EFFECTS OF ESSENTIAL OIL MIXTURE SUPPLEMENTATION TO BASAL DIET ON FATTENING PERFORMANCE, BLOOD PARAMETERS AND ANTIOXIDANT STATUS OF TISSUES IN JAPANESE QUAILS EXPOSED TO LOW AMBIENT TEMPERATURE

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ABSTRACT

In this study, effects of essential oil mixture (MEO) [Thyme (Origanum vulgare): 50%; orange peel (Citrus sinensis): 25%; bay leaf (Laurus nobilis): 12.5%; eucalyptus (Eucalyptus camaldulensis): 12.5%] supplementation to basal diets on performance, carcass characteristics, some blood parameters and antioxidant parameters in Japanese quails (Coturnix coturnix Japonica) exposed to low ambient temperature were investigated. Totally 90, 15-day-old quail chicks were divided into 3 groups with 3 repetitions and each repetition involved 10 quails. All groups were balanced in terms of initial live weight and gender. The quails were kept in the wire cages at 22°C for 16 hours/day and at 6-8°C for 8 hours/day (22:00-06:00) in the temperature controlled rooms during the study. The quails were fed with basal diet (Control) and basal diet in which mixture of 50 ppm (MEO-50) and 100 ppm (MEO-100)MEO were supplemented into basal diet. In the study, performance and carcass parameters were similar among groups ($P>0.05$).MEO supplemented into basal diet in quails raising in low ambient temperature considerably decreased serum glucose, triglyceride, total cholesterol, uric acid, and total protein levels compared to the control group ($P<0.05$). It was found that while malondialdehyde (MDA) levels in liver and heart tissues ($P<0.001$) were the lowest in MEO-50 group, glutathione peroxidase (GSH-Px) enzyme activity ($P<0.01$) was the highest inMEO-100 group. Levels of poly-unsaturated fatty acids (MUFA, PUFA, omega 3, omega-6/omega3) were higher in groups supplemented with MEO compared to the control group ($P<0.05$).
Consequently, the supplementation of MEO into basal diets of quails did not affect performance parameters, on the other hand, decreased negative effects of low ambient temperature on blood parameters and meat quality.

Keywords: Essential oil mixture, fattening performance, meat quality, oxidative stress, quail.

INTRODUCTION

Sudden changes of temperatures and/or low temperatures cause a response also reaching up to cellular dimensions in all living creatures and stress-induced death. Especially temperatures below 16˚C may cause serious decreasesin performance in poultry sector. Therefore, animal husbandry would be far from being economical with yield decrease. Cold stress displays an immunosuppressant in animals (Hangalapura et al., 2006), especially causing cellular and tissue damages as a result of lipid peroxidation of cell membranes (Lowry et al., 1951).Reactive oxygen species (ROS)forms in mitochondria during normal metabolism. Toxicity and lipid peroxidation in biomembranes occur in cases that cellular mechanisms cannot eliminate these reactive metabolites (Berzinska-Slebodzinska, 2001). Normally, these active metabolites are rapidly transformed into harmless metabolites by antioxidant pathways at mitochondrial level. However, the amount of ROS produced by mitochondria is more than antioxidant capacity in cases of oxidative stress or deficiencies of endogenous antioxidant systems. Therefore, ROS escaping from antioxidant system causes deformation of cellular functions and even cell and tissue injury by inducing lipid peroxidation (Berzinska-Slebodzinska, 2001). Antioxidants and various additives supplementing immune system and antioxidant system are used in the diet in order to reduce negative effects of cold stress in animals(Ciftci *et al.,* 2016).

Natural and reliable alternative sources have attracted attention of researchers after antibiotics, growth factors like growth hormone, insulin like growth factors etc. and productive additive substances were prohibited in

animal feeds.In this context, aromatic plants and their essential oils have acquired currency. There were studies indicating that supplementation of essential oils alone and as a mixture into diet increased fattening performances of broilers and their synergistic effects were observed when they were supplemented as mixture(Ciftci*et al*., 2009;Ciftci*et al.,* 2013). It was reported that aromatic plants and essential oils obtained from them increase the amount and activity of enzymes in the digestive system, provide regulation of intestinal microbial flora, strengthen immune system, and extend shelf life of products due to its antioxidant effect; thus they can be used as feed supplement in feeding animals(Bilgin and Kocabagli, 2010;Giannenas*et al.,* 2013).

