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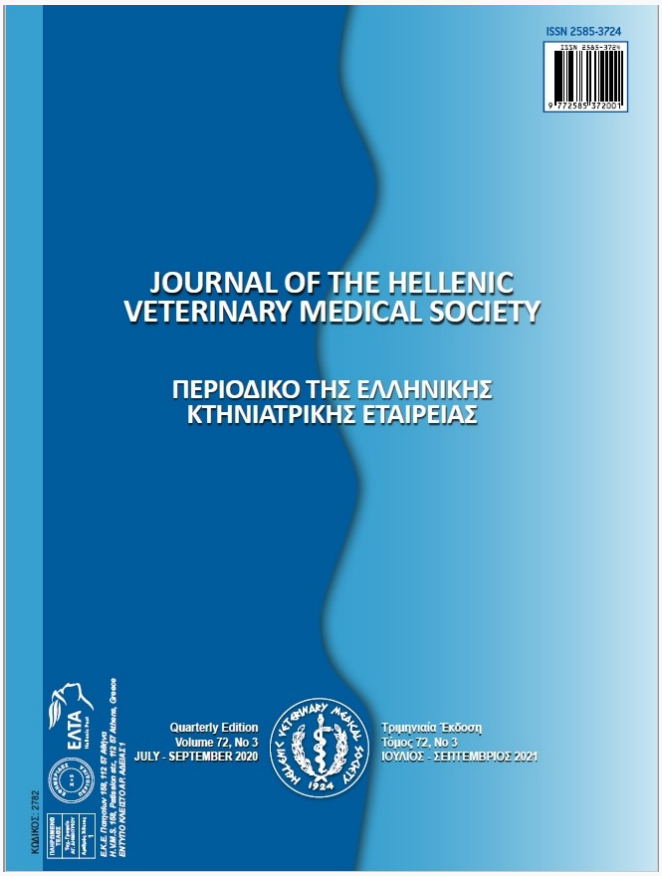

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Effects of pennyroyal (*Mentha pulegium* L.) dietary supplementation on performance, carcass quality, biochemical parameters and duodenal histomorphology of broilers

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ABSTRACT: In this study, the effects of (*Mentha pulegium* L.) dietary supplementation on performance, carcass characteristics, some biochemical parameters, and intestinal histology were investigated in broiler chickens. Four groups were formed as control and groups treated with pennyroyal at different levels (0.25%, 0.50%, and 1.00%). In the experiment. Each group had eight replicates. A total of 192 broilers were used in the study with six broilers in each replicate. Water and feed were *ad libitum* provided. Adding different levels of pennyroyals to broiler rations significantly affected performance parameters; an increase in final body weight and carcass yield and a decrease in total feed intake and the conversion rate was observed ($P<0.05$). Serum cholesterol, malondialdehyde, and glutathione values were also affected by the addition of pennyroyal ($P<0.05$). Pennyroyal was effective in vitro against S. enteritidis, E. coli, S. aureus, S. abortus ovis, B.anthracis sterne strains. Besides, it increased the duodenum villus' length compared to the control group ($P<0.05$). On the other hand, pennyroyal did not affect carcass and visceral organ weights, several serum biochemical values ($P>0.05$). As a result, it was concluded that pennyroyal at the level of 0.50% in ration was effective on health and growth performance of broilers.

***Keywords*:** Broiler; pennyroyal; performance; serum parameters; villus.

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INTRODUCTION

The essential amino acids that are necessary for the human nutrition can be found in foods of animal products (meat, milk, eggs). Most of the essential amino acids needed in the people's healthy nutrition can be met with foods of animal origin (Tuncer, 2007). Healthy and balanced nutrition are possible by covering the animal protein deficit. To bridge the gap in animal production, several studies were implemented in the late 1940s (Emborg et al., 2001; Tuncer, 2007). The effects of antibiotics that were first used in animal health for treatment and protection have been discovered concerning supporting growth and decreasing death rates in farm animals (Emborg et al., 2001; Barreto et al., 2008; Abedini et al., 2017) and have been used in mixed feed as feed additives since then (Hashemi and Davoodi, 2011; Abedini et al., 2017; Kostadinović and Lević, 2018). Research on broiler chickens also coincides with these periods (Moore et al., 1946; Dibner and Richards, 2005; Popović et al., 2019).

