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Effects of Dietary Peppermint and Thyme Powders on Growth, Immunity, Rumen Fermentation, Carcass, and Meat Quality in Sanjabi Fattening Lambs

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ABSTRACT

Medicinal herbs have attracted considerable attention as natural edibles to enhance growth performance and meat quality in ruminants. This study was conducted to evaluate the impact of dietary supplementation with medicinal herbs, peppermint (*Mentha piperita*) or thyme (*Thymus vulgaris*), on production performance and meat quality in Sanjabi fattening lambs. The experimental diets were control diet without medicinal herbs, basal diet contained 3% peppermint powder, and basal diet contained 3% thyme powder. Results showed that peppermint supplementation in lambs improved average daily gain (298.15 g/day), while the control group showed the lowest daily gain (228.70 g/day, $P < 0.05$). Dietary supplementation with peppermint and thyme powders lowered rumen pH and reduced ammonia-nitrogen concentrations compared to the control group ($P < 0.05$). The peppermint-supplemented group exhibited significantly higher final live weight, rumen capacity, and both hot and cold carcass weights compared to the thyme and control groups ($P < 0.05$). Overall, incorporating peppermint and thyme powders into the diet enhanced growth performance, decreased ruminal ammonia-nitrogen levels, and improved carcass traits and meat quality in Sanjabi fattening lambs. These findings highlight the potential of medicinal herbs as effective natural feed additives to boost small ruminant production efficiency.

Keywords: Sanjabi lamb, Peppermint, Thyme, Growth performance, Meat quality

INTRODUCTION

Ensuring the availability of safe, affordable, and nutritious food remains one of the most critical global challenges, especially in developing countries where protein and energy sources are fundamental nutritional pillars influencing both animal productivity and human health. Small ruminants are a vital source of animal protein worldwide, with their population density in many developing regions highlighting their importance in local food security and economies. Traditionally, antibiotics such as ionophores (e.g., monensin) have been widely used in ruminant diets to enhance feed efficiency, modify rumen microbial populations, and prevent metabolic disorders. These compounds improve ruminal fermentation by reducing ammonia concentrations and increasing propionate production, thereby improving dry matter intake and feed conversion efficiency. However, concerns about antibiotic residues and the emergence of antimicrobial resistance have led to regulatory restrictions and bans in several regions, including the European Union [1]. In this context, medicinal herbs and phytogenic feed additives have emerged as promising natural alternatives to antibiotics in ruminant nutrition. These plant-derived compounds contain bioactive substances such as flavonoids, saponins, and essential oils that exhibit antimicrobial, antioxidant, and immunomodulatory properties. Recent studies demonstrate that herbal supplementation can enhance rumen microbial balance, improve nutrient digestibility, boost immune function, and increase animal performance while reducing environmental impacts like methane emissions [2-4]. For example, a recent study on heat-stressed dairy cows showed that a herbal formula improved antioxidant capacity, immune responses, and modulated rumen microbiota, ultimately supporting better production performance under stress conditions [5]. Another comprehensive review highlighted that phytobiotics increase feed palatability and intake, stimulate digestive enzymes, and provide immunostimulatory benefits, contributing to healthier and more efficient ruminant production systems [6]. Collectively, these findings underscore the potential of medicinal herbs as sustainable, effective feed additives that can enhance ruminant health and productivity, and support the reduction of antibiotic use in livestock systems. Their integration into animal nutrition strategies, particularly in developing countries, offers a feasible approach to improving animal-derived food quality and safety while addressing public health concerns. Essential oils from medicinal plants contain diverse bioactive compounds that can stimulate appetite and enhance the secretion of digestive enzymes in the gastrointestinal tract, subsequently influencing the concentration of blood metabolites in ruminants [7]. Recent studies demonstrated that essential oils and herbal powders possess antimicrobial activity by disrupting microbial cell membranes, inhibiting the growth of certain gram-positive and gram-negative bacteria. This shift in rumen microbial populations reduces amino acid deamination and methanogenesis processes, leading to lower ruminal ammonia, methane, and acetate concentrations, while increasing propionate and butyrate levels [8]. In addition to numerous reports highlighting the positive effects of medicinal plants on animal performance and production volume, several studies have also documented improvements in meat quality traits. These improvements include reduced postmortem meat acidity and microbial load, decreased oxidation of hemoglobin which preserves meat color during storage, reduced oxidation of unsaturated fatty acids in body lipids, and enhanced flavor and aroma of meat [9,10,11]. Such effects are crucial in intensive production systems where limited access to fresh forage reduces antioxidant intake, diminishing tissue antioxidant capacity of the animals. Therefore, exploring natural compounds like medicinal plants to improve antioxidant capacity in tissues is essential for sustaining animal health and consequently human health, especially given rising concerns about synthetic antioxidants' carcinogenic potential [12]. One notable concern regarding red meat consumption is its association with cardiovascular diseases, primarily due to an imbalance in saturated versus unsaturated fatty acid ratios relative to human dietary requirements. This highlights the importance of investigating fatty acid profiles in red meat and developing strategies to enhance nutritional value by adjusting these ratios. Fatty acid composition remains a critical qualitative trait in meat science research. Recent studies reported that some essential oils can reduce hepatic cholesterol-synthesizing enzyme activity, lowering cholesterol concentration in tissues and increasing the ratio of unsaturated to saturated fatty acids in meat [13]. Despite these promising findings, limited research has specifically investigated the effects of essential oils and powdered medicinal herbs on growth performance, immunity, rumen fermentation, carcass traits, and meat quality in small ruminants. Accordingly, the present study aimed to evaluate the effects of dietary supplementation with powdered peppermint (*Mentha piperita*) and thyme (*Thymus vulgaris*) on growth performance, humoral immunity, rumen fermentation metabolites, carcass characteristics, and meat quality of Sanjabi fattening lambs.

