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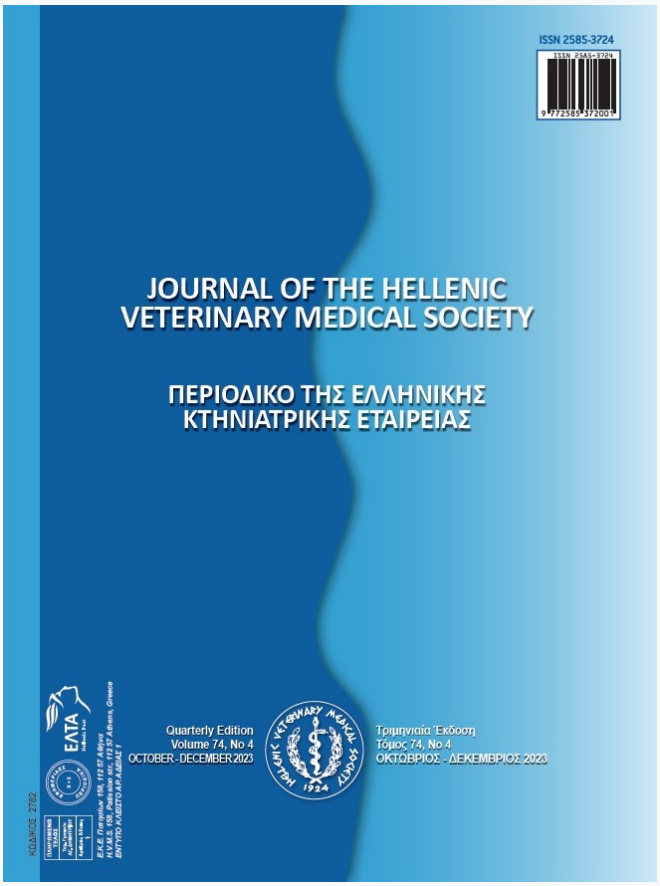

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Research article
Ερευνητικό άρθρο

The effects of rosemary essential oil supplementation on growth performance, rumen flora and antioxidant blood parameters in growing Merino lambs

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ABSTRACT: Thirty-two male growing Merino lambs (78 ± 5 days of age) were used in a 45-day randomized complete block desing to determine the effects of rosemary essential oil (REO) on the growth performance, rumen fermentation, rumen protozoa population, plasma antioxidant enzyme parameters and fecal microbiology. The control group was fed a diet consisting of concentrate and alfalfa hay. The lambs in control group did not receive REO whereas each lamb in experimental groups supplemented with 250 mg/d (R250), 500 mg/d (R500) and 750 mg/d (R750) REO throughout the study. There weren't statistically differences (P>0. 05) in average initial weight, average final weight, live weight gain, average daily gain, daily feed intake, daily dry matter intake and feed conversion ratio between the control and experimental groups. There were also no differences (P>0. 05) in the rumen pH, fecal pH, NH_3N and rumen volatile fatty acid (VFA) profile of the control and experimental groups. At the end of the study, although the plasma superoxide dismutase (SOD) value increased significantly (P<0. 05) in the experimental groups (R500 and R750), glutathione peroxidase (GP_X) and catalase values did not differ between REO groups and control. In addition, the fecal microorganism profile did not differ statistically (P>0. 05) between the control and experimental groups. The results of this study show that the addition of REO to Merino lamb rations is more effective on antioxidant parameters than on performance parameters and may be used as a natural antioxidant product.

