TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2018) 42: 581-589
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doi:10.3906/vet-1604-58

Effects of the supplementation of lamb rations with oregano essential oil on the antimicrobial and antioxidant metabolism in meat

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Received: 18.04.2016 • **Accepted/Published Online:** 18.09.2018 • **Final Version:** 10.12.2018

Abstract: Although oregano essential oil has common use in the cosmetics, pharmaceutical, and food sectors, information on its effects on meat quality and shelf life, when incorporated into lamb feed rations, is scarce. In this study, the effects of oregano essential oil (OEO) in feed on the water activity, pH, lipid peroxidation, color parameters, and microbial counts of the *Musculus longissimus dorsi* (MLD) were investigated. The control group was fed a basal ration and the OEO1 and OEO2 groups were fed a basal ration supplemented with 200 mg/kg and 400 mg/kg OEO, respectively. Akkaraman lambs were fed experimental rations for a period of 56 days and were slaughtered at the end of the fattening period. Lipid peroxidation of the MLD was significantly affected by storage time (ST), group (G) and ST × G, while pH was affected by ST. Lightness was significantly affected by ST, G, and ST × G; redness by ST and G; and yellowness by ST and ST × G. The *Micrococcus*/*Staphylococcus*, total mesophilic aerobic bacteria, and total psychrophilic aerobic bacteria counts were significantly affected by ST, G, and ST × G; *Pseudomonas* spp. counts by ST; and coliform counts by ST and ST × G. The antioxidant and antimicrobial effects of OEO were confirmed.

Key words: Oregano essential oil, antioxidant, antimicrobial, lamb, meat quality

1. Introduction

For many years, antibiotics have been used as feed additives. However, the use of the majority of these substances as feed additives bears risk for human health due to the development of resistance in microorganisms against antibiotics, which has resulted in the prohibition of their incorporation into animal feed in many countries. Therefore, the practice of using plant extracts as feed additives has started to become commonplace. Plant extracts are already commonly used in the pharmaceutical, cosmetics, perfumery, and food sectors due to their various biological effects. Both oregano itself and its extracts (oregano essential oil - OEO) contain substances that induce more than 60 effects, such as antiseptic, antioxidant, antimicrobial, and aroma-regulating effects (1).

Antioxidant additives are used in the food sector to prolong the shelf life of food by preventing oxidation reactions, which result in rancidity, color change, and microbial deterioration. As indicated in several studies, oregano and thyme leaves are known to be used for their antioxidant and antimicrobial effects in the long-term storage of meat (2–5). The antioxidant effect of essential oil has been attributed to its thymol content reducing the generation of hydrogen peroxide, while its antimicrobial effect is considered to be related to its lipophilic property and chemical structure (6).

It is well known that antioxidant substances supplemented in animal feed, such as vitamin E, vitamin C, and α-lipoic acid, have an influence on the postmortem metabolism and quality of meat (7–9). This study was aimed at demonstrating in detail the effects of OEO supplemented into feed on the postmortem metabolism and thus the quality of meat. For this purpose, the effects of OEO on the water activity, pH value (acidification), lipid peroxidation (thiobarbituric acid-reactive substances - TBARS), color parameters (L*, a*, and b*) and microbial varieties and shelf life of the *Musculus longissimus dorsi* (MLD) were investigated.

2. Materials and methods

2.1. Animals and experimental design

The study was conducted as a 4 × 3 factorial design at the Faculty of Veterinary Medicine of Atatürk University on 24 male Akkaraman lambs, which were weaned when they

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were 3 months old on average. This study was approved by the Ethics Committee of the Experimental Medical Research and Application Center of Atatürk University (Decision No: 2012/4/53). The control group was fed a basal ration, the OEO1 group was fed a basal ration + 200 mg/kg oregano oil, and the OEO2 group was fed a basal ration + 400 mg/kg oregano oil (Table 1). Orego-Stim was added in place of bran (Orego-Stim was obtained from Ecopharm Hellas S.A., Kilkis, Greece, and contains 5% essential oil of *Origanum vulgare* subsp. *hirtum* plants and 95% natural feed grade inert carrier). Concentrate feed was specially produced on a monthly basis in a factory in order to prevent the spoiling of OEO while it was stored.

