Effects of dietary supplementation with rosemary oil on methanogenic bacteria density, blood and rumen parameters and meat quality of fattening lambs

Mehtap Güney, Serhat Karaca, Sibel Erdogan, Askin Kor, Cagri Kale, Sukru Onalan, Murat Demirel and Nuriye Tugba Bingol

Department of Animal Science, Van Yuzuncu Yil University, Van, Turkey; Department of Animal Nutrition and Nutritional Diseases, Van Yuzuncu Yil University, Van, Turkey; Department of Fish Diseases, Van Yuzuncu Yil University, Van, Turkey

ABSTRACT

This study aimed to determine the effect of rosemary oil (Rosmarinus officinalis) essential oil (REO) in lamb fattening diets on blood, rumen parameters, fattening performance and meat quality. Thirty Norduz male lambs weaned at 4 months of age with average body weight 22.0 ± 4.41 kg were used. Lambs were divided into three groups: no rosemary oil (control, R0), with 250 mg/kg DM (R250) and with 500 mg/kg DM (R500) rosemary oil added to the basal ration, and fed for 70 d. The daily feed intake of the R500 lambs (1.63 kg) was lower than other groups (1.70 kg) (p = .01). Rosemary oil did not change the density of methane-producing bacteria in the rumen fluid. Rumen pH was 6.31 in R250 lambs, while it was 6.16 in control (p < .04). The proportion of propionic acid (PA) increased in R250 (26.5 molar%) and R500 (26.0 molar%) lambs compared to control lambs (22.7 molar%) (p < .001). Serum glucose levels increased with REO dose (p < .01) and serum IGF-1 levels were significantly higher in R250 lambs (p < .001). The dose rates of REO used in fattening lambs had limited effect on fattening performance, carcase and meat quality. The results showed that although it does not affect the final live weight, the negative effect of REO on feed intake at 500 mg/kg was considered as a limiting factor and 250 mg/kg dose of REO may have positive effects on ruminal fermentation. Hence, it may be beneficial to try doses lower than 500 mg in further studies.

HIGHLIGHTS

- Rosemary oil may improve rumen pH and the propionic acid (PA) concentration at 250 mg of REO/kg DM of the diet.
- Serum IGF-1 levels were significantly increased with 250 mg/kg dose of REO.
- Feed intake and feed conversion ratio were negatively affected by REO at 500 mg/kg DM.
- Lambs supplemented with 500 mg REO/kg DM in the diet had the lowest CLA.
- The effect of rosemary oil on slaughter-carcase characteristics and meat quality was limited.

Introduction

As the world population increases, the demand for animal products is increasing day by day (Thornton 2010). Considering the number of animals in the world today, CO₂ and methane-based greenhouse gas effects that occur as a result of the consumption of nutrients by each animal lead to significant energy losses in ruminants as well as ecological pollution (Jensen 1996). Therefore, there is increasing interest to finding natural nutritional alternatives in order to reduce energy losses and environmental pollution. For instance, various nutritional strategies were studied to change the fermentation pathways in the rumen. The addition of essential oils (EO) is one of these strategies, and rosemary essential oil (REO) is an example (Cobellis et al. 2015a).

Rosemary oil contains antioxidant compounds including polyphenolic extract, phenolic diterpenes, active carcinol, carnosic acid, rosmanol, epirosmanol, isorosmanol, methyl camosate and other phenolic acids, such as rosmarinic acid (Cuvelier et al. 1996). These active components in rosemary oils may be highly effective as a natural dietary supplementation...
option to manipulate ruminal fermentation (Benchaar et al. 2008).

Rosemary is now widely used for antioxidant purposes to increase the shelf life of animal products (Raadt et al. 2015). It was stated that REO has antimicrobial effects in addition to antioxidant effects and that it can change the rumen fermentation direction in favour of the ruminant (Faixova and Faix 2008). EOs are a potential feed additive that could be used as a natural antioxidant in animal nutrition to support and enhance animal performance (Kotsampasi et al. 2018). Recently, many in vivo and in vitro studies were conducted to investigate the effects of EOs in ruminants. But the studies did not evaluate the effect of EOs on both the density of methane-producing bacteria, rumen and blood parameters, and meat quality in lambs. We think that the effects on rumen fermentation and meat quality should be evaluated together in lambs. Therefore, this study was performed to investigate the effect of rosemary EO on fattening performance, rumen methanogenic bacterial density, rumen fermentation, and meat quality of lambs during a 70-day fattening period.

Material and methods

**Animals, diet and experimental procedure**

In the study, 30 Norduz male lambs with an average body weight of 22.0 ± 4.41 kg at the age of 4 months were used. The lambs were housed in the experimental farm at the Van Yuzuncu Yil University, in Van. Lambs were divided into three groups. All groups received the same basal diet containing barley and cottonseed meal. The first group (Control) did not receive REO supplementation, whereas the second group was supplemented with 250 mg/kg DM of REO (R250), and the third group was supplemented with 500 mg/kg DM of REO (R500), and all were fed for 70 d.

Rosemary EO was supplied in daily doses mixed with 0.1 kg of concentrate before feeding. Feeds were offered to the animals twice daily at 08:00 and 20:00 h for ad libitum intake. Animals were weighed every 14 d in the morning and the diets were not distributed for 12 h before each weighing. The daily weight gain and feed utilisation were calculated within the weighing period. The daily dry matter (DM) intake (DDMI) was calculated as difference between the quantity distributed diet and leftovers everyday. The experimental diets were formulated to meet nutrient requirements according to National Research Council (NRC 1985). In the study, the experiment ration was gradually increased for 20 d to accustomed to the fattening feed (before trial) and the experiment was started on the 0th day.