***Keywords*:** antioxidant, fecal microbiology, growth performance, merino lambs, rosemary essential oil

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INTRODUCTION

The use of aromatic plants and their extracts and essential oils for the livestock has expanded after the ban of the use of growth promoting antibiotics in the European Union since 2006. In recent years, the use of essential oils as a natural alternative feed additive has gained importance (Yesilbag et al.,2017). Generally, essential oils seem to benefit the digestive system and improve nutrient absorption, reduce the number of pathogens in the gut, have antimicrobial, antiviral, antiparasitic, antifungal, immunomodulating, antioxidant and anti-inflammatory activities (Zeng et al., 2015). Also, Calsamiglia (2007) announced that essential oil modifies rumen fermentation patterns with effects on volatile fatty acid production, protein metabolism, or both. Rosemary (*Rosmarinus officinalis* L.) has several natural bioactive compounds with antioxidative capacity, mainly the phenolic di-terpenes, such as carnasol, rosmanol, 7-methyl-epirosmanol, isorosmanol and carnosic acid; and the phenolic acids, such as caffeic and rosmarinic acids (Cuvelier et al., 1996). On the other hand, the main components of REO are mono-terpenes such as 1,8-cineole, α-pinene, myreceneand borneol which possess strong antimicrobial activities (Okoh et al., 2010). It has been reported that the daily addition of 200 mg of REO to the diet of Ghezel sheep has a positive effect on rumen fermentation (Sahraei et al.,2014). Furthermore, various benefits such as improved calf starter intake, feed efficiencies and live weight gains (Hill et al., 2007) and improved beneficial bacteria in the gut flora (Santos et al., 2015) by supplemented essential oil have been declared. There are many studies on the effects of herbal essential oils on performance parameters. However, most of these studies were conducted without bioactive ingredient analysis results. In this study, the structure of the bioactive components of rosemary essential oil was revealed and its antimicrobial and antioxidant effects were investigated. Due to the strong antioxidant effect of rosemary essential oil, which is the subject of our research, its effects on blood antioxidant parameters were examined, in addition to the rumen and fecal microorganism populations were evaluated. As a result, the planned study, aimed to examine the effects of adding rosemary essential oil to lambs after the suckling period on performance parameters, as well as on rumen parameters, blood antioxidant parameters and fecal microbiology. In addition, it aimed to reveal the possible effects of a natural and residue-free feed additive that has the potential to improve animal performance and digestive system health.

MATERIALS AND METHODS

Studydesign, animals and diets

This research project carried out at a private agricultural company in Kırklareli province, Turkiye during January to March 2020. All procedures (ethical statement) were approved by Tekirdag Namık Kemal University Animal Care and Use Committee before the start of the study (protocol ID: T2020-430). Thirty-two male merino lambs were used in this study. Each group formed by 8 merino lambs (78 ± 5 days of age) with an average initial live weight of 24. 06±2. 69 kg. Lambs were randomly selected from 1 of 4 treatments considering their birth and initial live weights. The rations were performed according to the

Table 1. Nutrient composition of grower concentrate and alfalfa hay on a dry matter basis*.

Item	Grower concentrate†,‡	Alfalfa hay	TMR
Dry matter, %	89. 2	93	89. 72
CP, %	17. 94	17. 04	17. 88
Ether extract, %	3. 39	1. 52	3. 35
NDF, %	28. 27	42. 43	28. 92
ADF, %	12. 21	32. 91	15. 22
ADL, %	4. 15	10. 63	4. 67
NFC§, %	41. 5	28. 17	40. 02
Ash, %	8. 9	10. 84	9. 12
Ca, %	1. 72	1. 42	1. 54
P, %	0. 61	0. 31	0. 53

*Nutrient analyses of the feeds were performed according to AOAC (2016).
†C. P. Feed Industry, Tekirdag, Turkey.
‡Contained the main ingredients: wheat brain, ground corn grain, corn gluten meal, sunflower meal, palm meal, canola meal, ground barley grain, corn meal, molasses, calcium carbonate, vinasse, soya bean meal, vegetable oil, ammonium chloride, vitamin and mineral mix, salt.
§NFC (Non-Fibre Carbonhydrate): 100 - (% NDF + % CP + % Ether extract + % Ash).

NRC (2007) nutrient requirements for sheep. The ingredients and chemical composition of the rations are illustrated in Table 1. Ration was prepared to contain isocaloric and isonitrogenic as total mixture ration, approximately 90% concentrate and 10% alfalfa hay.

