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Effects of oregano essential oil supplementation to diets for broiler chicks with delayed feeding after hatching. 1. Performances and digestibility of nutrients

Einfluss eines Zusatzes von essentiellen Ölen aus Oregano zu Broilerrationen bei verzögerter Anfütterung nach dem Schlupf. 1. Leistung und Nährstoffverdaulichkeit

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Introduction

In the last few decades, broiler chicks weighing 45–50 g at one-day of age, show 40–45 times increase in body weight at 40 days of age or an even shorter period of time (Van Den Brand et al., 2010). The magnitude of this growth indicates that every day immediately after hatch in the broiler's life becomes more important in reaching target weight (Panda et al., 2009). However, under practical conditions, a fasting period of 24 to 72 h after hatch during transportation to the broiler farm is generally common, due to variation in hatching time and logistics (Willemsen et al., 2010). This delay in start of feed and water intake leads to dehydration and negatively affects yolk utilization, gastrointestinal development, digestive enzyme stimulation, immune system activation, slaughter weight and breast meat yield (Van Den Brand et al., 2010; Willemsen et al., 2010). Newly hatched broiler chicks become more susceptible to pathogens, restricting the development of critical tissues and increasing weight loss when access to feed and water is delayed (Nielsen et al., 2010).

Studies have shown that offering nutrients immediately after hatch to chicks and poults results in increased growth (Kadam et al., 2009). In an attempt to decrease the negative effects of delayed feeding, recent researches have evaluated the use of feed supplements. Many researches suggest that early feeding of certain nutrients or feed additives has a great effect on digestive enzyme activity and growth performance (Henderson et al., 2008; Kadam et al., 2009; Basmacioglu Malayoglu et al., 2010).

The limited use of antibiotic growth promoters has accelerated investigations on alternative feed additives for animal diets. Herbs, spices and products derived thereof, mainly essential oils, have gained interest as phytogenic feed additives in recent years (Steiner, 2009). The antimicrobial effects (Jamroz et al., 2003; Mitsch et al., 2004), the stimulation of digestive enzyme secretion and the increase in gastric and intestinal motility as well as the improvement in performance by essential oils or plant extracts have been shown *in vivo* studies (Hernandez et al., 2004; Zhang et al., 2005; Garcia et al., 2007; Jang et al., 2007).

Oregano, is a characteristics spice in Turkey which is obtained by drying leaves and flowers of *Origanum vulgare* and *onites* spp. grown in wild and cultivated. Oregano essential oil extracted from oregano herb has two major phenols, which are carvacrol and thymol comprising about 78–85% of essential oil (Roofchaee et al., 2011). Thymol and carvacrol can disrupt membrane integrity, which affects pH homeostasis and equilibrium of inorganic ions (Lambert et al., 2001). Scientific evidence exists that herbs and plant extracts stimulate the growth of beneficial bacteria and limit numerous pathogenic bacterial activities in the gut of poultry (Cross et al., 2007; Jang et al., 2007; Roofchaee et al., 2011).

The aim of this study was to investigate the effects of the supplementation of oregano essential oil at different levels (250 or 500 mg kg^{-1}) to diet of broiler chicks with immediate, 24, 48 or 72 h delayed feeding on growth performance, digestive organs, nutrient composition of carcass, the ileal digestibility of nutrients and bacterial population of small intestine from hatching to 21 days of age.

Material and Methods

Seven hundred and twenty male broiler chicks were taken from the hatchery where time of hatch was defined as time of clearing the shell. Then, the chicks were wing-banded, weighed and randomly assigned to twelve groups of similar mean weight with three replicates of 20 chicks, each. In a 4 × 3 factorial arrangement, broiler chicks had access to water and diet at four different feeding times after hatch (immediate, delayed by 24, 48 or 72 h posthatching) and were fed one of 3 different all-mash diets, which were based on corn-soybean meal: diet 1 (CONT) – a commercial basal diet which contained no oregano essential oils, diet 2 (OO250) – supplemented with oregano essential oil at a level of 250 mg kg^{-1} and diet 3 (OO500) – supplemented with oregano essential oil at a level of 500 mg kg^{-1}. The ingredients and nutritional composition of the commercial basal diet are given in Table 1. The diets were formulated to meet or exceed minimum NRC (1994) standards for all ingredients. The basal diet contained 230 g kg^{-1} crude protein and 13.0 MJ kg^{-1} metabolisable energy (ME).

