Iranian Journal of Applied Animal Science

# Effects of Sweet Marjoram (*Origanum majorana*) Powder on Growth Performance, Nutrient Digestibility, Rumen Fermentation, Meat Quality and Humoral Immune Response in Fattening Lambs

Research Article

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Received on: 15 Jul 2020
Revised on: 14 Oct 2020
Accepted on: 15 Nov 2020
Online Published on: Sep 2021

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Online version is available on: www.ijas.ir

## ABSTRACT

The use of natural compounds such as herbs and herbal preparations because of their effects on animal health and product quality, is becoming a new goal in livestock production. Therefore, an experiment was carried to evaluate the effects of sweet marjoram on growth performance, rumen fermentation, meat quality and humoral immune response in Kermani lambs. A total of 24 Kermani lambs (27.5±0.450 kg) were distributed randomly into 24 individual cages in 3 experimental groups. The experimental diets consisted of the control and two experimental diet contained 2 and 4 % *Origanum majorana* leaf (OML) powder. The lambs were weighed and slaughtered after a feeding period of 80 days and meat quality were determined. OML powder had no effects on dry matter intake, but the apparent protein digestibility was higher linearly ($P<0.05$) for the diets containing OML powder. Live weight gain, final and carcass weight increased ($P<0.05$) with adding of OML in diet linearly. $NH_3$-N concentration and protozoa population were less ($P<0.05$) with 2 and 4% OML than control. Fat and total protein linear decreased and increased ($P<0.05$) respectively in meat by adding of OML powder in diet, and longissimus dorsi of lamb fed OML powder had lower ($P<0.05$) malonaldehyde and cooking loss than the control diet linearly. The results suggest that OML is useful for improvement ruminal fermentation and is a suitable natural antioxidant in the meat production industry.

KEY WORDS meat quality, performance, rumen, sweet marjoram.

## INTRODUCTION

The increase of public health concern about antibiotic residues have led to restrictions on the use of these compounds worldwide. The use of natural compounds such as herbs, herbal preparations and other botanicals because of their effects on human and animal health and on product quality and safety, is a goal in livestock production (Makkar *et al.* 2007). The plant or herb extracts have been shown to manipulate ruminal fermentation and improve nutrient utilization in ruminants (Busquet *et al.* 2005; Patra *et al.* 2006). Bioactive plants and plant compounds, when included as feed components have a range of effects on digestibility and blood parameters in the ruminant.

*Origanum majorana* (family Lamiaceae) is commonly known as sweet marjoram and found in Mediterranean regions particularly temperate regions. Sweet marjoram is an aromatic and perennial plant that main components of its essential oil are carvacrol, thymol, P-cymene, gamma-terpinene, α-pinene and myrsene (Raina and Negi, 2012).

Many studies have shown that antifungal, antibacterial and antioxidant activity of various species of marjoram (Aureli *et al*. 1992; Muller *et al*. 1995). The antibacterial, antiseptic and antifungal effects of sweet marjoram are attributed to thymol and carvacrol (Kelly, 2004; El-Ashmawy *et al*. 2005). According to the analysis report of Hafez (2012), the composition of sweet marjoram is as follows: crude protein 12.80%; crude fat 4.32; ash 5.62%; crude fiber 19.52%; carbohydrates 72.18 and moisture 5.66% marjoram powder.

Many additives and herbs have been used as powders (Hajalizadeh *et al*. 2019) or essential oils (Simitzis *et al*. 2008) in animal diets. Some researchers showed various bioactivity of carvacrol alimentary, inclusive antioxidant, anti-inflammatory, antiseptic, antibacterial, antiviral, growth stimulus, antifungal and immunomodulatory as well as converter of rumen microbial fermentation and depression of methane production (Hashemipour *et al*. 2013; Bravo *et al*. 2014). Also this herb improved the activity of superoxide dismutase enzyme which acts as an anti-inflammatory agent (Opara and Chohan, 2014). Abd El-Mola *et al*. (2019) reported that marjoram by-products up to 40% improved rumen fermentation condition and nutrient digestibility in lactating buffalo's rations. The study aimed to investigate the effect of marjoram powder on meat quality, rumen characteristics and humoral immune response in fattening lambs.