The extract obtained from the rosemary plant by the hydrodistillation method was obtained from Antalya province in Turkey (İnan Tarım, Ecobab). Rosemary extract was placed into vials after the EO extract was diluted and filtered. With liquid injection, 1 microliter of extract was injected into a 60/C20.25 mm i.d., 0.2 lm SGE Analytical-BPX70 column. The identification of volatile compounds was carried out with a gas chromatography device by comparing the total molecular weights, fragmentation ions and arrival times with the National Institute of Standards and Technology (NIST) library and the mass spectrum data and current literature data defined by Dool van den and Kratz (1963) and Dalar et al. (2015). According to this, major compounds and concentration values of REO are given in Table 1, and ingredients and chemical composition of the basal diet are shown in Table 2.

### Table 1. Major compounds of REO and their concentrations (%).

| Active compound | Concentration, % |
|-----------------|------------------|
| 1,8 cineol      | 32.0             |
| Camphor         | 8.78             |
| Caryophyllene   | 7.30             |
| Borneol         | 6.05             |

REO: Rosemary oil.

### Table 2. Ingredients and chemical composition of the basal diet.

| Item                        | Amount       |
|-----------------------------|--------------|
| Ingredient (% DM basis)     |              |
| Alfalfa hay                 | 19.7         |
| Barley grain                | 59.9         |
| Cottonseed meal             | 13.3         |
| Molasses                    | 0.25         |
| Vitamin-mineral premix      | 0.24         |
| Dicalcium phosphate         | 0.02         |
| Limestone                   | 0.16         |
| Salt                        | 0.06         |
| Chemical composition (%)    |              |
| Crude protein               | 17.29        |
| Crude fat                   | 1.02         |
| Crude ash                   | 8.27         |
| Acid detergent fibre (ADF)  | 13.02        |
| Neutral detergent fibre (NDF)| 29.84        |
| Energy (kcal)               | 2522         |

**Microbial DNA isolation, quantitative real-time PCR**

In the study, primers were used for methanogenic bacteria. Therefore, 2 mL of rumen fluid was taken from all animals at the beginning and end of the
experiment for DNA isolation. Rumen liquid samples were collected in sterile tubes, and DNA isolation with the residual precipitate was carried out by means of a QIAmp DNA Stool Kit with a QIACube automated isolation device according to the manufacturer’s instructions. In order to identify bacteria following DNA isolation, both the presence of bacteria and the desired bacteria were determined by using specific primers with a real-time PCR test. Total genomic DNA obtained in the study was used as a template. DNase with RT2 qPCR SYBRGreen Master Mix and Forward-Reverse primers, PCR mix with RNase free water was created. Forward primers 16S rRNA were used for Tokura et al. (1999) U8F AGAGTTGATCATGGCTCAG and 1492 R GGTTCACTTGTTACGACTT.

Feed analysis
During the experiment, weekly samples were taken from the distributed diet and orts. The samples were ground in a 1-mm sieve, and then dry matter (DM), crude protein (CP) and crude ash (CA) contents were determined according to the Weende analysis method (AOAC 2000). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analysed as described by Van Soest et al. (1991), and the crude fat (CF) level was measured according to ANKOM (2008) using an ANKOM XT15 device.

Rumen fluid analysis
Rumen fluid was taken from all animals with a ruminal probe at the beginning of the trial period (10 d before the beginning of the experimental period), and at days 0, 35 and 70 of fattening. Rumen pH was immediately measured with a 0.01 pH digital metre. Ammonia nitrogen analysis was performed with Kjeldahl distillation as reported by Markham (1942). Rumen volatile fatty acid (VFA) analysis was performed in the rumen fluid taken by HPLC using a Schimadzu UV detector and an Alltech IOA-1000 column (Spanghero et al. 2008).

Blood analysis
Blood samples were taken from the vena jugularis of all lambs at the same day and hour of rumen fluid sampling. A volume of 10 mL of blood samples was placed into anticoagulant-free tubes. The serum was centrifuged at 4000 × g for 10 min then stored at −18 °C until testing. Serum glucose, insulin, blood urea nitrogen (BUN), total protein and triglyceride levels were determined by using Bioanalytic™ kits on an Architect 1600 model device. The analysis of malondialdehyde (MDA) level in blood samples was done by first testing the samples with trichloroacetic acid (TCAA) and thiobarbituric acid (TBA), and then the results were read on a Shimatzu UV-1280 instrument at 535 nm wavelength in the spectrophotometer. Serum IGF-1 was determined with a commercial animal kit (lot no: 20180323066) using a micro ELISA device according to the manufacturer’s package insert.

Carcass characteristics and meat-quality analysis
At the end of the fattening period, all lambs were slaughtered, and hot carcass weights were determined. Carcasses were stored at +4 °C for 24 h. Hot carcass and offal weights were determined following slaughter. Then, carcasses were chilled at 4 °C for 24 h and jointed according to Colomer-Rocher et al. (1987).

For meat quality analysis, samples were taken from the left half of the carcasses (m. Longissimus thoracis (LT); between 6th and 12th ribs; Longissimus lumbarum (LL); L1–L5). After sampling, they were placed in polystyrene trays, wrapped in oxygen permeable PVC film, and maintained at 4 degrees for 72 h. The samples were then stored in vacuum bags at −18 °C until texture analysis. The pH of the meat was determined at two different times 45 min (pH45m) and 24 h (pH24h) after slaughter. The pH measurements were measured at 12–13 over LT in the left half between the ribs on the carcass using a Hanna brand meat pH metre (HI 99163), and the probe tip was placed approximately 3 cm deep in the muscle.