Turkey is considered as the centre of Lamiaceae family. This family is found mountainous areas of Mediterranean region and is observed as endemic at the rate of 44.2%. All of the commonly used thyme species in Turkey belong to this family and these species belong to the genera Origanum, Thymbra, Coridotyhmus, Satureja, and Thymus (Baydar *et al.,*2004). In different thyme species, concentrations of thymol and calvacrolun, which are two of the most important components of essential oil in thyme, vary between 3% and 60% (Kamel, 2000). Essential oils and flavonoids are concentrated on pigment glands found in coloured (flavedo) portion of orange peels and are approximately 0.2-0.5% of this fruit. Being the major component of peel extract,limonene is almost 95% of it(Botsoglou*et al.,*2002). Previous studies have reported that supplementation of orange peel essential oil to poultry feed have positive effects on performance, egg production and some blood parameters(Basmacioglu*et al.,* 2004;Ciftci *et al*., 2016). Being one important species of Laurus genus, one of 40 genera from Laureceae family, Mediterranean bay(*Laurus nobilis L*.), is naturally grown in coastal parts of Aegean, Mediterranean, and Black Sea Regions. Essential oil (1-4%), tannin, and bitter substances are found in composition of bay leaves. Rate of essential oil varies depending on growth region and sineol is foundat the rate of 35-50% in its composition (Karaogul*et al*., 2012).Eucalyptus trees belong to Myrtaceae family and it has nearly 3800 species in the world. Eucalyptus trees have green parts all the year round, are tall, and grow in almost all regions of the world (Boland *et al*., 1991;Mabberly, 1997). Eucalyptus (Eucalyptus camaldulensis) appears also as the common aromatic plant of Mediterranean region.

This study was conducted in order to determine the effects of essential oil mixture supplementation (thyme, orange peel, bay leaf and eucalyptus) to basal diet onperformance, carcass characteristics, some blood parameters, antioxidant parameters of liver and heart and meat quality among Japanese quailsexposed to low ambient temperature.

MATERIALS AND METHODS

Experimental design and diet regimens: In the study, 90 Japanese quails (Coturnix coturnix Japonica) with equal number of male-female (in mixed gender), supplied from a business firm were used as animal material. Approval of Firat University Animal ResearchEthics Committee **(Protocol Number: 2014/133)** was received for the study. The study was conducted at the Poultry Unit, Faculty of Veterinary, Firat University. In the study, a basal diet containing 24.10% crude protein, 3121 kcal/kg metabolic energy based on corn and soybean meal according to the requirements stated in NRC (1994) standards was prepared by a private feed factory(Table 1). The formula stipulated by Carpenter and Clegg (1956)was used in order to calculate metabolic energy value in diet. Any kind of supplement promoting growth was not added into diet mixtures. For the animal welfare to decrease the mortality of birds, 8 day-old quails were purchased from a commercial firm, fed all together for 7 days to accommodate the environment, and when they were 15 days old, they were divided into 3 groups.The groups were further divided into 3 sub-groups involving 10 quails. Initial weights of the groups were set in a way that there was no statistical difference among the groups. The quails were kept in the wire cages at 22°C for 16 hours/day and at 6-8°C for 8 hours/day (22:00-06:00) in the temperature controlled rooms during the study. In the experiment, presence and levels of essential oil mixture (MEO) in diets were the main factors tested. In the control group the birds were fed a standard diet (Control group). Two different levels of MEO was added to the standard diets to generate the other two treatment groups. For the MEO treatments, 50 ppm (MEO-50 group) and 100 ppm (MEO-100 group) MEO were added to the standard diets.Premixed MEO prepared by saturating zeolite was supplied from a commercial firm (Agromiks Yem Katki Maddeleri Hayvancilik Gida San. ve Tic. Ltd. Sti, Izmir).Then,these premixtures were added into diet of 100 kg. Table 2 illustrates gas chromatography analysis result given by producing company for MEO supplementedintobasal diet.