## MATERIALS AND METHODS

Twenty-four male Kermani lambs with average initial body weight of 26 ± 0.450 kg and eight months old, were obtained and used in this study. The lambs were drenched against parasites and enterotoxaemia (Razi Vaccine and Serum Research Institute, Karaj, Iran) and housed in individually pens (1.3 m×1 m). The lambs divided into three experimental diets for 80 days with feeding and watering facilities (including 2 weeks of adaptation period). The *Origanum majorana* leaf (OML) powder samples were collected from a local market and used in experimental diets after chemical analysis. The OML protein was carried out following standard methods of AOAC (2000). Also, samples were analyzed for neutral detergent fibre (NDF) and acid detergent fibre (ADF) according to Van Soest *et al*. (1991).

The experimental ration formulated according to the NRC (2007) recommendations. Diets in this study were: 1) control diet (without OML powder), 2) diet containing 2% OML dry powder and 3) diet containing 4% OML dry powder (Table 1). The average initial body weights in experimental groups were 26.02, 26.45 and 26.00 kg, respectively.

The animals were individually fed, twice per day, at 08:00 and 16:00, for *ad libitum* intake to allow 10% orts.

From day 5 until the day before slaughter, samples of feces were taken from rectal twice daily and stored at −20 ˚C for apparent digestibility estimation based on acid insoluble ash procedure according to the Van Keulen and Yang (1977). Rumen fluid samples at the end of the finishing period was collected via an esophageal tube approximately 2-3 h after the feeding. The samples were filtered through three layers of cheesecloth and pH of the ruminal fluid immediately was determined using a digital pH meter (AZ, Model 8601). After the pH measuring, 10 mL sample from each animal was mixed with 0.1 mL 50% sulphuric acid and stored at −20 ˚C for later determination of the NH3-N concentration (Broderick and Kang, 1980). To determine the number of protozoa, 5 mL of the ruminal fluid were added to 5 mL solution of methyl-green formalin saline (MFS) and stored in a dark place. The protozoal population was determined as described by Ogimoto and Imai (1981).

On days 70 of the experimental period and before the morning feeding, blood samples were collected from the jugular vein into the CBC experimental tube containing anticoagulant (K2 EDTA). Complete blood counts including red blood cell count (RBC), hematocrit (Ht), total white blood cell count (WBC), lymphocyte count, neutrophil count, eosinophil count and monocyte count were performed in an automatic counting set. For immune response, the blood sample was made for each animal into heparinized collection tube and its serum separated after centrifuging at 2500 rpm for 10 minutes. Immunoglobulins (IgG, IgA, and IgM) were determined using an ELISA test kit.

At the end of the experimental period, animals were weighed, and slaughtered on the same day at a slaughterhouse in Kerman city following an overnight fasting. The carcasses were weighed after slaughter which was designated as warm carcass weight. Cold carcass weight of each animal was calculated after 24 h chilling at 4 ˚C. Eye muscle area ($cm^2$) is determined by cutting the area of the longissimus dorsi muscle between the $12^{th}$ and $13^{th}$ ribs and placing on graph paper. Back fat depth was measured with a caliper at the cross section of the $12^{th}$ and $13^{th}$ ribs. Then, longissimus dorsi sample ($6^{th}$-$13^{th}$ ribs) was separated into two parts.

The first portion (about 5 g), were homogenized with 25 mL distilled water and the pH of the muscle was measured using a portable pH meter (AZ, Model 8601) after filtering (Jang *et al*. 2008). Another part was stored at −20 ˚C until subsequent determination of muscle chemical composition, dripping and cooking loss after 30 days.

Dripping loss was determined by the method described by Christensen (2003).

