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Effects of dietary oregano oil supplementation on performance parameters and intestinal morphology of Japanese quails (*Coturnix coturnix japonica*) reared under normal and cold stress situation

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ABSTRACT

This study aimed to assess the effects of dietary oregano oil supplementation on growth characteristics in Japanese quails reared in normal and cold temperatures. Three hundred 1-day old Japanese quail were randomly divided into five groups: One control group and two treatment groups of 100 and 200 mg of essential oil of oregano/kg in normal temperature (groups 1, 2, and 3); One control and a treatment group of 100 mg Oregano essential oil/kg in a cold stress situation (groups 4 and 5). All the groups were subjected to the same temperature regimen until the 14th day. At the age of 14, the cold stress groups were transferred into a different house in which the temperature ranged between 10 to 13°C until the 35th day of age. At the end of the experiment, performance parameters and intestinal morphology were analyzed; performance parameters were not affected by supplementing oregano oil at normal temperature. On the other hand, dietary supplementation of oregano essential oil at the level of 100 mg/kg under cold stress situations improved Japanese quail's body weight, FCR, and intestinal villus morphology. This study indirectly states that the supplementation of the oregano essential oil components to the Japanese quail's diet would delay the adverse effects caused by cold stress on the birds' performance.

Keywords: Cold stress, Intestinal morphology, Japanese quail, Oregano oil supplementation

For several years, antibiotics have been used as growth promoters. However, the use of antibiotics as feed additives due to cross-resistance and multiple resistance in bacteria is risky (Erik and Knudsen 2001, Sathishkumar *et al.* 2001), Therefore antibiotics have been discredited by consumers as well as by scientists and a lot of efforts have been made to ban the use of most growth-promoting antibiotics in animal feeds. Today, many studies focus on other feed additives to find replacements for antibiotics in the diet (Hertrampf 2001, Humphrey *et al.* 2002). These substitutes are diverse and include enzymes, vitamin metabolites, yeast components, competitive exclusion products, organic acids, fatty acids, bacteriophages, plant extracts, probiotics, prebiotics, and antimicrobial peptides. These additives often have anti-bacterial and/or stimulation effects in the gastrointestinal tract. The main active compound of oregano oil is carvacrol. In addition, it contains bioactive components such as thymol, γ-terpinene, and p-cymene and exhibits antimicrobial effects (Dorman and Deans 2000), antifungal activities (Daouk *et al.* 1995) and contains molecules that have intrinsic properties on animal physiology and metabolism. It appears that oregano oil compounds could improve growth performance in pigs (Namkung *et al.* 2004) and chickens (Bampidis *et al.* 2005). The use of oregano compounds in animal production may therefore have a promising potential as growth promoters without the adverse effects of antibiotics. However, the value of this oil in Japanese quail have not been well investigated yet. On the other side, in quails, the comfortable ambient temperature is between 18-30°C, but nobody has worked on the profits of oregano oil use against deleterious effects of cold stress on the performance and morphology of the intestinal mucosa in Japanese quail. Therefore, this study was designed to demonstrate the effectiveness of supplementation of an oregano oil substance on performance parameters and intestinal mucus morphology of Japanese quail in optimal temperature and cold stress situations.

MATERIALS AND METHODS

Experimental design: This study was conducted as a portion of the Animal Science Research Program at the Animal Science Research Center of Shahrekord University, in the southwest of Iran. In this study, three hundred 1-d old Japanese quail (mean body weight of 8 g) were reared on a deep litter system with wood shavings as the bedding material. All the birds were fed from day 1 to day 35 with an equally prepared quail diet containing 25.3% crude protein and 2850 kcal/kg metabolizable energy. The composition

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Table 1. Ingredients of the experimental diet (g/kg as fed basis)

Ingredient	Base diet
Corn seed	509.6
Soybean meal	438.4
Soybean oil	20.6
Di-calcium phosphate	8.3
Calcium carbonate	12.6
Salt	1.6
Mineral-Vitamin Premix #	5
DL-methionine	1.4
Vit D3 *	1
Vit E ♦	1.5

#Each kg of the mineral-vitamin premix contains vitamin A, 200,000 IU; vitamin D_3, 80,000 IU; vitamin E, 1600 mg; vitamin K_3, 35 mg; vitamin C, 1200 mg; vitamin B_1, 30 mg; vitamin B_2, 130 mg; vitamin B_{12}, 700 mcg; nicotinic acid 1300 mg; Pantothenic acid, 225 mg, Choline Chloride, 8200 mg, biotin, 3300 µg; and Mn, 1200 mg; Zn, 1000 mg; Fe, 1800 mg; Cu, 400 mg; Se, 8 mg; Iodine, 38 mg; and Ca 180 g. * Each kg of Vit D3 (Cholecalciferol) contains 100,000 IU. ♦ Each kg of Vit E (Alpha Tocopherol) contains 100,000 IU.