Mean live weights of the quails were weekly and individually determined by the help of 1 g precise balance. Differences between live weight measurements in individually two weeks were recorded as live weight gain. Feed consumption of the quails was determined by subtracting amount of dietsremaining in feeder on the days, in which the quails were weighed, from total amount of diet given by weighing every day during that period. Mean feed consumption per quail was detected by dividing the amount of diet consumed between two weighings to the number of days and number of the quails belonging to that group. Dead quails were not taken into consideration in determining the mean feed consumption. Weekly feed conversion ratios of the quails

were calculated by dividing total diet amount consumed between two weighingsas from the beginning to total live weight increase determined between these two weighings. Six quails (3 males, 3 females) with mean group weightwere slaughtered from each group while selecting the birds, repetitions were taken into consideration. Blood samples were taken and examined by using biochemical analyser (Architect i2000, Germany). Hot carcass weights of the quails were determined by removing their internal organs (except for kidneys and lungs) after their feathers wereplucked andtheir heads and legs were cut. Yield was calculated in percentage by dividing hot carcass, liver, spleen, and heart weights to slaughter weight (Anonymous, 2009).

Chemical analyses: Raw nutrient content of basal diet wereanalysed in Feed Analysis Laboratory, Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Science, Fırat University. While raw nutrient (dry matter, crude ash, crude protein, and ether extract) compositions of basal diet were determined according to analysis methods stated in AOAC(2000). Amount of crude fiber was determined according to Crampton and Maynard (1983).

Tissues lipid peroxidation (MDA) levels were determined according to spectrophotometric method defined by Placer et al. (1966).GSH-Px activity level was specified as stated by Lawrence and Burk (1976). GSH level was determined based on the method stated by Sedlak and Lindsay (1968). In this method, SOD activity measurement was performed according to the method reported by Sun *et al.* (1988) based on the fact that superoxide radical produced by xanthine- xanthine oxidase system renders coloration by reducing nitroblue tetrazolium (NBT). Tissue protein content were determined by the method of Lowry *et al.* (1951).

Lipids were extracted from tissue samples and feed (Table 3) with Hara and Radin (1978)method in which 3:2 (v/v) hexane isopropanol mixture was used. In order to carry out gas chromatographic analysis of fatty acids found in lipids, they were transformed into derivatives such as methyl esters with non-polar volatile and stabile structure.This analysis was performed as stated by Christie (1992).After fatty acids in lipid extract were transformed into methyl esters, they were analysed by usingSHIMADZU GC 17 Ver. 3 gas chromatography. For this analysis, 25m long Machery-Nagel (Germany) capillary colon having 0.25 µm inner diameter, and PERMABOND 25 micron film thickness was used. Colon temperature was kept as 120- 220 ºC, injection temperature as 240 ºC, and detector temperature as 280 ºC during the analysis. Colon temperature program was adjusted from 120 ºC up to 220 ºC, the temperature increase was determined as 5 ºC/min up to 200 ºC and 4 ºC/min from 200 ºC up to 220 ºC. It was determined as 8 minutes at220 ºC and 35 minutes for total duration. Nitrogen gas was used as carrying gas. Before analysis of fatty acid methyl esters belonging to samples, retention time of each fatty acid was determined by injecting standard mixtures of fatty acid methyl esters during analysis. After this process, analysis of mixtures of fatty acid methyl esters belonging to samples was performed by adjusting required programming.

Statistical analysis:Significance of all datas were determined by using Statistical Packages for the Social Sciences for Windows (23). (SPSS, 2002).Data were given as mean±standard error of means.While the analysis of variance was used todetermine difference amongthe groups for performance, carcass, blood parameters, antioxidant parameters and fatty acids structures of tissues.Tukey HSDtestwas used to compare sub-groups and chi-square analysis was used to compare survival parameter. $P \leq 0.05$ was considered as statistically significant.

RESULTS

Table 4 shows the data of performance. As Table was examined, any statistical difference was not determined among the groups in terms of live weight, live weightgain, feed consumption, and feed conversion ratios ($P>0.05$). In the study, no statistical difference was found between the groups in terms of data ofsurvival (Table 5) and carcass characteristics (Table 6) ($P>0.05$).

It was found that serum glucose, triglyceride, total cholesterol, uric acid, and total protein level were higher in the control group compared to experimental groups(Table7). MEO supplemented into basal diet positively affected these parameters ($P<0.05$). While liver and heart MDA level increased in control group(Table 8), MDA level decreased in the group in which 50 ppm supplement was added ($P<0.001$). GSH-Px activity was found to be high in the group added with 100 ppm supplement ($P<0.01$).