MATERIALS AND METHODS

Ethical Statement

This study was approved by the Ethics Committee of the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and conducted in accordance with international guidelines for animal welfare."

Study Site and Experimental Design

This study was conducted at the Mehrgan Animal Husbandry Research Station, affiliated with the Agricultural and Natural Resources Research and Education Center of Kermanshah Province. The experiment consisted of both laboratory and on-farm components. Eighteen male lambs with an average initial body weight of 22.5 ± 2.5 kg were used in a completely randomized design for a 90-day fattening period to investigate the effects of medicinal herbs on growth performance and some physicochemical properties of meat.

Animal Management and Experimental Diets

Before the initiation of the fattening period, lambs were treated with antiparasitic tablets to control internal and external parasites and vaccinated against enterotoxemia. The animals underwent an adaptation period before the fattening trial commenced. During the 90-day trial, water and feed were provided ad libitum, and feed was distributed three times daily. Lambs were weighed monthly following a 14-hour fasting period, and feed intake was recorded every 15 days. The basal diet was formulated according to the National Research Council [14] guidelines using UFFDA ration formulation software, with a concentrate to forage ratio of 70:30. Table 1 provides the detailed composition of experimental diets. Treatments included a control diet (basal diet without herb additives), a basal diet contained 3% peppermint (*Mentha piperita*) powder, and a basal diet contained 3% thyme (*Thymus vulgaris*) powder. The peppermint and thyme plants were collected from a local farm in Kermanshah/Iran. Dried leaves of peppermint (Mentha piperita L.) and thyme (Thymus vulgaris L.) were ground into a fine powder and incorporated into the experimental diets. Then, the trial diets were pelleted and fed to lams.Each experimental group consisted of 16 Sanjabi lambs, with a total of 48 lambs across all groups. The experiment was conducted over a period of 90 days. The herbal powders were thoroughly mixed and pelleted with the basal diet before being offered to lambs throughout the experiment.

Table 1 Composition of experimental diets and proportion of different feed ingredients in the diets (% dry matter).

Ingredient Composition (%)	Peppermint Treatment	Thyme Treatment	Control Group
Peppermint Powder (%)	3	-	-
Thyme Powder (%)	-	3	-
Corn (%)	25	25	25
Alfalfa (%)	27	27	30
Barley (%)	22.5	22.5	22.5
Soybean Meal (%)	15	15	15
Beet Molasses (%)	5	5	5
Salt (%)	0.5	0.5	0.5
Mineral Supplement* (%)	0.5	0.5	0.5
Vitamin Supplement* (%)	0.5	0.5	0.5
Sodium Bicarbonate (%)	0.5	0.5	0.5
Limestone (%)	0.5	0.5	0.5

Ingredient Composition (%)	Peppermint Treatment	Thyme Treatment	Control Group
Metabolizable Energy (Mcal/kg DM)	2.47	2.44	2.45
Crude Protein (%)	16.10	16.00	16.23
Calcium (%)	0.75	0.65	0.64
Phosphorus (%)	0.44	0.45	0.43

*Each kilogram of vitamin premix contains 600,000 IU beta-carotene, 200,000 IU cholecalciferol, 200 mg tocopherol, 2500 mg antioxidant, 195 g calcium, 80 g phosphorus, 210,000 mg magnesium, 2200 mg manganese, 3000 mg iron, 300 mg copper, 300 mg zinc, and 100 mg cobalt.

Humoral Immunity

In this study, changes in the white blood cell counts of fattening lambs following enterotoxemia vaccination. Blood samples were collected on 14, 21, 35, and 42 days post-vaccination in Sanjabi breed lambs. Differential white blood cell counts including lymphocytes, neutrophils, eosinophils, and total leukocytes were performed using the method described by Palhares Campolina et al. [15].

Rumen Fermentation Metabolites

Volatile fatty acids (VFAs) and rumen pH were measured at the end of the feeding trial. Rumen fluid samples (~50 mL) were collected via vacuum and stomach tubing before feeding and at 2 and 4 hours post-feeding. Initial rumen fluid was discarded to avoid saliva contamination. Samples were immediately measured for pH using a portable pH meter (Testo model 230). Filtered rumen fluid was mixed with 20% metaphosphoric acid (5:1 ratio) and stored at -20°C. After thawing, samples were centrifuged and supernatants analyzed for acetate, propionate, and butyrate concentrations by gas chromatography (GC) equipped with a DB-1701 column and flame ionization detector (FID). Ammonia nitrogen concentration was determined by mixing rumen fluid with 0.2 N hydrochloric acid, centrifuged, and analyzed using the phenol-hypochlorite method [16].

