Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Effects of Dietary Slow-Release Urea Supplementation on Rumen Fermentation Parameters and Bacterial Populations in Lambs: Implications for Meat Production Efficiency

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ARTICLE INFO

Article History:

Received: 2026/02/13
Review: 2025/03/15
Accepted: 2026/04/16

Keywords:

Slow-release urea,

Arabi lambs,

rumen fermentation,

volatile fatty acids,

cellulolytic bacteria,

meat quality,

feed efficiency

DOI: 10.48311/fsct.2026.119317.83063

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ABSTRACT

Slow-release urea (SRU) offers a strategic approach to sustainable protein nutrition in ruminant production systems, with direct implications for feed efficiency, production economics, and the environmental footprint of meat production. This study investigated the effects of dietary SRU supplementation at varying concentrations on rumen fermentation parameters and bacterial populations in Arabi lambs, thereby addressing knowledge gaps relevant to both animal nutrition and subsequent meat quality attributes. Sixteen male Arabi lambs (initial body weight 22.5 ± 1.1 kg; age 4–5 months) were randomly assigned to four dietary treatments over a 105-day experimental period, including a 15-day adaptation phase. Lambs received either a control diet (no SRU) or diets supplemented with SRU at 1.0%, 1.5%, or 2.0% of dry matter intake. Rumen fluid pH remained unaffected by SRU inclusion across all treatment levels ($P > 0.05$), indicating maintained rumen homeostasis. However, total bacterial counts increased significantly in lambs receiving 2.0% SRU (8.12 $\log_{10}$ CFU mL^{-1}) compared to control and lower supplementation groups ($P < 0.05$). Similarly, populations of cellulolytic and proteolytic bacteria were significantly elevated in the 2.0% SRU treatment. Conversely, amylolytic bacterial counts exhibited a declining trend with increasing SRU concentration, decreasing from 6.74 $\log_{10}$ CFU mL^{-1} in controls to 6.64 $\log_{10}$ CFU mL^{-1} at 2.0% SRU. Total volatile fatty acid (VFA) concentration increased progressively with SRU inclusion level. Acetic acid concentration reached 43.09 mmol L^{-1} in the 2.0% SRU treatment, representing a significant elevation relative to controls; parallel increases were observed for propionic and butyric acids. These findings demonstrate that dietary SRU supplementation, particularly at 2.0% inclusion, enhances rumen fermentative capacity through selective stimulation of cellulolytic and proteolytic bacterial populations and elevated VFA production, without compromising rumen pH stability. From a food science perspective, optimized rumen fermentation profiles are positively associated with improved growth performance, carcass composition, and meat quality attributes—including protein deposition, intramuscular fat content, and sensory characteristics. This study supports the application of SRU as a cost-effective, non-protein nitrogen source in lamb production systems, with potential benefits extending from feed efficiency to the nutritional and technological quality of ovine meat products.

1- INTRODUCTION

The global livestock sector faces mounting pressure to expand production in response to unprecedented population growth and rising demand for high-quality animal protein. According to United Nations projections, the world population is anticipated to reach 8.5 billion by 2030 and approximately 9.7 billion by 2050, up from 7.3 billion in 2000 [1, 2]. This demographic transition, coupled with urbanization and increasing disposable incomes, has fundamentally shifted dietary patterns toward greater consumption of meat and dairy products [3]. Ruminants occupy a uniquely strategic position within this context, owing to their physiological capacity to convert human-inedible plant biomass and agricultural by-products into nutritionally dense protein sources [4].

Notwithstanding this adaptive advantage, contemporary ruminant production systems are constrained by persistent inefficiencies in nitrogen utilization. Conventional dietary protein sources, particularly soybean meal, are characterized by extensive ruminal degradation that exceeds the temporal capacity of rumen microbes to capture liberated ammonia. This temporal asynchrony between nitrogen release and fermentable energy availability results in substantial nitrogen excretion—with concomitant economic losses and environmental consequences, including ammonia volatilization, nitrous oxide emissions, and nitrate contamination of water systems [5, 6]. Consequently, nutritional strategies that synchronize nitrogen and energy release kinetics within the rumen have emerged as a research priority for sustainable livestock production [7, 8].

Urea, as a non-protein nitrogen (NPN) source, has long been employed in ruminant diets to partially supplant expensive plant proteins. However, conventional urea undergoes rapid and complete hydrolysis in the rumen, releasing ammonia at rates that frequently exceed microbial assimilation capacity, thereby elevating the risk of ammonia toxicity and diminishing nitrogen retention efficiency [9]. Slow-release urea (SRU) formulations have been developed to address this limitation through encapsulation or chemical modification technologies that attenuate hydrolysis kinetics, achieving closer temporal alignment with ruminal carbohydrate fermentation [10, 11]. This synchronization enhances microbial protein synthesis, improves nitrogen utilization efficiency, and reduces environmental nitrogen excretion, while simultaneously lowering feed costs given urea's superior cost-per-nitrogen ratio relative to conventional protein meals [12].

Previous investigations have demonstrated that rumen-modulating feed additives—including bentonite and probiotics—can significantly improve digestibility coefficients and fermentation parameters in Iraqi lambs, confirming that targeted manipulation of the ruminal microenvironment enhances nutrient utilization efficiency and growth performance [13, 14]. These findings provide empirical precedent for evaluating SRU as a strategic intervention within comparable production systems.

