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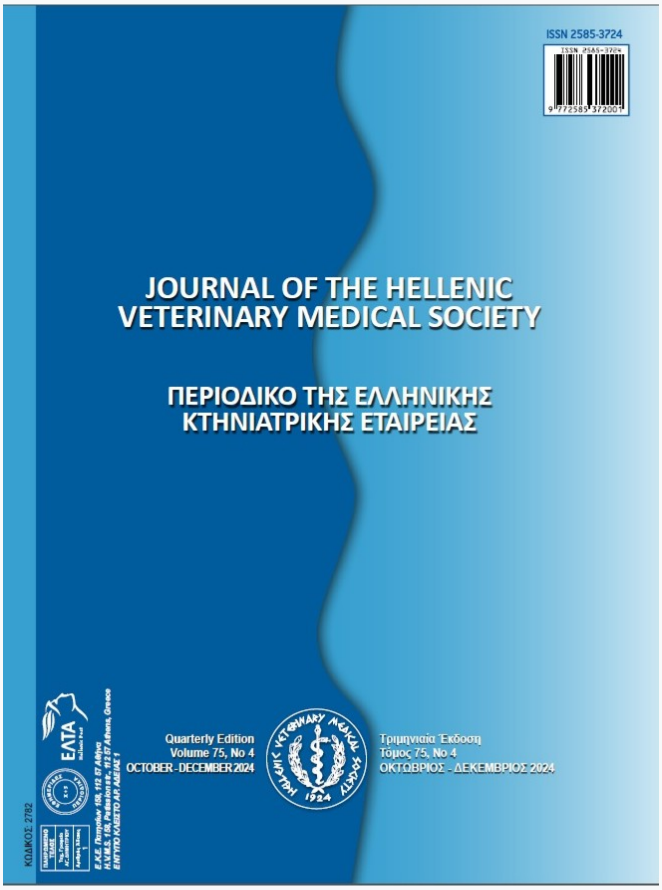

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Research article
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Feeding a blend of thymol and carvacrol to coccidiosis challenged broilers: Effect on performance and gut health

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ABSTRACT: Carvacrol and thymol were used in the diets of coccidiosis-challenged broilers to investigate their performance and gut health. The experiment was conducted using a completely randomized design. There were three phases of feeding for the chickens: starters (1-10 days), growers (11-24 days) and finishers (25-42 days). It was found that chicks were split into three experimental groups: 1) negative control diets without challenges or additives; 2) positive control diets with *Eimeria tenella* challenges and without additives; and 3) diets with the mentioned challenge and thymol and carvacrol (0.01 g/kg). The experimental groups consisted of five replicates, each with 12 chicks. As part of the trial, body weight gain (BWG), feed intake (FCR), and coccidial lesion scores were assessed in two birds/replicate, which were selected randomly and euthanized by cervical dislocation. When thymol and carvacrol were blended, the results indicated improved BWG and FCR ($P<0.05$) when compared to PC. Supplementing the diet with phytogenic essential oil increased the percentage of beneficial bacteria in the ileum, including *lactobacilli* and *bifidobacteria*, while decreasing the number of pathogenic *E.coli* bacteria ($P<0.05$). Compared with PC-treated birds, birds with coccidiosis treated with essential oils had a longer and wider villus, deeper crypts, and more goblet cells ($P<0.05$). Including this additive in PC diet suppressed intestinal lesion scores ($P<0.05$). Because the NC group was not exposed to the challenge, it caused the highest BWG and lowest FCR scores, the best microflora in the ileum, optimal villus growth, and the lowest intestinal lesion scores in birds. Researchers concluded that coccidiosis-challenged birds with high levels of carvacrol and thymol experienced improved performance and gut health ($P>0.05$).

