

Effects of oregano essential oil and capsicum extract supplementation on slaughter characteristics, meat quality, and fatty acid composition of lambs

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(Submitted 18 July 2022; Accepted 5 September 2022; Published 6 February 2023)

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Abstract

The aim of this study was to evaluate the effect of oregano essential oil (OEO) and capsicum oleoresin extract (CAO) supplementation on lamb slaughter characteristics, meat quality, and fatty acid composition. In the study, 18 male and 18 female lambs were divided into three equal groups for a 56-day feeding period. The first group was fed the control diet, while the other groups were fed a control diet containing either 300 mg OEO/kg or 300 mg CAO/kg of diet. Feeding OEO or CAO had no effect on the slaughter and carcass quality of the fattening lambs. However, when compared to the female lambs, the male lambs were found to have higher slaughter weights, hot and cold carcass weights, and dressing percentage, while having lower back fat thickness. In addition, OEO substantially increased the intramuscular fat. Dry matter, protein, pH24, meat colour (L^* , a^* , and b^*), drip loss, and cooking loss were not affected by dietary treatment or sex. The addition of OEO or CAO to the diets did not change the meat lipid oxidation or sensory quality characteristics at different storage times. However, meat from the female lambs was found to have thiobarbituric acid reactive substances (TBARS) values lower than that of the meat from the male lambs after 2 d and 4 d of storage. However, the female lamb meat was more preferred in terms of flavour and general acceptance. The Σ MUFA, Σ PUFA, Σ UFA, Σ n-6, Σ n-3, and Σ n-6/ Σ n-3 contents of the lamb meat were not affected by the dietary treatment. As a result, the inclusion of 300 mg of oregano essential oil/kg of DM in lamb diets is considered appropriate because of its significant contributions to the fat composition of lamb meat.

Keywords: oregano oil, capsicum oleoresin, lamb, meat quality

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Introduction

Feed supplements have been widely used for years to enhance the development of animal production or to improve product quality. These supplements have not only improved performance criteria, such as body weight gain and the utilization of feed but have also substantially improved the carcass weight, content, and composition, as well as the shelf life and stability of meat (Dikeman, 2007). These strategically functional products primarily meet the needs of the animal production industry, while also affecting end users of meat production, such as the industrial sector and consumers. In this context, these supplements provide a variety of benefits, such as increased carcass yield and less intramuscular fat accumulation, resulting in lean slaughter that meets the demands of modern consumers (Hao *et al.*, 2014). However, the increased demand for animal protein worldwide has led to more widespread concerns about human health, animal welfare, and environmental protection; stricter legal regulations in the production chain; and prohibition and limitation of their use in many countries (EC, 2003). In addition, today's increasing interest in healthy animal foods has increased the demand for reliable meat and meat products, grown naturally or organically, while also leading the animal feeding industry and researchers to search for alternative natural (non-synthetic) feed supplements. In this context, the bioactive compounds of plant origin (phytochemicals) have attracted great attention as an alternative to traditional growing supplements, due to their antimicrobial properties as well as their great potential in changing the rumen characteristics of ruminant animals (Oh *et al.*, 2017).

Oregano essential oil, one of the best-known and valuable phytochemicals, has antioxidant and antimicrobial properties mainly because of its active phenolic components, such as thymol and carvacrol (Applegate *et al.*, 2010). These components are antimicrobials with a potent effect on a wide range of bacteria, protozoa, and fungi (Walsh *et al.*, 2003). They can also change rumen fermentation in ruminant animals by affecting the microbial population (Calsamiglia *et al.*, 2007). Changes in the population of rumen microorganisms alter rumen fermentation, which is the main factor for the conversion of the nutrients assimilated with feed into muscle tissue (Valenzuela-Grijalva *et al.*, 2017). Likewise, Garcia-Galicia *et al.* (2020) reported that, specifically in sheep, carvacrol reduces the intra-rumen acetate concentration, while increasing the amount of propionate that becomes involved in energy metabolism (gluconeogenesis) and is therefore responsible for live weight gain. Both are among the volatile fatty acids that enable the formation of muscle and fat components in ruminants (Koyuncu and Canbolat, 2010). In addition, phytochemicals reduce the content of saturated fatty acids (SFA) in meat, show beneficial effects on antioxidant enzyme activity, and can affect lipid metabolism in animal tissues by inhibiting the biohydrogenation in the rumen as a result of their high redox and antimicrobial properties (Calsamiglia *et al.*, 2007).

Capsicum extract (derived from the capsicum pepper) is another important phytochemical that draws attention due to its stimulating and heating effect. Although its effect is felt as a burning and warming sensation, it is described as hot. When its effects on animals are evaluated, it can be defined as an herbal extract, which has antimicrobial activity in the rumen and can alter rumen fermentation through its main active ingredient, capsaicin. Capsaicin stimulates the immune and endocrine systems and exhibits antioxidant properties (Calsamiglia *et al.*, 2007). It is also reported to be an important herbal extract that reduces the rumen ammonia N concentration in beef cattle fed an intensive diet, increases the production of the total volatile oil acids to propionate ratio, and reduces the acetate content and acetate–propionate ratio (Cardozo *et al.*, 2006). For these reasons, oregano and capsicum oil seem to have potential properties that may affect not only the animals feeding on them, but also indirectly affect meat consumers.

