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# Evaluation of Pennyroyal as A Feed Additive on Growth Performance, Some Biochemical Parameters and Antioxidant Status in Weaned Tuj Lambs

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**ABSTRACT**

This study was conducted to evaluate the effect of pennyroyal (Mentha pulegium L.) leaf powder supplementation in ration on performance, some blood serum biochemical parameters, and antioxidant status in weaned Tuj lambs. Twenty-four Tuj male lambs with an average body weight of 11.5±0.3 kg were allocated to three groups. While no supplementation was added to the basal ration of the control group, dried leaves of pennyroyal at the levels of 0.1% (P1) and 0.2% (P2) were added to the basal ration of treatment groups, respectively. During the experimental period (56 days) the lambs were to be weighted individually every two weeks and body weight gain (BWG), feed consumption (FC), and feed conversion ratio (FCR) were estimated. Also, blood biochemical parameters and activity of antioxidants were determined. According to the study results, BWG, FC, and FCR were not affected by pennyroyal supplementation. Moreover, there was no difference between the control and pennyroyal treatment groups regarding serum biochemical parameters such as glucose, triglyceride, and total protein in the study. Mineral substances such as calcium and phosphorus levels were not influenced by supplementation of pennyroyal to the basal ration. Malondialdehyde and glutathione were affected by Pennyroyal supplementation on the 14th day. In conclusion, the supplementation of pennyroyal to lamb ration did not have a significant act on growth performance.

# Yem Katkı Maddesi Olarak Yarpuz Tozunun Sütten Kesilmiş Tuj Kuzularında Büyüme Performansı, Bazı Biyokimyasal Parametreler ve Antioksidan Durumun Değerlendirilmesi

**Araştırma Makalesi**

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**ÖZ**

Bu çalışma, sütten kesilmiş Tuj kuzularında rasyona kurutulmuş yarpuz (Mentha pulegium L.) tozu ilavesinin performans, bazı kan serumu biyokimyasal parametreleri ve antioksidan durumu üzerine etkisini değerlendirmek amacıyla yapılmıştır. Çalışmada ortalama canlı ağırlığı 11,5±0,3 kg kg olan 24 adet Tuj erkek kuzu üç gruba ayrılmıştır. Kontrol grubunun bazal rasyonuna herhangi bir katkı maddesi ilave edilmezken, muamele gruplarının bazal rasyonlarına sırasıyla %0,1 (P1) ve %0,2 (P2) seviyelerinde yarpuzun kurutulmuş yaprakları ilave edilmiştir. Deneme süresi boyunca (56 gün) kuzular iki haftada bir ayrı ayrı tartılarak, canlı ağırlık artışı (CAA), yem tüketimi (YT) ve yem dönüşüm oranı (YYO) belirlenmiştir. Ayrıca serum biyokimyasal parametreleri ve antioksidan aktivitesi tespit edilmiştir. Çalışma sonuçlarına göre, CAA, YT ve YYO yarpuz ilavesinden etkilenmemiştir. Ayrıca, çalışmada glukoz, trigliserit ve total protein gibi serum biyokimyasal parametreleri açısından kontrol ve

yarpuz muamele grupları arasında fark bulunmamıştır. Rasyona yarpuz ilavesinden kalsiyum ve fosfor gibi mineral maddelerin seviyeleri etkilenmemiştir. Yarpuz, 14. günde serum malondialdehit ve glutatyon seviyeleri üzerine etkili olmuştur. Sonuç olarak, kuzu rasyonlarına yarpuz ilavesinin kuzuların büyüme performansı üzerinde önemli bir etkisi olmadığı tespit edilmiştir.

