Research Article

Gas Production, Volatile Fatty Acids and *In vitro* Digestibility in Response to Replacing Native Grass with Fermentation Cavendish Banana Stems (*Musa acuminata Cavendish*)

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Abstract | This study aims to determine the production of gas, volatile fatty acids (VFA) and digestibility *in vitro* in response to replacing native grasses with fermented banana stems (*Musa acuminata Cavendish*) (BPF). The research feed consisted of 30% concentrate and 70% native grasses. The use of native grass was partially replaced with fermented banana stems as a treatment. A completely randomized design with 4 treatments and 4 replications was used. Treatment P0 = 50% native grasses + 50% fermented banana stems (BPF), P1 = 40% native grasses + 60% BPF, P2 = 30% native grasses + 70% BPF and P3 = 20% native grasses + 80% BPF. *In vitro* research was conducted by taking rumen fluid from the slaughterhouse and filtering it. The research parameters were VFA, dry matter digestibility, organic matter digestibility and total gas production, ammonia, methane, and rumen pH. Data processing was performed using SAS 9.0. The results showed that increasing the replacement of native grasses with BPF reduced dry matter digestibility and organic matter digestibility ($P<0.05$) *in vitro*. Treatment P3 was lower than P0, P1, and P2. The treatment significantly affected ammonia ($P<0.01$), methane ($P<0.05$), but did not affect total gas production or rumen pH ($P>0.05$). The treatment also significantly affected VFA, acetate, propionate, butyrate, and iso valerate ($P<0.05$) *in vitro*. The study concluded that replacing native grasses with BPF can be done up to 70% in the ration for *in vitro* digestibility and gas production.

Keywords | *In vitro*; Fermented banana stems, VFA, Native grass, Digestibility, Gas production

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INTRODUCTION

One of the challenges facing beef cattle farming in Indonesia is the fluctuating availability of forage (grass), especially during the dry season. This condition results in a greater diversity of green plants in the dry season than in the rainy season, primarily derived from agricultural waste. The dry matter content of native grasses contributes 41.31% of the forage in the dry season and 54.63% in the rainy season (Despal *et al*., 2014). Difficulties in providing grass certainly require addressing alternative feed sources from agricultural waste that are inexpensive, readily available, and have adequate nutritional content (Sokchea *et al*., 2018; Elahi *et al*., 2018; Darlis *et al*., 2023). One potential agricultural waste source is the stem of the Cavendish banana (*Musa acuminata Cavendish*).

Banana plantation waste that can be used as feed includes banana stems at harvest time and stems from banana shoots that interfere with growth. Direct observations of banana plantations indicate that per hectare, there are 55.5-66.6 tons of banana stems per season, obtained from 1.111 trees, each weighing 50-60 kg. According to Salehizadeh *et al.* (2017), banana stem waste accounts for 40% of the total, or 60–80 tons per hectare per year. Banana stems are rich in nutrients and abundantly available in rural areas, making them a potential alternative to grass for cattle feed (Handayani *et al.*, 2023). Challenges in utilizing banana stems include their high water and crude fiber content, and low protein content. Banana stems contain 4.46-6.5% protein, 1.5-4.12% fat, 18.48% crude fiber, and 66.42% nitrogen free extract (Handayani *et al.*, 2023; Tuan *et al.* (2004), ash content of 14%, consisting of K 33.4%, Ca 7.5%, Mg 4.3%, Si 2.7%, and P 2.2%, and lignin 12% (Codiero *et al.*, 2004). The low nutritional content of banana stems is a constraint, so it cannot be used as a single feed ingredient (Yang *et al.*, 2012). To improve the nutritional quality and digestibility of banana stems, fermentation can be carried out. Fermented feed can extend shelf life, improve nutritional quality, preserve agricultural waste biologically (Sokchea *et al.*, 2018; Elijayanti *et al.*, 2021). Fermenting banana stems can increase protein content from 3.04% to 9.12% (Haryantika *et al.*, 2020).

