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Rumen Fermentation Parameters of Awassi Lambs Fed Diets Containing Sesame and Sunflower Meals as Alternatives to Soybean Meal

Salih N. Hussein[1], Afrah M. Mohammad[1], Ashraf K. Azeez[1] and Asuman DURU[2]
[1] Department of Animal Production, College of Agriculture, University of Tikrit, Iraq
[2] Department of Animal Science, Faculty of Agriculture & Natural Sciences, Uşak University, 64200, Uşak, Turkey.

***Correspondence: salihnjim@tu.edu.iq**

KEY WORDS:
Replacement ; Protein sources ; Rumen fluid characteristics ; Growing lambs

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ABSTRACT

The increasing cost of soybean meal (SBM) has created an urgent need for economically viable alternative protein sources that maintain equivalent nutritional efficiency in ruminant diets. So this study aimed to provide cost-effective feed alternatives to expensive soybean meal by replacing it with cheaper materials while maintaining the same nutritional efficiency. The experiment was conducted at the Animal House, College of Veterinary Medicine, Tikrit University. Eighteen male Awassi lambs (3-4 months old, 20-26 kg initial weight) were randomly divided to three dietary treatments: T1, containing 8% soybean meal (SBM); T2, sesame meal replacement at 13% inclusion (SSM); and T3, sunflower meal replacement at 8% inclusion (SFM).. Rumen fluid samples were collected before feeding and 4 hours post-feeding to evaluate fermentation parameters, including pH, volatile fatty acids (VFAs), and ammonia nitrogen (NH3-N). Results demonstrated that both alternative protein sources significantly enhanced rumen fermentation activity compared to the control group. Post-feeding measurements revealed that the sesame and sunflower meal groups achieved higher VFA concentrations (67.14 and 67.46 mg/100 mL, respectively) compared to soybean meal (63.94 mg/100 mL). Similarly, ammonia nitrogen levels were significantly elevated in the alternative protein groups (25.11 and 25.17 mg/100 mL) versus the control (23.87 mg/100 mL). The pH values remained within optimal ranges across all treatments, indicating healthy rumen function. These findings suggest that sesame meal and sunflower meal can effectively replace soybean meal in growing lamb diets, offering economically advantageous alternatives without compromising rumen fermentation efficiency or nutritional performance.

معايير تخمرات الكرش في الحملان العواسية التي تتغذى على علائق تحتوي على كسبتي السمسم وزهرة الشمس كبدائل لكسبة فول الصويا

صالح حسين [1] ، أفراح محمد [1] ، أشرف عزيز [1] ، عصمان دورو [2]

[1]قسم الانتاج الحيواني ، كلية الزراعة ، جامعة تكريت ، العراق

[2] قسم علوم الحيوان ، كلية الزراعة والعلوم الطبيعية ، جامعة يو إرماك ، 64200 ، يو إرماك ، تركيا