Aromatic plants or their extracts are used directly in meat processing or added to animal feed to improve or maintain meat quality. Similar strategies can also be intended to increase the low consumption rate in human diets, with the advantage of the aroma of lamb meat and its relatively high saturated fatty acid (SFA) content (McAfee *et al.*, 2010). Lamb meat is known to have a high fat content and low polyunsaturated fatty acid (PUFA)/SFA ratio, despite its favourable content, consisting of protein, iron, zinc, vitamins, micro-elements, a very long-chain of n-3 PUFAs, and its total n-6/n-3 ratio (Sinclair, 2007). In fact, high SFA and a low PUFA/SFA ratio are associated with coronary cardiovascular disease (McAfee *et al.*, 2010). Today, many researchers are carrying out studies with the intent of improving the aroma of lamb meat and extending its shelf life by reducing the SFA content while increasing the PUFA content (Saçlı, 2018). However, the direct affinity of such natural antimicrobial and antioxidant supplements to the meat matrix causes considerable difficulties in the food industry (Valenzuela-Grijalva *et al.*, 2017). Therefore, giving such supplements to animals through dietary manipulation, rather than postmortem exogenous supplementation, is considered the most effective method. It is claimed that this method provides greater benefits by increasing the accumulation of phenolic compounds in meat throughout the life of the animal (Valenzuela-Grijalva *et al.*, 2017).

The objectives of this study were to contribute to the literature by investigating the effects of the dietary inclusion of OEO (carvacrol and thymol) and CAO (hot pepper oil) on the carcass characteristics, physio-chemical properties, oxidative stability, fatty acid profile, and sensorial properties of lamb meat (*M. Longissimus dorsi*).

Materials and Methods

The experiment was conducted at Ege University Experimental Farm (38°27'26" N, 27°13'47" E; İzmir, Turkey). This study protocol for care and use of animals used in the experiment was approved by the Ege University Animal Care and Use Committee (No: 2014-091). All experimental procedures involving animals were conducted in accordance with guidelines for the care and use of animals for research purposes. A total of 36 Menemen (Ille de France x Kivırcık) male and female lambs, aged 8 weeks, were used for the study. Prior to the experiment, the lambs were adapted to the diets for 14 days before the growth performance trial. Initially the lambs were fed a diet composed of 50% alfalfa hay and 50% the lamb grower concentrate feed (based on as-fed weight). Over the 14-day adaption period, the alfalfa hay was replaced by gradually by the concentrate diet. After the adaptation period, the lambs were fasted for 16 h and then weighed. At the beginning of the experiment, the mean initial body weight (IBW) of male and female lambs for the three treatments was 19.15 ± 0.79 and 19.21 ± 0.79 kg,

respectively. After being weighed, the animals were divided according to live weight into three groups of 12 (six male and female) and randomly housed in individual pens (2m²/lamb) for the duration of the experiment. At the beginning and end of the trial the BW of each animal was measured twice. During the experiment, the BW of the lambs were determined after 16 h of fasting at 14-day intervals.

Oregano essential oil obtained by steam distillation from selected *Oregano onites* L. growing wild in Turkey was used in the study. The carvacrol and thymol contents, which are the most active compounds of oregano essential oil, were determined at 85.87% and 7.81%, respectively (total 93.68%). Capsicum extract naturally produced from hot pepper, which contains 99% *Capsicum oleoresin*, was obtained by steam distillation from selected *Capsicum annuum* L. The basal diet was offered to meet the nutrient requirements of animals for both roughage feed and concentrate feed. One group was given a basal diet and served as control. The experimental diets given to the other two groups were based on the basal diet but contained an additional 300 mg oregano oil or capsicum oleoresin extract/kg of total mixed ration. The experimental diets were prepared considering the nutrient requirements for the lambs as referenced by the NRC (2007). The ingredients and nutritional compositions of the diets were shown in Table 1. Feed was provided twice daily, in the morning and evening. The feed and fresh water were available *ad libitum*. The study was conducted over a feeding period of 56 days. All lambs were slaughtered at a commercial slaughterhouse at end of the experiment.

Table 1. Ingredients and chemical composition of the experimental diets (g kg⁻¹, as fed), nutritional substance contents (%), and metabolizable energy (kcal/kg) level (as fed)

Ingredient, g kg ⁻¹	Control	OEO	CAO	Nutrient, %	Method of Analysis	g kg ⁻¹
Alfalfa pellets	103.1	103.1	103.1	Dry matter	AOAC 934.01	896.9
Barley	257.7	257.7	257.7	Crude protein	AOAC 990.03	165.0
Soybean hulls	124.1	124.1	124.1	Ether extract	AOAC 920.39	27.9
Wheat bran	113.5	113.5	113.5	Crude fibre	AOAC 962.09	110.0
Corn grain	103.1	103.1	103.1	NDF	Van Soest <i>et al.</i> (1991)	344.0
Wheat	103.1	103.1	103.1	ADF	Van Soest <i>et al.</i> (1991)	139.0
Soybean meal	102.5	102.5	102.5	Crude ash	942.05	72.7
Cotton meal	51.9	51.9	51.9	Starch	AOAC 14031.32	275.9
Soybean oil	5.2	5.2	5.2	Sugar	EC (2009)	43.1
Limestone	23.6	23.6	23.6	Calcium		12.0
Salt	5.7	5.7	5.7	Phosphorus		4.0
Ammonium chloride	5.2	5.2	5.2	**ME kcal/kg	TSI, 1991	2600
Vit-min premix ^a	1.5	1.5	1.5			
OEO	-	0.3	-			
CAO	-	-	0.3			

^a Each kilogram of the diets contains 11000 I.U. Vitamin A; 3 mg Vitamin B1; 5000 I.U. Vitamin D3; 0.069 mg 25-OH-D3; 8 mg Vitamin B2; 150 mg Vitamin E; 3 mg Vitamin K3; 4 mg Vitamin B6; 0.02 mg Vitamin B12; 60 mg Niacin; 15 mg D-Pantothenic; 2 mg Folic acid; 0.2 mg Biotin; 100 mg Vitamin C; 400 mg Co; 4000 mg Cu; 500 mg I; 5000 mg Fe; 500 mg Mn; 200 mg Se; 5000 mg Zn; OEO: Oregano essential oil, CAO: Capsicum oleoresin oil, **ME (kcal/kg): TSI-9610 (1991)

