Research Article

Probiotic-Fortified Cassava Peel Enhances Ruminal Fermentation in Complete Feed: An *In Vitro* Evaluation

Raguati Raguati*, Afzalani Afzalani, Raden Abdul Muthalib, Rahmi Dianita

Department of Animal Science, Faculty of Animal Science, Jambi University, Jambi, Indonesia.

Abstract | This study evaluated the use of cassava peel fortified with probiotics as a forage source on *in vitro* ruminal fermentation products. The experimental diets maintained a forage-to-concentrate ratio of 60:40. The concentrate consisted of rice bran (58%), corn meal (25%), soybean meal (6%), coconut cake (9%), mineral mix (1%), and salt (1%). The experiment was arranged in a Completely Randomized Design (CRD) with five treatments and five replications: T1 (60% native grass + 40% concentrate); T2 (30% native grass + 30% cassava peel + 40% concentrate); T3 (T2 + 1% probiotic); T4 (15% native grass + 45% cassava peel + 40% concentrate); and T5 (T4 + 1% probiotic). The probiotic used was *Bacillus pumilus* st. L1 (1% = 28x10^{10} CFU/g). Parameters observed included proximate analysis, HCN content, gas production, dry matter digestibility (DMD) and organic matter digestibility (OMD), ammonia (NH_3), volatile fatty acids (VFA), and methane (CH_4). Results showed that the nutritional content of the complete feed included 6.59–12.75% crude protein and 3,776–3,922 kcal/kg energy. The treatments had a significant effect ($P<0.05$) on total gas production (143.03–190.68 ml), pH (5.45–6.34), DMD (77.27-85.05%), OMD (76.10-80.45%), and ammonia levels (5.90–15.05 mM). In conclusion, utilizing T3(30% cassava peel fortified with probiotics in a complete feed as a forage substitute is recommended as the optimal formulation for practical application, in relation to the estimated pH content that is still tolerable for application to livestock.

Keywords | *Bacillus pumilus* St. L1, Cassava peel, Complete feed, *In vitro* fermentation, Rumen metabolism

Received | January 17, 2026; **Accepted** | August 01, 2026; **Published** | August 21, 2026
***Correspondence** | Raguati Raguati, Department of Animal Science, Faculty of Animal Science, Jambi University, Jambi, Indonesia; **Email:** raguati_iding@unja.ac.id
Citation | Raguati R, Afzalani A, Muthalib RA, Dianita R (2026). Probiotic-fortified cassava peel enhances ruminal fermentation in complete feed: An *in vitro* evaluation. Adv. Anim. Vet. Sci., 14(9):2043-2051.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.9.2043.2051
ISSN (Online) | 2307-8316

INTRODUCTION

Indonesia is among the global cassava producers, after Nigeria and Thailand. The cassava production in Indonesia reached 19,053,748 tons in 2017 (Central Bureau of Statistics, 2018). In 2018, cassava production in Jambi Province reached 56,605 tons and is forecasted to keep increasing. The highest productivity was in 2022 with 28.54 tons of cassava per hectare (Mu'min *et al*., 2024). Cassava is a high-yielding crop that produces a significant amount of agricultural waste, particularly in the form of peels and leaves. Cassava peels serve as a viable alternative feedstuff, a water content of 14.62%, 73.29% starch and 21.02% amylose and 52.27% amylopectin (Wasistha *et al*., 2021).

The crude protein content of cassava leaves varies between 16.01% and 39.90%, while the protein content of the peels ranges from 3.45% to 6.50%. Additionally, the tannin content is higher in the leaves, ranging from 1.17% to 21.60%, compared to peels, which contain only 0.12% to 0.31% (Fasae and Yusuf, 2022). However, the utilisation of cassava peels and leaves is limited due to the presence of hydrocyanic acid (HCN), a potent anti-nutritional factor.

Hydrogen cyanide (HCN) interferes with cellular respiration by blocking the transport of oxygen in the blood, which can lead to rapid cell death due to asphyxia (Jadav *et al.*, 2019). The safe threshold for HCN in livestock feed is 500 parts per million (ppm) based on fresh weight and 200 ppm based on dry weight (Karthika and Kalpana, 2017). Fresh cassava peels in this study contained HCN levels of 404.41 mg/kg, after the cassava peel is dried, the HCN content becomes 230 mg/kg. Processing methods such as drying, silaging, and extrusion have been shown to significantly reduce HCN levels (Raguati *et al.*, 2022; Qi *et al.*, 2024).

To optimize the utilization of cassava peels and enhance rumen fermentability, probiotics are essential. Probiotics provide a viable solution by stabilizing the rumen ecosystem, improving digestibility, and boosting the immune response (El-Tawab *et al.*, 2016). *Bacillus pumilus* St. L1 has been identified as a highly effective probiotic strain that can increase feed intake and rumen fermentation capacity while also eradicating pathogenic microorganisms in the rumen (Raguati, 2016; Raguati *et al.*, 2022).

Although there are benefits to using cassava peel, further evaluation is needed to understand the synergy between high inclusion levels of cassava peel and probiotic fortification in a complete feed system. This study aimed to assess the impact of cassava peel, used as a forage source and fortified with *Bacillus pumilus* St. L1, on ruminal digestibility, ammonia (NH_3) levels, and volatile fatty acid (VFA) production, using an *in vitro* method. The hypothesis of this research is that the higher the replacement of green fodder with cassava peel in rations enriched with probiotic supplementation, the higher the digestibility, ammonia, VFA and total rumen gas production values.

MATERIALS AND METHODS

Materials

This study was conducted at the Laboratory of Animal Feed and Nutrition within the Faculty of Animal Science at Jambi University. The *in vitro* method was used to estimate feed degradability and to determine the optimal formulation for complete feed (Tilley and Terry, 1963).