2.2. Determination of meat quality

The eight animals allocated to each group, with a total of 24 animals, were slaughtered at the end of the fattening period after being fasted for 10 h. After the carcasses were maintained at 4 °C for 24 h, the MLD was removed from the carcasses and divided into 7 equal portions. The muscle portions were placed onto polyethylene plates, covered with stretch film, and stored at 4 ± 1 °C during the storage period. On the 1st, 3rd, 5th, 7th, 9th, 11th, and 13th days the meat samples were analyzed for water activity, pH, TBARS, and color [L* (relative lightness), a* (relative redness), b* (relative yellowness)], and microbial counts (TMAB, TPAB, *Micrococcus*/*Staphylococcus*, *Pseudomonas* spp.,

Table 1. Composition of lamb diets used in the study (%).

Ingredient	Groups		
	Control	OEO1	OEO2
Barley	30	30	30
Corn	20	20	20
Sunflower seed meal	13.33	13.33	13.33
Cotton seed meal	13.2	13.2	13.2
Bran	9.70	9.68	9.66
Corn gluten	5	5	5
DDGS[1]	5	5	5
Marble powder	2.05	2.05	2.05
Molasses	1.12	1.12	1.12
Salt	0.5	0.5	0.5
Vitamin-mineral premix[2]	0.1	0.1	0.1
Oregano essential oil[3]	-	0.02	0.04
Rates of nutrient			
Crude protein	18.53	18.52	18.51
Crude fiber	12.74	12.74	12.73
Crude ash	7.2	7.2	7.2
Calcium	1	1	1
Phosphorus	0.68	0.68	0.68
Acid detergent fiber	13.67	13.67	13.67
Neutral detergent fiber	27.3	27.3	27.3

[1]DDGS: Dried distillers grains with solubles.

[2]The vitamin and mineral premix provided the following (per kg): 4,000,000 IU vitamin A, 800,000 IU vitamin D_3, 5000 IU vitamin E, 400 mg vitamin B_2, 2 mg vitamin B_{12}, 5000 mg vitamin PP, 1000 mg D-pantothenic acid, 20,000 mg choline, 50 mg Co, 5400 mg Fe, 185 mg I, 6900 mg Mn, 800 mg Cu, 6400 mg Zn, 14 mg Se.

[3] Oregano essential oil was added in place of marble powder (oregano essential oil was obtained from Ecopharm Hellas S.A., Kilkis, Greece). Oregano oil was in the form of a powder called Orego-Stim that contains 5% essential oil of *Origanum vulgare* subsp. *hirtum* plants and 95% natural feed grade inert carrier.

and coliform bacteria) were determined. Microbiological analyses were performed first.

Water activity was measured with a water activity meter of the Aqualab 4TE series (USA). For this purpose, the samples were placed in the container of the device to measure the a_w value.

The pH values of the samples were determined as described by Gökalp et al. (10). Accordingly, 10-g portions of the homogenized samples were weighed and each portion was added to 100 mL of distilled water. Following homogenization with an Ultra-Turrax blender (IKA Werk T 25, Germany) for 1 min, the pH values were measured using a pH-meter (WTW Inolab, Germany).

For the determination of TBARS values, a 2-g portion of the homogenized samples was added to 12 mL of TCA solution [7.5% TCA, 0.1% EDTA, 0.1% propyl gallate (dissolved in 3 mL of ethanol)], homogenized for 15–20 s with an Ultra-Turrax blender, and filtered through Whatman #1 filter paper. Three milliliters of the filtrate was transferred to a test tube, 3 mL of TBA solution (0.02 M) was added, and these were mixed thoroughly. The test tubes were first maintained in a water bath at 100 °C for 40 min and then cooled in cold water for 5 min. Following centrifugation (2000 × *g*, 5 min), the absorbance was measured at 530 nm with a spectrophotometer (AQUAMATE, Thermo-Electron Corporation, UK). A standard was prepared using TEP (1,1,3,3-tetraethoxypropane) and the k value was calculated as 0.06. The results are given in μmol malondialdehyde/kg (11).

TBARS = [(absorbance/ k (0.06) × 2/1000) × 6.8] × 1000/sample weight

The color concentration of the cross-sections (L*, a*, b*) was determined using a Minolta colorimeter (CR-200, Minolta Co., Japan).

