Effects of Dietary Slow-Release Urea Supplementation on Rumen Fermentation and Blood Biochemical Parameters in Sheep

Ghassan Hameed Shehab Al-Jubouri and Mwaffuk Hussain Aljumaily
Department of Animal Production, College of Agriculture, Tikrit University, Iraq.
Corresponding author's email: gs231158pag@st.tu.edu.iq
Email addresses of coauthors: drmwaffuk75@tu.edu.iq

Abstract

This study was conducted at the Sheep Farm of the Department of Animal Production, College of Agriculture, Tikrit University, from **October 5, 2025, to January 5, 2026**, to evaluate the effect of dietary supplementation with **slow-release urea (SRU)** on the productive and physiological performance of Awassi lambs. Fifteen Awassi lambs were randomly assigned to three treatments, with five lambs per treatments, aged **3 – 4 months** with an initial body weight of **(26 kg)**,The first treatment served as the control group (without slow-release urea supplementation), whereas the second and third treatments received diets supplemented with **(3%)** and **(5%)** slow-release urea, respectively. The experiment lasted for **(12 weeks)** and was arranged according to a **Completely Randomized Design (CRD).**

The results indicated that ruminal ammonia concentration increased significantly (**P ≤ 0.05**) in the third treatment compared with the second and first treatments, recording values of **(17.40, 15.00, and 13.60)**, respectively. Regarding ruminal pH, no significant differences were observed among the three treatments before feeding. However, one hour after feeding, the second treatment exhibited a significantly higher ruminal pH than the third and first treatments, recording values of **(7.30, 7.02, and 6.20)**, respectively.

Keywords: Slow-release urea; Awassi lambs; Rumen fermentation; Ruminal pH; Ruminal ammonia; Volatile fatty acids; Blood biochemical parameters

Introduction

The use of **non-protein nitrogen (NPN)** as a dietary nitrogen source for ruminants has increased considerably over the past decades. Previously, it was widely used as an alternative to true protein without causing adverse effects on animal health or productive performance. Replacing plant or animal protein sources with non-protein nitrogen can reduce feed costs by lowering the cost of dietary nitrogen, thereby decreasing the overall cost of feed production **[17].**

Urea is one of the most commonly used non-protein nitrogen sources in ruminant diets.

ISSN 2072-3857

However, it undergoes rapid hydrolysis in the rumen due to the activity of the microbial enzyme urease, resulting in ammonia release at a rate that often exceeds the capacity of ruminal microorganisms to utilize it efficiently for microbial protein synthesi. **[24]** The efficiency of urea utilization largely depends on the rate at which it is hydrolyzed into ammonia in the rumen. Consequently, several approaches have been developed to reduce the rate of urea degradation, including coating or blending urea with substances such as gelatin or other protective materials to produce **slow-release urea (SRU) [24]** . This technology provides a gradual supply of nitrogen while simultaneously supplying energy derived from the coating material, thereby enhancing microbial protein synthesis. Previous studies have demonstrated that incorporating SRU into ruminant diets can improve milk production and nutrient digestibility, although its effects on digestion rate and carcass characteristics in animals fed low-concentrate diets have been limited **[24].**

The use of slow-release nitrogen sources has also been reported to maintain stable blood urea nitrogen concentrations, indicating improved synchronization between ammonia release and microbial utilization. This synchronization enhances microbial protein synthesis efficiency while preventing excessive elevations in blood ammonia concentrations, thereby improving the safety of nitrogen supplementation compared with conventional urea **[16].**

Given the importance of slow-release urea and the limited information available regarding its use as an alternative protein source in ruminant nutrition, the present study was conducted to evaluate the effects of dietary slow-release urea supplementation on the ruminal environment and selected blood parameters of Awassi lambs.