The feed materials used in the study included native field grass, concentrates, cassava peel, and probiotics (*Bacillus pumilus* St. L1). The concentrate was formulated with the following components: 58% rice bran, 25% milled corn, 9% coconut dreg, 6% soybean dreg, 1% mineral mix, and 1% salt. The probiotic used was at a concentration of 1%, which means 1 millilitre contains 2.8 x 10^10 CFU/g (Colony Forming Units). This 1% of probiotic was inoculated into 100 g of rice bran, which acted as a carrier. From the 100 grams of inoculated rice bran, 1% (or 10 g) was then mixed with 1000 g of the complete feed.

Methods

The experiment utilized a Completely Randomized Design (CRD) with five treatments and five replications. The treatments were as follows:

- P1: 60% native grass + 40% concentrate (Control)
- P2: 30% native grass + 30% cassava peel + 40% concentrate
- P3: 30% native grass + 30% cassava peel + 40% concentrate + 1% probiotics
- P4: 15% native grass + 45% cassava peel + 40% concentrate
- P5: 15% native grass + 45% cassava peel + 40% concentrate + 1% probiotics

Cyanide content measurement (Sudarmadji, 1997)

Weigh 10-20 g of finely ground cassava peel silage, add 100 ml of distilled water to a Kjeldahl flask, and let stand for 2 hours. Add another 100 ml of distilled water and steam distillate. The distillate is collected in an Erlenmeyer flask filled with 20 ml of 2.5% NaOH. After the distillation (collected in the Erlenmeyer flask) reaches a volume of 150 ml, the distillation process is stopped. 5 ml of 5% KI and 8 ml of NH_4OH are added. The distillate mixture is titrated with 0.02 N $AgNO_3$ solution until turbidity appears.

Then, the hydrocyanic acid content is calculated using the formula:

$$HCN = \frac{(\text{ml AgNO3} \times 0.54)}{(\text{sample weight (g)})}$$

Note: 1 ml 0.02 N $AgNO_3$ = 0.54 mg HCN

Drying cassava peels

First, wash the cassava peels thoroughly. After washing, dry the peels in an oven at a temperature of 60°C, then grind them into a fine powder. Next, prepare a complete feed formula. The forage portion of the feed will consist of elephant grass. The concentrate will include the following ingredients: 58% rice bran, 25% corn, 6% soybean meal, 9% coconut meal, 1% mineral mix, and 1% salt. Finally, assemble the complete feed according to the specified experimental design.

In vitro procedures

Rumen fluid is collected in a plastic bottle, kept in a 39°C water bath, and transported to the lab. It is then strained through cheesecloth and mixed with McDougall buffer in a 1:4 v/v ratio (Tilley and Terry, 1963). A total of 2000 ml of rumen fluid-buffer mixture is transferred into a dark

2500 ml CO_2-saturated bottle with an automatic dispenser. Approximately 1 g of each diet is placed in pre-warmed serum bottles, followed by the addition of 40 ml of the mixture. Each bottle is sealed, with 53 bottles prepared for five treatments and three blanks. They are incubated at 39°C for 48 hours. To indicate the start of incubation, gas bubbles are released from each bottle. Gas production is measured at 3, 6, 9, 12, 24, and 48 hours using a 10 ml piston glass syringe, assessing gas volume through piston displacement. Methane production is quantified using a syringe connected to a gas washing vessel containing 4 M NaOH to absorb CO_2, with total gas and CH_4 production calculated by subtracting blank values. Total cumulative gas production is shown in Table 2, while hourly gas changes/kinetics can be seen in Figure 1.

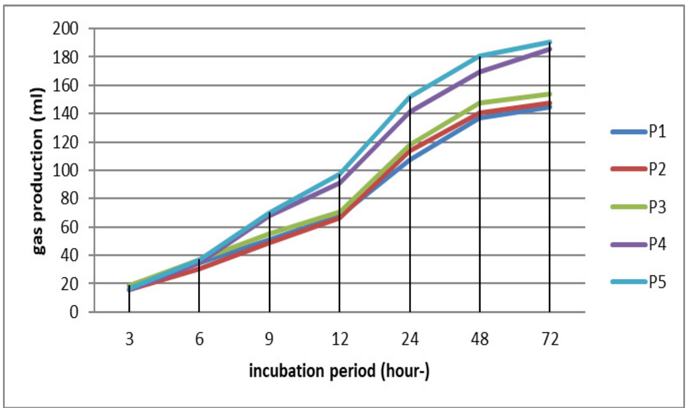

Figure 1: The kinetics of gas production during *in vitro* incubation period for 72 hours.

Ammonia and volatile fatty acids (VFAs) were measured after a 48-hour incubation period. Samples were centrifuged at 3,500 rpm for 10 minutes to separate the liquid from the precipitate. Ammonia concentration was determined using the Conway micro-diffusion technique. VFAs were analysed with a Hewlett-Packard 5890 gas chromatograph with a Flame Ionization Detector (FID), using nitrogen as the carrier gas at a flow rate of 0.5 ml/sec, and burning gases of oxygen and hydrogen at 5 ml/sec and 0.5 ml/sec, respectively. The column temperature was set to 125°C, with the injector and detector at 160°C and 200°C, respectively (Afzalani *et al*., 2015).

Data collection and parameters

The chemical composition of the diets was determined through proximate analysis, and the hydrocyanic acid (HCN) content was measured. Ruminal fermentability parameters were assessed using the *in vitro* gas production technique. The observed variables included:

- Fermentability: Total gas production, ammonia (NH_3) concentration, and volatile fatty acid (VFA) production.
- Digestibility: Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD).
- Environmental impact: Estimated methane (CH_4) production.