**Table 1** The ingredient and nutrient composition of experimental diets (DM basis)

| Ingredients (%) | *Origanum majorana* leaf (OML) level in diet (%) | | |
|---|---|---|---|
| | 0 | 2 | 4 |
| Alfalfa hay, chopped | 30 | 30 | 30 |
| Wheat straw, chopped | 10 | 10 | 10 |
| *Origanum majorana* leaf | 0 | 2 | 4 |
| Barley grain, ground | 28 | 28 | 28 |
| Corn grain, ground | 9 | 7 | 5 |
| Soybean meal | 8 | 8 | 8 |
| Wheat bran | 13 | 13 | 13 |
| Vitamin A, D and E premix[1] | 0.6 | 0.6 | 0.6 |
| Trace-mineralized salt[2] | 0.6 | 0.6 | 0.6 |
| Sodium bicarbonate | 0.5 | 0.5 | 0.5 |
| Limestone | 0.3 | 0.3 | 0.3 |
| **Chemical composition[3]** | | | |
| Dry matter (g/kg) | 899.6 | 900.1 | 900.6 |
| Metabolizable energy (Mcal/kg DM) | 2.52 | 2.52 | 2.52 |
| Crude protein (g/kg DM) | 13.98 | 13.99 | 14 |
| Organic matter (g/kg DM) | 926.1 | 924.3 | 922.5 |
| Ether extract (g/kg DM) | 22.5 | 23.4 | 24.3 |
| $NDF_{om}$ (g/kg DM) | 393.3 | 401.4 | 413.3 |
| $ADF_{om}$ (g/kg DM) | 240.2 | 248.7 | 240.2 |

[1] Contains per kg: vitamin A: 5000000 IU; vitamin D: 5000000 IU and vitamin E: 500000 IU.
[2] Composition: NaCl: 75.15%; Dynamad: 20.5%; Mn: 3.046%; Cu-sulphate: 1.025%; Zn-sulphate: 0.253%; EDDI-80: 0.015% and Na-selenide: 0.011%.
[3] ME value of the experimental diets was calculated from the tables of feed specification (NRC, 2007).
$NDF_{om}$: ash free neutral detergent fiber (NDF) and $ADF_{om}$: ash free acid detergent fiber (ADF).

The samples were cut into 2.5-cm thick pieces and after weighing, transferred into a plastic bag at 4 ˚C. After 24 h, each piece of meat was dabbed with a soft tissue and weighed again. The 1-$cm^3$ meat samples were cooked in a preheated water bath set at 85 ˚C and calculated cooking loss (Bertram *et al.* 2003).

To measure the water-holding capacity (WHC), 1g meat samples were placed on Whatman filter paper inside an experimental tube and centrifuged for 4 min. The water remaining after centrifugation was quantified by drying the samples at 70 ˚C overnight (Castellini *et al.* 2002). The chemical composition of meat including the crude protein, ether extract, DM and ash was determined by AOAC methods (AOAC, 2000).

Thiobarbituric acid reactive substances (TBARS) assay performed as described by Tarladgis *et al.* (1960). The samples (0.5 g) were mixed with 2.5 mL of 0.375% thiobarbituric acid (Sigma T5500), 15% trichloroacetic acid (Merck k46451107), and 0.25 N-HCl (Merck k36816017). The mixture was heated in a boiling water bath set 100 ˚C (for 10 min). Then, samples were cooled, centrifuged at 2000 g for 25 min and the absorbance measured spectrophotometrically (HALO, model XB10) at 532 nm. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient ($1.56\times10^5$ $M^{-1}$ $cm^{-1}$), and the absorbance values were converted to MDA (in ppm) by using the following equation:

$$\text{TBA (mg MDA/kg meat)= sample } A_{532} \times 2.77$$

**Statistical analyses**

The experimental design was completely randomized design and the experimental data were analysed by using the MIXED procedure of the SAS Version 9.1 (SAS, 2005). Data on digestibility, blood and ruminal parameters, etc. were analyzed using the following model:

$$Y_{ij}= \mu + T_i + e_{ij}$$

Where:
$Y_{ij}$: variable.
μ: population mean.
$T_i$: treatment effect.
$e_{ij}$: random error.

The Duncan's multiple range test used to compare means. The initial live body weight was used as the covariate for final BW and the carcass. The effects were considered significant at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Dry matter intake (DMI) and nutrient digestibility**

The results of feed intake and nutrient digestibility are shown in Table 2. The addition of OML powder in fattening lamb's diets had no effect on DMI, dry matter (DM), organic matter, ether extract, NDF and ADF digestibility but increased crude protein digestibility linearly (P<0.05).

