Diyala Agricultural Sciences Journal, 2025, Vol. (17) No. 1: 131-139
ISSN: 2073-9524
eISSN: 2310-8746

# Effect of Dietary Inclusion of Azolla (*Pinnata*) on Some Rumen Fermentation Characteristics of Awassi Lamb

**Afraah Mustafa Mohammad[1]*, Salih Najem Hussein[2], Amer Ibrahim Ali[2]*, Umar Mohammed Sani[3]**

[1]Department of Animal Production, Faculty of Agriculture, University of Tikrit, Iraq.
[2]Department of public of health, Faculty of veterinary medicine, University of Tikrit, Iraq.
[3]Department of Animal Science, Faculty of Agriculture, Federal University of Lafia, P.M.P. 146, Lafia, Nigeria.

*Corresponding Author: ameribr@tu.edu.iq

***Article history:***

Received: 26 November 2024
Accepted: 19 April 2025
Published: 30 June 2025

***Keywords:*** *Azolla concentration, Rumen fermentation, Awassi lambs, Rumen liquid.*

**Abstract**

This study aimed to evaluate the effect of the inclusion of different levels of Azolla on the rumen fermentation characteristics of Awassi lambs. Fifteen local Awassi lambs, aged 5-6 months and weighing on average 23.2 ± 0.3 kg, were randomly divided into three treatment groups: T1 (control), T2 (3%), and T3 (5%) based on body weight. Rumen liquid was collected at three different periods (before feeding, 2 hours, and 4 hours post-feeding). Results showed no significant difference ($P \leq 0.05$) in rumen pH and total volatile fatty acids (TVFAs). However, there was a significant increase ($P \leq 0.05$) in N-NH3 concentration with increasing Azolla level in the diet. Rumen parameters were significantly affected by the time of sampling after feeding; pH value decreased while the concentrations of ammonia and volatile fatty acids increased during the periods of 2 and 4 hours after feeding. The interaction between Azolla levels and time of sampling indicated that the pH value of rumen liquid from lambs in T2 and T3, at sample times of 2 and 4 hours after feeding, significantly ($P \leq 0.05$) decreased. Furthermore, lambs fed 5% Azolla showed a significant increase in TVFAs concentration after 4 hours and in N-NH3 concentration at both 2 and 4 hours post-feeding. The use of Azolla as a protein source in the feeding of Awassi lambs, as a partial replacement for wheat bran, has been associated with an improved rumen environment.

https://dx.doi.org/10.52951/dasj.25170110



## Introduction

The cost of feeding is a significant barrier to the advancement of livestock production. The increasing global population has created substantial pressure on the livestock industry to ensure sufficient human nutrition. This situation has created a necessity for improved animal productivity and an enhanced requirement for natural resources to produce animal feed. Feed manufacturers are at the forefront, continually improving their formulations to meet the changing

requirements of livestock, largely motivated by the fact that over 50% of costs are linked to feed production (Akintan *et al.,* 2024). Concentrate and green fodder shortage have prompted livestock producers to seek for cost-effective alternatives. Azolla, an invasive plant species, presents an intriguing solution. It thrives in freshwater environments such as lakes, rivers, wetlands, and ditches, in both temperate and tropical regions (El Naggar and El-Mesery, 2022). Azolla have a rapid growth rate, doubling its biomass in two to three days. It is considered one of the locally available aquatic plants, rich in protein and almost all essential amino acids (Mandal *et al.,* 2012). Azolla consists of a slender, branched stem that floats on the water, with its roots suspended in the water (Ahirwar and Leela, 2012). Kathirvelan *et al.* (2015) reported that, Azolla is highly nutritious, containing high quality proteins, essential amino acids, vitamins (such as vitamin A and vitamin B12), β-carotene, and important minerals like calcium, phosphorus, potassium, iron, copper, and magnesium. Azolla composition as dry matter contains 25-35% protein, 10-15% minerals, and 7-10% amino acids, low in soluble carbohydrate and oil and high digestible fiber content, in addition to the bioactive compounds, and biopolymers (Sujatha and Jeyakumar, 2009). Thus, Azolla has been widely used in the feeding of ruminants, particularly in Asia and Africa (Rana *et al.,* 2017).

