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Research Article

Effects of Urea Levels and Lime Supplementation during Rice Straw Ammoniation on Rumen *In Vitro* Fermentation, Methane Production, and Volatile Fatty Acid Profiles

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Abstract | This study evaluated the effects of urea-based ammoniation, with or without lime supplementation, on the chemical composition, methane production, fermented dry matter (DM), and volatile fatty acid (VFA) profiles of rice straw during *in vitro* rumen fermentation. Rice straw was treated with aqueous urea at levels of 2, 3, or 4% DM, either alone or in combination with a fixed lime level of 3% DM. Compared with untreated rice straw (UTRS), ammoniated rice straw (ARS) showed increased crude protein content ($p < 0.001$) and reduced neutral detergent fiber ($p < 0.05$) and acid detergent lignin concentrations ($p < 0.05$), while acid detergent fiber was unaffected. Increasing urea level further reduced acid detergent lignin, whereas lime supplementation did not provide additional improvement in fiber fractions. *In vitro* fermentation demonstrated that ammoniation and increasing urea levels reduced methane proportion in association with increased carbon dioxide proportion, without affecting total gas production. Methane production per unit of fermented DM was not influenced by treatment. However, ammoniation significantly increased fermented DM and total VFA concentrations ($p < 0.05$), accompanied by greater propionic acid production and a lower acetate-to-propionate ratio ($p < 0.05$). A urea inclusion level of 3% DM was identified as optimal for improving fermentation efficiency and mitigating methane production, with no additional benefits observed at 4% DM. Lime supplementation and its interaction with urea level had no effect on methane production and VFA profiles.

Keywords | Ammoniation, Rice straw, Methane production, Enteric fermentation, Propionic acid

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INTRODUCTION

Rice straw is one of the most abundant fibrous feed resources for ruminant production systems in Asia. However, its utilization in ruminant diets is constrained by low crude protein content, high concentrations of structural carbohydrates, and strong lignocellulosic associations that limit microbial degradation in the rumen. These characteristics result in poor digestibility and relatively high methane emissions per unit of degradable dry matter (Li *et al*., 2025). In addition, harvesting during the rainy season often compromises straw quality and increases collection costs, thereby reducing its feeding value.

Urea-based ammoniation has been widely applied to improve the nutritive value of rice straw. During ammoniation, urea is hydrolyzed to ammonia, which penetrates the plant cell wall, disrupts ester linkages between lignin and polysaccharides, and enhances microbial accessibility. Both urea alone treatment and lime-urea treatment showed increases in non-protein nitrogen content, enhancing rumen microbial protein synthesis (Ma *et al*., 2020; Sarnklong *et al*., 2010). Trach *et al*. (2001) suggested a combination of 3% quick lime is better than 6% with 2% urea for the treatment of rice straw and would provide a source of N and calcium in cattle on poor nutritional diets. Urea functions as the primary source of ammonia during ammonification through the action of urease, resulting in higher crude protein (CP) content, reduced neutral detergent fiber (NDF), and hemicellulose contents significantly in rice straw (Fadel Elseed *et al*., 2003). Alkaline additives such as lime (calcium hydroxide) have been proposed as complementary agents to urea ammoniation. Lime may increase treatment pH and facilitate partial delignification, potentially enhancing fiber solubilization (Polyrach and Wanapat, 2015). Although lime may confer certain physical advantages during processing, the dual effects of lime combined with varying urea levels remain unclear regarding the substantial nutritional benefits of ammoniated rice straw.

Methane emissions from enteric fermentation account for approximately 17% of global greenhouse gas emissions and particularly cause energy waste of 2-12% of feed intake in ruminants (Xie *et al*., 2025). Consequently, feed treatment strategies aimed at reducing methane production are considered an effective approach to improving animal productivity. Prior studies have reported that ammoniated rice straw lowers methane production and improves digestibility compared with untreated straw (Polyrach and Wanapat, 2015; Gunun *et al*., 2013; Jayanegara *et al*., 2017; Nguyen *et al*., 2025), generally attributed to improved digestibility, which often leads to lower methane emissions per unit of digested feed. Furthermore, the addition of alkaline agents, such as lime, promotes fiber degradation by effectively disrupting the lignocellulosic matrix. However, increasing overall fermentability without diverting hydrogen flow may not reduce methane proportionally. Therefore, whether lime and urea act synergistically to influence *in vitro* ruminal methanogenesis beyond the effects of urea alone remains unclear. Clarifying this relationship is essential to determine if lime inclusion provides a significant fermented-feed effect in livestock systems.

