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ENHANCING NUTRIENT DIGESTIBILITY AND MICROBIAL FERMENTATION IN RICE STRAW-BASED DIETS USING CASSAVA, COPRA, AND PALM KERNEL MEALS

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ABSTRACT: This study assessed the effects of cassava-urea and protein meal supplementation at different proportions on the *in vitro* fermentation characteristics and digestibility of rice straw-based diets. Five diets were prepared with 20% rice straw as the base, and cassava-urea to protein meal ratios of 80:0 (T1), 70:10 (T2), 60:20 (T3), 50:30 (T4), and 40:40 (T5). *In vitro* digestibility and fermentation traits were analyzed using a two-stage method. The results showed that higher levels of protein meal improved crude protein digestibility from 67.5 to 81.1%. However, this increases reduced dry matter and organic matter digestibility, dropping from 82.8 to 75.0% and from 83.4 to 74.4%, respectively ($P < 0.05$). The pH remained within the optimal range of 7.29 to 7.41, with no significant change in total volatile fatty acids (VFA) production, which ranged from 40.9 to 44.8 mM. The ammonia nitrogen (NH_3-N) levels rose significantly from 7.86 to 11.4 mg/100 mL. This led to a decrease in the VFA:NH_3-N ratio, which changed from 5.30 to 4.05 ($P < 0.05$). This suggests an energy-to-nitrogen imbalance at higher protein meal levels. Microbial protein synthesis improved from 1.17 to 1.35 mg/mL ($P < 0.05$), likely due to increased nitrogen availability despite the imbalance. Including a moderate amount of protein meals, specifically 60% cassava-urea and 20% protein meal, provided a better balance in digestibility and fermentation efficiency. This highlights the importance of optimizing the energy-to-protein ratio in ruminant feed formulations.

Keywords: Cassava meal, *In vitro* digestibility, Microbial protein synthesis, Rice straw, Rumen fermentation.

INTRODUCTION

The rising demand for animal protein has created a greater need for sustainable and cost-effective feed resources, especially for ruminants. Rice straw, a common agricultural by-product, is often used as a basic feed ingredient because it is abundant and inexpensive. However, rice straw has its drawbacks. It has low crude protein content, which ranges from 4.0 to 4.7%, high lignocellulose levels, and low digestibility (Hasan et al., 2006, Kumar et al., 2021). These factors limit its use as the only feed source. To address these issues, it is crucial to supplement rice straw-based diets with energy- and protein-rich feed components. This will improve their nutritional value and fermentation efficiency.

Cassava-urea, a composite feed made from cassava mixed with urea, is a high-energy supplement used in ruminant nutrition. It provides easily fermentable carbohydrates and non-protein nitrogen, promoting microbial growth in the rumen (Wanapat and Khampa, 2007). Additionally, protein sources like copra and palm kernel meal act as bypass proteins and supply fiber, complementing the energy from cassava-urea. Finding the right balance between energy and protein is essential for improving fermentation efficiency, nutrient digestibility, and overall animal performance (Ortega et al., 2022), highlighting the importance of precise formulation in ruminant diets.

Despite these advantages, the ideal levels of cassava-urea and protein meals for maximizing digestibility and fermentation in rice straw-based diets are still uncertain. Excessive protein addition may cause nitrogen wastage, while insufficient energy can impair microbial activity and feed utilization. This research investigates how different amounts of cassava-urea and protein meal affect *in vitro* fermentation and digestibility of rice straw-based diets. By identifying the optimal energy-protein ratio, the study offers valuable insights to enhance feed formulation for ruminant nutrition.