Meat colour was measured within 2 h after sampling. A sample was removed from LT between the 11th and 12th ribs from the sample taken from the left half carcass. The surface of the meat samples was allowed to come into contact with oxygen for 30 min at +4 °C (Karaca et al. 2016). Colour measurement was performed in three different areas on the surface of freshly cut LT oil-free samples, and the average of values for luminosity (L*), redness (a*) and yellowness (b*) were recorded. The flesh colour was measured with the Lovibond RT-300 portable spectrophotometer in daylight (CIELAB-illuminant D65/10°). Water holding capacity of meat samples was measured using the printed filter paper method (Wierbicki and Deatherage 1958). The water content of meat was determined according to AOAC (2000). The samples stored at −18 °C for texture analysis were thawed and then analysis was performed as described by Hoffman et al. (2003).
Thiobarbituric acid reactive substances (TBA(RS) analysis)

TBA(RS) analysis was performed on meat samples taken from the left half carcass. LL samples stored at −18 °C were thawed for 24 h at +4 °C. Then, the amount of MDA in a 10 g sample taken from muscle tissue was measured according to Yoshioka et al. (2006).

Fatty-acid analysis

Fatty-acid analysis was performed on LT samples (6th–11th ribs). For muscle and fat extraction, samples were homogenised according to Folch et al. (1957). The formation of methyl esters in fats was analysed in the tissue samples. Samples were then injected into the chromatograph (GC) and the results were determined as % methyl esters (Karaca et al. 2016).

Sensory analysis

A sensory panel test was performed on the samples taken from the left half carcass (LL; L1–L5 ribs). The meat samples were thawed at 4 °C overnight and stored at −18 °C, and then they were placed on aluminium foil and cooked in an electric oven at 180 °C until the internal temperatures reached 80 °C. The internal temperatures of the meat were monitored at the geometrical midpoint of the meat using probes connected to a Testo 175 T3 data logger. Each cooked sample was cut into pieces of approximately 1-cm cubes, kept at 60 °C until evaluation and served to panellists. The 60 semi-educated panellists were asked to evaluate the characteristics of the softness, juiciness, flavour and general taste. The panellists were asked to evaluate these characteristics from 1 to 9 for soft, excessively juicy, extremely good flavour and extremely good taste (Adnoy et al. 2005).

Statistical analysis

GLM and GLM repeated-measures ANOVA procedures were used to determine the differences in the means of the groups from the general mean using SPSS version 23.0 software (SPSS Inc. Chicago, IL). The Duncan Multiple Comparison Test was used to determine the differences between groups. The Kruskal–Wallis Test, which is a non-parametric method, was used to compare the differences in the samples for methanogenic bacterial density and the results of the sensory panel between the groups.

Results

Fattening performance of lambs in each group is in Table 3. The final weights were similar in all groups, and ranged from 42.2 to 45.7 kg. The average daily gain (ADG) of the groups was higher for R250 dose (0.23 kg/d) rather than R500 dose (0.19 kg/d) rosemary oil used during fattening, whereas the rate of feed conversion was higher at R500 dose (8.82) compared to R250 dose (7.44), but similar to the control group (7.93) during the fattening period.

The changes in total bacterial DNA concentrations in rumen fluid and sensory analysis in the meat samples:

\[ y_{ij} = \mu + a_i + e_{ij} \]

where \( y_{ij} \) is the value of the examined characteristic for animal at the \( j \)th time from the \( i \)th group; \( \mu \) is overall mean; \( a_i \) is fixed effect of diet \( (a_i = R0, R250, R500) \) and \( e_{ij} \) is residual random error.

Rumen, blood parameters and shelf life of the subcutaneous fat data were evaluated by repeated-measures one-way ANOVA (RANOVA) using the following equation:

\[ y_{ijkl} = \mu + I_i + a_{ij} + b_k + (ab)_{jk} + e_{ijkl} \]

where \( y_{ijkl} \) is the value of the examined characteristic for the \( k \)th animal at the \( j \)th time from the \( i \)th diet; \( \mu \) is overall mean; \( I_i \) is random effect of animal; \( a_{ij} \) is fixed effect of diet \( (a_{ij} = R0, R250, R500) \); \( b_k \) is fixed effect of sampling time \( (k = 0d, 35d, 70d \) or 3d, 14d and 28d); \( (ab)_{jk} \) is interaction of the effects; and \( e_{ijkl} \) is residual random error.

### Table 3. The least squares means of weights of fattening performance in feeding groups.

|                          | R0  | R250 | R500 | SEM | p Value |
|--------------------------|-----|------|------|-----|---------|
| Weaning weight (kg)      | 21.9| 21.3 | 21.7 | 1.42| .69     |
| Initial weight (kg)      | 28.9| 29.7 | 29.2 | 1.51| .93     |
| Final weight (kg)        | 43.9| 45.7 | 42.2 | 1.88| .43     |
| Average daily gain (kg/d)| 0.21 | 0.23 | 0.19 | 0.01| .05     |
| Dry matter intake/live weight (%)| 3.69 | 3.66 | 3.54 | 0.20| .87     |
| Feed intake (kg)         | 1.70 | 1.70 | 1.63 | 0.01| .01     |
| Feed conversion ratio    | 7.93 | 7.44 | 8.82 | 0.21| .02     |
| Dry matter intake (kg/d) | 1.39 | 1.41 | 1.26 | 0.09| .53     |

**a,b**Values within a line with different superscripts differ significantly.
before the experiment. However, after the experiment, the density of methanogenic bacteria was 0.20 ng/µl in the control group, 0.14 ng/µl with 250 mg/kg dose and 0.14 ng/µl with 500 mg/kg DM dose, and no significant effect was obtained on total bacterial load (Table 4).

