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ORIGINAL PAPER

Fermentation profile and *in vitro* ruminal digestibility of rice straw silage with papaya peel

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ABSTRACT. This study investigated the effects of papaya peel addition on the fermentation quality, chemical composition, and *in vitro* ruminal digestion of rice straw silage. Silage was prepared using a small-scale fermentation system, with four treatments: a control and rice straw supplemented with 15, 30, and 45% papaya peel. The results showed that the addition of papaya peel significantly reduced dry matter, neutral detergent fibre, and acid detergent fibre contents ($P < 0.05$), while increasing crude protein, organic matter, water-soluble carbohydrates, total digestible nutrients, and relative feed value ($P < 0.05$). In all experimental groups, silage containing 30% papaya peel had the lowest pH value, ammonia-N concentration, yeast content, and acetate/propionate ratio, while showing the highest concentrations of lactate, acetate, propionate, and other fatty acids ($P < 0.05$). These findings indicate that papaya peel supplementation significantly improves chemical composition and fermentation quality of rice straw silage, with 30% papaya peel inclusion producing the best overall quality and nutritional value for ruminants.

Introduction

Rice straw is a by-product of rice production and is commonly used as roughage for ruminants due to its availability and low cost. Ensiling is a common preservation method employed to ensure a year-round supply of this feedstock, and it has been shown to significantly improve the feeding value of rice straw (Liu et al., 2024). However, producing high-quality silage using conventional methods remains difficult due to the rice hollow stems, low content of water-soluble carbohydrates (WSC), and a limited population of naturally occurring lactic acid bacteria (LAB). In recent years, co-ensiling has emerged as an innovative approach to preserve nutrients and improve silage quality, offering advantages over single-forage fermentation (Larsen et al., 2017).

Among various co-ensiling materials, papaya (*Carica papaya* L.) peel has attracted interest as a potential silage ingredient (Yang et al., 2016). As a by-product of papaya processing, its use in silages offers a sustainable approach to waste reduction while providing an additional feed resource.

Papaya peel contains high levels of WSC that promote rapid lactic acid production during fermentation (Muzaffar et al., 2022). This process supports the growth of LAB, particularly *Lactobacillus plantarum* and *L. casei*, which effectively lower the pH and inhibit spoilage microorganisms, thereby preserving the silage (Yang et al., 2016). Another study showed that papaya peel contains approximately 18.1% crude protein (CP), making it a valuable protein source for animal feed formulations (Muzaffar et al., 2022). It has also been shown that papaya by-products, including peels, are rich in vitamins, minerals, and antioxidants, which improve the nutritional quality of feed (Vinha et al., 2024). As a result, incorporating papaya peel in animal diets has been associated with improved feed efficiency, growth performance, and nutritional properties.

Although silage production is a widely applied method for preserving forage, the impact of adding papaya peel on the fermentation and nutrient preservation of rice straw silage has not been extensively studied, as most existing research has focused on other additives or silage materials. Therefore, the present study was conducted to evaluate these effects systematically, with the objective of providing valuable information regarding silage production optimisation and livestock feed improvement.

Material and methods

Ensiling materials and silage preparation

Rice straw was collected directly after harvest from Fusui County. Fresh papaya peel was obtained from Huichuang Animal Husbandry Co. Ltd. (Chongzuo, GX, China). The peel was of high-quality, free from mould, pests and diseases, and was washed before use to remove impurities such as soil, dust, and plastic residues. Both materials were cut into approximately 2 cm pieces. The experiment included 4 groups with 6 replicates each, based on fresh matter (FM): (1) a control (100% rice straw), (2) 85% rice straw + 15% papaya peel, (3) 70% rice straw + 30% papaya peel, and (4) 55% rice straw + 45% papaya peel. After thorough mixing, approximately 1 kg of each mixture was packed into a fermentation bag (160 mm × 250 mm), vacuumsealed using a DZ500 vacuum sealer (Gzrifu Co. Ltd., Guangzhou, GD, China), and stored at room temperature in the dark for 45 days of fermentation.

Silage quality sensory investigation

Upon opening the silage bags, the quality of the silage was assessed by evaluating its odour, colour, and texture. The assessment was conducted following the established evaluation standards described by Abdelrahman et al. (2022), and presented in Table 1.