**Growth performance**

Growth performance and meat production are shown in Table 3. The average daily weight gain and final weight (kg) in both groups received OML powder were greater linearly than the control (P<0.05). The warm and cold carcass weight were greater (P<0.05) in lambs fed diet with 2 and 4% OML powder than those fed the control diet (P<0.05). In the present study, 4% OML powder in the lamb's diets increased linearly (P<0.05) lean meat weight. The back fat depth (mm) in lambs fed OML powder was linearly lower (P<0.05) than the control group. There were no significant differences in eye muscle area among lambs fed experimental dietary.

**Ruminal pH, ammonia-N and protozoa population**

The supplementation of OML powder in experimental diets had no effects on ruminal pH (Table 4). Compared with the control, in 2 and 4% OML powder containing diets, ruminal ammonia nitrogen concentrations were reduced linearly (P<0.05) significantly. The total protozoa, *Entodinium* and *Cellulolytica* species population were linearly decreased (P<0.05) by OML powder. Conversely, ruminal *Holotricha* population among dietary treatments did not differ significantly.

**Blood biochemical parameters**

The result of fattening lamb's immunity blood parameters is shown in Table 5. The total white blood cells and lymphocyte count were affected linearly by OML powder which were significantly higher than the control group. Cell blood counts and other white blood cells including monocyte, neutrophil and eosinophil were not affected by adding OML powder in experimental diets. Humoral immune indicators IgG and IgA were higher (P<0.05) in blood lambs fed 4% OML powder than those fed 2% OML and control diets. But blood IgM concentration was not affected by experimental diets.

**Meat quality**

Meat DM and ash in lambs were not affected by the experimental diets (Table 6). Total meat fat in lambs fed 2 and 4% OML powder in the diet was lower than the control diet (P<0.05). Total protein increased linearly in meat by adding OML to the diet. After one-month storage, pH, water holding capacity (WHC) and dripping loss were not affected significantly (Table 7). But lowest cooking loss observed in the meat of lambs that received 4% OML powder as compared with the control diet and 2% OML powder. Malondialdehyde concentration was decreased linearly (P<0.05) in the meat lambs by adding OML powder to diets.

OML powder in lamb's diet had no effect on feed intake (Table 2). This can be due to the same in the percentages of NDF, ADF and moisture of the experimental diets as NDF concentration (Mertens, 2009) has correlation with DMI. The herbal additives and essential oil on DMI might impressed by type of diet, diet interaction or adaptation of rumen microbial population to additives, management factors and animal growth stage (Yang *et al*. 2010; Geraci *et al*. 2012). Bampidis *et al*. (2005) reported that feeding dried *oregano* leaves inclusion diets (850 mg/g of carvacrol per kilogram of diet DM) had no effect on DMI of growing lambs. Heifers that received *oregano* extract had no an impact on DMI (Kolling *et al*. 2016). In study, Ruiz Garcia *et al*. (2011) noted that feed intake in lambs was increased with the herbal extract in diet. In agreement with this study, chamomile and chicory powder in Dalagh sheep diet not effected DMI and feed conversion ratio (Ghasemifard *et al*. 2017). In a research Hajalizadeh *et al*. (2019) suggested that adding of fennel powder to lamb's diet had positive effect on DMI. Organic matter, NDF, ADF digestibility were no different in lambs fed experimental diets. This can be explained by the same percentages of NDF, ADF and moisture in the experimental diets. The lambs fed 2 and 4% OML powder have greater crude protein digestibility in this study. Hart *et al*. (2008) stated that the effect of essential oil and their components on the ruminal protein degradation is selective because these compounds affect certain species of bacteria. In the present study, improve crude protein digestibility in OML containing treatments can be related to the further digestion and absorption of certain types of proteins. In Zamiri *et al*. (2015) study, carvacrol did not modify nutrient digestibility in rams. In an *in vivo* study, Khalesizadeh *et al*. (2011) noted that there are no effects on apparent digestibility in sheep that feeding garlic oil or tumeric powder. Therefore, when peppermint was fed to steers, the digestibility of nutrients tended to be higher than that of the control (Ando *et al*. 2003). Kongmun *et al*. (2010) showed that garlic powder supplementation at 16 mg/d did affect *in vitro* true digestibility. This contrast to the present study may be due to the use of herb supplementation in the powder and oil form and the concentration of the compounds. In this study, the use of in lamb's diet increased final weight and daily weight gain significantly. It seems that OML powder improve the ruminal fermentation due to its high protein digestibility that results in enhanced daily weight gain and final weight. It has been reported that the carvacrol in the Origanum species has decreased rumen methane production (Tekippe *et al*. 2011) and increased volatile fatty acids (VFA) concentration (Chaves *et al*. 2008).