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## Introduction

Antibiotics have been used for many years ensure a healtyhy growth for animals and increase yield and quality. However, since microorganisms develop resistance to antibiotics and leave residues in edible tissues, it has been revealed that these substances pose a risk for human health (Gümüş and Imik, 2016; Ölmez et al., 2021). After the prohibition of antibiotics as growth promoters in livestock, the search for alternative feed additives has focused on medicinal and aromatic plants (MAP) (Westendarp, 2005; Şahin et al., 2020). MAPs and their extracts have affected on digestibility and blood serum parameters in ruminant animals when added as feed ingredients. It has been determined that it regulates insulin metabolism by affecting serum glucose levels in sheep and calves and has positively effect total protein, calcium (Ca), and phosphorus (P) levels in sheep (Aslani et al., 2007; Mahgoub et al., 2008). It has also been stated that MAPs and their extracts show antioxidant activity (Gümüş et al., 2017).

Pennyroyal (*Mentha pulegium* L.), which belongs to the Mentha genus of the Lamiaceae family, is an aromatic plant used worldwide in food, cosmetics, and health found free in nature. Pennyroyal leaves are rich in polyphenols, such as linalool, menthone, menthan, pulegon, piperitenone and other flavonoids. It has been determined that pennyroyal has antioxidant and cytotoxic properties besides these effects (Goodarzi and Nanekarani, 2014; Ölmez and Yörük, 2021).

This study aims to determine the influence of pennyroyal on growth performance, some serum biochemical parameters, and antioxidant status in weaned lambs.

## Materials and Method

*Feed additive*

In the experiment, from Kars (40 ◦48’21.2 "N 42◦53’37.8" E) volunteer pennyroyal (*Mentha pulegium* L.) was collected in the summer season and powdered by drying and grinding leaves were used. Pennyroyal leaves were dried at room temperature without exposure to the sunshine. Pennyroyal essential oil was obtained for the determination of major compounds. The essential oil was obtained from pennyroyal by the water vapor distillation method. The essential oil components were determined using a gas chromatography device. Measurements were made with TR-FAME MS model, 5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter x 60 m length, 0.25 µm film thickness

column. Helium (99.9%) was used as the carrier gas. Mass spectra (Wiley 9) were identified with the Xcalibur program, using the Scan Mode in data collection (Table 1) (Ölmez and Yörük, 2021).

**Table 1.** Phenolic composition of pennyroyal essential oil

| *Compounds* | *%* |
|---|---|
| Linalool | 13.61 |
| p-Menthone | 10.56 |
| Terpinen-4-ol | 0.28 |
| p-Menthan | 6.19 |
| Levomenthol | 0.20 |
| α-Terpineol | 0.13 |
| Isopulegone | 0.11 |
| Pulegone | 4.45 |
| Piperitenone oxide | 3.07 |
| Thymol | 0.49 |

*Ethical approval*

The research was conducted with permission from the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK / 2020-010).

*Animals and trial design*

The research was conducted at Kafkas University Research Farm. Twenty-four weaned Tuj lambs were used as animal material. This race is bred in the northeastern region of Anatolia in Turkey. The lambs were 8-10 weeks old and had an approximate body weight of 11.5±0.35 kg. In the study, the lambs were divided into a control and two pennyroyal groups, each consisting of eight lambs. Lambs were fed as an individual. While no supplementation was made to the control group, dried pennyroyal leaf powder was added to the treatment groups' concentrate at the levels of 0.1% (P1) and 0.2% (P2), respectively. After the seven-day adaptation period, the animals were subjected to a 56-days experimental period. The lambs were fed with forage and concentrate feed twice a day (08.00 am and 04.00 pm). Ration was prepared according to the daily nutritional requirements of the lambs (NRC 1985). The daily feed consumption (FC) of the groups was determined during the adaption and the treatment period. The animals were fed ration containing approximately 30% forage (oat forage) and 70% concentrate. The feed was increased according to the body weight (BW) determined by two-week weighing. Fresh and clean water was provided *ad libitum*. Concentrate and oat hay (89.00% dry matter, 9.00% crude protein) were analyzed according to the methods specified in AOAC (2019). The Metabolic energy of concentrate was calculated according to the TSE (1991). The Concentrate was set

as isonitrogenic and isoenergetic. The composition of the concentrate were presented in Table 2.