However, the use of antibiotics as feed additives has led to the development of antibiotic-resistant bacteria in animals. Many reports state that pathogenic bacteria have gained antibiotic resistance worldwide (Ferket, 2004). Taking antibiotics together with feed, even at low doses, causes drug residues to be left in animal products (meat, milk, eggs) and bacteria to cross immune against the antibiotics used, as well as transferring the resistance between bacteria (Özkan and Açıkgöz, 2007; Adiyaman and Ayhan, 2010; Erhan, 2015). Thus, the use of all antibiotics as feed additives is prohibited with the decision made by the European Committee in 2002 (directive 70/524 / EEC, directive 82/471 / EEC and regulation 1831/2003 / EC) (Cheli et al., 2013). Following the banning of antibiotics, research on new and alternative growth factors which will increase growth performance, reduce costs and prevent the colonization of pathogenic bacteria in animals' digestive system, protect against diseases and improve digestion have accelerated (Karasu and Öztürk, 2014; Erhan, 2015; Abedini et al., 2017). Alternatives to antibiotics in the livestock sector, especially natural yield enhancers have recently found many areas of use (Goodarzi and Nanekarani, 2014; Karasu and Öztürk, 2014). These substances mainly are probiotics, prebiotics, enzymes, and medical aromatic plants (Yörük and Bolat, 2003; Yörük et al., 2008; Christaki et al., 2012; Vuong et al., 2016; Şahin et al., 2020). These substances have been used as drugs, food, and incense since ancient times and according to WHO, there are around 70.000 species in the world, and Turkey's vegetation is composed of 12.000 species and subspecies of the genus in 1251 from 174 families. The use of these substances as feed additives have brought the Medicinal and Aromatic Plants (MAP) forward (Kirici, 2015). These plants have gained importance as alternative feed additives in food animal nutrition, particularly poultry, due to their natural characteristics that do not harm the animal and human health (Çetin, 2016; Ghaly et al., 2017). Studies have shown that MAP increases feed conversion, body weight gain and protect intestinal health (Duru and Şahin, 2015; Erhan, 2015). One of the most widely used plants is Mentha from the Labiatae (*Lamiaceae*) family with 20 different subspecies. One of the most well-known types is Pennyroyal (*Mentha pulegium* L.) (Erhan et al., 2012; Goodarzi and Nanekarani, 2014; Abedini et al., 2017). It is also known by different names such as squaw mint, Mosquito plant, and Pudding grass. It has been determined that the plant spreads from the Mediterranean region to Iran, and its appearance, leaf shape, and hairiness vary as it has many varieties (Davis, 1982). In Turkey, it has been recognized as one of the most easily distinguished species of mint with its morphological structure and its peculiar smell. The chemical structure of pennyroyals includes terpenes and phenolic compounds. Due to these compounds, the plant has antioxidant and antimicrobial effects (Erhan and Ürüşan, 2015). Also, when compared with antibiotics and probiotics, (*Mentha pulegium* L.) was determined to have a positive effect on growth performance and feed conversion values and that it could support growth as an alternative to antibiotics (Abedini et al., 2017).

The purpose of this study was to examine the effects of the aromatic herb pennyroyal that can be obtained naturally and in an inexpensive way on body weight, body weight increase, feed intake, feed conversion rate, carcass traits, some internal organ weight, serum biochemical and oxidative stress parameters and intestinal histology as a growth factor added to the broiler rations at different levels.

MATERIAL AND METHODS

Ethical approval

The experimental protocol and animal care in this study were approved by animal experiments from the local ethics committee (KAÜ-HADYEK/2016-136) of Kafkas University.