Materials and Methods

This experiment was conducted at the Sheep Farm, Department of Animal Production, College of Agriculture, Tikrit University, from **October 5 to January 5, 2026**. Fifteen Awassi lambs, approximately three months of age and weighing **26 kg**, were used in the experiment. The animals were housed in three semi-open pens with concrete floors and walls and metal roofs. Each pen was equipped with individual feeders and drinking troughs to facilitate the monitoring of daily feed intake. The dimensions of each pen were **2.0 m × 1.5 m**.

The lambs were randomly assigned to three experimental treatments, with **five animals per treatment**, according to a **Completely Randomized Design (CRD)**. Feed allowance was standardized among treatments and offered in two equal meals daily, at **08:00 a.m.** and **03:00 p.m.**

The experimental treatments were as follows:

- **Control:** Basal diet without slow-release urea supplementation.
- **T1:** Basal diet supplemented with **30 kg of slow-release urea per ton of feed**.
- **T2:** Basal diet supplemented with **50 kg of slow-release urea per ton of feed**.

The experimental treatments were supplemented with slow-release coated urea (SRU). This type of urea is produced by coating urea granules with layers of fats or

ISSN 2072-3857

fatty acids, specifically palm oil, which restricts the penetration of water and enzymes into the urea granules, thereby reducing the rate of urea hydrolysis in the rumen.

Blood Sampling

Blood samples (10 mL) were collected from the jugular vein of each lamb according to the procedure described by **[11]**. The samples were placed into heparinized tubes to prevent coagulation. Serum was separated by centrifugation at **3,000 rpm for 20 min**, after which it was transferred into glass vials and stored at **−20°C** until biochemical analyses were performed.

Measured Parameters

Rumen Fluid Characteristics

Rumen pH

Immediately after collection, the pH of rumen fluid was determined using **pH indicator paper**. The indicator strip was immersed in the rumen fluid sample, and the resulting color was compared with the manufacturer's reference color chart to determine the corresponding pH value **[21].**

Determination of Ruminal Ammonia Concentration

Ruminal ammonia concentration was determined according to the method described by **[8].** Rumen fluid samples were first filtered through filter paper after thawing to room temperature. Subsequently, **0.5 mL** of the filtrate was transferred into a clean test tube, followed by the addition of **3 mL** of a solution containing **4 g sodium ethylene diamine tetraacetate (Na_2 EDTA)** and **13 g sodium hydroxide (NaOH)** dissolved in **1 L of distilled water**. Thereafter, **5 mL** of a reagent containing **10 g phenol** and **0.05 g sodium nitroprusside** dissolved in **1 L of distilled water** was added. Finally, a solution consisting of **5 g sodium hydroxide (NaOH), 10 mL sodium hypochlorite solution, and 31.8 g trisodium phosphate dissolved in 1 L of distilled water** was added.

The test tubes were tightly sealed, shaken twice, and incubated in a water bath at **35°C for 15 min**. Subsequently, the tubes were transferred to another water bath maintained at **20°C for 5 min**. Sample absorbance was then measured using a **spectrophotometer** at a wavelength of **589 nm**.

Standard solutions of **ammonium sulfate** with different concentrations were prepared and analyzed following the same procedure to construct a standard calibration curve, which was subsequently used to determine the ammonia concentration of rumen fluid samples.

Total Volatile Fatty Acids (TVFA)

Total volatile fatty acid concentration was calculated according to the following equation**:[6]**

Total VFA = Volume of standardized sodium hydroxide (NaOH) used in titration × 10

Statistical Analysis

The experimental data were analyzed using the **Statistical Analysis System [25].** Treatment means were compared using **Duncan's Multiple Range Test** as described by **[11]** . Differences were

ISSN 2072-3857

considered statistically significant at $P \leq 0.05$.