The objective of this study was to evaluate the effects of urea levels (2, 3, and 4% DM), with or without lime addition, on methane output, fermented dry matter, and VFA profiles during *in vitro* rumen fermentation of ammoniated rice straw.

MATERIALS AND METHODS

Experimental design

The experiment was designed to compare untreated rice straw (UTRS) with ammoniated rice straw prepared using two additive strategies: (i) aqueous urea solutions applied at 2, 3, and 4% of substrate dry matter (DM); and (ii) aqueous lime–urea solutions, in which lime was applied at a fixed level of 3% DM while urea levels were adjusted to 2, 3, and 4% DM. Each treatment was prepared in three independent replicates and arranged in a completely randomized design.

Ammoniation procedure

Rice straw was manually chopped into lengths of approximately 5–6 cm prior to treatment. For each replication, 500 g of chopped straw (fresh weight) was placed in a polyethylene container and thoroughly mixed with an aqueous urea solution. Urea was added at 2, 3, or 4% of DM and dissolved in 500 mL of water to achieve a final moisture content of approximately 40–45%, which is considered optimal for ammoniation.

For lime–urea treatments, lime was incorporated at a constant level of 3% DM across all treatments, while urea concentration varied from 2 to 4% DM. Mixing and moisture adjustment procedures were identical to those used for urea-only treatments. The treated rice straw was compressed in nylon bags to expel air, sealed, and stored at ambient temperature (approximately 25 °C) for 21 days. Bags were rotated weekly to ensure uniform distribution of urea and/or lime throughout the material.

Each treatment was prepared in duplicate, corresponding to two independent ammoniated batches. After the ammoniation period, the bags were opened and aerated prior to chemical analysis and *in vitro* incubation.

In vitro rumen incubation

A thermos flask was pre-warmed with warm water (approximately 40 °C) prior to rumen fluid collection. Rumen contents were obtained from beef cattle that had been fasted overnight before slaughter at a local abattoir. Immediately after slaughter, rumen contents were removed, thoroughly mixed, and strained through two layers of cheesecloth to remove residual feed particles. During filtration, the filtrate container was maintained at approximately 40 °C using warm water from the thermos flask. The strained rumen fluid was transferred to the pre-warmed thermos flask, sealed, and transported to the laboratory within 30 min. *In vitro* incubation was conducted following the procedure described by Inthapanya *et al*. (2011).

For each incubation bottle, 12 g of substrate (DM basis) was weighed and mixed with 0.24 L of strained rumen fluid and 0.96 L of buffer solution (Table 1). After addition of the incubation medium, bottles were flushed with carbon dioxide to establish anaerobic conditions and incubated in a water bath at 39 °C for 24 h. Fermentation was terminated by placing the bottles in an ice bath for 10–15 min to inhibit further microbial activity.

Table 1: The composition of the buffer solution.

	$CaCl_2$	$NaHPO_4 \cdot 12H_2O$	NaCl	KCl	$MgSO_4 \cdot 7H_2O$	$NaHCO_3$	Cysteine
g/L	0.04	9.30	0.47	0.57	0.12	9.80	0.25

Source: Tilley và Terry (1963).

Analytical methods and data collection

Samples of untreated and treated rice straw were analyzed for dry matter (DM) and crude protein (CP) according to AOAC (2011) procedures. Fiber fractions, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), were analyzed using ANKOM Technology procedures GE030.2021, GE029.2021, and GE234.2021, respectively, with an ANKOM200 Fiber Analyzer. Prior to analysis, samples were oven-dried at 60 °C, ground to pass a 1-mm sieve, and defatted following standard ANKOM sample preparation guidelines. For pH measurement, weigh 10 grams of the sample and blend it with 100 ml of distilled water for 30 seconds. Record the pH value after the reading has stabilized for 30 seconds using a pH meter (AD1020- Adwa Instruments, Hungary). Each sample was analyzed in duplicate, and each treatment analyzed two independent ammoniated batches.

Total gas production after 24 h of incubation was measured by water displacement using a graduated receiving bottle suspended in water. After gas volume measurement, gas samples were analyzed for methane and carbon dioxide concentrations (% vol) using a Crowcon gas analyzer equipped with an infrared sensor (Crowcon Instruments Ltd., UK).