The oregano essential oil was provided by the Altes Agricultural Products Ltd Company (Antalya, Turkey). The Oregano essential oil used in the study was obtained by steam-distillation using Clevenger distillation apparatus and was

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Table 1. Compositions (g kg^{-1}) of the basal diet
Zusammensetzung der Basisration (g/kg)

Ingredients	Starter (0–3 weeks)
Corn	510.00
Soybean meal	285.00
Fullfat soybean	140.00
Sunflower oil	24.90
Limestone	10.50
Dicalcium phosphate	20.50
Salt	3.50
Vitamin premix[1]	2.50
Trace mineral premix[2]	1.00
DL-methionine	2.10
Total	1000.00
Calculated chemical composition, (g kg^{-1})	
Dry matter	901.0
Crude protein	230.0
Metabolisable energy, MJ kg^{-1}	12.99
Calcium	10.1
Phosphorus (avail.)	4.5
Methionine	6.2
Met. + Cys.	9.4
Lysine	12.9
Analysed chemical composition, (g kg^{-1})	
Dry matter	898.20
Crude protein	230.09
Crude fat	69.80
Crude fiber	39.60
Crude ash	19.60

[1] Vitamin premix provides per kg of diet: *trans*-retinol 3600 µg, cholecalciferol 15.0 µg, α-tocopherol acetate 50 mg, vitamin K_3 5 mg, vitamin B_1 3 mg, vitamin B_2 6 mg, vitamin B_6 5 mg, vitamin B_{12} 0.03 mg, niacin 25 mg, Ca-D-pantothenate 12 mg, folic acid 1 mg, D-biotin 0.05 mg, apo-carotenoic acid ester 2.5 mg, choline chloride 400 mg
[2] Trace mineral premix provided the following (per kg diet): Mn 80 mg, Fe 60 mg, Zn 60 mg, Cu 5 mg, Co 0.20 mg, I 1 mg, Se 0.15 mg.

derived from *Origanum onites* spp. growing wild in Turkey. The carvacrol and thymol contents, which are the most active compounds of oregano essential oil were determined as 84.0% and 1.78%, respectively. The essential oil obtained was diluted with n-hexane (1 100^{-1}) and injected into a GC MS (HP 6890GC 5973MSD) system (injection temperature: 250°C; injection split: 1 100^{-1}; column: DB-17 30 m, 0.25 µm, 0.32 mm (Agilent); oven programme: initial temperature, 70°C, rate 8°C min^{-1}, final temperature: 200°C, injection vol., 1µl).

Oregano essential oil was supplemented to an amount of sunflower oil and homogenised by mixer and then the mixture was blended with corn. Corn with essential oil was added to pre-mixture. Finally, the pre-mixture was supplemented to the main diet, prepared weekly and stored in airtight containers. Prior to experimental diet formulation, feed ingredients were analyzed for their dry matter, crude protein, crude fat, crude fiber, starch and total sugar content according to the methods of the Association of Analytical Chemists (AOAC, 2007). ME of feed ingredients was calculated based on analyzed values of feedstuffs (WPSA, 1989). All values were expressed on a dry matter basis.

During the rearing period which lasted 21 days the experimental diets and drinking water were offered *ad libitum*. The chicks were kept in wire cages equipped with nipple drinkers (Cimuka, Ankara, Turkey) under standard environmental conditions throughout the experiment. A continuous lighting program was provided. Temperature and humidity in the room were controlled throughout the experiment. Ambient temperature was gradually decreased from 32°C on d 0 to 25°C on d 21.

Body weights of broilers in each experimental treatment were measured on d 0, 4 and 21. Chicks were weighed to ± 0.001 g at hatch and ± 1 g thereafter. During the period of 14–21 d, feed intake was recorded to the nearest g and feed conversion ratio was calculated as feed intake (g)/body weight gain (g). Mortality was recorded daily.

On d 4 and 21, nine male chickens whose body weights were similar to the group average were selected from each experimental groups and slaughtered by severing the jugular vein to determine remained egg yolk' weight and organ development. Digestive organs such as liver, bile bladder, pancreas, proventriculus, gizzard and small intestine and egg yolk were dissected and weighed to the nearest 0.001 g and values. Relative organ weights are presented (g 100 g^{-1} body weight).

On d 21, additional nine male chickens whose body weights were similar to the group average were selected from each experimental groups and slaughtered to determine the nutrient composition of carcass and bacterial populations of the small intestine. Carcasses were analyzed for their dry matter, crude protein, crude fat and crude ash content according to the methods of the Association of Analytical Chemists (AOAC, 2007).

On d 21, the small intestine (from the distal end of the duodenum to the ileocaecal junction) of slaughtered chickens was removed from each bird and put on ice until they were transported to the laboratory to determine the number of coliform and total aerobic bacteria. The small intestine was rapidly opened longitudinally, the mucosal surface and digesta were scraped with a sterile surgical knife and then samples of the small intestine were transferred under aseptic conditions into steril tubes. One gram intestinal content was diluted 1 to 9^{-1} (wt vol^{-1}) with physiological salt water (log_{10}). Samples were serially diluted from 10^{-1} to 10^{-9} to determine total aerobic bacteria colony forming units (cfu) in the intestine and enumerated on Nutrient Agar after incubation at 37°C after 48 h. Samples for coliform concentration were diluted serially from 10^{-1} to 10^{-9} to determine coliform concentration in the small intestine by enumeration on EMB (Eosine Methylene Blue) after incubation at 37°C after 48 h (Arda et al., 1997).