**Table 2** Dry matter intake and nutrient digestibility in lambs fed diets containing *Origanum majorana* leaf (OML)

| Item | OML concentration (% DM) | | | SEM | P-value | Contrast | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.02 | 0.04 | | | Linear | Quadratic |
| Dry matter intake (kg) | 1.45 | 1.43 | 1.42 | 0.023 | 0.720 | 0.226 | 1.00 |
| **Digestibility (%)** | | | | | | | |
| Dry matter | 70.68 | 72.46 | 73.96 | 1.224 | 0.212 | 0.075 | 0.925 |
| Crude protein | 64.06[b] | 67.14[a] | 67.74[a] | 0.799 | 0.016 | 0.0153 | 0.293 |
| Organic matter | 62.69 | 64.96 | 63.24 | 1.260 | 0.583 | 0.757 | 0.208 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

**Table 3** Growth and carcass characteristic of lambs fed diets containing *Oraganum majorana* leaf (OML)

| Characteristic | OML concentration (% DM) | | | SEM | P-value | Contrast | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.02 | 0.04 | | | Linear | Quadratic |
| Initial weight (kg) | 26.02 | 26.48 | 26.00 | 0.693 | *0.460* | 0.978 | 0.252 |
| Final live weight (kg) | 42.55[b] | 45.26[a] | 46.05[a] | 0.740 | 0.008 | 0.003 | 0.290 |
| Live daily gain (kg) | 0.19[b] | 0.23[a] | 0.24[a] | 0.006 | 0.014 | 0.0007 | 0.901 |
| Warm carcass weight (kg) | 20.38[b] | 21.85[ab] | 22.70[a] | 0.380 | 0.034 | 0.022 | 0.707 |
| Cold carcass weight (kg) | 19.65[b] | 21.25[ab] | 22.19[a] | 0.110 | 0.049 | 0.016 | 0.828 |
| Lean meat (kg) | 13.74[b] | 14.99[a] | 15.75[a] | 0.513 | 0.036 | 0.010 | 0.070 |
| Back fat (cm) | 5.07[a] | 3.62[b] | 3.73[b] | 0.422 | 0.049 | 0.050 | 0.176 |
| Eye muscle area ($cm^2$) | 18.78 | 18.89 | 19.45 | 0.546 | 0.703 | 0.459 | 0.688 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

**Table 4** Ruminal pH, $NH_3$-N and protozoa in lambs fed diets containing *Origanum majorana* leaf (OML)

| Parameters | OML concentration (% DM) | | | SEM | P-value | Contrast | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.02 | 0.04 | | | Linear | Quadratic |
| Ruminal fluid pH | 5.36 | 5.88 | 5.82 | 0.222 | 0.238 | 0.628 | 0.932 |
| $NH_3$-N concentration (mg/dL) | 15.13[a] | 12.94[b] | 12.04[b] | 0.496 | 0.0031 | 0.0006 | 0.290 |
| **Protozoa populations (×$10^5$ cells/mL)** | | | | | | | |
| Entodinium | 14.20[a] | 11.20[b] | 10.80[b] | 0.844 | 0.031 | 0.012 | 0.220 |
| Holotricha | 1.86 | 1.65 | 1.45 | 0.067 | 0. 0133 | 0.540 | 0.720 |
| Cellulolytica | 0.78[a] | 0.56[b] | 0.44[b] | 0.09 | 0.09 | 0.01 | 0.74 |
| Total protozoa | 16.84[a] | 13.41[b] | 12.69[b] | 1.305 | 0.033 | 0.0097 | 0.161 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