Fermentation pH was measured immediately after opening the incubation bottles using a pH meter (AD1020- Adwa Instruments, Hungary). Unfermented dry matter was recovered by filtering incubation contents through cloth and non-absorbent cotton wool. The residue was dried to constant weight, and fermented DM was calculated as the difference between the initial substrate (12 g DM) and the recovered residue. Methane production per unit of fermented DM (ml/g) was calculated as methane volume divided by fermented substrate DM. Filtrate samples were acidified with 1 M H_2SO_4 at a ratio of 10:1 (v/v) and centrifuged at 10,000 × g for 15 min. The supernatant was stored at −20 °C for determination of total volatile fatty acids (VFA) and molar proportions of acetic, propionic, and butyric acids following the method described by Thanh *et al.* (2022).

Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedure of Minitab software version 19. The statistical model included treatment type (untreated rice straw, ammoniated rice straw with urea alone, ammoniated rice straw with lime–urea), urea level (2, 3, and 4%), lime addition (absence or presence), and the interaction between urea level and lime addition. Mean comparisons were performed using Tukey's test, and statistical significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition

Table 2 presents the chemical composition of untreated and treated rice straw. Overall, ammoniation markedly increased the crude protein content of rice straw ($p < 0.001$), which is consistent with previous findings indicating that urea treatment enriches non-protein nitrogen and enhances ammoniation retention within the straw matrix (Preston and Leng, 1987; Wanapat *et al*., 2009). Ammoniated rice straw showed lower neutral detergent fiber (NDF) and acid detergent lignin (ADL) contents than

Table 2: Chemical composition of untreated and treated rice straw.

Items	UTRS	Ammoniated rice straw (ARS)						SEM	Significant[3]			
		Urea level[1], %			Lime-urea level[2], %				Overall	U	L	UL
		2	3	4	2	3	4					
pH value	7.05^{e}	7.75^{d}	7.95^{cd}	8.30^{ab}	7.95^{cd}	8.30^{ab}	8.45^{a}	0.04	**	**	*	ns
Dry matter	89.10^{a}	59.12^{b}	57.28^{b}	57.23^{b}	56.78^{bc}	50.67^{bc}	47.67^{c}	1.58	**	*	ns	ns
CP	2.3^{d}	3.67^{c}	4.75^{ab}	5.44^{a}	4.33^{bc}	5.34^{a}	5.75^{a}	0.16	***	***	*	ns
NDF	74.50^{a}	70.97^{abc}	71.94^{ab}	67.98^{bc}	68.36^{bc}	67.46^{bc}	66.55^{c}	1.40	*	ns	ns	ns
ADF	56.27	53.12	52.07	50.27	52.32	47.89	49.49	2.33	ns	ns	ns	ns
ADL	7.38^{a}	7.42^{a}	6.64^{ab}	6.49^{ab}	6.85^{ab}	6.23^{ab}	5.97^{b}	0.20	*	*	ns	ns

Note: UTRS (Untreated Rice Straw), CP (Crude Protein), NDF (Neutral Detergent Fiber); ADF (Acid Detergent Fiber; ADL (Acid Detergent Lignin). [1]No lime additive-urea levels only; [2]Lime additive at 3% and urea levels. [3]Overall (all treatments including ARS *vs* UTRS), U (urea levels as 2, 3 and 4%), L (ammoniation with *vs* whithout lime); UL (urea level × lime additive). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = non-significant (p>0.05).

Table 3: Effect of ammoniated rice straw versus untreated rice straw on total gas and methane prodution in rumen *in vitro* incubation