MATERIALS AND METHODS

Treatments and experimental design

Five dietary treatments utilized rice straw as the main fiber source, constituting 20% of the total diet. The other 80% consisted of a mixture of cassava-urea and protein meal. This ratio was chosen to enhance the rapid availability of carbohydrates and protein in the ration. The cassava-urea was prepared by combining 99% cassava flour with 1% urea, offering fermentable carbohydrates and non-protein nitrogen. The protein meal included 75% copra meal and 25% palm kernel meal, providing crude protein and structural fiber. The ratios of cassava-urea to protein meal were 80:0 (T1), 70:10

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(T2), 60:20 (T3), 50:30 (T4), and 40:40 (T5). This mixture was then mixed with 20% rice straw to create the dietary treatments. All treatments were assessed using a one-way completely randomized design. The composition of the dietary treatments is shown in Table 1, and the chemical composition is presented in Table 2.

Table 1 - Formulation of rice straw-based diets supplemented with cassava-urea and protein meal in varying proportions

Feedstuffs	T1	T2	T3	T4	T5
Rice straw (%)	20	20	20	20	20
Cassava-urea (%) [1]	80	70	60	50	40
Protein meal (%) [2]	0	10	20	30	40

[1] Cassava-urea = 99% cassava + 1% urea. [2] Protein meal = 75% copra meal + 25% palm kernel meal

Table 2 - Chemical composition of rice straw-based diets supplemented with cassava-urea and protein meal at varying proportions

Nutrients	T1	T2	T3	T4	T5
Dry matter (%)	94.3	93.5	92.5	92.8	92.3
Organic matter (%DM)	93.4	93.0	92.8	92.1	91.8
Crude protein (%DM)	5.16	7.38	8.82	8.97	10.7
Ether extract (%DM)	2.00	2.38	2.40	2.90	2.90
Crude fiber (%DM)	9.19	10.0	12.0	13.0	13.6
Nitrogen-free extract (%DM) [1]	77.1	73.2	69.6	68.3	64.7

T1 = 20% rice straw + 80% cassava-urea, T2 = 20% rice straw + 70% cassava-urea + 10% protein meal, T3 = 20% rice straw + 60% cassava-urea + 20% protein meal, T4 = 20% rice straw + 50% cassava-urea + 30% protein meal, T5 = 20% rice straw + 40% cassava-urea + 40% protein meal. [1] Nitrogen-free extract (NFE) = dry matter - (crude protein + ether extract + crude fiber + ash).

Chemical composition analysis

The analyses of chemical composition followed the methods described by the Association of Official Agricultural Chemists (AOAC, 2005). Samples from each treatment group were dried at 55°C and ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), both before and after *in vitro* incubation. The analyses included measurements of dry matter (DM) (method 934.01), ash (method 942.05), crude protein (CP) (method 984.13), ether extract (EE) (method 920.39), and crude fiber (CF) (method 978.10). The nitrogen-free extract (NFE) was also calculated using the following formula:

NFE = dry matter – (crude protein + ether extract + crude fiber + ash) (1)

***In vitro* digestibility and fermentation characteristics analysis**

Two Ongole crossbred cows served as donors of ruminal fluid for *in vitro* incubations. The cows received a diet of Napier grass (*Pennisetum purpureum*) and commercial concentrate in an 80:20 ratio. Rumen fluid was collected before morning feeding, filtered with four layers of cheesecloth, and mixed with artificial saliva following the method described by Tilley and Terry (1963).

The *in vitro* digestibility was assessed using Tilley and Terry's two-stage method, focusing only on rumen degradation; thus, only the first stage was conducted. Each dietary treatment was tested in seven replications. At the end of the incubation period, fermentation characteristics were measured, which included pH, total volatile fatty acids (VFA), individual VFAs (acetic, propionic, and butyric acids), ammonia nitrogen (NH_3-N), and microbial protein synthesis. Ruminal VFAs were separated and quantified using gas chromatography (GC 2010 Plus, Shimadzu Corporation, Kyoto, Japan) following the methods described by Filípek and Dvořák (2009). Ammonia nitrogen concentration was determined using the spectrophotometric method of Chaney and Marbach (1962). Microbial protein synthesis was analyzed using a method described by Plummer (1987).