**Table 2.** Nutrient content and composition of concentrated feed.

| Ingredients | % |
|---|---|
| Concentrate | |
| Maize | 21.00 |
| Barley | 11.50 |
| Wheat | 25.25 |
| Wheat bran | 17.64 |
| Cottonseed meal | 6.34 |
| Soybean meal | 11.00 |
| Vegetable oil | 3.25 |
| Dicalcium phosphate | 0.50 |
| Limestone | 2.77 |
| Salt | 0.50 |
| Vit-min mix* | 0.25 |
| Metabolized Energy kcal/kg | 2800 |
| Crude protein | 16.00 |

*: Each 1 kg of the vitamin-mineral mix: It contains 16.000.000 mg vitamin A, 4.000.000 mg vitamin D3, 25.000 mg vitamin E, 50.000 mg zinc, 40.000 mg iron, 40.000 mg manganese, 8.000 mg copper, 700 mg iodine, 450 mg selenium, 200 mg cobalt.

*Performance*

During the study, lambs were weighed every two weeks before morning feeding. The differences between the two weights were recorded as body weight gain (BWG). The FC of lambs was calculated by weighing the feeds given and leftover. The feed conversion rate (FCR) was determined by dividing the FC for two weeks by BWG.

*Biochemical Parameters*

Blood samples were taken from the vena jugularis of all lambs at the end of the study. Blood samples were centrifuged at 3000 rpm for 5 minutes. All samples were kept at -20 °C in the freezer. On the day of analysis, after the samples were thawed at + 4 ° C, glucose, triglyceride, total protein, Ca, and P-values were measured using commercial kits (MyBioSource® Company, San Diego/USA).

*Antioxidant Status*

Malondialdehyde (MDA) and glutathione (GSH) levels were measured in a spectrophotometric analyzer according to Yoshioka et al. (1979) and Beutler (1963) methods, respectively.

*Statistical Analysis*

The data were analyzed using the ANOVA test (Graphpad Prism 8 / San Diego, CA). Tukey's range test was adopted for the significance of the difference between the results. Results were given as mean ± Standard error mean (SEM).

## Results

*Effect of Pennyroyal on Growth Performance*

The results obtained regarding the BWG of Tuj lambs were shown in Table 3. BWG was not affected by supplementation of dietary pennyroyal (P>0.05).

**Table 3.** Effect of pennyroyal on BWG in Tuj lambs (Mean±SEM, g/day)

| Groups / Days | C | P1 | P2 | P-value |
|---|---|---|---|---|
| 0-14 | 211.29±1.98 | 211.86±2.34 | 211.90±2.56 | 0.702 |
| 14-28 | 217.57±1.29 | 218.29±1.95 | 217.32±1.85 | 0.706 |
| 28-42 | 213.71±1.41 | 213.20±1.38 | 216.14±1.42 | 0.891 |
| 42-56 | 212.14±2.54 | 213.30±1.32 | 213.71±1.57 | 0.430 |
| 0-56 | 213.68±1.16 | 214.1±1.24 | 214.76±1.45 | 0.610 |

C: Control, P1: 0.1% Pennyroyal, P2: 0.2% Pennyroyal

Also, the FC and FCR were supplied in Tables 4 and 5. It was determined that the differences between FC and FCR between groups were insignificant during the trial (P>0.05).

**Table 4.** Effect of pennyroyal on FC in Tuj lambs (Mean±SEM, g/day)

| Groups / Days | C | P1 | P2 | P value |
|---|---|---|---|---|
| 0-14 | 985.71±26.62 | 983.29±19.43 | 985.71±21.91 | 0.996 |
| 14-28 | 1068.43±14.53 | 1054.86±14.85 | 1050.43±10.52 | 0.623 |
| 28-42 | 1079.00±11.61 | 1071.29±9.14 | 1083.86±10.33 | 0.695 |
| 42-56 | 1200.86±9.63 | 1204.14±10.81 | 1208.86±11.34 | 0.859 |
| 0-56 | 1083.50±9.82 | 1078.39±8.32 | 1082.21±7.15 | 0.908 |