Management and experimental design

The lambs were housed in individual paddocks with approximately 2. 0 m^2 with wheat straw bedding. All 32 paddocks were in the same condition. Total mix ration and water were offered *ad libitum.* The nutrient compositions of grower concentrate feed and alfalfa hay are shown in Table 1. The lambs in the experimental groups were given rosemary essential oil orally with a syringe. The lambs in the control group did not receive REO oil whereas each lamb in the experimental groups was supplemented with 250 mg/d (R250), 500 mg/d (R500) and 750 mg/d (R750) REO throughout the study. Rosemary essential oil was offered to the lambs at 9:00 in the morning after feeding. The REO doses used in the study were determined by looking at the research results of the previous study (Yesilbag et al., 2017). After a 15-day adaptation period, the lambs were tested for 45 days and the study lasted for 60 days in total. The control and experimental groups underwent the same type of feeding and management standards. Rosemary essential oil used in the study was obtained from a private company (Nativital, ISTANBUL). Active ingredient analyzes of rosemary essential oil were carried out in Ege University Center for Drug and Pharmacokinetic Applications Environmental &Food Analysis Laboratories Environmental Control Laboratory.

Feed intake and gain

The current study, feed deliveries were recorded daily and refusals were weighed fortnightly, on an individual lamb basis, to define feed intake. Animals were weighed weekly and average daily gain was determined by dividing the weight gain. Feed conversion was calculated as the ratio between daily dry matter intake and average daily gain.

Rumen parameters

At the end of the study, rumen fluid was collected from all lambs with an oesophageal tube 4 h after morning feeding. Rumen pH was immediately assessed with a previously calibrated portable pH meter (Metler-Toledo, Switzerland). Ruminal fluid was immediately filtered using 4 layers of cheesecloth with a mesh size of 250 μm. 5 ml of filtrate sample was preserved by adding 1 ml of 1% $H_2SO_4$for NH_3-N and 2 ml of filtrate was acidified with 0. 04 ml of 50% H_2SO_4 for volatile fatty acid (VFA) concentrations. These samples were stored at -20°C for further analysis of nitrogen ammonia and VFA. The NH_3-N and VFA concentration were quantified using the method proposed by Annino (1964). Samples were prepared for VFA analysis and transferred into gas chromatography (GC) sample vial for analysis by GC (Hewlett Packard Agilent Technologies 6890N Network GC System, Serial CN10447002, China) with GPx 10% SP-1200/1% H3PO4 on 80/100 Chromosorb and using a 6'∞2 mm ID glass column (Supelco, Bellefonte, PA, USA).

Protozoa were counted and identified in the rumen fluid using a Fuchs-Rosenthal hematocytometer under the light microscope, according to the method described by Ogimoto andImai (1981).

Fecal pH and fecal microbiology

Fecal samples were gathered from each lamb by retrieval from the rectum at the end of the study. According to the method described by Verlinden et al. (2006), fecal pH was measured immediately using the electronic pH meter (Metler-Toledo, Switzerland).

In fecal microflora analysis, a serial dilution of the samples was made with saline peptone water for isolation and enumeration attempts. For *Lactobacillus* spp. isolation we used Man Rogosa and Sharpe (MRS, Merck, 1. 10660) agar and after plates were incubated for 3 days at 35°C under 5% CO_2 (Anaerobik Jar, Merck, 1. 16387 and Anaerocult C, Merck, 1. 16275). *Bifidobacterium* spp. was grown and counted on Bifidobacterium Selective Medium agar (BSM agar, SIGMA, 88517). The plates were incubated at 37°C for 48 to 72 hours in an anaerobic jar under anaerobic conditions(Anaerobic Jar, Merck, 1. 16387 and Anaerocult C, Merck, 1. 16275). For coliforms and *E. coli* isolation and enumeration, samples were plated onto relevant selective media. Coliform bacteria were grown on Violet Red Bile (VRB, Merck, 1. 01406) agar at 37 °C for 24-48 hours. After incubation, typical colonies were inoculated in Lactose Broth (LB, Merck, 1. 07661) in a Durham tube at 44°C for 24 h. After that, the colonies positive for acid and gas formation were confirmed to be *E. coli* using the IMVC series of tests (indole, methyl red, Voges-Proskauer, citrate).

Salmonella spp. isolation and identification were carried out using ISO 6579/A1: 2007 (ISO 2007).