Key words: broiler; carvacrol; coccidiosis; performance; thymol

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INTRODUCTION

The intestinal parasitic disease coccidiosis is the most widespread among poultry producers, causing economic losses. The disease is caused by protozoans of the genus *Eimeria* multiplying in the intestine, causing damage to the intestinal tract. Modifying the microflora and altering the physiology and morphology of the intestinal epithelium causes ulcers, nutrient malabsorption, poor growth, and impaired FCR (Ahmed et al., 2024). It has been shown that cecal coccidiosis results in the proliferation of *Clostridia* and *Enterobacteria* (*coliforms*), while lactic acid-producing bacteria decline (*lactobacilli* and *bifidobacteria*) (Dec et al., 2020). In addition to its anticoccidial properties, dietary supplements with anticlostridial drugs may be beneficial (Williams, 2005). But according to the drug-resistant *Eimeria* strains and with the recent increased emphasis on non-antibiotic broiler production, no-side-effects antibiotic alternatives, *e.g.* phytogenics, have been increasingly substituted for chemotherapeutic control of coccidiosis (Abbas et al., 2012). In other words, adding plants and their extracts as feed additives to treat or prevent coccidiosis is effective in maintaining the health and growth performance of poultry (Oelschlager et al., 2019).

The ecosystem of gut microflora and histomorphology is directly or indirectly affected by essential oils claimed to be alternatives to antibiotics (Taylor, 2001). Optimal animal health and performance require an optimal gut microbiota. Moreover, the gastrointestinal tract acts as a protective barrier against potentially harmful pathogens and environmental allergens. The essential oils; thymol and carvacrol are known as the active components of many medicinal plants such as oregano and thyme to have antiprotozoal activity. It has been shown that dietary supplementation of them reduces clinical signs of coccidiosis in birds and maintains flock health (Abbas et al., 2012). Moreover, oregano essential oil's (thymol and carvacrol) antioxidant activity and gastrointestinal absorption have been shown to improve during Eimeria infections (Ahmad et al., 2024). Furthermore, adding 500 ppm of oregano extract (thymol and carvacrol) reduced the severity of coccidiosis infection in broilers suffering from coccidiosis (Mohiti-Asli and Ghanaatparast-Rashti, 2015).

It is thought that once these active compounds cross the protozoan cell wall, they interact with periplasmic enzymes, and then interact with membrane proteins once they penetrate the lipid-rich interior of the cytoplasmic membrane, causing protons to backflow across the membrane, which affects proton motive force-driven cell activities (Lambert et al., 2001). A flock's health, or more specifically, gut health, can positively affect performance (Abbas et al., 2012).

In view of these factors, the present study investigated the effect of mixing carvacrol with thymol on coccidiosis-challenged broilers, both in terms of performance and health of their digestive tracts.

MATERIALS AND METHODS

Birds, diets and management

A research poultry farm located in Kashmar city was used for the study (35°14′ N and 58° 27′ E) for 42 days. All experimental procedures with the sheep were performed according to regulations established by the Animal Care Committee of AREEO (Agricultural Research, Education and Extension Organization; No. IR. AREEO.14365.125.264).

180 day-old male Ross 308 broiler chicks (average weight 45 g) were obtained from Saleh broiler Breeder Company (Kashmar, Iran). Using the pen system, chickens were raised under hygienic conditions in a ventilated and temperature-controlled environment. Additionally, three experimental groups were randomly assigned: T1) a negative control diet (NC) with no challenge or additive; T2) a positive control diet (PC) with an Eimeria tenella challenge via feed and without additives; T3) a thymol and carvacrol blend (0.01 g/kg) along with the challenge on day 12. The experimental groups consisted of 12 chicks divided into five replicates. All animals received an isocaloric and isonitrogenous diet. All pens had a feeding trough and nipple drinker to provide the birds with free access to food and water. For starters (1-10 days), growers (11-24 days) and finishers (25-42 days), the birds were fed according to the Ross 308 recommendations (Aviagen, 2007), meeting the following metabolic energy (ME) (kcal/kg): 3025, 3150 and 3200, crude protein, CP (%): 23.5, 22 and 20.5, Lys (%): 1.45, 1.26 and 1.10, Met+Cys (%): 1.08, 0.94 and 0.85, Ca (%): 1.04, 0.90 and 0.86, and Ava. P (%): 0.51, 0.46 and 0.42, respectively. As shown in Table 1, the ingredients of the basal diet and its chemical composition can be found. Approximately (1 x 1.5 m^2) of wood shaving litter covered the floor of the pen. In the first week, the birds were subjected to 24-hour lighting, followed by 23-hour light: 1-hour darkness until they were 42 days old. After three days at 32°C, the temperature was gradually lowered by 3°C per week until

Table 1- Ingredients and nutrient composition of the experimental diets (as-fed basis).