Carcass Characteristics

To compare the effects of experimental treatments on carcass traits, a total of 12 lambs (4 lambs per treatment, one from each replicate) were selected. These lambs had body weights closest to the average weight of their respective replicates. After a 14-hour fasting period, animals were weighed and slaughtered. Carcass traits were studied according to Ellison et al. [17].

Meat Quality

After slaughter and measurement of other carcass parameters in the laboratory, samples were collected from the leg muscle for the evaluation of meat quality parameters and fatty acid composition. Proximate analysis, including fat, crude protein, ash, and moisture content, was performed according to the standard AOAC method [18]. To assess the level of oxidation in the loin, shoulder, and leg muscles, the standard TBARS assay was used, which measures malondialdehyde (MDA) as an indicator of lipid oxidation in meat. For this purpose, the loin sample between the 11th and 12th ribs, as well as the leg and shoulder muscles were homogenized. Tissue oxidation levels were then determined using the method of Domínguez *et al.* [19] at 0, 4, and 7 days post-slaughter. The measurement of intramuscular fatty acid composition was done according to Ozdemir et al. [20]. Analysis was conducted at the National Animal Science Research Institute. The quantity of each fatty acid was calculated based on the known concentration of standards and the relative proportions of fatty acids detected.

Statistical Analysis

The experiment was conducted as a completely randomized design with 18 lambs assigned to three treatments (control, 3% peppermint, and 3% thyme) in three replicates with two observations per replicate. Data were analyzed using the General Linear Model (GLM) procedure in SAS software version 9.1 (2000). Means were compared using Duncan's multiple range test. The statistical model used was:

$$Y_{ijk}=\mu+T_i+e_{ij}+\varepsilon_{ik}$$

where Y_{ijk} represents the observed value of the dependent variable for the k-th observation within the j-th replicate under the i-th treatment. μ denotes the overall mean response across all treatments and replicates. T_i is the fixed effect of the *i*-th treatment (e.g., control, peppermint supplementation, thyme supplementation). This term captures the systematic variation attributable to the different dietary treatments applied in the experiment. e_{ij} signifies the random error associated with the j-th replicate within the i-th treatment, accounting for variability among replicates that is not explained by treatment effects. ε_{ik} refers to the residual random error term for the k-th observation within the j-th replicate and i-th treatment, representing within-replicate variability and measurement error**.**

RESULTS AND DISCUSSION

Growth Performance (Feed Intake, Average Daily Gain, and Feed Conversion Ratio)

The effects of essential oils and their active compounds on ruminant performance, dry matter intake (DMI), and rumen fermentation remain incompletely understood. Some studies suggest that essential oils enhance performance by increasing feed intake, altering rumen microbial fermentation, and improving nutrient utilization [21]. Additionally, certain essential oils may selectively inhibit bacteria involved in biohydrogenation of unsaturated fatty acids, thereby increasing beneficial fatty acids, such as conjugated linoleic acid, in animal products [22].

In this study, ANCOVA was used to assess the influence of initial body weight on final weight outcomes with peppermint or thyme supplementation. The covariate had no significant effect ($P = 0.313$), indicating that differences in average daily gain (ADG) resulted primarily from dietary treatments. Consequently, performance traits were analyzed using ANOVA without adjusting for initial weight. As shown in Table 2, ADG varied significantly among treatments ($P < 0.05$), with the peppermint group exhibiting the highest ADG and the control group the lowest. Both peppermint and thyme supplementation increased DMI compared to the control, with the peppermint group showing the highest intake. This contrasts with Yang et al. [23], who found no effect of 200 mg cinnamaldehyde or carvacrol supplementation on DMI, ADG, or feed efficiency in barley- and corn-based diets. These discrepancies may stem from differences in supplement form (powder vs. essential oil), active compound concentration, extraction methods, or environmental conditions at plant growth sites.

Lambs fed peppermint exhibited the best feed conversion ratio (FCR), followed by the control and thyme groups. The observed improvements in growth performance and rumen fermentation may be attributed to the antimicrobial and antioxidant properties of peppermint and thyme, which could modulate ruminal microbiota and enhance enzymatic activity, as reported in previous studies [24, 25]. The lower FCR in the thyme group may reflect antagonistic effects of certain thyme compounds on rumen microorganisms, reducing feed efficiency [26, 27]. Previous studies on essential oil blends report inconsistent effects on feed intake, ADG, and FCR, likely due to variations in herb species, active ingredient concentrations, and environmental factors [28]. For instance, garlic or wild berry essential oils had no effect on DMI in dairy cows [29], whereas a 5% herbal blend including peppermint increased DMI in calves [30]. The increased ADG and DMI in this study likely result from the higher inclusion levels and powder form of peppermint and thyme, which may deliver more bioactive compounds compared to lower doses or essential oil extracts used elsewhere. Overall, peppermint supplementation outperformed thyme in enhancing the growth performance of Sanjabi lambs, likely due to improved palatability and favorable modulation of rumen microbiota.