Nutrient contents of ingredients and concentrate growing lamb feed were analysed according to the methods reported in AOAC, (2005). All samples were analysed for dry matter (DM) (method 934.01), ash (method 942.05), crude protein (CP) (method 990.03), ether extract (EE) (method 920.39), and crude fibre (CF) (method 962.09). The sugar content of the materials was determined using the Luff-Scroll method and the starch determination using the polarimetric method (No: 14031.32) (AOAC, 2005). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined using the methods of Van Soest *et al.* (1991). Phosphorus (P) content of the materials was read by spectrophotometer (model PE General TU-1880 Model Double Beam UV-V15) using calorimetric methods. Atomic absorption spectroscopy (Ultrospec 2100 pro UV/visible106 spectrophotometer, USA) was used for determining calcium (Ca) concentration. Metabolizable energy (ME) (kcal/kg DM) was estimated based on the nutrient content (protein (CP), fibre (CF), and fat (EE) levels) using the prediction equation, TSI-9610, (1991):

$$\text{ME (kcal/kg)} = 3260 + (0.455 \times \text{CP} + 3.517 \times \text{EE} - 4.037 \times \text{CF})$$

where CP, EE, and CF quantities are in DM (g kg⁻¹)

At the end of the experiment, all of the male and female lambs were fasted for 16 h, weighed, and then transported to a commercial abattoir 30 km from the experimental unit where they were

slaughtered the same day. Hot carcass weight was recorded immediately after dressing. Dressing percentage (DP) was calculated as the ratio of HCW to slaughter BW. After slaughter, the carcasses were kept in cold storage at 2 °C for 24 h. Back fat thickness was measured near the longissimus dorsi (LD) on the left half of the carcasses between the 12th and 13th ribs using a BTS 12043 digital calliper. Then, the left and right longissimus dorsi muscle (MLD) of each left carcass from the end of the 12th costa was cut and removed (approximately 2.5-cm thick) and analysed for meat nutrient content and sensory analyses. Whole carcasses were defrosted at a temperature of 2 °C for a period of 24 h for the purpose of deboning and the removal of the heart, spleen, kidneys; omental and mesenteric fat were separated and weighed individually. All traits measured were expressed as a percentage of body weight.

Approximately 2.5-cm thick steaks were cut from the right MLD at the 12th rib for all lamb carcasses. These steaks were kept initially at 4 °C and then frozen and stored at -20 °C until analysis for meat quality. They were then homogenized using a blender and analysed for dry matter, crude protein, and ether extract for intramuscular fat content. Dry matter content of the MLD samples was determined by oven-drying at 105 °C for 18 h. Ether extract content of MLD samples was obtained by the Soxhlet extraction method using anhydrous diethyl ether. The Kjeldahl method was used for the analysis of total nitrogen content of MLD samples, and crude protein was expressed as nitrogen \times 6.25.

The 24-h pH of the MLD muscle from the left half of the carcasses from both groups was measured (Hanna, HI 8314 model). Objective measurement of colour (L^* , a^* , b^*) was performed at the surface of LTL meat using a Colour Flex Hunterlab-USA spectrophotometer to measure CIE Lab values (L^* : relative lightness, 0 = black to 100 = white; a^* : redness, -50 = green to +50 = red; and b^* : yellowness, -50 = blue to +50 = yellow) were measured on the surface of *longissimus thoracis et lumborum*. Before each measurement, the apparatus was standardized against a white plate. Three readings were obtained from each sample.

For determining drip loss and cooking loss of the all left (MLD) samples were defrosted for 24 h at a temperature of +4 °C, wrapped individually in cooking bags, and placed fat-side up on the rack of an open roasting pan. The samples were cooked at 160 °C in one electric oven connected to a computerized electronic temperature control system to an internal temperature of 70 °C (AMSA, 1978). Immediately after cooking, all visible subcutaneous fat was removed from each sample. Three 1.5 \times 1.5-cm cubed samples were taken from the middle of each sample and wrapped immediately in aluminium foil. The samples were placed in preheated glass ramekins in a preheated oven of 100 °C and evaluated within 10 min.

A trained taste panel was organized for sensory analyses and 20 panellists were informed about how the tasting and assessment would be carried out before the tasting panel. After removal from the freezer, the steaks were thawed at 4 °C for 24 h and cooked on a grill for 5 min. Each panellist scored the cooked samples using an 8-point hedonic scale. The panellists assigned a score for appearance, juiciness, flavour, and overall acceptability (Keeton, 1983). For appearance: 8 = extremely good, 7 = very good, 6 = moderately good, 5 = slightly good, 4 = neither good nor bad, 3 = moderately bad, 2 = very bad, and 1 = extremely bad. For juiciness: 8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry, and 1 = extremely dry. For intensity of flavour: 8 = extremely intense, 7 = very intense, 6 = moderately intense, 5 = slightly intense, 4 = slightly bland, 3 = moderately bland, 2 = very bland, and 1 = extremely bland. For overall acceptability: 8 = extremely acceptable; 7 = very acceptable; 6 = moderately acceptable, 5 = acceptable, 4 = slightly unacceptable, 3 = moderately unacceptable, 2 = very unacceptable, and 1 = extremely unacceptable. Oxidized flavour was scored based on the 3-point scale with 1 = least liked and 3 = most liked (Carr *et al.*, 1999).

The thiobarbituric acid (TBA) test for the left MLD muscles of 36 lamb-meat samples (according to the method of Tarladgis *et al.*, 1960) was used to determine the extent of oxidative rancidity on the 0, 2, 4, 6, and 8th day of storage at +4 °C. The results were expressed as mg malonaldehyde/kg meat.