and nutrient content of the basal diet is given in Table 1. The light was provided 24 h/day. The birds were randomly allocated to five treatments with 60 birds/treatment. Each treatment consisted of 3 replicates and 20 samples/replicate. The differences between the treatments were only the per cent of essential oil of oregano. Treatment groups were: treatment 1: control group (basal diet+ no additives); treatment 2: basal diet + essential oil of oregano, 100 mg/kg diet; treatment 3: basal diet + essential oil of oregano 200 mg/kg diet (two-fold dose); treatment 4: control group experiencing cold stress and treatment 5: essential oil group (basal diet + essential oil of oregano, 100 mg/kg diet experiencing cold stress. Initially, the temperature of the room was 36°C and reduced by 3°C/week until reached 24°C. All groups were subjected to the same temperature regimen until the 14th day of age. At the age of 14, the cold stress groups (4 and 5) were transferred into a different house, in which the temperature ranged between 10 to 13°C until the 35th day of age.

Data collection: Both feed and water were provided *ad lib.* Feed intake was monitored daily. All the birds in each replicate were weighed weekly and live weight, live weight gain, and feed conversion ratio were calculated on a per-bird basis. At the end of the experiment (d 35), 8 birds whose body weights were close to the group average were selected from each of the replicate groups of each treatment. These birds were slaughtered to measure carcass yield, selected internal organs, and abdominal fat. The weights of these internal organs were expressed as percentages of live body weight. The hot carcass yields were calculated as percentages of the pre-slaughter live body weight of quails. Then, 2 cm segments of the midpoint of the duodenum, the midpoint between the bile duct entry and Meckel's diverticulum (jejunum), and the distal end of the lower ileum (ileum) were dissected. The segments were flushed with phosphate-buffered saline (PBS, *p*H 7), fixed in Clark fixative for 45 min, and then left in ethyl alcohol for storage. Each segment was left in PAS for staining, and muscle layers were separated from the mucosa, rows of villi were cut in the thickness of the sections, transferred over the glass slides, and covered with a cover slip. These samples were examined by a microscope with eyepiece graticules (Zamani Moghaddam *et al.* 2009, Hassanpour *et al.* 2013). The villus height was measured from the top of the villus to the top of the lamina propria. Villus surface area was calculated using the formula ¼(p)_(VW)_(VL), where VW¼, villus width and VL¼, villus length (Sakamoto *et al.* 2000). The lamina propria thickness was measured at the space between the base of the villus and the top of the muscularis mucosa (Aptekmann *et al.* 2001).

Data analysis: The data collected were expressed as means and standard deviations and were subjected to statistical analysis using the sigma plot computer software according to a completely randomized design. Means were separated by T-test. The level of significance was determined at $p<0.05$.

RESULTS AND DISCUSSION

The effects of the oregano oil on carcass yield, the content of breast and leg, intestinal weight, abdominal fat, and weight of some inner organs of Japanese quails

Table 2. Effect of inclusion of different concentrations of an oregano oil mixture on food conversion ratio (FCR), carcass yield (%), content of breast and leg (%), intestinal weight (g), abdominal fat (g), internal organs weight (g) of 35–d old Japanese quails

Item	Groups					Pooled SEM
	1	2	3	4	5	
FCR	4.46[ab]	3.81[ab]	3.71[a]	5.56[c]	4.62[b]	0.18
Carcass yield [%]	58.474	55.789	57.181	55.907	58.131	1.074
Breast [%]	39.881	42.253	40.389	40.684	41.920	0.73
Abdominal fat [%]	1.724	1.589	2.201	1.522	1.611	0.256
Intestinal weight [g]	13.696	13.099	11.801	12.465	12.167	0.369
Heart [g]	1.484	1.424	1.521	1.545	1.519	0.065
Liver [g]	6.108	5.905	5.894	6.705	6.469	0.593
Gizzard [g]	4.690	4.249	4.100	4.756	4.682	0.204
Leg [g]	24.662	26.197	25.543	25.228	25.084	0.408

[a,b] significant difference within each row *(P<0.05).*

Table 3. Effect of inclusion of different concentrations of oregano oil mixture on morphologic parameters of duodenal villi of 35–d old Japanese quails

Item	No.[#]	Height[*] (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
1	8	0.585	0.127	0.115	0.236
2	8	0.711	0.124	0.107	0.259
3	8	0.720	0.115	0.100	0.264
4	8	0.545	0.111	0.0728	0.189
5	8	0.611	0.124	0.0763	0.245
Pooled SEM		0.052	0.0085	0.0054	0.024

[#] Total number of quails.[*]Height, from top of the villus to top of the lamina propria; width, the width of villus at the base; the lamina propria, the space between base of the villus and top of the muscularis mucosa; and villus surface area, calculated as [(π)×(height)×(width)].