Table 2 Effect of peppermint or thyme supplementation in the diet on daily feed intake, average daily gain, and feed conversion ratio in fattening lambs

Parameter	Peppermint	Thyme	Control	SEM	P-Value
Initial Weight (kg)	22.75a	22.80a	18.81b	0.836	0.124
Final Weight (kg)	49.58a	46.57a	39.64b	1.293	0.026
Feed Intake (g/day)	1494.37a	1488.53a	1185.55b	40.571	0.001
Average Daily Gain (g/day)	298.15a	265.83b	228.70c	11.870	0.046
Feed Conversion Ratio	5.01	6.05	5.20	0.250	0.612

SEM: Standard error of mean. Different superscript letters (a–c) in each column indicate significant differences ($P < 0.05$).

The effects of essential oils and their active compounds on ruminant performance, dry matter intake (DMI), and rumen fermentation remain partially understood. Some studies indicate that essential oils enhance performance by increasing feed intake, modulating rumen microbial fermentation, and improving nutrient utilization [31]. Additionally, certain essential oils may inhibit bacteria responsible for biohydrogenation of unsaturated fatty acids, thereby increasing beneficial fatty acids, such as conjugated linoleic acid, in animal products [32]. In a study evaluating wild thyme leaves (4 and 8 kg/ton), containing carvacrol and thymol, no significant differences were observed in DMI, final body weight, average daily gain (ADG), feed conversion ratio (FCR), or carcass traits compared to the control group in fattening lambs [29]. In contrast, dietary supplementation with essential oils rich in cinnamaldehyde and carvacrol (up to 120 mg/kg dry matter) improved final body mass and ADG in growing lambs ($P = 0.04$), aligning with the findings of this study [32]. Uyarlar *et al.* [33] reported increased DMI in dairy cows receiving 1 g/day of a mixed herbal essential oil blend (approximately 42 mg/kg diet). Similarly, garlic oil supplementation enhanced feed intake, digestibility, performance, and rumen function in goats, suggesting its potential as an alternative additive for poor-quality feeds [34]. The positive effects of essential oils and plant extracts on feed intake and growth are primarily attributed to their aromatic compounds, which stimulate gustatory and olfactory nerves, enhance appetite, and promote digestive enzyme activity. These compounds also improve intestinal nutrient absorption. However, their efficacy is dose-dependent, with lower concentrations often yielding insignificant improvements [34, 35].

Humoral Immunity

Medicinal plants, such as peppermint and thyme, have gained attention for their beneficial effects on the immune system of sheep and lambs, particularly on white blood cells and humoral immunity. Their active compounds, including thymol and carvacrol, exhibit antioxidant, anti-inflammatory, and antimicrobial properties that bolster immune responses [36]. These compounds enhance the activity of key immune cells, such as lymphocytes and neutrophils, and stimulate antibody production, thereby strengthening disease resistance [37]. Incorporating medicinal plants into diets offers a sustainable approach to improving livestock health and reducing antibiotic use, enhancing productivity in ruminant farming. In this study, dietary inclusion of peppermint or thyme powders did not significantly affect the percentages of white blood cells (neutrophils, lymphocytes, monocytes, and eosinophils) at 7 and 14 days post-vaccination ($P > 0.05$; Table 3). However, at 21 days post-enterotoxemia vaccination, the peppermint group showed a significant reduction in neutrophil percentage compared to the thyme and control groups ($P < 0.05$). This decrease likely reflects the immunostimulatory effect of peppermint, which enhances humoral immunity, leading to a relative increase in lymphocyte numbers and a corresponding reduction in neutrophils, the first line of defense, by day 21. No significant differences in white blood cell counts were observed at 35 and 42 days post-vaccination ($P > 0.05$).

Table 3 Effect of Incorporating Peppermint or Thyme into the Diet on Blood Cell Percentages at Different Time Points Following Enterotoxemia Vaccination in Fattening Lambs