The total fatty acid compositions of MLD were measured using the method of Wachira *et al.* (2002). Values for total fatty acids were obtained from the sum of the fatty acids of the phospholipids and the neutral lipids. These lipids were extracted from the left MLD meat samples (10 g) using chloroform: methanol (2:1). Silicic acid solid phase extraction was used to prepare pure fractions of each type of lipid, which were then hydrolysed and cleaned up. Fatty acid methyl esters were prepared from both types of lipid using diazomethane, and were analysed using gas chromatography (Agilent Technologies 6890N, China). Triplicate analyses were performed on the left MLD samples of all 36 animals.

Data were subjected to univariate analysis of variance (UNIANOVA) using General Linear Models. Differences among treatments were determined using Duncan's multiple range test using

SPSS 25 (SPSS, 2019). The model included essential oil as the main effect. Differences were considered to be significant based on a 0.05 level of probability.

Results

Slaughter and carcass quality characteristics of the fattening lambs: The effects of the inclusion of 300 mg of OEO and CAO/kg DM on the slaughter and carcass characteristics of fattening lambs are shown in Table 2. No differences were observed in the slaughter weight, hot/cold carcass weight, dressing percentage, relative heart, liver, spleen, kidney, omental, and mesenteric fat weight, and back fat thickness after dietary OEO or CAO supplementation in the lambs ($P > 0.05$). However, the slaughter weight, hot carcass weight, cold carcass weight, and relative kidney weight were higher in the male lambs (42.24 kg, 21.63 kg, 21.24 kg, and 0.73%, respectively) than in the female lambs (39.32 kg, 20.58 kg, 20.23 kg, and 0.64%) ($P < 0.05$). Back fat thickness, on the other hand, was higher in the female lambs (3.71 mm) than in the male lambs (2.61 mm) ($P < 0.05$). In addition, it was observed that no slaughter or carcass parameters were affected by the dietary treatment \times sex interaction ($P > 0.05$).

Table 2 Slaughter and carcass quality traits of fattening lambs

	Dietary Treatment ¹ (T)			SEM ²	Sex (S)		SEM ²	P-value		
	Control	OEO	CAO		Male	Female		T	S	T \times S
Slaughter weights, kg	40.46	41.80	40.09	0.95	42.24 ^a	39.32 ^b	0.77	0.378	0.001	0.666
Hot carcass, kg	21.15	21.52	20.64	0.61	21.63 ^a	20.58 ^b	0.50	0.529	0.034	0.252
Cold carcass, kg	20.80	21.15	20.26	0.60	21.24 ^a	20.23 ^b	0.49	0.511	0.039	0.265
Dressing percentage, %	52.27	51.48	51.48	0.65	51.20	52.27	0.53	0.502	0.170	0.285
Heart, %	0.80	0.79	0.81	0.02	0.82	0.78	0.01	0.858	0.154	0.158
Liver, %	4.26	4.09	4.27	0.13	4.37	4.04	0.11	0.600	0.052	0.875
Spleen, %	0.48	0.48	0.47	0.03	0.47	0.47	0.02	0.963	0.939	0.433
Kidney, %	0.69	0.72	0.65	0.02	0.73 ^a	0.64 ^b	0.02	0.149	0.011	0.342
Omental–mesenteric fat, %	1.11	1.25	1.08	0.12	1.07	1.23	0.10	0.591	0.266	0.743
Back fat thickness, mm	3.24	2.99	3.25	0.34	2.61 ^a	3.71 ^b	0.28	0.845	0.010	0.831

¹Oregano essential oil (OEO) and Capsicum oleoresin (CAO) were supplemented at 300 mg/kg of diet DM.

²SEM = standard error of the mean.

^{a,b}Mean values with different superscripts within a row and main factor are significantly different for $P < 0.05$

Meat chemical composition and physical quality characteristics: The nutrient composition and physical characteristics of the lamb meat were mainly unaffected by the dietary treatment and sex (Table 3). However, the intramuscular fat contents of the meat tended to increase in the CAO group (2.63%) when compared to the control group (2.58%), and increased in the OEO group (2.73%) ($P < 0.05$). Other measured chemical and physical properties of meat were not affected by diet, sex, and their interaction (Table 3).

Meat lipid oxidation: The lipid oxidation values of the lamb meat at 4 °C and at different storage times (on days 0, 2, 4, 6, and 8) are shown in Table 4. From day 0 to 8, the thiobarbituric acid reactive substance (TBARS) values in all of the groups numerically increased when compared to fresh meat. However, no differences were observed between the groups at any storage time, in terms of lipid oxidation ($P > 0.05$). After 8 d of storage, the mean TBARS value was 1.96 mg MDA/kg meat in the control and OEO groups, and 2.01 mg MDA/kg meat in the CAO group ($P > 0.05$). After 2 and 4 days of storage, the TBARS values (0.81 and 1.37 mg MDA/kg meat, respectively) in the female lamb groups were lower than in the male lamb groups (1.01 and 1.63 mg MDA/kg meat, respectively) ($P < 0.05$). On days 6 and 8, the TBARS values were not found to be affected by sex. The effect of the dietary treatment \times sex interaction on lipid oxidation of the meats was insignificant as well.