The microbiological analyses of the samples were performed as described by Baumgart et al. (12). Accordingly, a 25-g portion of each meat sample was homogenized in 225 mL of sterile Ringer's solution. Subsequently, the other solutions intended for use were prepared. Inoculations were performed using the spread plate technique. The TMAB count was determined using plate count agar (PCA, Merck) as a growth medium. The petri dishes were incubated under aerobic conditions at 30 ± 1 °C for 72 ± 1 h. The TPAB count was also determined using PCA (Merck) as a growth medium. The petri dishes were incubated under aerobic conditions at 7 ± 1 °C for 10 days. Coliform counts were determined by inoculating 0.1 mL of the appropriate sample dilutions onto violet red bile agar (VRBA, Merck) plates. The petri dishes were incubated under anaerobic conditions at 30 °C for 2 days. *Micrococcus/ Staphylococcus* counts were determined by inoculations made onto mannitol-salt agar (MSA). For this purpose, the petri dishes were incubated under aerobic conditions at 30 ± 1 °C for 48 ± 1 h. On the other hand, *Pseudomonas* spp. counts were performed by inoculation onto *Pseudomonas* agar base medium (Oxoid) supplemented with cetrimide-fucidin-cephaloridine (CFC) selective supplement. The petri dishes were incubated aerobically at 30 ± 1 °C for 48 ± 1 h. The bacterial counts were given in log cfu g^{-1}.

2.3. Statistical analysis

The statistical analyses of the water activity, pH, TBARS, color parameters (L*, a*, and b*) and microbial counts (log cfu g^{-1}) were performed using the general linear model procedure with the model given below. Differences between the groups were determined by Duncan's test ($P < 0.05$). All statistical analyses were performed using SPSS 10.00 software (13).

$$Y_{ijk} = \mu + d_i + g_j + dg_{ij} + e_{ijk}$$

Here, Y_{ijk} = response variable, μ = population mean, d_i = storage time (1, 3, 5, 7, 9, 11, and 13 days) (water activity, pH, TBARS, L*, a*, b*, microorganisms), g_j = group (control, OEO1, OEO2), dg_{ij} = storage time × group interaction, and e_{ijk} = experimental error.

3. Results and discussion

Since it is known that synthetic additives are involved in the development of several diseases, today the use of natural additives has emerged as an essential principle of healthy nutrition. For this reason, consumers tend to prefer healthy and wholesome natural foods as well as food products that contain natural additives. Previously conducted studies have pointed out the antioxidant effect of OEO (3–5,14), but studies on the effects of the supplementation of ruminant feed rations with OEO on meat quality are very limited in number and do not provide much detail. In this study, the effects of oregano essential oil on the water activity, pH value, TBARS, color parameters, and microbial counts of the MLD and shelf life were investigated. Postmortem metabolism, which defines meat quality, is affected by both internal factors (i.e. water activity, natural antioxidants, antimicrobial substances, and free radicals) and external factors (i.e. storage period of meat, storage temperature and atmospheric pressure, and type of storage).

The water contained in food, depending on the structure of the particular foodstuff, is involved in several biochemical and microbiological reactions. Based on the findings obtained in the present study, it was observed that neither OEO supplementation nor storage period had an effect on water activity (Table 2). The water activities of the MLD in the different groups being similar to each other bears significance for the assessment of the alterations observed in postmortem metabolism, as this would imply that any differences between the groups for postmortem metabolic changes do not arise from water activity.

Table 2. The effect of group and storage period on water activity, pH, and TBARS in lamb *musculus longissimus dorsi*.

Storage time (h)	Treatment groups	Parameters		
		Water activity	pH	TBARS
1	Control	0.99 ± 0.00	5.61 ± 0,09	1.72 ± 0.94
	OEO1	0.99 ± 0.00	5.69 ± 0.05	1.13 ± 0.56
	OEO2	0.99 ± 0.00	5.52 ± 0.02	1.17 ± 0.46
3	Control	0.98 ± 0.02	5.54 ± 0.06	3.01 ± 1.74
	OEO1	0.99 ± 0.00	5.62 ± 0.07	1.79 ± 0.67
	OEO2	0.99 ± 0.00	5.66 ± 0.02	2.01 ± 0.80
5	Control	0.99 ± 0.00	5.69 ± 0.12	4.30 ± 1.85
	OEO1	0.99 ± 0.00	5.74 ± 0.09	3.40 ± 1.91
	OEO2	0.99 ± 0.00	5.74 ± 0.03	3.46 ± 1.15
7	Control	0.99 ± 0.00	5.58 ± 0.10	5.47 ± 2.39
	OEO1	0.99 ± 0.00	5.58 ± 0.06	5.44 ± 2.03
	OEO2	0.99 ± 0.00	5.61 ± 0.05	8.08 ± 2.44
9	Control	0.99 ± 0.00	5.51 ± 0.07	10.72 ± 2.52
	OEO1	1.00 ± 0.00	5.51 ± 0.08	10.06 ± 1.57
	OEO2	0.99 ± 0.00	5.55 ± 0.03	10.04 ± 2.08
11	Control	0.99 ± 0.00	5.74 ± 0.09	14.19 ± 2.20
	OEO1	0.99 ± 0.00	5.64 ± 0.13	12.47 ± 5.35
	OEO2	0.99 ± 0.00	5.72 ± 0.09	13.07 ± 2.36
13	Control	0.99 ± 0.00	5.67 ± 0.07	17.75 ± 3.00
	OEO1	0.99 ± 0.00	5.71 ± 0.12	8.17 ± 3.86
	OEO2	0.99 ± 0.00	5.79 ± 0.12	15.52 ± 2.95
P-values	ST	0.310	0.000	0.000
	G	0.200	0.311	0.000
	ST × G	0.394	0.084	0.000