Chemical analysis

All silage samples were dried at 65 °C in a forced-air oven (LABO-250; STIK Co. Ltd., Shanghai, China) until constant weight, defined as a mass difference of <0.0002 g between two consecutive weighings. They were then ground to pass through a 1 mm sieve using an FS200 sample mill (Guangzhou Bomin Mechanical & Electrical Equipment Co. Ltd., Guangzhou, GD, China). Dry matter (DM; method 934.01), crude protein (CP; method 976.05), and ash (method 942.05) contents were determined according to AOAC (1990) procedures. Organic matter (OM) was calculated as the weight loss after ashing. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed following Van Soest et al. (1991), and were determined colorimetrically after reaction with anthrone reagent (Thomas, 1977). Total digestible nutrients (TDN) and relative feed value (RFV) were calculated as described by Bali et al. (2008). The results of the chemical analysis are presented in Table 2.

Table 2. Chemical compositions of rice straw and papaya peel prior to ensiling

Item	Rice straw	Papaya peel
DM, %	54.31	5.77
CP, % DM	11.32	32.83
OM, % DM	86.28	89.49
NDF, % DM	60.56	34.17
ADF, % DM	33.22	16.40
WSC, % DM	6.96	12.05
TDN, % DM	63.02	76.12
RFV	96.81	207.30

DM – dry matter, CP – crude protein, OM – organic matter, NDF – neutral detergent fibre, ADF – acid detergent fibre, WSC – water-soluble carbohydrate, TDN – total digestible nutrient, RFV – relative feed value

Fermentation profile analysis

Cold-water extraction was employed to determine the fermentation products in silage mixtures (Cai, 2004). Briefly, 20 g of fresh silage was homogenised with 180 ml of sterile distilled water and incubated at 4 °C overnight. Organic acid concentrations were measured using high-performance liquid chromatography (HPLC) with the 1260 Infinity II System (Agilent Technologies, Waldbronn, Germany) and a Shodex RSpak KC-811 column

Table 1. Silage quality evaluation standards

Parameter	Total score	Excellent	Good	Medium	Poor
Odour	25	Sour scent, acceptable (25)	Light sour (9–17)	Pungent wine sour (1–8)	Mouldy (0)
Colour	20	Turquoise (14–20)	Yellow-green (8–13)	Brownish-yellow (1–7)	Dark brown (0)
Texture	10	Loose and soft Soft and non-sticky (8–10)	Middle (4–7)	Slightly viscosity (1–3)	Sticky (0)
Grade		Excellent	Good	Medium	Poor quality

(8.0 × 300 mm; Showa Denko K.K., Tokyo, Japan). Detection was performed at 210 nm, using a 3 mmol/l $HClO_4$ eluent. The flow rate was maintained at 1 ml/min, the column temperature at 50 °C, and the injection volume was 5 µl. Ammonia-N (NH_3-N) concentration was determined following the procedure of Broderick and Kang (1980).

Microbiological analysis of the ensiled forage was performed using the plate count method (Cao et al., 2011). Silage samples (10 g) were mixed manually with 90 ml of distilled water, and serial dilutions (10^{-1} to 10^{-5}) were prepared. All plates were incubated at 30 °C for 48 h. LAB counts were determined using De Man-Rogosa-Sharpe agar (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, SD, China), and yeasts and moulds were cultivated on potato dextrose agar (Qingdao Hope Bio-Technology Co., Ltd.). Yeasts were distinguished from moulds and bacteria based on colony morphology and cell structure. Microbial populations were expressed as $\log_{10}$ colony-forming units per g of fresh matter (log CFU/g FM).

In vitro fermentation

Rumen fluid was obtained from three healthy, ruminally cannulated Murrah water buffaloes (average body weight 650 ± 50 kg) prior to the morning feeding. Animal care and management adhered to the guidelines of the Ethics Committee of the Buffalo Research Institute, Guangxi Zhuang Nationality Autonomous Region, China (Approval Number BRI-20241212). The buffaloes were fed a basal diet (Table 3) twice daily at 08:00 and 15:00, and had *ad libitum* access to water throughout the study. Before morning feeding, rumen contents were collected and pooled in equal volumes from each buffalo, and filtered through two layers of gauze. The filtrate was then mixed with CO_2-saturated artificial saliva at a 1:4 ratio (McDougall, 1948), prepared as described by Guo et al. (2022). For *in vitro* fermentation, 50 ml of the buffered rumen fluid was added to a 180 ml incubation bottle containing 1 g of the ground sample. To maintain anaerobic conditions, bottles were flushed with CO_2 and sealed with butyl rubber stoppers and aluminium caps. Fermentation was conducted at 39 °C for 72 h in a water bath equipped with a shaker set at 100 strokes per min.

