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RESEARCH ARTICLE

The Effects of Dietary Rosemary (*Rosmarinus officinalis L.*) Oil Supplementation on Performance, Carcass Traits and Some Blood Parameters of Japanese Quail Under Heat Stressed Condition

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Summary

In this study, the effects of rosemary (*Rosmarinus officinalis L.*) oil supplementation to diet were investigated on performance, carcass traits and some blood parameters of Japanese quails exposed to a high ambient temperature of 34°C. A total of 180 fifteen-day-old quails were divided into 6 treatments consisting of 10 birds of 3 replicates. All groups were balanced according to initial live weight and gender. Birds were kept in wire cages in temperature-controlled room at either 22°C for 24 h/d (thermo neutral-TN) or 22°C for 16 h/d and 34°C (heat stress-HS) for 8 h/d (from 9:00 to 17:00) during the study. Trial was conducted as a 2x3 factorial arrangement. Birds were fed either a basal (control) diet (TN and HS) or the basal diet supplemented with 125 or 250 ppm of rosemary oil. The highest final live weight was observed in 250 ppm rosemary oil under the TN condition and the lowest was in control group of HS. HS condition affected negatively on quail live weight ($P<0.05$), but the effect of rosemary oil on this parameter was not significant ($P>0.05$). Live weight gain and feed intake were not significantly different among the treatment groups ($P>0.05$). Feed conversion ratio was better in rosemary oil groups than control groups in both environmental conditions ($P<0.01$). Heat stress deteriorated carcass yield ($P<0.01$). The highest hot and cold carcass yield (g/100 g of body weight) was observed in 250 ppm rosemary oil added group under the TN condition, but this difference did not significant. Birds kept in HS conditions had greater glucose level than hens kept in TN conditions ($P<0.01$). Rosemary oil decreased blood glucose level, especially in 250 ppm group of HS ($P<0.05$). Total, HDL, LDL cholesterol and triglyceride levels were not significantly different among the treatment groups ($P>0.05$). In conclusion, rosemary oil supplementation reduced the negative effects of heat stress. Rosemary oil could be considered as a potential natural feed additive, following further studies.

Keywords: *Rosemary oil, Performance, Carcass traits, Blood parameters, Quail, Heat stress*

Sıcaklık Stresi Altındaki Japon Bıldırcınlarında Karma Yeme İlave Edilen Biberiye (*Rosmarinus officinalis L.*) Yağının Performans, Karkas Özellikleri ve Bazı Kan Parametreleri Üzerine Etkileri

Özet

Bu çalışmada, karma yeme ilave edilen biberiye (*Rosmarinus officinalis L.*) yağının yüksek çevre sıcaklığına (34°C) maruz bırakılan Japon bıldırcınlarında performans, karkas özellikleri ve bazı kan parametreleri üzerine etkileri araştırılmıştır. Toplam olarak 180 adet 15 günlük bıldırcın, her tekerrürde 10 bıldırcının bulunduğu 3 tekerrürlü 6 gruba ayrılmıştır. Tüm gruplar başlangıç canlı ağırlıkları ve cinsiyet bakımından dengelenmiştir. Bıldırcınlar, çalışma süresince sıcaklık kontrollü odalarda 22°C'de 24 saat/gün (termo nötral-TN) ve 22°C'de 16 saat/gün 34°C'de 8 saat/gün (9:00-17:00) (sıcaklık stresi-HS) tel kafeslerde barındırılmıştır. Deneme 2x3 faktöriyel deneme düzenine göre yapılmıştır. Bıldırcınlar temel yem (kontrol) (TN ve HS) ve temel yeme 125 ve 250 ppm biberiye yağı ilave edilen karma yemler ile beslenmiştir. En yüksek canlı ağırlık TN bölümünde 250 ppm biberiye yağı ile beslenen grupta, en düşük canlı ağırlık ise sıcaklık stresi uygulanan tarafta kontrol grubunda gözlenmiştir. Sıcaklık stresi (HS) bıldırcınların canlı ağırlığını olumsuz yönde etkilemiş ($P<0.05$), biberiye yağının bu parametre üzerine etkisi önemsiz bulunmuştur ($P>0.05$). Canlı ağırlık artışı ve yem tüketimi bakımından araştırma grupları arasında önemli farklılıklar tespit edilmemiştir ($P>0.05$). Yemden yararlanma oranı her iki çevre koşulunda da biberiye yağı ilave edilen gruplarda, kontrol gruplarından daha iyi bulunmuştur ($P<0.01$). Sıcaklık stresi karkas verimini kötü yönde etkilemiştir ($P<0.01$). En yüksek sıcak ve soğuk karkas randımanı (g/100 g canlı ağırlıkta) TN şartlarında 250 ppm biberiye yağı ile beslenen grupta gözlenmiş, fakat bu farklılık istatistiksel olarak önemli çıkmamıştır. Sıcaklık stresi grubundaki bıldırcınların kan glikoz seviyesi termo-nötral gruba göre daha yüksek tespit edilmiştir ($P<0.01$). Sıcaklık stresi uygulanan tarafta özellikle 250 ppm biberiye yağı verilen grupta kan glikoz seviyesi düşmüştür ($P<0.05$). Toplam HDL, LDL kolesterol ve trigliserit düzeylerinde deneme grupları arasında önemli farklılık tespit edilmemiştir ($P>0.05$). Sonuç olarak, biberiye yağı ilavesi sıcaklık stresinin olumsuz etkilerini azaltmıştır. Biberiye yağı müteakip çalışmalardan sonra, potansiyel bir doğal yem katkı maddesi olarak düşünülebilir.

