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Research Article

Potential of Ammoniated Citronella Distillation Waste as an Alternative Fiber Source to Replace Grass in Ruminant Diets

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ABSTRACT

This study evaluated the effect of substituting grass with ammoniated citronella waste supplemented with cassava leaves, phosphorus, and zinc minerals on nutrient digestibility and rumen fluid characteristics in vitro. The study used a completely randomized design with five treatments and four replications: R0 = 100% grass; R1 = 75% grass + 25% ammoniated citronella waste; R2 = 50% grass + 50% ammoniated citronella waste; R3 = 25% grass + 75% ammoniated citronella waste; and R4 = 100% ammoniated citronella waste. The variables observed included the digestibility of dry matter, organic matter, and crude protein, as well as pH, NH_3, and VFA. The results showed that the treatments significantly affected the digestibility of dry matter, organic matter, crude protein, NH_3, and VFA, but did not significantly affect rumen fluid pH. The highest digestibility of dry matter, organic matter, and VFA was obtained in treatment R3, while the highest digestibility of crude protein and NH_3 was obtained in treatment R4. The pH value of the rumen fluid in all treatments remained within the normal range. It was concluded that ammoniated citronella waste supplemented with cassava leaves, phosphorus minerals, and zinc could be used as an alternative fiber source to replace grass, with an optimal substitution level of 75% based on dry matter and organic matter digestibility and rumen fermentability.

Keywords: ammoniated citronella waste, grass substitution, in vitro digestibility, rumen fluid, ruminants

INTRODUCTION

Feed is a key factor determining ruminant livestock productivity because it affects nutrient consumption, rumen fermentation, and nutrient utilization efficiency. However, the availability of forage, the primary source of fiber, especially grass, is often limited in both quantity and quality due to seasonal fluctuations, land conversion, and increased competition for land use by other sectors. Under these conditions, the utilization of agro-industrial waste as an alternative feed ingredient has become an increasingly important strategy, as it not only reduces feed costs but also supports a more sustainable livestock system. Various studies have shown that agro-industrial byproducts have the potential to serve as feed sources for ruminants if their nutritional quality is adequate and their digestibility can be improved through appropriate processing technology (Jamarun et al., 2017; Pazla et al., 2018; Suyitman et al., 2021; Elihasridas et al., 2025).

Citronella grass (Cymbopogon nardus) distillation waste is an agro-industrial waste product with potential as an alternative feed. This waste is readily available at essential oil production centers and has potential as a fiber source for ruminant livestock. However, its direct use is limited

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because it generally contains high levels of crude fiber and lignin, which reduces digestibility and limits its utilization by rumen microbes (Kamoga et al., 2023). Previous research has indicated that citronella waste has potential as a feed ingredient; however, processing is required to improve its nutritional quality and digestibility (Elihasridas et al., 2023a).

Ammoniation is a technology widely used to improve the quality of fibrous feed ingredients. This treatment increases the nitrogen content of the material, loosens lignocellulose bonds, and improves the accessibility of the fiber fraction to rumen microbial degradation. Several studies on various fibrous wastes have shown that ammoniation increases the digestibility of dry matter and organic matter, while also improving rumen fermentation characteristics (Ningrat et al., 2019; Ningrat et al., 2020). In citronella waste, the combination of ammoniation with nutrient supplementation that promotes rumen microbial growth, such as cassava leaves (a source of branched-chain amino acids), phosphorus, and zinc, was also reported to significantly increase nutritional value and in vitro digestibility, thus strengthening its potential as an alternative feed source for ruminants (Elihasridas et al., 2023b).

However, most previous studies have focused on improving the quality of citronella waste through chemical or biological treatments. Meanwhile, information on the optimal substitution level of this material as a grass substitute in rations remains relatively limited. The success of an alternative feed ingredient is determined not only by increased nutrient content but also by its ability to maintain or enhance nutrient digestibility and rumen fermentation stability when used to replace conventional forages.