TBARS: thiobarbituric acid-reactive substances.
Control: Basal ration alone, OEO1: basal ration + 200 mg/kg of oregano essential oil, OEO2: basal ration + 400 mg/kg of oregano essential oil.
ST: Storage time, G: group.

The postmortem metabolism of meat depends mainly on the conversion of the glycogen content of muscles into lactic acid and the reduction of the pH value. While Vergara and Gallego (15) reported the pH value of lamb meat to range between 5.54 and 5.81, Hopkins and Fogarty (16) indicated that the pH value of lamb meat varied with breed and type of muscle (5.0–5.96). Teixeria et al. (17) determined that the pH value of meat measured 24 h after the slaughter of lambs varied with breed and body weight at slaughter (5.5–5.9). The pH values detected in the this study (5.52–5.79) were found to be within the reference ranges reported in literature (15,17). Feeding is one of the primary factors that affect the pH value of meat. It is known that the effect of feeding on meat pH value is related to the muscle glycogen level (18,19) and the conversion mechanism of glycogen into lactic acid. The increase of metabolic activity in the organism brings about an increase in the generation of free radicals. This, in return, disrupts the balance between antioxidants and free radicals in favor of the latter (20). Free radicals increase the activity of the adenosine monophosphate-activated protein kinase (AMPK) enzyme in tissues and thus exhibit a negative effect on glycogen synthesis and the conversion of glycogen into lactic acid in muscle tissue (21,22).

Antioxidants incorporated into feed rations show an effect on meat pH value by scavenging free radicals and reducing the activity of the AMPK enzyme.

In the present study, no statistically significant difference was detected between the different groups for the pH value of the MLD (Table 2), demonstrating that the glycogen content and lactic acid formation of the MLD were similar in the different groups. It was thus ascertained that the incorporation of oregano into feed rations did not have any effect on the pH value (and thus on glycogen synthesis and AMPK activity) of the longissimus dorsi muscle, and that the pH values remained within the reference range indicated in literature. The effect of storage period on meat pH value having been determined to be significant demonstrated the occurrence of postmortem metabolic changes in the longissimus dorsi muscle.

In a healthy organism, there is an approximate balance between the metabolic generation of free radicals and the free radical scavenging activity of antioxidants. Oxidative stress occurs when the balance between oxidation and antioxidation changes in favor of the former (20). The first products of oxidative reactions are peroxides, followed by the formation of hydrocarbons, aldehydes, ketones, alcohol, and organic acids. These products negatively affect the nutritional value, sensorial properties, and shelf life of food of animal origin (23). Essential oils, including OEO, contain substances with antioxidant properties (1,24). It is known that essential oil induces an antioxidant effect owing to its thymol content, which reduces the formation of hydrogen peroxide (25) and increases the level of polyunsaturated fatty acids in the phospholipid fraction of various tissues (14). Several studies point to the prevention of lipid peroxidation in meat by means of the use of oregano leaves in the packaging of meat (2,26,27). Nieto et al. (4,5) reported that lipid peroxidation in lamb meat was significantly decreased by supplementing the feed ration of lambs with oregano leaves. In the present study, it was ascertained that OEO incorporated into the feed ration exhibited free radical scavenging activity by means of the antioxidant substances found in its structure and thus significantly reduced the level of lipid peroxidation in the MLD (Table 2). However, the level of lipid peroxidation in Group OEO2 having been determined to be higher than that of Group OEO1 revealed that the supplementation of the feed ration with high levels of OEO triggered oxidation in the MLD. The development of lipid peroxidation in tissues is attributed to the disruption of the balance between free radicals and antioxidants. The level of oxidation in the OEO2 group having been determined to be higher than that of the OEO1 group demonstrated this balance to have been disrupted in favor of antioxidants. It was determined that the inhibitory effect of OEO on lipid peroxidation in the MLD continued throughout the storage period. In the OEO1 group, the TBARS levels of the MLD measured on day 13 of the storage period being lower than the levels measured on days 9 and 11 of the storage period is attributable to the variations observed in the animals from the antioxidant properties of oregano incorporated into the feed ration. Furthermore, the significant effect of OEO on group × storage period was attributed to its strong antioxidant effect. These findings are in agreement with previously conducted studies that also reported the antioxidant effect of oregano (2,4,5,26,27).