Pennyroyal

Pennyroyal (*Mentha pulegium* L.), used as a feed additive in the study was collected in July 2016, from the Kars province (40 ◦ 48'21.2 "N 42 ◦ 53'37.8" E / Google Earth) in Turkey. Botanists performed the species determination of the pennyroyal. The essential oil was obtained by water vapor distillation from the material obtained after drying and grinding the pennyroyal. The essential oil composition of the pennyroyal was determined by the gas chromatography method. Each compound's structure was defined using mass spectra with the Xcalibur program (Wiley 9) (Table 1).

Table 1. Bioactive compounds of Pennyroyal essential oil.

Compounds	Retention time (RT)	International code (Cas)	%
Linalool	21.10	78-70-6	13.61
p-Menthone	24.01	89-80-5	10.56
Terpinen-4-ol	24.71	562-74-3	0.28
p-Menthan	25.36	89-80-5	6.19
Levomenthol	25.53	89-78-1	0.20
α-Terpineol	27.53	470-08-6	0.13
Isopulegone	28.82	29606-79-9	0.11
Pulegone	29.39	89-82-7	4.45
Piperitenone oxide	33.38	35178-55-3	3.07
Thymol	39.36	499-75-2	0.49

Table 2. Nutrient composition and chemical analysis of rations

Ingredients, %	Starter	Grower	Finisher
Corn	55.45	62.60	69.95
Soybean meal (44%)	22.50	10.65	5.00
Corn gluten (60%)	16.25	20.55	20.60
Marble dust	2.38	2.40	1.10
DCP (dicalcium phosphate)	1.93	2.13	1.78
Salt	0.25	0.25	0.23
Vit (K_3A)	0.15	0.15	0.15
Soda	0.10	0.10	0.08
Vit E	0.63	0.65	0.63
Lysine	0.33	0.48	0.50
Methionine	0.05	0.05	-
Chemical Analysis			
ME, Kcal/kg	3025	3150	3210
Dry matter %	89.20	87.90	87.7
Crude protein %	23.80	21.20	20.4
Lysine %	1.20	1.20	1.10
Methionine %	0.66	0.50	0.49

Animals and experimental design

One hundred and ninety-two Ross-308 broiler chicks were used in the study. Control and experimental groups were created by allocating 48 chicks to each group homogeneously. Each group consisted of eight replicates with six chicks. While the broilers in the control group were fed only with mixed feed, pennyroyal was added at the rate of 0.25%, 0.50%, 1.00% in the other experimental groups. Feed and water were *ad libitum* offered. In the experiment, after all the broilers were allotted into groups during the acclimatization period (1 week), they were fed with a starter diet (I) for a week, then fed with a grower diet (II) between the 15th and 28th days, and finally with a finisher diet (III) between the 29th and 42nd day. The nutrient content and chemical analysis results of the feeds are shown in Table 2. All the chicks in the experiment groups excluding those in the control group were provided feed mixed with pennyroyal from the 7th day. The pennyroyal, dried, and powdered was added to the diets by mixing through manual methods. The coop heating was provided with radiators, and electric radiants were used when needed.

The ambient temperature was kept at 32-33˚C for

the first two days, while it was decreased by 1-2°C every week starting on the third day and set to 22-23°C which was maintained until the end of the study. Lighting was provided in the coop 23 hours a day. Wood shavings were used as the bedding. The broilers were observed daily during the study period, mortality was recorded, and their survival rate was determined at the end of the study.

Performance

All broilers were weighed weekly on days 7, 14, 28, 35 and 42 and body weights (BW) were recorded. The daily body weight gain (DBWG) of the broilers were calculated by calculating the difference of total weights between two weighings and dividing by the number of days and broilers. Feed intake (FI) was measured together with weekly weighings, and the daily average feed intake was determined by dividing the weekly average feed intake (AFI) by the number of broilers and days. Using the daily feed intake and daily body weight gain values of the broilers in the subgroups, the feed conversion rate (FCR) was calculated.