Table 3. Composition and nutrient content of the buffalo diet

Items	Content, % DM basis
Ingredient	
distiller's grains	30.00
maize straw silage	52.00
peanut straw	7.00
marigold meal	10.00
NaCl	0.35
Premix[1]	0.65
Total	100.00
Nutrient content*	
CP	13.22
NDF	52.26
ADF	33.98
Ash	8.27
Ca	0.73
P	0.30
GE, MJ/kg	15.48

DM – dry matter, CP − crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, GE – gross energy; [1] the premix provided the following per kg of the diet: IU: vit. E 2 000, vit. D 85 000, vit. A 300 000; mg: Cu (as copper sulphate) 80, Fe (as ferrous sulphate) 250, Mn (as manganese sulphate) 200, I (as potassium iodide) 1, Zn (as zinc sulphate) 150, Co (as cobalt sulphate) 2, and Se (as sodium selenite) 0.5; * nutrient content were measured values

Gas production was determined using air syringes (Guo et al., 2022). Hydrogen (H_2) and methane (CH_4) concentrations were analysed using gas chromatography (GC) (Model 8860; Agilent Technologies Co., Ltd., Shanghai, China). Cumulative H_2 and CH_4 production over a 72-h incubation period was calculated by summing the volumes measured at all sampling time points (3, 6, 12, 24, 36, 48, and 72 h).

Following the 72-h incubation, fermentation bottles were immediately cooled in an ice-water bath for 15 min to terminate microbial activity. The pH was measured directly after opening the bottles, using the same procedure as for the silage filtrates. Separate subsamples of the supernatant were collected to determine total volatile fatty acid (TVFA) fractions by GC (model 7890A; Agilent Technologies Co., Ltd.) (Azizi et al, 2020).

In vitro dry matter digestibility (IVDMD) was assessed by filtering the fermentation contents through pre-weighed nylon bags, followed by thorough washing with distilled water. The bags containing the washed residue were dried at 105 °C until constant weight. IVDMD was calculated using the formula:

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

Statistical analyses

Data were analysed using a general linear model in SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) was performed to evaluate differences between treatment means, and Duncan's multiple range test was used for post-hoc comparisons. Statistical significance was defined as $P < 0.05$.

Results

The chemical compositions of rice straw and papaya peel is presented in Table 2. Compared with rice straw, papaya peel contained higher CP, OM, WSC, RFV, and TDN contents but lower levels of DM, ADF, and NDF.

Based on the established criteria for silage quality, the effect of varying proportions of papaya peel on silage characteristics was assessed using a scoring system (Table 4). The sensory characteristics evaluation results showed that, compared to the control group, silage supplemented with 15, 30, and 45% papaya peel received higher colour scores, yet lower odour and texture scores. With increasing papaya peel proportion, colour scores gradually increased, while odour and texture scores decreased. All silages were classified as excellent quality.

Table 4. Sensory evaluation of the effect of papaya peel addition on rice straw silage quality

Item	Control group	85% rice straw + 15% papaya peel	70% rice straw +30% papaya peel	55% rice straw +45% papaya peel
Odour	22.00	21.42	20.67	17.83
Colour	16.17	17.00	17.17	17.33
Texture	9.67	9.00	8.33	8.00
Grade	Excellent	Excellent	Excellent	Excellent

As the proportion of papaya peel in the silage mixture increased, the contents of DM, NDF, and ADF showed a decreasing trend, while CP, OM, WSC, TDN, and RFV contents increased (Table 5). The control group had the highest contents of DM, ADF, and NDF, followed by the silages containing 15, 30, and 45% papaya peel, respectively ($P < 0.05$). Conversely, the 45% papaya peel treatment resulted in the highest contents of CP, OM, RFV, TDN, and WSC, followed by the 30% and 15% treatments, with the control group showing the lowest values ($P < 0.05$).