The natural color of meat is produced by the myoglobin and hemoglobin pigments. These pigments are composed of globin, a porphyrin ring, and a ferrous ion. These three components that define the color of meat are all highly susceptible to oxidation (28,29). Nieto et al. (5) reported that the supplementation of the ration fed to lambs with 3.7% and 7.5% oregano leaves produced statistically significant effects on the L*, a*, and b* values among meat color parameters. These researchers indicated that as the storage period was prolonged, the L* and b* values increased and the a* value decreased. Simitzis et al. (30) suggested that the supplementation of rations provided to lambs with oregano oil resulted in significant effects on meat color parameters L*, a*, and b*. In lamb meat, Teixeira et al. (17) measured the L* value as 34.1–53.2, the a* value as 11.5–21.5, and the b* value as 6.5–12.5 and indicated that these values varied with body weight, sex, and breed. Sante-Lhouteiller et al. (31) suggested that, of the lamb color parameters, the L*, a*, and b* values were not affected by the composition of roughage and concentrated feed provided to the animals, but they were influenced by storage period. Hopkins and Fogatry (16) indicated that the color parameters of the *M. longissimus thoracis* varied with breed, while the color parameters of the *M. semimembranosus* did not display any differences between animal breeds. Hernández-Hernández et al. (32) ascertained that incorporation of rosemary and oregano extracts into pig rations resulted in the difference of the L* values of the meat of the treatment groups compared to the control group. Camo et al. (27) reported that the packaging of lamb meat using rosemary and oregano extracts resulted in the difference of the a* values of the treated animals compared to the controls. In this study, it was determined that storage time had a significant effect on the L*, a*, and b* values of the MLD (Table 3). The incorporation of OEO at a level of 200 mg/kg into feed increased the L* value of the treated animals, excluding day 13 of storage, to a level higher than that of the control group, while supplementation with 400 mg/kg decreased the same value, excluding the 5th and 11th days of storage. It was ascertained that 200 mg/kg OEO significantly decreased the a* value on days 1, 5, 9, 11, and 13 of storage, while the effects of 400 mg/kg OEO

on the same value showed variability. Furthermore, it was observed that the effects of group, storage time, and storage time × group on b* also displayed variability. Thus, the effects of the supplementation of the ration with OEO doses of 200 and 400 mg/kg induced different effects on the color parameters of lamb meat. Based on the findings obtained in this study, the effect of storage period on meat color parameters was found to be in agreement with the literature (5,31) and within the reference ranges (17).

In foodstuffs, resistance against microbial growth is maintained by means of certain substances naturally found in food or certain compounds that exhibit antimicrobial activity in the food during storage. As synthetic antioxidants used for food storage pose carcinogenic health risks for humans, consumers tend to prefer food containing natural additives. Several studies are available that suggest that plant leaves and extracts could be used to prolong the shelf life of meat, owing to their bactericidal and bacteriostatic effects (2,3,26,27). The mode of action of carvacrol and thymol involves the breakdown of the bacterial membrane, while terpenoids and phenyl propanoids penetrate the bacterial wall given their lipophilic effects. Phenolic compounds lead to the degradation of the proteins of the bacterial cell membrane and alter the permeability of cations like H+ and K+, resulting in the failure of the main functions of the cell (6). It is known that, when incorporated into feed rations, plants and their extracts either limit or inhibit the growth and development of

Table 3. The effect of group and storage period on color parameter in lamb *musculus longissimus dorsi*.