Sampling and analysis

The broilers were fasted the day before the end of the study and ten broilers from each group were selected (near to mean weight) for slaughtering. Serums extracted from blood drawn during slaughter were kept at +4°C until the day of analysis. The broilers were sacrificed by cervical dislocation method and their blood was shed, and their internal organs were removed and weighed on a sensitive scale. After removing the internal organs, hot and cold carcass (after kept at -20°C for 24 hours) weighings were made.

After the serums were thawed, some biochemical (cholesterol, HDL, LDL, VLDL, triglyceride, glucose, total protein, albumin, globulin, uric acid) parameters were determined by a spectrophotometric device using commercial ELISA kits (Erba Mannheim-Germany). Malondialdehyde (MDA) from antioxidant parameters was analyzed according to Placer et al. (1966) and glutathione (GSH) was analyzed according to Sedlak and Lindsay (1968).

The antimicrobial effect of the essential oil obtained from the pennyroyal was determined in vitro, and minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were calculated again by spectrophotometric reading.

The measurements of the small intestine duodenum taken during the slaughtering process were examined and photographed under the imaging microscope after detection, blocking, cross-sectioning, and staining (Hematoxylin-Eosin), and villus measurements were performed using the ImageJ (1.46r-Wayne Rasband National Institutes of Health, USA) program.

Statistical analysis

SPSS 18 (IBM SPSS, Chicago, IL) program was used for statistical analyses. Results are presented as means and standard errors (±). A one-way variance (ANOVA) analysis was used to determine the differences between the groups in performance, slaughter and carcass characteristics, serum biochemical measurements, oxidative stress parameters, and histological villus lengths. A Duncan multiple comparison test was used to check the significance of the differences. The significance of the factors in the statistical results was tested at the level of $P<0.05$.

RESULTS

The survival rate of the control and pennyroyal supplemented (0.25%, 0.50%, 1.00%) groups were 91.67%, 89.52%, 89.58% and 95.83%, respectively.

The effects of pennyroyal on performance parameters of broilers are presented in Table 3. It has been shown that the addition of pennyroyal at all levels increases the BWG, lowers the daily AFI, and lowers the FCR. Moreover, pennyroyal dietary supplementation increased total body weight gain (TBWG) and decreased total feed intake (TFI) compared to the control group. At the end of the study, it was determined that the highest BW was in the group with the addition of pennyroyal at the level of 0.50%.

Table 3. Effect of Pennyroyal dietary supplementation on growth performance parameters (g)

Items	Control	0.25% Pennyroyal	0.50% Pennyroyal	1.00% Pennyroyal	P
IBW[1]	178.83± 1.15	176.88± 2.66	180.84± 0.85	180.75± 0.88	0.258
FBW[2]	2165.53± 11.80[b]	2230.78± 19.65[a]	2264.48± 19.81[a]	2259.42± 27.38[a]	0.007
BWG[3]	56.76± 0.32[b]	58.68± 0.53[a]	59.53± 0.57[a]	59.39± 0.77[a]	0.007
AFI[4]	110.25± 1.28[a]	106.71± 0.48[b]	105.70± 0.72[b]	106.63± 1.20[b]	0.002
FCR[5]	1.94± 0.02[b]	1.82± 0.01[a]	1.79± 0.02[a]	1.81± 0.02[a]	0.000
TBWG[6]	1986.71± 11.33[b]	2053.90± 18.59[a]	2083.63± 19.94[a]	2078.66± 27.12[a]	0.014
TFC[7]	3858.93± 44.76[a]	3734.83± 14.74[b]	3699.57± 25.16[b]	3732.22± 42.14[b]	0.014

[1]IBW: Initial body weight
[2]FBW: Final body weight
[3]BWG: Body weight gain
[4]AFI: Average feed intake
[5]FCR: Feed conversion ratio
[6]TBWG: Total body weight gain
[7]TFI: Total feed intake
[a-b:] The difference between means is significant at the level of P <0.05.