The addition of papaya peel significantly affected the microbial composition of rice straw silage (Table 6). Among the four experimental silage groups, the treatment containing 30% papaya peel

Table 5. Chemical composition of rice straw silage supplemented with papaya peel

Item	Control group	85% rice straw + 15% papaya peel	70% rice straw +30% papaya peel	55% rice straw +45% papaya peel	SEM	*P*-value
DM, %	53.32^{a}	40.01^{b}	37.16^{b}	29.00^{c}	0.9632	<0.0001
CP, % DM	10.77^{d}	13.16^{c}	14.32^{b}	15.11^{a}	0.1644	<0.0001
OM, % DM	83.73^{c}	84.59^{b}	84.87^{ab}	85.46^{a}	0.1611	<0.0001
NDF, % DM	58.34^{a}	56.71^{a}	54.03^{b}	51.81^{c}	0.5135	<0.0001
ADF, % DM	31.32^{a}	30.77^{a}	30.09^{ab}	28.34^{b}	0.5242	0.0041
WSC, % DM	5.14^{c}	6.17^{b}	6.94^{a}	7.25^{a}	0.1353	<0.0001
TDN, % DM	64.50^{b}	64.93^{b}	65.46^{ab}	66.82^{a}	0.4083	0.0041
RFV	103.03^{c}	106.56^{c}	112.73^{b}	119.99^{a}	1.5128	<0.0001

DM – dry matter, CP – crude protein, OM – organic matter, NDF – neutral detergent fibre, ADF – acid detergent fibre, WSC – water-soluble carbohydrate, TDN – total digestible nutrient, RFV – relative feed value, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 6. Fermentation quality and microbial composition of rice straw silage supplemented with papaya peel

Item	Control group	85% rice straw + 15% papaya peel	70% rice straw +30% papaya peel	55% rice straw +45% papaya peel	SEM	*P*-value
pH	4.55^{a}	4.41^{b}	4.15^{c}	4.24^{c}	0.0472	<0.0001
Lactic acid, g/kg DM	36.59^{c}	45.57^{b}	52.79^{a}	42.85^{b}	1.7141	<0.0001
Acetic acid, g/kg DM	0.81^{b}	2.79^{a}	3.07^{a}	2.52^{a}	0.5284	0.0292
Propionic acid, g/kg DM	0.35^{c}	0.65^{ab}	0.81^{a}	0.47^{bc}	0.0685	0.0168
Butyric acid, g/kg DM	ND	ND	ND	ND	-	-
NH_3-N, g/kg DM	0.71^{a}	0.69^{a}	0.64^{b}	0.68^{a}	0.018	0.0447
LAB, CFU/g FM	6.27	6.38	6.41	6.22	0.1661	0.8391
Yeasts, CFU/g FM	5.86^{a}	5.67^{a}	4.92^{b}	5.42^{ab}	0.2136	0.0316
Moulds, CFU/g FM	ND	ND	ND	ND	–	–

DM – dry matter, LAB – lactic acid bacteria, CFU – colony-forming unit, FM – fresh matter, ND – not detected, NH_3-N – ammonia-N, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

showed the lowest pH, NH_3-N concentration, and yeast counts, followed by the 45, 15%, and control treatments, respectively ($P < 0.05$). In contrast, the concentrations of lactic acid, acetic acid, and propionic acid were highest in the 30% papaya peel silage, followed by the 15% 45%, and control groups ($P < 0.05$). Butyric acid and moulds were not detected in any of the silages.

The addition of papaya peel significantly affected the *in vitro* fermentation products of the rice straw silage (Table 7). The IVDMD was highest in the 30% papaya peel treatment, followed by the 45% papaya peel, 15% papaya peel, and control groups, respectively ($P < 0.05$). The pH value and the acetate/propionate ratio were lowest in the silage with 30% papaya peel supplementation, intermediate for 15 and 45%, and highest in the control group ($P < 0.05$). The concentrations of TVFA, acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate were highest in the 30% papaya peel treatment, followed by the 15%, 45%, and control groups ($P < 0.05$). The NH_3-N concentration increased with the proportion of papaya peel, reaching the highest level in the 45% papaya peel treatment, and the lowest in the control group ($P < 0.05$).