Using different levels of Azolla in dairy cows' diets can enhance body weight and milk production (Yadav *et al.,* 2014). Supplementing dairy buffalo with 1.5 kg of Azolla daily resulted in a 16.25% increase in milk production, averaging 1.3 liters more than traditional feeding practices that rely on straw, grasses, and cottonseed cake (Meena *et al.,* 2017). Senthil Kumar *et al.* (2020) reported that using fresh Azolla as a supplement for dairy cows up to 1000 grams per day increased milk production by 7 to 13%. Supplementing 300 g of fresh Azolla with concentrate feed enhances the growth rate of Osmanabadi goat kids in India, including improvements in chest girth, body height, and body length, compared to other treatments that included 100 g or 200 g of Azolla with concentrate feed (Toradmal *et al.,* 2017). Furthermore, Kumari *et al.* (2021) reported that feeding Azolla at a 20% level resulted in greater body weight changes for goats compared to the control group and proved to be economically beneficial. Additionally, incorporating Azolla at a 6% level as a replacement for 25% of linseed showed positive growth performance in Corriedale sheep (Ahmed *et al.,* 2016). This study investigated the impact of different levels of Azolla inclusion on the rumen fermentation parameters of Awassi lambs.

## Materials and Methods

### Ethical approval

This study was conducted in accordance with the ethical standards set forth by the scientific ethical committee of Collage of Veterinary Medicine at University of Tikrit which is in line with all national and international guidelines and approved under the Ethical Number (Tu.vet.100/ 2025).

## Study Location

The study was conducted in the animal field of the College of Veterinary Medicine at Tikrit University, from 13 October 2021 to 12 January 2022, preceded by a preparatory period of 10 days.

## Management of Experimental Animals

The study involved fifteen (15) local Awassi lambs (5-6 months old, average weight 23.2 ± 0.3 kg), obtained from local markets in Salah al-Din Governorate. The lambs were weighed on an electronic scale and divided into three groups according to their body weight, with each group consisting of five lambs. For the experiment, metabolic cages were set up with three plastic containers to hold concentrated feed, roughage in the form of wheat hay, and 5 liters of drinking water. Mineral salt cubes were available in the concentrated feed trough for the entire duration**.**

## Experimental design

The experimental rations and chemical composition based on dry matter are shown in Table 1. The first ration (T1) contained 0% Azolla, the second and third rations (T2 and T3) contained 3 and 5% of air-dry Azolla, respectively. Azolla was added to the rations as an alternative to wheat bran. The proximate of the experiment feed has been done according to (AOAC, 2005).

**Table 1. Ingredient and nutrients composition of basal diet (DM basis)**

| Ingredients (%) | Treatments | | |
|---|---|---|---|
| | T1 | T2 | T3 |
| Barely | 61 | 61 | 61 |
| Wheat Bran | 30 | 27 | 25 |
| Corn | 8 | 8 | 8 |
| Azolla | 0 | 3 | 5 |
| Salt | 0.5 | 0.5 | 0.5 |
| Limestone | 0.5 | 0.5 | 0.5 |
| Nutrients composition (%) | | | |
| Dry matter | 91.16 | 91.76 | 91.65 |
| Organic matter | 91.39 | 92.33 | 93.18 |
| Crude protein | 15.03 | 16.57 | 17.44 |
| Crude fibers | 8.61 | 7.67 | 6.82 |
| Ether Extract | 6.95 | 6.49 | 7.04 |

## Feeding system

The feeding regimen was formulated using a mix of ingredients, including barley grains, yellow corn, wheat bran, Azolla, salt, and limestone. All the raw materials were procured from local markets and prepared at the feed factory of the College of Agriculture, Tikrit University. As a source of roughage, the lambs were provided with wheat straw at a rate of 1.5 kg per day per lamb.

**Collection of rumen fluid**

Samples of rumen fluid were collected at the end of the experiment. A plastic rumen cannula was used in the collection of rumen fluid. A large syringe with a capacity of 50 ml was attached to the tip of the cannula tube. Fluids were aspirated at three intervals: zero-time (before feeding), two (2) hours after feeding, and four (4) hours after feeding. Each sample was collected in a plastic test tube labeled with the corresponding animal identity number and collection time. The rumen samples were then filtered through a dull cloth, following the method outlined by (Shamoon, 1983).

