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## Effects of papaya peel addition on fermentation quality, nutritional value and *in vitro* ruminal digestion of napier grass silage

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**Abstract:** The objective of this study was to investigate the effect of papaya peel addition on the quality and *in vitro* ruminal digestion of napier grass silage. Fresh napier grass was mixed with papaya peel at 0% (control), 15%, 30%, and 45% of fresh weight and ensiled under anaerobic conditions. After fermentation, silages were analysed for pH, organic acids, ammonia-N, and nutrient composition. *In vitro* ruminal fermentation was conducted using the rumen fluid collected from buffaloes to assess gas production, volatile fatty acid profiles, and hydrogen release. The results showed that papaya peel addition significantly affected silage fermentation and nutrient composition. The inclusion at 15% lowered pH and increased lactic acid concentration compared with the control ( $P < 0.05$ ). Higher inclusion levels (30% and 45%) reduced neutral detergent fibre and acid detergent fibre content, increased total digestible nutrients and relative feed value ( $P < 0.05$ ). *In vitro* ruminal fermentation showed that, compared with the control, hydrogen production was significantly reduced in the 15% treatment, and the acetate-to-propionate ratio was highest in the 45% treatment as a result of decreased propionate acid concentration ( $P < 0.05$ ). Our results suggested that co-ensiling napier grass with papaya peel improved fermentation quality and feed value, with 15% addition yielding the most desirable balance of fermentation characteristics and ruminal fermentation outcomes. This approach provides a practical strategy for enhancing the forage preservation and utilising fruit-processing by-products in tropical livestock systems.

**Keywords:** *Carica papaya* L. peel; co-ensiling; fruit by-products; *Pennisetum purpureum* Schumach; silage quality

Napier grass (*Pennisetum purpureum* Schumach) is an essential roughage source for ruminants due to its adaptability and high biomass yield (Islam et al. 2024). However, its seasonal growth necessitates effective conservation strategies to ensure

a consistent year-round feed supply and optimise local resources. Ensiling is a preservation method for high-quality forage, driven by a diverse microbial community under anaerobic conditions. Lactic acid bacteria (LAB) rapidly dominate during silage

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production, converting water-soluble carbohydrates (WSC) into organic acids, reducing the pH value, inhibiting the growth of harmful bacteria, and reducing the risk of secondary fermentation, thus ensuring long-term storage, nutrient retention, and enhanced feed palatability (Yang et al. 2016). Nevertheless, when ensiled alone, napier grass often exhibits poor fermentation quality due to its low dry matter (DM) content, limited WSC concentration, and high cell wall content (Sanchez-Guerra et al. 2024). Additionally, the insufficient presence of epiphytic LAB is also a contributor to its suboptimal fermentation quality (Jaipolsaen et al. 2022). To address these challenges, incorporating fruit by-products into napier grass silage has been explored as a potential solution in tropical and subtropical regions. For instance, Neiva et al. (2006) observed increases in both DM and crude protein (CP) with the inclusion of passion fruit by-products. Additionally, Figueiredo et al. (2022) found that passion fruit by-products can improve the nutritional value and digestibility of napier grass silage by increasing CP content and improving *in vitro* DM digestibility.

Papaya (*Carica papaya* L.), native to tropical South America, is a fruit abundant in southern China (Yang et al. 2016). Papaya peel, a by-product of papaya fruit processing, has gained attention as a potential additive in silage production due to its beneficial fermentation and preservation properties. Papaya peel is rich in WSC, which is critical for silage fermentation as it provides the necessary substrate for the LAB (Yang et al. 2016). Furthermore, papaya peel contains epiphytic LAB, including strains like *Lactobacillus plantarum* and *Lactobacillus casei* (Yang et al. 2016). These bacteria help rapidly lower the silage pH, suppressing undesirable microbial activities (Pathak et al. 2019). In addition to facilitating fermentation, papaya peel contributes valuable nutrients to the silage, including CP, vitamins, minerals, and antioxidants (Jiao et al. 2023). These nutrients not only improve the nutritional profile of the silage but also they provide additional health benefits to the livestock. Moreover, the use of papaya peel in silage production serves as an effective waste management strategy, offering a sustainable approach to utilising a by-product. Currently, the application of papaya peel in napier grass silage has not yet been reported.