Results and Discussion :

Table (1). Effect of Slow-Release Urea on Selected Blood Parameters of Awassi Lambs (Mean ± Standard Error)

Treatments	LDL	Total Protein (g/100 mL)	Albumin (g/100 mL)	Globulin (g/100 mL)	AST Activity (IU/L)	ALT Activity (IU/L)
CONTROL	b46.400 ± 1.630	**0.930±67.600 a**	**c0.970±42.800**	**1.503±23.600**	**a1.030±32.400**	**b1.330±13.600**
T2	a63.600 ± 2.461	**a1.210±69.600**	**b1.160±45.800**	**2.782±24.800**	**b2.480±27.200**	**a0.840±15.00**
T3	a63.00 ± 1.790	**b0.860±61.800**	**a0.710±49.00**	**1.503±23.600**	**a2.204±37.400**	**a0.812±17.400**
Significance level	*	*	*	N.S	*	*

Note: Different letters within the same column indicate significant differences among treatments ($P \leq 0.05$).

The results showed significant differences ($P \leq 0.05$) in serum total protein among treatments. T1 and T2 recorded higher concentrations than T3, with values of 67.60, 69.60, and 61.80 g/L, respectively. This finding is consistent with [4], who reported a significant decrease in serum total protein following slow-release urea supplementation

Regarding serum albumin, T3 showed a significantly higher concentration ($P \leq 0.05$) than T1 and T2, with values of **49.00, 45.80, and 42.80 g/L**, respectively. This finding agrees with **[15],** who reported increased albumin concentration in lambs receiving polymer-coated urea. The gradual release of nitrogen may improve synchronization between nitrogen availability and microbial requirements, thereby enhancing microbial protein synthesis and amino acid absorption. No significant differences were observed in serum globulin concentration among treatments.

The results showed significant differences ($P \leq 0.05$) in serum AST activity among treatments. Treatment T3 recorded the highest activity (37.40 IU/L) compared with T2 (27.20 IU/L), while it did not differ significantly from the control (T1; 32.40 IU/L). AST is an important indicator associated with liver and tissue function; therefore, changes in its activity should be

ISSN 2072-3857

interpreted together with ALT and other blood parameters.

Previous studies reported no significant differences in serum AST and ALT activities among dietary treatments. However, the positive correlation between both enzymes suggests a similar physiological response [30]. The absence of significant differences in AST indicates that slow-release urea supplementation did not adversely affect liver function.

Alanine Aminotransferase (ALT)

The results in Table (1) showed that T3 had significantly (P≤0.05) higher ALT activity than T1 and T2, with values of 13.60, 15.00, and 17.40 IU/L, respectively. The lower ALT activity in T1 and T2 may reflect improved nitrogen utilization and reduced hepatic metabolic stress due to the gradual ammonia release from slow-release urea, consistent with [20]. However, the present AST results differed from those reported by [4].

Similarly, the present findings are inconsistent with those reported by [3], [23] , [22], [1], and [13] regarding the effects of coated slow-release urea supplementation in ruminant diets. According to [18], excessive urea intake may increase blood ammonia concentrations, thereby increasing the utilization of α-ketoglutarate for glutamate synthesis. This may interfere with energy metabolism and affect the Krebs cycle, potentially increasing reliance on glycolysis and contributing to altered lactate metabolism.

The results showed significant differences (P≤0.05) in serum LDL concentration among treatments. T2 recorded the highest value (63.60), followed by T3 (63.00), whereas the control group (T1) showed the lowest value (46.40). These changes in LDL concentration, particularly in T2 and T3, may indicate alterations in lipid metabolism in response to dietary slow-release urea supplementation.

Overall, T2 produced notable changes in protein, enzyme, and lipid-related parameters, whereas **serum globulin** was not significantly affected. The response was not dose-dependent, suggesting that the effects of slow-release urea may vary according to the supplementation level**.**

Rumen Fermentation

The results in Table (2) showed that pre-feeding ruminal NH_3 -N concentration was higher in the control (24.31 mg/100 mL) than in T2 (21.64) and T3 (20.12). After feeding, the control and T3 recorded higher concentrations (31.23 and 29.99 mg/100 mL, respectively) than T2 (28.09 mg/100 mL).