الخلاصة

بالنظر لتكاليف كسبة فول الصويا المتزايدة أدى ذلك الى ظهور حاجة ملحة لمصادر بروتين بديلة ذات جدوى اقتصادية وتحافظ على القيمة الغذائية لعلائق المجترات. لذا هدفت هذه الدراسة إلى توفير بدائل علفية فعالة لكسبة فول الصويا غالية الثمن من خلال استبدالها بكسب أقل تكلفة مع الحفاظ على نفس الكفاءة الغذائية. تم إجراء التجربة في بيت الحيوان، كلية الطب البيطري، جامعة تكريت، استخدم فيها ثمانية عشر حمل من نوع العواسي بعمر 3-4 أشهر، وبوزن ابتدائي 20-26 كجم. قسمت عشوائيًا إلى ثلاثة معاملات تغذوية المعاملة الأولى تحتوي على 8% كسبة فول الصويا , المعاملة الثانية تحتوي على 13% كسبة السمسم , واحتوت المعاملة الثالثة على كسبة زهرة الشمس بنسبة 8 % .تم جمع عينات من سائل الكرش قبل التغذية وبعد 4 ساعات من التغذية لتقييم معايير التخمر، التي تضمنت الاس الهيدروجيني، وتركيز كل من والأحماض الدهنية الطيّارة والامونيا, وقد أظهرت النتائج أن كلا المصدرين البديلين للبروتين حسّنا بشكل كبير فعالية تخمرات الكرش مقارنة بالمعاملة الأولى. حيث أظهر النتائج ان المعايير ما بعد التغذية في المعاملتين الثانية والثالثة انتجت تراكيز أعلى من الأحماض الدهنية الطيّارة (67.14 و67.46 ملغ/100 مل) على التوالي مقارنة بالمعاملة الاولى (63.94 ملغ/100 مل). وبذات التأثير، كانت تراكيز الامونيا مرتفعة بشكل ملحوظ في مجموعات البروتين البديل (25.11 و25.17 ملغ/100 مل) على التوالي مقارنة بالمعاملة الاولى (23.87 ملغ/100 مل). في حين بقيت قيم الاس الهيدروجيني ضمن المدى المثلي في المعاملات الثلاث، مما يشير إلى ان بيئة الكرش كانت متزنة. نستنتج من هذه النتائج إلى أن كسبتي السمسم وزهرة الشمس يمكن أن يحلان محل كسبة فول الصويا بشكل فعال في علائق الحملان النامية، مما يجعلها بدائل اقتصادية دون أن تؤثر على كفاءة تخمرات الكرش أو الأداء التغذوي.

الكلمات المفتاحية: استبدال ؛ مصادر البروتين ؛ خصائص سائل الكرش ؛ نمو الحملان

INTRODUCTION

The Awassi breed of sheep represents a vital element of global agriculture and food security, as small ruminant farming meets the needs of food production, especially in arid and semi-arid regions where these animals are naturally adapted (Van Soest, 1994). Protein serves as an essential macronutrient for ruminants because it supports their growth, maintenance needs, milk production capabilities, and helps regulate their physiological functions (NRC, 2001 and Ma & Tu 2022). The high protein content and favorable amino acid profile of soybean meal (SBM), combined with its consistent availability, have made it the leading protein supplement for livestock feeds around the world (Lambo *et al*., 2024). The dependence on SBM generates multiple difficulties, such as unstable pricing due to global market forces, competition with human food supplies, environmental damage from soybean cultivation, like deforestation, and high import costs in many regions (Samtiya *et al*., 2020).

Research interest has grown significantly to find alternative protein sources that are locally available and sustainable for ruminant diets, which are also cost-effective (Besharati *et al*., 2022). Oilseed meals from sesame (*Sesamum indicum* L.) and sunflower (*Helianthus annuus* L.) represent promising alternatives among protein sources (Alagawany *et al*., 2021; El-Hack *et al*., 2020). Sesame meal, which results from sesame oil production, contains significant levels of protein and may offer advantageous fatty acids (Wacal *et al*., 2024). Sunflower meal, which is produced after oil extraction from sunflower seeds, serves as a valuable protein source; yet, its nutritional profile varies based on the extraction method and the extent of dehulling (Laudadio & Tufarelli, 2020).

Understanding how sesame meal and sunflower meal affect rumen function and nutrient utilization is essential for their successful inclusion in lamb diets (Patra, 2021). The rumen serves as a complex microbial ecosystem that plays a fundamental role in digesting nutrients and metabolizing them in ruminant animals (Van Soest, 1994). Proteins from different dietary sources create distinct fermentation patterns in the rumen that affect the production of volatile fatty acids alongside ammonia levels, microbial protein synthesis, and the activity of protozoa within the rumen environment (Zhou *et al*., 2020). When these parameters change, they influence nutrient digestibility and energy supply, leading to variations in animal performance, such as growth rates and feed efficiency (Górka & Penner, 2020).

MATERIALS AND METHODS

Ethical Approval

Every animal handling and sample collection procedure followed the guidelines set by the Institutional Animal Care and Use Committee that approved the experiment.