Antioxidant blood parameters

At the beginning and end of the experiment, blood samples were taken from a total of 32 animals, 8 from each group, from the jugular vein using an injector. Samples were collected into ethylenediaminetetraacetic acid (K_3 EDTA) anticoagulant tubes and were centrifuged on the same day at 3000 rpm for 10 minutes to separate the plasma. The plasma samples were transferred into microtubes and were stored at -80°C until the analysis day. Antioxidant parameters such as Superoxide Dismutase (SOD ELISA Kit Catalogue No: 201-07-1566), Glutathione Peroxidase (GP_X ELISA Kit Catalogue No: 201-07-2374) and Catalase (CAT ELISA Kit Catalogue No: 201-07-1836) were defined with commercial kits (Sunred Biological Technology Co., Ltd., China) using by ELISA method. All antioxidant parameter changes were determined using a microplate reader (Biotek, Epoch, USA).

Statistical analysis

The effects of REO were statistically analyzed with the ANOVA procedure with SPSS 20 (SPSS Inc., Chicago, IL, USA). All the data (except performance data) were analyzed by the Kruskal-Wallis H-test and the Mann-Whitney U-test was used for paired comparisons; the Exact Test was chosen to be only asymptotic for both tests. Performance data were tested for normal distribution by an F-test, and one-way ANOVA was used to compare data according to groups. Homogeneity of variances was tested, and the Tukey HSD test was chosen as the post hoc multiple comparison test. Descriptive statistics were expressed as MEANS ± standard error of the mean. Statistical differences were considered to be significant when P<0. 05.

RESULTS

REO composition is presented in Table 2. The 1,8 cineol (39. 95%), trans caryophyllene (23. 75%), camphore (7. 84%), borneol (6. 42%), α-pinen (5. 98%) and α-terpinol (5. 68%) were analyzed to be main bioactive components for REO used in this study. REO supplementation did not create obvious effects on final weight, live weight gain, average daily gain, daily feed intake, daily dry matter in take and feed conversion ratio (Table 3). The initial weights of the lambs placed in the groups of this study were distributed close to each other and did not create a statistical significance between the groups. The fecal pH, rumen pH and NH_3N values did not differ (P>0. 05) between the control and experimental (REO) groups (Table 4). No significant changes (P>0. 05) were observed in the rumen VFA profile (Table 4) and rumen protozoa population (Table 5). Statistical differences of volatile fatty acid values were evaluated as acetic acid, propionic acid, Butyric acid, AA/PA and total VFA respectively. The statistical differences of rumen protozoa population values were determined as entodinium, epidinium, dastyrichia and total protozoa counts in a row. In the study, the effect of REO on blood antioxidant parameters is shown in Table 6. In

Table 2. Essential oil components of rosemary (*Rosmarinus officinalis*).

Components	(%)
Borneol	6. 42
1,8 cineol	37. 95
α-humulene	2. 63
α-pinen	5. 98
α-terpineol	5. 68
β-myrcene	0. 9
β-pinen	1. 78
Bornylacetate	3. 27
Camphene	1. 28
Camphore	7. 84
Limonen	0. 9
Para Cymen	0. 74
Undefined	0. 85
Trans Caryophyllene	23. 75

Table 3. The effects of rosemary essential oil supplemantation on performance parameters of Merino lamp.

	n	Control	REO250	REO500	REO750	SEM	***P*-value**
Average birth weight (kg)	8	4. 39	3. 89	3. 98	4. 28	0. 19	0. 77
Average initial weight (kg)	8	24. 22	24. 71	22. 87	24. 45	0. 47	0. 66
Average final weight (kg)	8	35. 20	35. 25	35. 42	37. 37	0. 71	0. 67
Live Weight Gain (kg)	8	10. 97	10. 54	12. 55	12. 92	0. 64	0. 51
Average daily gain (kg)	8	0. 24	0. 20	0. 27	0. 28	0. 001	0. 26
Daily DM intake (kg)	8	1. 39	1. 42	1. 37	1. 50	0. 03	0. 46
Feed Conversion Ratio (FCR)	8	6. 66	6. 54	5. 24	5. 40	0. 35	0. 35

REO250: added 250 mg/d rosemary essential oil; REO500: added 750 mg/d rosemary essential oil; REO750: added 750 mg/d rosemary essential oil FCR: daily DM intake/average daily gain

Table 4. The effects of rosemary essential oil supplementation on rumen pH, faecal pH, rumen NH_3N levels and rumen VFA profile of Merino lamp.

