Impact of Dietary Thyme Extract (Thymus vulgaris) on Rumen Fermentation, Methane Production, and Growth Performance in Awassi Lambs

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Abstract

This study aimed to evaluate the effects of adding thyme extract (*Thymus vulgaris*) at two different concentrations (250 and 500 mg/kg dry matter) on rumen fermentation, methane production, and growth performance in Awassi lambs. The experiment included 45 lambs (average initial weight 35.2 ± 0.5 kg) randomly allocated to three groups: a control group (0 mg/kg), T1 (250 mg/kg), and T2 (500 mg/kg). The results showed that the addition of the extract at 500 mg/kg significantly improved ($P < 0.05$) rumen fermentation by increasing total volatile fatty acids (104.3 ± 5.2 mmol/L) and the proportion of propionate (24.5 ± 1.3%), while it decreased the acetate-to-propionate ratio (2.45 ± 0.1) and ammonia concentration (9.8 ± 0.5 mg/dL). Methane production was reduced by 42.4% in the T2 group compared to the control. Growth performance improved significantly, with treated lambs achieving higher average daily gain (205 ± 4.8 g/day) and better feed conversion ratio (6.90 ± 0.18). Carcass traits were also improved, including higher dressing percentage (58.4 ± 1.3%) and reduced fat thickness (2.2 ± 0.3 mm). These results are attributed to the bioactive properties of thymol and carvacrol, which modulate the rumen microbiota and inhibit methanogenesis. The study concludes that thyme extract at 500 mg/kg dry matter can improve sheep productivity while reducing environmental impact, supporting its use as a sustainable feed additive.

Keywords: Thyme extract, rumen fermentation, methane reduction, Awassi sheep, growth performance, phytogenic additives

1. Introduction

The livestock sector faces a critical challenge in balancing productivity with environmental sustainability. Ruminants, particularly sheep, contribute significantly to global methane (CH_4) emissions—a greenhouse gas with 28–34 times the global warming potential of CO_2 over a 100-year horizon (IPCC, 2022). Methanogenesis in the rumen represents not only an environmental liability but also a 2–12% loss of dietary energy, undermining feed efficiency (Hristov et al., 2018). With global demand for meat and dairy projected to rise by 70% by 2050 (FAO, 2021), sustainable strategies to mitigate enteric methane while enhancing animal performance are urgently needed.

Synthetic feed additives like monensin have demonstrated methane-reducing effects but face growing restrictions due to antimicrobial resistance concerns and consumer preference for organic production systems (Thamsborg et al., 2018). This has spurred interest in phytogenic feed additives, particularly essential oils, which modulate rumen fermentation through selective antimicrobial activity (Cobellis et al., 2016). Among these, thyme (Thymus vulgaris) extract—rich in thymol (42.3 mg/g) and carvacrol (28.7 mg/g)—has emerged as a promising candidate due to its potent inhibitory effects on methanogenic archaea and protozoa (Benchaar et al., 2008).

In vitro studies report that thyme extract can reduce methane production by 15–30% by redirecting hydrogen away from methanogenesis toward propionate synthesis, a more energetically efficient pathway (Patra & Yu, 2014). However, translational data from in vivo trials—especially in arid-adapted breeds like Awassi sheep, which are pivotal to Middle Eastern and Mediterranean livestock systems—remain limited. Existing field studies often lack dose-

response analyses or fail to evaluate carcass and meat quality impacts (Ribeiro et al., 2020), creating a gap between experimental evidence and practical application.

This study addresses these limitations by investigating the dose-dependent effects of thyme extract (0, 250, and 500 mg/kg DM) on rumen fermentation dynamics, methane emissions, and growth performance in Awassi lambs, while also evaluating carcass traits and meat quality parameters.

2. Materials and Methods

2.1. Location and Experimental Design

The experiment was conducted on a private farm in Shaqlawa, Erbil Governorate, Iraq. A total of 45 Awassi lambs (average body weight 35.2 ± 0.5 kg) were used in the trial. The lambs were randomly divided into three treatment groups (15 lambs per group):

- Control (C): basal diet without thyme extract
- T1: basal diet + 250 mg thyme extract/kg DM
- T2: basal diet + 500 mg thyme extract/kg DM

The experimental period lasted 12 weeks, including a 2-week adaptation period.

2.2. Diets and Thyme Extract

The basal diet consisted of 60% barley straw and 40% commercial concentrate, with an estimated metabolizable energy of 2.6 Mcal/kg DM.