**Collection of feed and fecal samples for digestive coefficient**

A sample was taken from each feed type and ground well, then stored until the chemical analysis was conducted. Fecal samples from the digestion trials were collected during the last three days of the experiment. Feces from each animal were collected for three consecutive days in the morning before feeding. The collected feces were weighed, and a sample representing 10% of the total weight was taken and placed in plastic bags, then stored in the refrigerator. On the final day of the experiment, the three fecal samples from each animal were combined well, and a sample representing 20% of this mixture was taken. This sample was dried in an electric drying oven at 65 degrees Celsius for a full day, then ground well and stored in bags until chemical analysis was conducted. Samples were analyzed according to (AOAC, 2005).

**Measurement of rumen fluid characteristics**

The pH levels of the rumen fluid samples were immediately measured using an electronic pH meter (HORIBA, LAQUAtwin, Japan) upon collection. The total volatile fatty acid (TVFA) concentration in the rumen liquid was determined at the Tikrit University College of Agriculture laboratories, utilizing the methodology outlined by: Warner (1964), using the following formula:

$$\text{TVFA (mg /100 mL)} = \frac{volume\ NaoH \times 0.1 \times 100}{sample\ volume\ (ml)}$$

The ammonia nitrogen concentration (N- $NH_3$) in rumen liquid samples was measured after dissolving the 2 ml of the collected samples, following the guidelines outlined in AOAC (2005), as described below:

$$\text{N- NH}_3\text{ (mg/100 mL)} = \frac{volume\ HCL \times 0.05 \times 100 \times 14008}{sample\ value}$$

**Statistical analysis**

The experimental data were analyzed using software (SAS, 2005) for a 3 x 3 factorial experiment, following a complete randomized design (CRD) based on the mathematical model outlined below:

$$Yij = \mu + Ff + Tj + AB\ (ij) + eijk$$

It represents all of:

(Yij) is the view j of transaction i.

(μ) is the general mean of the studied variables.

(Ff) represents the effect of the first factor, which is the azolla level (0%, 3%, and 5%).

(Tj) represents the effect of the second factor, which is the time of withdrawal (before feeding, 2 hours after feeding, and 4 hours after feeding).

**Result and Discussion**

Result in Table 2 shows that Azolla inclusion has no significant effect on rumen pH, TVFA. However, N- NH3 has significantly been affected by Azolla inclusion levels. Where T3 record the highest N-NH3 concentration compare to the other treatments. Also, N-NH3 concentration in T2 has significantly higher than T1. In addition, TVFA increased with higher azolla concentration. This may be due to microbial activity and fermentation in rumen which led to increase in quantity of soluble carbohydrates in rumen. Furthermore, increase in degradation of protein may led to increase in N-NH3 concentration (Castillo-González *et al.,* 2014). This finding in line with Abou El-Fadel *et al.* (2020) who reported similar findings.

**Table 2. Effect of Azolla plant pH, TVFAs, and N-NH3 of the rumen fluid before feeding (mean ± standard error)**

| Treatments | Parameters | | |
|---|---|---|---|
| | pH | TVFAs (mg/100 mL) | N-$NH_3$ (mg/100 mL) |
| **T1** | 5.92 a ± 0.24 | 62.81 a ± 2.88 | 20.66 c ± 1.05 |
| **T2** | 5.93 a ± 0.21 | 61.31 a ± 3.94 | 22.48 b ± 0.94 |
| **T3** | 5.91 a ± 0.22 | 60.66 a ± 3.64 | 25.21a ± 1.34 |
| **Sign.** | N.S | N.S | * |

T1 = 0% Azolla, T2 = 3% Azolla, T3 = 5% Azolla.

[a–c]Means within a column with different superscripts differ (P ≤ 0.05).