Experimental Animals and Housing

This study involved eighteen male Awassi lambs, aged between 3 and 4 months, which initially weighed between 20 - 26 kg. The experiment was conducted at the Animal House, College of Veterinary Medicine, Tikrit University. The lambs experienced a 15-day adjustment phase to their housing conditions and basal diet before starting the trial (NRC, 2001). After the adaptation phase, the lambs were weighed and then distributed randomly into three experimental research groups, each containing six lambs, while maintaining comparable average initial body weights for all groups. The study used individual pens measuring 1.5 meters by 1.5 meters, along with feeders and waterers, to house each lamb. All pens featured concrete flooring with bedding that was suitable for lamb housing.

Experimental Diets and Feeding

Three experimental diets were created to potentially maintain consistent nitrogen levels while fulfilling the nutritional needs of growing lambs (NRC, 2001). Barley grain was used together with wheat bran to create the study's experimental diets. The primary protein supplement in the first diet consisted of 8% soybean meal (SBM). The second diet (SSM) replaced all soybean meal with sesame meal at a proportion of 13%. Sunflower meal (SFM) replaced soybean meal entirely in the third diet (SFM) at an 8% inclusion rate. The composition of ingredients used in the experimental diets. The lambs received their diets twice daily in equal portions at specified times. Each lamb received a daily feed allowance that represented 3% of its body weight. The lambs had access to fresh drinking water at all times during the entire experimental period(Table 1).

Rumen Fluid Sample Collection

On the final day of the experiment, each lamb had a rumen fluid sample collected before and four hours after the morning feeding using oro-ruminal intubation with a flexible stomach tube attached to a manual suction pump. Approximately 50 mL of rumen fluid was obtained from each lamb. Immediately after collection, the pH of the rumen fluid was measured using a calibrated portable pH meter. The samples were then filtered through four layers of cheesecloth, and aliquots were prepared for subsequent analyses. A 20 mL aliquot was preserved for ammonia-nitrogen (NH_3-N) analysis using standard preservation methods. Another 25 mL was mixed with 1 mL of concentrated hydrochloric acid (HCl) for protozoa enumeration. For volatile fatty acid (VFA) analysis, a 1 mL aliquot was combined with 9 mL of a 10% formalin solution. All prepared samples were immediately frozen and stored at -20°C until laboratory analysis.

Table 1: Ingredient and Chemical Composition of Experimental Diets (% Dry Matter Basis)

Ingredient	**T1 (SBM 8%)**	**T2 (SSM 13%)**	**T3 (SFM 8%)**
Barley Grain	54	54	54
Wheat Bran	21	21	21
Corn	15	10	15
Soybean Meal	8	---	---
Sesame Meal	---	13	---
Sunflower Meal	---	---	8
Limestone	1	1	1
Salt	1	1	1
Total	100	100	100
Chemical composition%			
Dry matter	89.76	90.38	90.06
Crude protein	13.99	14.24	14.39
Ether Extract	2.37	3.94	2.70
Crude fiber	2.83	3.56	2.83
Ash	3.96	5.57	3.94
Nitrogen free Extract	62.69	56.69	60.26
Metabolizable energy mj\Kg	1.15	2.98	1.13

Rumen Fluid Parameters

The concentration of ammonia-nitrogen in rumen fluid was evaluated using the colorimetric technique developed by Broderick and Kang (1980), which requires absorbance measurement at 630 nm. Protozoa counts were then conducted with a microscope and counting chamber based on the methods from Boyne *et al*. (1957). Standard laboratory protocols guided the determination of volatile fatty acid concentrations (including acetate, propionate, and butyrate) through gas chromatography analysis, with reference to established methods. The chemical makeup of both feed materials and fecal matter was analyzed during the digestibility measurements. The research team gathered samples from the experimental diets and feed ingredients throughout the entire trial duration. The chemical analysis followed the standardized methods developed by AOAC (2002).

Statistical Analysis

The analytical model we used correctly matched the requirements of a Factorial Experiment 3×2. The effect of dietary treatment was assessed by performing a GLM for repeated measures analysis of variance. The dietary treatments (T) (T = SBM, SSM, and SFM) were defined as the fixed factor, and the withdrawal time (W) was defined as the repeated measure, while their interactions (TW) were also assessed, according to the following model:

$Yij = \mu + Ti+Wj + (T*W)\ ij + eijk$

Where Yij is the dependent variable, the overall mean, Ti the effect of dietary treatment (i = 3; SBM, SSM, and SFM), Wj the effect of withdrawal time (j = 2; 0h, and 4h after feeding), (T*W) ij the interaction between dietary treatments and withdrawal time, and eij the residual error. For all tests, the significance level was set at p = 0.05. Simplifying the visualization of the results, GraphPad Prism 8.0.1(244) depicted bars.