To determine the ileal digestibility of nutrients, all chickens from 16 to 21 d were fed diets supplemented with 0.4 g kg^{-1} of chromium oxide (Cr_2O_3) as a marker. On the last day of this period, 2 h after feeding, 8 animals per treatment group were slaughtered and the intestinal contents from the Meckel's diverticulum until 10 cm anterior to the junction of the caecum and large intestine were taken out and the ileal contents of chickens were immediately collected, lyophilized and ground to pass through a 0.5 mm sieve and analyzed for organic matter and crude protein according to the methods of the Association of Analytical Chemists (AOAC, 2007).

Statistical analysis

Linear Model using the SPSS (17.0)® statistic package (SPSS, 2007) was applied to data with a model including

essential oil and accessing time to diet and water and interaction between essential oil and accessing time to diet and water. Significant differences between treatment means were separated using Duncan's multiple range test (Duncan, 1955). Results were presented at least square means and standard error of means. All statements of significance were based on $P \leq 0.05$.

Results and Discussion

Performance parameters

Average body weight of chicks at hatching day was not significantly different among experimental treatments (Table 2).

The effects of dietary treatments and accessing time to diet and water on the body weight gain of broilers from 0 to 21 d are summarized in Table 2. Dietary treatments did not significantly affect the body weight gain of male chickens during the period of 21 d. However body weight gain of broilers from 0 to 21 d was significantly decreased by delayed access to diet and water for 24, 48 or 72 hours compared with those of broilers which had an immediate access ($P < 0.01$). As indicated in Table 2, especially broilers fasted for 72 h had the significantly lowest body weight gain from 0 to 21 d ($P < 0.01$). Our results on body weight gain concur with the findings of Bigot et al. (2003a) and Abed et al. (2011) who reported that feed deprivation for 2 d posthatching of broilers was not sufficient to compensate for the retardation of body weight gain. Delayed access to diet and water may have caused an increased susceptibility to pathogens and a weight loss leading to poorly starting flocks with reduced weight gains (Henderson et al., 2008). Supplementation of oregano essential oil to broiler diets (Botsoglou et al., 2002; Basmacioglu et al., 2004; Cross et al., 2007; Roofchaee et al., 2011) at levels of 50 and 100, 150 and 300 mg kg^{-1}, 1000 mg kg^{-1}, 1200 mg kg^{-1}, respectively, had no beneficial effect on growth performance. In

Table 2. Effects of experimental treatments on initial body weight (IBW), body weight gain (BWG) from 0 to 21 d, feed intake (FI) and feed conversion ratio (FCR) as average/day from 4 to 21 d and ileal digestibility coefficient of organic matter and crude protein in broilers at 21 d

Effekt der Behandlungen auf das Gewicht der Eintagsküken (IBW), die Gewichtszunahmen (BWG) zwischen dem 0. und 21. Lebenstag, auf die tägliche Futteraufnahme (FI) und die Futterverwertung (FCR) im Zeitraum 4. bis 21. Lebenstag sowie auf die präcecalen Verdaulichkeitskoeffizenten für die Organische Substanz und das Rohprotein am 21. Lebenstag

DT	AT	IBW (g)	BWG (g)	FI (g)	FCR (g g^{-1})	Organic Matter	Crude Protein
CONT	Immediate	37.6	41.8	59.6	1.45	0.850	0.910
	24 h	37.2	37.8	62.1	1.64	0.620	0.790
	48 h	37.0	33.3	66.1	2.00	0.611	0.791
	72 h	37.5	31.5	61.5	1.96	0.500	0.701
OO250	Immediate	37.3	37.7	60.7	1.61	0.821	0.841
	24 h	37.3	35.7	58.2	1.63	0.831	0.900
	48 h	37.1	36.6	62.4	1.71	0.810	0.912
	72 h	37.7	29.4	57.9	1.96	0.690	0.810
OO500	Immediate	37.1	40.3	58.3	1.48	0.880	0.931
	24 h	37.0	33.7	57.4	1.71	0.882	0.930
	48 h	37.2	37.0	60.0	1.67	0.850	0.921
	72 h	37.6	32.6	61.9	1.90	0.691	0.861
DT	CONT		36.1	62.3	1.77	0.645^{b}	0.800^{b}
	OO250		34.9	59.8	1.73	0.788^{a}	0.866^{ab}
	OO500		35.9	59.4	1.69	0.826^{a}	0.911^{a}
AT	Immediate		39.9^{a}	59.5	1.51^{c}	0.850^{a}	0.894^{a}
	24 h		35.8^{b}	59.2	1.66^{bc}	0.778^{ab}	0.873^{ab}
	48 h		35.6^{b}	62.8	1.79^{ab}	0.757^{ab}	0.875^{ab}
	72 h		31.2^{c}	60.4	1.94^{a}	0.627^{b}	0.791^{b}
Pooled SEM		0.097	0.823	0.771	0.040	0.029	0.017
P value							
DT			NS	NS	NS	*	*
AT			**	NS	**	*	*
DTxAT			NS	NS	NS	NS	NS

