

«Research Note»

Effects of Thyme Essential Oil on Performance, Some Blood Parameters and Ileal Microflora of Japanese Quail

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The ban on the use of antibiotics as growth promoters has stimulated the search for alternative feed supplements in poultry production. The active principles of thyme essential oil act as a digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (Lovkova *et al.*, 2001; Williams and Losa, 2001). A study was performed to investigate the effects of thyme essential oil (TEO) on performance, carcass characteristics, some blood parameters and ileal microflora of Japanese quail. This study lasted 35 days of age. One hundred and fifty day-old male Japanese quail chicks in a completely randomized design with two treatments (with or without 1 g/kg TEO) and five replicates of 15 birds each were used. All parameters were measured at the end of the experiment. The supplementation of TEO significantly ($P < 0.05$) increased live body weight and relative weights of carcass and breast, lowered serum triglyceride, total cholesterol and glucose and improved ileal microflora. It was concluded that the addition of 1 g/kg TEO might offer some beneficial effects on Japanese quail to increase live body weight and improve some blood parameters and gut microflora.

Key words: blood parameters, microflora, quail, performance, thyme essential oil.

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Introduction

Antibiotics have been used to reduce the spread of diseases and more recently as growth promoter to enhance performance (Waldroup *et al.*, 2003). The mode of action of antibiotics may be explained by an inhibiting effect on certain intestinal bacteria that produce toxins or compete with the host for available nutrients. However, many countries tended to prohibit these feed additives because of increased antibiotic resistance in bacteria and their residual effects on poultry products and harmful effects on human health. Consequently, nutritionists and production managers have to find alternatives that have potential to alleviate the problems related to the withdrawal of antibiotics from diets and reduce enteric disease in poultry (Fritts and Waldroup, 2003; Ayed *et al.*, 2004).

Therefore, phytobiotics including herbs, spices and various plant extracts have received increased attention as possible antibiotics replacements due to their effects on microflora. The ideal situation throughout the life of any animal

would be to maintain specific numbers of beneficial bacteria in the digestive tract. This would ensure that at all times the animal would have the proper microbial balance. This, of course, cannot be guaranteed under natural field conditions. Scientific evidences suggest that herbs, spices, and various plant extracts have appetite- and digestion-stimulating properties and promotes the growth of beneficial bacteria and inhibits the growth of unfavorable bacteria in the gut (Wenk, 2000; Kamel, 2001). Thymol and carvacrol, the main bioactive components of thyme essential oil have considerable antimicrobial and antifungal activity (Twetman and Peterson, 1997; Basílico and Basílico, 1999). Thymol and carvacrol disintegrate the membrane of bacteria, which affects pH homeostasis and equilibrium of inorganic ions, leading to the release of membrane associated material from the cells to the external medium (Lambert *et al.*, 2001). Cross *et al.* (2007) reported that the inclusion of thyme oil had a positive effect on broiler body weight gain (BWG). Genedy and Zeweil (2003) used the medicine plant thyme flowers in growing Japanese quail diets as a growth promoter. They found that thyme flowers at the level of 1 g/kg diet increased body weight, improved feed conversion ratio (FCR) and economic efficiency.

Zataria, an important genus of the family Lamiaceae (previously called Labiatae), is widely distributed in Iran, Af-

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ghanistan and Pakistan and *Zataria multiflora*, an aromatic member of genus *Zataria*, was considered in this study to investigate the effects of its essential oil (thyme essential oil) on performance, carcass characteristics, some blood parameters and ileal microflora of Japanese (*Coturnix coturnix japonica*) quail.

Materials and Methods

Birds, Experimental Design and Diets

One hundred and fifty day-old male Japanese quail chicks (*Coturnix coturnix japonica*) of similar mean weight were weighed and randomly distributed into a completely randomized design considering 2 treatments, each of which included five replicates of 15 chicks. All isonitrogenous and isocaloric diets in the form of mash were formulated to meet nutrient requirements according to the National Research Council (NRC, 1994). The control group received a corn-soybean meal basal diet and the experimental group received basal diet plus thyme essential oil at the rate of 1 g/kg. The thyme essential oil was first dissolved in the vegetable oil component of the ration, and then this oil mixture was gently mixed with the basal diet. This experimental diet was prepared weekly and stored in airtight containers. The ingredients and calculated nutrient contents of the Japanese quail control and experimental diets are presented in Table 1. There were no antimicrobials, coccidiostats, or enzymes in the diets.

Bird Housing and Management

This study lasted 35 days. All chicks were reared on floor under similar standard environmental and management conditions from hatch to 35 days of age. The temperature was maintained at 32°C initially and reduced by 3°C per week until the temperature 20°C was reached at 5 weeks of age. A continuous lighting program was provided during the experiment. Birds had free access to feed and water.

Extraction of Essential Oil and GC/MS Analysis

The fresh plants were collected at the flowering stage and processed immediately after harvest. Essential oil was distilled from the ground plant material using Clevenger distillation apparatus. The samples were distilled for two hours, and the oils obtained were dried with anhydrous sodium sulfate, and stored in dark sealed glass vials at +4°C until required. The main active compositions of the thyme essential oil were determined by GC/MS and contained thymol and carvacrol, 48.32 and 7.48 % of oil, respectively.