RESULTS AND DISCUSSION

Figure 1. Indicate that the three protein sources used in the concentrate diet (soybean meal, sesame meal, and sunflower meal) did not significantly affect the chemical properties of the rumen fluid before feeding. The pH was observed to be stable at close values ranging from 6.56 to 6.62, which are within the normal range for rumen equilibrium. These values fall within the normal range for rumen pH, which typically ranges between 5.5 and 7.0 (Golder & Lean, 2024). The absence of significant differences between treatments indicates that different protein sources did not affect the acid-base balance in the rumen during fasting conditions. These results agree with what Jo *et al*. (2024) indicated in their study on the effects of protein and energy levels on rumen fermentation, where they found that rumen pH remains relatively stable in the absence of active feeding. Golder & Lean (2024) also confirm that pH alone is insufficient to assess rumen condition. No significant differences were observed in the concentration of volatile fatty acids (VFAs).

Volatile fatty acids are considered the main product of carbohydrate fermentation in the rumen and provide 70-80% of energy requirements for ruminants (Lv &Wang. 2020 and Chen *et al*., 2024). During fasting, VFA levels naturally decrease due to reduced fermentation activity and absorption of remaining acids from the previous meal. Additionally, the level of nitrogenous ammonia (N-NH3) showed values close to 58.28 and 59.72 mg/100 ml for volatile acids and about 20 mg/100 ml for ammonia in all treatments. These levels fall within the acceptable range for rumen ammonia, which typically ranges between 15 and 30 mg/100 mL (Wang & Zhang. 2021 and Kim *et al*., 2025). Rumen ammonia is primarily produced from protein degradation by microorganisms and is used as a nitrogen source for microbial protein synthesis (Kim *et al*., 2025). In pre-feeding conditions, ammonia levels are relatively low due to continued utilization by microorganisms and the absence of a new source of degraded protein.

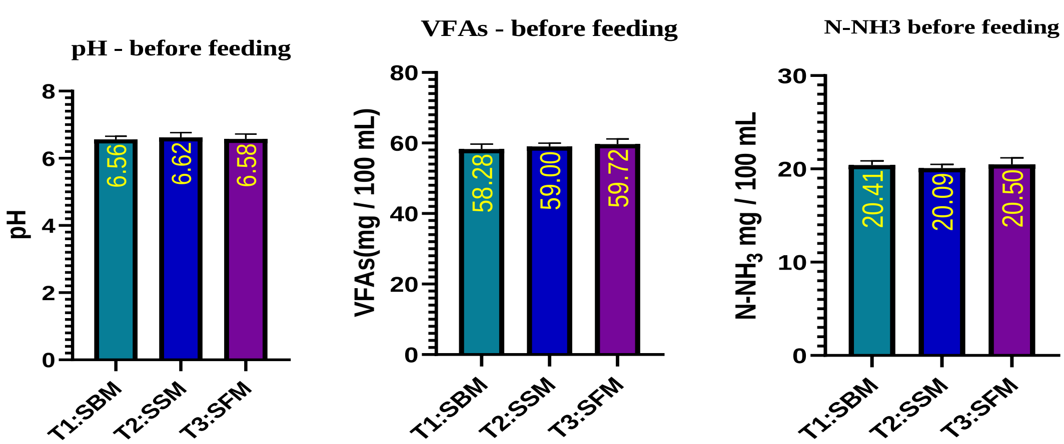


Figure 1. pH value, Volatile Fatty Acids concentration (VFAs) (mg/100mL) , and ammonia concentration (N-NH$_3$) (mg/100mL), in the rumen fluid of Awassi lambs, fed the three diets (SBM, SSM, , and SFM) before feeding.