The effects of rosemary oil on rumen parameters are presented in Table 5. EO doses used in lamb fattening did not influence ammonia, acetic acid, acetic acid/propionic acid (AA/PA) and total VFA levels, but rumen parameters in all groups changed significantly according to the sampling time (p < .001). The interactions between treatment groups and sampling time were significant for all parameters except AA.

Blood parameters of the groups are presented in Table 6. Serum glucose levels in R0 lambs (55.5 mg/dL) were significantly lower than R250 (61.9 mg/dL) and R500 lambs (64.6 mg/dL) (p < .01); total protein content was higher in R500 (70.0 g/L) lambs compared to R250 lambs (66.7 g/L) (p < .03); and serum IGF-1 levels were significantly higher in R250 lambs compared to the other groups (Table 6; p < .001). However, insulin, BUN, triglyceride and MDA levels were similar between the groups. Serum glucose levels increased significantly for all sampling times after consumed the fattening ration (p < .001). As the sampling time progressed, the serum BUN level increased, reaching the highest level on day 70 (p < .001). Total protein and triglyceride levels were found to be higher in samples taken on the 35th and 70th days when sampling times are compared (Table 6). Moreover, the effect of the interaction between feeding groups and sampling time on other parameters, except for insulin and serum IGF-1 levels, was not statistically significant.

Main lamb slaughter and carcass characteristics are presented in Table 7. There was no difference in terms of all of the parameters examined. Rosemary oil had no significant effect on hot carcass weights, dressing (%), cold-carcass weights, chilling loss (%), MLD area (cm²), cold-carcass indices and quality percentage categories. In the study, the effect on meat quality

| Feeding groups | Before experiment | After experiment |
|----------------|-------------------|------------------|
| R0             | 0.45 ± 0.06       | 0.20 ± 0.03      |
| R250           | 0.57 ± 0.08       | 0.14 ± 0.03      |
| R500           | 0.45 ± 0.03       | 0.14 ± 0.02      |
| p Value        | .33               | .28              |

R0: Rosemary oil without additives; R250: added 250 mg/kg DM Rosemary oil; R500: added 500 mg/kg DM Rosemary oil.

Table 5. Effect of rosemary oil on rumen parameters in lambs.

| Treatment (T) | pH     | NH₃ (mL/100, mL) | Acetic acid (Molar, %) | Propionic acid (Molar, %) | Butyric acid (Molar, %) | AA/PA (mmol/mL) | Total VFA  |
|---------------|--------|-----------------|------------------------|---------------------------|-------------------------|-----------------|------------|
| R0            | 6.16   | 24.2            | 63.5                   | 22.7                      | 13.9                    | 3.03            | 4104.9     |
| R250          | 6.31   | 24.1            | 62.4                   | 22.5                      | 11.2                    | 2.69            | 4026.3     |
| R500          | 6.26   | 24.9            | 61.7                   | 26.0                      | 12.0                    | 2.70            | 3974.9     |
| p Value       | .04    | .09             | .82                    | .87                       | .51                     | .13             | 100.1      |

Sampling time (St, d)

| Treatment (T) | pH     | NH₃ (mL/100, mL) | Acetic acid (Molar, %) | Propionic acid (Molar, %) | Butyric acid (Molar, %) | AA/PA (mmol/mL) | Total VFA  |
|---------------|--------|-----------------|------------------------|---------------------------|-------------------------|-----------------|------------|
| R0            | 6.64   | 18.1            | 71.2                   | 20.0                      | 8.9                     | 3.61            | 3936.1     |
| R250          | 6.37   | 27.1            | 62.6                   | 20.5                      | 16.6                    | 3.16            | 4510.1     |
| R500          | 5.98   | 26.4            | 60.3                   | 28.4                      | 11.4                    | 2.50            | 4061.5     |
| p Value       | .04    | .04             | .30                    | .91                       | .50                     | .13             | 100.1      |

Table 6. Rumen methanogenic bacteria density before and after the experiment (ng/µl).

| Treatment (T) | Before experiment | After experiment |
|---------------|-------------------|------------------|
| R0            | 0.45 ± 0.06       | 0.20 ± 0.03      |
| R250          | 0.57 ± 0.08       | 0.14 ± 0.03      |
| R500          | 0.45 ± 0.03       | 0.14 ± 0.02      |
| p Value       | .33               | .28              |

R0: Rosemary oil without additives; R250: added 250 mg/kg DM Rosemary oil; R500: added 500 mg/kg DM Rosemary oil.
The characteristics of rosemary oil added to lamb fattening diets was similar between groups except for yellowness ($b^{a}$). Shelf life, colour stability and fatty acid composition of longissimus dorsi intramuscular fat in lambs were not affected by rosemary oil (Tables 8–10).

### Table 6. Effect of rosemary oil on some blood parameters in lambs.

| Glucose (mg/dL) | Insulin (uU/mL) | BUN (mg/dL) | Total protein (g/L) | Triglyceride (mg/dL) | MDA (nmol/mL) | IGF1 (ng/mL) |
|----------------|----------------|-------------|---------------------|----------------------|--------------|--------------|
| Treatment (T)  | R0             | R250        | R500                | SEM                  | p Value      | R0           | R250 | R500 |
|                | 55.5$^{ab}$    | 61.9$^{a}$  | 64.6$^{a}$          | 1.30                 | 1.13          | 1.20     | 28.2   | 27.4  | 27.2  | <.01  | .001  |
|                | 66.0$^{a}$     | 66.7$^{a}$  | 70.0$^{a}$          | 0.97                 | 0.97          | 1.07     | 16.1   | 1.61  | 0.74  | <.001 |
|                | 10.8           | 10.9        | 10.9$^{c}$          | 0.03                 | 0.03          | .88      | .99    | 1.68  | <.001 |