The essential data was obtained on fatty acid profile of breast meat of quails subjected to cold stress as shown at Table 9. The essential fatty acid deposition was significantly increased by MEO supplementation. Although total SFA and omega 6 levels were similar among groups, MUFA ($P<0.01$), omega-3 ($P<0.05$) and PUFA ($P<0.05$) levels which were valuable for functional food, were increased by MEO supplementation, especially 50 ppm supplementation. In addition, omega-6/omega-3 ($P<0.01$) ratio was significantly decrased in 50 ppm MEO supplementation group. It was magnificant that the essential omega-3 fatty acids were highly deposited in MEO-50 group.

Table 1. Ingredients and chemical composition of standard and experimentaldiets[*] (%)

Ingredients	%	Nutritional composition	%
Maize	40.00	Dry matter	89.41
Wheat	9.00	Crude Protein	24.10
Soybean meal (48% CP)	29.00	Crude cellulose	3.38
Corn Gluten	11.50	Ether extract	6.30
Soybean oil	4.00	Crude ash	6.25
DL-Methionine	0.34	Calcium	1.00
Dicalcium phosphate	2.91	Available phosphorus	0.49
Ground limestone	1.00	Sodium	0.18
L-Lysine Hydrochloride	0.33	Meth+Cysteine	1.09
L-Treonine	0.09	Lysine	1.41
L- Tryptophan	0.09	Threonine	0.96
$NaHCO_3$	0.10	Tryptophan	0.37
Salt	0.30	ME, kcal/kg[****]	3121
Vitamin-Mineral Mix[**]	0.34		
Zeolite[***]	1.00		
TOTAL	**100**		

[*] Experimental diets were differentiated with essential oil impregnated Zeolite supplementation.
[**]Vitamin premix supplied per kg; Vitamin A 15.500 IU; vitamin D_3 3.500 IU; Mineral premix supplied per kg; Mn 120 mg; Fe 40 mg; Zn 100 mg; Cu 16 mg; Co 200 mg; I 1.25 mg; Se 0.30 mg,
[***]Group Control (1000 g zeolite); Group MEO 50 (5 g MEO + 995 g zeolite); Group OPE 100 (10 g MEO + 990 g zeolite)
[****]Calculated, ME (kcal/kg) = 53+38 B used formula. B= (% Crude protein) + (2.25) (%Ether extract) + (1.1) (% Starch) + (% Sugar)

Table 2. The concentrations of the volatile components in mix essential oil (%)

Analysis	Result[*]
Carvacrol	48.25
1,8 Cineol	15.74
Limonen	10.13
5-Methyl-2,4-Diisopropyl Phenol	7.58
Thymol	4.41
Para Cymen	3.29
Gamma Terpinen	2.28
Trans Caryophyllene	1.70
Propylene Glycol	1.48
2-Cresol	1.18
Alpha Pinen	0.94
Beta Myrcene	0.71
Alpha Terpinen	0.59
Solanone	0.46
Linalool	0.35
Alpha Terpineol	0.23
Sabinen	0.22
Undefined	0.46

[*]: obtained by GC-MS analysis

Table 3. Fatty acid composition of basal diet (%).

Fatty acids	%
ΣSFA	14.52
ΣMUFA	25.42
ΣPUFA	60.06
Σ ω-6	55.07
Σ ω-3	4.63
Σ ω-6 / ω-3	11.89

SFA: Saturated Fatty Acid, MUFA: Monounsaturated Fatty Acid, PUFA: Polyunsaturated Fatty Acid

Table 4. Effect of mix essential oil (MEO) supplementation on performance of Japanese quails reared under low ambient condition