$^{a-c}$ Row means with common superscripts do not differ (** $P > 0.01$)

DT: dietary treatments; AT: accessing time to diet and water; CONT: no contained essential oil; OO250: oregano essential oil, 250 mg kg^{-1}; OO500: oregano essential oil, 500 mg kg^{-1}; SEM: Standard Error of Means

contrast to these results, other studies showed that inclusion of oregano essential oil to drinking water at 150 or 399 ml ton^{-1} improved broiler performance (Bassett, 2000).

Experimental treatments did not significantly affect feed intake of chickens in the period 4 to 21 d (Table 2). The findings of this study concur with the results of Tabeidian et al. (2011) who pointed out that broilers fasted for 24 or 48 h had the same feed intake compared to broilers with immediate access to feed. On the other hand, Gonzales et al. (2003) and Abed et al. (2011) reported that broilers fasted for 24 or 48 h, respectively, consumed significantly less feed compared to broilers with immediate access to feed. Likewise, Bouvarel et al. (2007) pointed out that sequential feeding negatively affected growth performance by decreasing feed intake of broiler chicks.

The results of this study are in agreement with the findings of Lee et al. (2003a, b) and Hernandez et al. (2004) who reported that no difference in feed intake was observed by dietary supplementation of essential oil components. Feed conversion ratios of broilers fasted for 48 or 72 h from 4 to 21 d were significantly increased compared with that of chickens with immediate accessed to feed ($P < 0.01$). However, dietary treatments did not significantly influence feed conversion ratio from in the period 4 to 21 d. Our results on feed intake and feed conversion ratio concur with the finding of Saki (2005) and Tabeidian et al. (2011) who reported that no significant differences were observed in feed conversion ratio of broilers fed starter diet immediately or after 24 h fasting throughout the experiment.

These results are not in agreement with the findings of Kadam et al. (2009) who reported that supplementation of broiler diets with phytobiotics has a significant impact on early growth, on weekly weight gain, and on feed efficiency in contrast to the control indicating an overall beneficial effect of polyherbal product in young broiler chicks. *In vivo* studies showed that essential oils act by their dose, by the concentrations of active components, by the intensity of infection, by the diet composition and by the environment (Lee et al., 2003a; Cross et al., 2007).

In the present experiment, the diet contained highly digestible ingredients so that bacterial growth in the intestine probably may have been limited. Likewise, it is known that well-nourished, healthy chicks do not respond to antimicrobial growth promoters such as oregano essential oil when they are housed under clean and disinfected conditions, as it was the case in our study.

Ileal nutrient digestibility

The effects of experimental treatments on the ileal nutrient digestibility in broilers at 21 d of age are summarized in Table 2. Both, dietary treatment and accessing time to feed and water significantly affected the ileal digestibility of organic matter and crude protein at 21 d ($P < 0.05$). Delayed access to diet and water for 72 h significantly aggravated the ileal digestibility of organic matter and crude protein when compared to those of chickens with immediate access to diet and water ($P < 0.05$). As a result of this, body weight gain in the period 0 to 21 d ($P < 0.01$) and feed conversion ratio from 4 to 21 d ($P < 0.05$) were negatively affected by delayed access to diet and water for 72 h posthatch.

The ileal digestibility of organic matter was significantly lower in the CONT diet compared to diets supplemented with oregano essential oil at 250 or 500 mg kg^{-1}. Likewise, the dietary addition of oregano essential oil at 500 mg kg^{-1} significantly improved the ileal digestibility of crude protein compared to CONT diet ($P < 0.05$). The positive effect of oregano essential on the digestive enzyme activity has been reported previously, assuming stimulating effects on the output of digestive enzymes from the pancreas, gut mucosa and increased bile flow (Amad et al., 2011). This improvement in the ileal digestibility of crude protein may result from the increased activity of chymotrypsin in pancreas and in digesta of small intestine of broilers due to supplementation of oregano essential oil to diets (Basmacioglu-Malayoglu et al., 2010) and from an increased absorption surface area in the intestine (Amad et al., 2011).