Statistical analysis

All data were analyzed using Analysis of Variance (ANOVA). Significant differences between treatment means were further evaluated with Duncan's Multiple Range Test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

Nutritional quality of cassava peel and probiotic-fortified feed

The nutrient composition of the complete feed formulations used in this study is presented in Table 1. The crude protein (CP) content ranged from 6.59% to 12.75%. These values indicate that the formulated complete feed meets the nutritional requirements for breeding cows, beef cattle, buffalo meat, and goats. This complete ration with a crude protein content of 6.59% to 12.75% is not suitable for lactating goats. Despite the low protein content in the feed, the presence of probiotics enhances fermentation in the rumen. This is evidenced by an increase in total gas and volatile fatty acid (VFA) production. The observed increase in total gas indicates improved digestibility and a higher VFA yield in the *in vitro* process (Silva *et al*., 2024; Dhakal *et al*., 2022). Gas production serves as a key parameter of rumen microbial activity, which is essential for the synthesis of energy and protein from microbes. The higher the microbial population in the rumen fluid, the more BO feed can be degraded and the gas produced will increase. (Ramdani *et al*., 2017).

Table 1: Nutrient content of complete feed (%).

Nutrients	Treatmens				
	T1	T2	T3	T4	T5
Dry Matter	92.390	92.422	92.107	92.099	92.035
Crude Protein	12.747	10.989	8.351	6.593	7.912
Crude Fat	3.28	2.78	2.37	2.31	1.875
Crude Fiber	22.435	17.605	16.260	14.780	15.920
Ash	9.531	7.199	7.422	6.227	6.551
GE (Kcal/kg)	3922	3840	3883	3768	3776

T1: 60% native grass + 40% concentrate, T2: (30% native grass + 30% cassava peel) + 40% concentrate, T3: (30% native grass + 30% cassava peel) + 40% concentrate + probiotics, T4: (15% native grass + 45% cassava peel) + 40% concentrate, T5: (15% native grass + 45% cassava peel) + 40% concentrate + probiotics.

A major limiting factor in the utilization of cassava peels is their hydrocyanic acid (HCN) content. In this study, fresh cassava peel contained 401.40 mg/kg of HCN. However, after the drying process, the HCN levels were significantly reduced to 201.60 mg/kg, bringing them closer to safe consumption thresholds for livestock. In this study, cassava peels were not used alone but were mixed with other feed ingredients, which is not an issue. Although the hydrogen cyanide (HCN) content in the feed remains high, the addition of probiotics facilitates effective fermentation in the rumen, leading to a reduction in HCN levels. This decrease in HCN is attributed to the presence of the probiotic *Bacillus pumilus* St. L1. *Bacillus pumilus* is known to produce two key enzymes: Cyanide Dihydratase (CynD), also known as Cyanidase, and β-glucosidase, both of which are essential for hydrolyzing cyanogenic glucosides. This bacterium is effective in breaking down the chemical bonds in Linamarin, a cyanogenic glycoside found in cassava, which releases HCN. The Cyanide Dihydratase enzyme from *Bacillus pumilus* helps to neutralize cyanide poison (Jandhyala *et al*., 2003).

The incorporation of *Bacillus pumilus* St. L1 into animal feed enhances nutrient utilization. This cellulolytic bacterium is known for its high tolerance to carbohydrate-rich media and its ability to improve nutrient absorption in the digestive tract (Raguati *et al*., 2022). *Bacillus pumilus* St. L1 can produce xylanase, an enzyme that breaks down xylan polymers by cleaving internal bonds to yield xylooligosaccharides (XOS). According to Lee *et al*. (2018), rumen-derived xylanases are crucial for fiber degradationand the fermentation of xylan hydrolysates by beneficial microbes highlights the potential of xylanase in producing prebiotics, which further stabilize the ruminal environment.

Rumen fermentation

Total gas and methane production

The total gas production is an indicator of the efficiency of ruminal fermentability. Higher total gas production suggests more favourable ruminal fermentation. *In vitro* data related to cassava peel in a complete feed can be found in Table 2.

A significant increase in total gas production was observed in the T4 and T5 treatments compared to T1 ($P < 0.05$). Both T4 (45% cassava peel, no probiotic) and T5 (45% cassava peel plus probiotic) displayed statistically similar high gas volumes, measuring 188.20 ml and 190.68 ml, respectively. This indicates that at high inclusion levels, the abundance of readily fermentable carbohydrates in cassava peel is the primary factor driving the volume of fermentation gas, which in turn stimulates rapid microbial activity. We acknowledge the relatively high SEM for total gas production. This variability is likely attributed to the biological nature of the rumen liquor used as inoculum in the *in vitro* system, which can exhibit inherent fluctuations in microbial activity despite standardized collection. However, despite this variability, the treatment effects (T4 and T5) remained statistically significant ($P < 0.001$) compared to the control, suggesting a robust response to the probiotic-fortified cassava peel. The high gas production in these treatments aligns with the VFA production pathways; typically, fermentation of fibrous carbohydrates that yields acetate and butyrate results in higher gas (CO2 and CH4) compared to propionate production (Jonker *et al*., 2020).

Although the total gas content in T4 and T5 varied, their fermentation efficiencies differed as well. T5 achieved a significantly higher Organic Matter Digestibility (OMD) compared to T4. This suggests that while the cassava peel substrate produced a high total gas content, the presence of probiotics played a crucial role in optimizing the breakdown of carbohydrates into microbial biomass and absorbable nutrients. The gas composition typically includes CO2 and CH4, which are natural byproducts of this anaerobic fermentation activity (Raguati, 2016). The total *in vitro* fermentation gas production was measured at various incubation hours: 3, 6, 12, 24, 48, and 72 (Figure 1).