Accordingly, this study aimed to evaluate the effect of replacing grass with ammoniated citronella waste enriched with microbial growth factors (cassava leaves, phosphorus, and zinc minerals) in ruminant rations in vitro, in terms of nutrient digestibility and rumen fluid characteristics, to determine the most effective level of use as an alternative feed ingredient to replace grass.

MATERIAL AND METHODS

Experimental Design

This experiment was conducted in a laboratory to test the *in vitro* digestibility of rations. The experimental design used was a 5 × 4 completely randomized design, with five rations as treatments and four replications. The treatment arrangement is as follows, and the nutrient content is as stated in Table 1.

Rations (R0) = 100% Field Grass
Rations (R1) = 75% Field Grass + 25% Ammoniated citronella waste
Rations (R2) = 50% Field Grass + 50% Ammoniated citronella waste
Rations (R3) = 25% Field Grass + 75% Ammoniated citronella waste
Rations (R4) = 100% Ammoniated citronella waste

Preparation of Ammoniated Citronella Waste (Elihasridas et al., 2023b)

The citronella waste was cut into 3–5 cm pieces and mixed evenly with dry chicken manure at a ratio of 10% citronella waste dry matter. Urea was dissolved in water, with a ratio of water to the dry matter of the citronella waste of 1:1. The amount of urea used was 4% of the dry matter of the citronella waste (40 g of urea dissolved in 1 L of water for 1 kg of dry citronella waste). The citronella waste was placed in a 50-L plastic bucket layer by layer and sprayed with the urea solution and compacted to create an anaerobic environment. After the bucket was filled, it was covered with two layers of plastic and tied with a rope. The bucket was stored in a safe and shaded

place for 10 days. After 10 days, the bucket lid was opened, and the citronella waste was removed and air-dried for 2 days to eliminate excess ammonia gas. It was then dried and ground, and cassava leaves (as a source of branched-chain amino acids) were added at a rate of 5%, along with 0.4% phosphorus minerals and 100 ppm zinc minerals. The product was then ready for use.

Table 1. Chemical composition of experimental rations

Component	R0	R1	R2	R3	R4
Dry matter	90.23	89.23	88.23	87.23	86.25
Organic matter	79.42	82.88	86.35	89.81	93.28
Crude protein	8.29	8.93	9.55	10.19	10.83
Crude fat	0.87	1.16	1.44	1.74	2.03
Crude fiber	25.14	25.77	26.40	27.03	27.67
TDN	50.87	51.60	52.33	53.08	53.81
Ash	20.48	17.04	13.60	10.16	6.72
NDF	58.61	60.65	62.68	64.73	66.77
ADF	37.79	39.08	40.38	41.68	42.98
Cellulose	31.54	31.46	31.38	31.30	31.23
Hemicellulose	20.82	21.56	22.30	23.04	23.79
Lignin	4.20	5.27	6.35	7.42	8.50
Silica	2.05	2.35	2.64	2.95	3.22

The variables observed were Digestibility of Dry Matter, Organic Matter, and Crude Protein of the Ration, pH of rumen fluid, NH_3 concentration in rumen fluid, and Rumen fluid VFA concentration

In vitro Incubation

The ammoniated citronella waste sample was dried in an oven at 60°C and then ground. The sample was then incubated *in vitro* according to the procedure described by Tilley and Terry (1963). A total of 2.5 g of the sample was weighed and placed in a fermenter tube, after which 50 mL of rumen fluid and 200 mL of McDougall buffer solution were added. Subsequently, CO_2 gas was flushed into the tube for approximately 30 s to maintain in vitro anaerobic rumen conditions. The tube was then sealed with a vented rubber stopper to allow gas to escape. The tube was immediately placed in a shaker water bath at 39°C for 48 h. After 48 h of incubation, fermentation was stopped by adding 1 mL of $HgCl_2$. The tube containing the sample was centrifuged at 1200 rpm for 30 min to separate the supernatant and sediment. The supernatant was used to determine the concentrations of VFA, NH_3, and rumen microbial protein synthesis. In contrast, the sediment was used to determine the digestibility of the ration (DM, OM, CP, and fiber fractions).