	n	Control	REO250	REO500	REO750	SEM	*P*-value
Rumen pH	8	5. 98	5. 88	5. 83	5. 85	0. 08	0. 94
Faecal pH	8	7. 57	7. 90	7. 48	7. 50	0. 06	0. 69
NH_3N (mg/dl)	8	18. 79	19. 31	17. 58	17. 44	0. 32	0. 11
Volatile fatty acid profile							
Acetic acid (mmol/L)	8	38. 55	34. 45	29. 82	35. 07	1. 64	0. 09
Propionic acid (mmol/L)	8	22. 27	22. 67	22. 98	27. 99	1. 77	0. 32
Butyric acid (mmol/L)	8	15. 23	14. 21	10. 07	12. 16	1. 02	0. 11
AA/PA	8	2. 12	1. 68	1. 41	1. 36	0. 13	0. 07
Total VFA (mmol/L)	8	89. 67	80. 24	71. 53	84. 58	4. 29	0. 19

REO250: added 250 mg/d rosemary essential oil; REO500: added 750 mg/d rosemary essential oil; REO750: added 750 mg/d rosemary essential oil NH_3N: ammonia nitrogen; VFA: volatile fatty acid; AA/PA: acetic acid/propionic acid

Table 5. The effects of rosemary essential oil supplementation on rumen protozoa population of Merino lamp.

Protozoa	n	Control	R250	R500	R750	SEM	*P*-value
Entodinium (%)	8	91. 67	90. 19	90. 91	87. 60	1. 97	0. 91
Epidinium (%)	8	8. 32	9. 41	4. 68	12. 22	1. 95	0. 62
Dastyrichia (%)	8	0. 00	0. 21	4. 40	0. 17	0. 82	0. 17
Protozoa count/ml rumen fluid (Log_{10})	8	5. 88	6. 13	5. 98	6. 04	0. 08	0. 78

REO250: added 250 mg/d rosemary essential oil; REO500: added 750 mg/d rosemary essential oil; REO750: added 750 mg/d rosemary essential oil

Table 6. The effects of rosemary essential oil supplementation on antioxidant blood parameters of Merino lamp (n:8).

Parameters	Experiment period (days)	Control	R250	R500	R750	SEM	*P*-value
SOD (ng/ml)	1st	2. 59	2. 48	2. 68	2. 40	0. 13	0. 89
	45th	2. 31^{b}	2. 18^{b}	3. 41^{a}	3. 15^{a}	0. 16	0. 006
GP_X (ng/ml)	1st	26. 39	26. 95	26. 89	26. 55	1. 07	0. 99
	45th	28. 45	29. 24	34. 71	32. 90	1. 74	0. 56
Catalase (ng/ml)	1st	2. 22	2. 16	2. 47	2. 25	0. 09	0. 71
	45th	2. 67	2. 36	2. 62	2. 47	0. 14	0. 87

R250: added 250 mg/d rosemary esential oil; R500: added 750 mg/d rosemary essential oil; R750: added 750 mg/d rosemary esesntial oil; SOD: superoxide dismutase; GP_X: glutathione peroxidase
Different superscripts in each row show the significant difference between the groups p<0. 05

Table 7. The effects of rosemary essential oil supplementation on faecal microbiyology of Merino lamp (n:8).

Parameters	Control	R250	R500	R750	SEM	*P*-value
Coliform (cfu Log_{10}/g)	3. 62	2. 91	2. 52	3. 04	0. 19	0. 07
Lactobacillus (cfu Log_{10}/g)	3. 47	3. 93	4. 08	3. 92	0. 20	0. 36
Bifidobacterium (cfu Log_{10}/g)	4. 60	4. 83	5. 32	4. 87	0. 23	0. 35
Presence of E. coli and Salmonella in groups						
E. coli positive (Fresh feces)	8/5	8/3	8/6	8/8		
Salmonella positive (Fresh feces)	8/0	8/0	8/0	8/0		

R250: added 250 mg/d rosemary essential oil; R500: added 750 mg/d rosemary essential oil; R750: added 750 mg/d rosemary essential oil

the study, the addition of 750 and 500 mg/day REO to lamb rations caused a significant increase (P=0. 006) in plasma SOD levels, one of the blood antioxidant parameters, at the end of the study. At the end of the study, there was no significant difference (P>0. 05) between the groups in plasma GP_X and catalase levels. Among the last parameters evaluated in the study, *Coliform*, *Lactobacillus* and *Bifidobacterium* were examined in fecal samples, and the microbiology results in fecal content did not differ (P>0. 05) between the control and experimental groups (Table 7). *Salmonella* and *E. coli* values were evaluated as positive/negative in fecal microbiology and are presented in Table 7.