The effect of pennyroyal on hot and cold carcass yields was significant (P<0.05), and the highest yield rates were observed in groups with added pennyroyal at the levels of 0.25% and 0.50%. No significant differences were found among groups on thigh, breast, wing, and back-neck relative weights (P>0.05). In addition, pennyroyal did not affect the weights of heart, gizzard, liver, and spleen (Table 4).

Table 4. Effect of Pennyroyal dietary supplementation on carcass traits

Items	Control	0.25% Pennyroyal	0.50% Pennyroyal	1.00% Pennyroyal	P
Hot Carcass, (%)	71.01±0.14[c]	73.95±0.56[a]	72.82±0.25[b]	71.97±0.30[bc]	0.000
Warm Carcass, (%)	70.28±0.14[c]	72.76±0.65[a]	71.95±0.23[ab]	71.19±0.32[bc]	0.001
Thigh, (g)	453.47±6.05	458.17±14.70	455.14±10.24	453.96±8.57	0.989
Breast, (g)	483.83±21.39	502.52±15.12	502.11±13.11	492.97±22.46	0.705
Wings, (g)	170.62±2.87	172.50±4.01	177.25±1.98	169.85±2.47	0.299
Back-Neck, (g)	426.13±6.47	429.99±7.86	449.40±7.07	436.86±6.36	0.116
Heart, (g)	16.56±0.64	16.41±0.64	17.15±0.91	17.15±0.65	0.835
Gizzard, (g)	40.68±1.88	47.64±3.41	44.23±1.95	42.60±2.13	0.237
Liver, (g)	44.68±1.17	41.35±1.71	46.63±2.23	44.17±1.22	0.171
Spleen, (g)	2.99 ± 0.26	3.43 ± 0.30	3.57 ± 0.26	2.95 ± 0.24	0.267

[a-c:] The difference between means is significant at the level of P<0.05.

The effects of pennyroyal on biochemical parameters are presented in Table 5. As indicated, pennyroyal dietary supplementation at the level of 0.50 and 1.00% increased the high-density lipoprotein (HDL) values, while it decreased the low-density lipoprotein (LDL) and the very low-density lipoprotein (VLDL) at the level of 0.25 and 1.00% (P<0.05).

Table 5. Effect of Pennyroyal on blood serum parameters

Parameters	Control	0.25% Pennyroyal	0.50% Pennyroyal	1.00% Pennyroyal	P
Cholesterol mg/dl	123.00 ± 1.22[a]	115.70 ± 2.27[b]	123.00 ± 1.25[a]	122.20 ± 1.57[a]	0.070
Triglycerides mg/dl	56.20 ± 3.53	57.10 ± 4.40	53.50 ± 1.83	58.20 ± 3.92	0.812
HDL[1] mg/dl	55.90 ± 1.36[b]	56.60 ± 1.20[b]	58.60 ± 1.23[a]	61.80 ± 1.72[a]	0.023
LDL[2] mg/dl	41.55 ± 1.65[a]	33.15 ± 1.73[b]	40.08 ± 1.45[a]	33.94 ± 1.97[b]	0.002
VLDL[3] mmol	55.86 ± 1.56[a]	47.68 ± 1.98[b]	53.70 ± 1.26[a]	48.76 ± 1.50[b]	0.002
Total Protein g/dl	3.02 ± 0.05	2.97 ± 0.11	3.22 ± 0.05	2.96 ± 0.16	0.292
Albumin mg/dl	0.93 ± 0.03	0.96 ± 0.05	1.07 ± 0.03	1.03 ± 0.06	0.137
Globulin mg/dl	2.09 ± 0.04	2.01 ± 0.08	2.15 ± 0.04	1.94 ± 0.11	0.217
Glucose mg/dl	235.10 ± 4.91[a]	223.20 ± 2.03[b]	220.70 ± 2.79[b]	227.00 ± 4.23[a]	0.046
Uric acid mg/dl	6.48 ± 0.65	6.19 ± 0.56	7.15 ± 0.56	9.82 ± 1.79	0.070

[1]HDL: High-density lipoprotein
[2]LDL: Low-density lipoprotein
[3]VLDL: Very low-density lipoprotein.
[a-b:] The difference between means is significant at the level of P<0.05.