Table 2: Effects of complete feed fortified with probiotic on dry and organic matters digestibility. pH. Ammonia. and Partial VFA

Parameters	Treatments					SEM	P value
	T1	T2	T3	T4	T5		
Total gas (ml)	143.03[b]	157.45[b]	154.62[b]	188.20[a]	190.68[a]	14.599	0.0004
CH_4 (ml)	74.35	71.63	71.13	68.45	67.90	2.610	0.1037
DMD (%)	77.27[b]	81.35[ab]	85.05[a]	81.06[ab]	83.52[a]	2.945	0.0180
OMD (%)	76.10[c]	80.45[a]	81.75[a]	78.25[b]	80.36[a]	2.223	<.0001
pH	6.34[a]	5.72[b]	5.70[b]	5.51[c]	5.45[c]	0.352	<.0001
NH_3 (mM)	15.05[a]	8.20[bc]	9.10[b]	10.35[b]	5.90[c]	3.395	<.0001

Note: a. b. c. d Different superscript letters in each row indicate significant differences at $p<0.05$. DMD; Dry matter digestibility. OMD: Organic matter digestibility, T1: 60% native grass + 40% concentrate, T2: (30% native grass + 30% cassava peel) + 40% concentrate, T3: (30% native grass + 30% cassava peel) + 40% concentrate + probiotics, T4: (15% native grass + 45% cassava peel) + 40% concentrate, T5: (15% native grass + 45% cassava peel) + 40% concentrate + probiotics.

An analysis of variance indicated that incorporating cassava peel fortified with probiotics into the complete feed did not significantly affect methane (CH4) production (P > 0.05). The treatments had no detectable effect on methane production. It suggests a potential improvement in rumen fermentation efficiency The decrease in methane indicates that more energy is being conserved rather than lost as gas. This conserved energy likely contributes to the synthesis of volatile fatty acids (VFAs), particularly propionate, which is beneficial for the host animal (Ku-Vera *et al*., 2020). According to Beschkov and Angelov (2025), methanogens actively use metabolic hydrogen (H_2) produced during the formation of acetate and butyrate. Therefore, a methane reduction suggests that H_2 utilization has shifted toward pathways that do not produce methane, such as propionate production.

The longer the incubation period, the greater the methane produced. This study demonstrated a significant volume of methane up to hour 48, remaining relatively stable until hour 72 (Figure 2).

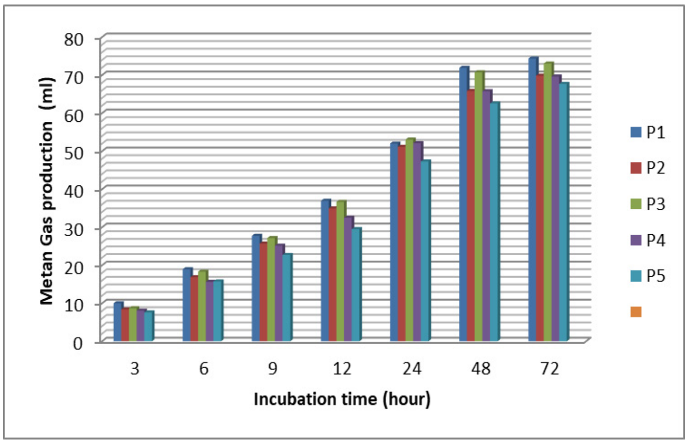

Figure 2: Methane production during *in vitro* incubation for 72 hours.

In vitro digestibility

The inclusion of cassava peel and probiotics in complete feed significantly influenced dry matter digestibility (DMD) and organic matter digestibility (OMD), with statistical significance at P<0.05. The data indicate that treatments T3 and T5 exhibited the highest levels of digestibility. Both cassava peels and probiotics help optimize the rumen ecosystem. Probiotics contribute by supplying extracellular enzymes that facilitate the hydrolysis of feed. This increase in digestibility is directly linked to the total gas yield, meaning that more nutrients from cassava peels are being absorbed by the digestive system. Moreover, feed mixtures containing cassava peel have been shown to enhance the metabolism of short-chain fatty acids (SCFAs) and improve the digestibility of rumen organic matter in small ruminants (Kalio, 2017). Additionally, research by Niayale *et al*. (2019) found that sheep fed ensiled cassava peel had superior dry matter intake, digestibility, and average daily gain (ADG) compared to those fed dried cassava peel.

The average DMD and OMD observed in this study were 77.27% to 85.05% and 76.10% to 81.75%, respectively. These results are higher than those reported by Dagaew *et al*. (2021), who found that cassava pulp fermented with yeast waste, when used as a replacement for soybean meal (SBM) and in various roughage-to-concentrate (R:C) ratios, yielded DMD and neutral detergent fiber degradability (NDFD) values ranging from 42.70% to 64.70% and 43.00% to 63.70%.

The high digestibility of dry matter and organic matter is attributed to the availability of nitrogen and carbon skeletons in complete feed, which optimises microbial growth and leads to increased feed degradation. Treatments T3 and T5, which include cassava peel and probiotics, showed higher digestibility values compared to T1 (which did not contain cassava peel or probiotics). This increased digestibility suggests a better nutrient supply for the animals. Additionally, cassava peels can mitigate the negative impacts on the growth and health of West African Dwarf Rams (Adegun *et al*., 2023). The effectiveness of probiotics on *in vitro* digestibility depends on the type of feed substrate used (Sheikh *et al*., 2017). Supplementing with probiotics can enhance the microbial population and activity, thereby improving feed digestibility (Raguati *et al*., 2022). Probiotics, whether in powder or liquid form, have been shown to increase *in vitro* dry matter digestibility, ultimately benefiting fattening calves and nutrient absorption (Maamouri and Salem, 2021). Specific probiotics, such as *Bacillus pumilus* St. L1, are capable of degrading the components of complete feed, including cassava peel. Diets supplemented with probiotics and their metabolites positively influence animal productivity by enhancing nutrient digestibility and stabilizing the rumen ecosystem (El-Thawab *et al*., 2016).

pH

The pH values recorded in this study ranged from 5.45 to 6.34. These results are consistent with findings from Ajagbe *et al*. (2022), who reported that the pH level in the diet of West African Dwarf goats, supplemented with pineapple waste and cassava peel concentrates, ranged from 5.08 to 6.14.