Traits	Control	MEO-50	MEO-100	SEM	P
Live Weight, g					
15. day	46.90	46.90	46.91	0.99	NS
22. day	78.83	77.40	81.31	1.63	NS
29. day	118.74	120.64	120.76	2.47	NS
36. day	155.19	159.70	160.37	2.67	NS
43. day	184.42	187.90	191.87	1.97	NS
Live Weight Gain, g / bird / day					
15 - 22 days	4.56	4.36	4.91	0.11	NS
22 - 29 days	5.71	6.18	5.64	0.23	NS
29 - 36 days	5.21	5.58	5.66	0.22	NS
36 - 43 days	4.18	4.03	4.50	0.18	NS
15 - 43 days	4.74	4.86	5.00	0.08	NS
Feed Intake, g / bird / day					
15 - 22 days	13.40	13.68	15.58	0.39	NS
22 - 29 days	19.79	20.80	19.69	0.44	NS
29 - 36 days	24.97	25.91	25.69	0.31	NS
36 - 43 days	29.14	29.42	29.60	0.41	NS
15 - 43 days	21.83	22.45	22.64	0.24	NS
Feed Conversion Ratio, g feed / g gain					
15 - 22 days	2.94	3.14	3.17	0.05	NS
22 - 29 days	3.47	3.37	3.49	0.07	NS
29 - 36 days	4.79	4.64	4.54	0.16	NS
36 - 43 days	6.97	7.30	6.58	0.18	NS
15 - 43 days	4.61	4.62	4.53	0.03	NS

MEO-50: Supplemented 50 ppm Mix Essential Oil (MEO); MEO-100: Supplemented 100 ppm Mix Essential Oil (MEO)
P: Statistical significance, SEM: Standard error mean, NS: No significant

Table 5. Effect of mix essential oil (MEO) supplementation on mortality and viability rates of Japanese quails reared under low ambient condition (%)

Days	Control	MEO-50	MEO-100	Chi-Square
15 - 22	-	-	-	-
22 - 29	2	2	1	-
29 - 36	-	-	1	-
36 - 43	1	-	-	-
Total	3	2	2	-
Mortality rate	10.0	6.7	6.7	-
Viability	90.0	93.3	93.3	P: 0.856 X^2: 0.310

MEO-50: Supplemented 50 ppm Mix Essential Oil (MEO); MEO-100: Supplemented 100 ppm Mix Essential Oil (MEO)

Table 6. Effect of mix essential oil (MEO) supplementation on carcass characteristics of Japanese quails reared under low ambient condition

Traits	Control	MEO-50	MEO-100	SEM	P
Slaughter Weight, g	173.18	177.02	177.60	3.50	NS
Carcass Weight, g	116.98	122.58	123.04	2.86	NS
Carcass Yield, %	67.46	69.58	68.61	1.28	NS
Liver Weight, g	5.91	5.73	5.58	0.25	NS
Liver Ratio, %	3.41	3.22	3.11	0.12	NS
Heart Weight, g	1.85	1.94	1.99	0.06	NS
Heart Ratio, %	1.07	1.10	1.11	0.03	NS

Spleen Weight, g	0.12	0.11	0.11	0.01	NS
Spleen Ratio, %	0.07	0.06	0.06	0.00	NS

MEO-50: Supplemented 50 ppm Mix Essential Oil (MEO); MEO-100: Supplemented 100 ppm Mix Essential Oil (MEO)
P: Statistical significance, SEM: Standard error mean, NS: No significant

Table 7. Effect of mix essential oil (MEO) supplementation on blood biochemical parameters of Japanese quails reared under low ambient condition

Traits, (mg / dL)	Control	MEO-50	MEO-100	SEM	P
Glucose	274.17^{a}	248.00^{b}	254.33^{b}	3.64	*
Triglyceride	88.67^{a}	61.83^{b}	70.83^{b}	4.36	*
Total Cholesterol	184.00^{a}	159.33^{b}	158.83^{b}	4.85	*
Uric Acid	5.52^{a}	4.25^{b}	3.43^{b}	0.36	*
Total Protein	3.28^{a}	2.93^{b}	2.87^{b}	0.07	*

MEO-50: Supplemented 50 ppm Mix Essential Oil (MEO); MEO-100: Supplemented 100 ppm Mix Essential Oil (MEO)
P: Statistical significance, SEM: Standard error mean, *: $P < 0.05$, ^{a,b}: Mean values with different superscripts within a row differ significantly

Table 8. Effect of mix essential oil (MEO)supplementation on antioxidant status of liver and heart tissues in Japanese quails reared under low ambient condition