Regarding the protein source Figure 2. There was no significant change in the acidity level of the rumen fluid 4 hours after feeding the feed. A decrease in the pH was observed to values between 5.55 and 5.56, this decrease even it was non-significant but this decrease in pH is explained by increased volatile fatty acid production resulting from fermentation of carbohydrates consumed with feed (Golder & Lean, 2024). As Jo *et al*. (2024) indicated, high temperatures and intensive fermentation activity lead to increased acid production, which is reflected in decreased pH in rumen fluid. It is important to note that the recorded values (5.55-5.56) fall within the lower limit of the normal range for rumen pH, indicating active fermentation without reaching subclinical acidosis SARA levels, which typically occur when pH drops below 5.5 for extended periods (Abo-Donia et. al. 2021 and Golder & Lean, 2024)

Meanwhile, the levels of volatile fatty acids (VFAs) witnessed a clear significant increase, reaching their highest levels in the T2 (sesame meal) and T3 (sunflower meal) groups, which reached 67.14 and 67.46 mg/100 ml, compared to the T1 (soybean meal) group, which reached 63.94 mg/100 ml, First, differences in carbohydrate and fiber composition between different protein sources. As Wencelová *et al*. (2024) indicated, secondary compounds in seeds such as saponins and flavonoids can affect the microbial community in the rumen and fermentation patterns. Second, protein and carbohydrate degradation rates may differ between different protein sources, affecting the availability of raw materials for microbial fermentation. As Kim *et al*. (2025) explained, the ratio of rumen undegradable protein to rumen degradable protein significantly affects fermentation patterns in the rumen.

In addition, the values of ammonia nitrogen (N-NH3) increased significantly, as the T2 and T3 groups outperformed (25.11 and 25.17 mg/100 ml) compared to the control group (23.87 mg/100 ml), this increase in ammonia levels (from approximately 20 mg/100mL to 24-25 mg/100mL) reflects intensive protein activity in the rumen after feeding. Protein degradation by microorganisms produces peptides, amino acids, and finally ammonia, which is used as a nitrogen source for microbial protein synthesis (Kim *et al*., 2025).