**Table 5** Effect of diets containing *Origanum majorana* leaf (OML) on serum immunity of lambs

| Parameters | OML concentration (% DM) | | | SEM | P-value | Contrast | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.02 | 0.04 | | | Linear | Quadratic |
| White blood cell (WBC) | 8.40[b] | 9.70[b] | 11.25[a] | 0.633 | 0.030 | 0.0073 | 0.896 |
| Red blood cell (RBC) | 7.40 | 8.75 | 9 | 0.537 | 0.127 | 0.0384 | 0.371 |
| Neutrophil (%) | 3.57 | 4.20 | 4.67 | 0.32 | 0.19 | 0.290 | 0.858 |
| Lymphocytes (%) | 7.70[b] | 10.20[a] | 10.60[a] | 0.76 | 0.044 | 0.0169 | 0.268 |
| Monocyte (%) | 8.70 | 9.26 | 10.02 | 0.36 | 0.076 | 0.0397 | 0.834 |
| Eosophil (%) | 0.15 | 0.16 | 0.15 | 0.008 | 0.83 | 0.0744 | 0.456 |
| Hematocrit (%) | 34.56 | 34.02 | 39.85 | 1.80 | 0.081 | 0.052 | 0.159 |
| IgA | 0.21[b] | 0.21[b] | 0.25[a] | 0.009 | 0.0162 | 0.012 | 0.0817 |
| IgG | 0.15[b] | 0.16[b] | 0.19[a] | 0.005 | 0.009 | 0.0046 | 0.167 |
| IgM | 0.058 | 0.052 | 0.068 | 0.010 | 0.540 | 0.494 | 0.3888 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Since the VFA are the main sources of metabolizable energy for ruminants, then increasing diet fermentability can be nutritionally beneficial. However, Hajalizadeh *et al.* (2019) observed the highest DMI, final BW and live daily gain in lambs fed the 1.5% fennel seed powder diet which was in agreement with the results of this study. There was no difference in fasted body weight among lambs fed oregano inclusion levels (Bampidis *et al.* 2005). In the other study, Payvastegan *et al.* (2013) suggested that savory dry powder (up to 20 g/d) had no effects on dry matter intake, average daily gain and feed conversion of kids. The highest lean meat and lowest back fat found in lambs fed 4% and 2% OML, respectively. This increase in lean meat is probably due to the higher live weight of these lambs.

Totally, it was investigated that OML powder in lamb's diet had a positive impact on muscularity while lowering fatness significantly. The dietary containing essential oil of *Oreganum vulgare* increased the measure of muscles longissimus dorsi depth and decreased back-fat thickness in a study by Dudko *et al.* (2018).

Ruminal pH was not affected by experimental diets, whereas rumen liquid ammonia nitrogen decreased by increasing OML powder in fattening lambs diet. Variable results have been reported for the effects of herbs and essential oil on ruminal pH. Peppermint powder (Hosoda *et al.* 2006) and chamomile and chicory dry powder (Ghasemifard *et al.* 2017) had no effect on rumen pH in steers and sheep diet respectively. Very little *in vivo* information is available on the effect of herbal supplement on rumen fermentation. A portion of observed reduction in ammonia nitrogen concentration in the current study may be due to decreasing protozoa population. Because rumen protozoa was increased bacterial protein breakdown and feed protein degradability (Williams and Coleman, 1992). Beside, several reports indicated that addition of essential oils resulted in a reduction in the number of hyper-ammonia producing bacteria (HAB) (McEwan *et al.* 2002; Wallace *et al.* 2002). Generally, origanum oil contains a high content level of carvacrol that inhibited HAB. This result agrees with Hristov *et al.* (2013). These authors reported that oregano leaves supplementation had no effect on rumen pH, but decreased ammonia concentration. In addition, ammonia nitrogen was decreased with oregano oil at 30 and 300 mg and cinnamon oil at 0.3-300 mg (Cardozo *et al.* 2005). In contrast, *in vitro* study by Busquet *et al.* (2005) showed that carvacrol increased and decreased ruminal ammonia nitrogen and large peptide concentrations respectively. Also, Tekippe *et al.* (2011) observed increase in ammonia concentration in lactating dairy cows by feeding 500 g/day *Origanum vulgare* leaves.

In general, the protein metabolism by microorganism in the rumen is a complex process and so on essential oils effects on protein degradation and hydrolysis varies depending on their component, dose and structure (Castillejos *et al.* 2006). In a study, feeding of 500 mg ropadiar (containing essential oil of marjoram) to sheep showed higher concentration of protein in the rumen fluid without affecting the nutrient digestibility (Kozelov *et al.* 2001).