Table 3 Physical–chemical quality traits of *Longissimus dorsi* muscle in fattening lambs fed essential oils

	Dietary Treatment ¹ (T)			SEM ²	Sex (S)		SEM ²	P-value		
	Control	OEO	CAO		Male	Female		T	S	T x S
Dry matter, %	50.84	49.72	49.10	0.84	49.08	50.69	0.68	0.349	0.110	0.474
Protein, %	21.22	21.47	22.12	0.45	21.91	21.28	0.36	0.357	0.235	0.946
Intramuscular fat, %	2.58 ^b	2.73 ^a	2.63 ^{ab}	0.09	2.66	2.69	0.32	0.030	0.077	0.071
pH ₂₄	5.62	5.62	5.57	0.03	5.62	5.59	0.39	0.605	0.525	0.196
L*	42.30	41.25	42.15	0.48	41.85	41.96	0.27	0.271	0.848	0.964
a*	16.01	16.24	16.66	0.33	16.21	16.40	0.32	0.391	0.632	0.789
b*	6.97	7.06	7.13	0.39	6.89	7.21	0.02	0.960	0.489	0.870
Drip loss, %	1.70	1.70	1.81	0.08	1.78	1.69	0.07	0.578	0.368	0.650
Cooking loss, %	36.36	36.77	37.14	0.71	37.00	36.52	0.58	0.745	0.562	0.826

¹Oregano essential oil (OEO) and Capsicum oleoresin (CAO) were supplemented at 300 mg/kg of diet DM

²SEM = standard error of the mean. Abbreviations: L*, Lightness; a*, redness; b*, yellowness

^{a,b}Mean values with different superscripts within a row and main factor are significantly different for $P < 0.05$

Table 4 Lipid oxidation (TBARS, mg MDA/kg meat) of *Longissimus dorsi* muscle in fattening lambs

Storage period (days, at 4 °C)	Dietary Treatment ¹ (T)			SEM ²	Sex (S)		SEM ²	P-value		
	Control	OEO	CAO		Male	Female		T	S	T x S
0	0.51	0.43	0.56	0.05	0.50	0.50	0.04	0.267	0.972	0.582
2	0.87	1.00	0.85	0.07	1.01 ^b	0.81 ^a	0.06	0.308	0.032	0.940
4	1.52	1.50	1.47	0.10	1.63 ^b	1.37 ^a	0.08	0.948	0.048	0.449
6	1.47	1.54	1.44	0.11	1.40	1.57	0.09	0.845	0.212	0.428
8	1.96	1.96	2.01	0.12	1.89	2.07	0.10	0.949	0.250	0.522

¹Oregano essential oil (OEO) and Capsicum oleoresin (CAO) were supplemented at 300 mg/kg of diet DM

²SEM = standard error of the mean; TBARS, Thiobarbituric acid reactive substances; MDA, Malondialdehyde

^{a,b}Mean values with different superscripts within a row and main factor are significantly different for $P < 0.05$

Meat sensory quality characteristics: The effect of the dietary inclusion of OEO or CAO on the sensory properties of the lamb meat is presented in Table 5. Accordingly, no difference was observed between the dietary treatment groups in terms of appearance, juiciness, flavour, oxidized flavour, and general acceptance ($P > 0.05$). On the other hand, the effect of sex on the flavour and general acceptance was significant, and these values were lower in the male lambs (5.30 and 5.69, respectively) than in the female lambs (6.15 and 6.51, respectively) ($P < 0.05$). In addition, it was determined that the appearance and juiciness values were affected by the dietary treatment x sex interaction ($P < 0.05$).

Table 5 Sensory properties of *Longissimus dorsi* muscle in fattening lambs fed essential oils

	Dietary Treatment ¹ (T)			SEM ²	Sex (S)		SEM ²	P-value		
	Control	OEO	CAO		Male	Female		T	S	T x S
Appearance	6.36	6.00	6.40	0.19	6.18	6.33	0.15	0.263	0.496	0.014
Juiciness	5.45	5.27	5.59	0.25	5.57	5.30	0.21	0.685	0.365	0.025
Flavour	5.54	6.00	5.63	0.30	5.30 ^b	6.15 ^a	0.25	0.542	0.019	0.174
Oxidised Flavour	1.22	1.09	1.36	0.11	1.30	1.15	0.09	0.260	0.263	0.385
General Acceptance	5.86	6.31	6.13	0.24	5.69 ^b	6.51 ^a	0.19	0.408	0.004	0.074

¹Oregano essential oil (OEO) and Capsicum oleoresin (CAO) were supplemented at 300 mg/kg of diet DM

²SEM=standard error of the mean

^{a,b}Mean values with different superscripts within a row and main factor are significantly different for $P < 0.05$

Meat fatty acid composition: The fatty acids in the lambs feeding on the OEO- or CAO-treated diets were found in the carbon range of 14–22 with medium to long chain lengths (Table 6). No significant differences were observed between the dietary treatment groups or sex groups in terms of the fatty acid composition and fatty acid groups (i.e., Σ -SFA, UFA, MUFA, PUFA, n-6, and n-3 PUFA). On the other hand, the Σ UFA and Σ MUFA values tended to increase in the OEO groups (53.27% and 44.99%, respectively) when compared to the control and CAO groups (50.43%, 41.34%; and 49.04%, and 41.52%, respectively) ($P > 0.05$). It was also observed that the ratio of n-6/n-3 PUFA was not affected by the dietary treatment or sex ($P > 0.05$). In addition, no fatty acid profiles in the meat were affected by the dietary treatment \times sex interaction.