Parameter / Treatment	Peppermint	Thyme	Control	SEM	P-Value
7 days post-vaccination					
Neutrophils (%)	47.66	43.50	40.80	3.73	0.779
Lymphocytes (%)	52.00	56.33	59.00	3.78	0.776
Monocytes (%)	0.33	0.59	0.20	0.12	0.577
Eosinophils (%)	-	0.16	-	0.05	0.427
14 days post-vaccination					
Neutrophils (%)	51.66	47.66	55.66	2.23	0.366
Lymphocytes (%)	48.33	52.33	44.00	2.22	0.331
Monocytes (%)	-	-	0.33	0.11	0.391
Eosinophils (%)	-	-	-	-	-
21 days post-vaccination					
Neutrophils (%)	25.00 c	39.50 ab	41.50 a	3.22	0.065
Lymphocytes (%)	74.33 a	60.50 ab	58.00 c	3.13	0.063
Monocytes (%)	-	-	-	-	-
Eosinophils (%)	0.66	-	0.50	0.22	0.391
35 days post-vaccination					
Neutrophils (%)	46.00	49.00	47.83	1.80	0.821
Lymphocytes (%)	45.00	50.66	51.33	2.01	0.807
Monocytes (%)	-	-	-	-	-
Eosinophils (%)	-	0.33	-	0.11	0.427
42 days post-vaccination					
Neutrophils (%)	46.00	49.00	47.83	1.80	0.821
Lymphocytes (%)	54.00	50.66	51.33	2.01	0.807
Monocytes (%)	-	-	-	-	-
Eosinophils (%)	-	0.33	-	0.11	0.427

SEM: Standard Error of the Mean.
Different superscript letters (a–c) within each row indicate statistically significant differences ($P < 0.05$).

Dietary supplementation with chamomile essential oil (30–100 mg/kg body weight) did not alter blood lymphocyte concentrations compared to the control group [38], consistent with the present findings. Similarly, Zhang *et al*. [39] reported that essential oils, including cinnamaldehyde, garlic oil, and juniper berry oil, had no significant effect on white blood cell counts in dairy cows.

Rumen Fermentation Metabolites

Rumen fermentation parameters are presented in Table 4. No significant differences in rumen fluid pH were observed among treatments ($P > 0.05$), although significant variations occurred at 0, 2, and 4 hours post-feeding. Treatments with medicinal plants exhibited lower pH values than the control group. Yu *et al*. [40] found that thymol supplementation had minimal impact on rumen pH in goats during in vitro experiments, aligning with these results. Similarly, BenJemaa *et al*. [42] reported no significant pH changes with Thymbra capitata essential oil (rich in carvacrol) in batch cultures. However, Alabi *et al*. [43] observed slight pH reductions with essential oil blends containing oregano components, contrasting with the current findings. Variations in outcomes may arise from differences in essential oil dosage, form, diet composition, animal breed, experimental conditions (in vivo vs. in vitro), and adaptation period length [41, 45].

Ammonia nitrogen concentrations, measured at various post-feeding intervals, are shown in Tables 4–5. Significant differences among treatments were observed ($P < 0.05$), with peppermint and thyme reducing ammonia nitrogen levels, likely by enhancing nitrogen efficiency in the rumen. Active compounds in Lamiaceae family plants, such as peppermint and thyme, reduce rumen ammonia concentrations and increase protein flow to the lower digestive tract [44]. Protein degradation occurs in two stages: initial hydrolysis by gram-positive bacteria releases peptides and amino acids, followed by deamination, producing ammonia. This ammonia is either converted to microbial protein or absorbed and transformed into urea in the liver [40]. Excessive deamination leads to protein and energy loss, but controlling protein-degrading microbes can enhance protein bypass to the intestine. Carvacrol and thymol in essential oils (e.g., thyme, peppermint) exhibit antimicrobial properties that promote protein-to-peptide conversion while inhibiting peptide-to-amino acid breakdown, reducing ammonia production [41]. BenJemaa *et al*. [42] noted that carvacrol selectively modulates microbial activity, inhibiting specific rumen bacteria at certain concentrations. In this study, reduced ammonia nitrogen in treatments with medicinal plants suggests improved nitrogen absorption and utilization [43]. Roy *et al.* [44] reported that thyme oil at 600 ppm lowered ammonia nitrogen in buffalo rumen liquor without affecting branched-chain volatile fatty acids. Similarly, Foggi *et al.* [45] and Ramos-Morales *et al.* [41] found that carvacrol and thymol reduced ammonia nitrogen in vitro while increasing peptide and amino acid concentrations, though higher doses occasionally showed no effect. These inconsistencies may stem from differences in methodology and experimental conditions.

Table 4 Effect of Adding Peppermint or Thyme Medicinal Plants to the Diet on pH, Ammonia Nitrogen, and Volatile Fatty Acid Composition of Rumen Fluid at the End of the Fattening Period

Parameter / Treatment	Control	Thyme	Peppermint	SEM	P-Value Treatment × Time	P-Value Time	P-Value Treatment
pH	6.05 b	6.12 b	7.05 a	0.083	0.0001	0.502	0.376
Ammonia Nitrogen (mg/dL)	9.94 b	14.01 a	7.95 c	1.35	0.0001	0.145	0.192
Acetate (mmol/L)	24.24	24.92	23.01	0.678	0.678	0.068	0.678
Propionate (mmol/L)	19.56	20.56	16.46	0.750	0.446	0.077	0.382
Butyrate (mmol/L)	2.09	3.09	2.75	0.381	0.835	0.191	0.068
Valerate (mmol/L)	0.25 a	0.30 b	0.25 b	0.106	0.004	0.377	0.106
Total Volatile Fatty Acids (mmol/L)	48.10	48.11	42.88	0.833	0.541	0.850	0.388
Total Fatty Acids (mmol/L)	52.20	50.94	54.02	0.646	0.382	0.007	0.646
Acetate (%)	52.20	50.94	54.02	0.748	0.468	0.474	0.383
Propionate (%)	38.59	38.89	40.91	0.733	0.210	0.349	0.591
Butyrate (%)	1.37	1.21	1.47	0.584	0.217	0.009	0.011
Acetate to Propionate Ratio	1.68 b	1.22 a	1.16 a	—	—	—	—