In disagreement with our result, Hernandez et al. (2004) noted that essential oil extract from oregano, cinnamon and pepper at level of 200 mg kg^{-1} did not significantly affect crude protein ileal digestibility. Jamroz et al. (2003) showed that the apparent ileal digestibility of nutrients (crude protein, fibre and amino acids) was not significantly better in birds fed diet supplemented with 100 mg kg^{-1} plant extract containing capsaicin, cinnamaldehyde and carvacrol than in the control group. Our result on the ileal digestibility of crude protein did not concur with the findings Lee et al. (2003b) who showed that the dietary supplementation of thymol, cinnamaldehyde and a commercial preparation of essential oil components (CRINA® Poultry) did not significantly influence the ileal digestibility of protein in broiler chickens for 21 d.

Weight of the egg yolk and digestive organs

As indicated in Table 3, the relative weight of egg yolk of chicks at 4 or 7 d was not significantly affected by experimental treatments. This finding concurs with the results of Abed et al. (2011) who reported that absolute and relative weight of residual yolk sac to body weight at three days of age was not influenced by duration of diet and water deprivation. The results are in agreement with Noy and Sklan (1997) who concluded that the effect of feed intake on yolk utilization may be attributed to enhanced transport of yolk to the gastrointestinal tract due to increased intestinal motility and activity after feed and water are ingested. Residual yolk sac is used up more quickly in chicken that have access to feed immediately after hatch than those fasted for 48 h (Kadam et al., 2009). Otherwise, egg yolk was completely depleted until 7 d.

The effects of the experimental treatments on the relative weights of liver, bile bladder, pancreas, proventriculus, gizzard and small intestine at 4 and 21 d are shown in Table 4. Dietary treatments and delayed access to diet and water did not significantly affect relative weights of liver, bile bladder, pancreas and small intestine at 4 d. However, access to diet and water for 72 h posthatch significantly decreased the relative weight of proventriculus of chicks at 4 d ($P < 0.05$) compared to that of chicks with immediate or 24 h posthatch access to diet and water, respectively. In addition, the relative weight of gizzard of chicks at 4 d was significantly increased as accessing time to diet and water was extended ($P < 0.01$). This finding concurs with that reported by Abed et al. (2011) who indicated that a delayed access to diet and water for 48 h posthatch had no adverse effect on relative weights of pancreas, proventriculus and gizzard of broilers at 3 and 21 d of age. In contrast, Corless and Sell (1999) pointed out that delaying access to feed and water (a 54-h placement time) resulted in depressed relative weight of the pancreas at 4 d of age. On the other hand, many studies (Hernandez et al., 2004; Lee et al., 2004a, b; Jang et al., 2007) noted that essential oils or their components supplementation alone did not affect organ (liver, pancreas etc.) weights

Neither dietary treatments nor delayed access to diet and water did significantly influence the relative weights of liver, bile bladder, pancreas, proventriculus and small intestine of chickens at 21 d (Table 4). Only access to diet

Table 3. Effects of oregano essential oil supplementation at three different levels to diets and delayed access to diet and water at four different times on relative weight of egg yolk at 4 and 7 d

Einfluss der Zulage von essentiellen Ölen des Oregano zu den Rationen in drei verschiedenen Höhen und des verzögerten Zugangs zu Futter und Wasser in vier Stufen auf das relative Gewicht des Dottersacks am 4. und am 7. Lebenstag

DT	AT	4 d	7 d
CONT	Immediate	0.720	0.081
	24 h	0.653	0.163
	48 h	1.68	0.222
	72 h	1.34	0.524
OO250	Immediate	0.844	0.080
	24 h	1.08	0.052
	48 h	1.13	0.142
	72 h	0.991	0.391
OO500	Immediate	0.483	0.041
	24 h	0.942	0.050
	48 h	2.05	0.413
	72 h	3.25	0.052
DT	CONT	1.10	0.248
	OO250	1.01	0.166
	OO500	1.68	0.139
AT	Immediate	0.682	0.067
	24 h	0.892	0.088
	48 h	1.62	0.259
	72 h	1.86	0.322
Pooled SEM		1.582	0.257
P value			
DT		NS	NS
AT		NS	NS
DTxAT		NS	NS

DT: dietary treatments; AT: accessing time to diet and water; CONT: no contained essential oil; OO250: oregano essential oil, 250 mg kg^{-1}; OO500: oregano essential oil, 500 mg kg^{-1}; SEM: Standard Error of Means

and water for 72 h posthatch significantly increased the relative weight of the gizzard at 21 d ($P < 0.05$). Palo et al. (1995) reported that the broiler chickens with delayed access to diet and water exhibited a higher proportional gizzard weight on days 14 and 21 compared to the chickens with immediate access to diet and water. The supply organs such as gizzard of previously restricted broiler chickens might need to "catch-up" first when exceed in absolute weight those of the controls before compensatory growth can occur. These findings concur with the results of Abed et al. (2011) who reported that the relative weight of the pancreas, proventriculus and gizzard at 21 d of age were not affected by diet and water deprivation. This result is not in agreement with the findings of Lee et al. (2004a) who showed that dietary thymol supplementation significantly increased relative liver weight in broilers at 21 d.