Total protozoa, *Cellulolytica* and *Entodiniomorpha* populations decreased in this study by 2 and 4 % OML powder to the experimental diet. The effect of essential oil on protozoa reduction was most likely due to its main active compounds and structures (Talebzadeh *et al.* 2012). This components permeated across cell membrane and inhibited enzymes activity (Goel *et al.* 2005). Therefore, like with this study, Ando *et al.* (2003) reported that 200 g peppermint to Holstein steers ration, decreased the total protozoa concentrations. Protozoa numbers decreased by increasing oregano oil in an *in vitro* study (Patra and Yu, 2012). Khalilnezhad (2013) found that marjoram essential oil did not affect the ruminal protozoal. Overall, essential oil and components have no marked effects on numbers of ruminal protozoa.

Total white blood cells and lymphocyte numbers were greater in lambs fed OML powder than lambs fed control diet. In some livestock studies, including goats, the effect of herbs on improving the immune system has been reported (Grossi and Lacetera, 2004; Moradpour *et al.* 2018). Carvacrol has been reported to have antibacterial, antiviral, and antioxidant activities, an increase in immune responses of chicks is anticipated (Botsoglou *et al.* 2002). The improvement of blood parameters in animals fed OML powder than control group, is maybe due to the antioxidant properties of carvacrol that protects blood cells from oxidative effects (Zare Mehrjerdi *et al.* 2018). In agreement with the findings of the present study, Hajalizadeh *et al.* (2019) reported that fennel seed powder increased the level of total white blood cell and lymphocyte counts in fattening lambs. Fennel powder in Holstein calves' starter diets, was increased blood eosinophil but not effected on white blood cells (Saeedi *et al.* 2016).

Immune response and immunity status were affected by adding 4% OML powder in lamb's diet. It is shown that carvacrol has an anti-inflammatory effect and was inhabitation of the prostaglandin production (Luna *et al.* 2010) which modulated animal immunity status. Dietary curcumin supplement can increase the serum concentration of IgG in lactating cows reared under high ambient temperature environment (Liu *et al.* 2014). Another study reported an increase in the sheep serum concentration of IgA, IgG, and IgM after feeding curcumin supplement (Jiang *et al.* 2019). Plant extracts such as thyme and oregano caused to enhancing the concentration of IgG in mice and pig's serum (Namkung *et al.* 2004).

**Table 6** Chemical composition of *Longissimus dorsi* muscle in lambs fed diets containing *Origanum majorana* leaf (OML)

| Traits (%) | OML concentration (% DM) | | | SEM | P-value | Contrast | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.02 | 0.04 | | | Linear | Quadratic |
| Dry matter | 22.83 | 23.04 | 22.91 | 0.609 | 0.97 | 0.875 | 0.849 |
| Ash | 1.03 | 1.09 | 1.04 | 0.03 | 0.54 | 0.584 | 0.561 |
| Fat | 5.38[a] | 3.82[b] | 3.54[b] | 0.259 | 0.0008 | 0.0002 | 0.0562 |
| Total protein | 19.99[b] | 22.56[a] | 24.12[a] | 0.528 | 0.0006 | 0.0001 | 0.433 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

**Table 7** Malonaldehyde content and meat quality of lambs fed diets containing *Origanum majorana* leaf (OML)

| Traits | OML concentration (% DM) | | | SEM | P-value | Contrast | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.02 | 0.04 | | | Linear | Quadratic |
| Malondialdehyde (MDA) (nmol/mg protein) | 0.703[a] | 0.493[b] | 0.482[b] | 0.0317 | 0.0006 | 0.0002 | 0.0206 |
| Cooking less (%) | 24.98[a] | 24.02[ab] | 23.27[b] | 0.363 | 0.0216 | 0.0927 | 0.0191 |
| Dripping less (%) | 2.07 | 2.06 | 2.07 | 0.046 | 0.98 | 0.976 | 0.876 |
| Water holding capacity (%) | 25.04 | 25.64 | 25.83 | 0.365 | 0.317 | 0.150 | 0.665 |
| pH | 5.40 | 5.56 | 5.64 | 0.356 | 0.885 | 0.110 | 0.458 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Redoy *et al.* (2020) noted that the sheep fed herbal supplemented including plantain or garlic leaf had higher serum immunoglobulin concentration and antioxidant capacity. The elevation of IgG and IgA serum concentrations in lambs fed OML powder indicated that B lymphocytes increased production of antibodies which beneficial for the improvement of lamb's humoral immunity.