- Volatile fatty acid concentrations are expressed in mmol/L; ammonia nitrogen in mg/dL.
- SEM: Standard Error of the Mean.
- Different superscript letters (a-c) within each row indicate significant differences ($P < 0.05$).
- Measurements were taken at 0, 2, and 4 hours relative to feeding time.

This study did not directly investigate changes in ruminal microbiota or enzymatic activity, which warrants further exploration in future research.

Carcass Characteristics

Carcass trait results are presented in Table 5. Peppermint powder supplementation increased live weight, skin weight, tail fat weight, mesenteric fat weight, and rumen capacity compared to the thyme and control groups ($P < 0.05$). Both peppermint and thyme powders enhanced hot and cold carcass weights, intramuscular fat, heart weight, and lung weight in fattening lambs compared to the control ($P < 0.05$). Other measured traits showed no significant differences among treatments ($P > 0.05$). In contrast, Marcel et al. [46] found no significant effects on carcass traits—including hot and cold carcass weights, fat tissue, and internal organs (liver, lungs, heart, spleen, kidneys, testes, small and large intestines, and cecum)—when supplementing fattening lamb diets with a blend of essential oils (thyme leaf, daphne leaf, tea sage, phenol, orange cortex, and myrtle) at 1 g/kg dry matter. Similarly, Yang et al. [29] reported that adding 200 mg/kg cinnamaldehyde or carvacrol to barley- and corn-based diets did not affect carcass traits in growing lambs. These discrepancies may result from variations in plant species, active compound concentrations, methods of dietary incorporation, or other experimental factors.

Table 5 Effect of Adding Peppermint or Thyme Medicinal Plants to the Diet on Warm Carcass Components in Fattening Lambs

Parameter / Treatment	Peppermint	Thyme	Control	SEM	P-Value
Live Weight (kg)	53.83 a	50.31 b	44.83 c	13.405	0.001
Warm Carcass Weight (kg)	23.41 a	22.79 a	19.84 b	5.854	0.002
Full Gastrointestinal Tract Weight (g)	533.5	530.5	441.4	386.0	0.184
Intramuscular Fat (g)	771.00 b	825.00 a	790.00 b	53.816	0.049
Mesenteric Fat Weight (g)	128.33 a	105.00 ab	90.00 c	107.77	0.025

Parameter / Treatment	Peppermint	Thyme	Control	SEM	P-Value
Carcass Yield (%)	43.50	45.47	42.24	0.455	0.200
Front and Hind Limb Percentage (%)	2.18	2.15	2.32	0.047	0.421
Liver Percentage (%)	3.793	4.26	3.99	0.182	0.641
Spleen Percentage (%)	0.36	0.68	0.51	0.092	0.424
Heart Percentage (%)	0.83	0.85	0.84	0.186	0.912
Lung Percentage (%)	2.52	2.48	0.24	0.082	0.385
Kidney Percentage (%)	0.6049	0.6218	0.67	0.175	0.208
Rumen Capacity (mL)	4491.66 a	3451.66 ab	2813.33 c	309.529	0.053

Note: Similar letters in each row indicate no significant difference between experimental treatments (P > 0.05).

The results related to cold carcass composition are presented in Table 6. The percentage of loin, weight of the back, weight of the leg, and cross-sectional area of the loin meat were affected by the medicinal plants and were higher than those in the control treatment (P < 0.05). Other measured traits showed no significant differences among the three experimental treatments (P > 0.05).

Table 6 Effect of Adding Peppermint or Thyme Medicinal Plants to the Diet on Cold Carcass Components in Fattening Lambs

Parameter / Treatment	Peppermint	Thyme	Control	SEM	P-Value
Hot Carcass Weight (kg)	23.41 a	22.79 a	19.84 b	0.581	0.002
Cold Carcass Weight (kg)	22.90 a	21.99 a	19.30 b	0.572	0.002
Cold Carcass Yield (%)	43.50	45.47	42.24	0.451	0.200
Front Limb Percentage (%)	18.48	18.81	18.58	0.137	0.666
Neck Percentage (%)	8.70	7.90	7.79	0.401	0.668
Back Percentage (%)	21.50	17.99	21.28	1.271	0.513
Loin Percentage (%)	3.74 a	4.51 a	4.89 b	0.200	0.023
Backfat Percentage (%)	13.27	13.47	12.49	0.324	0.493
Leg Percentage (%)	29.51	28.68	28.98	0.397	0.749
Brisket Percentage (%)	17.91	17.98	17.73	0.355	0.967
Kidney Fat Percentage (%)	3.92	4.74	4.55	0.233	0.370
Backfat Thickness (cm)	0.413	0.453	0.413	0.156	0.425
Loin Cross-sectional Area (cm^2)	19.38 a	18.00 ab	15.52 c	0.726	0.062

Notes:

- SEM: Standard Error of the Mean.
- Different superscript letters (a-c) within each row indicate statistically significant differences (P < 0.05).

