a covariate.

Results

The current study revealed a wide variability in tibia morphology and mineral composition (Table 1). Farm, hatchery and season affected significantly all morphological traits, ash, Ca and P content of tibia (P < 0.001). Influence of sex on tibia length, width, weight and ash content was significant (P < 0.001). Age affected significantly tibia length, width, weight and ash content (P < 0.001). Age×season affected significantly all morphological traits and tibia content of Ca and P (P < 0.001). All morphological traits and mineral composition of tibia were significantly affected by age×hatchery (P < 0.001). At age of 14 days, the range of tibia length (mm/kg), width (mm/kg) weight (g/kg) and strength (N/kg), ash (%), Ca (%) and P (%) were 158, 11.2, 4.01, 639, 11.9, 26.4, 11.4 respectively. At age of 28 days, range of tibia length (mm/kg), width (mm/kg) weight (g/kg) and strength (N/kg), ash (%), Ca (%) and P (%) was 20.3, 3.31, 3.49, 3.49, 337, 13.7, 5.66 respectively. However, at slaughter age, the

Table 1. Tibia morphology and mineral composition (ash, Calcium and Phosphorus) of broilers at different growth stages

Parameters		Day 14		Day 28		Slaughter	
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Non-normalised	Length (mm)	61.2(52.5)	52.5–70.3	89.8(3.8)	78.8–99.1	103(5.3)	90.2–121.0
	Width (mm)	4.22(3.21)	3.21–5.74	6.65(0.65)	4.88–8.19	7.73(0.82)	6.15–9.79
	Weight (g)	1.21(0.70)	0.70–2.13	4.11(0.68)	2.51–6.00	6.39(1.22)	3.73–9.72
	Strength (N)	104.0(37.3)	37.3–283.0	225.0(64.1)	124.0–461.0	281.0(65.4)	134.0–489.0
Normalised (live weight)	Length (mm/kg)	127.0(81.1)	81.1–239.0	62.8(9.5)	46.8–96.8	48.7(6.7)	35.5–66.5
	Width (mm/kg)	8.72(5.27)	5.27–16.50	4.62(0.63)	3.48–7.02	3.62(0.40)	2.64–4.88
	Weight (g/kg)	2.48(1.19)	1.19–5.20	2.82(0.33)	1.63–4.83	2.95(0.21)	2.45–3.55
	Strength (N/kg)	214.0(63.8)	63.8–703.0	155.0(42.6)	92.3–371.0	132.0(30.9)	82.0–211.0
Mineral composition	Ash (%)	39.6(32.0)	32.0–43.9	40.2(1.8)	36.0–44.6	39.2(1.8)	35.0–44.1
	Calcium (%)	13.2(1.6)	1.6–28.0	13.1(2.0)	4.6–18.3	12.8(2.3)	3.0–18.3
	Phosphorus (%)	5.13(1.14)	0.61–12.00	4.99(0.76)	1.21–6.87	4.84(0.83)	1.23–6.44

range in tibia length, width, weight, and strength (N/kg), ash, Ca and P range was 30.8 mm/kg, 2.24 mm/kg, 1.1 g/kg, 129 N/kg, 9.1%, 15.3%, 5.21% respectively.

Conclusion

Wide variation in tibia morphology and mineral composition of healthy broilers tibia due to effect of age, sex, farm, hatchery and season. This data is key to diagnose tibia anomality.

Acknowledgements

Poultry unit in NTU and Cargill are acknowledged for their support.

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Lack of genetic correlation between laying hen bone quality and egg production suggests egg number may not explain bone quality

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Application

Improving bone quality, especially the keel, in laying hens is an important welfare goal. However, to do that we must understand what influences bone quality. This study examines the facts rather than the dogma.

Introduction

Bone fractures including Keel Bone damage are a challenge for laying hens, as the physiological adaptations for egg laying make them susceptible to osteoporosis. There is also a welfare paradox in laying hens; cage free production that allows greater movement and loading of the bone is consistently associated with increased bone quality but at the same time these alternative systems are associated with greater incidence of bone damage, often featuring the keel. We are also starting to see the ‘Long Life Layer’, which has great benefits for sustainability but with a longer period of production could be a greater risk for bone quality at the end of lay. In this context it is frequently stated that poor bone quality is a result of selection for increased egg production. However, the literature is not entirely supportive of this hypothesis. Whilst laying eggs can be related to potential issues of poor bone quality, it is not clear that the relationship between numbers of eggs laid and bone quality is negative.

Material and methods

To test the hypothesis of a relationship between egg production and bone quality we estimated genetic correlations between the traits towards the end of lay in a White Leghorn (WL, 53 weeks of age) and a Rhode Island Red (RIR, 68 weeks of age) pure line using ASREML. Egg numbers at the commencement of lay, related to age at first egg, were analysed separately (early egg number) and the remaining periods (egg number) represented post peak production. Tibia and humerus breaking strength and the radiographic density of the tibia, humerus and keel bone were measured (>900 per line).

Results

Heritability estimates for bone quality traits were 0.19 ± 0.07–0.48 ± 0.08 for WL, 0.35 ± 0.08–0.59 ± 0.09 for RIR. Heritability of keel bone density was near zero. Genetic correlations between egg number and bone quality were not significant. Correlation between egg production and tibia bone density was 0.24 ± 0.21and −0.05 ± 0.22 for WL and RIR respectively. In the WL there were negative genetic correlations between bone quality and early egg number (−0.50 ± 0.11). Genetic correlations were also negative between early egg number and body weight in the WL line (−0.64 ± 0.13). This may reflect the interaction between bone quality and puberty which also affects mature body weight.

Conclusion

These results and previous data (Bishop, Fleming, McCormack, Flock, & Whitehead, 2000) has demonstrated the potential exists to improve bone quality by selection, assuming a simple phenotype can be developed. We can find no evidence for a relationship between post peak egg production and bone quality. However, in one line there is an effect of age at first egg. This suggests the move towards longer laying periods will not necessarily impact bone quality but will need to be carefully monitored. These results will inform and guide future efforts to improve bone quality and, in some cases, merits an examination of puberty onset.

Acknowledgements

Funding ERANET grant BB/M028291/1; COST Action CA15224 Keel Bone Damage.

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