Nutrient composition of carcass

The effects of experimental treatments on the nutrient composition of broiler carcass at 21 d are summarized in Table 5. In this study, neither dietary treatments nor delayed access to diet and water significantly affected the nutrient composition of carcass of chickens on d 21. The results of our study concur with the findings of Pinchasov and Noy (1993) who reported that carcass composition of newly-hatched broilers did not differ from those held for 24 h.

On the other hand, Bigot et al. (2003b) reported that chicken muscle ribosomal protein S6 kinase (S6K1) is a key regulator of protein synthesis. According to these authors, the S6K1 pathway was stimulated to the same level in the early-feeding and 48 h-delayed-feeding chicks, which indicates that post-hatching starvation did not increase S6K1 activation. As a result, the protein content of broiler carcass was not significantly changed because muscle ribosomal protein S6 kinase extent was not affected by the early-feeding and 48 h-delayed-feeding of broiler chicks.

Bacterial populations of small intestine

Effects of experimental treatments on bacterial populations of small intestine of chickens at 21 d are given in Table 6. The total aerobic and coliform bacteria contents of small intestine at 21 d were significantly increased by extending the access to diet and water of broilers ($P < 0.01$). However, dietary treatments did not significantly influence the total aerobic and coliform bacteria contents of small intestine in broilers at 21 d.

The lacking effect of oregano essential oil supplementation may result from using corn-based diets contained highly digestible ingredients in diets of broiler raised under a cage environment which provided good hygienic conditions. As a result, it was suggested that the antimicrobial activity of oregano essential oil may be masked by diet composition and/or environment.

Conclusions

The negative effects of delayed access to diet and water for 72 h on growth performance, ileal digestibilities of nutrients and bacterial populations of small intestine could not be improved by dietary supplementation of oregano essential oil at the different levels. There is need for further researches to determine the optimal dietary inclusion level and the mode of actions of the active compounds in oregano essential oil to remove the negative effects on growth performance, digestive organ weights, the digestibility of nutrients and bacterial content of small intestine of broilers with delayed access to diet and water.

Summary

The study was conducted to investigate the effects of dietary supplementation of oregano essential oil at different levels (0, 250 or 500 mg kg^{-1}) on growth performance, digestive organs, nutrient composition of carcass, nutrient digestibility and bacterial populations of the small intestine of broiler chicks with immediate, 24, 48 or 72 h delayed feeding from d 0 to 21. The following diets were used from 0 to 21 d. Diet 1 (control, CONT): a commercial diet containing no essential oil, diet 2 (OO250): supplemented with oregano essential oil at 250 mg kg^{-1} and diet 3 (OO500): supplemented with oregano essential oil at 500 mg kg^{-1}.

Table 4. Effects of experimental treatments on relative weight of digestive organs of chicks at 4 and 21 d (g 100 g^{-1} body weight)
Einfluss der Behandlungen auf das relative Gewicht der Verdauungsorgane der Broiler am 4. und am 21. Lebenstag (g/100 g Körpergewicht)

		Liver		Bile bladder		Pancreas		Proventriculus		Gizzard		Small intestine	
DT	AT	4 d	21 d	4 d	21 d	4 d	21 d	4 d	21 d	4 d	21 d	4 d	21 d
CONT	Immediate	3.41	3.90	0.240	0.171	0.514	0.421	1.51	0.620	7.46	3.36	7.42	5.43
	24 h	3.30	3.08	0.220	0.191	0.473	0.402	1.47	0.631	8.56	3.59	8.16	4.52
	48 h	4.31	2.77	0.171	0.120	0.413	0.361	1.40	0.690	9.39	3.53	8.29	4.09
	72 h	3.60	3.37	0.902	0.153	0.312	0.394	1.27	0.732	10.2	3.27	6.75	4.73
OO250	Immediate	3.45	3.71	0.300	0.134	0.472	0.422	1.40	0.601	7.92	3.47	7.07	4.77
	24 h	3.46	2.91	0.141	0.132	0.551	0.383	1.66	0.654	9.39	3.21	8.86	3.99
	48 h	3.78	4.17	0.171	0.221	0.403	0.614	1.44	1.05	10.3	4.01	8.41	5.48
	72 h	4.00	3.39	0.230	0.110	0.582	0.444	1.27	0.751	9.68	4.59	8.37	5.34
OO500	Immediate	3.50	3.17	0.231	0.151	0.461	0.421	1.55	0.651	7.20	3.38	7.58	4.11
	24 h	3.57	3.89	0.170	0.104	0.450	0.413	1.46	0.644	8.53	4.02	6.83	5.58
	48 h	3.59	3.34	0.141	0.192	0.523	0.402	1.40	0.693	9.94	3.73	8.09	4.51
	72 h	3.29	3.22	0.121	0.132	0.321	0.451	1.30	0.702	11.1	4.06	6.53	5.31
DT	CONT	3.66	3.28	0.383	0.159	0.428	0.395	1.41	0.668	8.90	3.69	7.65	4.69
	OO250	3.67	3.55	0.211	0.149	0.502	0.466	1.44	0.765	9.32	3.82	8.18	4.89
	OO500	3.49	3.41	0.166	0.145	0.439	0.422	1.43	0.673	9.18	3.80	7.26	4.88
AT	Immediate	3.45	3.59	0.257	0.152	0.482	0.421	1.49^{a}	0.624	7.52^{c}	3.41^{b}	7.36	4.77
	24 h	3.45	3.30	0.177	0.142	0.491	0.399	1.53^{a}	0.643	8.83^{b}	3.61^{b}	7.95	4.70
	48 h	3.89	3.43	0.161	0.178	0.446	0.459	1.41^{ab}	0.812	9.88^{a}	3.75^{b}	8.26	4.69
	72 h	3.63	3.33	0.418	0.132	0.405	0.430	1.28^{b}	0.728	10.3^{a}	4.31^{a}	7.22	5.13
Pooled SEM		0.520	0.134	0.378	0.068	0.130	0.120	0.186	0.183	1.477	0.103	0.175	0.199
P value													
DT		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
AT		NS	NS	NS	NS	NS	NS	*	NS	**	*	NS	NS
DTxAT		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