Ruminal ammonia is the primary nitrogen source for microbial protein synthesis [12]. Its peak concentration generally occurs about 1 h after feeding with NPN sources and 3–5 h with true or protected protein sources [17]. Consistent with [3], the present findings indicate that ruminal ammonia concentration decreased with increasing slow-release urea supplementation.

Previous studies have shown variable effects of slow-release urea on ruminal **NH_3 -N** concentration. Some studies reported a slight or significant increase after feeding,

ISSN 2072-3857

attributed to the gradual release of nitrogen and sustained ammonia availability for ruminal microbial growth **[27, 18, 2, 26, 17, 22]**. In contrast, other studies reported a reduction in ruminal ammonia concentration with increasing slow-release urea supplementation **[3]**. These differences may be related to the level of supplementation, timing of sampling, and rate of urea degradation in the rumen.

Total Volatile Fatty Acids (VFA)

The results showed that pre-feeding total VFA concentration was slightly lower in T3 (7.07 mg/100 mL) than in T1 (7.67) and T2 (7.23). After feeding, T3 showed a marked increase in VFA concentration (12.98 mg/100 mL) compared with T1 (9.11) and T2 (8.37).

Previous studies have reported that slow-release urea supplementation increased ruminal volatile fatty acid (VFA) concentration, possibly due to improved synchronization between nitrogen availability and fermentable energy, thereby enhancing microbial activity and carbohydrate fermentation [17]. Elevated ruminal VFA concentration generally reflects increased fermentation activity and energy production in the rumen.

(The present findings agree with those of **[18] ,** who suggested that elevated circulating VFA concentrations reflect increased fat mobilization caused by an energy deficit, in which energy expenditure exceeds dietary energy intake. Likewise, **[6]** reported that VFA concentration is closely associated with energy intake, energy expenditure, and adipose tissue mobilization, thereby supporting the observed increase in VFA concentration with higher levels of slow-release urea supplementation. In contrast, these findings differ from those reported by **[25] ,** who concluded that protected urea improved nitrogen utilization without adversely affecting metabolic balance or the overall health status of ruminants.)

The present findings agree with [19] and [7], who associated increased VFA concentrations with energy status and nutrient utilization. In contrast, [27] reported that protected urea improved nitrogen utilization without adversely affecting metabolic balance or overall animal health

No significant differences (P>0.05) were observed among treatments in pre-feeding ruminal pH. Ruminal pH is influenced by dietary composition, with concentrate-rich diets generally lowering pH, whereas roughage-based diets tend to maintain higher values. The observed pH values were within the range suitable for cellulolytic bacterial activity [14]. Similarly, [29] reported that increasing slow-release urea supplementation increased ruminal pH and improved feed intake in sheep.

ISSN 2072-3857

Ruminal PH

Table 2. Effect of Slow-Release Urea on Selected Rumen Fluid Characteristics of Awassi

Treatments	NH_3 -N Before Feeding (mg/100 mL)	NH_3 -N After Feeding (mg/100 mL)	**VFA** Before Feeding (mg/100 mL)	**VFA** After Feeding (mg/100 mL)	**pH** Before Feeding	**pH** After **Lambs** Feeding
Control	a 1.114±24.31	A 0.316±31.23	A 0.125±7.67	B 0.380±9.11	0.050±6.84	B 0.083±6.20
T2	Ab 0.855±21.64	B 1.112±28.09	Ab 0.170±7.23	B 0.264±8.37	0.06±6.90	A 0.130±7.30
T3	B 1.400±20.12	ab 0.575±29.99	B 0.200±7.07	A 1.770±12.98	0.06±7.02	A 0.070±7.02
Significance level	*	*	*	*	N.S	*

Note: Different letters within the same column indicate significant differences among treatments ($P \leq 0.05$).