| Sampling time (St) | BT  | 0 | 35 | 70 | SEM | p Value |
|--------------------|-----|---|----|----|-----|---------|
|                    | 46.3$^{ab}$ | 63.6$^{a}$ | 68.6$^{a}$ | 64.2$^{a}$ | 2.05 | <.001  |
|                    | 61.9$^{a}$  | 63.7$^{a}$ | 74.5$^{a}$ | 72.6$^{a}$ | 1.13 | <.001  |
|                    | 64.6$^{a}$  | 26.7$^{a}$ | 35.3$^{a}$ | 25.6$^{a}$ | 0.97 | <.001  |

| p Value | <.01  | <.001  | <.001  | <.001  | <.001  |

### Table 7. Effect of rosemary oil on some main slaughter and carcass traits in lambs.

| Treatment (T)   | R0 (without additives) | R250 (250 mg/kg) | R500 (500 mg/kg) |
|----------------|-------------------------|------------------|------------------|
| Slaughter weight (kg) | 43.6                    | 45.9             | 42.3             | 1.89 | .41    |
| Hot carcass (%)    | 21.4                    | 22.5             | 20.7             | 1.17 | .56    |
| Dressing (%)       | 49.0                    | 48.8             | 48.6             | 0.82 | .94    |
| Cold carcass (kg)  | 20.9                    | 21.9             | 20.1             | 1.16 | .56    |
| Chilling loss (%)  | 2.24                    | 2.52             | 2.73             | 0.25 | .39    |
| MLD area (cm$^2$)  | 14.3                    | 14.8             | 14.6             | 0.78 | .89    |
| Cold carcass indices | Carcass compactness (g/cm) | 337.1         | 356.0             | 328.1 | 14.1 | .37    |
|                    | Leg compactness (g/cm)   | 128.6           | 137.4             | 123.3 | 5.49 | .20    |
|                    | Chest compactness (g/cm) | 0.63            | 0.63              | 0.61 | 0.01 | .68    |

### Table 8. Effect of Rosemary oil on some meat quality characteristics in lambs.

| Treatment (T) | R0 (without additives) | R250 (250 mg/kg) | R500 (500 mg/kg) |
|---------------|-------------------------|------------------|------------------|
| pH45          | 6.24                    | 6.19             | 6.21             | 0.05 | .83    |
| pH24          | 5.69                    | 5.63             | 5.76             | 0.03 | .09    |
| pH72 (Ultimate pH) | 5.48                | 5.57             | 5.54             | 0.04 | .42    |
| a$^*$         | 43.2                    | 42.8             | 41.4             | .60 | .11    |
| b$^*$         | 9.59                    | 9.66             | 9.35             | .35 | .81    |
| C$^*$         | 13.1$^{a}$              | 13.1$^{a}$       | 12.1$^{a}$       | .24 | <.01  |
| h$^*$         | 16.3                    | 16.3             | 15.2             | .35 | .08    |
| WHC (%)       | 15.0                    | 15.5             | 14.4             | .50 | .33    |
| Cooking loss (%) | 26.3               | 26.3             | 27.2             | .88 | .69    |
| WBSF (N)      | 40.0                    | 40.7             | 38.8             | 1.46 | .64    |

### Nutrient contents (%)
- Moisture: 77.2 ± 7.6 ± 76.8 ± 0.31 ± .48
- Crude protein: 20.9 ± 21.2 ± 21.2 ± 0.31 ± .61
- Ether extract: 0.52 ± 0.72 ± 0.65 ± 0.13 ± .55
- Crude ash: 1.34 ± 1.29 ± 1.20 ± 0.06 ± .29
- Sensory analysis:
  - Juiciness: 4.86 ± 4.58 ± 5.12 ± 0.20 ± .23
  - Tenderness: 5.40 ± 5.22 ± 5.64 ± 0.21 ± .32
  - Flavour: 5.42 ± 5.48 ± 5.72 ± 0.22 ± .75
  - Overall liking: 5.90 ± 5.72 ± 5.90 ± 0.19 ± .85

### Sensory analysis:
- Juiciness: 4.86 ± 4.58 ± 5.12 ± 0.20 ± .23
- Tenderness: 5.40 ± 5.22 ± 5.64 ± 0.21 ± .32
- Flavour: 5.42 ± 5.48 ± 5.72 ± 0.22 ± .75
- Overall liking: 5.90 ± 5.72 ± 5.90 ± 0.19 ± .85

*Values within a column with different superscripts differ significantly.

R0: Rosemary oil without additives; R250: added 250 mg/kg DM Rosemary oil; R500: added 500 mg/kg DM Rosemary oil.

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*BL: Back-loin; HL: hind leg; S: shoulder; FL: Fore leg; N: neck; F: flank.
Table 9. MDA (mg/1000g) amounts in subcutaneous fat at different storage times and colour parameters in LD.

| Treatment* (T) | MDA (mg/1000g) | pH | L* | a* | b* | C* | h* |
|---------------|----------------|----|----|----|----|----|----|
| R0            | 0.44           | 5.48 | 43.2b | 9.59e | 13.1a | 16.3a | 54.1c |
| R250          | 0.22           | 5.69b | 44.3a | 6.70de | 13.1a | 14.7bc | 62.9a |
| R500          | 1.02ab         | 6.05a | 39.3cd | 8.09bed | 11.0b | 13.8bcd | 55.4abc |
| SEM           | 0.08           | 0.04  | 0.46  | 0.31  | 0.22  | 0.30  | 1.04  |