The effect of rumen collection time of rumen fermentation characteristics was presented in Table 3. The time of samples collection has significant effect on all studied parameters. pH value significantly decreased at 2 and 4 hours after feeding when compare to pH value of samples collected before feeding. Similarly, there was significant increase in TVFAs concentration at 2 and 4 hours after feeding compared to its concentration before feeding. Time of sampling collection also had a significant effect on rumen N-NH3, where N-NH3 concentration at 2 and 4 hours after feeding was higher than its concentration before feeding. High carbohydrate fermentation has been found to lower pH levels because of increased production of total volatile fatty acids (TVFAs) and improved digestibility of organic matter (El-Ashry *et al.,* 2003). This observation aligns with our findings.

**Table 3. Effect of withdrawal time on pH, TVFAs, and N-NH3 in the rumen fluid (mean ± standard error)**

| Withdrawal time | Parameters | | |
|---|---|---|---|
| | pH | TVFAs (mg /100 mL) | N-$NH_3$ (mg/100 mL) |
| **P1** | 6.79 a ± 0.01 | 50.11 b ± 0.97 | 19.45 c ± 0.32 |
| **P2** | 5.51 b ± 0.04 | 65.80 a ± 2.48 | 22.88 b ± 1.24 |
| **P3** | 5.64 b ± 0.01 | 68.87 a ± 2.23 | 26. 04 a ± 0.82 |
| **Sign.** | * | * | * |

P1 = before feeding, P2 = 2 hours after feeding, P3 = 4 hours after feeding.

[a–c]Means within a column with different superscripts differ (P ≤ 0.05).

Results of the interaction between azolla inclusion levels and time of rumen sample collection were presented in Table 4. The results showed a significant interaction effect, as the rumen pH fluid decreased significantly in samples collected from lambs fed 3 and 5% Azolla, 2 and 4 hours after feeding. However, there was no significant interaction effect on the rumen liquid pH of lambs fed 0% level Azolla (Control group) and samples collected before feeding. It is worth noting that all the values recorded in the current study fall within the normal range of pH in the rumen fluid 5.5-7.3 (Hungate, 1966).

There was a significant interaction (P<0.05) between Azolla level and rumen fluid collection period, affecting the concentration of total volatile fatty acids in the rumen fluid. A significant increase in the concentration of total volatile fatty acids (TVFAs) was observed in samples collected from lambs fed 5% Azolla at 4 h after feeding. This increase in the concentration of total volatile fatty acids in rumen fluid was consistent with the decrease in pH values.

Regarding to concentration of ammonia nitrogen in the rumen fluid, the results of the interaction between the level of Azolla and the time of collection of rumen fluid showed a significant increase in ammonia concentration in the rumen fluid. This effect was particularly noted in samples taken from lambs fed 5% Azolla at 2 and 4 hours post-feeding compared to other interactions. Khadr (1990) attributed high levels of rumen ammonia to the increase in crude protein intake, the removal of amino acids, and the increase in free ammonia available in the food decomposed in the rumen.

**Table 4. Effect of interaction between Azolla level and rumen fluid withdrawal time on pH, TVFAs, and N- $NH_3$ in the rumen fluid (mean ± standard error)**

| Treatment * time | Parameters | | |
|---|---|---|---|
| | pH | TVFAs (mg/100 mL) | N-$NH_3$ (mg/100 mL) |
| **T1P1** | 6.84 a ± 0.01 | 52.00 cd ± 0.88 | 18.88 d ± 0.94 |
| **T1P2** | 5.61 b ± 0.02 | 65.55 ab ± 0.42 | 18.86 d ± 1.29 |
| **T1P3** | 5.32 c ± 0.13 | 70.88 a ± 1.99 | 24.24 bc ± 1.07 |
| **T2P1** | 6.77 a ± 0.01 | 47.12 d ± 1.81 | 19.32 d ± 0.42 |

| | | | |
|---|---|---|---|
| **T2P2** | 5.54 b ± 0.01 | 72.02 a ± 4.24 | 23.02 c ± 0.91 |
| **T2P3** | 5.46 bc ± 0.02 | 64.70 ab ± 1. 67 | 25.12 bc ± 0.90 |
| **T3P1** | 6.77 a ± 0.00 | 51.10 cd ± 0.79 | 20.14 d ± 0.51 |
| **T3P2** | 5.38 c ± 0.01 | 59.83 bc ± 4.29 | 26.75 ab ± 0.94 |
| **T3P3** | 5.60 b ± 0.02 | 71.03 a ± 6.32 | 28.74 a ± 0.70 |
| **Sign.** | * | * | * |

T1 = 0% Azolla, T2 = 3% Azolla, T3 = 5% Azolla, P1 = before feeding, P2 = 2 hours after feeding, P3 = 4 hours after feeding.