C: Control, P1: 0.1% Pennyroyal, P2: 0.2% Pennyroyal

**Table 5.** Effect of pennyroyal on FCR in Tuj lambs (Mean±SEM, g/g)

| Groups / Days | C | P1 | P2 | P value |
|---|---|---|---|---|
| 0-14 | 4.67±0.15 | 4.65±0.13 | 4.66±0.11 | 0.993 |
| 14-28 | 4.91±0.07 | 4.83±0.08 | 4.83±0.06 | 0.662 |
| 28-42 | 5.05±0.05 | 5.02±0.05 | 5.01±0.05 | 0.876 |
| 42-56 | 5.66±0.05 | 5.65±0.07 | 5.66±0.04 | 0.981 |
| 0-56 | 5.07±0.06 | 5.03±0.06 | 5.04±0.04 | 0.780 |

C: Control, P1: 0.1% Pennyroyal, P2: 0.2% Pennyroyal

*The Effect of Pennyroyal on The Biochemical Parameters*

The results regarding the glucose, triglyceride, total protein, calcium, and phosphorus in the serum obtained during the trial were given in Figure 1. The measurements every two weeks were determined that the differences among the groups in serum glucose were not significant ($P>0.05$). While the differences in serum triglyceride among the groups on the 1st, 14th, 28th, 42nd, and 56th days were not significant ($P>0.05$), it was found that the difference was significant in the P1 group compared to the control group on the 14th day ($P<0.01$). There was no difference between the P1 and P2 groups ($P>0.05$). The differences among the groups in serum total protein were not significant, except for the 42nd day ($P>0.05$). There was no significant difference between the pennyroyal groups' calcium and phosphorus compared to the control group ($P>0.05$). Also, there was no difference between the P1 and P2 groups ($P>0.05$).