Ingredients (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	55.00	55.94	59.94
Wheat	0.00	5.00	7.00
Soybean meal (44%)	37.50	29.65	23.76
Corn gluten (62%)	0.00	3.00	3.00
Soybean oil	3.20	2.06	2.23
Calcium carbonate	1.30	0.98	0.91
Dicalcium phosphate	1.50	1.68	1.52
Sodium chloride	0.20	0.30	0.30
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
L-Lysine HCl	0.25	0.34	0.35
DL-Methionine	0.38	0.28	0.26
L-Threonine	0.00	0.11	0.09
Sodium Bicarbonate	0.12	0.10	0.10
Choline chloride (60%)	0.07	0.06	0.06
Calculated nutrient levels[3]			
Metabolizable energy (kcal/kg)	2950	3000	3050
Crude protein	23.00	21.00	18.89
Lysine	1.00	1.25	1.12
Methionine + Cystine	1.04	0.96	0.88
Threonine	0.94	0.85	0.76
Calcium	1.00	0.84	0.77
Available Phosphorus	0.50	0.42	0.38
Sodium	0.16	0.16	0.16
Potassium	0.85	0.79	0.70
Chlorine	0.31	0.30	0.30

[11]Vitamin supplement provided per Kg of diet: vitamin A (retinylacetate, 9500 IU; vitamin E (DL-α-tocopherol acetate, 30 IU; vitamin K3 (menadione sodium bisulfate), 2.65 mg; vitamin D3 (cholecalciferol), 2500 IU; vitamin B12 (cyanocobalamin), 0.025 mg; biotin, 0.30 mg; nicotinic acid, 50 mg; folic acid, 1.25 mg; d-pantothenic acid, 12 mg; riboflavin, 6.5 mg; pyridoxine hydrochloride, 6.0 mg; thiamine mononitrate, 3 mg.

[2]Mineral supplement provided per Kg of diet: zinc, 80 mg; manganese, 100 mg; copper 8 mg; iron, 80 mg; iodine, 0.35 mg; selenium, 0.15 mg.

[3]Calculated values according to the analyzed data of experimental diets

23°C was reached, and then remained constant. At the beginning of the experiment, the humidity was 60-65%, and it decreased to 55-60 by the end.

In intensively reared poultry, *Eimeria tenella* is a common species and infects chicks worldwide, causing considerable economic losses (McDougald and Fitz-Coy, 2008; Shirley et al., 2005). *Eimeria tenella* infection was administered on day 12 via feed (at a dosage of 100,000 oocysts/chick) in this experiment.

Thymol and carvacrol are known as the active components of many medicinal plants such as oregano and thyme. In the ration, thymol and carvacrol were dissolved in vegetable oil and homogenized by a mixer before being blended with wheat bran. A gentle mix of the pre-mixture and the basal diet was then performed. An airtight container was used to store the diet in this experiment, which was prepared weekly. Blending thymol and carvacrol at 0.01 grams/kg of diet was used to measure effectiveness.

Samples collection and measurements

Upon completion of the experiment, the body weight (BW) of each pen was measured. Each bird's feed intake was measured on a pen basis over the same period. In this experiment, bird deaths and culled birds' weights were recorded. In addition, the FCR was calculated for this experiment.

On d 7 post-challenge (d 19), two birds per replicate were randomly selected, euthanized by cervical dislocation and total intestinal coccidial lesion scores were measured according to the method of Amerah and Ravindran (2015) which were recorded as 0, 1, 2, or 3, from 0 (no lesion) to 4 (most severe lesion). There are three segments of the small intestine: the duodenum (from the gizzard to the pancreo-biliary ducts), the jejunum (from the meckel's diverticulum to the ileo-caecal junction), and the ileum (from the ileo-caecal junction to the pancreas-biliary ducts). A coccidiosis score was taken at four different sites in the intestines: duodenum, jejunum, ileum, and ceca. Based on the individual scores of the four intestine sections, we calculated total lesion scores.