[a–d]Means within a column with different superscripts differ ($P \leq 0.05$).

The results of the nutritive value followed the same trend of the digestion coefficients (Table 5) were increased by Azolla level increased and there was no significant effect ($p \leq 0.05$).

**Table 5. Effect of Azolla plant in the digestion coefficient % (mean ± standard error)**

| **Treatments** | **parameters** | | | | |
|---|---|---|---|---|---|
| | **Dry matter** | **Organic matter** | **Crude protein** | **Either extract** | **Crude fibers** |
| **T1** | 76.54 ± 0.57 | 78.39 ± 0.81 | 73.94 ± 1.84 | 75.58 ± 1.50 | 71.55 ± 1.16 |
| **T2** | 76.96 ± 0.88 | 78.63 ± 0.68 | 74.45 ± 0.60 | 75.41 ± 2.48 | 72.37 ± 0.83 |
| **T3** | 77.48 ± 0.83 | 79.44 ± 1.40 | 75.25 ± 1.51 | 76.49 ± 0.85 | 72.10 ± 1.43 |
| **Sign.** | N.S | N.S | N.S | N.S | N.S |

T1 = 0% Azolla, T2 = 3% Azolla, T3 = 5% Azolla.

## Conclusions

The findings of the present study conclude that using azolla as a protein source in the feeding of Awassi lambs at 3 and 5 % inclusion levels as a partial replacement for wheat bran has been associated with improved rumen environment through enhancing optimal pH value, TVFAs and N-NH3 concentration and digestive coefficient. Furthermore, time of sampling has a significant effect on all rumen fermentation parameters.

## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

## Acknowledgments

We would like to express our sincere gratitude to all the staff of College of Veterinary Medicine, university of Tikrit for their invaluable assistance.

## References

Abou El-Fadel, M. H., Hassanein, H. A. M., and El-Sanafawy, H. A. (2020). Effect of Partial Replacement of Protein Sun Flower Meal by Azolla Meal as Source of Protein on Productive Performance of Growing Lambs. *Journal of Animal and Poultry Production*, *11*(4), 149–153. https://doi.org/10.21608/jappmu.2020.95833

Ahirwar, M. K., and Leela, V. (2012). Nutritive value and in vitro degradability of Azolla pinnata for ruminants. *The Indian veterinary journal, 89*(4), 101-102. http://krishikosh.egranth.ac.in/handle/1/68398

Ahmed, H. A., Ganai, A. M., Beigh, Y. A., Sheikh, G. G., and Reshi, P. A. (2016). Performance of growing sheep on Azolla based diets. *Indian Journal of Animal Research*, *50*(5), 721-724. https://www.indianjournals.com/ijor.aspx?target=ijor:ijar1&volume=50&issue=5&article=016

Akintan, O., Gebremedhin, K. G., and Uyeh, D. D. (2024). Animal feed formulation-connecting technologies to build a resilient and sustainable system. *Animals*, *14*(10), 1-16. https://doi.org/10.3390/ani14101497

AOAC International, (2005). *Association of Official Analytical Chemists*, Gaithersburg, MD, United States.