The incorporation of cassava peel and probiotics had a significant impact on pH levels (P < 0.0001). As the proportion of cassava peel increased (specifically in treatments T4 and T5), the pH decreased notably to 5.51 and 5.45, respectively. While a rumen pH below 5.8 is typically recognized as a risk factor for sub-acute ruminal acidosis (SARA) and can inhibit fibrolytic bacteria *in vivo* (Ogata *et al*., 2020), it is important to interpret the low pH observed in this study within the context of an *in vitro* closed system.

In this batch culture system, organic acids (volatile fatty acids, or VFAs) are continuously produced through the rapid fermentation of highly digestible carbohydrates found in cassava peel. This fermentation process leads to an accumulation of acids in the medium, which naturally lowers the pH. An inverse relationship was observed between pH and gas production, with treatments T4 and T5 demonstrating the highest gas production and the lowest pH levels.

Interestingly, despite the acidic environment in treatment T5 (pH 5.45), Dry Matter Digestibility (DMD) remained significantly high at 83.52%, the highest among all treatments. This finding challenges the common assumption that low pH levels hinder digestion (Maamouri and Salem, 2021). It suggests that probiotic supplementation may have enhanced the rumen microbiome's tolerance to acidity or optimised fermentation pathways, allowing effective feed degradation even at lower pH levels. Our pH results, ranging from 5.45 to 6.34, are consistent with those reported by Ajagbe *et al.* (2022), who noted pH levels between 5.08 and 6.14 in goats fed with cassava peel concentrates. Nevertheless, considering physiological balance, treatment T3 is highlighted as the optimal formulation, offering high digestibility (81.75% OMD) while maintaining a safer pH buffer (5.70) compared to the more acidic T5. Statistically, T3 and T5 were not significantly different, but the pH of T3 was much lower, this indicates the potential for a compensatory mechanism or greater adaptation of rumen microbes.

Ammonia (NH_3-N) concentration

In this study, the use of cassava peel and probiotics in complete feed significantly impacted ammonia concentration ($P < 0.05$). Ammonia concentration can influence the fermentation outcomes of organic feed ingredients by affecting the production of volatile fatty acids (VFA), which serve as energy sources for ruminants. Adequate ammonia availability ensures a favorable balance of nitrogen and energy vital for the growth of rumen microbes. Ammonia levels decreased markedly in T5 (5.90 mM) compared to T1 (15.05 mM). The NH3 concentration in T5 decreased significantly to 5.90 mM. A significant drop in ammonia levels can sometimes indicate nitrogen limitation, especially in low-protein diets. The lower NH3 levels in T5 were related to the protein levels in the feed, indicating good utilization potential by microbes, thus providing a more balanced analysis.However, the data from this study suggest a different explanation. The observed ammonia level of 5.90 mM is above the critical threshold of 3.6 mM (5 mg/dL), which is necessary for optimal microbial growth, as proposed by Satter and Slyter (1974).

Additionally, the relatively low ammonia concentration in treatment T5 corresponded with the highest Dry Matter Digestibility (83.52%) and Organic Matter Digestibility (80.36%). This inverse relationship suggests that the available nitrogen was not lacking; instead, it was being efficiently absorbed and converted into microbial protein to support the intense fermentation activity triggered by the probiotic. If nitrogen had been insufficient to limit synthesis, feed digestibility would have been adversely affected. Therefore, these results indicate a closely linked balance between energy and nitrogen, where the rate of ammonia utilization by microbes matched the high availability of fermentable energy from the cassava peel. Moreover, the supplementation of probiotic and fibrinolytic enzyme mixtures can enhance ammonia concentration, as noted by Sheikh *et al.* (2017).

Partial VFA

The use of cassava peel and probiotics significantly($P<0.05$). influenced the profile of volatile fatty acids (VFAs). As shown in Table 3, the supplementation contributed to an overall increase in partial VFAs. The highest concentration was observed in T5 (127.63 mMol/L), which was significantly higher than the control T1. The high production of acetic acid in T5 indicates that the probiotic effectively stimulates cellulolytic bacteria to degrade the high fiber content present in the 45% cassava peel inclusion, despite the lower pH observed in this treatment. Treatment T3 recorded the highest concentration of propionic acid (116.75 mMol/L) and was significantly different from T1 and T4 ($P < 0.05$). High levels of propionic acid are metabolically advantageous as it serves as the primary precursor for gluconeogenesis, supporting tissue growth and weight gain. Microorganisms in the rumen of ruminants can break down feed and produce metabolites such as volatile fatty acids (VFAs), proteins, and vitamins (Zhu *et al.*, 2025).

The elevated VFA concentrations in the probiotic-treated groups (T3 and T5) compared to their non-probiotic counterparts (T2 and T4) align with the higher digestibility (OMD and DMD) reported in Table 2. This suggests that probiotics enhance the fermentation rate and microbial population density. The concentrations of acetate and propionate in the studies ranged from 191.48 to 238.67 mMol/L. These values are significantly higher than those reported by Dagaew *et al.* (2021), who observed VFA concentrations of approximately 166.0 to 167.5 mMol/L. The optimal VFA requirement for rumen microbial growth is between 70 and 130 mM, according to France and Dijkstra (2005). This difference may be attributed to variations in feed composition, particularly the replacement of green fodder with cassava peel, along

Table 3: Average partial VFA content of complete feeds fortified with probiotics.