Sampling time (St)

| 3  | 0.09d | 5.53c | 42.5b | 9.53a | 12.8a | 16.0a | 53.4bc |
| 14 | 0.44b | 5.72b | 43.3a | 6.76b | 12.5a | 14.2b | 61.5a |
| 28 | 1.19a | 6.07a | 38.6b | 7.80b | 10.7b | 13.4b | 55.0b |
| SEM| 0.08  | 0.04  | 0.46  | 0.31  | 0.22  | 0.30  | 1.04  |

Table 10. Effect of rosemary oil on fatty acid composition of longissimus dorsi intramuscular fat in lambs.

| Fatty acids | Treatment* |
|-------------|------------|
| C18:0       | R0         | R250       | R500       | SEM | p Value |
| 0.02        | 0.02       | 0.01       | <0.01      |     | .16     |
| 0.18        | 0.19       | 0.16       | 0.01       |     | .19     |
| 0.18        | 0.17       | 0.14       | 0.02       |     | .56     |
| 2.37        | 2.34       | 2.04       | 0.21       |     | .49     |
| 0.09        | 0.11       | 0.08       | 0.01       |     | .25     |
| 0.23        | 0.17       | 0.19       | 0.05       |     | .77     |
| 0.08        | 0.08       | 0.08       | 0.01       |     | .97     |
| 23.00       | 23.87      | 21.97      | 0.56       |     | .09     |
| 1.37a       | 1.37a      | 1.16h      | 0.06       |     | .04     |
| 1.02b       | 1.31h      | 1.01h      | 0.07       |     | .01     |
| 0.33        | 0.32       | 0.31       | 0.08       |     | .98     |
| 19.19       | 18.64      | 19.59      | 0.48       |     | .40     |
| 2.50        | 3.05       | 2.78       | 0.35       |     | .54     |
| 32.50       | 31.39      | 29.79      | 0.89       |     | .36     |
| 0.42        | 0.36       | 0.31       | 0.06       |     | .48     |
| 11.44       | 10.88      | 12.88      | 0.59       |     | .26     |
| 0.09        | 0.08       | 0.10       | 0.01       |     | .43     |
| 0.49        | 0.47       | 0.50       | 0.03       |     | .72     |
| 0.35        | 0.33b      | 0.26b      | 0.02       |     | .04     |
| 0.10        | 0.09       | 0.08       | 0.01       |     | .74     |
| 0.10        | 0.11       | 0.13       | 0.01       |     | .55     |
| 0.54b       | 0.54b      | 0.73a      | 0.05       |     | .03     |
| 0.32ab      | 0.29b      | 0.53b      | 0.06       |     | .03     |
| 3.80ab      | 3.35b      | 4.55b      | 0.33       |     | .04     |
| 0.15        | 0.17       | 0.18       | 0.03       |     | .65     |

R0: Rosemary oil without additives; R250: added 250 mg/kg DM Rosemary oil; R500: added 500 mg/kg DM Rosemary oil. a,b, c,d Values within a column with different superscripts differ significantly. *Different superscript letters in the same row represent significant difference.

Discussion

Feed intake and performance

The use of rosemary oil in lamb fattening diets does not improve fattening performance, and also the doses of REO do not produce positive results, especially at the 500 mg/kg dose (Table 3). Yagoubi et al. (2018) found an improved ADG and decreased FCR. Unfortunately, the present study did not change the daily DM intake, and these results were explained by an insufficient dose of rosemary oil (Smeti et al. 2018). High dose of EO (400 mg/kg) was considered an appropriate supplementation level for sheep, since nutrient intake was not affected (Soltan et al. 2018). The doses used in this study ranged from those used in the previous studies above. The feed intake of lambs in the R500 group was lower, and this may be due to the intense aroma effect of EO, but the results for similar DM intake should also be evaluated. Low doses were noted to stimulate appetite and increase total feed intake per body weight (Patra et al. 2019). Moreover, in vivo experiments are required to decide optimum dose levels, and low doses that have a stimulating effect (Patra 2016). The variable effects on fattening performance in different studies might be due to strong differences in type, source and concentrations of EO between experiments.

Methanogenic bacteria density

In the study, the main active compounds and concentration % values in REO were 1,8-Cineole at 32%, carophyllene at 7.30%, camphor at 8.78% and borneol at 6.05%, which are secondary metabolites. These monoterpenes are used to change the direction of rumen fermentation (Cobellis et al. 2015b). In this study, however, the antimicrobial effect of monoterpenes on methanogenic bacteria density in rumen was not observed (Table 4). This result may be below the level of monoterpenes substances in rosemary oil used in our study. It is stated that the most decisive
effect of EOs in reducing the production of methane in the rumen is the decrease in AA or acetate/propionate production (Lin et al. 2013). It is possible to say that these parameters did not change with the contribution of REO in our study, except for PA.

**Rumen fermentation characteristics and blood metabolites**

One of the most important issues about the addition of EO to ruminant diets is undoubtedly to positively change VFAs without suppressing rumen fermentation. Thus, it will be possible to determine the species that contribute to this change and the optimum dose of additives. In this study, 250 mg/kg dose of rosemary oil increased rumen pH compared to the control, however, pH was similar between control and R500 diet (Table 5). The increase in rumen pH at 250 mg/kg dose did not change with respect to the concentration of rumen AA and total VFAs. This may be related to the antimicrobial effect of EO against rumen microflora, which could be more effective at this dose. It was reported that the effects of rumen pH may vary due to the main chemical constituents depending on the concentrations of EO or compounds used in the diets (Benchaar et al. 2008). In addition, some bacterial and archae populations have different sensitivities to EO (Cobellis et al. 2016). In this study, it is thought that the polyphenols in the EO used at 250 mg/kg dose increased the pH level of rumen by causing an increase in saliva production. Rumen pH was at the highest level before the trial and, decreased as fattening progressed. This was also seen in interactions between all treatment groups and the sampling time. Moreover, while rumen pH increases, did not cause any change in the concentration of rumen AA and the total VFAs in this study. This can be associated with the insufficient inhibitory effect of rosemary oil used against the rumen microflora, and it is possible to argue that the optimum nutrient use efficiency was realised. The ammonia concentration in rumen fluid with REO supplementation to diet was similar in all groups.

