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ORIGINAL PAPER

Evaluation of silage quality, rumen fermentation dynamics, degradability, and methane emissions of total mixed rations formulated from agricultural by-products: an *in vitro* analysis

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ABSTRACT. This study evaluates the silage fermentation quality, rumen fermentation dynamics, degradability, and methane emissions of total mixed rations (TMRs) formulated primarily with varying ratios of pineapple peel and maize husk alongside other agricultural by-products (tofu waste 30%, soy sauce waste 15%, rice bran 10%, and cassava pulp 5%). The TMR formulations differed in crude protein (CP) and neutral detergent fibre (NDF) content due to the changing proportions of pineapple peel (40% to 0%) and maize husk (0% to 40%), with TMR-1, TMR-2, TMR-3, TMR-4, and TMR-5 containing pineapple peel to maize husk ratios of 40:0, 30:10, 20:20, 10:30, and 0:40%, respectively. All TMR silages were well-preserved, as evidenced by low pH (4.06–4.18) and high lactic acid content (1.89–2.25% dry matter). TMR-1 and TMR-2, with lower NDF and higher total digestible nutrients (TDN), demonstrated superior fermentation quality, greater total short-chain fatty acid (TSCFA) production, and lower methane emissions (15.98–15.87% of TSCFA) compared to TMR-4 and TMR-5 (17.85–17.98%). The higher *in vitro* degradability observed in TMR-1 and TMR-2 was associated with balanced CP levels (14.02–14.86%) and moderate NDF content (46.06–46.36%), which supported efficient microbial fermentation. In contrast, TMR-4 and TMR-5, with higher NDF content (47.98–48.98%), showed reduced degradability and increased methane production. These results highlight the potential of TMR-1 (40:0) and TMR-2 (30:10) as promising options for beef cattle. However, the lack of vitamin and mineral supplementation is a limitation that should be addressed, and further *in vivo* studies are necessary to validate nutrient absorption, utilisation, and overall animal performance.

Introduction

Agricultural and agro-industrial by-products can serve as alternative feed substitutes for ruminant livestock. However, their use is restricted due to limited nutritional value, requiring various enhancement processing technologies (Vastolo et al., 2022). Another significant issue is their high moisture content, which reduces storage capacity and complicates direct incorporation into feeding systems. While drying technology is commonly employed, it has not fully resolved these challenges, as it incurs high costs when applied to waste materials such as tofu waste, soy sauce waste, and cassava pulp (Dentinho et al., 2023).

Tofu and soy sauce waste are solid by-products derived from soybean processing during the manufacture of tofu and soy sauce (Sadarman et al., 2020). These agro-industrial residues can be utilised as concentrated mixtures in ruminant feed, at inclusion rates of 10 to 65% (Pulungan et al., 2024). Their protein content varies between 12 and 24%, while their total digestible nutrient (TDN) content ranges from 70 to 78%, depending on processing methods and the quality of raw materials (Sadarman et al., 2020; Pulungan et al., 2024).

Meanwhile, cassava pulp, a by-product of the tapioca industry, is often discarded despite its potential as a high-energy feed ingredient for livestock (Ramadhanti et al., 2024). Typically produced in wet form, cassava pulp constitutes about 10–40% of the total cassava used in tapioca production (Kosugi et al., 2009). While commonly preserved through sun-drying, this method proves inconsistent and frequently results in fungal contamination during the drying process. Both processed and unprocessed cassava pulp can be effectively incorporated into animal feed formulations at inclusion rates of 10–40% for both ruminants and poultry (Ramadhanti et al., 2024; Pongsub et al., 2024).

Pineapple peels and maize husks are often used as grass substitutes in ruminant feed, though their low chemical quality, resulting from high lignocellulose content, limits digestibility (Sukri et al., 2023). This constraint requires the improvement of their nutrient profile to meet the dietary demands of ruminant livestock. As fibre sources, pineapple peels and maize husks can replace grass as their crude fibre ranging from 20 to 33%, and a total digestible nutrient (TDN) value of approximately 55–60% (Mehraj et al., 2024).

Fermentation technology offers an effective method for developing total mixed rations (TMR) that simultaneously preserve high-moisture by-products (including tofu waste, soy sauce waste, and cassava pulp) while enhancing the nutritional quality of fibrous materials (such as pineapple peels and maize husks) (Dentinho et al., 2023). This approach provides a practical and cost-efficient solution that can be directly implemented at production sites. The production and preservation of nutritionally complete feed can be achieved through the integration of various agricultural and agro-industrial by-products, including tofu waste, soy sauce waste, pineapple peels, and maize husks using fermentation technology (Tuoxunjiang et al., 2020; Ridla et al., 2023)

The present study aimed to assess the fermentation quality, nutrient content, methane emissions, and degradability of mixtures of agricultural and agro-industrial by-products processed using TMR fermentation technology. The findings are expected to contribute to reducing environmental pollution while simultaneously developing nutritionally improved feed alternatives for ruminant production systems.