Items	UTRS	Ammoniated rice straw (ARS)						SEM	Significant[3]			
		Urea level[1], %			Lime-urea level[2], %				Overall	U	L	UL
		2	3	4	2	3	4					
Total gas, ml	167	180	197	193	183	190	193	13.68	ns	ns	ns	ns
Methane, % in gas	11.67[a]	11.00[ab]	9.33[b]	9.33[b]	10.67[ab]	9.67[b]	10.00[ab]	0.32	*	*	ns	ns
Methane volume, ml	20.70	19.80	18.37	18.07	19.67	18.50	19.20	1.75	ns	ns	ns	ns
Carbon dioxide, % in gas	15.40[b]	13.72[b]	18.73[ab]	22.35[a]	15.71[ab]	16.90[ab]	18.83[ab]	1.19	*	*	ns	ns
Carbon dioxide volume, ml	27.72	24.59	36.80	43.29	29.15	32.44	36.6	3.85	ns	*	ns	ns
Fermented DM, %	42.71[b]	44.38[ab]	52.50[a]	52.92[a]	47.50[ab]	51.45[ab]	51.25[ab]	1.70	*	*	ns	ns
Methane per unit fermented DM, ml/g	6.09	5.56	4.38	4.29	5.26	4.51	4.72	0.51	ns	ns	ns	ns
Ratio of CO_2 and CH_4	1.25[c]	1.25[c]	2.01[ab]	2.40[a]	1.47[bc]	1.75[abc]	1.90[abc]	0.14	*	*	ns	ns

Note: UTRS (Untreated Rice Straw). [1]No lime additive-urea levels only; [2]Lime additive at 3% and urea levels; [3]Overall (all treatments including ARS *vs* UTRS), U (urea levels as 2, 3 and 4%), L (ammoniation with *vs* whithout lime); UL (urea level × lime additive). * $p<0.05$, ns = non-significant ($p>0.05$).

untreated rice straw (UTRS), whereas acid detergent fiber (ADF) was not affected. The reductions in NDF and ADL are consistent with reports demonstrating that ammoniation disrupts ester linkages between lignin and hemicellulose, thereby improving fiber degradability (Gunun *et al*., 2013; Balan *et al*., 2025).

Increasing urea concentration during ammoniation further reduced ADL content, indicating that higher ammoniation availability enhances lignin disruption relative to untreated rice straw (Figure 1). Urea inclusion levels of 3% or greater are commonly adopted in ammoniation protocols, as they provide sufficient ammoniation release to achieve effective delignification and improve accessibility of structural carbohydrates (Gunun *et al*., 2013; Wanapat *et al*., 2013; Bai *et al*., 2025). In the present study, however, no significant differences in NDF or ADF were observed among urea levels, although a slight reduction in both fractions was noted at the 4% urea level.

Lime addition did not further improve fiber fractions compared with urea treatment alone, although a higher pH was observed in ammoniated rice straw treated with lime (Table 2). While lime is expected to increase alkalinity and potentially enhance solubilization of the rice straw matrix (Sirohi and Rai, 1999), the present results indicate that alkaline agents such as lime provide limited additional benefit when adequate urea levels are applied. Under these conditions, urea appears to be the primary factor responsible for structural modification of rice straw. Accordingly, no additive or interactive effects of urea level and lime supplementation were observed for neutral detergent fiber (NDF), acid detergent fiber (ADF), or acid detergent lignin (ADL) across treatments, whereas ammoniation overall resulted in improved nutritive value compared with untreated rice straw (UTRS).

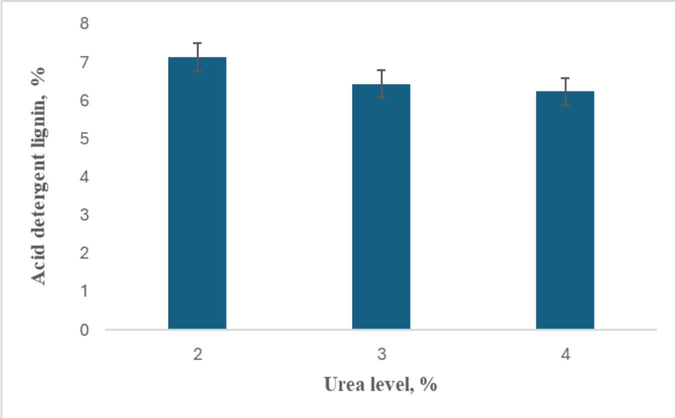

Figure 1: Effect of urea level (2, 3 and 4% as DM basis) on acid detergent lignin (ADL) of rice straw after 21 days ammoniation.

In vitro GAS, METHANE AND CARBON DIOXIDE PRODUCTION

The effect of ammoniated rice straw versus untreated rice straw on total gas and methane production in rumen *in vitro* incubation is presented in Table 3. The total gas volume did not differ significantly across treatments; however, the lower methane percentage responses were almost linear with higher CO_2 percentage responses and finally generate ratio of CO_2/CH_4 was higher when increasing levels of urea with $p < 0.05$ (Table 4) and was greater for ammoniated rice straw than for untreated rice straw (Figure 2). It reflects a shift in fermentation pathways rather than a reduction in overall fermentative activity, improved fiber degradability and increased soluble carbohydrate availability can reduce hydrogen sink for methanogenesis (Beauchemin *et al*., 2020; Ungerfeld, 2020).