Storage time (h)	Treatment groups	Parameters		
		L*	a*	b*
1	Control	43.18 ± 2.04	17.63 ± 1.55	4.10 ± 0.80
	OEO1	44.36 ± 3.12	16.27 ± 1.34	4.23 ± 1.73
	OEO2	42.57 ± 3.32	16.32 ± 0.88	3.44 ± 0.77
3	Control	44.81 ± 2.26	17.71 ± 1.72	4.63 ± 1.34
	OEO1	47.27 ± 2.35	17.84 ± 1.81	4.85 ± 1.21
	OEO2	43.68 ± 1.60	18.01 ± 1.11	4.19 ± 0.86
5	Control	43.88 ± 5.22	18.67 ± 1.65	4.11 ± 2.19
	OEO1	48.15 ± 3.53	18.08 ± 1.68	5.79 ± 1.64
	OEO2	44.73 ± 2.40	19.34 ± 1.48	4.93 ± 1.05
7	Control	46.95 ± 6.12	18.12 ± 2.94	7.04 ± 3.38
	OEO1	48.51 ± 4.00	18.11 ± 2.64	5.85 ± 1.14
	OEO2	44.46 ± 3.18	18.83 ± 1.65	4.53 ± 1.10
9	Control	44.47 ± 2.69	17.40 ± 1.25	4.60 ± 1.31
	OEO1	44.57 ± 3.41	16.58 ± 1.47	3.85 ± 0.84
	OEO2	44.25 ± 2.74	17.30 ± 1.62	4.62 ± 1.38
11	Control	42.15 ± 2.76	16.48 ± 1.09	3.56 ± 1.24
	OEO1	45.31 ± 2.25	16.40 ± 1.57	4.02 ± 0.92
	OEO2	44.12 ± 3.87	18.01 ± 1.94	4.96 ± 1.56
13	Control	45.03 ± 3.13	18.17 ± 1.13	4.81 ± 1.19
	OEO1	44.27 ± 2.33	16.90 ± 1.51	4.33 ± 0.99
	OEO2	41.85 ± 2.68	17.20 ± 1.94	3.46 ± 1.32
P-values	ST	0.000	0.000	0.000
	G	0.000	0.005	0.066
	ST × G	0.021	0.091	0.000

Control: basal ration alone, OEO1: basal ration + 200 mg/kg of oregano essential oil, OEO2: basal ration + 400 mg/kg of oregano essential oil.
ST: Storage time, G: group.

microorganisms in the digestive tract of animals (33). On the other hand, the effect of feed supplementation with plants and plant extracts on the microbial quality of meat has been investigated only to a very limited level. Nieto et al. (5) reported that feeding lambs on 3.5% and 7.5% oregano leaves resulted in an antibacterial effect against psychrotrophic and lactic acid bacteria, while an effect against the growth of mold and yeasts was observed in the event of the incorporation of oregano levels at a level of 7.5% into the feed ration. In this study, it was observed that OEO prevented the growth of bacteria belonging to the genera *Micrococcus* and *Staphylococcus* in meat, and that this antibacterial effect was maintained throughout the storage period (Table 4). The antibacterial effect of 200 mg/kg OEO against *Pseudomonas* spp. was confirmed on days 1, 5, 7, 11, and 13 of the storage period, while the effect of 400 mg/kg OEO was detected on only day 11 of the storage period. In previously conducted researches, it was determined that the antimicrobial effect of OEO varied with its active substance content (type and amount) (2,3,5,27), the packaging of meat (2,26), and the storage temperature and period (2,5,26,27). The antimicrobial effect of OEO observed in this study was in agreement with the literature.

In conclusion, OEO was observed to significantly reduce lipid peroxidation in the MLD, while it did not have

Table 4. The effects of group and storage time on *Micrococcus/Staphylococcus*, *Pseudomonas* spp., coliform, total mesophilic aerobic bacteria (TMAB), and total psychrophilic aerobic bacteria (TPAB) counts in lamb *musculus longissimus dorsi*.