The use of fermented banana stems can increase palatability in ruminants, improve digestion, and reduce waste odor (Do-Espírito *et al.*, 2012; Alvarez *et al.*, 2015; Mitiku *et al.*, 2023). During 3 months of research, it was found that cattle preferred banana stem silage (*Musa paradisiaca forma typica*) compared to fresh banana stems (Anwar *et al.*, 2020). Banana stem silage can also improve pig growth (Ty et al., 2014; Manivanh and Preston, 2016; Tien *et al.*, 2013). *In vitro* digestion can be used to determine the digestibility of banana stems.

In vitro digestion is a method of measuring digestibility outside the body by creating conditions similar to those inside the body for gas production and feed digestibility (Pashaei *et al.*, 2010; Foster *et al.*, 2023). This condition can predict feed digestibility, FVA, and gas production (Wang *et al.*, 2020). The profile of key VFAs, such as acetate, propionate, and butyrate, is influenced by nutrient content. High dietary fiber content will increase acetate production and decrease propionate production (Lu *et al.*, 2020). Volatile fatty acids (VFAs) are the main energy source for livestock, and ammonia is a source of protein for rumen microbial synthesis. VFA and NH_3–N levels in the rumen can affect feed digestibility and livestock productivity (Elahi *et al.*, 2018).

Based on these considerations, we wanted to determine the effect of replacing ruminant with fermented Cavendish banana (*Musa acuminata Cavendish*) stems on gas production, VFA, and *in vitro* digestibility.

MATERIALS AND METHODS

Diet

The forage consisted of native grass and fermented banana stems. Native grass was collected from the research pen and dried in a 60°C oven for 24 hours. Once dry, the grass was ground using a blender. The ground grass was mixed with a concentrate containing 70% native grass, 12% rice bran, 12% palm kernel meal, and 6% soybean meal. This feed mixture was blended again and sieved using a 16-mesh sieve. It was then used as a substitute for fermented banana stems according to the treatment percentages (Table 1). The results of the proximate analysis of the study showed that the dry matter content of native grass was 23.12%, crude protein 9.11%, fat 3.39%, crude fiber 43.00%, while the dry matter content of the Cavendish banana stem was 11.02%, crude protein 6.14%, fat 3.29% and crude fiber 29.38%.

Table 1: Feed composition and proximate analysis results of the treatment of replacing native grasses with fermented banana stems.

Ration materials	Treatment			
	P0	P1	P2	P3
Native grasses (%)	50	40	30	20
Cavendish Banana Stems (%)	50	60	70	80
Amount	100	100	100	100
Feed nutritional content				
Water (%)	76.35	76.79	77.03	77.53
Dry matter (%)	23.65	23.34	22.97	22.47
Crude protein (%)	13.71	13.53	13.47	13.22
Crude fat (%)	2.64	3.19	3.43	3.62
Energy (kcal/kg)	3799	4015	4040	4064
Crude fiber (%)	22.82	22.24	23.42	23.67
Nitrogen free extract (%)	41.63	42.34	41.75	41.52
Ash (%)	8.92	9.07	9.23	9.35
Ca (%)	0.52	0.50	0.48	0.43
P (%)	0.31	0.32	0.32	0.32

Description: Laboratory analysis results from the Poultry and Various Livestock Assembly and Testing Center, Bogor.

The banana stems were taken from a Cavendish banana plantation in Jambi Province. Fermented banana stems (BPF) were made by chopping them into 5-10 cm pieces. Banana stems are pressed to reduce the air content with humidity reaching ± 60%. The pressed stems were mixed with 12% rice bran, 12% palm kernel meal, 6% soybean meal. Then given 2% Produfer-Plus activator (lactic acid bacteria 20-35%, cellulolytic bacteria 30-40% and mananolytic bacteria 30-40%). The percentages of feed ingredients used were calculated based on the dry matter content. All ingredients were mixed until homogeneous.