For evaluating gut health, including ileal microbiota and jejunal histomorphology, two birds were randomly selected from each replicate and euthanized. After excising the ileums, gentle fingers were used to collect the contents into tubes. Microbes were enumerated by pooling digesta with a replication and keeping them on ice until they were transported to the laboratory. In 9 ml of sterile water, one gram of ileal contents was homogenized. Several dilutions were conducted on each sample. A De Man-Rogosa-Sharpe (MRS) agar was used to count Lactobacilli, while Mac Conkey (MC) agar was used to count E coli following incubation at 37°C for 48 hours in an anaerobic chamber and 24 hours in an aerobic chamber (Guban et al., 2006). The standard laboratory method was used to determine Bifidobacteria populations in ileal samples (Ibrahim and Salameh, 2001). In order to surface plate onto modified BIM 24 agar, a 10-g ileal sample was homogenized in 90 ml sterilized 0.1% peptone water and homogenized for two minutes on the Stomacher 400 Lab System 4 (Seward, Norfolk, UK) before being diluted in sterilized 0.1% peptone water. The level was measured in this study at a serial dilution of 10−5. At 37°C, the plates were incubated for three days.

A 10% buffered formalin solution was immersed in jejunum samples at the midpoint for 72 hours. Their excision was followed by a physiological saline solution wash. A tissue processor apparatus was used to embed the samples in paraffin wax (Bancroft and Gamble, 2002). A rotary microtome was used to cut sections under a light microscope and calculate the morphometric indices (LEICA RM 2145). A software program called Image Pro Plus v 4.5 was used to measure the morphological parameters. The morphological variables measured by Aptekmann et al. (2001) and Sakamoto et al. (2000) were the following: villus height (VH), measured from the junction between villus and crypt; villus width (VW), measured from the midvillus height; crypt depth (CD), calculated from the junction between villus and crypt; and goblet cells.

Statistical analysis

In a completely randomized study, SAS (2001) generated statistical analyses of the gut health and performance of coccidiosis-challenged broilers fed a coccidiostat or a blend of thymol and carvacrol. Using Tukey's test, multiple ranges were analyzed. Significant values ($P<0.05$) were considered.

RESULTS AND DISCUSSION

Tables 2 and 3 present data from coccidiosis-challenged broilers fed a diet supplemented with thymol and carvacrol. An NC group without exposure to the challenge had the highest BWG and lowest FCR, the best ileal microflora balance, and optimal villus growth, despite not having been challenged by the challenge.

Interestingly, we obtained similar results to those found in other research that coccidiosis-challenged broilers fed thymol/carvacrol diets showed improvements in performance and gut health.

Performance

According to the Table 2 data, body weight gain (BWG) and feed conversion ratio (FCR) improved compared with the PC group (coccidiosis-challenged group without additive) ($P<0.05$) as well as feed intake (FI) increased ($P<0.05$) when the blend of thymol and carvacrol was added. There is a lack of optimal BWG and FCR than in the NC group probably due to damage to the gut wall, leaving nutrients inaccessible and not being absorbed. Additionally, repairing the gut wall requires energy and protein, which cannot be used for growth (Williams, 2005). As a dietary supplement, thymol and carvacrol may improve poultry health and performance due to their mode of action, which includes improving feed flavor and intake, stimulating digestive enzyme production, increasing the motility of the stomach and intestines, gut development and health, and antimicrobial activity (Jamroz et al., 2006). According to Tsinas et al. (2011), broilers fed oregano essential oil at levels of 300 and 600 mg/kg were significantly more likely to develop BWG than those in a control group challenged with Eimeria. In another research, it was shown that the addition of herbal supplements containing thymol and carvacrol to poultry under the challenge of coccidiosis increases feed consumption (Lee et al., 2020). On the other hand, the reduction of the negative effects caused by coccidiosis in intestinal morphology has

Table 2. Performance of chickens challenged with coccidiosis and fed with blend of thymol and carvacrol in diet (TC)

Parameters	NC	PC	PC+TC	SEM	P-value
	Treatments*				
Performance, d 42					
Body weight gain (g/b/day)	66.9[a]	61.0[c]	65.7[b]	0.303	0.0001
Feed intake (g/b/day)	115.0[a]	111.5[b]	115.9[a]	0.621	0.0007
Feed conversion ratio	1.72[c]	1.83[a]	1.77[b]	0.011	0.0001

[a,b,c]Means within the same row with uncommon superscript differ significantly (P<0.05).