Table 6. Fatty acid composition (g/100 g fat) of *Longissimus dorsi* muscle in fattening lambs fed essential oils

	Dietary Treatment ¹ (T)			SEM ²	Sex (S)		SEM ²	P-value		
	Control	OEO	CAO		Male	Female		T	S	T x S
C _{14:0}	0.17	0.38	0.18	0.13	0.20	0.29	0.11	0.452	0.557	0.442
C _{14:1}	0.21	0.18	0.21	0.03	0.19	0.21	0.12	0.791	0.516	0.257
C _{15:0}	0.13	0.10	0.09	0.02	0.12	0.10	0.02	0.543	0.492	0.357
C _{15:1}	0.12	0.16	0.14	0.02	0.14	0.14	0.04	0.552	0.890	0.921
C _{16:0}	22.15	20.31	23.95	4.54	20.27	24.01	3.62	0.847	0.476	0.050
C _{16:1}	0.31	0.24	0.22	0.07	0.24	0.28	0.05	0.673	0.629	0.758
C _{17:0}	3.01	3.20	3.13	0.32	3.17	3.05	0.25	0.910	0.738	0.770
C _{17:1}	0.28	0.35	0.33	0.09	0.35	0.29	0.07	0.857	0.550	0.572
C _{18:0}	20.51	18.59	17.37	1.75	18.82	18.83	1.40	0.453	0.994	0.767
C _{18:1n9t}	20.11	20.92	18.04	2.57	19.46	19.92	2.05	0.714	0.878	0.941
C _{18:1n9c}	20.18	23.01	22.44	3.53	23.07	20.68	2.89	0.835	0.558	0.521
C _{18:2n6t}	0.34	0.23	0.31	0.05	0.31	0.28	0.04	0.274	0.713	0.996
C _{18:2n6c}	7.72	6.94	6.09	0.87	7.20	6.64	0.73	0.458	0.590	0.516
C _{18:3n3}	0.81	0.80	0.81	0.01	0.81	0.80	0.01	0.884	0.402	0.216
C _{20:0}	0.17	0.24	0.21	0.05	0.20	0.21	0.04	0.653	0.840	0.865
C _{20:1}	0.13	0.13	0.14	0.04	0.13	0.13	0.03	0.982	0.948	0.927
C _{20:2}	0.05	0.08	0.12	0.03	0.06	0.11	0.03	0.494	0.265	0.409
C _{20:3n6}	0.06	0.11	0.10	0.01	0.06	0.12	0.03	0.576	0.169	0.601
C _{20:3n3}	0.11	0.12	0.09	0.01	0.10	0.10	0.01	0.330	0.919	0.307
C _{21:0}	0.07	0.14	0.15	0.06	0.06	0.17	0.04	0.603	0.109	0.496
C _{22:0}	0.09	0.16	0.11	0.01	0.11	0.13	0.02	0.401	0.566	0.322
Σ SFA	46.39	43.24	45.58	5.19	43.09	47.06	4.14	0.902	0.508	0.171
Σ UFA	50.43	53.27	49.04	2.41	52.12	49.70	2.01	0.460	0.383	0.113
Σ MUFA	41.34	44.99	41.52	2.22	43.58	41.65	1.76	0.419	0.449	0.154
Σ PUFA	9.09	8.28	7.52	0.94	8.54	8.05	0.75	0.444	0.594	0.399
Σ n-6	8.12	7.28	6.50	0.91	7.57	7.04	0.76	0.422	0.574	0.430
Σ n-3	0.92	0.92	0.91	0.02	0.91	0.90	0.01	0.960	0.454	0.640
Σ n-6/ Σ n-3	8.83	7.91	7.14	0.86	8.32	7.82	0.69	0.240	0.538	0.353

¹Oregano essential oil (OEO) and Capsicum oleoresin (CAO) were supplemented at 300 mg/kg of diet DM

²SEM = standard error of the mean; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Σ n-6/ Σ n-3, total omega 6/total omega 3

^{a,b}Mean values with different superscripts within a row and main factor are significantly different for $P < 0.05$

Discussion

With their strong antimicrobial activities, phytochemicals can lead to positive results in the conversion of dietary nutrients into muscular tissue, by manipulating the rumen microbial population or by modulating ruminal fermentation (Cardozo *et al.*, 2006; Calsamiglia *et al.*, 2007; Benchaar *et al.*, 2008). However, contrary to expectations in this study, the inclusion of OEO and CAO in the lamb diets did not affect the slaughter body weight, hot or cold carcass weight, or dressing percentage. As a matter of fact, these results were consistent with the results of another study conducted by the same research group using the same experimental design, where OEO and CAO had no effects on the daily weight gain, total DMI, or FCR (Ünlü *et al.*, 2021). In the same study, the acetate–propionate ratio among the

ruminal fermentation characteristics was found to be substantially lower in the OEO group (1.68) when compared to the CAO and control groups (1.79 and 1.81, respectively) and this result may have supported the numerical increase in the slaughter weights of the lambs in the OEO group (Ünlü *et al.*, 2021). There is evidence that particularly carvacrol, one of the main bioactive components of OEO, in sheep, potentially reduces intra-ruminal acetate concentrations, while increasing the propionate ratio that is involved in energy metabolism (gluconeogenesis) and is therefore responsible for the gaining of live weight (Garcia-Galicia *et al.*, 2020). Studies in agreement with the present study report that final body weights and carcass traits were not affected by the inclusion 0.15, 0.30, or 0.45 g carvacrol/kg of DM (Koyuncu and Canbolat, 2010), or 100 or 300 mg/kg of carvacrol, thymol or their mixtures (Biricik *et al.*, 2016). In addition, the lack of effect of OEO or CAO supplementation on slaughter traits observed in the present study was consistent with other *in vivo* studies using different phytogetic feed supplements. Similarly, Chaves *et al.*, (2003) reported that the inclusion of 200 mg/kg of cinnamaldehyde, garlic, or juniper berry essential oils in lamb diets did not change the final live weight or hot dressed weights.

In the current study, the slaughter body weight, and hot and cold carcass weights, and dressing percentage values in the male lambs were substantially higher than in the female lambs. These findings can be explained by the results obtained by Ünlü *et al.*, (2021) using the same experimental design, where the DMI values were numerically higher and FCR values were better in the male lambs. In addition, the dietary effect, as well as higher growth rate, in the male lambs when compared to the females, can be attributed to the greater number of muscle cells in male animals, and the effect of testosterone secreted from the gonads. In fact, testosterone accelerates muscle growth by increasing protein synthesis and by markedly reducing the amount of nitrogen excretion from the kidneys to urine (Lind *et al.*, 2011).