Data Collection

At the end of the experiment, the growth performance was evaluated by recording live body weight, cumulative feed consumption and cumulative feed conversion ratio. The feeds and feed residuals were weighed to determine the feed consumption. The feed conversion ratio was calculated as the amount of feed consumed per unit of live body weight. Then five quails per replicate whose body weights were similar to the group average were selected and their blood samples were collected from the wing vein by a syringe. Blood samples were collected in labeled sterile test tubes and centrifuged at 3000 x g for 10 min to isolate serum. After

Table 1. The ingredients and calculated nutrient contents of the Japanese quail control and experimental diets fed 0–35 days of age

Ingredients, %	Treatments	
	Control	Experimental
Corn	49.43	49.43
Soybean meal, 44% CP	44.80	44.80
Wheat bran	0.10	—
Vegetable oil	2.80	2.80
Limestone	1.31	1.31
Dicalcium phosphate	0.71	0.71
Salt (NaCl)	0.35	0.35
DL-Methionine	0.15	0.15
Vitamin mix ¹	0.25	0.25
Mineral mix ²	0.10	0.10
Thyme essential oil	—	0.10
Total	100	100
Calculated nutrient contents		
ME, kcal/kg	2900	2900
Crude Protein, %	24.00	24.00
Calcium, %	0.80	0.80
Available Phosphorus, %	0.30	0.30
Methionine, %	0.50	0.50
Lysine, %	1.30	1.30

¹ Vitamin mix provided the following per kilogram of diet: vitamin A, 1,000 IU; vitamin D₃, 3,500 IU; vitamin E, 100 mg; vitamin K₃, 3 mg; vitamin B₁, 3 mg; vitamin B₂, 6 mg; vitamin B₆, 5 mg; vitamin B₁₂, 0.03 mg; niacin, 45 mg; calcium pantothenate, 15 mg; folic acid, 1 mg; biotin, 0.15 mg; choline chloride, 400 mg; ethoxyquin (antioxidant), 150 mg.

² Trace mineral mix provided the following per kilogram of diet: iron, 60 mg; manganese, 100 mg; zinc, 60 mg; copper, 5 mg; iodine, 2 mg; cobalt, 0.2 mg; selenium, 0.15 mg.

centrifugation, serum was collected and stored at –20°C for later analysis. Serum biochemistry parameters were determined by an enzymatically method in an autoanalyzer (Selectra E vital scientific Netherlands). The birds had been sampled for blood parameters, were immediately plucked, and eviscerated of gastrointestinal tract, giblets and other inner organs to determine the carcass characteristics.

Ileal contents were collected in tubes and put on ice until they were transported to the laboratory for enumeration of microbial populations. One gram of ileal content homogenized in 9 mL sterile water. Samples were determined by serially diluted. Using these samples, *Lactobacillus* was enumerated on De Man-Rogosa-Sharpe agar and *E. coli* was counted on Mac Conkey agar after both incubated at 37°C for 24–48 h (Guban *et al.*, 2006).

Statistical Analysis

Prior to analysis, all percentage data were normalized by subjecting to arc sine transformation. The data obtained from the study were analyzed by t-test using SAS (1999). The threshold for significance was $P < 0.05$.

Results and Discussion

Performance and Carcass Characteristics

The effects of thyme essential oil on performance and carcass characteristics of Japanese quail are presented in Table 2. The values for live body weight were significantly influenced after the application of thyme essential oil ($P < 0.05$). The inclusion of this essential oil in the basal diet numerically decreased cumulative feed consumption and enhanced cumulative feed conversion ratio. In line with our study, Cross *et al.* (2007) reported that the inclusion of thyme oil had a positive effect on broiler body weight gain. Genedy and Zeweil (2003) used the thyme flowers in growing Japanese quail diets as a growth promoter. They found that thyme flowers at the level of 1 g/kg diet increased body weight, improved feed conversion ratio and economic efficiency. The active principles of essential oils act as a digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes, and thus improving growth performance in poultry (Lovkova *et al.*, 2001; Williams and Losa, 2001; Cross *et al.*, 2007). Lee *et al.* (2003a) however, pointed out that 200 ppm thymol in the diet did not affect the BWG, FI, and FCR of female broilers, whereas the same amount of carvacrol significantly decreased BWG, FI, and FCR. Possibly, carvacrol affected feed intake (FI) by modulating appetite. This is corroborated by a study of Deyoe *et al.* (1962) who showed that the flavor of chickens' diets can stimulate or depress feed intake.