*Treatments: negative control (NC) diet: without challenge and dietary additive; positive control (PC) diet: with *Eimeria tenella* challenge on day 12 via feed and without dietary additive; and PC+TC diet: with mentioned challenge+a blend of thymol and carvacrol (0.01 g/kg).

occurred by herbal supplements containing thymol and carvacrol, and this leads to improved digestion as well as absorption of feed with improved production performance (Lee et al., 2020). In addition, in one study, it was shown that infected chickens fed oregano essential oil had a lower number of *Eimeria* oocysts in the feces. Moreover, WG in chickens consuming essential oil was higher than control chickens (Yu et al., 2021). Also, broilers suffering from coccidiosis showed higher performance when fed with oregano and garlic essential oil, which was associated with WG, improved FCR, and lower levels of fecal oocyst excretion (Sidiropoulou et al., 2020).

In a study by Abbas et al. (2010), turmeric powder containing Curcuma longa L. was significantly more effective in improving FCR in broilers compared with the E. tenella challenged control group. The administration of essential oil-loaded microcapsules to broiler chickens infected with C perfringens enhanced growth parameters, total FI, FCR, antioxidant status, intestinal morphology, and intestinal bacterial population. Additionally, the treatment reduced genes associated with inflammation and antioxidant activity in the ileum (Moharreri et al., 2021).

Ileal microflora

In comparison with the PC group, phytogenic essential oil supplementation increased the beneficial population of ileal lactobacilli and bifidobacteria and reduced the number of pathogens such as E.coli (Table 3) (P<0.05). Carvacrol and thymol appear to have a high potential for promoting poultry growth and combating pathogens. It is thought that carvacrol and thymol are beneficial bacteria that grow in broiler feed, and that they inhibit the growth of harmful bacteria such as *E.coli* and *Clostridium* perfringens that are found in the gut of the chickens (Hashemipour et al., 2016). Furthermore, another study found broilers fed a mixture of oregano oil and garlic had a potentially more beneficial gut microbiome composition compared to controls. Moreover, a decrease in the number of *C. perfringens* in the jejunum was observed (Sidiropoulou et al., 2020).

Lactobacillus population in the ileum increased when thymol and carvacrol were combined (Akyurek and Yel, 2011). The active components (such as thymol and carvacrol) can decrease the pH in the gastrointestinal tract, thus preventing the growth of pathogenic bacteria and promoting the growth of non-pathogenic ones. Intestinal microflora can be improved by phytogenics by reducing coliform counts at 14 days of age and enhancing gut flora by 42 days of age with Lactobacillus and bifidobacteria (Mountzouris et al., 2011). Broiler chickens challenged with S enteritidis were found to benefit from microcapsules' antioxidant and antibacterial properties and intestinal microbial balance improved when 0.5, 1 and 2 kg/ton were applied dietaryly (Moharreri et al., 2022).

Jejunal histomorphology

It was noted that the villus of the coccidiosis challenged birds that were treated with the essential oil mixture had a longer and wider size as well as a deeper and larger crypt and more goblet cells than the birds that were treated with PC (Table 3). Coccidial challenge negatively affects intestine morphology, especially the shortening of villus (Kettunen et al., 2001). It has been suggested that intestinal histomorphology has a direct relationship with intestinal functions and the growth of broilers. Intestinal morphology and mucin secretion have been positively affected by carvacrol, an active component of thyme essential oil (Jamroz et al., 2006). Similar observations were made by Jamroz et al. (2007), in which carvacrol, cinnamaldehyde, and capsicum oleoresin were fed to broilers at a concentration of 5, 3, and 2 mg/kg to increase GC and mucin secretions at the jejunum villus surface. Compared to an unsupplemented diet, an

Table 3. Intestinal health and morphology of chickens challenged with coccidiosis and fed with blend of thymol and carvacrol in diet (TC)