Statistical analysis

All data were analyzed using the SAS software (SAS Institute Inc., Cary, NC). The statistical model applied was:

$Y_{ij} = \mu + T_i + E_j$ (2)

Yij represents the individual response variable measured, μ is the overall mean, Ti denotes the fixed effect of treatment (i = 1 to 5), and Ej is the error term. Mean comparisons were performed using Duncan's multiple range test when the model found significant treatment effects (P≤0.05). Significant differences were noted at P≤0.05.

RESULTS AND DISCUSSION

In vitro digestibility

The *in vitro* digestibility results in Table 3 show significant differences in dry matter digestibility (DMD), organic matter digestibility (OMD), and crude protein digestibility (CPD) among the five dietary treatments. The DMD decreased from 82.8% in T1 to 75.0% in T5. This change shows that increasing protein meal inclusion lowers dry matter digestibility. The high DMD in T1 comes from its high cassava-urea content (80%), which provides easily fermentable carbohydrates, as shown in the nitrogen-free extract (NFE) in Table 2. Cassava has a high NFE level and ferments easily in the rumen, giving a quick energy source for rumen microbes (Rosmalia et al., 2023). However, the higher fiber content in diets with more protein meals (T4 and T5; Table 2) reduces DMD by lowering the availability of digestible energy.

The OMD data also showed a decline, dropping from 83.4% in T1 to 74.4% in T5. This highlights the trade-off between energy availability and fiber inclusion as the levels of protein meal increase. These changes align with the lower NFE values observed in Table 2, decreasing from 77.1% DM in T1 to 64.7% DM in T5. Conversely, CPD showed notable improvement with increased inclusion of protein meal, rising from 67.5% in T1 to 81.1% in T5. This trend is related to the higher crude protein content in diets with more protein meals, with values increasing from 5.16% DM in T1 to 10.7% DM in T5, as detailed in Table 2. The findings suggest that higher cassava-urea levels (T1 and T2) enhance energy digestibility but may restrict protein availability. Studies have indicated that cassava tubers, rich in carbohydrates, can substantially boost the digestibility of organic matter and gross energy in ruminants (Thang et al., 2010, Putridinanti et al., 2019, Santos et al., 2019). In contrast, diets high in protein meals (T4 and T5) improve CPD but lower overall energy digestibility due to increased fiber content. Earlier research indicates that higher dietary protein, particularly rumen undegradable protein, can improve protein digestibility in ruminants. For instance, increasing rumen undegradable protein (RUP) in diets led to higher milk yield and fat content in lactating cows, even though nutrient digestibility declined with increased RUP levels (Akhtar et al., 2017, Araújo et al., 2020).

These *in vitro* digestibility results demonstrate a nutritional trade-off from the treatments. While increasing protein meals enhance crude protein availability and utilization, it simultaneously decreases carbohydrate availability and overall fermentability, as shown by DMD and OMD measurements. This emphasizes the need to balance fermentable energy, such as NFE from cassava, with nitrogen sources like true protein from meals. Maintaining such a balance is crucial for enhancing microbial activity and nutrient digestibility in ruminants. While T5 might provide better protein digestibility, intermediate formulations like T3 could offer a better balance of energy and protein use for practical feeding systems in tropical ruminant production. Diet T3, which contains 60% cassava-urea and 20% protein meal, shows improved results in DMD, OMD, and CPD. This suggests it could be a good option for balancing energy and protein in diets using rice straw. These results highlight the importance of modifying cassava-urea and protein meal levels to enhance nutrient utilization in ruminant diets.