Anahtar sözcükler: *Biberiye yağı, Performans, Karkas özellikleri, Kan parametreleri, Bıldırcın, Sıcaklık stresi*

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INTRODUCTION

Heat stress is one of the most important factors adversely affecting overall poultry production in tropical countries [1]. Heat stress has been associated with decreases on weight gain, feed intake, feed efficiency and digestibility of nutrients of birds [2]. The ideal temperature for poultry is 10-22°C for optimum body weight and 15-27°C for feed efficiency.

Different natural agents are used to minimize the harmful effects of heat stress on performance of poultry. For example some vitamins [3,4], minerals [5] and substances with anti-oxidant character such as tomato powder [6], Turkish propolis [7], different essential oils [8] and oil mix (*Thymus serpyllum, Laurus nobilis L., Myrtle oil, Foeniculum vulgare, Salvia officinalis*) [9].

Rosemary, (*Rosmarinus officinalis L.*), of the family Labiatae, is an aromatic shrub with an intense pleasant smell reminiscent of pine wood [10]. The essential oil volatile composition of rosemary has been the subject of considerable research in recent years. The principal volatile compounds in rosemary are camphor and 1,8-cineole, followed by borneol, verbenone, a-pinene and camphene [11-13]. Rosemary's oils from natural populations showed high variations in their antimicrobial and anti-oxidant activity [14,15].

The aim of this study was to evaluate the effects of dietary Rosemary oil (*Rosmarinus officinalis L.*) supplementation on the performance, carcass traits and some blood parameters in Japanese quail reared in thermo-neutral (TN) condition and exposed to high ambient temperatures (HS) of 22 and 34°C, respectively.

MATERIAL and METHODS

Experimental Design and Diet Regimens

A total of 180 fifteen-day-old Japanese quails *(Coturnix coturnix japonica)* obtained from a commercial company (Deva-Yum Marketing Company, Elazig, Turkey) were used after Firat University Animal Ethical Committee approval (Official form date and number: 20.01.2012 and 2012/06). The experiment was conducted at the Poultry Unit of Veterinary Faculty, Firat University, between February 15 to March 14. The birds which were exposed to two different ambient temperature [thermo-neutral (TN) and heat stress (HS)] and three different concentrations of rosemary oil (0, 125 and 250 mg/kg), were divided into 6 treatments consisting of 10 birds of 3 replicates according to 2x3 factorial order. All groups were balanced according to initial live weight and gender. Birds were kept in wire cages in temperature controlled room at either 22°C for 24 h/d (TN) or 22°C for 16 h/d and 34°C (HS) for 8 h/d (from 9:00 to 17:00 h). At both temperatures, birds were fed either a basal diet or the basal diet supplemented with 125 or 250 ppm rosemary oil. The rosemary oil was mixed in a carrier (zeolite), which was then added at one kg per hundred kg to the basal diet. For the rosemary oil treatments, 125 or 250 mg of commercial rosemary oil were added per kg of feed. The concentrations of the volatile components in rosemary oil were shown *Table 1*. Diets and fresh water were offered *ad libitum*. Light was provided continuously (24 h) throughout the experiment. Ingredients and chemical composition of the basal diet were shown in *Table 2*. The basal diets contained 23.87% CP and 2897 kcal/kg of ME.