Table 1. Composition and nutritional analysis of the basal diet (DM basis)

Component	Details	Value	Method/Analysis
Ingredients			
Barley straw	Chopped (2-3 cm), sun-cured	60%	-
Commercial concentrate*	Corn grain (35%), soybean meal (25%), wheat bran (20%), barley (15%), vitamin-mineral premix (5%)	40%	Manufacturer's data
Chemical composition			
Dry matter (DM, %)		90.2	AOAC (2005; Method 934.01)
Crude protein (% DM)		14.5	Kjeldahl (AOAC 984.13)
Neutral detergent fiber (NDF, % DM)		42.3	Van Soest et al. (1991)
Acid detergent fiber (ADF, % DM)		26.7	Van Soest et al. (1991)
Ether extract (EE, % DM)		3.1	AOAC (920.39)
Ash (% DM)		6.8	AOAC (942.05)
Energy & minerals			
Metabolizable energy (Mcal/kg DM)		2.6	NRC (2007)
Calcium (% DM)		0.8	AOAC (968.08)
Phosphorus (% DM)		0.5	AOAC (965.17)

*The vitamin-mineral premix per kg concentrate contained Vitamin A (8,000 IU), Vitamin D_3 (2,000 IU), Vitamin E (50 mg), and trace minerals (Cu, Zn, Mn, Se, I).

2.3. Extract Preparation

Thyme extract was prepared by hot water extraction followed by spray drying. GC-MS analysis identified the major compounds:

- Thymol (42.3 g/kg)
- Carvacrol (28.7 g/kg)

- p-Cymene (12.1 g/kg)
- γ-Terpinene (5.2 g/kg)

Purity was >98% (HPLC-PDA, Agilent 1260).

2.4. Laboratory Equipment

- GC-MS: Agilent 7890B/5977A
- Semi-automatic gas analyzer: ANKOM GF-2
- VFA analyzer: Agilent 1260 Infinity II HPLC
- Incubator: Kubota SE350

2.5. Sample Collection and Analysis

Rumen fluid samples were collected at the end of the trial (12 hours post-feeding). They were analyzed for pH, volatile fatty acids (VFA), ammonia nitrogen (NH_3–N), and methane production. Live weight was recorded weekly, and average daily gain (ADG) was calculated. Feed intake was measured, and feed conversion ratio (FCR) was calculated as feed intake/weight gain.

2.6. Carcass Evaluation

At the end of the trial, lambs were slaughtered to measure carcass characteristics, including hot and cold carcass weight, dressing percentage, subcutaneous fat thickness, and meat quality traits (moisture, protein, fat, meat color L*).

2.7. Statistical Analysis

Data were analyzed using one-way ANOVA (SAS version 9.4). When significant differences

were detected, Tukey's HSD test was applied to separate means. Statistical significance was declared at $P < 0.05$.

3. Results

3.1. Effect of Thyme Extract on Rumen Fermentation

Table 2. Effect of thyme extract supplementation on rumen fermentation parameters in Awassi lambs

Parameter	Control	T1 (250 mg/kg)	T2 (500 mg/kg)
Total VFA (mmol/L)	92.5 ± 3.2	98.7 ± 4.1*	104.3 ± 5.2**
Acetate (%)	65.2 ± 1.8	63.5 ± 1.6	60.1 ± 1.4**
Propionate (%)	18.3 ± 0.9	21.7 ± 1.1*	24.5 ± 1.3**
Acetate:Propionate	3.56 ± 0.2	2.93 ± 0.1*	2.45 ± 0.1**
Ammonia (mg/dL)	12.7 ± 0.8	11.2 ± 0.6*	9.8 ± 0.5**
Rumen pH	6.8 ± 0.1	6.7 ± 0.1	6.6 ± 0.1*

*Values are mean ± SD; *P < 0.05, **P < 0.01 vs. control.

Table (2) observed impact how adding different amounts of thyme extract to the diet of Awassi lambs changed their rumen (the first stomach of a sheep) fermentation.

- **Total VFA (Volatile Fatty Acids):** This is the main energy source for the animal. Both levels of thyme extract (T1 and T2) significantly increased total VFA, with the higher dose (T2) having a greater effect.
- **Acetate and Propionate:** These are individual types of VFAs. The percentage of **acetate** significantly decreased only at the high dose (T2), while the percentage of **propionate** significantly increased at both doses.
- **Acetate:Propionate Ratio:** This ratio significantly decreased with thyme extract. A lower ratio is often associated with more efficient energy metabolism.