The dietary treatments did not alter the relative omental and mesenteric fat weights. Similarly, it was reported that 250 or 500 ppm of oregano oil did not change the relative omental or mesenteric fat weights in the lambs (Demirel *et al.*, 2013). Scarpa *et al.* (2021) reported that the percentage of the abdominal region dropped markedly in lambs fed pelleted feed that contained 3% extruded linseed and 0.6% dried oregano (*Origanum vulgare* L.) inflorescences when compared to the control group. Moreover, in the present study, the back fat thickness was higher in the female lambs than in the male lambs. Simitzis *et al.*, (2008) attributed this difference to the greater precociousness and fatness in female lambs than in males, especially in the growing period after weaning.

In general, the doses of OEO and CAO, chosen based on their phytochemical components, possibly did not cause the lambs to suffer aversive post-ingestive effects. However, they were found to have no improving effects on the carcass quality or dressing percentage. The inability to achieve the expected effect can be attributed to the fact that the vast majority of such phytogetic supplements were degraded in the rumen before reaching the target site. They would have passed into the gas phase in the rumen and disappeared during eructation; or were absorbed from the rumen wall into the blood system and excreted in the urine; or the rumen flora became accustomed to these supplemented diets (Benchaar *et al.*, 2008). In addition, the mechanism of action recommended for a particular phytogetic feed supplement is largely dependent on the structure, dosage, and pharmacokinetics of the respective solid matter, as well as on the animal species, productive stage, and the administration period (Valenzuela-Grijalva *et al.*, 2017). However, Calsamiglia *et al.* (2007) reported that some of these phytogetic feed supplements have effects depending on pH and diet (roughage/concentrate feed), and that their use may only be beneficial under certain conditions and in certain production systems.

The physico-chemical properties, which are the nutritional and sensory aspects of meat, are the properties most valued by consumers to a large extent. In general, the MLD muscle, also known as rib eye, is considered as a reference in studies on meat quality in many countries. Accordingly, in the present study, the solid matter and protein content of the meat samples taken from the rib eye area were not affected by the OEO and CAO supplementation. Consistent with these results, it has been reported that rosemary essential oil (Smeti *et al.*, 2017); *Ferulago angulata* essential oil (Parvar *et al.*, 2018); and a mixture of dill, cinnamon, and eucalyptus essential oils (Ranucci *et al.*, 2019) had no effect on the moisture and protein content of lamb meat. When compared to the control group, CAO tended to increase the intramuscular fat content, while OEO increased it substantially. This finding may have been associated with the decreased acetate/propionate ratio, which contributes to the storage of intramuscular fat (marbling), as reported by Ünlü *et al.*, (2021) using the same experimental design. However, Chikwanha *et al.* (2019) reported that grape pomace did not affect the intramuscular fat content. The observed difference can be attributed to the type of phytoGENICS used, the dose and method of its administration, content of chemical components, biological activity, intra-rumen fermentation conditions, animal species, sex, and growing period.

The lamb L^* , a^* , and b^* values were not affected by dietary supplementation of OEO and CAO. However, they were found to be higher than the minimum L^* and a^* threshold values (34 and 9.5, respectively) of lambs visually accepted by consumers, which were reported by Khlijji *et al.* (2010). The b^* value is reported to have no effects on the consumers' visual preference for lamb meat (Khlijji *et al.*, 2010). Garcia-Galicia *et al.* (2020) stated that consumers do not expect to find high b^* in fresh meat. Accordingly, the colour parameters of the lamb meat obtained in the present study were within the appropriate reference ranges. In addition, the lack of colour differences between the dietary treatment groups 24-h postmortem was consistent with other studies on rosemary essential oil (Smeti *et al.*, 2017), grape pomace (Chikwanha *et al.*, 2019), and cinnamon essential oil (Simitzis *et al.*, 2014). Teixeira *et al.* (2005) reported that meat colour may also vary depending on the lamb body weight, breed, and sex.

Drip loss was not affected by the dietary treatment or sex, but it remained at an ideal level. Teixeira *et al.* (2005) reported that 1–3% wastage occurred in all muscles after slaughter and water retention capacity, muscle pH, and muscle cell gaps directly affected the drip loss during storage. Therefore, it can be said that the carcasses were cooled in the shortest time at the appropriate freezing temperature and that the pH decrease rate was ideal. Similarly, Chikwanha *et al.* (2019) reported that the increasing inclusion of grape pomace in lamb diets (0%, 5%, 10%, and 20%) did not change the drip loss and the values were in the range of 1.38–1.78%. The inclusion of OEO and CAO in lamb diets did not change the cooking loss. Consistent with this finding, cooking loss was not affected by the inclusion of oregano oil in lamb diets (Demirel *et al.*, 2013) and rosemary essential oil (Smeti *et al.*, 2017). Lipid oxidation (TBARS formation) is an important indicator of not only oxidative stress, but also tissue damage, and it is the main cause of meat quality losses (Shah *et al.*, 2014). In the current study, the inclusion of 300 mg of OEO/kg of DM in the lamb diets was observed to numerically reduce the TBARS value in the fresh meat (on day 0). This result can be partially explained by the report of Simitzis *et al.* (2010) stating that the conversion of lipid and hydroxyl radicals into stable products was caused by the effect of the bioactive components of OEO, such as carvacrol and thymol, on the permeability of cell membranes. The storage of lamb meat at 4 °C until day 2 remained below the threshold value of 1 mg MDA/kg of meat recommended by Ripoll *et al.* (2011) for lamb meat, beyond which rancid flavour overpowers lamb meat flavour. It was reported that this value can be considered to be 2 mg MDA/kg of meat for beef (Garcia-Galicia *et al.*, 2020). Similarly, in the present study, the TBARS values of the dietary treatment groups were found to be similar and ≤ 2 mg MDA/kg of meat during the storage period up to day 8. This similarity was probably caused by the degradation of OEO and CAO in the rumen, and their subsequent inability to accumulate in the muscles sufficiently. Several studies have concluded that the addition of a mixture of extruded linseed and dried oregano inflorescences; *Ferulago angulata* essential oil; a mixture of dill, cinnamon bark, and eucalyptus essential oils; and tanniferous bush oil to the diets exerts a protective effect against lipid oxidation and maintains low TBARS values in lamb meat (Francisco *et al.*, 2015; Parvar *et al.*, 2018; Ranucci *et al.*, 2019; Scarpa *et al.*, 2021). In the present study, the TBARS value in the meat of the female lambs was lower than in the meat of the males at the end of 2 and 4 days of storage, and the values were similar on other days. Simitzis *et al.* (2008) found that during a 6-day storage period, the MDA value in the female *Longissimus thoracis* muscles decreased when compared to the males, and then became similar again during the following storage period (on day 9). The higher TBARS value in male lambs may also have been associated with a numerically increased ratio of unsaturated fatty acids.