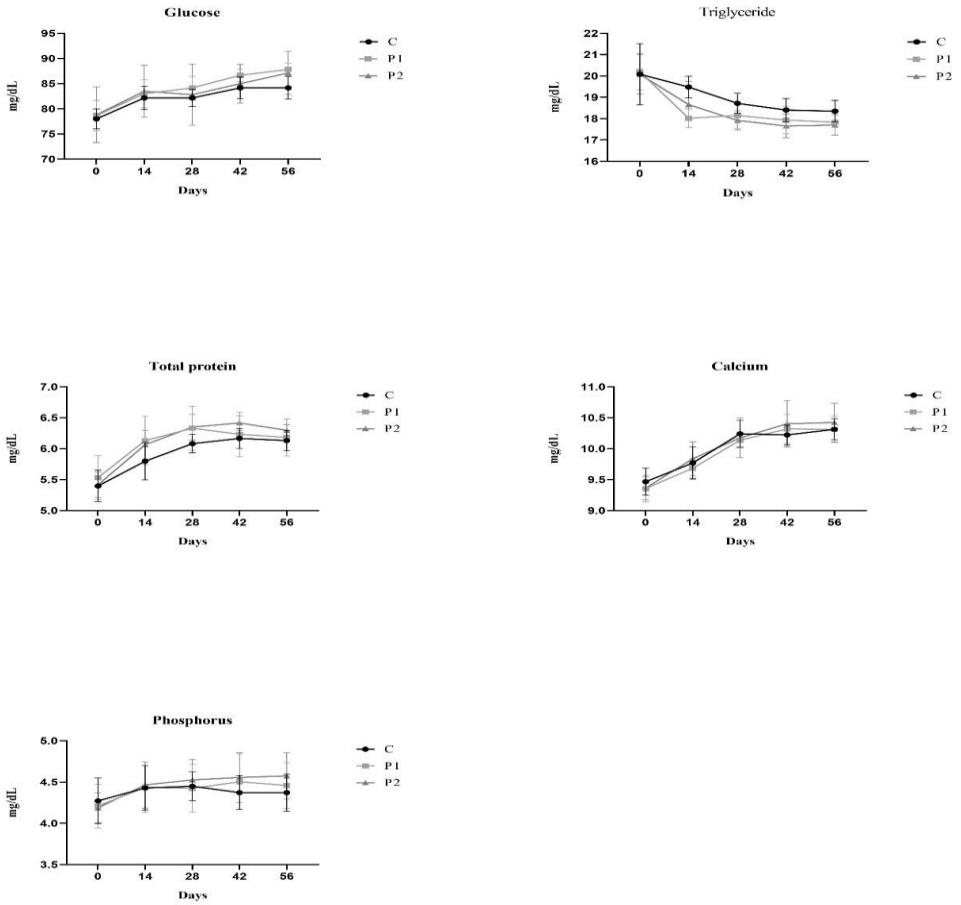


**Figure 1.** Effect of pennyroyal on serum biochemical parameters in Tuj lambs (n:8).
C: Control, P1: 0.1% Pennyroyal, P2: 0.2% Pennyroyal

*The Effect of Pennyroyal on Antioxidant Status*

The effects of pennyroyal on antioxidant parameters such as MDA and GSH were presented in Figure 2. While the differences in serum MDA on the 1st, 28th, 42nd, and 56th days of the study were not significant (P>0.05), it was concluded that the pennyroyal groups decreased serum MDA on the 14th day of the study (P<0.01). It was determined that the difference between the P1 and P2 groups was not significant (P>0.05). The differences among serum GSH of lambs on the 1st, 28th, 42nd, and 56th days were not significant (P> 0.05). But, it was concluded that the GSH in the P2 group was statistically different from the control group on the 14th day of the study (P<0.05).