- **Ammonia:** This is a waste product. Thyme extract significantly reduced ammonia levels, meaning less protein was wasted in the rumen.
- **Rumen pH:** The acidity level. Only the high dose (T2) caused a small but statistically significant decrease in pH.

For Significance:

- **Significant Effects:** Total VFA, Propionate, Acetate:Propionate ratio, and Ammonia were significantly different from the control at both T1 and T2 doses. Acetate was only significantly lower at the T2 dose. Rumen pH was only significantly lower at the T2 dose.
- **Non-Significant Effects:** Acetate in the T1 group and Rumen pH in the T1 group were not significantly different from the control.

3.2. Methane Production

Table 3. Effect of thyme extract on methane production and gas yield in Awassi lambs

Parameter	Control	T1 (250 mg/kg)	T2 (500 mg/kg)
Gas production (ml/g DM)	45.2 ± 2.1	42.8 ± 1.9*	40.5 ± 1.7**
Methane (ml/g DM)	12.5 ± 1.3	9.8 ± 0.9*	7.2 ± 0.7**
Methane reduction (%)	-	21.6%	42.4%

*Values are mean ± SD; *P < 0.05, **P < 0.01 vs. control.

In this table shows how impact of thyme extract affected gas and methane production in Awassi lambs.

- **Gas Production (ml/g DM):** This is the total gas produced from digesting one gram of dry matter (DM). Both doses of thyme extract (T1 and T2) caused a significant decrease in total gas.
- **Methane (ml/g DM):** This is the amount of methane produced from one gram of dry matter. Both doses of thyme extract significantly reduced methane production.

- **Methane Reduction (%):** This calculates the percentage decrease in methane compared to the control group. The low dose (T1) reduced methane by 21.6%, and the high dose (T2) reduced it by 42.4%.
- **Significant Effects:** All parameters measured (Gas Production and Methane Production) showed a statistically significant improvement at both the T1 and T2 doses compared to the control. The effects were stronger (indicated by **) at the higher 500 mg/kg dose.
- **Non-Significant Effects:** There are no non-significant effects reported in this table for the tested parameters. Both doses of thyme extract had a measurable and significant impact.

3.3. Growth Performance

Table 4. Effect of thyme extract supplementation on growth performance in Awassi lambs

Treatment	Initial weight (kg)	Final weight (kg)	ADG (g/day)	Total gain (kg)	Feed intake (kg)	FCR (kg feed/kg gain)
Control	35.2 ± 0.7a	46.8 ± 1.1a	180 ± 6.2a	11.6 ± 0.5a	90.5 ± 2.3a	7.80 ± 0.21a
T1	35.3 ± 0.6a	48.7 ± 1.0ab	192 ± 5.7ab	13.4 ± 0.4ab	97.8 ± 2.0ab	7.30 ± 0.19ab
T2	35.1 ± 0.5a	50.4 ± 1.3b	205 ± 4.8b	15.3 ± 0.6b	105.6 ± 2.7b	6.90 ± 0.18b

Different superscripts within a column indicate significant differences at $P < 0.05$ (Tukey's test).

In table (4) shows the effect of thyme extract on the growth performance of Awassi lambs over a feeding period.

- **Initial Weight (kg):** The starting weight of all lamb groups was the same, with no significant difference.

- **Final Weight (kg):** Lambs in the T2 group (500 mg/kg) had a significantly higher final weight than the Control group. The T1 group was intermediate and not statistically different from either.
- **ADG (Average Daily Gain) (g/day):** The T2 group grew significantly faster each day than the Control group.
- **Total Gain (kg):** The total weight gained over the entire period was significantly higher for the T2 group compared to the Control.
- **Feed Intake (kg):** Lambs in the T2 group consumed significantly more total feed than the Control group.
- **FCR (Feed Conversion Ratio):** This measures efficiency: how much feed is needed for one kg of weight gain. A lower FCR is better. The T2 group had a significantly better (lower) FCR than the Control.
- **Significant Effects:** The high dose of thyme extract (T2) led to significant improvements in **Final Weight, ADG, Total Gain, Feed Intake, and FCR** compared to the Control group.
- **Non-Significant Effects:** The **Initial Weight** was the same for all groups. The low dose (T1) showed a positive trend but its results for Final Weight, ADG, Total Gain, and FCR were **not significantly different** from either the Control or the T2 group, placing it in an intermediate position.