Table 7. *In vitro* ruminal dry matter digestibility, gas production and fermentation characteristics of rice straw silage supplemented with papaya peel

Item	Control group	85% rice straw + 15% papaya peel	70% rice straw +30% papaya peel	55% rice straw +45% papaya peel	SEM	*P*-value
IVDMD, %	40.74^b	43.34^{ab}	45.57^a	43.81^a	0.9036	0.0106
Total gas production, ml/g DM	69.58	70.95	71.40	70.07	1.3518	0.7739
H_2 production, ml/g DM	0.15	0.17	0.16	0.15	0.0156	0.6584
Methane production, ml/g DM	13.64	14.26	13.58	13.61	0.5887	0.8177
pH	6.63^a	6.61^a	6.59^b	6.62^a	0.0070	0.0026
TVFA, mmol/l	16.66^b	18.00^b	19.46^a	17.64^b	0.4815	0.0050
Acetate acid, mmol/l	8.75^b	9.10^b	9.74^a	8.91^b	0.1973	0.0108
Propionate acid, mmol/l	5.13^c	5.81^{ab}	6.35^a	5.68^{bc}	0.1943	0.0027
Isobutyrate acid, mmol/l	0.12^b	0.16^a	0.17^a	0.15^{ab}	0.0107	0.0202
Butyrate acid, mmol/l	2.12^b	2.28^b	2.52^a	2.26^b	0.0634	0.0023
Isovalerate acid, mmol/l	0.31^b	0.39^a	0.40^a	0.38^a	0.0202	0.0256
Valerate acid, mmol/l	0.22^b	0.26^a	0.27^a	0.25^{ab}	0.0119	0.0420
Acetate/propionate	1.72^a	1.57^b	1.54^b	1.57^b	0.0284	0.0011
NH_3-N, mg/l	56.33^b	59.70^a	60.49^a	61.94^a	1.1382	0.0162

IVDMD – *in vitro* ruminal dry matter digestibility, DM – dry matter, TVFA – total volatile fatty acid; NH_3-N – ammonia-N, SEM – standard error of the mean, ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Discussion

The characteristics of different raw materials directly determine the quality of mixed silage. Rice straw is characterised by high fibre and low protein contents, and feeding it alone to ruminants reduces digestibility. These two properties limit its nutritional value as a single silage material, which is consistent with previous findings on rice straw materials (Sarwono et al., 2022). The DM content of papaya peel was only 5.77%, indicating excessive moisture, whereas rice straw had a DM content of 54.31%, indicating very low moisture. High-quality silage generally requires a DM content between 30% and 40% (Liu et al., 2024). In this study, only the 30% papaya peel treatment achieved a DM content of 37.16%, which was within the indicated optimal range for effective fermentation. The higher moisture and WSC content of papaya peel compared to rice straw provide a suitable substrate for LAB fermentation, stimulating the acidification process in the silage (Yang et al., 2016). The high sugar level of papaya peel compensates for the insufficient fermentation substrate in rice straw. In contrast, the high DM content of rice straw may reduce effluent loss in papaya peel when ensiled alone, a finding consistent with studies on other fruit by-products, such as banana (Padam et al., 2014). In the present study, significant differences were observed in the contents of DM, CP, ADF, NDF, and WSC between rice straw and papaya peel, indicating that their chemical compositions are complementary when mixed for ensiling. These results support the conclusion of Wang et al. (2024) that incorporating various fruit and vegetable residues into maize stover can improve silage value.

However, variations in the types and proportions of mixed materials may lead to differences in final chemical composition. Here, increasing the proportion of papaya peel gradually reduced the NDF and ADF contents. Research has shown that papaya by-products contain various fibre-degrading enzymes, such as cellulases and hemicellulases (Vinha et al., 2024). The presence of these enzymes in papaya peel likely promotes fibre degradation, thereby improving its nutritional value and digestibility. Consequently, the NDF and ADF contents in the 45% papaya peel treatment were 6.53 and 2.98% lower, respectively, than in the control group, which could be related to the enzymatic breakdown of fibre (Patidar et al., 2016). Another study demonstrated that co-ensiling rice straw with whole sugar beet improves WSC availability, fermentation efficiency, and animal intake (Abo-Donia et al., 2024). In the current study, co-ensiling rice straw with papaya peel improved fermentation efficiency, although further animal trials are required to confirm these effects. While the chemical composition improved, the high moisture content of papaya peel could hinder proper compaction of the mixture and reduce the fermentation efficiency of LAB by limiting their access to WSC. Therefore, the proportion of papaya peel supplementation should be carefully optimised.