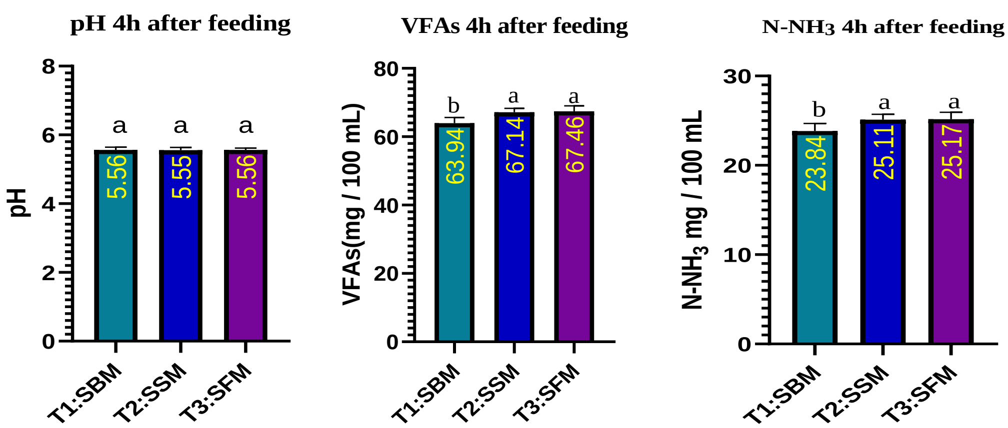


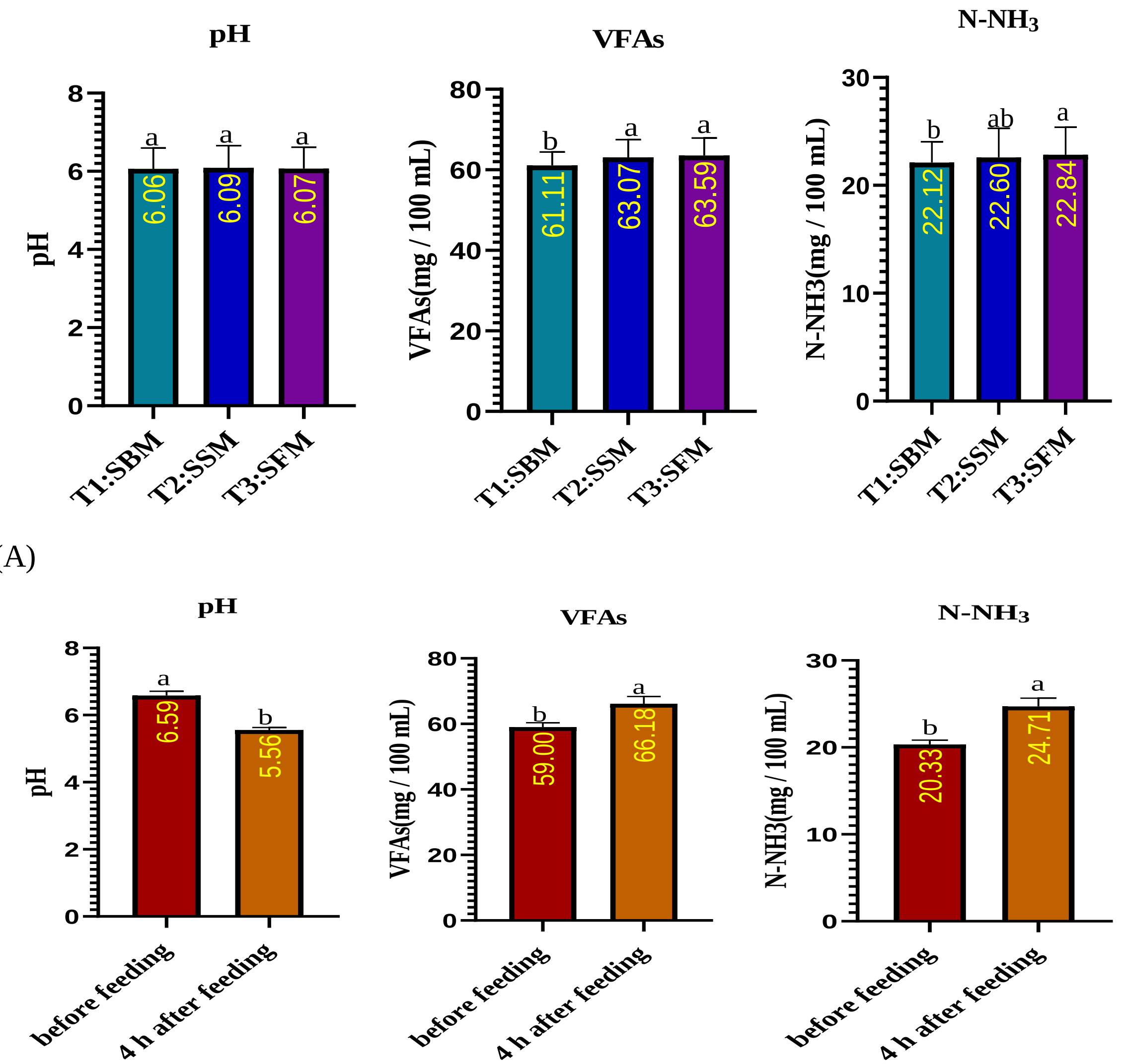
Figure 2. pH value, Volatile Fatty Acids concentration (VFAs) (mg/100mL) , and ammonia concentration (N-NH$_3$) (mg/100mL), in the rumen fluid of Awassi lambs, fed

the three diets (SBM, SSM, , and SFM) after feeding .with different Superscript (a, b) between dietary treatments differ significantly ($p \leq 0.05$).