Traits	Control	MEO-50	MEO-100	SEM	P
MDA (nmol / g prot)					
Liver	6.91^{a}	4.31^{b}	4.49^{ab}	0.34	***
Heart	5.45^{a}	2.96^{b}	4.00^{ab}	0.27	***
GSH (nmol / g prot)					
Liver	0.13	0.13	0.12	0.01	NS
Heart	0.25	0.24	0.25	0.01	NS
GSH-Px(U / mg prot)					
Liver	0.13	0.15	0.14	0.01	NS
Heart	0.28^{b}	0.28^{b}	0.37^{a}	0.01	**
SOD (U / g prot)					
Liver	39.79	39.06	39.21	1.14	NS
Heart	138.80	140.64	138.54	3.07	NS

MEO-50: Supplemented 50 ppm Mix Essential Oil (MEO); MEO-100: Supplemented 100 ppm Mix Essential Oil (MEO); MDA: Malondialdehyde; GSH: Glutathione; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase
P: Statistical significance, SEM: Standard error mean, NS: No significant, **: $P<0.01$, ***: $P<0.001$, ^{a,b}: Mean values with different superscripts within a row differ significantly

Table 9. Effect of mix essential oil (MEO)supplementation on the fatty acid composition of lipids isolated from breast meat under low ambient condition (%)

Fatty Acids	Control	MEO-50	MEO-100	SEM	P
ΣSFA	47.91	46.29	45.37	0.74	NS
ΣMUFA	2.86^{b}	4.53^{a}	3.34^{b}	0.27	**
ΣPUFA	49.23^{b}	49.18^{b}	51.29^{a}	0.41	*
Σ ω-6	42.31	41.42	43.63	0.69	NS
Σ ω-3	3.57^{b}	5.27^{a}	4.29^{ab}	0.26	*
Σ ω-6 / ω-3	11.85^{a}	7.80^{b}	10.17^{a}	0.44	**

MEO-50: Supplemented 50 ppm Mix Essential Oil (MEO); MEO-100: Supplemented 100 ppm Mix Essential Oil (MEO)
SFA: Saturated Fatty Acid, MUFA: Monounsaturated Fatty Acid, PUFA: Polyunsaturated Fatty Acid, P: Statistical significance, SEM: Standard error mean, NS: No significant, *: $P < 0.05$, **: $P<0.01$, ^{a,b}: Mean values with different superscripts within a row differ significantly.

DISCUSSION

In this study, effects of essential oil mixture supplementation to basal diet on performance, carcass characteristics, serum glucose, triglyceride, cholesterol, uric acid, total protein level, mortality rate, antioxidant parameters of liver and heartamong quails under cold stress were investigated. When performance parameters were examined (Table 4), any statistical difference was not determined between the groups in terms of live weight, live weightgain, feed consumption, and feed conversion ratios. Some of studies conducted to determine the effects of essential oils on live weight in broilers reported that volatile oils positively affected the live weight(Ciftci *et al.,* 2016; Dalkilic*et al.,* 2015); whereas,results of some other studies were statistically insignificant similar to results obtained in this study(Bahsi*et al.,* 2016).Isabel andSantos(2009)examined the effects of organic acid (calcium propionate and calcium formate) and essential oil (clove and cinnamon) supplementation into ration on performance and carcass characteristics in broilers and determined that supplements did not have any effect on live weight gain.Similarly, Ciftci*et al.*(2013)reported that addition of rosemary extract into ration did not affect the live weightgain in broilers. On the other hand, Ciftci et al.(2016) found that orange peel extract supplemented into basal diet had a positive effect on live weight gain. The results obtained in the present study regarding the effect of essential oil mixtures on feed consumption show similarities with studies reporting that essential oils did not affect feed consumption (Bahsi et al., 2016; Ciftci et al., 2013).The effect of used herbal mixture on feed conversion ratio was found to be statistically insignificant. Results obtained in the study are compatible withsome of studies conducted to determine the effects of essential oils on feed conversion in poultry. Tonbak and Ciftci (2012) found that the effects of both heat stress and cinnamon oil on food conversion were insignificant. Furthermore, in the study conducted by Karsli and Donmez (2007) it was reported that stress and herbal extract did not have any significant effect in general.It could be thought that differences between the studies were associated withdifferences of active substance or dose. Various essential oils were determined to show different bioactive properties due to antagonistic and synergistic effect when they were used as mixtures (Simsek*et al*., 2015).