A study examining oregano supplementation (3%, 5%, and 10% of the diet) in fattening calves found that the 10% oregano essential oil treatment increased loin cross-sectional area compared to other treatments, while other carcass traits remained unaffected [47]. Previous research highlights that medicinal plants improve both qualitative and quantitative carcass traits, consistent with the present study. Hot carcass weight differed among treatments ($P < 0.01$), with peppermint and thyme supplementation increasing weights of key body parts, including the leg, back, and brisket, compared to the control group ($P < 0.05$). Karami *et al.* [48] reported that essential oils (0.5% oregano, 0.5% turmeric, and 400 mg/kg alpha-tocopherol) had no effect on carcass weight loss during refrigerated storage at 4°C in fattening goat kids. In this study, peppermint supplementation enhanced live weight, carcass weight, and loin cross-sectional area. In contrast, Ornaghi *et al.* [49] found that cold carcass weight was greater in bulls fed essential oil-supplemented diets ($P < 0.05$), but no differences were observed between clove and cinnamon essential oil treatments ($P > 0.05$). Additionally, key carcass quality traits—such as muscle, fat, and bone proportions, fat thickness, marbling score, Longissimus muscle area, and meat pH—showed no significant differences across treatments. These discrepancies may stem from variations in animal species or essential oil inclusion levels.

Meat Quality

Herbal additives and essential oils effectively maintain or enhance meat quality parameters without altering proximate chemical composition, including moisture, protein, fat, and ash content [50]. Their antimicrobial properties help prevent protein and lipid degradation during storage, preserving meat's chemical composition and sensory qualities. Incorporating herbal powders or essential oils into diets or using them as natural preservatives in meat processing extends shelf life and offers a sustainable alternative to synthetic additives. The chemical composition of meat is shown in Table 7. No significant differences were observed in dry matter, crude protein, ash, or fat content among treatments in fattening lambs ($P > 0.05$). Castellanos *et al.* [51] reported that thyme leaf extract supplementation in ewes reduced loin muscle protein content and increased fat content compared to the control. Similarly, Ünlü *et al.* [52] found that adding 300 mg/kg oregano essential oil to lamb diets did not affect slaughter or carcass quality traits, such as dry matter, protein, or pH, but increased intramuscular fat content, enhancing meat flavor and tenderness. Other studies indicate that oregano essential oil delays lipid oxidation, improving meat quality and shelf life without altering chemical composition [53].

Table 7 Chemical Composition (%) of Meat from Fattening Lambs

Trait	Peppermint	Thyme	Control	SEM	P-Value
Dry Matter	25.94	27.19	25.97	0.320	0.240
Crude Protein	21.24	21.35	21.21	0.191	0.960
Ash	1.32	1.47	1.22	0.082	0.610
Fat	2.46	2.96	3.09	0.250	0.580

Note:
SEM = Standard Error of the Mean.
Different letters within each row indicate statistically significant differences ($P < 0.05$).

The fatty acid composition of leg meat from fattening lambs supplemented with peppermint or thyme is presented in Table 8. Incorporating these medicinal plants into the diet can improve the balance of fatty acids deposited in sheep muscles. For optimal human health, the ratio of long-chain unsaturated fatty acids to saturated fatty acids (PUFA/SFA) should exceed 0.45, and the omega-6 to omega-3 polyunsaturated fatty acid (PUFA) ratio should be below 4, ideally approaching 1. However, typical PUFA/SFA ratios in sheep and beef meats are approximately 0.15 and 0.11, respectively, while the omega-6 to omega-3 ratios are 1.32 and 2.11, respectively. These values indicate that the fatty acid profile of ruminant meat generally deviates from nutritional recommendations, potentially increasing the risk of cardiovascular diseases in consumers [54, 55]. Consequently, there is growing interest in using plants and organic feed additives to improve the omega-6 to omega-3 balance in meat.

Table 8 Effects of Dietary Supplementation with Peppermint or Thyme on Fatty Acid Composition (% of Identified Fatty Acids) of Cold Shoulder Meat in Sanjabi Fattening Lambs

Fatty Acid	Peppermint	Thyme	Control	SEM	P-Value
C10:0	-	-	-	-	-
C12:0	0.11	-	-	-	-
C14:0	2.57	2.66	2.68	0.026	0.079
C14:1	2.75	2.66	2.68	0.080	0.914
C16:0	16.28	16.41	18.12	0.739	0.591
C16:1	1.93	1.98	2.03	0.117	0.959
C18:0	11.05	10.23	11.34	0.272	0.242
C18:1	30.16	31.16	32.01	1.091	0.832
C18:2	14.15	12.95	11.67	0.841	0.553
C18:3	1.23	1.63	0.94	0.154	0.192
Total Saturated Fatty Acids	30.20	29.30	32.13	0.843	0.431
Total Unsaturated Fatty Acids	61.97	61.59	59.22	1.281	0.702
Saturated to Unsaturated Fatty Acids Ratio	0.49	0.47	0.54	0.040	0.490

Notes:

- Fatty acid composition is expressed as the weight percentage of identified fatty acids.
- SEM: Standard Error of the Mean.