Table 1. *The concentration of the volatile components in rosemary oil*
Tablo 1. *Biberiye yağının içindeki uçucu bileşenlerin konsantrasyonu*

Volatile Components	**Concentration** (%)
1,8 Cineole	39.31
Camphor	14.69
α-Pinene	13.85
β- Pinene	9.87
Camphene	6.17
Limonene	3.17
P-Cymene	2.58
Borneol	2.33
Myrcene	2.02
α-Terpineol	2.28
Bornyl Acetate	1.46
Others	2.27

Table 2. *Ingredients and chemical composition of standard diet*
Tablo 2. *Standart karma yemin bileşimi ve kimyasal kompozisyonu*

Feed Ingredients	**%**	**Nutritional Composition**	**%**
Maize	29.03	Dry matter	88.25
Wheat	25.00	Crude protein	23.87
Soybean meal (48 CP)	34.29	Crude fibre	2.55
Corn Gluten	4.10	Ether extract	4.75
Vegetable oil	2.92	Ash	5.45
Dicalcium phosphate	2.02	Calcium ****	1.00
Ground limestone	0.87	Available phosphorus****	0.79
$NaHCO_3$	0.12	Methionine ****	0.40
Salt	0.28	Lysine ****	1.18
DL-Metiyonin	0.02	ME, kcal/kg****	2897
Vitamin mix *	0.25		
Mineral mix**	0.10		
Additive***	1.00		

** Vitamin premix supplied per 2.5 kg; Vitamin A 12.000.000 IU; vitamin D_3 2.000.000 IU; vitamin E 35.000 mg; vitamin K_3 4.000 mg; vitamin B_1 3.000 mg; vitamin B_2 7.000 mg; Niacine 20.000 mg; Calcium D-pantotenat 10.000 mg; vitamin B_6 5.000 mg; vitamin B_{12} 15 mg; Folik Asit 1.000 mg; D-Biotin 45 mg; vitamin C 50.000 mg; Choline chloride 125.000 mg; Canthaxanthin 2.500 mg; Apo Karotenoik Acid Ester 500 mg, ** Mineral premix supplied per kg; Mn 80.000 mg; Fe 60.000 mg; Zn 60.000 mg; Cu 5.000 mg; Co 200 mg; I 1.000 mg; Se 150 mg, *** Group Rosemary 0 (1.000 g zeolit); Group Rosemary 125 (12.5 g rosemary oil+987.5 g zeolit); Group Rosemary 250 (25 g rosemary oil + 975 g zeolit), **** Calculated*

Feed intake and BW were determined at weekly intervals. The weight gain and feed conversion of birds were then calculated.

At the end of the study (43th day) six males and females quail from each group with an average body weight near the group average were slaughtered and blood samples were collected. Blood samples were centrifuged at 2260 × *g* for 5 min, and sera were collected. Following slaughtering, hot and cold carcass characteristics were evaluated according to Institute of Turkish standards rules [16].

Chemical Analysis

Serum cholesterol, triglyceride, and glucose concentrations were measured using a biochemical analyzer (Olympus AU-600) at University of Firat, Faculty of Medicine, Department of Biochemistry. Chemical composition of feed ingredients (dry matter, crude protein, ash and ether extract) were analyzed according to the AOAC [17] procedures and crude fiber was determined by the methods of Crampton and Maynard [18].

Statistical Analysis

Data were subjected to two-way anova by using GLM (General Linear Model) prosedure. Significant differences were further subjected to Duncan's multiple range test (SPSS [19]). The results were considered as significant when *P* values were lower than 0.05.

RESULTS

The effects of dietary rosemary oil on performance of quails are given in *Table 3*. As shown in *Table 3*, the highest final live weight was observed in 250 ppm rosemary oil under the TN condition, the lowest was obtained in control group of HS condition. Heat stress decreased live weight of quails (P<0.05), supplementation of rosemary oil on live weight was not significant and dose of rosemary oil was not important among the groups of both TN and HS conditions. Live weight gain and feed intake were not significantly different among the treatment groups both in TN and HS conditions (P>0.05). The worst feed conversion ratio was calculated in control groups of both TN and HS conditions. Feed conversion ratio was improved in rosemary oil groups of 125 and 250 ppm in both conditions (P<0.01). The highest hot and cold carcass yield (g/100 g of body weight) was observed in 250 ppm rosemary oil under the TN condition. The lowest was in control group of HS. Under HS condition, deterioration of carcass yield was found significant (P<0.01).

Heat stress affected blood glucose level (P<0.01). Birds kept in HS conditions had greater glucose level than hens kept in TN conditions *(Table 4)*. Rosemary oil decreased blood glucose level especially in 250 ppm rosemary oil group (P<0.05). Total, HDL, LDL cholesterol and triglyceride levels were not significantly different among the treatments (P>0.05).

DISCUSSION

In the present study, rosemary oil supplementation to diet had significant effects on the measured values under thermo-neutral (TN) and heat stress (HS) conditions in growing Japanese quails; it improved performance, positively. The improved performance of rosemary oil groups could be due to these positive effects of rosemary oil on digestive system. In agreement with these results, HERNANDEZ et al.[20] reported that a supplementation of essential oil extract (EOE) from oregano, cinnamon and pepper improved apparent whole tract and ileac digestibility of the nutrients in broilers. JANG et al.[21] showed that a supplementation of a blend of commercial essential oils

Table 3. *Effects of rosemary (Rosmarinus officinalis L.) supplementation in diet on performance, hot and cold carcass yield in Japanese quail reared under heat stress*

Tablo 3. *Sıcaklık stresi altındaki Japon bıldırcınlarında karma yeme ilave edilen biberiye (Rosmarinus officinalis L.) yağının performans, sıcak ve soğuk karkas üzerine etkileri*

Traıts	Rosemary Oil, ppm						Main Effects of Heat Stress and Feed Additive	
	HS			TN				
	0	125	250	0	125	250	HS	FA
Initial Live Weight, *g*	58.15±0.85	58.10±0.30	58.10±0.50	58.50±1.00	58.20±0.20	58.10±0.40	NS	NS
Final Live Weight, *g*	192.67±3.88	200.60±4.05	202.54±3.55	206.20±5.80	205.56±3.92	214.11±3.66	*	NS
Live Weight Gain, *g/bird/day*	4.64±0.12	4.91±0.40	4.98±0.16	5.09±0.17	5.08±0.13	5.38±0.12	NS	NS
Feed Intake, *g/bird/day*	17.04±0.06	17.15±0.12	17.28±0.15	18.61±0.10	17.76±0.63	18.60±0.37	NS	NS
Feed Conversion Ratio, *g feed/g gain*	3.68±0.01^{a}	3.50±0.04^{b}	3.47±0.01^{b}	3.66±0.03^{A}	3.50±0.05^{B}	3.46±0.01^{B}	NS	**
Hot Carcass, *g hot carcase wt/100 g live wt.*	64.73±0.75	65.97±1.03	66.55±1.92	69.92±1.29	69.31±0.90	70.64±1.15	**	NS
Cold Carcass, *g cold carcase wt/100 g live wt.*	62.70±0.86	64.32±1.09	64.24±1.96	67.71±1.19	67.85±1.27	69.04±1.04	**	NS

NS: *Non significant, * P<0.05,* ******* *P<0.01,* ^{a,b,A,B}***:*** *Mean values with different superscripts within a column differ significantly,* ***HS:*** *Heat stres,* ***TN:*** *Thermo-Neutral,* ***FA:*** *Feed Additive*

Table 4. *Effects of rosemary (Rosmarinus officinalis L.) supplementation in diet on serum glucose and lipid levels in Japanese quails reared under heat stress*

Tablo 4. *Sıcaklık stresi altındaki Japon bıldırcınlarında karma yeme ilave edilen biberiye (Rosmarinus officinalis L.) yağının serum glikoz ve lipit seviyelerinin üzerine etkileri*

Traits, mg/dl	Rosemary Oil, ppm						Main Effects of Heat Stress and Feed Additive	
	HS			TN				
	0	125	250	0	125	250	HS	FA
Glucose	289.67±5.53^{a}	273.67±7.27^{ab}	257.33±7.01^{b}	215.80±8.34^{B}	251.17±8.30^{A}	201.80±5.63^{B}	**	*
Total Cholesterol	209.00±32.75	175.00±21.99	165.33±16.71	152.67±29.01	154.60±25.95	149.83±18.56	NS	NS
Triglyceride	919.50±176.44	913.00±90.93	881.83±231.30	680.20±155.41	741.00±243.22	614.33±97.58	NS	NS
HDL Cholesterol	69.33±9.97	69.67±3.52	70.80±7.04	75.00±4.80	82.80±9.64	80.00±5.29	NS	NS
LDL Cholesterol	95.40±12.08	60.83±13.13	60.33±9.03	54.80±10.19	60.00±7.22	58.83±8.51	NS	NS

NS: *Non significant, * P<0.05,* ** *P<0.01,* ^{a,b,A,B}***:*** *Mean values with different superscripts within a column differ significantly,* ***HS:*** *Heat stres,* ***TN:*** *Thermo-Neutral,* ***FA:*** *Feed Additive*

combined with lactic acid increased trypsin and pancreatic amylase activity in broilers. Rosemary's oils obtained from natural plants showed high variations of their antimicrobial and antioxidant activity [14,15]. Previous studies reported that dietary antioxidants, such as vitamin C, E, flavonoids, and phenolic can reduce oxidative damage in animals which is generated by different stress sources [22,23]. Jamroz and Kamel [24] who observed improvements of 7.7% in feed conversation ratio fed a diet supplemented with a plant extract containing capsaicin, cinnamaldehyde and carvacrol in broilers. Improved feed conversion in groups with supplemented rosemary oil may be due to the combined effects of all these active ingredients in a positive manner.

At the inspection of the carcass characteristics *(Table 3),* there was significant effect of HS on this parameter. In stressed conditions, elevated concentrations of glucocorticoids exert catabolic effects. This demolition decreases the rate of muscle synthesis and thus results in muscle wasting and retardation in growth [25,26]. Supplementation of rosemary oil to diet did not affect the carcass yields at the present study. However, Simsek et al.[8] reported that adding anise oil in the ration had positive effects on the carcass yield in broilers. Also, Alcicek et al.[27] showed that supplementation essential oil (Herbomix™) in the ration had positive effects on the carcass yield in broilers. Possibility, the doses of rosemary oil could not have enough action on catabolic effects of glucocorticoids via anti-oxidant activity [28].

Effects of rosemary (*Rosmarinus officinalis L.*) oil supplementation in diet on blood levels of lipids and glucose were investigated in the present study *(Table 4)*. We observed that birds kept in HS conditions had greater glucose level than hens kept in TN condition and dietary rosemary oil had a positive effect on blood glucose level. This finding indicates that the rosemary oil might be producing its hypoglycemic activity by a mechanism independent from insulin secretion by the inhibition of endogenous glucose production or the inhibition of intestinal glucose absorption [29,30]. In a previous study, it has been suggested that 50% ethanol extract of *Rosmarinus officinalis*, in part, due to intestinal α-glycosidase (AGc) inhibitory activity of its active compound might play a role in controlling dietary glucose uptake in the small intestinal track [31]. In agreement with current results, BAKIREL et al.[32] investigated potential effect of ethanolic extract of *Rosmarinus officinalis* leaves on glucose homeostasis in rabbits. Results of that study showed that ethanolic extracts of leaves of *Rosmarinus officinalis* reduced blood glucose level in normoglycemic and glucose-hyperglycemic rabbits.

In conclusion, rosemary oil supplemented to diet especially at a level of 250 ppm level supplemented diet had positive effects on performance and blood glucose level. The rosemary oil could therefore be considered as a potential natural feed additive for growing quails, due to increasing consumer's demand for healthy animal production after further studies carried out in different stress conditions.

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