Parameters	Treatments					SEM	Pvalue
	T1	T2	T3	T4	T5		
Acetic (C_2) mMol/l.	103.58^{b}	113.02^{ab}	121.92^{ab}	114.22^{ab}	127.63^{a}	2.375	0 .066
Propionic(C_3) mMol/l.	87.90^{b}	108.01^{ab}	116.75^{a}	87.83^{b}	103.71^{ab}	3.338	0.125
Butyric(C_4) mMol/l.	10.88	17.73	29.07	20.87	23.98	2.906	0.116
Valerat mMol/l.	1.67	2.50	3.27	2.30	2.75	0.534	0.395
Iso valerat mMol/l.	3.81	3.18	3.32	3.58	2.88	0.604	0.654
Ratio C_2/C_3	1.18	1.05	1.05	1.33	1.18	0.043	0.106
C_2 (%)	49.33^{a}	45.77^{b}	45.06^{b}	49.47^{a}	47.63^{ab}	0.309	0.128
C_3 (%)	41.83	43.73	43.20	38.06	40.54	1.344	0.252
C_4 (%)	5.14	7.17	8.71	9.03	8.97	.866	0.227

Note: a. b. c. d Different superscript letters in each row indicate significant differences at p <0.05. T1: 60% native grass + 40% concentrate, T2: (30% native grass + 30% cassava peel) + 40% concentrate, T3: (30% native grass + 30% cassava peel) + 40% concentrate + probiotics, T4: (15% native grass + 45% cassava peel) + 40% concentrate, T5: (15% native grass + 45% cassava peel) + 40% concentrate + probiotics.

with the use of probiotic supplementation in this study. Additionally, the supplementation of cassava peel with cassava leaves and cowpeas in goat feed has been shown to significantly impact the levels of rumen acetic acid and propionic acid (Oni *et al*., 2017).

The inclusion of probiotics in the feed can enhance rumen fermentability. The concentration of volatile fatty acids (VFAs) is influenced by factors such as fermentation rate, soluble carbohydrate content, ruminal pH, feed digestibility, feed volume, and the types of ruminal microbes present. VFAs, produced from carbohydrate fermentation in the rumen, serve as a key energy source for ruminants and play a vital role in microbial protein synthesis. As the production of VFAs increases, along with the organic matter in the feed that rumen bacteria degrade, the rumen pH decreases (Wang *et al*., 2020). Supplementation with probiotics (CON-PRO) has been shown to increase total VFA concentrations (Maas *et al*., 2021). High VFA concentrations are related to the fermentation efficiency of the feed, the amount of soluble carbohydrates, and elevated rumen pH levels. It is important to note that acetic acid is the end product of fibre fermentation, whereas propionic acid is the end product of sugar and starch fermentation.

Ratio C2/ C3

The acetic to propionic acid ratio can be used to evaluate energy utilization efficiency in ruminants and the quality of the yield produced. In this study, no significant difference was found among the groups regarding the C2/C3 ratio (P > 0.05). The average C2/C3 ratio ranged from 1.05 to 1.33. A lower C2/C3 ratio indicates better energy utilization efficiency, with values close to 1 suggesting high propionate production. Propionate production is more efficient in rumen metabolism, as it reduces energy loss in the form of methane (CH_4). Previous studies reported that the C2/C3 ratios for cassava pulp processed with yeast waste ranged from 2.23 to 4.55 (Dagaew *et al*., 2021). Additionally, Rahayu *et al*. (2018) found that the C2/C3 ratio of complete feeds, where banana weevil replaced molasses, ranged from 3.32 to 3.45.

Treatments T2 (30% cassava peel, without probiotics) and T3 (30% cassava peel with probiotics) demonstrated the most efficient ratio of 1.05. The increase in the ratio for T2, when compared to T1 and T4, suggests that at the 30% level of addition, the composition of the substrate plays a significant role. Cassava peel contains non-structural carbohydrates, specifically starch, which naturally promote the growth of amylolytic bacteria and the production of propionate (Phesatcha *et al*., 2020). However, the probiotic in T3 is also crucial. Although T2 and T3 exhibited the same efficiency ratio, T3 achieved this with a higher total production volume of propionic acid (116.75 mM) compared to T2 (108.01 mM). Statistically, T3 was able to differentiate significantly from the lower-performing groups, while T2 remained intermediate. This indicates that while the cassava peel substrate directs the fermentation process (lowering the ratio), the probiotic enhances the magnitude of acid production, ensuring a greater total energy supply for the host.

The proportion of VFAs

Treatment significantly impacted (P < 0.05) the proportions of volatile fatty acids (VFA), particularly the percentage of acetic acid. Cassava peel is rich in easily fermentable carbohydrates, resulting in acetic acid making up a dominant proportion (45–49%) in all treatments. This abundance of acetic acid helps maintain the precursors required for lipogenesis. The percentage of C3 tended to decrease, with similar results observed across all treatments. This balance ensures that while energy efficiency is maximized through propionate, there is also enough acetate available for synthesizing milk fat or adipose tissue.

CONCLUSION

Probiotic-enriched cassava peels can effectively replace up to 30% of forage (T3) without disrupting rumen fermentation. In this study, T3 provided an optimal balance between dry matter degradability and VFA production *in vitro*.

ACKNOWLEDGMENTS

This research was funded by the PNBP University of Jambi, via the Bureau of the Research and Community Services of Jambi University. Additionally, we would like to thank the Department of Animal Science in the Faculty of Animal Husbandry for providing research facilities.

NOVELTY STATEMENT

Probiotics containing *Bacillus pumilus* St. L1 can be used in complete ration feed based on cassava peel and can increase rumen fermentability.