The Arabi lamb, indigenous to southern Iraq, represents a locally adapted genetic resource of considerable socioeconomic importance. Despite the recognized potential of SRU to improve nitrogen utilization in ruminants, limited information exists regarding its dose-dependent effects on rumen fermentation dynamics and specific bacterial functional groups in this breed. Furthermore, the relationship between SRU-induced modifications in ruminal fermentation profiles and subsequent meat quality attributes—including protein deposition, fatty acid composition, and sensory characteristics—remains insufficiently

characterized from a food science perspective.

Accordingly, the present study was designed to evaluate the effects of graded levels of dietary SRU supplementation on rumen fermentation parameters (pH, volatile fatty acid concentrations) and key bacterial populations (total bacteria, cellulolytic, proteolytic, and amylolytic bacteria) in Arabi lambs. By elucidating the dose-response relationships between SRU inclusion rate and rumen fermentation efficiency, this research aims to inform evidence-based feeding strategies that optimize growth performance, reduce production costs, and enhance the nutritional and technological quality of ovine meat products.

2-Materials and Methods

2.1 Experimental Location and Duration

The experiment was conducted at the Animal Production Department, College of Agriculture, University of Basrah, Iraq, over a 105-day period from 10 October 2024 to 22 January 2025. This study aimed to evaluate the dose-dependent effects of dietary slow-release urea (SRU) supplementation on rumen fermentation parameters and bacterial populations in Arabi lambs.

2.2 Animals and Experimental Design

Sixteen apparently healthy male Arabi lambs, aged 4–5 months with a mean initial body weight of 22.5 ± 1.1 kg, were purchased from local livestock markets in Basrah Governorate. Lambs were housed in a semi-closed barn and randomly assigned to four dietary treatments with four replicates per treatment. The experimental period comprised 15 days of adaptation followed by 90 days of data collection.

The dietary treatments were as follows:

- **T1 (Control)**: Basal diet without SRU supplementation
- **T2**: Basal diet supplemented with 1.0% SRU
- **T3**: Basal diet supplemented with 1.5% SRU
- **T4**: Basal diet supplemented with 2.0% SRU

2.3 Diets and Feeding Management

The basal concentrate diet consisted of 46% barley, 35% wheat bran, 10% crushed yellow corn, 8% soybean meal, and 1% vitamin–mineral premix. This formulation was designed to meet the nutritional requirements of growing lambs in accordance with National Research Council (NRC, 2007) recommendations.

Lambs were fed twice daily (morning and evening) throughout the experimental period. Feed residues were collected and weighed daily to determine actual feed intake. Clean drinking water was available ad libitum. Prior to the commencement of the experiment, all animals received veterinary examination and were administered anthelmintic agents to control internal parasites.

2.4 Rumen Fluid Collection and pH Measurement

Rumen fluid samples were collected 3 h post-feeding using a sterile oral-gastric tube. Ruminal pH was measured immediately using a pre-calibrated Hanna digital pH meter. Following pH determination, rumen fluid was filtered through four layers of sterile gauze and divided into two aliquots. The first aliquot was preserved under sterile conditions and frozen at −20°C for subsequent volatile fatty acid (VFA) analysis. The second aliquot was maintained at 4°C for immediate bacterial enumeration.

2.5 Volatile Fatty Acid Analysis

Total volatile fatty acid concentration was determined by titration. Frozen rumen fluid samples were thawed at room temperature; 5 mL of rumen fluid was transferred to a Kjeldahl digestion tube and mixed with 1 mL of orthophosphoric acid. The mixture was transferred to a titration flask, and 2–3 drops of phenolphthalein indicator (prepared by dissolving 1 g phenolphthalein in 50 mL absolute ethanol and 50 mL distilled water, titrated with 0.05 M sodium hydroxide until faint pink) were added. The solution was titrated against 0.1 M sodium hydroxide until a persistent pale pink endpoint was reached. Total VFA concentration was calculated using the following equation:

Total VFA (mmol 100 mL^{-1}) = [(V_sample − V_blank) × 0.1 M NaOH × 100] / 5 mL

where V_sample and V_blank represent the titration volumes (mL) for sample and blank, respectively.

2.6 Bacterial Enumeration

2.6.1 Serial Dilution Preparation

A tenfold serial dilution series was prepared by aseptically transferring 1 mL of rumen fluid into 9 mL of sterile peptone water. The suspension was vortexed thoroughly, and 1 mL was transferred to the subsequent dilution tube. This procedure was repeated sequentially to obtain seven consecutive dilutions (10^{-1} to 10^{-7}).

2.6.2 Total Bacterial Count

From each dilution, 1 mL was aseptically pipetted into sterile Petri dishes. Molten nutrient agar cooled to 45–50°C was poured into each dish and gently swirled in a figure-eight motion to ensure homogeneous distribution. After solidification, plates were inverted and incubated at 37°C for 24 h. Bacterial colonies were counted, and total bacterial count was calculated as:

Total bacteria (CFU mL^{-1}) = Number of colonies × Reciprocal of dilution factor

Results were expressed as $\log_{10}$ colony-forming units per milliliter ($\log_{10}$ CFU mL^{-1}).

2.6.3 Cellulolytic Bacteria

Cellulolytic bacteria were enumerated using the same pour-plate technique with carboxymethylcellulose (CMC) agar medium. Following incubation, plates were flooded with Gram's iodine solution; clear zones surrounding colonies indicated cellulase activity. Colonies exhibiting halo formation were counted as cellulolytic bacteria.