Therefore, this study aims to investigate the effects of adding papaya peel to napier grass silage,

particularly its impact on fermentation characteristics, nutrient retention, and overall feed quality. It is hypothesised that the papaya peel inclusion will enhance the silage fermentation quality and nutritional value, making it a viable and sustainable feed option for ruminants.

## MATERIAL AND METHODS

### Ensiling materials and silage preparation.

Napier grass was harvested from the Pasture Research Base of Buffalo Research Institute, Guangxi Zhuang Nationality Autonomous Region, China. Papaya peel was provided by Huichuang Animal Husbandry Company Limited (Chongzuo, P.R. China). The forages were cut into about 2-cm pieces, and the chemical analysis is shown in Table 1. The experiment consisted of four groups with six replicates: (i) the control group without papaya peel addition, (ii) 85% napier grass + 15% papaya peel, (iii) 70% napier grass + 30% papaya peel, (iv) 55% napier grass + 45% papaya peel. After mixing the napier grass and papaya peel evenly, about 1 kg of this material was put into a fermentation bag. Six replicates were set in treatment. The bag was sealed and stored at room temperature (25 ± 2 °C) away from light, and fermented for 45 days.

**Silage quality sensory investigation.** After the silage bag was opened, the quality of the silage was

Table 1. Chemical composition of papaya peel and napier grass before ensiling

Item	Papaya peel	Napier grass
DM (%)	5.77	23.6
CP (% DM)	32.8	8.33
OM (% DM)	89.5	92.9
NDF (% DM)	34.2	76.5
ADF (% DM)	16.4	48.5
WSC (% DM)	12.1	3.64
TDN (% DM)	76.1	51.1
RFV	207	62.2

TDN and RFV were calculated using the following equations (NRC 2001): TDN (%) = 88.9 – 0.779ADF (1) and RFV = (TDN × 120/NDF)/1.29 (2)

ADF = acid detergent fibre; CP = crude protein; DM = dry matter; NDF = neutral detergent fibre; OM = organic matter; RFV = relative feed value; TDN = total digestible nutrients; WSC = water-soluble carbohydrates

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assessed by evaluating its odour, colour, and texture. The evaluation of silage quality was conducted according to Abdelrahman et al. (2022). The odour was evaluated and scored as sour scent, acceptable (25); light sour (9–17); pungent wine/sour (1–8); or mouldy (0). The colour of the silage was scored as turquoise (14–20), yellow-green (8–13), brownish-yellow (1–7), or dark brown (0). The texture was assessed as loose and soft/soft and non-sticky (8–10); middle (4–7); slightly viscous (1–3); or sticky (0).

**Chemical analysis.** After drying the samples of napier grass, papaya peel, and their mixed silages at 65 °C to constant weight in a forced air oven (LABO–250; STIK Co. Ltd., Shanghai, P.R. China) and grinding through a 1-mm sieve using a sample mill (FS200; Guangzhou Bomin Mechanical & Electrical Equipment Co., Ltd., Guangzhou, P.R. China), compositional analyses were performed. DM, CP, and ash were quantified according to AOAC (1990) standards, while organic matter (OM) content was derived from weight loss upon ashing. Additionally, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured following Van Soest et al. (1991). WSC content was determined via the anthrone colorimetric method as described by Uden (2006). The total digestible nutrients (TDN) and relative feed value (RFV) were calculated according to NRC (2001).

$$\text{TDN (\%)} = 88.9 - 0.779\text{ADF} \quad (1)$$

$$\text{RFV} = (\text{TDN} \times 120/\text{NDF})/1.29 \quad (2)$$

**Analysis of fermentation characteristics.** Cold-water extraction served to determine silage mixture fermentation products. Fresh silage samples (20 g) were mixed with 180 ml sterilised distilled water, homogenised, and held overnight at 4 °C. The resulting filtrate pH was assessed with a glass electrode pH meter (HI 8424; HANNA® instruments, Woonsocket, RI, USA). For organic acid quantification (lactic, acetic, propionic, butyric acids), high-performance liquid chromatography was employed [(1260 Infinity II System; Agilent Technologies, Waldbronn, Germany); (Shodex RSpak KC-811 column, 8.0 × 300 mm; Showa Denko K.K., Tokyo, Japan)]. Chromatographic conditions comprised: detection wavelength 210 nm, eluent 3 mmol/l HClO<sub>4</sub> at 1 ml/min, column temperature 50 °C, and injection volume 5 µl. Ammonia-N (NH<sub>3</sub>-N)

was measured according to Broderick and Kang (Broderick and Kang 1980).