The results showing the effect of pennyroyal on MDA and GSH, one of the antioxidant parameters, are presented in Table 6. According to the results obtained, it has been determined that pennyroyal improved MDA and GSH levels (P<0.05).

Table 6. Effect of Pennyroyal on MDA and GSH (nmol/ml)

Parameters	Control	0.25% Pennyroyal	0.50% Pennyroyal	1.00% Pennyroyal	P
MDA[1]	2.40±0.07[b]	2.03±0.08[a]	1.97±0.08[a]	2.32± 0.09[b]	0.010
GSH[2]	0.17±0.01[b]	0.18±0.02[a]	0.18±0.03[a]	0.18± 0.02[a]	0.010

[1]MDA: Malondialdehyde, [2]GSH: Glutathione.
[a-c:] The difference between means is significant at the level of P<0.05.

It was determined that pennyroyal inhibited the reproduction of *Salmonella enteritidis*, *Escherichia coli* (0157h7), *Escherichia coli* (laboratory strain), *Staphylococcus aureus, Salmonella abortus ovis*, *Bacillus anthracis Sterne* bacteria. In terms of inhibitory effects, the bacteria that pennyroyal affects at the lowest concentration (2µl / ml) was *B. anthracis Sterne* and the bacteria that it inhibits at the highest concentration (128 µl / ml) was *S. enteritidis*. Pennyroyal did not have any inhibitory or bactericidal effect on *P. aeruginosa* (Table 7).

Table 7. The MIC ve MBC effects of Pennyroyal (µl/dl)

Bacteria	MIC[1]	MBC[2]
S. enteritidis ATCC 13076	128	128
E. coli O157H7	16	32
E. coli (laboratory strain)	16	32
S. aureus ATCC 6538	8	64
S. abortus ovis	32	32
B.anthracis sterne	2	32
P. aeriginosa	-	-

[1]MIC: Minimum inhibitory concentration.
[2]MBC: Minimum bactericidal concentration.

As shown in Figure 1, the addition of pennyroyal in different doses to the diet increased the duodenum villus lengths (Table 8) (P<0.05). Duodenum villus height of broilers fed diets supplemented with pennyroyal at the rate of 0.50% (1013.26 µm) and 1.00% (984.05 µm) were higher than that of the control group (830.78 µm) and the group with 0.25% pennyroyal additive (942.01 µm).

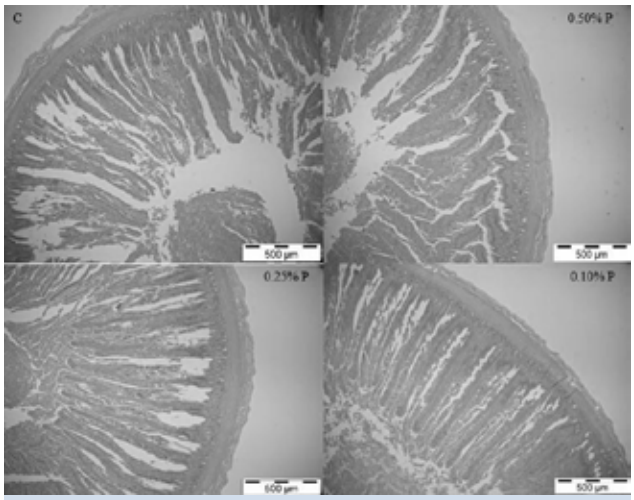

Figure 1. Duodenal villi of Broilers

C: Control group, 0.25% P: 0.25% Pennyroyal, 0.50% P: 0.50% Pennyroyal, 1.00% P: 1.00% Pennyroyal.