2.6.4 Proteolytic Bacteria

Proteolytic bacterial counts were determined using Skim Milk Agar medium incubated under anaerobic conditions at 39°C. Colonies surrounded by transparent halos—resulting from casein hydrolysis—were enumerated as proteolytic bacteria. Results were expressed as $\log_{10}$ CFU mL^{-1}.

2.6.5 Amylolytic Bacteria

Amylolytic bacteria were cultured on Starch Agar medium. Following incubation, plates were flooded with Lugol's iodine solution; clear zones around colonies indicated starch hydrolysis. Amylolytic bacterial counts were calculated and expressed as $\log_{10}$ CFU mL^{-1}.

2.6.6 Bacterial Identification

Representative isolates were subjected to phenotypic characterization, Gram staining, and functional assays to confirm bacterial identity where necessary.

2.7 Rumen Ammonia Nitrogen Determination

Ammonia nitrogen (NH_3–N) concentration in rumen fluid was determined

spectrophotometrically using the Nesslerization method. This procedure is based on the reaction of ammonium ions (NH_4^+) with Nessler's reagent to form a yellow–orange complex, the intensity of which is directly proportional to ammonia concentration. Absorbance was measured at 420 nm using a spectrophotometer. NH_3–N concentration was calculated from a standard curve prepared using ammonium chloride standards of known concentrations:

NH_3–N (mg 100 mL^{-1}) = C × Dilution Factor

where C represents the concentration read directly from the standard curve (mg 100 mL^{-1}), and Dilution Factor = Total volume / Original sample volume.

2.8 Statistical Analysis

2.8.1 Experimental Design

A completely randomized design (CRD) with repeated measures was employed. Data were analyzed using SPSS (Version 26, 2019). The statistical model included:

- **Treatment**: Fixed effect (four levels: T1–T4)
- **Week**: Fixed repeated effect (15 weeks)
- **Lamb (Treatment)**: Random effect (four lambs per treatment nested within treatment)
- **Treatment × Week interaction**: Fixed effect

Baseline measurements obtained during the 15-day adaptation period were incorporated as covariates (ANCOVA) for sensitive parameters including pH and VFA concentrations.

2.8.2 Linear Mixed Model

The following linear mixed model was applied:

$$\mathbf{Y_{ijk} = \mu + T_i + W_k + (T \times W)_{ik} + S_{j(i)} + \varepsilon_{ijk}}$$

where:

- Y_{ijk} = observed value of the dependent variable
- μ = overall mean
- T_i = fixed effect of treatment i
- W_k = fixed effect of week k
- $(T \times W)_{ik}$ = interaction between treatment i and week k
- $S_{j(i)}$ = random effect of lamb j nested within treatment i
- ε_{ijk} = residual error

Appropriate covariance structures—including first-order autoregressive [AR(1)] and unstructured (UN)—were selected based on model fit criteria.

2.8.3 Regression Analysis

General linear regression models were constructed as follows:

$$\mathbf{Y_{ijk} = \beta_0 + \beta_1(Week) + \beta_2(Week^2) + T_i + \beta_3(T_i \times Week_k) + \varepsilon_{ijk}}$$

where β_0, β_1, β_2, and β_3 represent estimated regression coefficients. First-order (linear) or second-order (quadratic) polynomial contrasts were applied depending on the significance of regression parameters. The optimal model was selected based on coefficient of determination (R^2) and statistical significance ($P < 0.05$).

2.8.4 Post-Hoc Comparisons and Graphical Presentation

Treatment means were compared using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Temporal trends in

rumen parameters across experimental weeks were visualized using slope line graphs generated in SPSS, illustrating the relationship between time and each dependent variable under different SRU supplementation levels.

3- Results and Discussion

3.1 Rumen Fluid pH

The effects of dietary slow-release urea (SRU) supplementation on rumen fluid pH are presented in Table 1. Mean pH values ranged from 6.79 (T1, control) to 6.72 (T4, 2% SRU), with no statistically significant differences observed among treatments ($P > 0.05$). These values fall within the optimal range for rumen microbial activity (6.5–7.0) and are consistent with the physiological pH range of 5.5–7.0 required for effective fiber digestion [33, 34]. The maintenance of rumen pH within the normal range of 6.1–6.8, as reported by Xu et al. [35], indicates that SRU supplementation did not disrupt rumen homeostasis.

The slight, non-significant decrease in pH with increasing SRU levels may be attributed to concomitant increases in ruminal ammonia concentration and stable total VFA concentrations, which collectively promote digestion and feed intake [36, 37]. Maintenance of stable pH is critical for preventing rumen disorders such as subacute ruminal acidosis and ammonia toxicity [37]. The absence of dramatic pH fluctuation in SRU-supplemented groups is consistent with the slow-release characteristics of encapsulated urea, which minimizes ammonia accumulation and provides adequate time for microbial assimilation [38].

These findings align with those of Sevim and Önol [39], who reported no significant differences in rumen pH following SRU supplementation at 0% and 10% in male sheep and goats. Similarly, Hallajian et al. [40] observed no significant pH differences (6.45, 6.38, 6.41, and 6.36) when substituting soybean meal with SRU at 0%, 50%, 75%, and 100% in Holstein dairy cows. Saro et al. [41] compared fast- and slow-release urea in sheep diets and found no significant differences in rumen pH values. Safavi and Chaji [42] also reported comparable pH values between SRU-treated and control sheep.