AUTHORS CONTRIBUTION

RR: Conceptualization, methodology, formal analysis, investigation, writing original draft, funding acquisition. AA: Supervision, validation, writing review and editing. RAM: Resources, data curation, supervision. RD: Software, formal analysis, visualization, writing review and editing.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Adegun MK, Ekundayo FG, Ajay DD (2023). Growth and blood indices of west african dwarf rams fed urea treated cassava peels with graded levels of *Gliricidia sepium* Fodder. J. Anim. Health Prod., 11(4): 420-429. https://doi.org/10.17582/journal.jahp/2023/11.4.420.429

Afzalani MZ, Novirman J, Endri M (2015). Effect of increasing doses of essential oil extracted from berastagi orange (*Citrus sinensis* L.) peels on performance, rumen fermentation and blood metabolites in fattening bali cattle. J. Pak. Nutr., 14(8): 480-486. https://doi.org/10.3923/pjn.2015.480.486

Ajagbe D, Mohammed BA, Abutusalami DI, Alfa VO (2022). Rumen characteristics and the thermo-physiological response of West African Dwarf goats supplemented with pineapple waste and cassava peel concentrates. J. Anim. Sci. Vet. Med., 7(3): 85-91. https://doi.org/10.31248/JASVM2022.321

Beschkov VN, Angelov IK (2025). Volatile fatty acid production vs. methane and hydrogen in anaerobic digestion. J. Ferment., 11(4): 172. https://doi.org/10.3390/fermentation11040172

Central Bureau of Statistics (2018). Quality of wet tuber production. Ministry Of Agriculture Of The Republic Of Indonesia. https://www.pertanian.go.id/home/pertanian.go.id

Dagaew G, Cherdthong A, Wanapat M, Sarong SO, Suntara C (2021). Manipulation of *in vitro* ruminal fermentation and feed digestibility as influenced by yeast waste-treated cassava pulp substitute soybean meal and different roughage to concentrate ratio. J. Fermentation. MDPI, 7(196): 1-13. https://doi.org/10.3390/fermentation7030196

Dhakal R, Ronquillo MG, Pérez EVB, Hansen HH (2022). Effect of autochthonous nepalese fruits on nutrient degradation, fermentation kinetics, total gas production, and methane production in *in-vitro* rumen fermentation. J. Anim., 12: 2199. https://doi.org/10.3390/ani12172199

El-Thawab MMA, Youssef IMI, Bakr HA, Fthenakis G , Giadinis ND (2016). Role of probiotics in nutrition and health of small ruminants. Review. J. Polish Vet. Sci., 19(4): 893–906. https://doi.org/10.1515/pjvs-2016-0114

Fasae, Yusuf (2022). Cassava leaves and peels: Nutritional value and potential productivity in West African dwarf breeds of sheep and goats– A review. Nigerian J. Anim. Prod., 49(3): 301-311. https://www.njap.org.ng/index.php/njap/article/view/3562/2801 https://doi.org/10.51791/njap.v49i3.3562

France J, Djikstra J (2005). Volatile Fatty acid production in: quantitative aspect of ruminant digestion and metabolism. Ed. C.A.B. Internasional, Cambridge, USA. https://doi.org/10.1079/9780851998145.0157

Jadav CN, Makwana RB, Parikh SS, Gamit PM, Murthy KS, Gajabhiye PU (2019). Hydrocyanic Acid (HCN) Estimation during Different Stages of Growth in Gundri jowar (Sorghum Vulgare) Fodder Crop. J. Int. Cur. Microb. Appl. Sci. 8(9): 1328-1333. https://doi.org/10.20546/ijcmas.2019.809.152

Jandhyala D, Berman M, Meyers PR, Sewell BT, Willson RC, Benedik MJ (2003). CynD, the Cyanide Dihydratase from Bacillus pumilus: Gene Cloning and Structural Studies. J. Appl. Envir. Microb., 69(8): 4794–4805. file:///C:/Users/ACER/Downloads/jandhyala-et-al-2003-cynd-the-cyanide-dihydratase-from-bacillus-pumilus-gene-cloning-and-structural-studies.pdf, https://doi.org/10.1128/AEM.69.8.4794-4805.2003

Jonker A, Hickey S, Boma P, Woju CW, Sandoval E, MacLean S, Calzada MGR, Lewis WYS, Janssen PH, McEwan JC, Rowe S (2020). Individual-level correlations of rumen volatile fatty acids with enteric methane emissions for ranking methane yield in sheep fed fresh pasture. J. Anim. Prod. Sci., 61(3): 300-305. https://pubag.nal.usda.gov, https://doi.org/10.1071/AN20128

Kalio GA (2017). Metabolizable energy and digestibility estimates of mixed diets of crop by-products as readily available feeds for small ruminants. J. Green. Agric. Sci., 7 (8): 210-215. https://doi.org/10.15580/GJAS.2017.8.101417150

Karthika N, Kalpana R (2017). HCN Content and forage yield of multi-cut forage sorghum under different organic manures and nitrogen levels. Chem. Sci. Rev. Lett., 6(23): 1659-1663.

Ku-Vera JC, Castelán-Ortega OA, Galindo-Maldonado FA, Arango J, Chirinda N, Jiménez-Ocampo R, Valencia-Salazar SS, Flores-Santiago EJ, Montoya-Flores MD, Molina-Botero IC, Pineiro-Vázquez AT, Arceo-Castillo JI, Aguilar-Pérez CF, Ramírez-Avilés L, Solorio-Sánchez FJ (2020). Review: Strategies for enteric methane mitigation in cattle-fed tropical forages. J. Anim., 14(3): 453–463. https://doi.org/10.1017/S1751731120001780

Lee KT, Toushik SH, Baek JY, Kim JE, Lee JS, Kim KS (2018). Metagenomic mining and functional characterization of a novel KG51 bifunctional cellulase/hemicellulase from black goat rumen. J. Agric. Food Chem., 66(34): 9034–9041. https://doi.org/10.1021/acs.jafc.8b01449

Maamouri O, Salem MB (2021). Effect of yeast culture feed supply on growth, ruminal pH, and digestibility of fattening calves. J. Food Sci. Nutr., 9(5): 2762–2767. https://doi.org/10.1002/fsn3.2238

Maas RM, Deng Y, Dersjant-Li Y, Petit J, Verdegem MCJ, Schrama JW, Kokou F (2021). Exogenous enzymes and probiotics alter digestion kinetics, volatile fatty acid content and microbial interactions in the gut of Nile tilapia. J. Nat., 11: 8221. https://doi.org/10.1038/s41598-021-87408-3

Mu'min K, Bakti AS, Lestari SP, Sari YE (2024). Cassava farming income in Madukoro Village, North Kotabumi Sub-District, North Lampung District. J. Agric. Anim. Sci., 4(1).