The homogenized material was compacted into a drum, then tightly closed, and anaerobic fermentation took place. After 21 days of fermentation, the BPF was used as a replacement for native grass. The research ration composition and proximate analysis results are shown in Table 1.

Experimental design

The study used a 4 x 4 completely randomized design, with 4 treatments and ach with 4 replications. Treatment P0 = 50% native grasses + 50% fermented banana stems (BPF), P1 = 40% native grasses + 60% BPF, P2 = 30% native grasses + 70% BPF, and P3= 20% native grasses + 80% BPF.

In vitro procedure

The *in vitro* study followed the procedures of Tilley and Terry (1963). The study used bovine rumen fluid collected from a slaughterhouse. Rumen fluid was taken from a 3.7-year-old male Bali cattle from a smallholder farm. One gram of ground feed was weighed and placed into a 120 ml fermenter tube. Then, 20 ml of rumen fluid and 30 ml of McDougall's solution were added. Fermenter tubes were randomly placed into an incubator at 39°C. Anaerobic conditions were created by circulating CO_2 gas. The incubation process was carried out for 72 hours in a shaker. Cumulative gas production measurements were carried out at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 hours. The total gas production for each sample bottle was measured using a meter velocity and recorded at each incubation time period. The cumulative gas volume was repeated three times on the same research day using 3 different tubes.

At the end of the incubation process, the pH was measured using a pH meter (Mettler-Toledo FE20/FG2 pH meter). After the pH measurement, two drops of saturated HgCl2 solution were added to the incubation sample. The supernatant and residue were then separated by centrifugation (Sigma, Type 22-5, Germany) for 30 minutes at 1200 rpm until separation occurred. The residue settled at the bottom, while the supernatant settled at the top. The residue sample was dried in an oven at 105°C for 24 hours and used for dry matter (DM) and organic matter (OM) analysis according to AOAC (2016).

The resulting supernatant was used for VFA analysis. VFA was determined using a gas chromatograph (Shimadzu GC-14A). NH3 analysis was performed using the Conway microdiffusion technique. *In vitro* dry matter digestibility was calculated as the difference between the initial sample dry matter and the residual dry matter. For *in vitro* organic matter digestibility, the dried residue was oven-dried at 550°C for 6 hours to determine ash content. The organic matter content of the residue was calculated by subtracting the ash weight from the dry matter of the residue. Organic matter digestibility was determined as the difference between the initial organic matter and the organic matter residue.

Statistical analysis

The research data were analyzed using Analysis of Variance. If differences between treatments were found, Duncan's range test was used. The research data were analyzed using SAS 9.0 (SAS, 2002).

RESULTS AND DISCUSSION

The average gas production during 72 hours of incubation, dry matter digestibility, and organic matter digestibility in response to replacing native grasses with fermented banana stems are shown in Table 2.

Replacing native grass with BPF does not affect gas production (P>0.05) *in vitro*. The average gas in the study was 109.88 mM with a range of 108.13-113.25 mM. Higher gas production indicates that more substrate is available for energy generation for the livestock consuming it (Makar, 2002). Total gas production during 72 hours of *in vitro* incubation according to treatment is shown in Figure 1.

Table 2: Average gas, ammonia, and methane production, dry matter digestibility (DMD), and organic matter digestibility (OMD) in response to replacing native grasses with fermented banana stems.

Parameter	Treatment				P value	Sig
	P0	P1	P2	P3		
Gas production (mM)	108.13 ±4.97	109.00 ±3.04	113.25 ±4.66	110.00 ±2.38	0.26	NS
Ammonia (mM)	10.15±1,26^{A}	11.44±2.17^{B}	9.08±1.19^{A}	5.94±0.24^{C}	0.00	**
Methane (mM)	21.52 ±1.84^{a}	27.79 ±0.93^{b}	21.85 ±2.10^{a}	22.82 ±2.27^{a}	0.02	*
pH	6.64 ±0.01	6.64 ±0.01	6.43 ±0.06	6.59 ±0.02	0.11	NS
DMD (%)	69.19±6.49^{a}	72.91±8.92^{a}	68.83±8.34^{a}	52.25±7.91^{b}	0.04	*
OMD (%)	52.41±9.67a	48.08±12.34a	48.08±9.99a	37.25±8.66b	0.04	*