Table 3 - *In vitro* digestibility of rice straw-based diets supplemented with cassava-urea and protein meal at varying proportions

Digestibility (%)	T1	T2	T3	T4	T5
Dry matter	82.8^{c} ± 0.34	79.6^{b} ± 0.79	78.7^{b} ± 1.39	75.3^{a} ± 0.63	75.0^{a} ± 1.45
Organic matter	83.4^{c} ± 0.78	80.3^{b} ± 0.64	79.1^{b} ± 1.25	75.5^{a} ± 0.78	74.4^{a} ± 0.64
Crude protein	67.5^{a} ± 0.52	73.8^{b} ± 0.80	80.3^{c} ± 1.02	74.8^{b} ± 0.52	81.1^{c} ± 0.82

^{a,b,c} Means within a column with different superscripts differ significantly (P<0.05). T1 = 20% rice straw + 80% cassava-urea, T2 = 20% rice straw + 70% cassava-urea + 10% protein meal, T3 = 20% rice straw + 60% cassava-urea + 20% protein meal, T4 = 20% rice straw + 50% cassava-urea + 30% protein meal, T5 = 20% rice straw + 40% cassava-urea + 40% protein meal.

In vitro fermentation characteristics

Table 4 presents the *in vitro* fermentation characteristics of rice straw-based diets with varying levels of cassava-urea and protein meal, as outlined in Table 1. The parameters assessed – such as pH, total and individual volatile fatty acids (VFAs), ammonia nitrogen (NH_3-N), the VFA:NH_3-N ratio, and microbial protein – offer valuable insights into rumen fermentation and nutrient utilization.

The pH levels ranged from 7.29 to 7.41 (Table 4), remaining within the optimal range for rumen microbial activity. This indicates effective buffering capacity despite dietary changes. Total volatile fatty acid (VFA) concentrations varied from 40.9 mM (T1) to 44.8 mM (T5) with no significant differences between treatments, suggesting consistent fermentation activity across all diets. Similarly, individual VFAs (acetic, propionic, and butyric acids) showed no significant variation, implying that alterations in cassava-urea and protein meal ratios did not adversely affect these fermentation end-products. The acetate-to-propionate (A:P) ratio ranged from 2.63 (T3) to 3.13 (T2), with no significant differences. Nevertheless, the numerically lower A:P ratio observed in T3 may indicate a tendency toward more energy-efficient fermentation. A lower A:P ratio is often associated with improved energy utilization due to higher propionate production, reduced methane formation, and enhanced fermentation stability, which collectively promote efficient microbial syntrophy and reduce competition among methanogens (Meng et al., 2013, Wagner et al., 2014, Zakaria et al., 2022). As a result, it creates a more effective and stable fermentation environment.

Table 4 - *In vitro* fermentation characteristics of rice straw-based diets supplemented with cassava-urea and protein meal at varying proportions

Item	T1	T2	T3	T4	T5
pH	7.34 ± 0.18	7.35 ± 0.14	7.29 ± 0.19	7.41 ± 0.21	7.40 ± 0.16
Total VFA (mM)	40.9 ± 0.78	42.6 ± 4.00	42.9 ± 2.27	43.5 ± 0.30	44.8 ± 0.11
Acetate (mM)	24.4 ± 0.34	26.3 ± 3.00	25.2 ± 1.16	26.3 ± 1.01	27.2 ± 1.30
Propionate (mM)	8.75 ± 0.99	8.40 ± 0.90	9.61 ± 0.96	9.12 ± 0.53	9.64 ± 1.06
Butyrate (mM)	7.84 ± 0.13	7.82 ± 0.14	8.04 ± 0.15	8.00 ± 0.18	8.04 ± 0.13
A:P	2.80 ± 0.36	3.13 ± 0.03	2.63 ± 0.14	2.88 ± 0.26	2.85 ± 0.45
NH_3-N (mg/100mL)	7.86^{a} ± 0.23	8.07^{a} ± 0.70	9.33^{a} ± 1.25	9.54^{a} ± 0.11	11.4^{b} ± 1.00
VFA:NH_3-N	5.30^{b} ± 0.06	5.27^{b} ± 0.24	5.22^{b} ± 0.06	4.72^{b} ± 0.30	4.05^{a} ± 0.53
Microbial protein (mg/mL)	1.17^{a} ± 0.07	1.17^{a} ± 0.09	1.23^{ab} ± 0.11	1.30^{ab} ± 0.11	1.35^{b} ± 0.05