(Mean ± SE

:References

1- Abdel-Raouf, E., Bassiouni, M., Ali, M., & Hassanien, H. (2017). Effect of using slow-release urea on milk production and its composition of lactating dairy cows. Journal of Sustainable Agriculture Science, 43, 17-26.

2 - Alizadeh, Z., Yansari, A. T., Chashnidel, Y., Kazemifard, M., & Azarpajouh, S. (2020). Effect of soybean meal replacement by slow-release urea on ruminal parameter, blood metabolites, and microbial protein synthesis in Zel ram. Acta Scientiarum. Animal Sciences, 43.

3- Al-Jandil, K. A. (2022). Effect of different levels of rapidly and slowly degradable urea in the rumen on productive performance and rumen fermentation in Awassi lambs (Master's thesis). Al-Qasim Green University, College of Agriculture.

4- Al-Khafaf, W. A. H. H. (2024). Effect of replacing urea with slow-release urea on

ISSN 2072-3857

nutrient digestion and productive performance in Awassi sheep (Doctoral dissertation). University of Mosul, College of Agriculture and Forestry.

5-Alves, E. M., Magalhães, D. R., Freitas, M. A., Santos, E. D. J. D., Pereira, M. L. A., & Pedreira, M. D. S. (2014). Metabolismo do nitrogênio e síntese microbiana em ovinos alimentados com dietas contendo ureia de liberação lenta em substituição à ureia convencional. Acta Scientiarum. Animal Sciences, 36, 55-62.

6- Barnett, A. J. G., & Reid, R. L. (1957). Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen: I. The volatile fatty acid production. Journal of Agricultural Science, 48, 315–321.

7- Block, S. S., Butler, W. R., Ehrhardt, R. A., Bell, A. W., Van Amburgh, M. E., & Boisclair, Y. R. (2001). Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. Journal of Endocrinology, 171(2), 339–348.

8 - Broderick, G. A. and J. H. Kang (1980). Automated simultaneous Golombeski determination of ammonia and amino acids in ruminal fluid and in vitro media. J. Diary Sci 33:64-75.

9-Devendra, C. (2007). Perspectives on animal production systems in Asia. Livestock Science, 106(1), 1–18.

10-Duncanson, G. R. (2025). *Veterinary Treatment of Sheep and Goats*. Wallingford, UK: CABI.

11-Duncan, D.B. (1955), Multiple range and multiple "F99 test Biometries.11: 1-2.

12-Devendra, C. (2007). Perspectives on animal production systems in Asia. Livestock Science, 106(1), 1–18.

13-El-Zaiat, M., Kholif, A. E., Khattab, I. M., & Sallam, S. M. A. (2022). Slow-Release Urea Partially Replacing Soybean in the Diet of Holstein Dairy Cows: Intake, Blood Parameters, Nutrients Digestibility, Energy Utilization, and Milk Production. Annals of Animal Science, 22(2), 723–30.

14-Frizzo, L. S., Soto, L. P., Zbrun, M. V., Bertozzi, E., Sequeira, G., Armesto, R. R., & Rosmini, M. R. (2010). Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. Animal Feed Science and Technology, 157(3–4), 159–167.

15- Fan, C., Li, H., Li, S., Zhong, G., Jia, W., Zhuo, Z., Xue, Y., Koontz, A. F.,&Cheng, J. (2024). Effect of Different Slow-Release Urea on the Production Performance, Rumen Fermentation, and Blood Parameters of Angus Heifer. Animals, 14(16), 2296

ISSN 2072-3857

16- Gao, X., & Oba, M. (2016). Effect of increasing dietary nonfiber carbohydrate with starch, sucrose, or lactose on rumen fermentation and productivity of lactating dairy cows. Journal of Dairy Science, 99(1), 291–300.