LAB, yeasts, and moulds in silage samples were quantified by plate counting. LAB were cultured on De Man-Rogosa-Sharp agar (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, P.R. China), and yeasts/moulds on potato dextrose agar (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, P.R. China), followed by 48 h incubation at 30 °C. Yeast identification was based on colony morphology and cell structure comparison with moulds or bacteria. Colony counts represented viable microorganism numbers.

**In vitro fermentation.** Three healthy buffaloes (body weight 610 ± 10 kg) equipped with permanent rumen cannulas were used as rumen fluid donors. The animals were provided with a basal diet consisting of 80% napier grass and 20% concentrate, fed twice daily at 8:00 and 15:00, with free access to water. The care and management of the buffaloes adhered to the guidelines of the Ethics Committee of the Buffalo Research Institute, Guangxi Zhuang Nationality Autonomous Region, P.R. China (Approval No. BRI-20241212). After pre-morning feeding, equal volumes of rumen fluid from each buffalo were pooled. The resulting mixture was homogenised, filtered, and diluted 1:4 with CO<sub>2</sub>-saturated artificial saliva (McDougall 1948), prepared as per Guo et al. (2022). For *in vitro* fermentation, 50 ml of the buffered fluid was dispensed into 180 ml bottles holding 1 g ground silage. Following CO<sub>2</sub> flushing to achieve anaerobiosis and sealing, incubation proceeded at 39 °C for 72 h in a shaking water bath (100 strokes/min). This process was performed in duplicate over two consecutive weeks under identical conditions.

Gas production from the serum bottles was assessed with air syringes, while H<sub>2</sub> and CH<sub>4</sub> levels were quantified by gas chromatography (GC) (Model 8860; Agilent Technologies Co., Ltd., Shanghai, P.R. China). At the end of incubation, fermentation bottles were immediately cooled in an ice-water bath for 15 min to halt fermentation. Upon opening, pH was measured according to the same protocol applied to silage filtrates.

For volatile fatty acid (VFA) profiling, separate supernatant subsamples were subjected to GC analysis (model 7890A; Agilent Technologies, Inc.), as outlined by Azizi et al. (2020). *In vitro* dry matter digestibility (IVDMD) was calculated using the following formula:

$$\text{IVDMD (\%)} = \left( 1 - \frac{\text{Weight of residue after digestion}}{\text{Weight of substrate before digestion}} \right) \times 100 \quad (3)$$

**Statistical analyses.** The data on chemical composition, fermentation parameters, and *in vitro* ruminal fermentation characteristics were subjected to statistical analysis using the SAS software (v9.2; SAS Institute Inc., Cary, NC, USA). One-way analysis of variance was performed to evaluate the differences between group means. Duncan's test was used to identify significant differences ( $P < 0.05$ ) between treatments.

## RESULTS

**Chemical composition of napier grass and papaya peel before ensiling.** The chemical composition of papaya peel and napier grass is shown in Table 1. The DM content of papaya peel and napier grass was 5.77% and 23.59%, respectively. Papaya peel contained more CP and WSC than napier grass, but less NDF and ADF. Additionally, papaya peel had higher RFV and TDN than napier grass.