Note: NS= no significant, a single asterisk (*) indicates a significant difference at P<0.05, whereas a double asterisk (**) indicates a significant difference at P<0.01. Lowercase superscripts in the column indicate a significant difference P<0.05 and uppercase superscripts in the column indicate a very significant difference P<0.01.

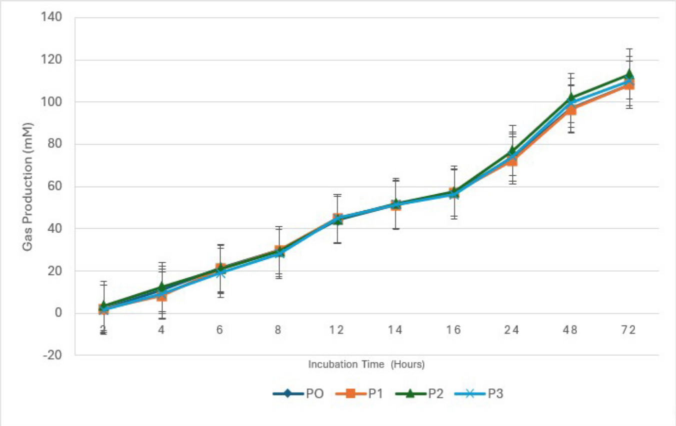

Figure 1: Gas production in response to replacing field grass with BPF for 72 hours of *in vitro* incubation.

Figure 1 shows that gas production increased in all treatments up to 72 hours of incubation, including treatments P0, P1, P2, and P3. The average gas production during incubation for P0 was 108.13 mM, P1 was 109.00 mM, P2 was 113.25 mM, and P3 was 110 mM. This indicates that the microbial digestion process was still running well after 72 hours of incubation, resulting in an increase in gas production. A decrease in gas production indicates a decrease in the digested substrate. According to Ansah *et al.* (2023), the decrease in gas production during *in vitro* incubation is due to a decrease in fermentables substrate.

Replacing native grass with BPF significantly affects ammonia production ($P<0.01$), Ammonia production in treatment P0 differed from P1, and P3, but not significantly from P2. Although the feed protein content was nearly identical, the ammonia production differed. This is thought to be due to the banana stems' low protein content and high fiber content, lignocellulose (Marques *et al.*, 2024). Therefore, using large amounts can reduce rumen fermentation activity, resulting in lower ammonia levels. Research by Li *et al.* (2010) using a Scanning Electron Microscope (SEM) revealed that banana stems have a unique morphological structure, where the fibers are covered by a non-cellulose membrane. In addition to the structural differences, the chemical composition includes up to 72% hemicellulose and 8.8% lignin.

Replacing native grass with BPF significantly affects methane production ($P<0.05$). Methane production in treatment P1 was different from P0, P2, and P3, but there was no difference between P0, P2, and P3. This condition is thought to be due to the increased use of fermented banana stems, which results in a higher fiber content in feed, thus reducing methane production. Furthermore, banana stems contain a high level of tannin (Ulvi *et al.*, 2023). Feeding with tannin can reduce methane production in the rumen (Cardoso-Gutierrez *et al.*, 2021). Methane reflects the loss of some of the livestock's energy, making it unusable for production. Approximately 6%-10% of the gross energy in feed consumed by ruminants is lost as methane (Pinares-Patiño *et al.*, 2023). Based on this, developing feeding strategies that can reduce methane emissions in ruminants will be beneficial both in the long term by reducing the rate of greenhouse gas accumulation and in the short term by reducing energy losses in livestock. A graph of methane production during 72 hours of *in vitro* incubation in response to replacing grass with BPF is shown in Figure 2.