Table 1. The effect of adding slow-releasing urea to the diet of Arabi lambs on rumen liquid pH and microbiology enumeration (log_{10} CFU/mL) (Mean± SE, n = 4)

Group / Treatment	T1 Control	T2 1% SRU	T3 1.5% SRU	T4 2% SRU	P-value
pH	6.79± 0.07	6.77± 0.14	6.74± 0.09	6.72± 0.02	**NS**
Total bacteria	8.02 ± 0.03^{c}	8.07 ± 0.03^{bc}	8.11 ± 0.02^{ab}	8.12 ± 0.02^{a}	<0.05
Cellulolytic	7.00 ± 0.04^{c}	7.11 ± 0.03^{b}	7.17 ± 0.03^{ab}	7.20 ± 0.03^{a}	<0.05
Amylolytic	6.74 ± 0.04^{a}	6.70 ± 0.03^{ab}	6.66 ± 0.03^{b}	6.64 ± 0.03^{b}	<0.05
Proteolytic	6.52 ± 0.04^{b}	6.56 ± 0.03^{b}	6.63 ± 0.03^{a}	6.66 ± 0.03^{a}	<0.05

^{abc} **Means within a row with different letters differ significantly at ($P < 0.05$).**

3.2 Bacterial Populations

3.2.1 Total Bacterial Count

Total bacterial counts exhibited a significant positive response to SRU supplementation ($P < 0.05$), with the highest value recorded in T4 (8.12 log_{10} CFU mL^{-1}) and the lowest in T1 (8.02 log_{10} CFU mL^{-1}) (Table 1). This progressive increase with ascending SRU levels reflects

enhanced microbial proliferation driven by sustained nitrogen availability.

The slow nitrogen release kinetics of SRU prevent rapid ammonia accumulation, thereby maintaining rumen ammonia concentrations within a range conducive to microbial protein synthesis [43]. The stabilized pH environment (6.7–6.8) further promoted cellulolytic bacterial activity, which is optimal within this pH range. Conversely, the slight but significant reduction in amylolytic bacterial counts suggests a shift in rumen microbial ecology toward fiber-digesting populations at the expense of starch-utilizing bacteria [35]. This transition indicates improved synchronization of nitrogen and energy availability, favoring cellulolytic over amylolytic fermentation [44].

Zhang et al. [45] demonstrated that SRU maintains consistent ruminal ammonia concentrations, thereby enhancing microbial activity and digestive efficiency. Zhu et al. [46] reported that bacterial growth is positively correlated with the availability of non-protein nitrogen (NPN), which SRU supplies in a sustained manner. Shi et al. [47] further confirmed that slow-release nitrogen sources stimulate microbial proliferation without reaching toxic thresholds. Wang et al. [48] emphasized that different bacterial species exhibit varying responses to ammonia concentration gradients, underscoring the importance of controlled nitrogen release.

3.2.2 Cellulolytic Bacteria

Cellulolytic bacterial counts increased significantly ($P < 0.05$) from 7.00 $\log_{10}$ CFU mL^{-1} in T1 to 7.20 $\log_{10}$ CFU mL^{-1} in T4 (Table 1). This enhancement is of particular nutritional significance, as cellulolytic bacteria are responsible for degrading plant cell wall components (cellulose and hemicellulose) into volatile fatty acids—the primary energy source for ruminants [49].

Rabee et al. [50] reported that cellulolytic activity is closely associated with the availability of microbially assimilable nitrogen, and that improved nitrogen supply enhances fiber digestion and feed utilization efficiency. The stable rumen pH observed in SRU-supplemented groups further supports cellulolytic bacterial proliferation and functionality [51].

These results are consistent with Guo et al. [52], who observed increased cellulolytic activity when soybean protein was replaced with SRU at 35% inclusion. Hashem and Tayeb [53] reported similar findings when substituting fast-release urea with SRU at 0%, 0.6%, 1.2%, and 1.8% in Awassi sheep diets. Amani-Yengejeh et al. [54] also documented enhanced bacterial growth in dairy cows supplemented with 60 g day^{-1} SRU.

3.2.3 Amylolytic Bacteria

A significant ($P < 0.05$) progressive decline in amylolytic bacterial counts was observed with increasing SRU levels. The control group (T1) exhibited the highest count (6.74 $\log_{10}$ CFU mL^{-1}), which decreased to 6.70, 6.66, and 6.64 $\log_{10}$ CFU mL^{-1} in T2, T3, and T4, respectively (Table 1).

Although the magnitude of reduction was modest, the consistent dose-dependent trend indicates a slight inhibitory effect on amylolytic populations. Amylolytic bacteria are primarily dependent on readily fermentable carbohydrates. The stabilization of rumen ammonia concentrations via SRU supplementation improves synchronization between carbohydrate fermentation and nitrogen availability. As nitrogen becomes less limiting, fiber-fermenting (cellulolytic) populations gain competitive advantage, potentially suppressing amylolytic activity through microbial competition or shifts in rumen ecological niches [35].

Xu et al. [35] reported that combining SRU with rapidly fermentable energy sources

(e.g., molasses) enhanced the growth of carbohydrate-fermenting bacteria, suggesting that coordination of NH_3-N availability (via SRU) and energy supply (via starch or sugars) optimizes starch-utilizing bacterial populations, including *Streptococcus bovis*, *Succinivibrio*, and *Prevotella* spp. Yan et al. [55] observed that SRU supplementation in male yaks induced compositional shifts in bacterial communities, particularly affecting carbohydrate-metabolizing taxa. Wang et al. [56] demonstrated that urea degradation rate, rather than absolute quantity, determines competitive outcomes among bacterial functional groups; the gradual, sustained ammonia release from SRU prevents abrupt NH_3-N spikes that may adversely affect pH-sensitive amylolytic bacteria.