In terms of odour, the control group received the highest score (22.00), while the 45% papaya peel treatment scored the lowest (17.83). Nevertheless, all samples had pleasant aromas characteristic of successful lactic acid fermentation. High-quality silage should have a fresh, acidic odour without signs of putrefaction, consistent with the sweet-acidic scent observed in all groups. Colour scores ranged from 16.17 to 17.33, with all silages retaining a yellowish-green hue similar to the original raw materials. This hue indicates minimal pigment degradation during fermentation, a key criterion for excellent-quality silage. Regarding texture, although scores slightly decreased with increasing papaya peel content, all samples retained structural integrity, with stems and leaves remaining distinguishable, as expected for high-quality silage. Papaya peel may contain volatile compounds, such as fatty acid oxidation products, which could be released during processing to form exudates, potentially affecting aroma and texture. However, its favourable colour profile can compensate for these deficiencies in comprehensive evaluations, positively influencing the total score (Manzoor et al., 2019). Moreover, sensory evaluation is inherently subjective; even if certain traits decline, the overall assessment can still be positive thanks to other favourable characteristics. Collectively, based on the combined evaluation of odour, colour, and texture, all experimental groups in this study met the 'excellent' standard, indicating that the fermentation of rice straw with papaya peel was generally successful. The pH value is an important indicator for evaluating the quality of mixed silages, reflecting the production of organic acids, such as acetic and propionic acids, during fermentation (Kumanowska et al., 2017). In this experiment, the 30% papaya peel treatment had the lowest pH and significantly higher concentrations of acetic and propionic acids than the other groups, suggesting more effective preservation. LAB conserves silage and reduces nutrient loss by converting WSC into organic acids and inhibiting degradation by other probiotics (Kim et al., 2021). Yeasts were detected in all experimental groups, with the 45% papaya peel treatment showing higher yeast counts than the 30% group, likely due to the high-moisture environment promoting excessive yeast proliferation. These yeasts consume WSC and may produce metabolic by-products such as ethanol and carbon dioxide, which can adversely affect silage fermentation quality. During ensiling, undesirable microorganisms, such as clostridia, can inhibit LAB growth, promote butyric acid production, impair lactic acid fermentation, degrade protein into NH_3-N, and reduce feed nutritional value (Muck, 2013). In the current experiment, the 30% papaya peel treatment had a significantly lower NH_3-N concentration and a significantly higher lactic acid level than the control. This effect may be due to the fibre-degrading activity of papaya peel, which releases WSC from rice straw, providing sufficient nutrients for LAB growth, and thus increased lactic acid generation, and inhibited growth of undesirable microorganisms (Guo et al., 2022). This conclusion is further supported by the fact that butyric acid was not detected in any of the experimental groups.

IVDMD is a measure of feed digestibility in the rumen, with higher values indicating greater digestibility and nutritional value (Holden, 1999). The significantly higher IVDMD observed in the 30% papaya peel treatment compared to the control group in this trial can be attributed to the nutrient composition of the 70% rice straw and 30% papaya peel mixture. This combination appears to support the growth and activity of rumen microorganisms, thereby improving the digestion and nutrient absorption. Additionally, the higher overall nutrient content in the 30% papaya peel silage may have further contributed to the improved IVDMD. However, *in vitro* models do not account for important physiological processes in ruminants, such as chewing and rumen emptying. Therefore, to fully validate the practical efficacy of papaya peel silage, subsequent *in vivo* digestion trials involving fistulated water buffaloes are necessary. Furthermore, feeding trials with 30% papaya inclusion should be conducted to assess its effects on key production performance parameters, such as body weight gain and feed intake. A 72-h fermentation period more accurately reflects rumen microbial fermentation dynamics, especially for high-fibre diets, allowing for complete gas production and VFA accumulation. This time is essential for evaluating the fermentation characteristics and energy potential of silage (Hidayah et al., 2023). Gas production is considered an important indicator of silage nutritional value, reflecting the extent of organic matter fermentation (Menke and Steingass, 1988). Total gas production was highest in the 30% papaya peel treatment, which also indicates that this proportion of papaya peel seems to be optimal for fermentation efficiency. Concentrations of TVFA and NH_3-N in silages are important markers of fermentation quality (Abd El-Hady et al., 2012). In the present study, 30% papaya peel inclusion resulted in a significantly higher TVFA content than in the other experimental groups, and significantly higher NH_3-N concentration compared to the control group, indicating improved fermentation quality. This mixture likely maintained optimal pH and oxygen conditions, promoting lactic acid bacterial activity and resulting in higher TVFA, lactate, acetate, and propionate levels in the 30% papaya peel silage compared with the 15 and 45% treatments. During rumen fermentation in ruminants, the acetate-to-propionate ratio is an important indicator of energy utilisation efficiency, as higher propionic acid production provides more readily usable energy and improves feed conversion (Wang et al., 2021). In the present experiment, both acetate and propionate concentrations were significantly higher in the 30% papaya peel treatment compared to the other experimental groups, suggesting that these proportions of rice straw and papaya peel can improve energy utilisation in ruminants.