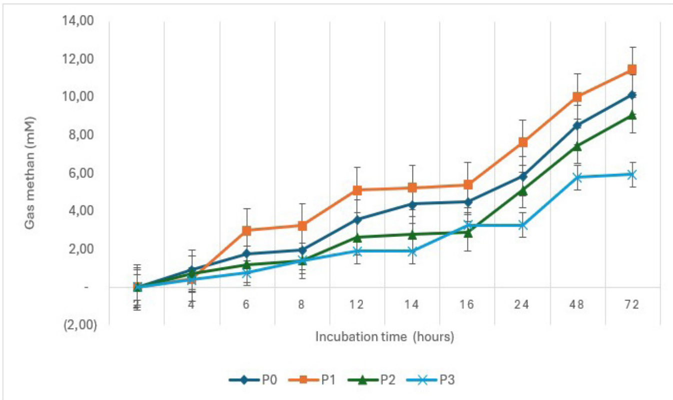

Figure 2: Methane gas production is in response to replacing field grass with BPF during 72 hours of incubation.

Table 3: Average total and partial VFA values for the *in vitro* treatment of replacing native grass with fermented banana stems.

Parameter	Treatment				P Value	Sig
	P0	P1	P2	P3		
VFA (mM)	244.68 ±38.63[a]	219.90 ±29.92[b]	213.08 ±19.69[b]	168.07 ±28.48[c]	0.03	*
Acetic acid (mM)	83.95 ±14.46[a]	80.71 ±15.51[b]	74.37 ±14.81[b]	61.17 ±14.58[c]	0.04	*
Propionic acid (mM)	70.03 ±10.73[a]	63.16 ±10.74[b]	53.13 ±8.49[c]	37.25 ±10.54[d]	0.02	*
Acetate propionate ratio	1.20±0.21	1.39±0.51	1.38±0.27	1.81±0.73	0.36	TN
Is butyric acid (Mm)	17.75 ±6.34[ab]	19.63 ±5.72[b]	25.00 ±9.78[a]	18.26 ±4.17[ab]	0.04	*
Butyric Acid (mM)	46.97 ±11.27[a]	41.53 ±10.86[b]	38.08 ±5.18[b]	33.22 ±10.90[c]	0.04	*
Iso valeric acid (mM)	14.40 ±3,37[a]	15.94 ±3,43[a]	12.29 ±1.84[ab]	9.26 ±2.74[b]	0.03	*
Valeric acid (mM)	11.77 ±3.05	8.83 ±3.46	9.11 ±0.74	8.91 ±3.68	0,46	TN

Note: NS= no significant, a single asterisk (*) indicates a significant difference at $P<0.05$, whereas a double asterisk (**) indicates a significant difference at $P<0.01$. Lowercase superscripts in the column indicate a significant difference ($P<0.05$) and uppercase superscripts in the column indicate a very significant difference ($P<0.01$).

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In general, methane production increased in all treatments up to the 72-hour incubation period *in vitro*. The increase began between the 2nd and 72nd hours of incubation. Methane gas production increased most significantly in treatment P1, followed by P0, P2, and P3 during the 72-hour incubation period.

The average pH in the study was 6.63, with a range of 6.43-6.64. This pH is considered normal for microbial activity. The optimum pH for rumen microbial activity ranges from 6 to 6.9 (Kamra *et al*., 2005). Increased rumen pH is usually associated with increased ammonia concentration. The results of a study by Comacho *et al*. (2019) showed that increased ammonia concentration resulted in an increase in rumen pH.