3.2.4 Proteolytic Bacteria

In contrast to amylolytic bacteria, proteolytic bacterial counts increased significantly ($P < 0.05$) with ascending SRU levels. The lowest value was recorded in T1 (6.52 $\log_{10}$ CFU mL^{-1}), while T3 and T4 exhibited significantly higher counts (6.63 and 6.66 $\log_{10}$ CFU mL^{-1}, respectively) (Table 1).

Proteolytic bacteria benefit from sustained ammonia-N supply, as nitrogen availability is synchronized with microbial growth requirements. SRU provides gradual ammonia liberation, enabling proteolytic and deaminating bacteria to proliferate more effectively, maintain higher enzymatic activity, and exhibit reduced post-prandial lag phases [57]. In the present study, proteolytic counts increased progressively up to 1.5% SRU, with marginal additional increase at 2% SRU, suggesting a plateau effect.

Fan et al. [58] reported that SRU supplementation in Angus steers increased the relative abundance of *Prevotella* and *Paraprevotella*—predominant proteolytic genera responsible for protein and amino acid digestion in the rumen. Cherdthong and Wanapat [38] demonstrated that inclusion of slow-release urea–calcium hydroxide (U-CaS) in feed blocks linearly increased total bacterial counts and microbial protein synthesis, promoting balanced development of proteolytic bacteria dependent on ammonia availability. Bezerra et al. [59] recently reviewed that SRU maintains NH_3-N concentrations within the optimal range of 7–15 mg dL^{-1}, supporting microbial protein synthesis while avoiding toxic ammonia peaks characteristic of conventional urea.

3.2.5 Temporal Microbial Growth Patterns

All microbial populations exhibited substantial proliferation between week 1 and week 15 (Table 2, Figure 1). Proteolytic bacteria demonstrated the greatest increase (+0.20 $\log_{10}$ CFU mL^{-1}; approximately 58.9% increase), reflecting enhanced nitrogen turnover and proteolytic activity. Amylolytic bacteria also increased substantially (+0.18 $\log_{10}$ CFU mL^{-1}; 51.5%), likely attributable to improved energy synchronization. Cellulolytic bacteria exhibited moderate enhancement (+0.16 $\log_{10}$ CFU mL^{-1}; 44.7%), consistent with gradual improvement in fiber degradation efficiency. Total bacterial counts increased by +0.12 $\log_{10}$ CFU mL^{-1} (31.6%), corroborating enhanced overall fermentation activity and feed digestibility [60].

Table (2). Microbial growth patterns (quantify percentage increase)

Bacteria type	Week 1 (log_{10} CFU/mL)	Week 15	Δ (log units)	% Increase (approx.)
Total bacteria	7.90	8.08	+0.12	+31.6 %
Cellulolytic	6.91	7.12	+0.16	+44.7 %
Amylolytic	6.93	7.11	+0.18	+51.5 %
Proteolytic	6.92	7.12	+0.20	+58.9 %

Percentages converted from log_{10} differences: 10^Δ – 1

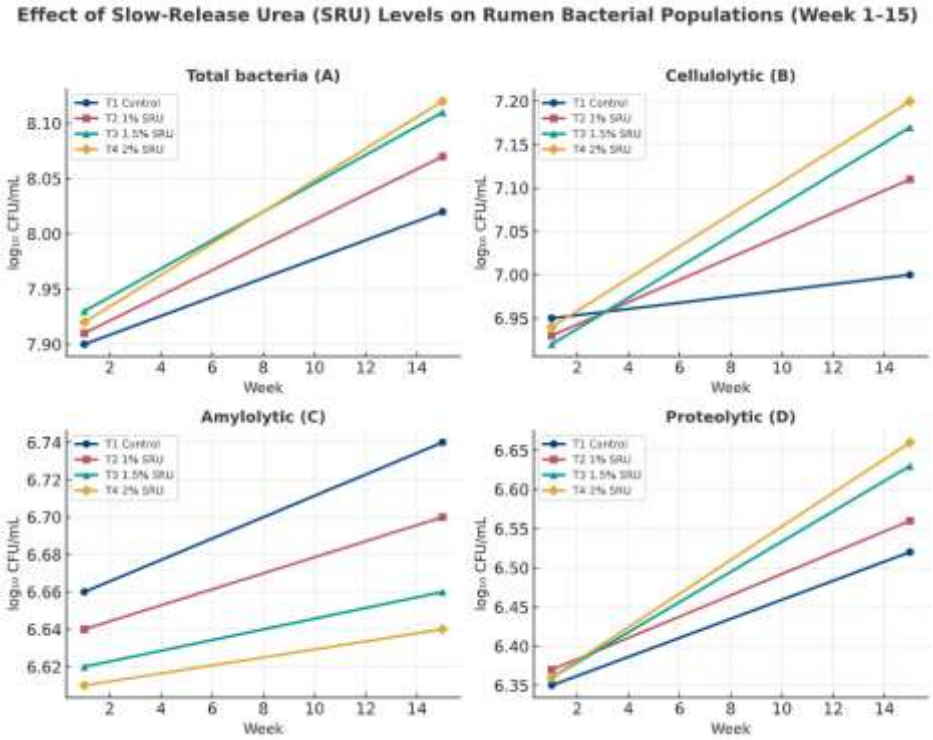

Fig. (1). Effect of slow urea (SRU) levels on rumen bacterial populations (week 1-15)

3.3 Volatile Fatty Acids

3.3.1 Total Volatile Fatty Acids

Dietary SRU supplementation significantly influenced rumen fermentation parameters, including total volatile fatty acids (TVFA) and individual VFA concentrations (Table 3). TVFA concentration increased progressively with SRU inclusion level: T1 (74.29 mmol L^{-1}), T2 (83.82 mmol L^{-1}), T3 (83.94 mmol L^{-1}), and T4 (81.66 mmol L^{-1}), with significant differences between control and treated groups ($P < 0.05$). The slight numerical decline at 2% SRU relative to 1.5% SRU suggests a potential plateau effect.