Meat total protein and fat content were influenced significantly by OML powder. While there was no difference between experimental diets in dry matter and ash meat, the highest proportion of meat crude protein was found in lambs fed 2 and 4% OML powder. According to observations on rumen ammonia nitrogen, OML inhibited the deamination process in the rumen resulting in higher nitrogen turnover in sheep which lead to an increase in body protein synthesis rates (Al-Mamun *et al.* 2008). More specifically, bioactive components in OML were increased protein digestibility in lambs. Besides, OML powder in lamb's diet was able to affect protozoa in the rumen and reduced unnecessary nutrient loss. However, a previous study indicated that fennel seed powder increased crude protein and decreased fat content in lamb's meat (Hajalizadeh *et al.* 2020). In contrast to this research, Khamisabadi *et al.* (2015) reported that peppermint or thymus had no effect on meat chemical composition of Sanjabi finishing lambs. The meat ether extract in lambs fed OML powder was lower than those fed control diet. The studies related to the effect of herbs and their components in fat content of meat was little on livestock cases. Hajalizadeh *et al.* (2020) noted the fennel seed powder was decline fat content in lamb's meat which attributed to fennel seed polyunsatur9ated fatty acids. Previous research has demonstrated that *Plantago lanceolata* supplementation in lamb's diet reduced muscle fat (Shuvo *et al.* 2017). Also, in a study, Redoy *et al.* (2020) reported that garlic leaf and plantain decreased mutton ether extract in lambs.

There were no differences between lambs fed experimental diets in muscle pH, but 4% OML powder in lamb's diet significantly decreased cooking loss. Meat pH is very important in measuring of meat quality, because it may directly affect texture, water holding capacity and color (Kirmizibayrak *et al.* 2011). Meat pH was influenced by many characteristics such as, animal age, breeding methods, feed additives, hormonal status, muscles morphology and glycogen content. Increasing dietary antioxidants has been shown to decrease the oxidation of cell membrane phospholipids and improve dripping and cooking loss (Jensen *et al.* 1998). Ipcak and Alcicek (2018) concluded that the addition of carvacrol and cinnamaldehyde are effective on the cooking loss. In contrast, herbal treatment diets including plantain and garlic leaf were no effect on cooking and drip loss (Redoy *et al.* 2020).

In this study, lambs that consumed OML powder had the lowest MDA content. Determination of MDA is an important indicator for lipidoxidation in meat and meat products. The lower TBARS values for lambs fed OML powder may be attributed to the lower fat content of these lambs which could speed up lipid oxidation. In addition to, carvacrol in marjoram has high antioxidant activity (Muller *et al.* 1995). It was demonstrated that dietary supplementation of lambs with Thyme (containing thymol and carvacrol) led to higher polyunsaturated fatty acid (PUFA) content in muscle (Nieto *et al.* 2012). Therefore, the lower MDA content in muscle lambs fed OML is associated with higher amounts of PUFA in muscles which play antioxidant role in animal body. Dietary supplementation of lambs with 1.5% fennel seed powder significantly decreased lipid peroxidation of meat during refrigeration storage (Hajalizadeh *et al.* 2020). Oregano essential oil supplementation caused to retardation lipid oxidation in raw sheep longissimus thoracis after storage (Simitzis *et al.* 2008).

Also, Rivaroli *et al.* (2016) fed crossbred young bulls with blend including oregano and found that a dose of 3.5 g essential oil per day, decreases lipid oxidation. The positive effects of essential oil in preventing oxidation in lamb meat has also been reported by many studies (Nieto *et al.* 2011; Garcia-Galicia *et al.* 2020).

## CONCLUSION

Feeding OML to fattening lambs improved the performance and meat yield and had a positive effect on meat quality, meat composition, and fat oxidation. It also decreased ruminal protozoa, nitrogen concentrations and increased protein digestibility. It is suggested that OML may be a good additive for improving the ruminal environment and a suitable natural antioxidant for the meat production industry.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge support for this work that was provided by, Science and Research Branch, Islamic Azad University, Tehran.

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