Castillo-González, A. R., Burrola-Barraza, M. E., Domínguez-Viveros, J., and Chavez-Martinez, A. (2014). Rumen microorganisms and fermentation. *Archivos de medicina veterinaria*, *46*(3), 349-361. http://dx.doi.org/10.4067/S0301-732X2014000300003

El Naggar, S., and El-Mesery, H. S. (2022). Azolla pinnata as unconventional feeds for ruminant feeding. *Bulletin of the National Research Centre*, *46*(1), 1-5. https://doi.org/10.1186/s42269-022-00752-w

El-Ashry, M. A., Kholif, A. M., Fadel, M., El-Alamy, H. A., El-Sayed, H. M., and Kholif, S. M. (2003). Effect of biological treatments on chemical composition, in vitro and in vivo nutrient digestibilities of poor quality roughages. *Egyptian Journal of Nutrition and Feeds, 6*(2), 113-126. https://www.cabidigitallibrary.org/doi/full/10.5555/20083091317

Hungate, R. E. (1966). *The rumen and its microbes Academic Press New York and London*. Academic Press, New York, Estados Unidos. 533 p. https://www.sciencedirect.com/book/9781483233086/the-rumen-and-its-microbes

Kathirvelan, C., Banupriya, S., and Purushothaman, M. R. (2015). Azolla-an alternate and sustainable feed for livestock. *International Journal of Science, Environment and Technology*, *4*(4), 1153-1157.

Khadr, N. E. A. (1990). *Effect of Non Protein Nitrogenous Supplements on Digestibility of Low Quality Roughages in Goats,* PhD Thesis, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Kumari, J., Kumar, S., Kumar, K., Singh, P. K., MONI, C., Kumar, P., and Kumari, R. (2021). Effect of different level of azolla meal on nutrient utilization and growth performance in goat kids: Influence of azolla meal on nutrient utilization and growth in goat kids. *Journal of AgriSearch*, *8*(3), 275-280. https://demo8.purnank.co.in/index.php/jas/article/view/883

Mandal, R. N., Pandey, B. K., Chattopadhyay, D. N., and Mukhopadhyay, P. K. (2012). Azolla-an aquatic fern of significance to small-scale aquaculture. *Aquaculture Asia Magazine, 17,* (1), 11-15. https://www.cabidigitallibrary.org/doi/full/10.5555/20123303668

Meena, G. S., Dhaka, B. L., Singh, B., Meena, R. K., and Meena, K. C. (2017). Effect of Azolla as feed supplement on milk yield in buffaloes. *International Journal of Current Microbiology and Applied Sciences*, *6*(12), 3490–3494. https://doi.org/10.20546/ijcmas.2017.612.406

Rana, D., Katoch, S., Mane, B. G., Rani, D., and Sankhyan, V. (2017). Biological evaluation of Azolla in ration of commercial chicken broiler. *Journal of Animal Research*, *7*(3), 601–607. http://dx.doi.org/10.5958/2277-940X.2017.00091.2

SAS. (2005). *SAS/STAT/ User's Guide for Personal computers, Release 6.12*, SAS Institute Inc., Cary, NC, USA.

Senthil Kumar, A., Murugesan, S., and Balamurugan, P. (2020). Feeding of Azolla as a green fodder feed supplement on productive performance and milk composition of crossbred dairy cows in Theni District of Tamil Nadu, India. *TNV_IJCMAS, 9*(6), 1382-1392. https://krishikosh.egranth.ac.in/items/c92ae2c6-28a8-4934-b166-52b12af6c7cc

Shamoon, S. A. (1983). *Amino acid supplements for ruminant farm livestock, with special reference to methionine*. University of Glasgow (United Kingdom). ProQuest Dissertations & Theses, 1-24.

Sujatha, T., and Jeyakumar, S. (2009). Azolla as feed supplement for livestock and backyard poultry. *Indian Farming*, *59*(12), 22–24.

Toradmal, S. D., Shelke, R. R., Kahate, P. A., and Bidwe, K. U. (2017). Studies on supplementation of green Azolla (*Azolla pinnata*) on growth performance of Osmanabadi goat kids. *Asian Journal of Animal Science, 12*(2), 124-128.

Warner, A. C. I. (1964). *Production of volatile fatty acids in the rumen: Methods of measurement. Nutrition Abstracts and Reviews, 34*(2), 339-52. https://www.cabidigitallibrary.org/doi/full/10.5555/19640402239

Yadav, R. K., Abraham, G., Singh, Y. V., and Singh, P. K. (2014). Advancements in the utilization of Azolla-Anabaena system in relation to sustainable agricultural practices. *Proceedings of the Indian National Science Academy*, *80*(2), 301–316. http://dx.doi.org/10.16943/ptinsa/2014/v80i2/55108