**Sensory evaluation of silage quality.** The scores for odour, colour and texture of silages decreased slightly with increasing papaya peel addition (Table 2). Odour scores decreased from 16.3 in the

Table 2. Effect of papaya peel addition on the napier grass silage quality sensory evaluation

Item	Inclusion of papaya peel (% FM)			
	0%	15%	30%	45%
Odour	16.3	16.0	15.7	15.2
Colour	14.3	14.0	13.5	13.3
Texture	7.00	6.83	6.67	6.50
Grade	good	good	good	good

Silage sensory quality was evaluated by odour, colour, and texture (Abdelrahman et al. 2022) and categorised into four grades: excellent, good, general and poor: (i) Odour: Sour scent and acceptable (25), light sour (9–17), pungent wine/sour (1–8), and mouldy (0); (ii) Colour: Turquoise (14–20), yellow-green (8–13), brownish-yellow (1–7), and dark brown (0); (iii) Texture: loose and soft/soft and non-sticky (8–10), middle (4–7), slightly viscous (1–3), and sticky (0) FM = fresh matter

control group to 15.2 in the 45% papaya peel addition group, colour scores decreased from 14.3 to 13.3, and texture scores decreased from 7.00 to 6.50. Overall, all treatment groups were rated as “good”.

**Silage nutritional composition.** The highest DM content was found in the control group, followed by the 15%, 30%, 45% papaya peel addition groups ( $P < 0.05$ ) (Table 3). The CP content significantly increased in the following order: 45% papaya peel addition group < 30% papaya peel addition group < 15% papaya peel addition group < the con-

Table 3. Chemical composition of napier grass silage supplemented with papaya peel

Item	Inclusion of papaya peel (% FM)				SEM	P-value
	0%	15%	30%	45%		
DM (%)	22.3 <sup>a</sup>	19.9 <sup>b</sup>	17.5 <sup>c</sup>	16.1 <sup>c</sup>	0.404	<0.001
CP (% DM)	6.94 <sup>d</sup>	8.20 <sup>c</sup>	9.40 <sup>b</sup>	10.8 <sup>a</sup>	0.150	<0.001
OM (% DM)	92.3 <sup>a</sup>	92.0 <sup>ab</sup>	91.7 <sup>b</sup>	90.8 <sup>c</sup>	0.104	<0.001
NDF (% DM)	75.6 <sup>a</sup>	71.9 <sup>a</sup>	63.9 <sup>b</sup>	56.1 <sup>c</sup>	1.15	<0.001
ADF (% DM)	47.6 <sup>a</sup>	46.7 <sup>ab</sup>	45.0 <sup>b</sup>	42.1 <sup>c</sup>	0.468	<0.001
WSC (% DM)	2.43 <sup>c</sup>	3.89 <sup>a</sup>	3.39 <sup>b</sup>	2.81 <sup>c</sup>	0.120	<0.001
TDN (% DM)	51.8 <sup>c</sup>	52.5 <sup>bc</sup>	53.9 <sup>b</sup>	56.1 <sup>a</sup>	0.365	<0.001
RFV	62.7 <sup>c</sup>	66.8 <sup>c</sup>	77.6 <sup>b</sup>	91.6 <sup>a</sup>	2.46	<0.001

<sup>a–d</sup>Means with unlike lower-case superscript letters in the same row differ ( $P < 0.05$ ) between the treatments

TDN and RFV were calculated using the following equations (NRC 2001): TDN (%) =  $88.9 - 0.779\text{ADF}$  (1) and RFV =  $(\text{TDN} \times 120/\text{NDF})/1.29$  (2)

ADF = acid detergent fibre; CP = crude protein; DM = dry matter; FM = fresh matter; NDF = neutral detergent fibre; OM = organic matter; RFV = relative feed value; SEM = standard error of the mean; TDN = total digestible nutrients; WSC = water-soluble carbohydrates

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trol group ( $P < 0.05$ ). Both OM and ADF contents were significantly elevated in silage treated with 30% and 45% papaya peel addition compared to the control group ( $P < 0.05$ ). For NDF, the 30% and 45% papaya peel addition groups demonstrated higher content than both the control and the 15% papaya peel addition group ( $P < 0.05$ ). WSC content was significantly higher in silage supplemented with 15% and 30% papaya peel than in the control group

( $P < 0.05$ ). Compared to the control group, the 30% and 45% papaya peel addition groups showed significantly improved contents of TND and RFV ( $P < 0.05$ ).