3.4. Carcass and Meat Traits

Table 5. Effect of thyme extract supplementation on carcass characteristics and meat composition in Awassi lambs

Trait	Control	T1	T2
Hot carcass (kg)	19.5 ± 0.9a	20.6 ± 1.0ab	22.1 ± 1.2b
Cold carcass (kg)	18.9 ± 0.8a	20.1 ± 0.9ab	21.6 ± 1.0b
Dressing (%)	54.3 ± 1.2a	56.4 ± 1.1ab	58.4 ± 1.3b
Fat thickness (mm)	3.2 ± 0.5a	2.7 ± 0.4ab	2.2 ± 0.3b
Meat color (L*)	48.1 ± 1.3a	50.3 ± 1.1ab	52.0 ± 0.9b
Moisture (%)	72.5 ± 1.0a	73.2 ± 0.8ab	74.0 ± 0.7b
Protein (%)	20.2 ± 0.6a	21.0 ± 0.7ab	21.9 ± 0.5b
Fat (%)	5.1 ± 0.4a	4.5 ± 0.3ab	4.0 ± 0.2b

Values with different superscripts differ significantly at $P < 0.05$.

This table shows how thyme extract affected the meat yield (carcass characteristics) and the nutritional composition of the meat from Awassi lambs.

- **Carcass Yield:** The T2 group had significantly higher **Hot Carcass** and **Cold Carcass** weights, and a better **Dressing Percentage** (the ratio of carcass weight to live weight) compared to the Control.
- **Fat and Meat Quality:** The T2 group had significantly **less back fat (Fat thickness)**. The meat was also significantly **lighter in color (higher L* value)**.
- **Meat Composition:** The meat from the T2 group had a significantly higher percentage of **Moisture** and **Protein**, and a significantly lower percentage of **Fat**.
- **Significant Effects:** The high dose of thyme extract (T2) led to significant improvements in all measured traits compared to the Control group. This includes higher carcass weights, better dressing percentage, reduced fat thickness, lighter meat color, and a superior nutritional profile (more protein/water, less fat).
- **Non-Significant Effects:** The low dose (T1) group, for all parameters, was **not significantly different** from either the Control group or the T2 group. Its values were consistently intermediate, showing a trend but not a statistically confirmed effect.

4. Discussion

Rumen Fermentation Parameters

The significant alterations in rumen fermentation parameters indicate that thyme extract acts as a potent rumen modulator. The dose-dependent increase in total VFA concentration suggests an overall enhancement of fermentative activity or a shift towards more efficient microbial populations (Cobellis et al., 2016). The most critical change is the significant shift in the VFA profile: a decrease in acetate and a marked increase in propionate, resulting in a lower acetate-to-propionate ratio. This shift is metabolically favorable because propionate formation is a hydrogen-sinking process that enhances the efficiency of dietary energy capture by the animal (Ungerfeld, 2020). Furthermore, the significant reduction in ammonia-nitrogen concentration is a key finding, indicating that thyme extract inhibits the activity of hyper-ammonia-producing bacteria, thereby decreasing protein deamination and increasing the flow of dietary protein to the small intestine—a phenomenon known as the "protein-sparing effect" (Benchaar and Greathead, 2011). The slight but significant decrease in pH at the high dose is likely a direct consequence of the increased total VFA production and is consistent with enhanced fermentation.

Methane Production and Gas Yield

The data presented demonstrate a potent anti-methanogenic effect of thyme extract. The significant, dose-dependent reduction in methane production, culminating in a 42.4% decrease at the 500 mg/kg dose, is a highly significant finding for both animal production and environmental

sustainability. This reduction is directly linked to the altered fermentation pattern observed in Table 2. The increase in propionate production serves as a competitive pathway for metabolic hydrogen (H_2) utilization, thereby diverting H_2 away from methanogenic archaea, which use it to reduce CO_2 to CH_4 (Ungerfeld, 2020). The concomitant decrease in total gas production supports the premise of a fundamental shift in fermentation end-products. By curbing methanogenesis, thyme extract reduces a major energy loss for the animal, which can represent 2-12% of gross energy intake, thereby contributing to the improved feed efficiency observed later (Hristov et al., 2013). This positions thyme extract as a viable natural strategy for mitigating enteric methane, a potent greenhouse gas, from ruminant livestock.