DISCUSSION

In the current study, merino lambs were fed different levels of REO containing 1,8 cineol as the major bioactive compound. REO used in this research, extracted from *R. officinalis L*. leaves which is rich in volatile oil as analyzed composition showing high proportion of 1,8-cineole (37. 95%), trans caryophyllene (23. 75%) and moderate proportion camphor (7. 84%), borneol (6. 42%), α-pinene (5. 98%) and α-terpineol (5. 68%). In studies with REO, the results of the bioactive component analysis show compatibility with each other. While Güney (2021) reported1-8 cineol level was 32% in their study, it was found to be slightly higher (37. 95%) in our study. The results of the bioactive ingredient analysis in the studies may vary depending on the plant species used, the season in which it is harvested and the extraction method. The effect of feeding essential oils on ruminant growth performance has been found with variable results. Similar to our results, Chaves (2008) observed that supplementation with a high-energy ration for growing lambs with cinnamaldehyde or carvacrol (200 mg/kg DMI) did not effect on dry matter intake, gain and feed efficiency. Moreover, Yesilbag (2017) reported that supplementing the ration for Saanen kids with 0, 0. 4, 0,8 or 2 ml/kg juniper essential oil had no effecton live weight, live weight gain and feed consumption but increased in feed efficiency in the 0. 8 ml/kg juniper oil addition. In contrast, supplementation with 500 mg/kg DM copaiba essential oils did not affect DMI, but markedly improved average daily gain and gain efficiency compared to a non-supplemented group (Moura et al., 2017). In their study, Smeti (2018) determined that the daily oral doses of 0. 3 and 0. 6 μmL/day rosemary oil addition to the lamb ration did not change the daily DM intake and stated that these results were due to insufficient dose. In this study, it can be said that the addition of REO to the lamb ration at different levels did not cause any difference in performance parameters in the control and experimental groups, but the addition,especially of 500 and 750mg/d to the ration caused positive improvements in performance values (live weight gain and feed conversion ratio). The effects of the essential oil used vary due to the dose amount, bioactive ingredient composition, animal age and ration composition. Considering the reasons mentioned, there may be very variable results regarding the effect of adding plant essential oils to ruminant diets on performance parameters. In this study, we can say that it did not have a negative effect on performance parameters and even caused improvements that were not statistically significant.

Bioactive substances, especially monoterpene structures, in the structure of aromatic plants and extracts affect rumen fermentation. They are added to the rations in order to more clearly reveal their effects on the microorganism population with their fermentation parameters (Cobellis et al., 2015). Various results have been reported for the effects of essential oil on ruminal flora. In the present study, it was determined that the addition of different doses of REO to lamb rations did not have a significant effect on rumen flora. Similary to our results, it has been declared the absence of effects on acetic acid concentration, total VFA concentration and AA/PA rate in the rumen in fattening lambs that were daily supplemented with 250 mg/kg or 500 mg/kg rosemary oil (Guney et al., 2021). Likewise, Campolina (2021) reported that the essential oil blend supplementation into the milk replacer did not affect the concentration of total VFA, acetate, propionate and butyrate in calves. In contrast, Lin (2013) observed that supplemented with 1g/d EOC (mixture of equal amount of oils from clove, oregano, cinnamon and lemon) and 0. 5 or 1 g/d EOAC (mixture of eugenol, carvacrol, citral and cinnamaldehyde in equal ratio) for Hu sheep had a decrease of the acetate-to-propionate ratio and ammonia nitrogen concentration, despite the addition of fumarate in control sheep. The effects of plant essential oils on rumen fermentation parameters are highly variable. It is necessary to continue studies in order to reveal the significant effects on these parameters and to reveal their mechanisms. Although the results of the research are not statistically significant, they can be a source for future studies.