Conclusions

The results of the present experiment indicate that ensiling rice straw with papaya peel improves its utilisation efficiency and fermentation quality. The optimum mixing ratio for rice straw and papaya peel was determined to be 30:70 (w/w). Silage prepared at this proportions had improved fermentation quality and *in vitro* rumen fermentation characteristics compared to the other experimental groups. Nevertheless, additional research is required to evaluate the feeding value of this mixed silage in animal trials, including feed intake and feed conversion rate.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

Abd El-Hady M., Gabr, A., El-Ayouty S., Shahin M., 2012. Effect of physical and chemical treatments on berseem silage quality, nutritive values, rumen fermentation and blood metabolites of rams. J. Anim. Poult. Prod. 3, 267–288, https://doi.org/10.21608/jappmu.2012.82793

Abdelrahman M., Wang W., Lv H. et al., 2022. Evaluating the effect of forage rape (*Brassica napus*) ensiling kinetics on degradability and milk performance as non-conventional forage for dairy buffalo. Front. Vet. Sci. 9, 926906, https://doi.org/10.3389/fvets.2022.926906

Abo-Donia F.M., Elsheikh H.A., Esh A.M.H., El-Shora M.A.H., Eldiahy Y.M.M., 2024. Co-ensiled rice straw with whole sugar beet and its effect on the performance of lactating cows. Trop. Anim. Health Prod. 56, 173, https://doi.org/10.1007/s11250-024-03945-9

AOAC, 1990. Official methods of analysis of AOAC International. 15th Edition. Association of official analytical chemists. Washington, DC (USA), https://doi.org/10.1016/0003-2670(91)87088-O

Azizi A., Sharifi A., Fazaeli H., Azarfar A., Jonker A., Kiani A., 2020. Effect of transferring lignocellulose-degrading bacteria from termite to rumen fluid of sheep on *in vitro* gas production, fermentation parameters, microbial populations and enzyme activity. J. Integr. Agric. 19, 1323–1331, https://doi.org/10.1016/S2095-3119(19)62854-6

Bali K., Canevari, M., Cangiano C. et al., 2008. Forage quality and testing. Irrigated alfalfa management in Mediterranean and Desert zones. In: D.H. Putnam, P. Robinson (Editors). Proceedings of the 37th California Alfalfa & Forage Symposium. Monterey (USA). The Irrigated Alfalfa Management publication. Chapter 16, http://alfalfa.ucdavis.edu/IrrigatedAlfalfa

Broderick G.A., Kang J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media1. J. Dairy Sci. 63, 64–75, https://doi.org/10.3168/jds.S0022-0302(80)82888-8

Cai Y., 2004. Analysis method for silage. Japanese Society of Grassland Science. Field and Laboratory Methods for Grassland Science. Tosho Printing Co., Ltd. Tokyo, (Japan), pp. 279–282

Cao Y., Cai Y., Hirakubo, T., Fukui H., Matsuyama H., 2011. Fermentation characteristics and microorganism composition of total mixed ration silage with local food by-products in different seasons. Anim. Sci. J. 82, 259–266, https://doi.org/10.1111/j.1740-0929.2010.00840.x

Guo Y., Hassan F., Li M., Xie H., Peng L., Tang Z., Yang C., 2022. Effect of sodium nitrate and cysteamine on *in vitro* ruminal fermentation, amino acid metabolism and microbiota in buffalo. Microorganisms 10, 2038, https://doi.org/10.3390/microorganisms10102038

Hidayah N., Noviandi C. T., Astuti A., Kustantinah K., 2023. Chemical composition and *in vitro* rumen fermentation characteristics of various tropical seaweeds. J. Adv. Vet. Anim. Res. 10, 751–762, https://doi.org/10.5455/javar.2023.j731