Data revealed that the supplementation of thyme essential oil in Japanese quail diet resulted in significantly ($P < 0.05$) higher carcass and breast percentages, but the other carcass characteristics such as relative weight of thighs, drumsticks, spleen, liver, heart and abdominal fat were not influenced by this feed additive. In agreement, the relative weights of pancreas, spleen, liver and heart were not affected by thyme

powder or essential oil (Hernandez *et al.*, 2004; Sarica *et al.*, 2005; Basmacioglu *et al.*, 2010). However, Lee *et al.* (2003a) determined an increase in relative weight of liver of birds given thymol, but this was seen only at the age of 21 d and not at 40 d. Thyme leaves increased ($P < 0.05$) the abdominal fat pad at 42 days of age (Ocak *et al.*, 2008).

Some Blood Parameters and Ileal Microflora

The effects of thyme essential oil on some blood parameters and counts of ileal *Lactobacillus* and *E.coli* of Japanese quail are summarized in Table 3. Data of this study showed that thyme essential oil significantly decreased ($P < 0.05$) serum triglyceride, total cholesterol and glucose, but did not affect serum calcium and phosphorus. Blood glucose level is a biochemical indicator of stress that depressed in birds fed thyme essential oil in this study and could be justified by the stress-lowering effect of this feed additive. There is evidence that dietary essential oils decreased serum cholesterol in chickens (Yu *et al.*, 1994) and carvacrol lowered plasma triglyceride and phospholipids (Lee *et al.*, 2003b). Case *et al.* (1995) reported that additional 150 ppm carvacrol or thymol to the diet showed a serum hypocholesterolaemic effect on Leghorn chicks. Various components of essential oils may exhibit a hypocholesterolaemic effect on chickens (Yu *et al.*, 1994; Elson, 1995). The cholesterol-lowering effect of essential oil constituents has been ascribed to inhibition of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a key regulatory enzyme in cholesterol synthesis (Crowell, 1999; Lee *et al.*, 2003a; b). However, it is known that the absence or presence of cholesterolaemic effect of essential oils in an animal depends on breed, gender, age and the composition of the feed (Lee *et al.*, 2003b).

Inclusion of thyme essential oil in the diet of Japanese quail significantly ($P < 0.05$) increased *Lactobacillus* and decreased *E.coli* in the ileum. Ground thyme has been shown to inhibit the growth of *S. typhimurium* when added to media

Table 2. Effects of thyme essential oil (TEO) at the rate of 1 g/kg on performance (during the 0–35 days of age) and carcass characteristics (at day 35) of Japanese quail

Treatments	Performance			Carcass characteristic (% ¹)							
	Live body weight (g)	Cumulative feed consumption (g/bird)	Cumulative feed conversion ratio (g feed/g gain)	Carcass ²	Breast ³	Thighs ³	Drumsticks ³	Spleen	Liver	Heart	Abdominal fat
Control	157 ^b	443	2.82	74.64 ^b	38.44 ^b	15.41	9.20	0.13	3.24	0.71	1.83
TEO	169 ^a	458	2.71	76.53 ^a	41.28 ^a	15.39	9.17	0.12	3.29	0.71	1.77
±SE	0.84	2.54	0.05	0.39	0.30	0.24	0.28	0.002	0.03	0.04	0.04

^{a,b} Means within each column with different superscripts are significantly different ($P < 0.05$).

¹ Given as % of live body weight.

² With skin on it, excluding head, feet, and inner organs.

³ With skin on them, including bones.

Table 3. Effects of thyme essential oil (TEO) at the rate of 1 g/kg on some blood parameters and counts of ileal *Lactobacillus* and *E.coli* of Japanese quail at day 35

Treatments	Some blood parameters (mg/dl)					Ileal microflora (Log cfu/g)	
	Triglyceride	Total cholesterol	Glucose	Calcium	Phosphorus	<i>Lactobacillus</i>	<i>E.coli</i>
Control	170.40 ^a	178.20 ^a	298.10 ^a	11.50	5.80	7.43 ^b	8.10 ^a
TEO	160.11 ^b	162.22 ^b	280.62 ^b	11.53	5.82	8.29 ^a	7.23 ^b
±SE	2.13	3.02	3.82	0.04	0.03	0.17	0.14

^{a,b} Means within each column with different superscripts are significantly different ($P < 0.05$).

(Aktug and Karapinar, 1986), and the essential oil of thyme has been shown to inhibit the growth of *E. coli* in media (Marino *et al.*, 1999). Other groups have shown that thymol can inhibit the growth of *S. typhimurium* and *E. coli* (Karapinar and Aktug, 1987). Dorman and Deans (2000) reported that thymol, a main component of the essential oil from thyme, has antimicrobial properties. However, there is a study that conversely reported that dietary thyme essential oil had no effect on the intestinal microflora populations (Cross *et al.*, 2007).

Overall, the observed different effects of thyme essential oil supplement on Japanese quail may be related to the feed quality, levels of the essential oils, flock status, and the environmental conditions. Botsoglou *et al.* (2004) elucidated that well-nourished healthy chicks may not positively respond to growth promoting supplements when they are housed under hygienic or normal management conditions. Therefore, as floor litter provides a substrate for pathogenic bacterial growth (Pope and Cherry, 2000), it is concluded that responses to essential oil may be greater in a more challenging environment, in agreement with Lee *et al.* (2003a).

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