$^{a,b:}$ Means within a column with different superscripts differ significantly (P<0.05). T1 = 20% rice straw + 80% cassava-urea, T2 = 20% rice straw + 70% cassava-urea + 10% protein meal, T3 = 20% rice straw + 60% cassava-urea + 20% protein meal, T4 = 20% rice straw + 50% cassava-urea + 30% protein meal, T5 = 20% rice straw + 40% cassava-urea + 40% protein meal.

Ammonia nitrogen concentrations increased significantly with higher protein meal inclusion, moving from 7.86 mg/100 mL in T1 to 11.4 mg/100 mL in T5. This change shows more protein breakdown as crude protein content rose (Table 2). However, the decreasing VFA:NH_3-N ratio, which dropped from 5.30 in T1 to 4.05 in T5, suggests an imbalance between energy and nitrogen at higher protein meal levels. This imbalance could limit microbial protein production. Microbial protein synthesis in the rumen works best when the degradation rates of dietary carbohydrates and proteins are synchronized (Shete et al., 2012, Brassard et al., 2015). This synchronization gives rumen microbes a steady supply of energy and nitrogen, which is essential for their growth and protein production.

Microbial protein concentrations increased from T1 (1.17 mg/mL) to T5 (1.35 mg/mL), with significant improvements observed in T4 and T5 ($P < 0.05$). These results reflect the combined effects of increased crude protein digestibility and NH_3-N availability (Table 3), which together support enhanced microbial growth. Although energy (NFE) levels declined with higher protein inclusion (Table 2), the slight increase in microbial protein indicates that true protein sources such as copra and palm kernel meal are more effective in supporting microbial synthesis than cassava-derived non-protein nitrogen (cassava-urea) alone. Previous researchers reported that true protein sources like copra and palm kernel meals support microbial synthesis more effectively than cassava-derived NPN alone. They provide higher protein content and better digestibility, leading to more efficient nitrogen utilization and microbial protein synthesis (Stein et al., 2015, Rebelo et al., 2019).

Overall, the fermentation characteristics shown in Table 4 confirm that changes in protein and carbohydrate sources, as outlined in Table 1, greatly affect ruminal fermentation patterns. Protein supplementation improves nitrogen use and microbial growth. However, too much protein compared to fermentable energy can lower fermentation efficiency, which is indicated by lower VFA:NH_3-N ratios. Thus, achieving the right balance of fermentable carbohydrates and degradable protein, as in T3, is essential for efficient rumen fermentation and to maximize microbial protein yield.

CONCLUSION

This study shows that rice straw diets with higher cassava-urea levels (T1 and T2) enhance fermentation efficiency by improving energy digestibility. Conversely, diets richer in protein meals (T4 and T5) increase crude protein digestibility but decrease overall efficiency. Diet T3, with 60% cassava-urea and 20% protein meal, strikes a good balance between digestibility, fermentation, and nutrient utilization. These findings highlight the importance of combining degradable protein with fermentable energy to improve rumen performance. This strategy also enhances the nutritional value of low-quality tropical forages like rice straw.

DECLARATIONS

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical Regulations
The authors confirm that the journal's ethical policies, as noted on the journal's author guidelines page, have been adhered to. Ethical approval was not required, as this was an *in vitro* study in which no animals were used in the main experiment.

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Authors' contribution
All authors worked together on the manuscript and contributed equally to its development. Each of them played an active role at different stages of the research, including generating ideas, collecting data, analyzing information, and writing. Their contributions were shared evenly, ensuring a balanced effort in shaping the final paper.

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Competing interests
The authors declare that there is no competing interest regarding the publication of this article.

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