Protozoa in the rumen environment can constitute approximately 50% of the biomass. However, their role in this ecosystem still remains unclear today (Williams et al., 2020). In this study, the addition of REO to lamb rations did not cause an effect on rumen protozoa counts. In many studies, the addition of different essential oils to ruminant rations did not have a significant effect on rumen protozoa (McIntosh et al., 2003; Newbold et al., 2004; Belverdy et al., 2014). When the studies are examined, it is clear that the effect of essential oils on rumen protozoa varies depending on the plant essential oil and dose.

There are three key enzymes that are important in preventing oxidation in the organism. These enzymes are mainly SOD, catalase and GP_X. These enzymes, in

turn, convert the superoxide radical, hydrogen peroxides and hydroperoxides into harmless molecules (Ighodaroab and Akinloyeb, 2018). The potential uses of essential oil as an antioxidant modifier have been reported (Giannenas et al., 2013; Peterfalvi et al.,2019). There is little information concerning the antioxidant enzymes in lambs. This study was planned to determine the effects of REO, which has a strong antioxidant effect, on blood antioxidant enzymes, added to the ration of Merino lambs under stress, especially during the weaning period. The SOD values increased with both R500 and R750 groups at the end of the study. The mean value of serum SOD was significantly higher in the experimental groups (R500 and R750) than in the control group. Supplementation of juniper oil to the growing Saanen kid's rations created significant effects on antioxidant blood parameters (Yesilbag et al., 2017). Especially superoxide dismutase, total antioxidant capacity and catalase values were significantly increased in the juniper oil added groups. In a recent study by Passetti et al. (2021) reported that the supplementation of essential oil mix added to lamb rations did not cause a significant effect on blood oxidation parameters such as total SOD and glutathione peroxidase enzyme activity. As previously reported (Govaris et al., 2007), the antioxidant effect of rosemary is due to the polyphenols present in the leaves (mainly rosmarinic acid, carnosol, carnosic acid and phenolic acids, flavonoids, and diterpenoids), which accumulate in the fatty membranes of cells where the antioxidant effect is required. In this study, a significant increase in SOD enzyme was detected due to the presence of antioxidant-effective phenolic compounds included in the ration. This significant increase has shown that the compound can have an antioxidant effect and can be added to rations to support the organism, especially under stress conditions.

Fecal microbiology in organisms is an important parameter in the conduct of ideas about the digestive system microorganism community and health (Celi et al., 2017). In our study,the fecal bacteria of both pathogens (*Escherichia and Salmonella*) and beneficial bacteria (*Bifidobacterium and Lactobacillus*) were similar between lambs fed all treatments groups. In our knowledge, there is no published research evaluating the effect of the REO on fecal microbiology in merino lambs. Muhl and Liebert (2007) evaluated blend of essential oil (carvacrol and thymol) at the levels of 500-1500 mg/kg for weaner pigs. Supplementation did not affect fecal microbial counts and intestinal microflora. On the other hand, Zhang (2012) noted that supplementation 2 g/kg phytoncide did not affectfecal *E. coli* counts and diarrhea scores but increased the fecal lactic acid bacteria counts of weaning pigs. In this study, when we examined the fecal microbiology in terms of intestinal health by adding rosemary essential oil to the ration, there was no difference in terms of pathogenic microorganisms, according to the data we have obtained a positive trend was noticed in the fecal content of the experimental group in terms of beneficial microorganisms.

CONCLUSION

Aromatic plants and extracts are added to rations to carry out various metabolic reactions through the bioactive substances in their structure. At the end of the study, it was determined that especially the additions of 500 and 750 mg/day caused significant increases in SOD activity, which is one of the blood antioxidant enzyme parameters. In this article, results were obtained had shown improvement in favor of beneficial microorganisms in fecal microbiology, without disturbing the rumen flora, without causing negative effects on performance parameters, and with REO, which has a strong antioxidant. As a result, the quality and safety of food offered for human consumption are related to how well animals are fed with the right rations. For this reason, aromatic plants and extracts, with their strong components, may be considered as important additives in animal nutrition for the future both in terms of animal health and product quality.

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