These elevations indicate that SRU supplementation enhanced rumen microbial fermentation efficiency. The concurrent increase in bacterial counts and VFA production reflects improved digestion of both structural and non-structural carbohydrates [50]. These findings corroborate those of Rabee et al. [50] and Ma et al. [61].

3.3.2 Acetic Acid

Acetic acid concentration increased significantly ($P < 0.05$) from 43.09 mmol L^{-1} in T1 to 46.98 mmol L^{-1} in T3 (Table 3). Acetic acid is typically the predominant VFA in ruminants fed roughage-based diets, and its elevation is directly attributable to enhanced cellulolytic bacterial activity [62]. The positive correlation between SRU level, cellulolytic

bacterial counts, and acetic acid concentration confirms that sustained nitrogen release stimulates fiber-degrading microbial populations [61, 63].

3.3.3 Propionic Acid

Propionic acid concentration increased significantly ($P < 0.05$) from 19.32 mmol L^{-1} in T1 to 22.56 mmol L^{-1} in T3 (Table 3). This elevation is nutritionally significant, as propionate is absorbed across the rumen epithelium and serves as the primary gluconeogenic substrate in ruminants, supplying up to 70% of hepatic glucose production [46]. The increase in propionic acid indicates enhanced fermentation of non-structural carbohydrates and improved energy metabolism efficiency.

3.3.4 Butyric Acid

Butyric acid concentration increased significantly ($P < 0.05$) from 12.00 mmol L^{-1} in T1 to 13.93 mmol L^{-1} in T3 (Table 3). Butyrate is the preferred energy substrate for rumen epithelial cells and promotes papillary development, thereby enhancing nutrient absorption capacity [46, 50]. Elevated butyrate concentrations in SRU-supplemented groups suggest improved rumen health and absorptive function.

3.3.5 Acetate:Propionate Ratio

The acetate:propionate (A:P) ratio decreased significantly ($P < 0.05$) with increasing SRU levels, from 2.23 in T1 to 2.08 in T2 and T3, with a slight increase to 2.19 in T4 (Table 3). The reduction in A:P ratio indicates a shift toward more energetically efficient fermentation, as propionate production is associated with lower methane emissions and greater energy retention [60]. This shift is attributable to SRU-mediated stabilization of rumen pH and proliferation of propionate-producing bacterial species [52, 61].

3.3.6 Regression Analysis of Volatile Fatty Acids

Linear regression analysis of VFA concentrations over time revealed significant positive temporal trends across all SRU-supplemented treatments (Table 4). The slope coefficients (β_1) increased with SRU level, indicating accelerated VFA production rates. Relative increases in TVFA concentration ranged from 31.1% (control) to 167.8% (2% SRU). High coefficients of determination ($R^2 > 0.7$) in SRU-treated groups indicate strong temporal consistency and confirm the efficacy of SRU supplementation in enhancing rumen fermentation dynamics [61, 52].

Figure 2 illustrates the significant effects of week and week × treatment interaction on VFA concentrations. The rate of increase was markedly greater in SRU-supplemented groups, particularly at 1.5% and 2% inclusion levels. Maximum values recorded at week 15 in the 2% SRU treatment confirm substantial enhancement of microbial fermentation efficiency and energy yield [64, 65].

Table (3). Effect of adding different levels of SRU on the mean concentration of volatile fatty acids in rumen fluid (Mean mmol/L ± SE)

Treatments	TVFA	Acetic acid	Propionic acid	Butyric acid	A:P ratio
T1 Control	74.29^{b}± 6.71	43.09^{b}±2.36	19.32^{b}±1.01	12.00^{b}±1.05	2.23^{a}± 0.06
T2 1% SRU	83.82^{a}±9.19	46.94^{a}±2.98	22.55^{a}±1.37	13.83^{a}±0.96	2.08^{b}± 0.07
T3 1.5% SRU	83.94^{a}±8.68	46.98^{a}±2.49	22.56^{a}±1.14	13.93^{a}±1.10	2.08^{c}± 0.07
T4 2% SRU	81.66^{a}±8.75	46.75^{a}±2.99	21.23^{ab}±1.82	13.39^{a}±1.16	2.19^{d}±0.08

^{abc} Means within a column with different letters differ significantly at ($P < 0.05$).