Growth Performance

The improvements in growth performance are a direct consequence of the enhanced ruminal environment and nutrient utilization documented in the previous tables. The significant increase in Average Daily Gain (ADG) and the improvement in Feed Conversion Ratio (FCR) at the 500 mg/kg dose are key zootechnical outcomes. The improved FCR indicates that the lambs supplemented with thyme extract converted feed into body mass more efficiently. This can be attributed to two primary factors from the earlier data: first, the increased production of total VFAs, particularly the glucogenic precursor propionate, provided more available energy for growth (Benchaar, 2020); and second, the protein-sparing effect (reduced ammonia) increased the supply of digestible protein for tissue synthesis (Cobellis et al., 2016). The observed increase in feed intake in the T2 group further contributed to the higher growth rates, suggesting the extract did not negatively affect palatability. The reduction in energy loss as methane (Table 3) also directly contributes to the improved energy availability reflected in the better FCR (Hristov et al., 2013).

Carcass Characteristics and Meat Composition

The superior carcass characteristics and meat composition confirm that the growth-promoting effects of thyme extract translated into higher-quality, more valuable end-products. The significant increases in hot and cold carcass weights and dressing percentage in the T2 group demonstrate that the weight gained was primarily high-value muscle tissue rather than non-carcass components (López-Campos et al., 2021). The most notable improvements are in the chemical composition of the meat. The significantly higher protein content and lower fat content (both subcutaneously and intramuscularly) are highly desirable for producing leaner meat that aligns with modern consumer demands for healthier food options (Jiang et al., 2021). This shift towards lean tissue deposition is a direct result of the more efficient nutrient utilization, where the increased supply of metabolizable protein and energy was partitioned towards muscle protein synthesis rather than adipose tissue (Smeti et al., 2018). The lighter meat color (higher L* value) is often associated with improved water-holding capacity and can enhance consumer appeal, while the higher moisture content supports the finding of a leaner tissue with a higher protein-to-fat ratio (Wood et al., 2008).

5. Conclusion

Based on the comprehensive data obtained from this study, it can be conclusively determined that dietary supplementation with thyme extract exerts a significant positive influence on rumen fermentation, methane emissions, growth performance, and carcass characteristics in Awassi lambs. The effects were demonstrably dose-dependent, with the higher dosage of 500 mg/kg (T2) consistently yielding the most pronounced benefits.

The primary mechanism of action appears to be the modulation of the rumen microbial ecosystem. This is evidenced by a significant shift in volatile fatty acid profiles, characterized by an increased propionate proportion and a decreased acetate-to-propionate ratio, indicating a more energetically efficient fermentation process. Concurrently, a substantial reduction in ammonia-nitrogen concentration suggests a protein-sparing effect, enhancing nitrogen utilization. These improvements in ruminal metabolism directly contributed to a remarkable suppression of enteric methane production, achieving a 42.4% reduction at the high dose.

The enhanced nutrient utilization was directly translated into superior zootechnical outcomes. Lambs in the T2 group exhibited significantly improved average daily gain and feed conversion ratio. Furthermore, these growth advantages culminated in higher-quality carcasses, marked by increased dressing percentage and a more favorable meat composition—significantly higher protein and lower fat content—which aligns with modern consumer demands for healthier animal products.

6.Recommendations

The following recommendations are proposed based on the findings of this study:

1. **For Implementation in Lamb Production:** It is recommended to incorporate thyme extract at a dosage of 500 mg/kg of dry matter in the diets of finishing Awassi lambs. This dosage has been proven to optimize feed efficiency, enhance growth rates, and improve the marketability of the carcass through superior lean meat yield.

2. **For Environmental Sustainability:** Farmers and producers aiming to reduce the environmental footprint of their operations should adopt thyme extract as a viable natural feed additive. Its proven efficacy in drastically lowering methane emissions contributes to sustainable livestock production by mitigating a potent greenhouse gas.

3. **For Future Research:** Subsequent investigations should focus on:

    - **Long-term Studies:** Evaluating the effects of thyme extract over a complete production cycle and across different animal physiological states (e.g., gestation, lactation).

    - **Bacterial Community Analysis:** Utilizing molecular techniques to precisely characterize the shifts in the rumen microbiome responsible for the observed fermentation changes.

- **Economic Analysis:** Conducting a full cost-benefit analysis to validate the economic viability of thyme extract supplementation at a commercial scale.
- **Product Quality:** Further exploring the impact on meat shelf-life, sensory attributes, and fatty acid profile.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.