Storage time	Treatment groups	Parameters				
		Micrococcus/Staphylococcus	*Pseudomonas* spp.	Coliform	TMAB	TPAB
1	Control	2.30 ± 0.26	2.54 ± 0.19	1.70 ± 0.00	3.41 ± 0.08	0.70 ± 0.00
	OEO1	1.70 ± 0.00	2.42 ± 0.22	1.70 ± 0.00	3.39 ± 0.11	0.70 ± 0.00
	OEO2	2.34 ± 0.13	2.61 ± 0.26	1.70 ± 0.00	3.25 ± 0.17	0.70 ± 0.00
3	Control	2.98 ± 0.09	3.36 ± 0.26	2.32 ± 0.06	5.15 ± 0.08	2.07 ± 0.14
	OEO1	2.18 ± 0.07	3.47 ± 0.18	2.44 ± 0.08	5.25 ± 0.16	3.30 ± 0.29
	OEO2	2.89 ± 0.08	3.43 ± 0.10	2.39 ± 0.07	5.26 ± 0.10	3.37 ± 0.18
5	Control	3.39 ± 0.24	4.53 ± 0.21	3.31 ± 0.16	5.23 ± 0.09	4.63 ± 0.05
	OEO1	2.55 ± 0.06	4.15 ± 0.14	3.09 ± 0.17	5.43 ± 0.24	4.33 ± 0.23
	OEO2	3.46 ± 0.14	4.45 ± 0.26	3.29 ± 0.04	5.45 ± 0.23	4.22 ± 0.11
7	Control	3.84 ± 0.07	5.34 ± 0.32	3.57 ± 0.20	6.24 ± 0.14	5.23 ± 0.08
	OEO1	2.88 ± 0.09	4.15 ± 0.14	3.29 ± 0.18	6.57 ± 0.29	5.37 ± 0.35
	OEO2	3.92 ± 0.06	5.62 ± 0.25	3.56 ± 0.15	6.65 ± 0.05	5.58 ± 0.21
9	Control	4.29 ± 0.18	6.68 ± 0.37	3.76 ± 0.23	6.66 ± 0.07	6.32 ± 0.33
	OEO1	3.16 ± 0.10	6.82 ± 0.03	3.71 ± 0.20	7.51 ± 0.32	6.67 ± 0.35
	OEO2	4.22 ± 0.13	6.74 ± 0.09	3.68 ± 0.12	6.91 ± 0.05	6.68 ± 0.08
11	Control	4.82 ± 0.09	8.23 ± 0.29	4.64 ± 0.25	7.66 ± 0.12	7.74 ± 0.23
	OEO1	3.40 ± 0.07	7.93 ± 0.11	5.34 ± 0.11	8.32 ± 0.17	7.45 ± 0.36
	OEO2	4.46 ± 0.12	7.84 ± 0.14	4.37 ± 0.08	8.27 ± 0.17	7.32 ± 0.20
13	Control	5.16 ± 0.18	8.67 ± 0.13	5.24 ± 0.18	8.48 ± 0.06	8.49 ± 0.21
	OEO1	4.45 ± 0.12	8.47 ± 0.26	5.53 ± 0.18	8.45 ± 0.16	8.32 ± 0.32
	OEO2	4.81 ± 0.16	8.78 ± 0.10	5.48 ± 0.26	9.56 ± 0.28	9.26 ± 0.36
P-values	ST	0.000	0.000	0.000	0.000	0.000
	G	0.000	0.075	0.060	0.000	0.000
	ST × G	0.000	0.078	0.000	0.000	0.000

Control: Basal ration alone, OEO1: basal ration + 200 mg/kg of oregano essential oil, OEO2: basal ration + 400 mg/kg of oregano essential oil.
ST: Storage time, G: group.

any effect on the pH value. Feed supplementation with 200 mg/kg OEO increased the L* value and decreased the a* value of the MLD, while feed supplementation with 400 mg/kg OEO decreased the L* value and increased the a* value of the MLD. The effect of both doses of OEO on the b* value was observed to vary with the length of storage time. OEO induced an antimicrobial effect against bacteria belonging to the genera *Micrococcus* and *Staphylococcus* in the MLD, and the most effective dose was found to be 200 mg/kg. An antimicrobial effect against *Pseudomonas* spp. was observed on certain days of the storage time. The present study demonstrated that the supplementation of lamb feed rations with OEO induced improvement in lamb meat quality most effectively at the dose of 200 mg/kg. In order to elucidate the effects of the supplementation of ruminant feed rations with oregano and its derivatives on meat quality, further studies are required to be carried out.