Table (4). The linear regression analysis of fatty acid concentrations (TFA, Acetic, Propionic, and Butyric acids) across weeks under different levels of Slow Release Urea

Acid	Treatment	Intercept (β_0)	Slope (β_1)	Relative Increase (%)	R^2
TVFA	T1 control	77.577	4.832	31.1	0.388
	T2 1%SRU	72.974	9.964	68.3	0.87
	T3 1.5%SRU	79.944	18.075	113.0	0.667
	T4 2%SRU	83.633	28.073	167.8	0.772
Acetic	T1 control	48.834	3.43	35.1	0.436
	T2 1%SRU	44.357	7.031	79.3	0.883
	T3 1.5%SRU	46.713	12.683	135.8	0.703
	T4 2%SRU	46.666	19.602	210.0	0.792
Propionic	T1 control	22.539	1.583	35.1	0.436
	T2 1%SRU	20.472	3.245	79.3	0.883
	T3 1.5%SRU	21.56	5.854	135.8	0.703
	T4 2%SRU	21.538	9.047	210.0	0.792
Butyric	T1 control	11.269	0.792	35.1	0.436
	T2 1%SRU	10.236	1.622	79.3	0.883
	T3 1.5%SRU	10.78	2.927	135.8	0.703
	T4 2%SRU	10.769	4.524	210.0	0.792

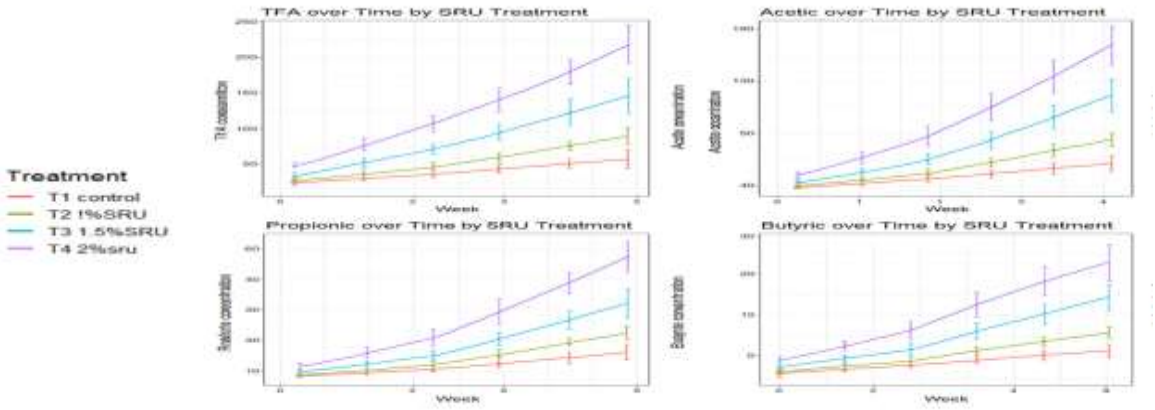

Fig. (2). Effect of slow urea (SRU) levels on rumen volatile fatty acids concentration (week 1-15)

Table (5). NH_3–N (mg/100 mL) concentration at different levels of SRU across weeks

Weeks	0% SRU (control)	1% SRU	1.5% SRU	2% SRU
0	5.20 ± 0.30	6.80± 0.42	7.50 ± 0.44	8.00 ± 0.54
1	5.00± 0.27	6.90 ± 0.48	7.60 ± 0.49	8.10 ± 0.58

5	5.00 ± 0.33	7.00 ± 0.45	8.00 ± 0.50	8.50 ± 0.57
9	5.00± 0.36	7.30 ± 0.43	8.40 ± 0.52	8.90 ± 0.61
13	5.80 ± 0.35	7.30 ± 0.44	8.60 ± 0.54	9.30 ± 0.63
15	5.70 ± 0.37	7.10 ± 0.49	8.60 ± 0.55	9.20 ± 0.66
SRU mean	5.06^{d}± 0.39	7.08^{c}± 0.48	8.21^{b}± 0.69	8.73^{a}± 0.57
Total Variation Between Week 1 and 15 (%)	9.61	4.41	14.67	15.00

^{abc} Means within a row with different letters differ significantly at ($P < 0.05$).

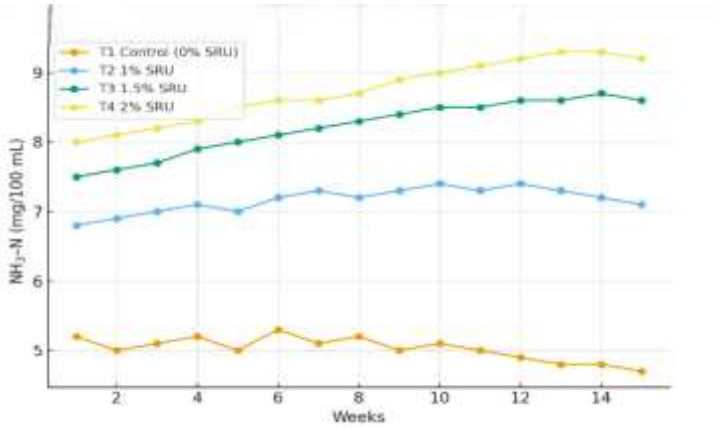

Figure 3. Evolution of ruminal NH 3 N concentration (mg/100mL) over time in lambs fed slow-release urea (SRU) diets with varying concentrations of urea. The data is in the form of mean values per week.