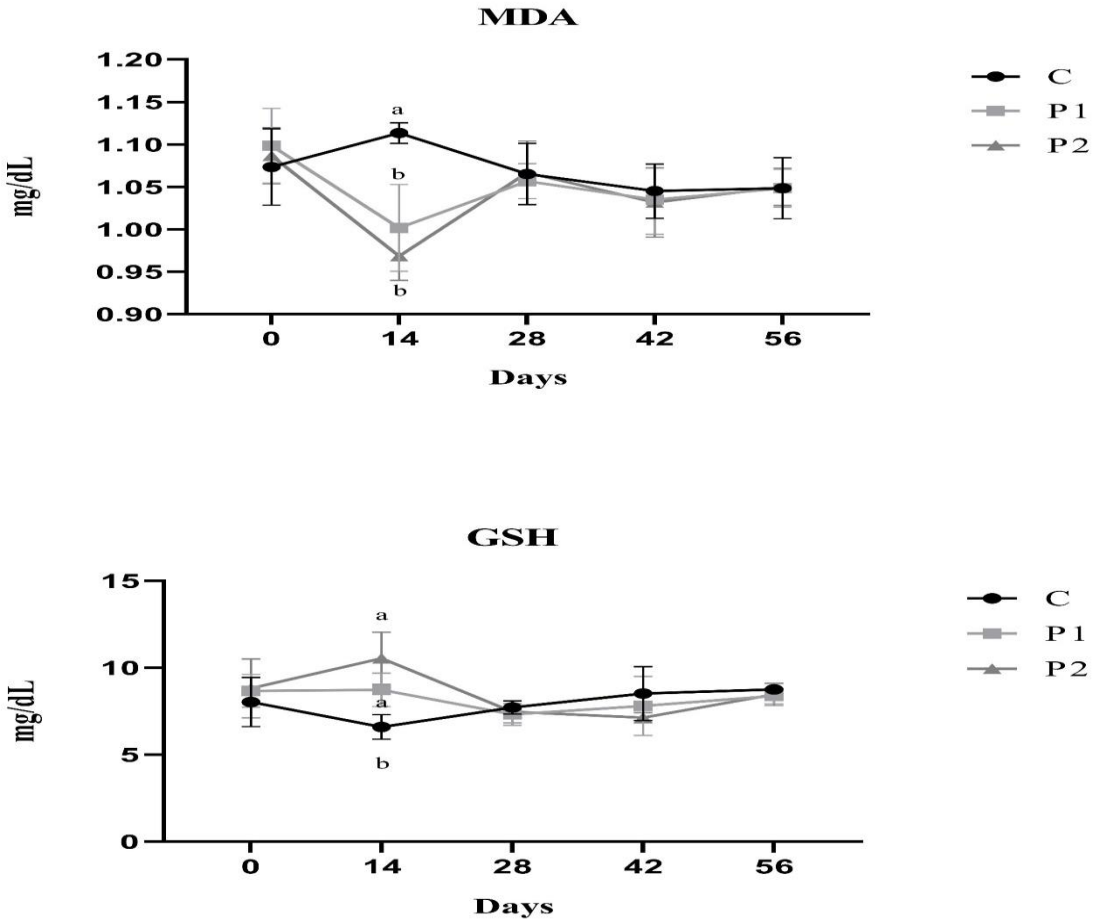


**Figure 2.** Effect of pennyroyal on antioxidant parameters in Tuj lambs (n:8).

C: Control, P1: 0.1% Pennyroyal, P2: 0.2% Pennyroyal

[a-c:] Means in the same column with different superscripts differ ($P < 0.05$)

**Discussion**

It has been stated that pennyroyal provides body weight gain by increasing nutrient absorption through major components such as Linalool and Menthone. In addition, it has been reported that it prevents the oxidation of nutrients and increases the yield by playing the role of antioxidant (Ölmez and Yörük, 2021). The present study results determined that pennyroyal supplemented at different levels did not affect the growth performance of Tuj lambs. Many studies that the effectiveness of aromatic herbs on performance parameters have pointed out similarities with this study's results (Chaves et al., 2011; Yang et al., 2007). In the studies conducted, Mohamadi et al. (2017) reported that pennyroyal had no effect on performance parameters in sheep, and Khamisabadi et al. (2016) stated that there was no change in FCR in the groups in which 3% mint and 3% thyme were added to lamb rations. The same researchers declared that the addition of 3% mint from the Mentha family increased the daily BWG. Contrary to this study's results, (Ölmez and Yörük, 2021) stated that pennyroyal improved BWG by increasing digestibility. Likewise, Lewis et al. (2003) argued that pennyroyal and canola oils increased

BWG in broilers. This result was due to the antimicrobial property of pennyroyal and its positive effect on the intestines. Another study affirmed that mint, one of the Mentha species, increased appetite and improved FC, and BWG in broilers (Asadi, 2017; Ölmez et al., 2021). As a result, differences in the aromatic plants, ration formulation, interaction between feeds, animal species, and compatibility with rumen microbial fermentation cause aromatic plants to yield varying results between studies (Ölmez and Yörük, 2021).

Serum glucose level was not affected by pennyroyal supplementation during the entire trial. In contrast to this study, Khamisabadi et al. (2016) stated that the supplementation of dietary mint significantly increased the serum glucose level in the middle of the study compared to the control group and decreased the glucose level at the end of the study. This decrease may be due to phenolic components such as menthol and menthone in the mint structure. In the present study, serum triglyceride level was not affected by pennyroyal supplementation, which decreased significantly in the P2 group on the 14th day. It did not differ in the following weeks. A similar study showed that the supplementation of thyme essential oil to calf ration (5 g/day/calf) did not change serum triglyceride levels (Vakili et al., 2013). This study's results were similar to some studies (Ahmadi Naghadehi et al., 2014; Mohamadi et al., 2017).

At the end of the study, the serum protein results were consistent with the results of Mohamadi et al. (2017) that it was not affected by the supplementation of 110 mg/day peppermint essential oil and 110 mg/day pennyroyal essential oil to sheep ration and the results of Ahmadi Naghadehi et al. (2014) where the addition of peppermint essential oil (100 and 200 mg) to sheep rations did not affect total protein values. Researchers have reported that aromatic plants depending on feed consumption may affect changes in serum parameters such as protein and triglycerides (Yang et al., 2010).