**Silage fermentation quality.** The pH of the napier grass silage decreased significantly ( $P < 0.05$ ) with an increasing proportion of papaya peel, with the lowest value in the 15% papaya peel addition group (Table 4). Lactic acid content was highest

Table 4. Fermentation quality and microorganism composition of napier grass silage supplemented with papaya peel

Item	Inclusion of papaya peel (% FM)				SEM	P-value
	0%	15%	30%	45%		
pH	4.48 <sup>a</sup>	4.35 <sup>b</sup>	4.52 <sup>a</sup>	4.51 <sup>a</sup>	0.019	<0.001
Lactic acid (g/kg DM)	13.3 <sup>b</sup>	15.9 <sup>a</sup>	13.7 <sup>b</sup>	12.8 <sup>b</sup>	0.510	0.002
Acetic acid (g/kg DM)	9.14 <sup>c</sup>	12.5 <sup>a</sup>	10.7 <sup>b</sup>	8.83 <sup>c</sup>	0.341	<0.001
Propionic acid (g/kg DM)	nd	nd	nd	nd	–	–
Butyric acid (g/kg DM)	nd	nd	nd	nd	–	–
NH <sub>3</sub> -N (g/kg DM)	1.98 <sup>c</sup>	2.02 <sup>c</sup>	2.39 <sup>b</sup>	2.68 <sup>a</sup>	0.059	<0.001
LAB (log cfu/g FM)	5.68	5.79	5.66	5.49	0.101	0.250
Yeasts (log cfu/g FM)	4.64	4.56	4.69	4.68	0.098	0.782
Molds (log cfu/g FM)	nd	nd	nd	nd	–	–

<sup>a–c</sup>Means with unlike lower-case superscript letters in the same row differ ( $P < 0.05$ ) between the treatments

DM = dry matter; FM = fresh matter; LAB = lactic acid bacteria; nd = not detected; NH<sub>3</sub>-N = ammonia-N; SEM = standard error of the mean

Table 5. IVDMD, gas production and fermentation characteristics of napier grass silage supplemented with papaya peel

Item	Inclusion of papaya peel (% FM)				SEM	P-value
	0%	15%	30%	45%		
IVDMD (%)	41.9	44.1	43.4	42.5	0.723	0.170
Total gas production (ml/g DM)	87.7	90.6	90.5	88.9	1.92	0.680
H <sub>2</sub> production (ml/g DM)	0.14 <sup>a</sup>	0.08 <sup>b</sup>	0.13 <sup>ab</sup>	0.16 <sup>a</sup>	0.017	0.036
Methane production (ml/g DM)	16.6	18.4	17.4	17.9	0.779	0.410
pH	6.57 <sup>b</sup>	6.58 <sup>ab</sup>	6.58 <sup>ab</sup>	6.60 <sup>a</sup>	0.009	0.028
Total VFA (mmol/l)	18.0	17.6	17.4	16.4	0.570	0.265
Acetate acid (mmol/l)	9.64	9.50	9.44	9.02	0.284	0.467
Propionate acid (mmol/l)	5.70 <sup>a</sup>	5.45 <sup>a</sup>	5.26 <sup>ab</sup>	4.83 <sup>b</sup>	0.193	0.032
Isobutyrate acid (mmol/l)	0.13	0.12	0.12	0.10	0.011	0.355
Butyrate acid (mmol/l)	2.04	2.02	2.06	1.98	0.063	0.816
Isovalerate acid (mmol/l)	0.31	0.31	0.29	0.28	0.020	0.677
Valerate acid (mmol/l)	0.20	0.20	0.20	0.20	0.010	0.992
Acetate/propionate	1.69 <sup>d</sup>	1.75 <sup>c</sup>	1.80 <sup>b</sup>	1.87 <sup>a</sup>	0.015	<0.001
NH <sub>3</sub> -N (mg/l)	55.9	56.4	56.1	56.8	3.43	0.998

<sup>a–d</sup>Means with unlike lower-case superscript letters in the same row differ ( $P < 0.05$ ) between the treatments

DM = dry matter; FM = fresh matter; IVDMD = *in vitro* dry matter digestibility; NH<sub>3</sub>-N = ammonia-N; SEM = standard error of the mean; VFA = volatile fatty acid

in the 15% papaya peel addition group and then it decreased with a further increase in the proportion of papaya peel ( $P < 0.05$ ), whereas acetic acid content decreased significantly with the increase in the proportion of papaya peel addition ( $P < 0.05$ ). Neither propionic acid nor butyric acid was detected in any treatment group.  $\text{NH}_3\text{-N}$  content increased significantly with the increasing proportion of papaya peel, from 1.98 g/kg DM in the control group to 2.68 g/kg DM in the 45% papaya peel addition group ( $P < 0.05$ ). LAB and yeast counts did not differ significantly between the groups ( $P > 0.05$ ). No moulds were detected in the silage concerned.