Table (6). Regression model coefficients between NH_3 and SRU level

Variable	Coefficient (β)	Standard Error	t value	P Value
(Intercept)	5.10	0.12	42.5	<0.001
SRU	+1.45	0.08	18.1	<0.001
SRU^2	-0.25	0.07	-3.5	0.003

- **Square Model: $NH_3 = \beta_0 + \beta_1(SRU) + \beta_2(SRU)^2 + \varepsilon$**

Table (7). The percentage of total change in ammonia concentration between the first week and the fifteenth week

Treatment (SRU level)	Average NH_3 at week 1 (mg/100 mL)	Total Variation Between Week 1 and 15 (%)
0 % SRU	5.2	+9.6
1 % SRU	6.8	+13.5
1.5 % SRU	7.5	+16.8
2 % SRU	8.0	+17.4

3.4 Rumen Ammonia Nitrogen Concentration

Rumen ammonia nitrogen (NH_3–N) concentration at 3 h post-feeding during week 15 differed significantly among treatments ($P < 0.001$), increasing progressively with SRU inclusion level: 5.52 mg 100 mL^{-1} (T1, control), 7.20 mg 100 mL^{-1} (T2, 1% SRU), 8.60 mg 100 mL^{-1} (T3, 1.5% SRU), and 9.18 mg 100 mL^{-1} (T4, 2% SRU) (Table 5). Relative to control, these values represent increases of approximately 32%, 58%, and 69%, respectively.

These findings confirm that SRU supplementation provides a sustained, controlled release of non-protein nitrogen to the rumen ecosystem, resulting in elevated but non-toxic ammonia concentrations. The gradual increase across treatment levels reflects the slow-release characteristics of SRU, which prevent rapid ammonia spikes characteristic of conventional urea [35, 52].

The temporal evolution of NH_3–N concentration across 15 weeks (Figure 3, Table 5) demonstrated that all SRU-supplemented treatments maintained stable or gradually increasing ammonia concentrations, whereas control treatment exhibited minimal fluctuation. The total percentage change between week 1 and week 15 was +9.61% (control), +4.41% (1% SRU), +14.67% (1.5% SRU), and +15.00% (2% SRU). The moderate increase at 1.5% SRU suggests optimal balance between ammonia production and microbial uptake, whereas the marginal additional increase at 2% SRU indicates potential saturation of microbial assimilation capacity [46, 60].

Xu et al. [35] reported that SRU optimizes temporal synchrony between energy and nitrogen supply in the rumen, enhancing microbial protein synthesis while reducing gaseous nitrogen losses. Wahyono et al. [60] and Zhu et al. [46] similarly emphasized that optimizing SRU inclusion level is essential to achieve equilibrium between ammonia production and microbial utilization, avoiding excessive nitrogen excretion.

Quadratic regression analysis (Table 6) revealed a significant curvilinear relationship between SRU level and NH_3–N concentration (NH_3–N = 5.10 + 1.45 SRU – 0.25 SRU^2; $P < 0.01$). The negative quadratic coefficient indicates diminishing returns at higher inclusion levels, with optimal NH_3–N concentration achieved at approximately 1.5% SRU. This finding is consistent with Guo et al. [52], who demonstrated that NH_3–N increases proportionally with NPN supply but plateaus when microbial assimilation capacity is exceeded.

Table 7 presents the percentage change in NH_3–N concentration between week 1 and week 15. The 1.5% SRU treatment exhibited the most balanced increase (+16.8%), achieving elevated ammonia concentrations without excessive accumulation. Dias et al. [66] emphasized that carbohydrate availability is critical for regulating NH_3–N utilization, as microbial growth and activity require balanced energy and nitrogen supply.

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Table (8). Correlation coefficients (r) between rumen fermentation variables and some bacterial populations under the influence of different levels of SRU.

Bacterial group / Variable	NH_3–N	TVFA	Acetic acid	Propionic acid	Butyric acid	pH
Total bacteria	0.65**	0.78**	0.72**	0.69**	0.66**	−0.48*
Cellulolytic bacteria	0.54*	0.69**	0.80**	0.55*	0.60*	−0.44*
Amylolytic bacteria	0.61**	0.74**	0.63*	0.81**	0.68**	−0.50*
Proteolytic bacteria	0.77**	0.72**	0.70**	0.67**	0.65*	−0.52**

* Statistically Significant at a probability level ($P < 0.05$) ** Statistically highly significant at a probability level ($P < 0.01$)

4-Conclusions

The findings of the present study show that the percentage addition of slow-releasing urea (SRU) of 0, 1, 1.5, and 2 percent did not influence the values of the pH in the rumen because the values were maintained within the normal physiological level during the experiment, which shows that SRU did not lead to an unbalanced change in the pH within the rumen.

The additions also exhibited a definite increase in the counts of total bacteria, cellulolytic, and proteolytic bacteria, and there was also a significant reduction in the counts of amylolytic bacteria as the concentration of added SRU augmented. Although this was different across the species, all the bacterial groups still rose over time, indicating that the microbial community had adapted to the diet and the rumen environment was stable throughout the experiment.

Concerning volatile fatty acids (VFAs), it has been noted that they rose in linear relationship with the level of SRU in the diet and rose in quadratic relationship with weeks that the experiment was being conducted. This indicates a better microbial fermentation and increased rate of carbohydrate degradation as a result of having a stable and graded supply of nitrogen.

On the whole, the findings indicate that SRU is a useful and safe nitrogen source, and it can stimulate the fermentation process in the rumen, as well as enhance the fermentation environment, without having any adverse effects on pH, which contributes to the adoption of this source of nitrogen in lamb or ruminant feeds in intensive feeding systems.

Acknowledgment

We are grateful to all staff in animal farm/ College of Agriculture/ university of Basrah.

Ethical consideration

Research ethics are based on the ethical rules approved by the College of Agriculture/University of Basrah.

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