Replacing field grass with BPF reduced dry matter digestibility (DMD) and organic matter digestibility (P<0.05) *in vitro*. The digestibility of DMD in treatments P0, P1 and P2 was different from P3, but there was no difference between P0, P1 and P3. The lower dry matter digestibility with increased banana stem use is thought to be due to the structure of banana stems, which contain more cellulose than protein and other nutrients. Digestibility is related to the chemical composition of the feed, namely protein. DMD increases linearly with increasing protein levels in the feed (Sultan *et al*., 2010). These results are higher than those of Amarnath and Balakrishnan (2007), who found that a ration containing 60% unfermented banana stems produced a dry matter digestibility of 35.33%, while a ration containing 30% banana stems produced a dry matter digestibility of 48.66%. Research by Triasari and Setiyawan (2021) found that the digestibility of dry matter from fermented banana stems in the form of complete feed was 71.56%.

The organic matter digestibility of treatment P0 differed from P3 but did not differ from P1 and P2. These digestibility results are lower than those of Santoso and Yakin (2026), who found organic matter digestibility of fermented banana stems at around 70.29-77-33%. Meanwhile, Kaswari (2016) found that the digestibility of organic material from banana stems without fermentation was 39.98%. Kaswari (2004) stated that rumen microorganisms play a role in the feed digestion process in ruminants. The growth of rumen microorganisms is primarily influenced by the availability of protein and energy in the feed. A lack of protein or energy in the feed results in suboptimal microbial growth and reduced feed digestibility.

The average total and partial VFA values in response to replacing field grass with fermented banana stems *in vitro* are shown in Table 2.

Replacing native grass with BPF reduced VFA production *in vitro* (P<0.05). Treatment P0 differed from P1, P2, and P3. P3 differed from P1 and P2, and but P1 and P2 did not. The greater the BPF replacement, the lower the VFA concentration produced. This condition is thought to be because VFA is the result of carbohydrate breakdown by rumen microbes. Carbohydrates entering the rumen undergo a degradation process into simple saccharides, then converted to pyruvate through the Embden-Meyerhof glycolytic pathway. Pyruvate is then converted by intracellular microorganisms into volatile fatty acids (VFA). VFA are the result of carbohydrate digestion in the rumen (Alvarez *et al*., 2015; Yanuartono *et al*., 2020). Changes in VFA concentration in the rumen are closely related to the physical form of the feed, feed nutrition, and the level and frequency of feeding (Wang *et al*., 2020). Feed entering the rumen is fermented to produce primary products in the form of VFAs, microbial cells, methane gas, and CO_2.

Replacing grass with fermented banana stems reduced acetic acid, propionate, butyrate, and isovalerate *in vitro* (P<0.05). However, it did not affect valeric acid or the propionate acetate ratio (P>0.05). This is likely due to increased use of fermented banana stems, which reduces nutritional quality. Acetic acid is a volatile fatty acid absorbed through the rumen wall. Acetic acid production indicates the ease with which feed nutrients, especially carbohydrates and protein, are digested by rumen microbes. The more forage is replaced with fermented banana stems, the lower the propionate production. These results are aligned with other studies that found that increasing the supply of banana stems can reduce propionate (Amarnath and Balakrishnan, 2007). However, the propionate acetate ratio was below 2, indicating that rumen digestion was functioning well. The ratio of acetate and propionate describes the efficiency of energy utilization and the quality of the resulting product (Hambakodu *et al*., 2019).

CONCLUSION

The study concluded that replacing native grasses with BPF can be done up to 70% in the ration for *in vitro* digestibility and gas production.

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NOVELTY STATEMENT

This study presents the replacement of native grasses with fermented Cavendish banana stalks as livestock feed and provides evidence that Cavendish banana stalks can replace field grass by up to 70%.

AUTHORS CONTRIBUTION

Adriani: Conceptualization, supervision, methodology, writing review and editing original draft. Yurleni: Methodology, writing review and editing original draft. Ika Armita sari: Formal analysis, visualization, writing review and editing original draft. Yusuf Amrulah Luber: Conceptualization, supervision, writing review & editing original draft.

Generative AI and AI-assisted technology statement

The authors declare that generative AI was used solely for language editing and grammar improvement. The authors take full responsibility for the content of the manuscript

Conflict of interest

The authors have declared no conflict of interest.

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