Sample Analysis

The chemical composition of feed materials and residues was analyzed according to the methods described by AOAC (2016), whereas fiber content (neutral detergent fiber [NDF] and acid detergent fiber [ADF]) was analyzed according to the method described by Goering and Van Soest (1991). Cellulose content was calculated as the fraction lost after soaking ADF in 72% H_2SO_4 for 3 h, whereas hemicellulose was determined as the difference between NDF and ADF. The concentration of VFA was measured using the steam distillation method, whereas NH_3 concentration was determined using the Conway microdiffusion method.

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Statistical Analysis

The data obtained were analyzed using a diversity test according to the experimental design. Differences in the mean values of each treatment were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The average effects of treatment on the digestibility of dry matter, organic matter, and crude protein are shown in Table 2.

Table 2. Average digestibility of food substances in treatment rations

Nutrients	Treatment Ration (%)					SE
	R0	R1	R2	R3	R4	
Dry matter	56.27^{c}	56.34^{c}	60.45^{b}	65.47^{a}	55.21^{c}	1.46
Organic matter	58.34^{c}	61.64^{c}	65.41^{b}	70.21^{a}	60.01^{c}	1.25
Crude protein	60.75^{c}	63.36^{c}	66.45^{b}	67.45^{ab}	69.08^{a}	0.67

Note: Values with different superscripts in the same row indicate a significantly different effect ($P<0.05$).

The results of the statistical analysis showed that the treatments had a significantly different effect ($P < 0.05$) on the digestibility of dry matter, organic matter, and crude protein in the ration. Further analysis using DMRT showed that the highest dry matter and organic matter digestibility was obtained with ration 3 (25% grass and 75% ammoniated citronella waste). The digestibility of dry matter and organic matter in the field grass (R0) was not significantly different from that of ammoniated citronella waste (R4). This indicates that ammoniated citronella waste supplemented with cassava leaves, phosphorus, and zinc minerals could be used as an alternative feed to replace grass.

However, the highest digestibility of dry matter and organic matter was observed in ration 3 (25% grass + 75% ammoniated citronella waste). This was presumed to be due to a balance in the supply of nutrients for rumen microbial growth, namely, VFA as an energy source and carbon source, and NH_3 as a nitrogen source for microbial protein synthesis. High nutrient digestibility provides greater benefits to livestock consuming the ration because it contains more nutrients that meet their requirements.

The use of 100% ammoniated citronella waste tended to decrease dry matter and organic matter digestibility, despite higher protein and TDN levels. This was attributed to the increased levels of NDF, ADF, cellulose, lignin, and silica. Crude protein digestibility tended to increase with increasing inclusion of ammoniated citronella waste because of the higher crude protein content of the ration.

A sufficient and balanced supply of energy and protein optimizes rumen fermentation conditions. Jamarun et al. (2017b) reported that when microbial requirements were met, the microbial population reached an optimal level, thereby accelerating the rate of feed degradation in the rumen. Rumen bioprocesses are strongly influenced by rumen microbial populations and activities, as well as feed fermentability. Rumen microbes are the main agents of feed digestion in the rumen; therefore, the higher the microbial population and activity, the higher the rate of feed degradation in the rumen (Zain et al., 2020). Maximum microbial growth rates were achieved when all nutrients required for microbial growth and development were available at optimal concentrations (Putri et al., 2021).

Crude protein digestibility of the rations also differed significantly ($P < 0.05$), with digestibility increasing as the proportion of ammoniated citronella waste increased. This was due to the higher protein content of the rations. Zain et al. (2023) stated that feed digestibility was influenced by its chemical composition. This was also supported by the increased NH_3 production as the proportion of citronella waste in the ration increased. Ammoniated citronella waste contained high levels of NPN derived from nitrogen fixation during the ammoniation process, making it easily hydrolyzed in the rumen. Statistical analysis also showed that crude protein digestibility of ammoniated citronella waste was higher than that of grass.