At the end of the study, any statistical difference was not found between the groups in terms of mortality rate(Table 6). This situation could be associated with good hygiene conditions inside the poultry house. Hence, the studies have reported that use of aromatic plant extract does not affect the mortality rate (Ciftci*et al*., 2005;Guler*et al*., 2006).

Essential oil mixture supplementation to diet did not have any significant effect on carcass characteristics. The obtained results have similarity with results of study ofCiftci *et al.*(2016).Any difference was not determined between the groups in terms of carcass characteristics in the study conducted by Ciftci et al. (2009) to investigate the effects of antibiotic (10 mg/kg) and cinnamon oil with different doses (500 and 1000 mg/kg).

In the study, it was found that glucose, triglyceride, total cholesterol, uric acid, and total protein levels increased with the effect of low ambient temperature,but essential mixture added into basal diet had positive effects on these parameters. Results of this study are compatible with some studies(Ciftci *et al.,* 2016; Mary andGomathy, 2008). Glucose from blood metabolites is an important energy source for cells. Since glucose consumption of cells would considerably increase in case that poultry are exposed to acute stress, liver releases the stored glycogen in the form of glucose in order to keep blood glucose level balanced(Garriga*et al*., 2006).Daneshyar *et al*.(2009)stated that cold stress increased blood glucose level from 189.8 mg/dl up to 205.0 mg/dl. In addition, the increased glucose level might be associated with the increased cortisol activity. The glucose production causes the production of non-protein nitrogen and decreases the incorporation of glucose carbon into protein and increases the uric acid excretion (Virden andKidd, 2009).Parallel tothese results, increased serum uric acid levels of control group obtained in the present study may be associated with the presence of cold stress. It was observed that birds kept under cold stress had higher level of total protein and, dietary MEO had a positive effect on blood total protein level. Similarly, Ciftci *et al.* (2016) indicated that cold stress increased the serum total protein level of Coturnix coturnix japonica reared under low ambient temperature.

In the study, cold stress increased MDA level in liver and heart tissue. Cold stress often causes damage to cell membranes. It is well known that environmental stress causes an increased production of free radicals and thus resulting in increased level of MDA. MEO supplemented to basal diet decreased MDA level in liver and heart tissue. This situation may be associated with phenolic compounds found in the structure of essential mixture. As a matter of fact, phenolic compound have properties such as eradicating free radicals (Pekkarinan*et al.,* 1999;Rice-Avans*et al.,* 1995), forming compounds with metal ions (metal chelation) and preventing or reducing singlet oxygen formation (Rice-Avans*et al.,* 1995). Similarly, Ciftci et al. (2016) indicated that orange peel extract supplementation decreased the serum MDA levels of quails exposed to low ambient temperature. In this study, 100 ppm MEO supplementation had an increase on Glutathione peroxidase (GSH-Px) activities of heart.Similarly, Ciftci *et al*. (2016) recorded that the dietary supplementation of orange peel essential oil

significantly changed the glutathione peroxidase activities compared to the control group.

When fatty acid composition of pectoral muscle was examined, 50 ppm MEO supplemented group was determined statistically different from the other two groups in terms of MUFA, omega-3 and omega-6/omega-3 rate. PUFA level in the 100 ppm MEO supplemented group was found to be at higher level.The reason may be decreased oxidation of fatty acids because MEO had antioxidant properties. Similarly, in a study conducted by adding coriander that has antioxidant effects into rat rations it was determined that activities of antioxidant enzymes (catalase and glutathione peroxidase) increased and lipid peroxidation decreased (ChithraandLeelamma1999).In the study conducted by Ertas *et al*., (2005) by adding coriander seed into broiler basal diets, they reported that with addition of coriander, SFA level decreased andMUFA and PUFA levels increased. On the other hand, in the study conducted by Elmali *et al.* (2014) to investigate the effects of herbal extracts and oil mixture (mint, thyme, and anise) supplementation into drinking water on composition of brisket quality in quails, it was determined that composition of pectoral muscle fatty acid was not affected.

Conclusions: Supplementationof essential oil mixture especially at 50ppm dose to basal diets had a positive contribution to serum glucose, triglyceride, cholesterol, uric acid, total protein, liver and heartMDA levels, pectoral muscle MUFA, omega-3 and omega6/omega3 levels. As these results, In this sense, it is evident from the study that we think that essential oil mixture can be a potential supplement in stressful conditions to basal diets of growing quails.

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