$^{a–c}$ Row means with common superscripts do not differ (* P > 0.05; ** P > 0.01)
DT: dietary treatments; T: accessing time to diet and water; CONT: no contained essential oil; OO250: oregano essential oil, 250 mg kg^{-1}; OO500: oregano essential oil, 500 mg kg^{-1}; SEM: Standard Error of Means

Especially, with extended delay of access to diet and water body weight gain during the period of 0 and 21 d was significantly decreased and feed conversion ratio from 4 to 21 d-old broilers was significantly aggravated. Experimental treatments did not affect the relative weights of yolk sac and digestive organs except gizzard and the nutrient composition of carcass in broilers at 21 d. CONT group or the group with delayed access to diet and water for 72 h showed a significantly reduced ileal digestibility of organic matter and crude protein. The extension of the delayed access to diet and water significantly increased total aerobic bacteria and coliform bacteria contents of chickens' small intestine at 21 d. The supplemented levels of oregano essential oil to the diet have not been sufficient to compensating the negative effects on growth performance. The reason was probably the aggravated bacterial content of small intestine and the nutrient ileal digestibility by the delayed access to diet and water. There is need for further research to determine the effects of the optimal level or of the combined use of the active compounds in oregano essential oil in diet to remove the negative effects on growth performance, digestive organs, nutrient digestibility and bacterial populations of small intestine of broilers with delayed access to diet and water.

Key words

Broiler chicks, delayed access, oregano essential oil, performance, bacterial content, small intestine

Zusammenfassung

Einfluss eines Zusatzes von essentiellen Ölen aus Oregano zu Broilerrationen bei verzögerter Anfütterung nach dem Schlupf. 1. Leistung und Nährstoffverdaulichkeit

Das Ziel der Studie war die Untersuchung der Effekte einer Zugabe von essentiellen Ölen aus Oregano in verschiedener Höhe zum Broilerfutter (0, 250, 500 mg/kg) auf die Wachstumsleistung, die Verdauungsorgane, den Nährstoffgehalt des Schlachtkörpers, die Nährstoffverdaulichkeit und die

Table 5. Effects of oregano essential oil supplementation at three different levels to diets and delayed access to diet and water at four different times on nutrient composition of carcass of broiler chickens at 21 d of age (%)
Einfluss der Zulage von essentiellen Ölen des Oregano zu den Rationen in drei verschiedenen Höhen und des verzögerten Zugangs zu Futter und Wasser in vier Stufen auf den Nährstoffgehalt des Schlachtkörpers am 21. Lebenstag (%)

DT	AT	Crude Matter	Crude Ash	Crude Fat	Crude Protein
CONT	Immediate	28.0	1.21	3.99	20.0
	24 h	26.2	1.14	3.33	21.6
	48 h	25.8	1.31	3.04	19.3
	72 h	26.2	1.68	4.33	19.8
OO250	Immediate	25.2	1.14	3.42	20.3
	24 h	27.0	1.21	4.45	20.1
	48 h	27.3	1.26	4.01	20.3
	72 h	25.0	1.24	3.36	20.8
OO500	Immediate	24.8	1.42	3.15	20.0
	24 h	23.9	1.70	3.17	20.5
	48 h	24.9	1.36	2.34	20.6
	72 h	25.3	1.51	5.98	21.0
DT	CONT	26.5	1.33	3.68	20.2
	OO250	26.1	1.21	3.81	20.4
	OO500	24.7	1.50	3.66	20.5
AT	Immediate	26.0	1.26	3.52	20.1
	24 h	25.7	1.35	3.65	20.7
	48 h	26.0	1.31	3.13	20.1
	72 h	25.5	1.48	4.56	20.5
Pooled SEM		0.353	0.071	0.248	0.403
P value					
DT		NS	NS	NS	NS
AT		NS	NS	NS	NS
DTxAT		NS	NS	NS	NS