In this study, molar AA concentration, total UYA concentration and AA/PA ratio in the rumen did not change with additive doses. However, while PA concentration increased with both doses of EO, BA concentration decreased. VFA, which is an important energy source for animals was not suppressed in the rumen. According to the sampling time, while AA concentration tended to decrease as the fattening period progressed, PA concentration increased. This effect on AA and PA concentrations is thought to be related to the transition from roughage used at the beginning of fattening to concentrated feed. Yesilbag et al. (2016) found that an EO supplement containing 89.7% α-pinene had no effect on rumen fluid total and individual VFA concentrations in Saanen goats, but there were significant differences between sampling times. They also reported that the responses of EOs to VFA concentrations may depend on the type of substrate consumed by ruminants and the pH of the rumen fluid. Patra et al. (2019) found that doses of EO did not change the results of ruminal microbial fermentation, and that stable fermentation is maintained during minor microbial alterations as a result of metabolic redundancy of the ruminal ecosystem. More in vivo studies are needed to confirm our results.

Blood parameters are important indicators of the effect of consumed diets on blood biochemistry. Blood glucose level increased with both REO doses in animals consuming the same diet (Table 6). Blood and rumen parameters should be evaluated together in this study, because carbohydrate metabolism is affected by PA produced in the rumen, and PA absorbed from the rumen is converted to glucose in the liver. The level of PA produced in the rumen was also increased, and this is due to the antimicrobial effect of the active compounds of REO in the rumen. In addition, blood glucose levels before the trial were significantly lower than other sampling times (day). This result is due to the dietary intake of lambs in this period consisting completely of roughage. Harmon (2009) stated that feed containing high starch increases blood glucose levels in ruminant, but there was no significant effect of rosemary oil supplementation doses on serum insulin, BUN and triglyceride levels. Moreover, it is stated that the addition of 80–160 mg/d EO to diets does not change the blood biochemical profile (Patra et al. 2019). BUN concentration is a result of the NH$_3$–N concentration produced in the rumen. BUN concentration showed a similar trend with NH$_3$–N concentration in this study, and did not change in fattening groups with REO supplementation (Table 6). Serum IGF-1 concentration in livestock is an indicator of nutrient balance, performance and production efficiency of animals (Breier et al. 1988). In this study, serum IGF-1 levels increased significantly with the addition of 250 mg/kg rosemary oil to the diet compared to other groups. There is a positive correlation between IGF-1 level and nutrition intake (Whitney and Muir 2010) and it was stated that serum IGF-1 concentration increases in lambs as a result of the consumption of protein preserved in the rumen.
(Davenport et al. 1995). Hassan and Hassan (2009) reported that growth hormone increases with the contribution of rosemary, and these differences are related to the level and source of feed additive intake rather than dietary energy and N intakes. In this study, although the nutrition intake was similar in the fattening groups, high serum IGF-1 levels in lambs given low dose (R250) rosemary oil could be associated with nutrient use, amino acid from rumen to abomasum, and the differing effects of total N sources.

**Meat quality**

The study showed that post-mortem glycolysis maintained the normal course for all groups, and the final pH was within the accepted limits. Moreover, luminosity (L*) which is the colour property that directly affects consumer preferences, was above 38 (Page et al. 2001) which was accepted as appropriate in all groups, and was not affected by rosemary supplementation. In general, the effect of rosemary additive on the main meat quality characteristics, such as water holding capacity, hardness and nutrient content, except for b*, was not significant. In previous studies, the aim was to generally determine the effect of rosemary oil supplementation and other EO in rations, and the rate of substitution on slaughter carcase characteristics of lambs. The effects on meat quality characteristics of lambs were investigated in very few studies. The results obtained in these studies were in parallel with this study, and the effect of rosemary additives on these meat quality features was not significant (Nieto 2013; Smeti, Atti, Mahouachi 2013; Smeti et al. 2018; Yagoubi et al. 2018).

Lipid peroxidation is undesirable as it causes bitterness and abnormal taste during the aging of meats. The two phenolic diterpenes that have main antioxidant effect in rosemary oil are carnisic acid and carnosol (Genena et al. 2008). In this study, the amount of MDA (mg/1000 g) and colour stability in subcutaneous fat was similar at different storage times, and the effect of the rosemary additive was not significant. However, in the R500 lambs, the L* value was lower on the 28th day compared to the other groups. The difference (%) between the 3rd–28th day changes in the groups was similar, and this difference may be the cumulative effect due to the lower L* value of R500 lambs on the 3rd day. However, the fact that the L* value on the 3rd day tended to be low in R500 lambs may be associated with the negative effects of high doses of EO. High doses of EO can cause mitochondria permeability and damage, and produce more free radicals, such as reactive oxygen species (ROS). Antioxidants that interact with ROS become prooxidants that cause oxidation of lipids and proteins. This does not occur in EO at low concentrations and antioxidant activity is maintained (Rivaroli et al. 2016). The effect of adding 1000 mg of rosemary oil daily to the ration in cattle was not significant with regards to the shelf life and colour stability of the meat, and it was stated that the dose used may have been insufficient for adequate storage of antioxidant compounds in the cell membrane and muscle tissue (O’Grady et al. 2006).