Table 8. Effect of Pennyroyal on duodenal villus lengths (µm)

Groups	Villus Lengths
Control	830.78± 26.04[b]
%0.25 Pennyroyal	942.01 ± 41.56[ab]
%0.50 Pennyroyal	1013.26 ± 60.03[a]
%1.00 Pennyroyal	984.05 ± 27.05[a]
P	0.021

[a-b:] The difference between means is significant at the level of P <0.05.

DISCUSSION

The similarity of body weights on the seventh day (start of the experiment) was expected due to the random distribution of chicks in groups. At the end of the experiment, the mean body weights in the control and pennyroyal supplemented groups were 2165.53, 2230.78, 2264.48 and 2259.42 g, respectively, and the body weight values of the groups with additives at different levels were determined to increase by 3.02%,

4.56% and 4.33%, respectively, in comparison to the control group (P<0.05). Average daily body weight gain values compared to the control group were found to increase by 3.38%, 4.88%, and 4.63%, respectively, and this increase was also significant (P<0.05). Feed intake was higher in the control group compared to pennyroyal supplemented groups(P<0.05). The feed conversion rates in the pennyroyal groups decreased significantly compared to the control group (P<0.05). The feed conversion rates were not affected by the addition of the pennyroyal according to the weekly values, and the differences between the groups remained at the numerical level (P>0.05). However, when the overall study was taken into consideration, pennyroyal dietary supplementation has significantly improved the feed conversion rate (P<0.05).

It is suggested that the positive effects of pennyroyal addition to broiler rations on body weight, body weight increase and feed conversion rate are due to improvement of nutrient digestibility, increase of lactic acid bacteria in the environment and inhibition of coliform bacteria, increase of the absorption of feed substances and stimulation of bile retention capacity (Erhan et al., 2012; Abedini et al., 2017). In the present study, feed conversion rate was improved as an expected result of the significant decrease in the rate of feed intake and the to be increased in body weight.

The pennyroyal additive significantly improved both hot and cold carcass yields (P<0.05). The results obtained from the present study are in agreement with previous researchers (Durrani et al., 2008; Erhan et al., 2012; Mondal et al., 2015). It has been reported that the pennyroyal has stimulating effects on pancreas by increasing the secretions of digestive enzymes, and it causes nutrients and especially amino acids to be digested and absorbed more efficiently, thereby increasing feed efficiency (Aminzade et al., 2012). On the other hand, there are also studies that provide contradictory results (Nobakht et al., 2011; Abedini et al., 2017). It was found that thigh, breast, wing, and back-neck ratios did not change with the addition of pennyroyal (P> 0.05). While the results obtained from this study are in agreement with that of a previous one (Shafiei et al., 2014), the majority of the existing literature provides different results (Goodarzi and Nanekarani, 2014; Shamlo et al., 2014; Abedini et al., 2017). At the end of the study, no statistically significant difference was found between the groups in the weights of the heart, gizzard, liver, and spleen in the slaughtered broilers (P>0.05). The numerical increases observed in the pennyroyal-added groups resulted from the fact that the slaughter weights were also high as a result of the high increase in body weight in these groups. The current results are similar to the results of studies showing that the effects of pennyroyal and similar aromatic plants on heart, liver, gizzard, and spleen weights are not significant (Erhan et al., 2012; Ghalamkari et al., 2012; Shokraneh et al., 2016). However, in contrast to the current study, there are studies reporting that different types of mint have changed internal organ weights (Al-Kassie, 2010; Aminzade et al., 2012).