Ileal microbial population (Log CFU/g digesta), d 42					
Lactobacilli	8.51[a]	6.88[b]	8.47[a]	0.111	0.0001
Bifidobacteria	8.40[a]	7.39[b]	8.37[a]	0.104	0.0001
E. coli	6.68[b]	7.34[a]	6.38[b]	0.156	0.0008
Jejunal histomorphology, d 42					
Villus height (µm)	1386[a]	1188[b]	1315[a]	32.39	0.003
Villus width (µm)	265[a]	213[c]	239[b]	6.75	0.006
Crypt depth (µm)	236[a]	201[c]	223[b]	2.81	0.0001
Number of goblet cells	344[a]	301[b]	331[a]	5.57	0.0004
Total intestinal coccidial lesion scores in duodenum+jejunum+ileum+ceca, d 19					
Eimeria tenella	0.17[c]	5.19[a]	2.87[b]	0.056	0.0001

[a,b,c]Means within the same row with uncommon superscript differ significantly (P<0.05).

*Treatments: negative control (NC) diet: without challenge and dietary additive; positive control (PC) diet: with *Eimeria tenella* challenge on day 12 via feed and without dietary additive; and PC+TC diet: with mentioned challenge+a blend of thymol and carvacrol (0.01 g/kg).

essential oil blend made from oregano, anise, and citrus peel led to longer ileal VH and higher GC levels (Reisinger et al., 2011). In another study, researcher was stated that increasing the supplement containing thymol and carvacrol to the diet of broiler chickens increased the height of duodenal villi, the depth of the jejunum crypt and the ratio of the height of duodenal villi to the depth of the crypt (Lee et al., 2020).

With an increase in villus size, the number of GCs per villus increases (Tucker and Taylor-Pickard, 2004), and GCs are secreted throughout the digestive tract, forming an adherent gel that contributes significantly to epithelial cell repair (Ikeda et al., 2002; Sklan, 2004). Villus plays an essential role in digestion and absorption of nutrients because it is the first part of the small intestine to come into contact with them (Gartner and Hiatt, 2001). There is a significant difference between a large villus and a small crypt, where a large villus increases absorption surface and a small crypt indicates a lower requirement for tissue regeneration and replacement. In addition to increased epithelial cell turnover, Fan et al. (1997) found a direct correlation between increases in VH and CD. Increased intestinal nutrient absorption and faster enterocyte turnover may be responsible for the increments observed with coccididiostat or thymol and carvacrol mixture treatments. In addition to absorptive cells, GCs, and regenerative cells within the villus, which replace old cells, the crypts contain several specialized cells. In this study, increased GC concentrations in the crypt were also associated with increased mucus secretion. Mucin (combines with water and produces mucus) is synthesized and secreted by GC in the crypt (Duangnumsawang et al., 2021). During GC increase in the crypt, mucin secretion also enhanced (Schneider et al., 2018) and more mucin secretion indicates GC maturation (Duangnumsawang et al., 2021). Ileal morphology and inflammation genes are modulated in ileal tissue by microcapsules containing essential oils. SOD is upregulated as well as occludin expression when microcapsules containing essential oils are used (Moharreri et al., 2022).

Intestinal total coccidial lesion scores

PC diets containing this additive reduced intestinal lesion scores (P<0.05). In addition to its positive effects on the ileal microflora, the use of beneficial natural additives during a coccidiosis challenge may prevent secondary infection (Enteritis). Healthy intestinal epithelium prevents potential pathogens from entering the body. It is crucial for the bird's health and performance to have a healthy intestine, that is, with minimal damage to its epithelium (Ritzi et al., 2014).

CONCLUSIONS

As natural growth promoters, thymol and carvacrol have beneficial effects on broiler chickens exposed to coccidiosis if dietary added in the right amounts. Further, thymol and carvacrol may have antimicrobial effects to reduce intestinal pathogen pressure (*E.coli*) and improve populations of beneficial bacteria including *Lactobacillus* and *Bifidobacteria*. Moreover, Thymol and carvacrol have beneficial effects on intestinal morphology (villus height, villus width and crypt depth) and the overall performance of poultry

(Feed intake, body weight gain, feed conversion ratio). Besides, supplementation of this essential in birds challenged with coccidiosis improved intestinal lesion scores. Thymol and carvacrol have demonstrated that they can improve the gut health and performance of birds.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

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