In the present study, the sensorial properties analyses indicated no changes in the meat appearance, juiciness, flavour, oxidized flavour, or general acceptance between the dietary treatment groups. However, the panellists rated the meat flavour and general acceptance numerically higher in lambs feeding on OEO than in the control group. This numerical increase can be claimed to be associated with the accumulation of intramuscular fat. In fact, this finding can be supported by the fact that the OEO obtained in the study increased the meat fat content (marbling). Intramuscular fat has been reported to increase the tenderness of cooked meat by slowing down the rate of temperature drop, increasing the duration of active proteolysis, and lessening the extent of myofibrillar shortening (Garcia-Galicia *et al.*, 2020). In addition, the fatty acids in the meat may also have contributed to the enhancement of the flavour of the meat by providing volatile degradation products during cooking. Consistent with these results, the inclusion of oregano oil (Demirel *et al.*, 2013) in lamb diets did not change the sensory properties of the meat. Smeti *et al.* (2017) reported that rosemary essential oil increased the meat flavour and acceptability values. Aside from the subjective assessments of the panellists, these differences between the studies can be attributed to the meat pH, its chemical composition, sarcomere length, diameter of the fibrils, type of muscle, postmortem biochemical reactions, the age, sex, and nutrition of the animal, and the conditions before and after slaughter. The interaction of diet and sex on appearance and juiciness may be attributable to the effects of essential

oils on intramuscular fat deposition and the tendency of female lambs to gain fat earlier than male lambs, and the relatively darker female lamb meat than male lamb (Teixeira *et al.*, 2005).

Feeding OEO or CAO to the lambs had little numeric effect on the overall FA composition of the lamb meat. In general, it has been ascertained that more than 80% of the meat fatty acids belonging to all of the groups consisted of C16:0 (palmitic acid), C18:0 (stearic acid), and C18:1 (oleic acid); a small amount consisted of C18:2 (linoleic acid) and C18:3 (linoleic acid); and a trace amount consisted of other fatty acids. OEO numerically increased the Σ UFA value and decreased the Σ SFA value when compared to the control and CAO groups. Therefore, OEO can be claimed to be partially effective in protecting unsaturated fatty acids from microbial lipase, and in inhibiting their bio-hydrogenation in the rumen (Sinclair, 2007). Phytochemicals can inhibit the proliferation and reproduction of bacteria by damaging the glycolipid walls of bacterial cells responsible for bio-hydrogenation, through their powerful antimicrobial effects (Dorman and Deans, 2000). Thus, as a result of incomplete bio-hydrogenation, unsaturated fatty acids are absorbed in the small intestine through the provided duodenal outflow, and they then can be stored in the muscle, adipose tissue, or the liver. In another study, the oral administration of rosemary essential oil (0.6 mL/day) to lambs substantially improved the Σ PUFA, Σ PUFA/ Σ SFA, Σ n-6, Σ n-3, and Σ n-6/ Σ n-3 values (Smeti *et al.*, 2017). Scarpa *et al.* (2021) found that the mixture of extruded linseed and dried oregano inflorescences decreased the Σ SFA and Σ n-6/ Σ n-3 values in lamb meat, while not changing the Σ MUFA values, but increasing the Σ PUFA, Σ UFA, Σ n-6, and Σ n-3 values. These differences can basically be attributed to the bio-hydrogenation process in the rumen.

Conclusion

In conclusion, OEO and CAO did not affect the slaughter characteristics, meat quality, or fatty acid composition of the lamb meat, possibly as a result of their selected doses and bioconversion by the rumen microflora. However, 300 mg OEO/kg of DM can be included in lamb diets because of its remarkable contribution to intramuscular fat, meat flavour, and Σ UFA. However, there is a need for further *in vivo* studies to protect such supplements from ruminal microbial activity, to determine optimal doses, and to analyse the presence of residues in animal products because of the adaptation and degradation capacity of rumen microorganisms to the phytochemical additives used. In addition, future studies should focus on improving the PUFA/SFA ratio of lamb meat so as to allow people to eat healthier.

Conflict of interest

There is no conflict of interest.

Authors' contributions

HBÜ designed the study. HBÜ and HHI executed the study. HBÜ, HHI, and ÇK implemented the study and analysed the samples. HBÜ, HI, and ÇK drafted the manuscript.

Acknowledgements

This study was supported by Ege University Scientific Research Projects Directorate under Grant 2016-ZRF-070.

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