Hosoda et al. (2006) reported that the addition of 5% aromatic herb mixture to cattle rations did not affect serum glucose, triglyceride, and total protein levels. However, in a study on Oregano essential oil, it was stated that serum glucose, triglyceride, and total protein levels were not affected (Gümüş et al., 2017). In another study, it was determined that the serum glucose level increased with the supplementation of thyme essential oil. The glucose level was higher in the groups with the highest essential oil level (Ebrahimi et al., 2018). Amad et al. (2011) observed that the supplementation of thyme and anise to broilers and Soltan (2009) discovered that adding essential oil to milk replacer in calves increased serum total protein levels. It has also been established that the addition of laurel essential oil has a lowering effect on serum triglyceride levels in Holstein calves (Izzaddeen and Kaygısız, 2018).

In light of the statistical data gathered, it was concluded that pennyroyal addition did not significantly affect serum calcium and phosphorus concentration during the trial. Some researchers have reported that the differences in mineral metabolism may vary depending on the composition of aromatic plants used. No changes were found in serum calcium and phosphorus concentration in the present study. Limited studies have performed that the effects of pennyroyal supplementation on blood serum

mineral concentrations in Tuj lambs in the growing period. Gümüş et al. (2017) stated that the supplementation of essential oil (oregano) to lamb ration did not affect serum phosphorus levels. Also, they reported that 200 mg/kg oregano oil significantly decreased serum calcium level compared to the control group. El-Katcha et al. (2016) reported that garlic supplementation did not affect serum mineral levels in growing lambs. In addition, Khaksar et al. (2012) stated that the supplementation of thyme essential oil did not affect calcium and phosphorus levels in quails. Existing data were also supported by some researchers (Pirmohammadi et al., 2014; Zakeri et al., 2014). Another study stated that the essential oil mixture in broilers decreased the serum calcium level but did not change the serum phosphorus level (Cengiz et al., 2016). It is believed that the variety and form of aromatic plants, ration, and feeding conditions were affected the study results.

The organism keeps antioxidant functions in balance with agents that cause oxidative stress. Stress conditions lead to lipid peroxidation with the increase of free radicals. The peroxides formed are broken down to form reactive carbon compounds. The increase of MDA is directly proportional to oxidative damage. It has been reported that the effectiveness of pennyroyal on low MDA levels is higher than many medicinal aromatics (Karabulut and Gülay, 2016). GSH is the reduced state of glutathione, the most critical antioxidant substance in the cell. Glutathione protects the organism against oxidative damage by reacting with peroxides (Chen et al., 2013; Çöteli et al., 2013). In the present study, while there was no difference in serum MDA and GSH levels on the 1st, 28th, 42nd, and 56th days, pennyroyal affected the MDA and GSH levels on the 14th day of the study. While these data were similar to some study results (Marzouk et al., 2008; Ahmadi et al., 2014), they also differed from some study results (Mašek et al., 2014; Ri et al., 2017). Pennyroyal has phenolics, such as linalool and menthol. These compounds function as antioxidants which are very useful for improving the health of the body's system (Sunarno et al., 2019). In light of the study's data, more studies are needed to determine the effectiveness antioxidant activity of pennyroyal.

## Conclusion

Although it is thought that the effect of pennyroyal addition to the growing rations of Tuj lambs on the growth performance, blood serum biochemical, and antioxidant parameters are limited, pennyroyal supplementation of the lamb ration has been represented a similar effect in comparison to the control group.

## Conflict of Interest

The authors declare no conflict of interest.

**Authors' contributions**

MÖ designed the study. MÖ, TŞ, and MM performed the methodology. MÖ and MM collected the data. MM analysed the biochemical parameters. MÖ and MM performed statistical analysis. The original draft was written by MÖ and TŞ. All authors read and agreed the final version of manuscript.

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