From Figure 3(A,B). Regarding the effect of both protein source and withdrawal time independently on rumen characteristics, no significant effect was found on pH between different protein sources (A), as it ranged between 6.06 and 6.09. While the volatile fatty acids (VFAs) values were higher in the sesame and sunflower meal groups (63.07 and 63.59 mg/100 ml) compared to the soybean meal group (61.11 mg/100 ml), the sunflower meal group recorded the highest values of ammonia nitrogen (22.84 mg/100 ml) while the lowest values were in the soybean meal group (22.12 mg/100 ml). While the effect of withdrawal time (B) was more evident, as measurements before feeding were characterized by a higher pH (6.57) may be due to the soybean meal showed moderate response to feeding, indicating average degradation rate and gradual release of nutrients. This agrees with known characteristics of soybean protein as a high-quality and balanced-degradation protein source (Liu, & Zhang, 2024 and Kim *et al*., 2025). In addition, lower values for volatile fatty acids (59.00 mg/100 ml) and ammonia (20.33 mg/100 ml), while the pH decreased to 5.56 after feeding. The results also showed an increase in VFAs (66.18 mg/100 ml) and N-NH3 (24.71 mg/100 ml), VFA levels experienced a large increase from 59.00 ± 0.44 mg/100mL before feeding to 66.18 ± 0.56 mg/100mL after 4 hours. This increase of over 12% reflects intensive fermentation activity of consumed carbohydrates. These results agree with what Jo *et al*. (2024) indicated about the effect of high temperatures and fermentation activity on increasing total volatile fatty acid production. This increase also confirms the central role of volatile fatty acids as a main energy source for ruminants, providing 70-80% of energy requirements (Salman & Mohamed, 2021 and Dhakal *et al*., 2024). Also as Kim *et al*. (2025) explained, this increase in ammonia results from degradation of consumed protein by microorganisms, and the resulting ammonia is used as a nitrogen source for microbial protein synthesis. The final level (24.71 mg/100mL) falls within the optimal range for microbial growth and microbial protein synthesis.

From Figure 4. The mean values of the interaction between protein source and withdrawal time on rumen biochemical variables were shown. Before feeding, no significant differences were recorded between groups in pH, VFAs, or ammonia, with high pH values (approximately 6.56-6.62) and low VFA and ammonia levels (approximately 58-59 mg/100 ml and 20 mg/100 ml, respectively). Four hours after feeding, a sharp significant decrease in pH (5.55-5.56) was observed, along with a significant increase in volatile fatty acids and ammonia, especially in groups T2 and T3, reflecting a significant interaction between protein type and measurement time. As Dhakal *et al*. (2024) and Yin (2024) indicated, different feed sources contain diverse bioactive compounds such as flavonoids, saponins, and tannins. These compounds can affect: Microbial community composition: Stimulating growth of specific bacterial species beneficial for fermentation first, Enzyme activity: Modifying activity of protein and carbohydrate-degrading enzymes second and Degradation rates: Accelerating or slowing microbial degradation processes third. These results agree with Jo *et al*. (2024): Confirmed that high protein levels lead to increased ammonia and acetate concentrations, which agrees with the obtained results for sesame meal and sunflower meal. Pérez-Trejo (2022) and Al-Ghamdi *et al*. (2025) Found that different dietary supplements affect rumen development and morphology in Awassi lambs, , supporting the importance of protein source in rumen health, and Muratov *et al*.

(2025) Indicated that alternative protein sources from food industry byproducts can be effective in improving digestive performance.



(B)

Figure 3. (A) Effect of protein source. (B) Effect of withdrawal time independently on pH, (VFAs), and ($N\text{-}NH_3$) in the rumen fluid of Awassi lambs. With different Superscript (a, b) between dietary treatments differ significantly ($p \leq 0.05$).