- No statistically significant differences were observed among treatments for any fatty acid or derived parameter (P > 0.05), as indicated by the absence of different superscript letters within each row.
- Missing data for C10:0 and C12:0 (denoted by "-") indicate that these fatty acids were either not detected or not quantifiable in the respective treatments.

Dietary supplementation with peppermint or thyme powders improved the saturated to unsaturated fatty acid ratio and reduced lipid oxidation, as measured by thiobarbituric acid reactive substances (TBARS), particularly in leg meat. Both herbs increased omega-3 unsaturated fatty acids while lowering TBARS levels. These benefits likely stem from the antioxidant properties of active compounds, such as thymol, carvacrol, and menthol, which are abundant in peppermint and thyme. Higher linoleic acid content enhances meat flavor and organoleptic properties but increases susceptibility to lipid oxidation due to insufficient natural antioxidants [56]. Among long-chain unsaturated fatty acids, linoleic acid (C18:2 n-6) influences TBARS values, with higher proportions elevating oxidation levels [57]. Elevated TBARS values above 0.5 mg MDA/kg produce off-odors detectable by consumers, negatively affecting beef flavor, redness, and texture while increasing undesirable flavors and softness [58, 59].

In fattening animals, limited vitamin E levels exacerbate lipid and myoglobin oxidation during meat storage, leading to off-flavors and color changes. Thymol, carvacrol, and menthol in peppermint and thyme provide antioxidant and antimicrobial effects, reducing meat pH below 5.8 and lowering microbial load post-slaughter. Phenolic compounds in thyme inhibit hemoglobin oxidation and methemoglobin formation, preserving meat color [60]. The reduction in TBARS with thyme supplementation results from phenolic groups with conjugated rings and hydroxyl groups, which scavenge free radicals and chelate metal ions [61]. Additionally, essential oils from these herbs reduce hepatic cholesterol-synthesizing enzyme activity, lowering tissue cholesterol, and mitigate the age-related decline in the unsaturated to saturated fatty acid ratio [62, 63]. This study demonstrates that peppermint and thyme supplementation enhances fatty acid profiles and controls oxidative degradation in lamb meat, improving meat quality and supporting consumer health.

Table 8 Effect of Dietary Supplementation with Peppermint or Thyme on Lipid Oxidation Measured by TBARS (mg malondialdehyde/kg meat) in Fattening Lamb Meat

Trait / Treatment	Peppermint	Thyme	Control	SEM	P-Value
Leg					
At 0 days post-slaughter	0.21 a	0.23 ab	0.31 c	0.021	0.075
At 4 days post-slaughter	0.23 a	0.32 b	0.41 c	0.028	0.002
At 7 days post-slaughter	0.32 a	0.34 a	0.47 b	0.028	0.046
Shoulder and Neck					
At 0 days post-slaughter	0.20	0.15	0.16	0.014	0.350
At 4 days post-slaughter	0.23 a	0.25 a	0.32 b	0.016	0.025
At 7 days post-slaughter	0.25 a	0.27 a	0.43 b	0.028	0.001
Loin					
At 0 days post-slaughter	0.33	0.29	0.23	0.018	0.112
At 4 days post-slaughter	0.37 a	0.33 ab	0.43 c	0.019	0.080
At 7 days post-slaughter	0.43	0.37	0.43	0.018	0.355

SEM: Standard Error of the Mean.

Different superscript letters in each row indicate statistically significant differences (P < 0.05).

CONCLUSION

The inclusion of peppermint or thyme medicinal plants in the diet improved growth performance, feed conversion ratio, and carcass yield in fattening lambs, while also enhancing immune function during this stage. Additionally, supplementation with these herbs improved rumen fermentation by reducing ammonia nitrogen concentration and decreasing the acetate to propionate ratio in fattening lambs. Moreover, feeding peppermint and thyme led to extended meat shelf life by reducing lipid oxidation. Although positive effects were observed in the present study regarding the impact of these two medicinal plants, definitive conclusions cannot be drawn from a single study alone. Further research and replication of these results in different trials are necessary. Considering the economic benefits associated with the use of medicinal plants as observed in this research, final recommendations on their advantages in ruminant diets can be made once their efficacy is consistently confirmed. This study is the first to demonstrate that dietary supplementation with peppermint and thyme powders can serve as effective alternatives to antibiotics in Sanjabi fattening lambs, improving growth performance, immunity, and meat quality while supporting sustainable livestock production. Also, future research could compare these herbal additives with other natural (e.g., probiotics) or chemical additives (e.g., ionophores) to provide a more comprehensive understanding of their relative efficacy.

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