Niayale R, Addah WAA (2019). Utilization of cassava peels as feed for ruminants in Ghana: A review of alternative methods of improving their nutritional value. Book. Roots and tubers in Ghana: Overview and selected research papers. Printing Press KNUST, PMB, Kumasi Ghana. pp. 332-350.

Ogata T, Makino H, Ishizuka N, Iwamoto E, Masaki T, Kizaki K, Yo-Han Kim, Sato S (2020). Long-term high-grain di*et al*ters ruminal pH, fermentation, and epithelial transcriptomes, leading to restored mitochondrial oxidative phosphorylation in Japanese Black cattle during fattening. J. Sci. Rep ,10(1): 6381-6390. https://doi.org/10.1038/s41598-020-63471-0

Oni AO, Abatan, Adebayo A, Sowande K, Iposu OS, Onwuka SCF (2017). Effects of supplementing cassava peels with cassava leaves and cowpea haulms on the rumen environment and blood profile parameters of West African dwarf goats. J. Arch. Zootec., 66 (255): 395-402. https://www.uco.es/ucopress/az/index.php/az/

Ososanya TO, Inyang UA (2016). Assessment of *in vitro* gas and methane production of diet fortified with yeast and *Lactobacilli* spp. J. Br. Appl. Sci. Tech., 16(4): 1-8. http://sciencedomain.org/review-history/14900, https://doi.org/10.9734/BJAST/2016/19166

Phesatcha K, Phesatcha B, Wanapat M, Cherdthong A (2020). Roughage to concentrate ratio and saccharomyces cerevisiae inclusion could modulate feed digestion and *in vitro* ruminal fermentation. J. Vet. Sci., 7(15): 1-13. https://doi.org/10.3390/vetsci7040151

Qi M, Jialin S, Lijun J, Luxia L, Mei X, Yueming L, Shifeng Y, HongjunL (2024). Understanding the degradation mechanisms of cyanide and starch in cassava flour during extrusion processing. J. Innov. Food Sci. Emerg. Technol., 91: 103548. https://doi.org/10.1016/j.ifset.2023.103548

Raguati (2016). Exploration of probiotics bacteria from pineapple peel and its use in feed to increase milk production of etawah crossbreed goats. Dissertation. Unand, Padang. http://scholar.unand.ac.id/id/eprint/16083

Raguati R, Darlis D, Afzalani A, Ningsi Z, Hoesni F, Musnandar E (2022). Effect of ensiling and EM4 bio activator level on physical quality and content of cassava peel silage (*Manihot utilissima Pohl*). JIUBJ.22(1): 510-516. https://doi.org/10.33087/jiubj.v22i1.2152

Rahayu RI, Subrata A, Achmadi J (2018). *In vitro* ruminal fermentability of ammonified rice straw based feed with banana cob flour and molasses supplementation. J. Indo. Anim. Husb. 20(3): 166-174. https://doi.org/10.25077/jpi.20.3.166-174.2018

Ramdani D, Marjuki M, Chuzaemi S (2017). The effect of different types of solvents in the extraction process of noni fruit (*Morinda citrifolia* L.) in feed on protozoa viability and in-vitro gas production. J. Ilmu-Ilmu Pet. 27(2): 54–62. https://doi.org/10.21776/ub.jiip.2017.027.02.07

Satter, LD and Slyter LL (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. Brit. J. Nutr., 32;199-208. https://www.cambridge.org/core/services/aop-cambridgecore/content/view/, https://doi.org/10.1079/BJN19740073

Sheikh GG, Abdul MG, Ahmad I, Yasir A, Haider AA (2017). *In vitro* effect of probiotic mix and fibrinolytic enzyme mixture on digestibility of paddy straw. J. Adv. Anim. Vet. Sci., 5(6): 260.

Silva TH, Amancio BR, Magnani E, Meurer GW, Reolon HG, Timm TG, Cappellozza B I, Branco RH, Paula EM (2024). Evaluation of direct-fed microbials on *in vitro* ruminal fermentation, gas production kinetic, and greenhouse gas emissions in different ruminants' diet. J. Front. Anim. Sci., 5: 1-15. https://doi.org/10.3389/fanim.2024.1320075

Tilley JMA, Terry RA (1963). A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci., 18: 104-111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Wang L, Zhang G, Zhang YLY (2020). Effects of high forage/concentrate diet on volatile fatty acid production and the microorganisms involved in VFA production in cow rumen. J. Anim., 10(223): 1-12. https://doi.org/10.3390/ani10020223

Wasistha AW, Dika MRS,. Aulia AS, Samudra NM, Putri DN (2021). Physical and mechanical characteristics of the edible film based on cassava peel starch. Agrisiantifika J. Ilmu-Ilmu Pertan., 5(2): 149-158. https://doi.org/10.32585/ags.v5i2.1877

Zhu D, Hu Y, Liu Y, Zhou C, Tan Z, Kang J, Wang Z (2025). Multi-omics revealed the promoting effect of dietary inulin supplementation on hepatic gluconeogenesis in goats. J. Front. Anim. Sci., 6: 1614869. https://doi.org/10.3389/fanim.2025.1614869