Rumen Fluid Characteristics (pH, Total VFA, and NH_3 Concentration)

Optimal rumen conditions for microbial activity and growth were essential to support a high-quality fermentation process. In general, pH, VFA concentration, and NH_3 concentration in the rumen fluid are the main factors used to assess rumen conditions. Rumen metabolic products, such as VFA and NH_3, reflect the level of rumen fermentability. Higher levels of rumen metabolic products indicate that the ration is more readily degraded in the rumen. The average pH, VFA, and NH_3 levels in the rumen fluid are presented in Table 3.

Rumen pH reflects the interaction between buffer capacity and the acidity or alkalinity of fermentation products, which can affect the survival of rumen microorganisms. The results of the analysis of variance showed that the pH of each ration formulation was not significantly different ($P > 0.05$) and ranged from 6.82 to 7.06. The pH values obtained for each ration formulation were within the range required to support rumen microbial activity, with the ideal rumen pH for microbial development being 6.0–7.0; when pH decreases below 6, cellulolytic bacterial activity is disrupted (Jamarun et al., 2021).

Table 3. Average pH, total VFA, and NH_3 content of rumen fluid

Variable	Treatment					SE
	R0	R1	R2	R3	R4	
pH	6.84	6.86	6.87	6.89	7.06	1.26
NH_3 (mg/100 ml)	11.12^{d}	12.42^{b}	14.11^{bc}	15.63^{ac}	16.70^{a}	2.04
VFA (mM)	116.50^{c}	119.22^{c}	125.25^{b}	130.27^{a}	122.50^{b}	1.78

Note: Values with different superscripts in the same row indicate a significantly different effect ($P<0.05$).

The results of the analysis of variance showed that the treatments significantly affected ($P < 0.05$) VFA and NH_3 concentrations in the rumen fluid. The VFA production pattern was similar to that of dry matter and organic matter degradation of the ration. The greater the degradation of dry and organic matter in a feed ingredient, the higher the VFA concentration produced. The highest VFA concentration was obtained in ration 3 (25% grass + 75% ammoniated citronella waste).

The VFA concentration produced by ammoniated citronella waste was higher than that of field grass, indicating that ammoniated citronella waste was more readily degraded in the rumen than field grass. In general, the VFA concentration produced in each treatment ration met the requirements for optimal microbial growth. According to Van Soest (1982), the VFA range required for optimal rumen microbial growth was 80–160 mM.

NH_3 concentrations were also significantly different ($P < 0.05$), with NH_3 levels following the pattern of protein degradation. The highest NH_3 concentration was observed in ration treatment

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5. The high NH_3 concentration in this treatment was attributed to the higher crude protein content of the ration, particularly its NPN content. Ammonia (NH_3) levels in the rumen are an indicator of the relationship between protein degradation and microbial protein synthesis. However, ammonia concentration was not the only determining factor, as microbial growth and protein synthesis also depended on energy availability, as reflected in VFA production. NH_3 concentration in the rumen resulted from the degradation of protein and NPN by rumen microbes. Increasing protein and NPN levels in the ration also increased NH_3 levels in the rumen.

NH_3 production from each treatment ration ranged from 11.12 to 16.70 mg/100 mL of rumen fluid. The NH_3 content produced was sufficient to meet the needs of rumen microbes, with a minimum requirement of 5 mg/100 mL of rumen fluid (Satter & Slyter, 1974). The production of VFA and NH_3 reflects a material's fermentability; the higher the fermentability, the greater the production of VFA and NH_3 (Pazla et al., 2021; Putri et al., 2025).

The production of NH_3 and VFAs from ammoniated citronella waste was significantly higher than that from field grass, indicating that the fermentability of ammoniated citronella waste was better than that of field grass. Several studies have reported that fibrous feed materials processed through ammoniation have increased fermentability.

CONCLUSION

Ammoniated citronella waste supplemented with cassava leaves, phosphorus minerals, and zinc could be used as an alternative fiber source to replace grass, with an optimal substitution level of 75% based on dry matter and organic matter digestibility and rumen fermentability.

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