Fermented dry matter was significantly higher in ammoniated rice straw than in untreated rice straw (Figure 4), reflecting improved digestibility following ammonia.

Adding 3% urea enhanced fermented DM compared to 2% (Table 3; Figure 3), whereas no additional improvement was observed at 4%. This response occurred despite the absence of significant differences in neutral detergent fiber (NDF) and acid detergent fiber (ADF) among urea levels. The lower acid detergent lignin (ADL) content observed at urea levels above 3% may have facilitated greater access of fungi and cellulolytic bacteria to structural carbohydrates, thereby enhancing fiber degradation. Among the urea levels evaluated, 3% appeared to be the most effective for methane mitigation, as no further reduction was observed at 4%.

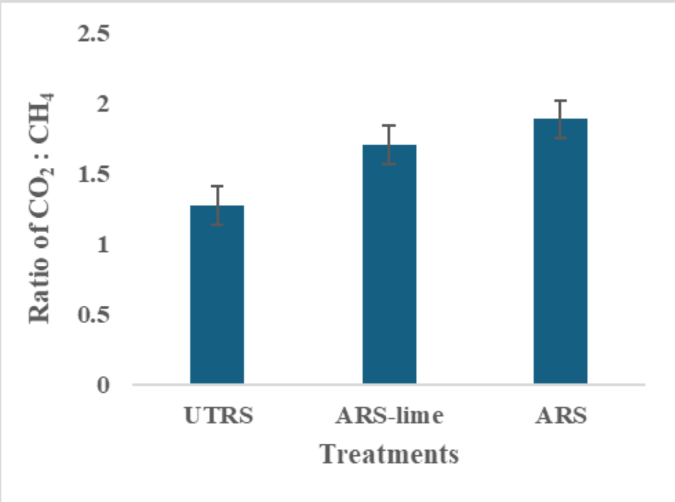

Figure 2: Effect of ammoniated rice straw (ARS) without lime and with lime (ARS lime) on ratio of carbon dioxide and methane compared to untreated rice straw (UTRS).

There were no differences in methane per unit of fermented DM among the treatments (Table 3), although fermented DM was positively improved by ammoniation than unammoniated rice straw and reached an optimum at 3% urea dose ($p < 0.05$). Conceptually, highly fermentable/soluble substrates would produce more methane than less fermentable/soluble substrates. However, methane mitigation is closely associated with altered hydrogen partitioning rather than solely reduced fermentation intensity (Pereira *et al*., 2022). In the present study, the increased carbon dioxide proportion and greater propionic acid production (Tables 3 and 4) indicate that a larger fraction of metabolic hydrogen was redirected toward alternative sinks instead of being utilized by methanogenic archaea. Propionic acid formation acts as a competitive hydrogen sink relative to methanogenesis, thereby reducing methane proportion even under conditions of increased fermented dry matter. This mechanism explains why methane production per unit of fermented dry matter did not differ significantly among treatments (Table 3), despite a clear reduction in methane proportion with ammoniation. From an energetic perspective, redirection of hydrogen toward propionic acid represents a metabolizable energy gain for the animal, as energy is retained in fermentation end-products rather than lost as methane (Migwi *et al*. 2013; Ungerfeld, 2020).

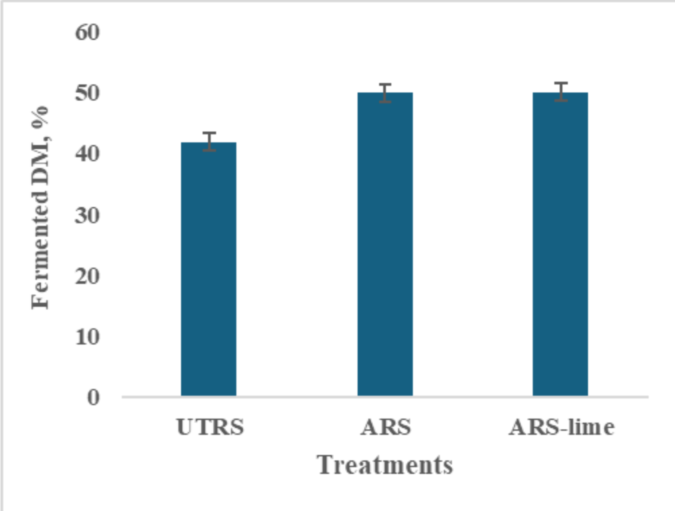

Figure 3: Effect of ammoniated rice straw (ARS) without lime and with lime (ARS lime) on fermented DM compared to untreated rice straw (UTRS).