17-Geron, L. J. V., de Paula, E. J. H., de Aguiar, S. C., de Carvalho, J. T. H., Juffo, G. D., da Silva, A. P., & Diniz, L. C. (2016). Effect of slow release urea in sheep feed on intake, nutrient digestibility, and ruminal parameters. Semina: Ciências Agrárias, 37(4), 2793–2805.

18-Hashem, W. A., & Tayeb, M. A. M. (2023). Effect of Using Slow-Release Urea on Food Compound Digestion Coefficient and some Rumen and Blood Fluid Traits in Awassi Lambs. IOP Conference Series: Earth and Environmental Science, 1213(1), 012086

19-Herdt, T. H. (2000). Ruminant adaptation to negative energy balance: Influences on the etiology of ketosis and fatty liver. Veterinary Clinics of North America: Food Animal Practice, 16(2), 215–230.

20-Nordlund, K. V., & Garrett, E. F. (1994). Rumenocentesis: A technique for collecting rumen fluid for diagnosis of subacute rumen acidosis in dairy herds. Bovine Practitioner, 28, 109–112.

21-Ma, S. W., & faciola ,A.P. (2024). Impacts of Slow-Release Urea in Ruminant Diets: A Review. Fermentation, 10(10), 527.

22- Manju, G. U., Nagalakshmi, D., Nagabhushana, V., Venkateswarlu, M., & Rajanna, N. (2022). Effect of feeding slow release non protein nitrogen sources on milk production and milk quality parameters in Cross bred dairy cows. Indian J Dairy Sci, 75(2), 173-180.

23-Reddy, R., K., P., Srinivasa Kumar, D., Raghava Rao, E., Venkata Seshiah, C., Sateesh, K., Pradeep Kumar Reddy, Y., & Hyder, I. (2019). Assessment of eco-sustainability vis-à-vis zoo-technical attributes of soybean meal (SBM) replacement with varying levels of coated urea in Nellore sheep (Ovis aries)., 14(8), 0220252.

24- Rimbawanto, E. A., Suhermiyati, S., & Hartoyo, B. (2018). Effects of slow release urea supplementation of sheep protein source feed protected with condensed tannin from Leucaena on protein degradation in rumen and post-rumen in vitro. Animal Production, 19(2), 119–126

25- SAS Institute Inc. (2010). *Base SAS® 9.2 procedures guide: Statistical procedures* (3rd ed.). SAS Institute Inc., Cary, NC.

ISSN 2072-3857

26 -Safvi, S., & Chaji, M. (2022). The effect of slow-release urea sources on digestibility of nutrients and growth performance of fattening lambs fed rations containing low-quality forage. Iranian Journal of Animal Science Research, 14(2), 189–200

27-Silva, A., Pereira Filho, J. M., Oliveira, J., Lucena, K., Mazza, P., Silva Filho, E., ... & Bezerra, L. (2023). Effect of slow-release urea on intake, ingestive behavior, digestibility, nitrogen metabolism, microbial protein production, blood and ruminal parameters of sheep. Tropical Animal Health and Production, 55(6), 414.

28-Taylor-Edwards, C. C., Elam, N. A., Kitts, S. E., McLeod, K. R., Axe, D. E., Vanzant, E. S., Kristensen, N. B., & Harmon, D. L. (2009). Influence of slow-release urea on nitrogen balance and portal-drained visceral nutrient flux in beef steers. Journal of Animal Science, 87(6), 209–221.

29- Wahyono, T., Sholikin, M. M., Konca, Y., Obitsu, T., Sadarman, S., & Jayanegara, A. (2022). Effects of urea supplementation on ruminal fermentation characteristics, nutrient intake, digestibility, and performance in sheep: A meta-analysis. Veterinary World, 15(2), 331.

30- Zhang, Y., Li, F., Wang, C.,&Chen, H. (2023). Effects of controlled-release

urea on nitrogen metabolism and blood biochemical parameters in sheep. Animal

Feed Science and Technology, 305, 115215.

ISSN 2072-3857