DT: dietary treatments; T: accessing time to diet and water;
CONT: no contained essential oil; OO250: oregano essential oil, 250 mg kg^{-1}; OO500: oregano essential oil, 500 mg kg^{-1};
SEM: Standard Error of Means

Table 6. Effects of experimental treatments on the bacterial populations of small intestine of broilers at 21 d
Einfluss der Behandlungen auf die Bakterienpopulation im Dünndarm von Broilern am 21. Lebenstag

DT	AT	Total aerobic bacteria (x10^6 cfu g^{-1})	Coliform (x10^5 cfu g^{-1})
CONT	Immediate	2.01	1.02
	24 h	3.50	2.00
	48 h	8.00	3.50
	72 h	10.0	8.01
OO250	Immediate	4.01	1.51
	24 h	6.02	2.01
	48 h	10.0	3.00
	72 h	10.0	4.50
OO500	Immediate	4.50	1.50
	24 h	5.00	1.50
	48 h	6.51	3.01
	72 h	10.0	3.51
DT	CONT	5.88	3.63
	OO250	7.51	2.76
	OO500	6.50	2.38
AT	Immediate	3.51^c	1.34^c
	24 h	4.84^c	1.84^c
	48 h	8.17^b	3.17^b
	72 h	10.0^a	5.34^a
Pooled SEM		0.534	0.361
P value			
DT		NS	NS
AT		**	**
DTxAT		NS	NS

$^{a–c}$ Row means with common superscripts do not differ (* P > 0.05)
DT: dietary treatments; T: accessing time to diet and water;
CONT: no contained
essential oil; OO250: oregano essential oil, 250 mg kg^{-1};
OO500: oregano essential
oil, 500 mg kg^{-1}; SEM: Standard Error of Means; cfu: colony forming units

Zusammensetzung der Bakterienflora im Dünndarm nach Anfütterung unmittelbar, 24, 48 oder 72 Stunden nach dem Schlupf in den ersten 21 Lebenstagen. Folgende Futterrationen wurden eingesetzt: Kontrolle (CONT) – kommerzielle Basisration ohne Zusatz von essentiellen Ölen, Ration 2 (OO250) – Basisration mit Zulage von 250 mg essentiellen Ölen/kg, Ration 3 (OO500) – Basisration mit Zulage von 500 mg essentiellen Ölen/kg.

Vor allem die Verlängerung des Zeitraums bis zur Anfütterung der Küken führte zu einer signifikanten Verminderung der Gewichtszunahme zwischen dem 1. und 21. Lebenstag und beeinträchtigte die Futterverwertung zwischen dem 4. und 21. Lebenstag. Die Zulagen an essentiellen Ölen hatten keinen Einfluss auf die relativen Gewichte des Dottersacks und der Verdauungsorgane, mit Ausnahme des Muskelmagens, sowie auf die Nährstoffzusammensetzung des Schlachtkörpers am 21. Lebenstag. In der Kontrollgruppe und in der Gruppe, die erst nach 72 Stunden angefüttert wurde, war die präcecale Verdaulichkeit der Organischen Substanz und des Rohproteins vermindert. Die Verlängerung des Zeitraums bis zur Anfütterung erhöhte die Gehalte an gesamten, aeroben Bakterien und an coliformen Bakterien im Dünndarm der Tiere am 21. Lebenstag signifikant. Die Zulage an essentiellen Ölen aus Oregano konnte die negativen Effekte der verzögerten Anfütterung auf die Wachstumsleistung nicht ausgleichen, da durch die verzögerte Nahrungsaufnahme die Bakterienzahlen im Dünndarm und die präcecale Verdaulichkeit der Nährstoffe verschlechtert wurde. Es besteht daher weiterer Forschungsbedarf hinsichtlich der optimalen Einsatzmengen der essentiellen Öle und der Kombination der aktiven Komponenten in den essentiellen Ölen des Oregano im Futter, um die nega-

tiven Effekte auf die Wachstumsleistung, die Verdauungsorgane, die Nährstoffverdaulichkeit und die Bakterienpopulation im Dünndarm von Broilern zu vermindern, die einen verzögerten Zugang zu Futter und Wasser haben.

Stichworte

Broiler, verzögerter Zugang, Essentielle Öle, Oregano, Leistung, Mikroflora, Dünndarm

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