References

1. Grosso C, Figueiredo AC, Burillo J, Mainar AM, Urieta JS, Barroso JG, José A, Coelho JA, António MFP. Composition and antioxidant activity of *Thymus vulgaris* volatiles: comparison between supercritical fluid extraction and hydrodistillation. J Sep Sci 2010; 33: 2211-2218.
2. Karabagias I, Badeka A, Kontominas MG. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat Sci 2011; 88: 109-115.
3. Mastromatteo M, Danza A, Conte A, Muratore G, Del Nobile MA. Shelf life of ready to use peeled shrimps as affected by thymol essential oil and modified atmosphere packaging. Int J Food Microbiol 2010; 144: 250-256.
4. Nieto G, Bañón S, Garrido MD. Effect of supplementing ewes' diet with thyme (*Thymus zygis* ssp. *gracilis*) leaves on the lipid oxidation of cooked lamb meat. Food Chem 2011; 125: 1147-1152.
5. Nieto G, Díaz P, Bañón S, Garrido MD. Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. *gracilis*) leaves in ewes' diet. Meat Sci 2010; 85: 82-90.
6. Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Leon GM, Wright A. Characterization of the action of selected essential oil components on gram-negative bacteria. J Agr Food Chem 1998; 46: 3590-3595.
7. Imik H, Atasever MA, Koc M, Atasever M, Ozturan K. Effect of dietary supplementation of some antioxidants on growth performance, carcass composition and breast meat characteristics in quails reared under heat stress. Czech J Anim Sci 2010; 55: 209-220.
8. Imik H, Atasever MA, Urcar S, Ozlu H, Gumus R, Atasever M. Meat quality of heat stress exposed broilers and effect of protein and vitamin E. Brit Poultry Sci 2012; 53: 689-698.
9. Imik H, Ozlu H, Gumus R, Atasever MA, Urcar S, Atasever M. Effects of ascorbic acid and alpha-lipoic acid on performance and meat quality of broilers subjected to heat stress. Brit Poultry Sci 2012; 53: 800-808.
10. Gökalp H, Kaya M, Tülek Y, Zorba Ö. Et ve Ürünlerinde Kalite Kontrolü ve Laboratuvar Uygulama Kılavuzu. Erzurum, Turkey: Atatürk Üniversitesi Ziraat Ofset; 2001. pp. 65-72 (in Turkish).
11. Lemon DW. An Improved TBA Test for Rancidity. New Series Circular, No: 51. Halifax, Canada: Fisheries and Marine Service; 1975.
12. Baumgart J, Firnhaber J, Spicher G. Mikrobiologische Untersuchung von Lebensmitteln. Hamburg, Germany: Behr's Verlag; 1986.
13. SPSS Inc. SPSS for Windows Release 10.01. Chicago, IL, USA: SPSS; 1996.
14. Youdim KA, Deans SG. Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. Brit J Nutr 2000; 83: 87-93.
15. Vergara H, Gallego L. Effects of gas composition in modified atmosphere packaging on the meat quality of Spanish Manchega lamb. J Sci Food Agr 2001; 81: 1353-1360.
16. Hopkins DL, Fogarty NM. Diverse lamb genotypes-2. Meat pH, colour and tenderness. Meat Sci 1998; 49: 477-488.
17. Teixeira A, Batista S, Delfa R, Cadavez V. Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight. Meat Sci 2005; 71: 530-536.
18. Immonen K, Ruusunen M, Hissa K, Puolanne E. Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. Meat Sci 2000; 55: 25-31.
19. Tikk K, Tikk M, Karlsson AH, Andersen HJ. The effect of a muscle-glycogen-reducing finishing diet on porcine meat and fat colour. Meat Sci 2006; 73: 378-385.
20. Halıcı M, Imik H, Koç M, Gümüş R. Effects of α-lipoic acid, vitamins E and C upon the heat stress in Japanese quails. J Anim Physiol An N 2012; 96: 408-415.
21. Ciobanu D, Bastiaansen J, Malek M, Helm J, Woollard J, Plastow G, Rothschild M. Evidence for new alleles in the protein kinase adenosine monophosphate-activated γ_3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics 2001; 159: 1151-1162.
22. Scheffler TL, Park S, Gerrard DE. Lessons to learn about postmortem metabolism using the AMPK gamma 3(R200Q) mutation in the pig. Meat Sci 2011; 89: 244-250.
23. El-Massry KF, El-Ghorab AH, Farouk A. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chem 2002; 79: 331-336.

24. Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem 2004; 85: 633-640.

25. Farag RS, Daw ZY, Aboraya SH. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J Food Sci 1989; 54: 74-80.

26. Bornez R, Linares MB, Vergara H. Microbial quality and lipid oxidation of Manchega breed suckling lamb meat: effect of stunning method and modified atmosphere packaging. Meat Sci 2009; 83: 383-392.

27. Camo J, Beltran JA, Roncales P. Extension of the display life of lamb with an antioxidant active packaging. Meat Sci 2008; 80: 1086-1091.

28. Barbut S. Primary processing of poultry. In: Barbut S, editor. Poultry Products Processing: An Industry Guide. Boca Raton, FL, USA: CRC Press; 2002. pp. 81-107.

29. Mancini RA, Hunt MC. Current research in meat color. Meat Sci 2005; 71: 100-121.

30. Simitzis PE, Deligeorgis SG, Bizelis JA, Dardamani A, Theodosiou I, Fegeros K. Effect of dietary oregano oil supplementation on lamb meat characteristics. Meat Sci 2008; 79: 217-223.

31. Sante-Lhoutellier V, Engel E, Gatellier P. Assessment of the influence of diet on lamb meat oxidation. Food Chem 2008; 109: 573-582.

32. Hernandez-Hernandez E, Ponce-Alquicira E, Jaramillo-Flores ME, Legarreta IG. Antioxidant effect rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters. Meat Sci 2009; 81: 410-417.

33. Garcia V, Catala-Gregori R, Madrid J, Hernandez F, Megias MD, Andrade-Montemayor HM. Potential of carvacrol to modify in vitro rumen fermentation as compared with monensin. Animal 2007; 1: 675-680.