Table 4: Effect of ammoniated rice straw versus untreated rice straw on total volatile fatty acid (VFA) and individual VFA in *in vitro* incubation.

Items	UTRS	Ammoniated rice straw (ARS)						SEM	Significant[3]			
		Urea level[1], %			Lime-urea level[2], %				Overall	U	L	UL
		2	3	4	2	3	4					
pH value	7.10	7.33	7.40	7.47	7.43	7.37	7.67	0.02	ns	ns	ns	ns
Total VFA, mM	21.33[b]	24.17[ab]	27.54[ab]	29.80[a]	25.50[ab]	28.67[ab]	27.50[ab]	1.63	*	ns	ns	ns
Individual VFA, %												
Acetic acid	68.73	68.06	63.6	64.58	68.10	67.00	67.50	1.26	ns	ns	ns	ns
Propionic acid	15.88[c]	17.11[bc]	23.13[a]	22.27[a]	17.26[bc]	18.99[abc]	21.32[ab]	0.95	*	*	ns	ns
Butyric acid	15.41	14.83	13.29	13.14	13.94	13.36	11.20	0.89	ns	ns	ns	ns
Ratio of Ac: Pr	4.38[a]	4.00[ab]	2.76[c]	2.94[bc]	3.80a[bc]	3.46a[bc]	3.17[bc]	0.22	*	*	ns	ns

Note: UTRS (untreated rice straw), Ac: Pr (ratio of acetic acid and propionic acid); [1]No lime additive-urea levels only; [2]Lime additive at 3% and urea levels; [3]Overall (all treatments including ARS *vs* UTRS), U (urea levels as 2, 3 and 4%), L (ammoniation with *vs* whithout lime), UL (urea level × lime additive). * p<0.05, ns = non-significant (p>0.05).

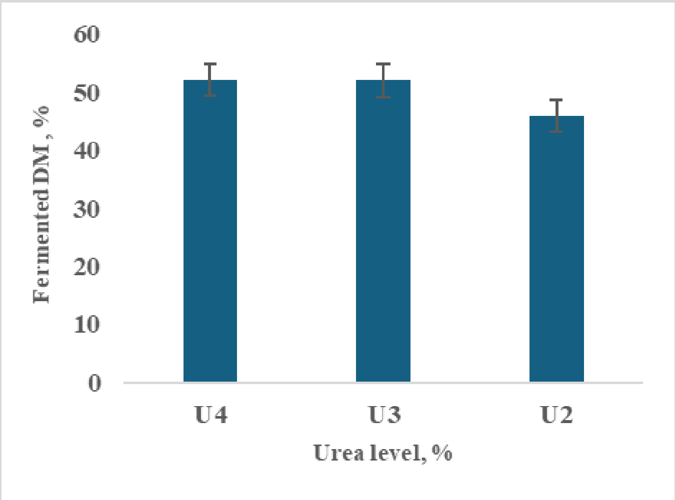

Figure 4: Effect of urea levels in ammoniated rice straw (2, 3 and 4%) on fermented DM.

Lime additive did not exert significant effects on total gas production, methane output, or methane-related indices, nor were interactive effects between urea level and lime detected (Table 3), indicating that the influence of urea on fermentation characteristics was independent of lime under *in vitro* rumen fermentation conditions. Although the CO_2:CH_4 ratio tended to be higher in lime-treated samples (Figure 2), this response was primarily associated with variation in carbon dioxide volume, while methane volume remained unchanged. Overall, no statistically significant differences were observed in methane or carbon dioxide production between treatments with and without lime, suggesting that lime may facilitate fiber processing during ammoniation but does not directly influence rumen fermentation characteristics under the conditions of the present study.