The sensory properties of meat are closely related to physical properties, as well as volatile compounds and fatty acid composition. In this study, the results obtained from the sensory panel test were in line with the results about the physical and chemical properties of the meat, and the effect of the rosemary additive on the sensory properties of meat was not significant. In similar studies, it was reported that the addition of rosemary extract to the ration did not cause significant changes in the sensory characteristics of beef (O’Grady et al. 2006) and lamb (Smeti, Atti, Mahouachi, Munoz 2013) meat. In contrast, Nieto et al. (2010) reported that the addition of rosemary oil to the ration did not cause a significant difference on the 7th day of storage, but significantly reduced the taste of meat on the 14th and 21st day. Smeti et al. (2018) also suggests that rosemary oil significantly improves flavour and general acceptability with regards to the sensory properties of meat. Researchers reported that the antioxidant compounds in rosemary that inhibit protein oxidation may play a role in improving the sensory properties of meat.

Most of the unsaturated fatty acids in ruminants are saturated after the hydrolysis of lipids, and this process varies significantly depending on the effectiveness of biohydrogenation (activity of rumen microorganisms) (Wood et al. 2008). Durmic et al. (2008) reported that some EO can inhibit the growth activity of bacteria, such as *B. fibrisolvens* and *B. proteoclasticus*, which play an important role in biohydrogenation. However, the results obtained in our study showed that the effect of rosemary oil supplementation was limited on the fatty acid composition in different tissues and did not cause a significant change in the general profile. The results obtained in some similar studies also showed that the rosemary additive does not cause a significant difference to the following fatty acids, which make up about 80% of the fatty acid profile; palmitic (C16: 0), stearic (C18: 0) and oleic (C18:1) fatty acids (Morán et al. 2013; Smeti et al. 2018).
However, Nieto (2013) reported that rosemary oil significantly reduced the C16:0 fatty acid level, and it caused an increase with regards to C18:0 content. Since C18:0, which is one of the major fatty acids in ruminant meats, is a product of biohydrogenation of unsaturated C18 fatty acids, it is possible to relate C18:0 with the biohydrogenation activity that varies depending on the level of microbial activity in the rumen.

CLA is very important for human health as a functional food, especially in terms of its carcinogenic effects. For this reason, researchers generally tend to investigate and modify the initiators/substrates of CLA fatty acid synthesis or the factors affecting desaturase enzyme activity (Bauman et al. 2000; Howes et al. 2015). Although the amount of CLA in fat in tissues varies significantly depending on ration, it varies between 0.2 and 2.0% (Howes et al. 2015). Although the results obtained from the study were within this range, it was determined that the amount of CLA in R500 lambs decreased in both tissues. CLA is an intermediate product of the metabolic pathway (C18:2–C18:0) in the biohydrogenation of unsaturated fatty acids by rumen bacteria, and some of it is stored in body fats by being unsaturated with vaccinic acid (C18:1t11). The second source of CLA is adipose tissues and CLA synthesis from C18:1t11 occurs in adipose tissue, especially during growth in ruminants. Although the bacterial (Butyrivibrio fibrisolvens) population in weighted forage feeding and CLA content increased due to the increase in its activity, the amount of CLA produced decreased in lambs fed with intensive feed due to higher C18:1t10 compared to C18:1t11 (Bessa et al. 2015). Thus, it can be said that the increase in the dose of rosemary additives negatively affected the conversion of C18:1t11 to CLA and may have limited the activity of bacteria involved in this process. The results for the main polysaturated fatty acids were also not affected significantly by rosemary, and the content of C18:2-n6 and C18:3-n3 fatty acids was similar. However, in some studies, the content of C18:2-n6 increased significantly with the addition of high-dose rosemary oil. Researchers explained this situation as due to the phenolic compounds in the rosemary extract protecting against saturation of polysaturated fatty acids in the cell membrane by inhibition of rumen bacteria involved in biohydrogenation (Nieto 2013; Smeti et al. 2018). In addition, in this study, C18:2n-6 and C20:4n-6 contents synthesised by metabolism of C18:2n-6 were higher in R500 lambs. This may be related to the fact that the C18:2n-6 content tended to be higher in R500 lambs, with the elongation of the fatty acid in question to longer chain fatty acids and a higher level of desaturation with conversion to CLA. In a similar study, Morán et al. (2013) reported that the content of DHA (C22:6n-3), which is an important omega-3, has an increasing tendency in lambs fed rations including high doses of carnosic acid.

PUFA/SFA should be above 0.45 in foods especially in terms of reducing coronary heart diseases and the n6/n3 ratio is recommended to be less than 4 (British Department of Health 1994). In this study, the ratios were outside of the values recommended for all groups. It was determined that the effect of rosemary oil on total fatty acids, ratios and some important indices was not significant. Contrary to the results obtained in this study, rosemary oil was reported to significantly improve PUFA/SFA and n6:n3 ratio (Smeti et al. 2018).

Conclusions

The addition of rosemary oil to fattening lamb diet improved rumen pH and the molar PA content at a dose of 250 mg/kg DM without suppressing rumen fermentation. Serum IGF-1 levels increased significantly at a dose of 250 mg/kg DM compared to the 500 mg/kg DM rosemary oil dose. The 250 mg/kg dose of rosemary oil can be recommended in fattening lamb diets for 70 d without adversely affecting fattening performance, slaughter and carcass characteristics or meat quality. The results need to be supported with experiments using different levels of REO supplementation.

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Ethical approval

In this study, animal research procedures were conducted with the approval of the Local Animal Ethics Committee of Van Yuzuncu Yildizi University in Turkey (Decision No. 2016/08).

Disclosure statement

The authors disclose that there is no conflict of interest.

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