Material and methods

TMR materials and production

The experimental TMRs were developed using specific ingredient categories: pineapple peels and maize husks as fibre sources, tofu waste, and soy sauce waste as protein sources, and rice bran and cassava pulp as energy-providing components. Five different TMR formulations (TMR-1 to TMR-5) were created with varying pineapple peel-to-maize husk ratios (40:0, 30:10, 20:20, 10:30, and 0:40%, respectively), while maintaining fixed proportions of other ingredients (30% tofu waste, 15% soy sauce waste, 10% rice bran, and 5% cassava pulp), totalling 100%. The formulations were designed to meet the primary nutritional requirements of ruminants for energy, protein, and fibre, though without additional vitamin or mineral supplementation. These materials were purchased locally in the vicinity of Bogor, IPB University. The complete nutritional composition of each agricultural by-product is listed in Table 1, and the specific TMR formulations are summarised in Table 2. The TMR production

Table 1. Nutrient content of agricultural waste used in the experiment

Nutrient content	Pineapple peel	Maize husk	Tofu waste	Soy sauce waste	Rice bran	Cassava pulp
DM, %	28.54	36.73	14.25	15.32	88.2	13.43
Crude ash, % DM	3.31	8.42	1.31	12.00	12.28	1.3
Crude protein, % DM	3.63	6.77	25.15	29.31	10.80	7.8
Ether extract, % DM	1.84	1.06	9.80	6.35	4.81	0.4
Crude fibre, % DM	23.76	30.21	15.55	14.79	14.86	14.9
N-free extract, % DM	67.46	53.54	48.19	37.55	57.25	75.6
NDF, % DM	53.13	62.32	42.34	40.27	25,86	46.43
ADF, % DM	42.33	50.23	36.65	34.98	14.67	32.45
Calcium, % DM	0.32	0.44	0.49	0.46	0.09	0.2
Total phosphorus, % DM	0.46	0.55	0.78	0.43	1.09	0.05
Predicted TDN, %	60.48	55.34	77.58	76.78	72.17	68.58

DM – dry matter, NDF – neutral detergent fibre, ADF – acid detergent fibre, TDN – total digestible nutrients

Table 2. Total mixed ration (TMR) ingredients, DM basis

Ingredients	TMR-1	TMR-2	TMR-3	TMR-4	TMR-5
Pineapple peel, %	40	30	20	10	0
Maize husk, %	0	10	20	30	40
Tofu waste, %	30	30	30	30	30
Soy sauce waste, %	15	15	15	15	15
Rice bran, %	10	10	10	10	10
Cassava pulp, %	5	5	5	5	5
Total, %	100	100	100	100	100
Calculated nutrient content					
DM, %	27.48	28.29	29.11	29.93	30.75
crude ash, % DM	4.81	5.32	5.83	6.34	6.85
crude protein, % DM	14.86	15.17	15.49	15.80	16.11
ether extract, % DM	5.51	5.43	5.35	5.27	5.19
crude fibre, % DM	18.61	19.26	19.90	20.55	21.19
N-free extract, % DM	56.57	55.18	53.79	52.4	51.01
NDF, % DM	44.90	45.82	46.74	47.66	48.58
ADF, % DM	36.26	37.05	37.84	38.63	39.42
calcium, % DM	0.36	0.37	0.38	0.39	0.41
total phosphorus, % DM	0.59	0.60	0.61	0.62	0.63
predicted TDN, %	69.62	69.11	68.60	68.08	67.57

TMR-1 – pineapple peel to maize husk ratio: 40:0, TMR-2 – pineapple peel to maize husk ratio: 30:10, TMR-3 – pineapple peel to maize husk ratio: 20:20, TMR-4 – pineapple peel to maize husk ratio: 10:30, TMR-5 – pineapple peel to maize husk ratio: 0:40%; DM – dry matter, NDF – neutral detergent fibre, ADF – acid detergent fibre, TDN – total digestible nutrients

process was adapted from the ensiling techniques described by Ridla and Uchida (1998), focusing on the preservation of high-moisture feed materials. The methodology also adhered to the protocols of Ridla and Uchida (1994) regarding fermentation quality and nutritional value in mixed silage. The experimental procedure involved ensiling mixed samples in 50-l high-density polyethylene (HDPE) containers under controlled conditions. Following thorough weighing and labelling, the containers were vacuum-sealed to remove air and incubated at ambient temperature (25 ± 2 °C) for a 40-day fermentation period. After the incubation period, the silos were reweighed and opened. Each sample was divided into two portions. The first portion was extracted with distilled water (1:10 dilution) for pH, lactic acid, and volatile nitrogen analyses, while the second portion was oven-dried at 60 °C for 48 h and subsequently ground to pass through a 1 mm sieve for further testing.

TMR silage analysis

Silage pH was measured using a digital pH meter (Horiba F-12, HORIBA Instruments (Singapore) Pte. Ltd.). