Romanian Biotechnological Letters *Vol. 23, No. 5, 2018*

ORIGINAL PAPER

The Response of Japanese Quails to Dietary *Thymbra spicata L.* Essential Oil

DOI:10.26327/RBL2017.62

Received for publication, July, 22, 2016
Accepted, May, 27,2017

TAYLAN AKSU[1], DEVRİM S. AKSU[2], DURMUŞ A. KAYA[3], NİZAMI DURAN[4], SÜLEYMAN E. ÖNEL[5], SİBEL CANOĞULLARI[6]

[1]*University of Yuzuncu Yıl, Faculty of Veterinary Medicine,Department of Animal Nutrition, Van, Turkey*
[2]*University of Yuzuncu Yıl, Faculty of Veterinary Medicine, Department of Physiology, Van, Turkey*
[3]*University of Mustafa Kemal, Faculty of Agriculture, Department of Field Crop, Hatay, Turkey*
[4]*University of Mustafa Kemal, Faculty of Medicine, Department of Clinical Microbiology, Hatay, Turkey*
[5]*University of Mustafa Kemal, Samandag Vocation High School, Department of Animal Production, Hatay, Turkey*
[6]*University of Niğde, Faculty of Agricultural Sciences and Technologies, Department of Animal Production Technologies, Niğde, Turkey*
Address for corresponding to: *Dr. Devrim Sarıpınar AKSU, PhD, Department of Physiology, Faculty of Veterinary Medicine, University of Yuzuncu Yıl, 65080, Van-Turkey; Tel: +904322251118; Fax: +904322251118; Email: aksuturkiye@gmail.com*

Abstract

In the present study, the response of Japanese Quails to dietary Thymbra spicata L. essential oil was investigated. A total of 192 Japanese quail chicks, 1-day old and of mixed sexes, were used in the feeding trial. Birds were randomly allocated to four dietary treatments comprising three experimental groups and one control group (basal diet). Each group was divided into four subgroups, with each containing twelve chicks. EO was added daily to the basal diet at levels of 200 (T1), 400 (T2) and 600 mg/kg (T3), respectively. There was a significantly increase in live weight at 35 days when quails were supplemented with increasing level of EO. Moreover, live weight gain significantly increased over ranges of 28-35 and 0-35 days in quails fed the EO at 600 mg/kg level compared to the control group ($P<0.05$). No differences were observed among treatments for feed intake. A significantly improvement in feed efficiency at 28-35 days ($P<0.001$) was observed in treated groups. Treatments significantly decreased the number of total bacteria and Escherichia coli in the small intestine. A significantly decrease was observed for TG levels in all treatment groups. TG/TC rate was significantly decreased in supplemented groups ($P<0.001$). Treatments did not alter serum HDL and LDL levels. In conclusion, dietary T. spicata L. EO enhanced the performance parameters by showing time-depended effects. This property could be important to recommend the implementation time of such compounds to the poultry diets.

Keywords: Thymbra spicata, essential oil, performance, cecal E. coli, quail.

1. Introduction

In recent years, different plant-derived agents have been explored for their usefulness in animal production systems to improve health and the quality of their products. However, they are not simple compounds, rather a mixture of various compounds (mainly terpenes and terpene derivatives). It has been shown that the inclusion of these agents in the diets of broiler chickens leads to improvement in growth performance and reduced pathogen load. For instance, it was demonstrated that a diet containing a blend of essential oils (**EO**) as a feed additive improved feed intake, BW, and carcass yield in poultry (KÜÇÜKYILMAZ et al., [1]). It was also reported that diets containing herbal extracts reduced the numbers of the foodborne pathogen *Escherichia coli* (ORAL et al., [2]). the animal pathogen *Clostridium* perfringens (SEÇİL EKİCİ et al., [3]), and the parasite *Eimeria tenella* (CHRISTAK et al., [4]) in the

digestive tract of poultry. In addition, plant-derived agents also appeared to improve the food quality of animal products by enhancing the oxidative stability of lipids in blood by depressing cholesterol synthesis (KIRKPINAR et al., [5]). As a result, it was suggested that plant-derived agents such as EO could be used as potential growth promotants in poultry. *Thymbra spicata L.* (*T. spicata*) from the Lamiaceae family is a perennial plant known as "Kekik, Zahter or Sater" in Turkey. The EOs are found in different parts of Thymbra plants. This oil is also popular among the herbalists because it contains carvacrol, which is phenols having important biological activities and pharmacological properties (İNAN et al. [6]). The efficacy of any plant extract or EO will be linked to certain active molecules at a clearly defined dosage, and variations in this concentration could be the reason for a lack of response sometime observed with these products. Variations can arise from production area, harvest period, processing, stabilization of products etc. (BASMACIOĞLU et al., [7]). Thus, numerous reports exist in the literature regarding the effect of EOs on the performance of poultry with varying and conflicting results. Some reports have demonstrated that EOs improved animal performance (KÜÇÜKYILMAZ et al. [1], HERTRAMPF, [8]), controlled pathogens in gut microflora (KIRKPINAR et al., [5]) decreased blood cholesterol and triglyceride levels (KIRKPINAR et al., [5]) and delayed lipid peroxidation in the blood and meat of poultry (AKSU et al., [9]), while some researchers (MANSOUB, [10]) reported that these additives were not effective in these regards. Thus, the objective of this study was to investigate the effects of different dosages of EOs extracted from *Thymbra spicata L.* on growth performance, antioxidant status, lipid metabolism and cecal *E. coli* counts of Japanese quails.

2. Materials and Methods

Plant material and characterisation of essential oil

In the study, *Thymbra spicata* L. var. *spicata* plants, which are found naturally in the province of Hatay, were used for EO extraction. The plants were collected by villagers in the blooming period. Samples were dried at 35 °C in the Laboratory of Medicinal and Aromatic Plants, Faculty of Agriculture, Mustafa Kemal University. EO was extracted by using steam distillation and chemical composition of the EOs was determined by Gas Chromatography-Mass Spectrometry (GC-MS). Analysis of the essential oil was carried out by using Thermo Scientific ISQ Single Quadrupole Gas Choromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 1.2-1100 amu. Data acquisition method was scan mode. MS transfer line temperature was 250°C, MS Ionization source temperature was 220°C, the injection port temperature was 220°C. The samples were injected with 250 split ratio. The injection volume was 1 µl. Oven temperature was programmed to increase from 50°C to 220°C at a rate 3°C/min. The structure of each compound was identified by comparison of their mass spectrum (Wiley) and data were handled using Xcalibur software program. The chemical component of thyme oil is presented in Table 1.

Diets and feeding regimens

A basal diet was formulated according to NRC, [11] recommendations. The ingredients and chemical composition of the basal diet is shown in Table 2. Experimental diets were prepared as (1) a commercial basal diet without EO (CTL), (2) basal diet supplemented with EO at 200 mg/kg (T1), (3) basal diet supplemented with EO at 400 mg/kg (T2) and (4) basal diet supplemented with EO at 600 mg/kg (T3). EO was added to diet freshly each day except for the EO-free control diets as pre-mixture. Feed and water were supplied ad-libitum during the experimental period.

Table 1. Ingredients and chemical composition of the basal diet

Ingredients	***Composition*** g kg^{-1}
Maize	515.0
Wheat	70.0
Wheat bran	45.0
Extracted soybean meal	275.0
Fish meal	55.0
Vegetable oil	15.0
Limestone	10.0
Dicalcium phosphate	5.5
Sodium chloride	2.5
Vitamin-mineral premix*	5.0
Calculated nutrients	
ME, (MJ kg^{-1})**	12.6
Crude protein (g kg^{-1})	221
Ca (g kg^{-1})	9.0
P (g kg^{-1})	6.0
Lysine (g kg^{-1})	11.0

*, Vitamin premix provides the following per kg, all-trans-retinyl acetate, 1·8 mg; cholecalciferol, 0·025 mg; all-rac-α-tocopherol acetate, 25 mg; menadione (menadione sodium bisulphate), 1·1 mg; thiamine (thiamine mononitrate), 1·1 mg; riboflavin, 4·4 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; pyridoxine, 2·2 mg; folic acid, 0·55 mg; cyanocobalamin, 0·02 mg; Mn, 74 mg (from MnO); Zn, 45mg (from ZnO); Cu, 4 mg (from CuO); Fe (from $FeSO_4$), 12·5 mg; I (from KI), 0·3 mg; Se (from NaSe), 0·15 mg. **ME, Metabolisable energy. The ME, crude protein, calcium, phosphorus and lysine contents were calculated based on their tabular values listed for the feeding ingredients (JURGENS, [33]).

Traits measured

Birds were weighed individually at weekly intervals. Mortality was recorded daily. Total feed intake was measured per pen at weekly intervals. Feed intake and feed efficiency were adjusted for mortality. At the end of the experiment, thirty-two quails (one male and one female in each subgroup) were selected balancing both sexes for blood parameters and enumeration of intestinal microbial populations After slaughtering, the small intestine was removed from the distal end of the duodenum to the ileocecal junction and put on ice until they were rapidly transported into sterile glass petri to the laboratory for enumeration of microbial populations. To evaluate bacterial count in fecal samples, approximately 1 g of the each fecal sample was diluted in 1 ml of PBS. And then, for a homogenous distribution, the tubes that contain fecal samples in PBS were vortexed for 30 seconds. And then a total of 100 ml from fecal dilution was inoculated to eosine-methylene-blue (EMB) agar and Mueller-Hinton agar. Plates were grown aerobically at 37°C for 24 h. After cultivation at 37°C for 24 hours, in every tested sample, the number of total bacteria and Escherichia coli were determined as CFU/ml (colony forming units per milliliter). In addition to conventional methods, VITEK 2 (bioMerieux, St. Louis, MO, ABD) automated system was used for identification of micro-organisms that were grown in these media. Blood samples were collected into test tubes without anticoagulants during decapitation of quail at 35 days of age for subsequent measurement of Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Total Triglyceride (TG). Serum samples were obtained following centrifugation at 1700 x g for 15 min. TAS and TOS were determined colorimetrically (PowerWave XS, BioTek Instruments, USA) by using a commercial kit (Rel Assay Diagnostic, Turkey) (EREL, [12, 13]). For TAS measurement, an automated measurement method was used (EREL, [12, 13]). The percent ratio of the TOS to the TAS indicated the OSI, an indicator of the degree of oxidative stress (EREL, [12-14]).

The concentrations of TG, TC, HDL and LDL were measured spectrophotometrically (Konelab PRIME-60 Chemistry Analyzer, USA) using commercially available assay kits (Thermo).

Statistical analysis

All parameters were based on the individual birds as the experimental unit. The effect of treatments on sex ratio in quails was evaluated by chi-squared test (DAWSON and TRAMP, [15]). The number of microbial colony-forming units (CFUs) was expressed as logarithmic (log10) transformation per milliliter of intestinal digest. All microbiological concentrations from each bird were subjected to log transformation prior to statistical analysis. For performance parameters (live weight, live weight gain, feed intake, and feed efficiency) treatment, time and the treatment*time interactions were examined. All values are expressed as means ± SEM. The data were analyzed using ANOVA (GLM) with Repeated Measures [16] by the use the SPSS (Version 15, SPSS, Chicago, IL). Differences were determined by the use of the Tukey test and considered significant at $P < 0.05$.

3. Results and Discussion

The chemical components of *T. spicata* EO are shown in Table 1. The EO consisted of 24 components of which the main components were carvacrol (60.43%), cymol (13.08%) and γ-terpinene (10.41%) respectively, and also formed a high level of phenolic components including 62.43% of phenol and 31.31% of hydrocarbon. Others certain chemical components of *T. spicata* were composed to alcohol (2.87%), ketones (0.12%), respectively.

Table 2. Chemical components of *Thymbra spicata L.* essential oil

Retention Time	***Rate (%)***	***Components***
3.65	0.67	α-pinene
3.72	0.78	α-phellandrene
4.36	0.18	Camphene
5.17	0.17	β-pinene
6.11	0.06	Δ-3-carene
6.52	1.03	β-myrcene
6.90	1.36	α-terpinene
7.44	0.83	Limonene
7.70	0.26	β-phellanderne
8.86	10.41	γ-terpinene
9.69	13.08	Cymol
10.05	0.23	α-terpinolene
16.18	0.31	Oct-1-en-3-ol
16.60	0.32	Trans sabinene hydrate
19.73	0.11	Cis sabinene hydrate
19.91	0.91	Linalool
21.39	1.48	Trans caryophyllene
21.79	0.66	4-terpineol
25.36	0.44	Isoborneol
26.56	0.12	Carvone
34.88	0.77	Caryophyllene oxide
39.57	0.12	Spathulenol
41.80	2.00	Thymol
42.62	60.43	Carvacrol

Table 3. Effect of *Thymbra spicata L.* essential oil on live weight and live weight gain

Live Weight, g			**Days**				***Significance***	
Treatment	**1d**	**7d**	**14d**	**21d**	**28d**	**35d**	***(GLM Repeated Measures)***	
Control	8.68 ±.11	34.71±.86	96.55±1.56	172.54±2.26	233.55±2.82	273.80±3.85^{b}	*Treatment*	*NS*
T_1	8.78 ±.08	32.95±.68	95.57±1.17	171.06±1.63	232.90±2.38	285.12±3.30^{ab}	*Time*	**
T_2	8.83 ±.11	34.24±76	97.29±1.37	176.13±2.01	236.67±2.50	284.00±3.85^{ab}	*Treatment-Time*	*
T_3	8.68 ±.11	33.52±.59	94.00±1.28	171.64±2.10	235.56±3.28	290.70±4.81^{a}		
Live Weight Gain, g			**Days**				***Significance***	
Treatment	**1-7d**	**7-14d**	**14-21d**	**21-28d**	**28-35d**	**1-35d**	***(GLM Repeated Measures)***	
Control	26.03±.88	61.85±1.63	75.87±2.88	61.05±3.95	40.25±.4.92^{b}	265.44±3.82^{b}	*Treatment*	*
T_1	24.17±.67	62.63±1.48	75.47±1.99	62.07±2.91	52.24±3.75^{a}	276.1±3.30^{ab}	*Time*	**
T_2	25.41±.75	63.05±1.62	78.57±2.50	60.53±2.95	47.33±4.33^{ab}	275.15±3.85^{ab}	*Treatment-Time*	*
T_3	24.84±.59	60.42±1.37	77.44±2.39	63.85±3.92	55.13±5.96^{a}	282.01±2.02^{a}		

^{a,b,c} Means within a column in each variable with no common superscript differ significantly (P<0.05).
*: P<0.05, **P<0.01, NS: Non-significant

Table 4. Effect of *Thymbra spicata L.* essential oil on feed intake and feed efficiency

Feed Intake, g			**Days**				***Significance***	
Treatment	**1-7d**	**7-14d**	**14-21d**	**21-28d**	**28-35d**	**1-35d**	***(GLM Repeated Measures)***	
Control	40.84±0.29	119.07±1.30	182.42±7.31	254.26±2.86	271.66±6.19	868.27±14.32	*Treatment:*	*NS*
T_1	40.59±1.35	120.03±2.73	173.78±2.19	254.13±2.56	268.46±2.17	856.99±7.53	*Time:*	**
T_2	38.77±0.99	119.57±3.25	178.09±3.50	254.70±4.13	273.35±5.43	864.48±14.40	*Treatment-time:*	*NS*
T_3	38.42±0.87	117.56±1.46	178.26±3.35	259.47±3.51	281.86±4.04	875.59±10.28		
Feed Efficiency			**Days**				***Significance***	
Treatment	**1-7d**	**7-14d**	**14-21d**	**21-28d**	**28-35d**	**1-35d**	***(GLM Repeated Measures)***	
Control	1.56±0.011	1.92±0.021	2.40±0.096	4.16±0.046	6.74±0.153^{a}	3.27±0.054^{a}	*Treatment*	**
T_1	1.67±0.055	1.91±0.043	2.30±0.029	4.09±0.041	5.13±0.041^{b}	3.10±0.027^{b}	*Time*	**
T_2	1.52±0.039	1.89±0.051	2.26±0.044	4.20±0.068	5.77±0.114^{b}	3.14±0.052^{b}	*Treatment-Time*	*
T_3	1.54±0.035	1.94±0.024	2.30±0.043	4.06±0.055	5.11±0.073^{b}	3.10±0.036^{b}		

^{a,b,c} Means within a column in each variable with no common superscript differ significantly (P<0.05).
*: P<0.05, **P<0.01, NS: Non-significant

A total of eight quails (4.16%) died during the entire experimental period. Mortality for quails fed the control diet was not different from that of quails fed with *T. spicata* EO diets (x^2:1.043, P>0.05). Distributions of gender were also not statistically different among the experimental groups (x^2:1.813, >0.05). The effects of treatments on quails' performance are given in Table 3. *T. spicata* EO has significantly important effect on live weight and live weight gain in different dosages and interval (weeks). In generally, final live weight was significantly higher in the quails fed the diet supplemented with the EO at 600mg/kg level compared to the control group (P<0.05). This time-depended differences arised from the improvement observed in live weight gain in 28-35 days (P<0.05). Thus, starting differences in live weight gain in those days was noticed a statistically improvement for live weight gain of the quails fed the diet supplemented with the EO at 600mg/kg level compared to the control group (P<0.05) for all over the experiment.

No differences were observed among treatments for feed intake. There were a significantly improvement feed efficiency for all treatments groups at 28-35 days (P<0.001); thus, significantly differences were observed at 0-35 days (P<0.05) in supplemented groups (Table 4). *T. spicata* EO has significantly important effect on feed efficiency in different dosages and interval (weeks). Dietary essential oil supplementation significantly improved feed efficiency in all supplemented groups compared to control (P<0.01). This time-depended effect arised from the improvement observed in feed efficiency in 28-35 days (P<0.05) and noticed a statistically improvement for feed efficiency the quails fed the diet supplemented with EO to the control group (P<0.05) for all over the experiment. A significantly time-depended increase was observed

in live weight of in the quails fed the diet supplemented with the EO at 600 mg/kg level. Moreover, live weight gain significantly increased over ranges of 28-35 and 0-35 days in quails fed the EO at 600 mg/kg level compared to the control group (P<0.05). No effects of the experimental diets on feed intake were noted (Table 3). There were significantly time-depended differences for feed efficiency in all supplemented groups with averages of 6.75, 5.13, 5.77, and 5.11 at 28-35 days (P<0.001); and with averages of 3.27, 3.10, 3.14 and 3.10 at 0-35 days (P<0.05), respectively. The results are in agreement with the results of studies involving broiler on supplementation of thyme oil (NAJAFI and TORKI, [17], ABOUBAKER, [18]). ABOUBAKER [18] studied the effects of different levels of thymol (0, 100, 200, 300, 400 or 500 mg/kg) on broiler performance and showed that different levels of thymol revealed quadratic effects on live weight gain (LWG) and feed intake (FI), as well as linear improvements on feed conversion ratio (FCR). Hoffman-Pennesi and Wu [19] who reported that supplementation of poultry feed with thymol (0.2, 0.4 and 0.8 g/kg) and thyme oil (2 and 4 ml/kg) had no significant effect on the broiler performance. On the other hand, Sengül et al. [20] who reported that thyme extracts supplementation at the 2.5 ml/kg level did not affect performance but slightly decreased the feed consumption of Japanese quails during the experiment.

All levels of the EO significantly decreased the total bacteria and *E. coli* counts in the small intestine compared to the control; however, a superior antibacterial effect was observed in the diet supplemented with the EO at 600 mg/kg compared to those others (Table 5). Antimicrobial action of EO is mediated by the lipophilic property to perforate the bacterial membrane, which releases membrane components from the cells to the external environment (HALENDER et al., [21]). Numerous *in vitro* studies demonstrated that EO from plants including thymol, carvacrol etc., displayed antimicrobial activity against intestinal microbes such as *C. perfiringens*, *S. thphimurium* and *E. coli* without negatively affecting their natural gut biota and in some cases improving the gut microflora (SI et al., [22]). In the present study, dietary *T. spicata* EO supplementation showed significantly antibacterial effects on the cecal total bacteri and *E. coli* counts depends on the EO's dosage. Total bacteria and *E. coli* counts were significantly decreased in line with increasing EO levels in the diet (P < 0.01). Aboubaker [18] reported that birds supplemented with different levels of thymol (100, 200, 300, 400 or 500 mg/kg) expressed quadratic effects for total viable count in crop and small intestine, as well as quadratic and linear effects for *Lactobacillus spp.* in the crop and a trend for quadratic effects in the small intestine. Moreover, quadratic effects have been observed on colony forming unit of *E. coli* in the small intestine and caecum. Similarly, Khaskar et al. [23] found that thyme oil from *Zataria multiflora* in broiler diet at 0.1% level significantly decreased colony forming unit of *E. coli* in the ileal microbial population compared to that of unsupplemented group. Superior antibacterial effect depends on increased T. spicata level was the one of the most remarkable findings observed from current study. These observations have been supported by recent in-vivo data by Tiihonen et al. [24] who reported that active components of some herbs have strong antibacterial effect enough to be compared with conventional antibiotics. Moreover, a study where antibacterial effects of ten different essential oil derived from herbs were investigated, it was reported that essential oils had more antibacterial effect than conventional antibiotic (*vancomycin)* on antibiotic-resistant staphylococcus [25].

Table 5. Cecal *E. coli* and total bacteri counts of Japanese quails (cfu/g digesta $^{-1}$)

Items	CTL	T1	T2	T3	P<
E.coli	3.95±0.08^{a}	2.71±0.08^{b}	2.03±0.02^{c}	1.69±0.07^{d}	0.000
Total bacteria	5.28±0.03^{a}	4.14±0.07^{b}	3.69±0.11^{c}	3.31±0.11^{d}	0.000

^{a,b,c} Means within a line in each variable with no common superscript differ significantly.

The effects of EO supplementation on some blood parameters of Japanese quails are given in Table 6. EO supplementation did not alter the total cholesterol levels but a significantly decrease was observed for TG levels in all groups. The lowest TG level was observed in the quails fed the diet supplemented with the EO at the 400 mg/kg level. Thus, TG/TC rate was significantly decreased in the groups supplemented with EO ($P<0.001$). No differences were observed among experimental groups for serum HDL and LDL levels.The reduction of triglycerides and cholesterol noticed with the supplementation of essential oil in animal studies was attributed to the lowering effect of thyme or carvacrol on hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-Co A) reductase, which is the rate-limiting enzyme of cholesterol synthesis (LEE et al., [26], LEE et al., [27]). Our results failed to show any superior hypocholesterolemic effect of depending on increasing levels of dietary *T. spicata* EO but a significantly decrease was observed in TG/TC rate, especially at the group supplemented with at 400 mg/kg level. As considers this finding, it may be speculated that after a certain level, *T. spicata* EO may have more impact on lipogenesis than on cholesterol biosynthesis. The main metabolic pathway of these compounds after a certain dose might be changed in the blood and, thus, the hypocholesterolemic effect of EOs might constitute a weakness. These findings are in agreement with (LEE et al., [26], LEE et al., [27]) who noted that dietary carvacrol and thymol did not promote hypocholesterolemic activity. On the other hand, Lee et al. [27] indicated that dietary carvacrol, but not thymol, lowered plasma triglyceride in the light of a later conducted study. On the other hand, Bolukbasi et al. [28] reported that thyme oil at 100 and 200 mg/kg in broiler diets, except to TC value for 100 mg/kg level of thyme oil, significantly increased TC, TG, HDL and LDL levels of broilers ($P<0.05$). Oxidative reactions are an important function for cellular activities. Blood contains many antioxidants that scavenge harmful radicals (RICE-EVEN, [29]). There is a critical balance between oxidants and antioxidant defenses. If cells are unable to maintain this redox balance, oxidative stress occurs (REITER et al., [30]).

In the present study, TAS values were not different among the experimental groups but there was a linear decrease in first two dosage of *T. spicata* EO while an increase was observed in TAS value for the group supplemented with at 600mg/kg level. TOS value significantly increased in the quails fed the diet supplemented with the EO at the 600 mg/kg level, while a decreasing tendency was observed in the quails fed the diet supplemented with the EO at both the 200 and 400 mg/kg levels. Except for this quadratic response between T2 and T3 groups there were no effect of experimental diets on OSI value. Considering the findings, it appears that low level supplementation of *Thymbra spicata* EO, especially 400 mg/kg, delayed the lipid peroxidation in quails. On the other hand, a slightly increase in the serum LDL in the quails fed the diet supplemented with EO at the 600 mg/kg level might be contributed to the formation of reactive oxygen species, thus lipid peroxidation might be induced. These results are in agreement with Hoffmann-Pennesi and Wu [19] who reported that hydrophilic and lipophilic oxygen absorbance capacity (ORAC) were decreased when supplementation level of thyme oil increased from 0.4 to 0.8 g/kg in broilers, with averages of 58.11-51.66, and 0.90-0.85, respectively. Although the antioxidant effect of different essential oils and other plant extracts is generally accepted in the literature (ERCISLI et al.[31], COSTEA et al [32, 33], ASAN-OZUSAGLAM et al [34], MOTHANA et el [35] , RASHID et al [36]), however, in some cases, antioxidants may cause production of additional radical species such as hydrogen peroxide, phenoxyl and superoxide radicals. The pro-oxidant effect depends on the density of the natural extracellular antioxidant components that have penetrated to the cell (SAKAGAMI et al., [37]). Moreover, the concentration of

reactive oxygen substances has a known correlation with the cholesterol and LDL-cholesterol concentration in plasma (SCHIMKE et al., [38]).

Table 6. Serum lipid profile and antioxidant status

	Lipid Profile				
Items	**CTL**	**T1**	**T2**	**T3**	**P<**
TC (mg/dl)	209.79±9.62	212.23±8.25	219.72±7.25	229.06±10.24	0.104
TG(mg/dl)	145.78±4.26^{a}	138.28±2.56^{ab}	124.12±3.60^{c}	133.40±3.44^{bc}	0.000
TG/TC	0.74±0.04^{a}	0.65±0.02^{ab}	0.56±0.02^{c}	0.65±0.03b^{c}	0.000
HDL (mg/dl)	112.71±8.29	93.34±7.92	92.65±7.81	95.81±6.72	0.139
LDL (mg/dl)	28.50±1.87	28.71±1.45	26.06±2.12	32.93±2.83	0.266
	Antioxidant Status				
TAS	1.56±0.06	1.55±0.07	1.57±0.07	1.60±0.07	0.680
TOS	37.68±3.20^{ab}	33.62±3.05^{b}	31.44±2.41^{b}	49.47±5.78^{a}	0.055
OSI	25.75±2.60^{ab}	24.25±2.84^{ab}	22.42±2.31^{b}	33.13±3.59^{a}	0.117

^{a,b,c} Means within a line in each variable with no common superscript differ significantly.
TAS (µmol, Trovolox Eq/L); TOS (µmol, H_2O_2 Eq/L)

4. Conclusion

Dietary *T. spicata* EO enhanced the performance parameters showing time-depended activity. This property could be important to recommend the implementation time of such compounds into the poultry diets.

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