Holden L.A., 1999. Comparison of methods of in vitro dry matter digestibility for ten feeds. J. Dairy Sci. 82, 1791–1794, https://doi.org/10.3168/jds.S0022-0302(99)75409-3

Kim D., Lee K.D., Choi K.C., Kim D., Lee K.D., Choi K.C., 2021. Role of LAB in silage fermentation: Effect on nutritional quality and organic acid production - An overview. AIMS Agric. Food 6, 216–234, https://doi.org/10.3934/agrfood.2021014

Kumanowska E., Uruñuela Saldaña M., Zielonka S., Oechsner H., 2017. Two-stage anaerobic digestion of sugar beet silage: The effect of the pH-value on process parameters and process efficiency. Biores. Technol. 245, 876–883, https://doi.org/10.1016/j.biortech.2017.09.011

Larsen S.U., Hjort-Gregersen K., Vazifehkhoran A.H., Triolo J.M., 2017. Co-ensiling of straw with sugar beet leaves increases the methane yield from straw. Biores. Technol. 245, 106–115, https://doi.org/10.1016/j.biortech.2017.08.117

Liu H., Li X., Hu J., Zhao J., Xu G., Dong D., Jia Y., Shao T., 2024. Fermentation quality and aerobic stability evaluation of rice straw silage with different ensiling densities. Ferment. 10, 20, https://doi.org/10.3390/fermentation10010020

Manzoor S., Yusof Y. A., Chin N. L., Mohamed Amin Tawakkal I. S., Ahmed M. F. M., Chang L. S., 2019. Quality characteristics and sensory profile of stirred yogurt enriched with papaya peel powder. Pertanika J. Trop. Agric. Sci. 42, 519–533, http://www.pertanika.upm.edu.my

McDougall E.I., 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43, 99–109, https://doi.org/10.1042/bj0430099

Menke K.H., Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 28, 7-55

Muck R.E., 2013. Recent advances in silage microbiology. Agric. Food Sci. 22, 3-15, https://doi.org/10.23986/afsci.6718

Muzaffar K., Sofi S.A., Mir S.A., 2022. Handbook of fruit wastes and by-products: chemistry, processing technology, and utilization. CRC Press. Boca Raton, FL (USA), https://doi.org/10.1201/9781003164463

Padam B.S., Tin H.S., Chye F.Y., Abdullah M.I., 2014. Banana by-products: an under-utilized renewable food biomass with great potential. J. Food Sci. Technol. 51, 3527–3545, https://doi.org/10.1007/s13197-012-0861-2

Patidar M.K., Nighojkar S., Kumar A., Nighojkar A., 2016. Papaya peel valorization for production of acidic pectin methylesterase by *Aspergillus tubingensis* and its application for fruit juice clarification. Biocatal. Agr. Biotech. 6, 58–67, https://doi.org/10.1016/j.bcab.2016.02.008

Sarwono K.A., Rohmatussolihat R., Watman M. et al, 2022. Characteristics of fresh rice straw silage quality prepared with addition of lactic acid bacteria and crude cellulase. AIMS Agric. Food 7, 481–499, https://doi.org/10.3934/agrfood.2022030

Thomas T.A., 1977. An automated procedure for the determination of soluble carbohydrates in herbage. J. Sci. Food Agric. 28, 639–642, https://doi.org/10.1002/jsfa.2740280711

Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vinha A.F., Costa A.S.G., Espírito Santo L., Ferreira D.M., Sousa C., Pinto E., Almeida A., Oliveira M.B.P.P., 2024. High-value compounds in papaya by-products (*Carica papaya* L. var. Formosa and Aliança): potential sustainable use and exploitation. Plants 13, 1009, https://doi.org/10.3390/plants13071009

Wang Q., Zeng Y., Zeng X. et al, 2021. Effects of dietary energy levels on rumen fermentation, gastrointestinal tract histology, and bacterial community diversity in fattening male hu lambs. Front. Microbiol. 12, 695445, https://doi.org/10.3389/fmicb.2021.695445

Yang J., Tan H., Cai Y., 2016. Characteristics of lactic acid bacteria isolates and their effect on silage fermentation of fruit residues. J. Dairy Sci. 99, 5325–5334, https://doi.org/10.3168/jds.2016-10952