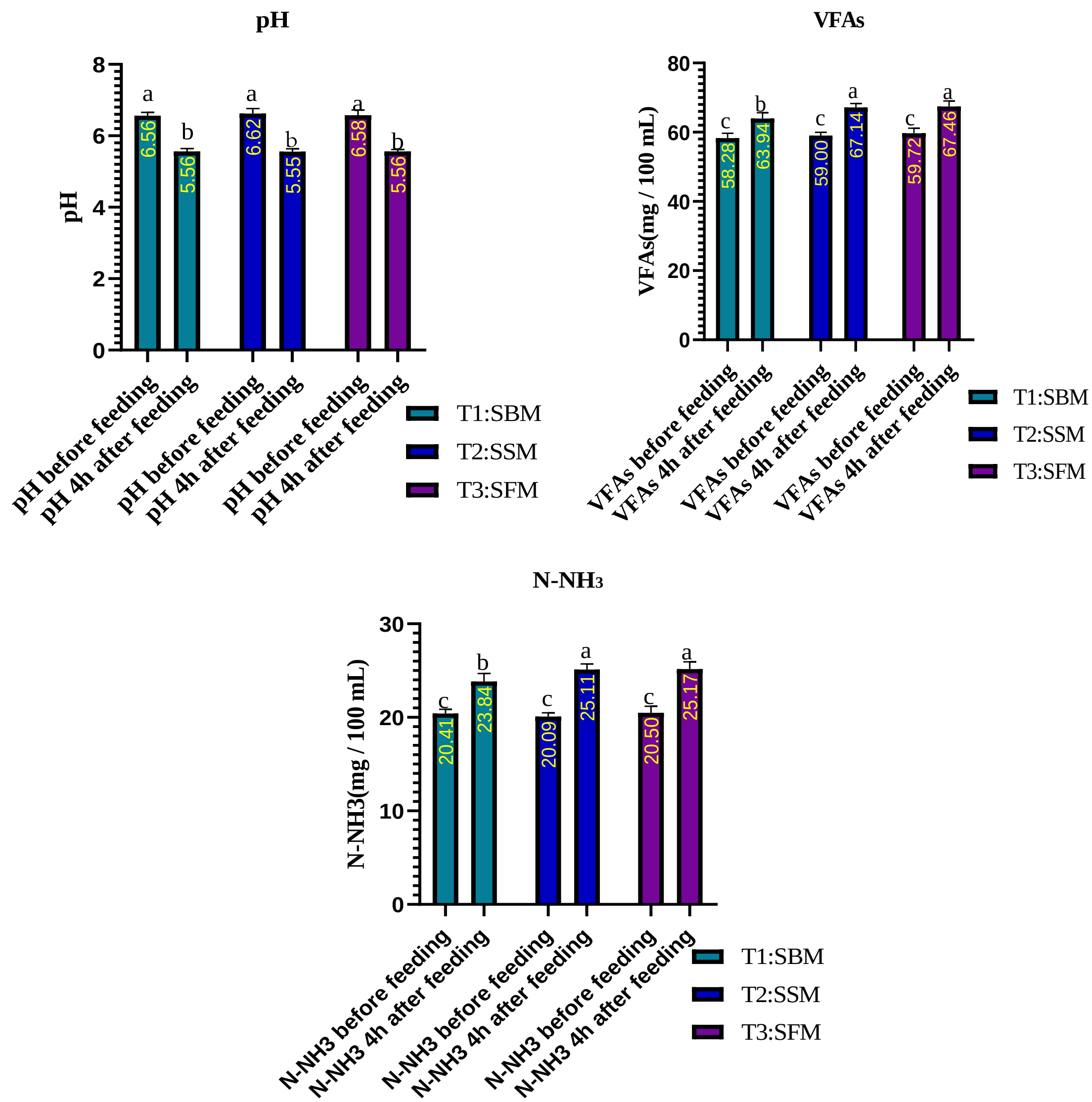


Figure 4. Effect of interaction the protein source and withdrawal time on the Rumen fluid parameters for Awassi lambs. With different Superscript (a, b, c) between dietary treatments, differ significantly ($p \leq 0.05$)**.**

CONCLUSION

This research successfully demonstrates that sesame meal and sunflower meal serve as viable and economically beneficial alternatives to expensive soybean meal in the diets of growing Awassi lambs. The enhanced volatile fatty acid production and ammonia nitrogen concentrations observed with both alternative protein sources indicate improved rumen fermentation efficiency compared to conventional soybean meal supplementation. The maintenance of optimal pH levels across all dietary treatments confirms that these substitutions do not compromise rumen health or digestive stability. From an economic perspective, the utilization of locally available and cost-effective protein sources like sesame and sunflower meals can significantly reduce feed costs while maintaining or potentially improving nutritional outcomes. The superior fermentation parameters achieved with these alternatives suggest enhanced microbial protein synthesis and energy availability for growing lambs. These findings provide practical evidence for livestock producers seeking to optimize feed formulations while managing production costs, particularly in regions where sesame and sunflower processing byproducts are readily accessible and affordable compared to imported soybean meal.

CONFLICT OF INTEREST

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

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