Table 4. Effect of inclusion of different concentrations of oregano oil mixture on morphologic parameters of jejunal villi of 35–d old Japanese quails

Item	No.[#]	Height[*] (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
1	8	0.386^{ab}	0.121	0.0595^{ab}	0.163
2	8	0.438^{a}	0.135	0.0819^{a}	0.171
3	8	0.426^{a}	0.132	0.0843^{a}	0.180
4	8	0.306^{b}	0.108	0.0491^{b}	0.121
5	8	0.406^{a}	0.122	0.0620^{a}	0.156
Pooled SEM		0.026	0.012	0.005	0.021

[#] Total number of quails.*Height, from top of the villus to top of the lamina propria; width, the width of villus at the base; the lamina propria, the space between base of the villus and top of the muscularis mucosa; and villus surface area, calculated as [(π)×(height)×(width)].
[a,b] significant difference within each column *(P<0.05).*

on the 35th day are given in Table 2. At the end of the experiment, there was no difference in feed intake among treated birds and the control birds (data not shown). The supplementation of 100 mg essential oil/kg of low environmental temperature group improved *(p<0.05)* the conversion ratio compared to the cold stress control group. However, there were no significant differences between the two supplemented essential oil groups (100 and 200 mg essential oil/kg) and the normal temperature control group at the end of the experiment. The results of this experiment showed that the addition of oregano oil to the diet did not affect the feed conversion ratio of japanese quails at 35 days of age. Similar to the results obtained in this survey, some researchers reported that oregano oil extract supplementation to diet did not affect the conversion ratio in broiler chickens (Demir *et al.* 2003, Hernandez *et al.* 2004) and in sheep (Bampidis *et al.* 2005). In contrast, some authors reported that oregano oil components could improve the FCR (Denli *et al.* 2004, Cabuk *et al.* 2006). No parameters were influenced by treatments and there were no differences in the carcass yield, intestinal weight, and inner organ weight among groups fed the diet supplemented with different levels of oregano oil and negative control.

The effects of the oregano oil on intestinal morphometric variables (villous sizes included length, width and surface area, and lamina propria thickness from the duodenum, jejunum, and ileum) are shown in Tables 3, 4, and 5.

The duodenal villus height and surface area were greater in quails fed the diet supplemented with oregano oil than in their controls, but they were not significant (Table 3). The Jejunal villus height and lamina propria thickness were improved *(p<0.05)* by the supplementation of 100 mg essential oil/kg in the low-temperature oregano oil group when compared to the cold control. The lack of

Table 5. Effect of inclusion of different concentrations of oregano oil mixture on morphologic parameters of ileal villi of 35–d old Japanese quails

Item	No.[#]	Height* (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
1	8	0.241	0.0788^{ab}	0.0596	0.0554^{ab}
2	8	0.285	0.123^{a}	0.0615	0.150^{a}
3	8	0.291	0.128^{a}	0.0676	0.137^{a}
4	8	0.218	0.0663^{b}	0.0480	0.0474^{b}
5	8	0.248	0.109^{ab}	0.0769	0.100^{a}
Pooled SEM		0.012	0.01	0.009	0.019

[#] Total number of quails.*Height, from top of the villus to top of the lamina propria; width, the width of villus at the base; the lamina propria, the space between base of the villus and top of the muscularis mucosa; and villus surface area, calculated as [(π)×(height)×(width)].
[a,b] significant difference within each column *(P<0.05).*

growth-promoting action of oregano essential oil observed in this study has been carried out in several investigations in other animals. Based on (Botsoglou *et al.* 2002) and (Papageorgiou *et al.* 2003), supplementation of oregano oil to broilers or turkeys at levels of 50 and 100 or 100 and 200 mg/kg respectively was not beneficial. In contrast, several studies have reported the beneficial effects of dietary essential oil as a growth-promoting agent (Bassett 2000, Hertrampf 2001). Surprisingly, in the low-temperature groups, the use of oregano oil improved bird performance and intestinal villus morphology *($p<0.05$)*. Although measurement of possible reasons for detecting this positive effect was not designed in this study, the antioxidant activity of oregano oil components that entered the circulatory system is important. Several studies have shown that the dietary essential oil of oregano has the potential to increase the antioxidant capacity in chickens (Botsoglou *et al.* 2002, Botsoglou *et al.* 2003a), turkeys (Botsoglou *et al.* 2003b, Botsoglou *et al.* 2003c) and rabbits (Botsoglou *et al.* 2007). Poultry growth, food intake, and physiological responses are changed by environmental temperature because birds are homoeothermic and can live only in a relatively narrow zone of thermoneutral (Yunianto *et al.* 1997, Aengwanich and Simaraks 2004). In this study, the effects of low environmental temperature on performance and intestinal villus morphology of Japanese quail were significant. However, based on our results, the supplementation of oregano oil improved bird performance and intestinal villus morphology of Japanese quail kept in a cold stress situation.

The results obtained from this study indicated that the supplementation of oregano oil to the diet significantly improved feed intake, feed conversion ratio, and intestinal villus morphology of Japanese quails kept under cold stress conditions. It can be stated that the environmental air temperature is one of the most significant abiotic factors that can significantly influence metabolism and subsequently the production of broiler chickens. Due to cold stress happenings in some cold environmental zones like the Shahrekord region and the possible deleterious effects of this stress, the oregano oil may be considered a growth promoter with the potential to achieve an environmentally friendly production system. However, more detailed research is required on the mode of action of oregano oil supplementation on Japanese quails.

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