Volatile fatty acid profile and fermentation characteristics

The volatile fatty acid profile and pH value in Table 4 contributed to clarify the effects of ammoniation on rumen fermentation characteristics and methane mitigation. Ammoniation of rice straw increased total VFA concentration (overall $p < 0.05$), indicating enhanced fermentative activity and improved substrate digestibility. This response reflects greater availability of fermentable nutrients, which supports increased VFA production. In addition, ammoniation increased the proportion of propionic acid (overall $p < 0.05$) and reduced the acetate-to-propionate (Ac:Pr) ratio (overall $p < 0.05$), a fermentation pattern commonly associated with reduced methane emissions. In contrast, butyric acid proportion was not affected by treatment.

Increasing urea level further influenced fermentation characteristics by slightly increasing total VFA concentration and significantly increasing propionic acid proportion ($p < 0.05$), accompanied by a reduction in the Ac:Pr ratio with $p < 0.05$ (Table 4). These findings agree that enhanced fiber degradation is associated with a shift in VFA profiles toward propionate production (Foiklang *et al.*, 2016). Greater nitrogen availability at higher urea levels may stimulate microbial activity and substrate degradation, thereby contributing to increased total VFA production. Moreover, elevated propionic acid concentration under higher urea treatments may be positively associated with improved nitrogen utilization and deposition, as discussed by Ma *et al.* (2020).

Lime inclusion during ammoniation did not significantly alter VFA profiles but resulted in a slightly higher fermentation pH compared with the no-lime treatment (Table 4), with borderline statistical significance ($p = 0.06$). Although elevated pH conditions are generally considered less favorable for VFA production, no differences in total VFA concentration were observed between treatments with and without lime. This suggests that lime supplementation at 3% of dry matter remained within a range suitable for active rumen fermentation, consistent with the findings of Trach *et al.* (2001). No interaction between urea level and lime addition was detected for VFA profiles, in agreement with the corresponding results for methane production and fermented dry matter (Tables 3 and 4), indicating that the effects of urea and lime were largely independent under *in vitro* conditions.

Overall, lime additive had no effect on the ammoniation of rice straw or on *in vitro* ruminal fermentation, whereas a 3% urea level appeared optimal, with no additional benefits observed at 4%. Although lime addition during urea ammoniation elevated batch pH (Table 2), its influence on methane production and VFA profile was not different. In practice, however, combining lime with urea remains beneficial during storage, as it increases pH and reduces the risk of mold development.

CONCLUSIONS AND RECOMMENDATIONS

In summary, ammoniation of rice straw increased crude protein content and reduced neutral detergent fiber (NDF) and acid detergent lignin (ADL) compared with untreated rice straw. Lime addition did not provide additional improvement in fiber fractions beyond that achieved with urea alone. Ammoniated rice straw enhanced *in vitro* rumen fermentation by reducing methane production, increasing fermented dry matter, and increasing total volatile fatty acid concentrations, accompanied by a shift toward greater propionic acid production. A urea dose of 3% was identified as optimal for improving fermentation efficiency and mitigating methane production, with no further benefits observed at 4%. In contrast, lime supplementation and its interaction with urea level did not influence methane production or volatile fatty acid profiles. These findings clarify the respective roles of urea and lime during ammoniation and support the efficient utilization of rice straw in ruminant feeding systems, contributing to improved feed resource use and methane mitigation within circular agriculture models.

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NOVELTY STATEMENT

This study enhances understanding of how urea dosage and lime supplementation affect the fiber quality of ammoniated rice straw and associate methane production and rumen fermentation. The findings support improved utilization of rice straw in ruminant diets and provide practical insights for integrating such strategies into circular agriculture models in Vietnam.

AUTHOR'S CONTRIBUTION

LTBP conceived the study, performed data processing, and drafted the manuscript. DTD conducted data collection and analysis. DTNA provided support during manuscript preparation.

Ethical approval

Rumen fluid was collected post-mortem from beef cattle at a licensed slaughterhouse. As no live animals were handled or subjected to experimental procedures, the study did not require approval from an institutional animal ethics committee.

Generative AI and AI-assisted technology statement

The authors used AI-assisted language editing tools to improve grammar and clarity of the manuscript. The authors do not use any AI tool that can produce various types of content including text, imagery, audio and synthetic data. The authors take full responsibility for the content of the manuscript, including the accuracy of the data, analyses, and conclusions.

Conflict of interest

The authors have declared no conflict of interest.

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