As indicated, pennyroyal dietary supplementation decreases LDL and increases HDL levels. It is thought that the inhibition of 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that controls cholesterol synthesis, by the plant extracts is effective in pennyroyal lowering the cholesterol levels (Elson and Qureshi, 1995). These results are similar to those obtained by Shamlo et al. (2014) and Abdulkarimi et al. (2012) in terms of cholesterol, LDL, and HDL levels. It is reported that pennyroyal decreases the serum glucose levels in comparison to the control group due to the stimulation of pancreatic activity by medicinal aromatic herbs including pennyroyal and thus, reducing the glucose levels (Tekce and Gül, 2015; Ulbricht, 2016). Nobakht et al. (2011), in their study conducted with pennyroyal, had similar results to the current study in terms of serum triglyceride, total protein, albumin, and uric acid values, but had different results in terms of glucose.

While the organism keeps the antioxidant functions in a balanced manner with the agents that create oxidative stress in physiological conditions, it causes lipid peroxidation by affecting the unsaturated fatty acids in the structure of membranes due to the increase of free radicals as a result of stress. The resulting lipid peroxides break down to form reactive carbon compounds. The increase in MDA, which is the most important of these, is directly proportional to the increase in oxidative damage. In previous studies (Çöteli et al., 2013; Meral et al., 2017), it has been determined that the effect of pennyroyal on MDA values is more intense than that of many medicinal plants. Due to this fact, pennyroyal is thought to decrease the level of MDA in the current research with its sweeping effect that inhibits lipid peroxidation. GSH is the reduced form of glutathione which is the most important antioxidant substance in the cell. GSH has important duties such as transport of amino acids,

reshaping some antioxidants, regulation of vitamins C and E. Glutathione defends the organism against oxidative damage by reacting with free radicals and peroxides (Çöteli et al., 2013; Karabulut and Gülay, 2016). In the current study, while the serum GSH level increased with the addition of pennyroyal, serum MDA level decreased. Therefore, pennyroyal is considered to be a good source of antioxidants. The current study results show similarities with the results of the studies conducted by Gumus et al. (2017) and Ri et al. (2017) reporting the positive effects on serum antioxidant parameters. There are also studies that show results that are not similar to that of the current in terms of antioxidant parameters (Alagawany and Abd El-Hack, 2015; Imaseun and Ijeh, 2017).

It was determined that the bacteria species in which the essential oil of pennyroyal is effective at the lowest concentration of inhibition activity is *B. anthracis Sterne* while *S. enteritidis* (ATCC13076) is the species that pennyroyal is effective at the highest concentration. It was also found that pennyroyal had no effects on P. aeruginosa. It is suggested that the resistance of this bacteria is caused by the lipopolysaccharide barrier structure on the outer membrane (Sbayou et al., 2014). The results related to the bacteria species affected by the antibacterial effect of the pennyroyal are compatible with the results of several studies using the pennyroyal (Abd El Azim et al., 2014; Abedini et al., 2017). When the results of the study were examined, it was found that the essential oil of the pennyroyal showed more efficacy on Gram (+) bacteria. It has been stated that the antimicrobial activity of the pennyroyal is due to the phenolic compounds it contains such as pulegone, piperitone, and piperitenone and that Mentha species can be a good alternative to antibiotics (Sbayou et al., 2014; Amalich et al., 2016).

When the villus lengths of the groups with pennyroyal additive was measured, an increase by 13.39%, 21.96%, and 19.45%, respectively, were found compared to the control group ($P < 0.05$). Erhan et al. (2012) reported that the pennyroyal increased the number of intestinal lactic acid bacteria linearly ($P<0.001$) in comparison to the control group, attributing the effect of the addition of pennyroyal to the increase of duodenal villus lengths in the current study. The results of the research were in accordance with the reports of Hamedi et al. (2017) and Rajput et al. (2013). There are also data that are not in accordance with the findings of the current study (Viveros et al., 2011; Yakhkeshi et al., 2011).

CONCLUSION

It was found that the pennyroyal improves performance, optimizes serum cholesterol values, has an antioxidant effect, increases the surface area by extending the intestinal villus, and can be safely used as an alternative feed additive to antibiotics due to its antimicrobial activity. However, it is suggested that there is a need to increase the studies that can serve as national and international resources in the future as there is limited literature information.

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