***In vitro* ruminal digestion.** There were no significant effects on most *in vitro* rumen fermentation parameters for the treatment ( $P > 0.05$ ) (Table 5). However, compared to the control group, silage with papaya peel supplementation exhibited an increased tendency in IVDMD, total gas production, methane production, and  $\text{NH}_3\text{-N}$  content, while total VFA, acetate, and isobutyrate showed a decreasing trend. Significant differences were observed in specific parameters: compared to the control group, the 15% papaya peel addition resulted in lower  $\text{H}_2$  production, whereas the 45% addition led to higher  $\text{H}_2$  production and lower propionate levels ( $P < 0.05$ ). Additionally, the acetate-to-propionate ratio increased across silages in the following order: control group < 15% papaya peel addition group < 30% papaya peel addition group < 45% papaya peel addition group ( $P < 0.05$ ).

## DISCUSSION

The fermentation quality of silage largely depends on the intrinsic properties of the forage crops used. Optimal preservation requires raw materials containing WSC exceeding 5% of DM, with concurrent moisture content below 75% (Wu et al. 2020). In this study, the moisture level of napier grass was at a marginal level coupled with suboptimal WSC concentration (3.64% of DM), which indicates that the silage may demonstrate compromised quality or potentially fail to preserve adequately. Contrastingly, papaya peel contributed to a remarkably high WSC content (12.05% of DM) suggesting its potential as an effective WSC supplement in napier grass-based mixed silage systems. This supplementation strategy was further supported by favourable sensory charac-

teristics and enhanced nutritional composition in papaya peel-supplemented silages. Chemical composition is a key to assessing feed nutritional and silage quality, especially CP. In this study, the inclusion of papaya peel significantly enriched the nutritional profile of napier grass silage, whose CP content increased from 6.94% in the control group to 10.8% in the 45% papaya peel addition group, demonstrating the high protein contribution of papaya peel (32.8% DM). This improvement aligns with Neiva et al. (2006) findings of increased CP when fruit by-products are added to silage. Notably, reductions in both NDF and ADF contents were observed in all papaya peel-supplemented silages, with particularly significant effects in the 30% and 45% treatment groups ( $P < 0.05$ ). This is likely because of lower NDF and ADF in papaya peel relative to napier grass. These findings suggest that co-ensiling napier grass with papaya peel can improve the feed nutritional profile, achieving the better nutritional balance than using the roughage components separately. In addition, the increases in TDN and RFV further highlight the positive effects of papaya peel on silage energy density. The significant rise in RFV from 62.7 in the control to 91.6 in 45% papaya peel suggests that papaya peel not only improved the nutritional composition but also it enhanced the overall feeding value of the silage, making it a more energy-dense feed for ruminants (Cunha et al. 2022).

High-quality silage is typically characterised by green to yellowish-green coloration, acidic and aromatic odour, soft yet slightly moist texture, and absence of mould growth (Muck 2013). In this study, the colour, odour and texture of silage were in the normal range, which indicated that the silage effect was better in all groups. During silage fermentation, LAB convert WSC into organic acids, reducing the pH value, and inhibiting the growth of harmful bacteria, thus ensuring the long-term storage of fresh forage. Among them, lactic acid is the most powerful organic acid capable of rapidly decreasing pH (Azizi et al. 2020). In this study, the 15% papaya peel supplementation group had significantly lower pH and higher lactic acid content than the control group. This is likely because papaya peel provides suitable fermentation conditions for silage, with the 15% supplementation level appearing to be most optimal. However, the fermentation quality declined slightly at higher papaya peel inclusion levels, as reflected by higher pH values

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and reduced lactic acid content. This phenomenon may be associated with the high moisture content of papaya peel. The excessively high moisture content can lead to unfavourable microbial growth or fermentation outcomes (Yin et al. 2021). The presence of moderate acetic acid concentrations in silage confers the advantage of yeast suppression during fermentation, leading to improved stability under aerobic conditions (Kung et al. 2018). In this study, the acetic acid in the 15% and 30% papaya peel addition groups was significantly higher than that in the control group ( $P < 0.05$ ). Although the yeast count in the 15% group was lower than that in the control group, the difference was not significant ( $P > 0.05$ ). These findings suggest that the higher acetic acid concentration in the 15% papaya peel group may contribute to inhibiting the growth of aerobic spoiling microorganisms, such as yeasts, thereby potentially enhancing the preservation quality upon aerobic exposure. The silage surface harbours diverse microorganisms, including beneficial ones like LAB and detrimental types such as yeasts, moulds and spoilage bacteria. To achieve optimal silage quality, the LAB population within the ensiled material needs to reach a level of 5 to 6 log colony-forming units per gram (cfu/g) of fresh weight (Li et al. 2010; Sifeeldein et al. 2019). In this study, all silage samples had lactic acid bacteria counts within this range.  $\text{NH}_3\text{-N}$  in silage, produced via protein hydrolysis and amino acid deamination, indicates proteolytic degradation and nitrogen loss, often correlating with reduced nutritional quality (Scherer et al. 2015). In this study, elevated  $\text{NH}_3\text{-N}$  levels were observed with increasing papaya peel supplementation, suggesting intensified proteolysis during ensiling. This phenomenon likely resulted from the higher pH in the 30% and 45% papaya peel addition groups, which insufficiently suppressed the activity of plant proteases and deleterious microorganisms responsible for protein breakdown. These findings imply that while a moderate papaya peel inclusion may confer beneficial effects, an excessive incorporation could destabilise the fermentation process and compromise silage quality.

The DM digestibility reflects the extent to which the substrate is degraded by microorganisms in the fermentation system and it is an important indicator of roughage utilisation rate in rumen fermentation. We observed that mixing napier grass with papaya peel tended to increase DM digestibility

although no significant differences were observed between the silages. The gas production is an indicator for predicting the degree of feed fermentation, which can reflect the digestion and degradation characteristics of ruminants to feed (Menke et al. 1979). The higher the content of fermentable nutrients in the diet, the stronger the fermentability and the higher the gas production (Menke et al. 1979). In this study, the increasing total gas production corresponded to increases in DM disappearance. During the microbial fermentation of dietary fermentable carbohydrates in the rumen, the production of VFA is often accompanied by  $\text{H}_2$  generation (Janssen 2010). To maintain the stable ruminal  $\text{H}_2$  partial pressure, methanogens convert  $\text{H}_2$  into  $\text{CH}_4$ , which is subsequently expelled from the rumen (Beauchemin et al. 2020). In this study, the silage with 15% papaya peel addition exhibited the lowest  $\text{H}_2$  concentration and the highest  $\text{CH}_4$  production. This suggests that the hydrogen generated during VFA synthesis was likely utilised by methanogens and subsequently emitted as methane (Janssen 2010). The pH of the ruminal environment influences the normal physiological activities of rumen microorganisms, with its normal range being 5.0~7.5 (Russell 1998). In this *in vitro* experiment, the pH remained within the normal range, indicating that papaya peel had no adverse effects on the growth and reproduction of rumen microorganisms. VFA are the primary metabolites generated during rumen microbial fermentation, supplying 70~80% of the energy required by ruminants (Reynolds et al. 2003). Acetate, propionate, and butyrate account for approximately 95% of the total VFA. Acetate is the main component for milk fat synthesis in ruminants, while propionate acts as a precursor for glucose production (Reynolds et al. 2003). In this study, as the proportion of papaya peel in the mixed silage increased, the propionate content gradually decreased, and the acetate-to-propionate ratio significantly increased. This may be attributed to variations in the nutritional composition, soluble carbohydrates, and fibre structure of the mixed silage.

## CONCLUSION

The addition of papaya peel to napier grass silage effectively enhances fermentation quality, nutritional composition, and ruminal fermentation

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characteristics. Based on the findings, an inclusion level of 15% papaya peel in napier grass silage is recommended as an optimal ratio. However, further optimisation and validation through animal trials are warranted to confirm its practical benefits.

### Conflict of interest

The authors declare no conflict of interest.

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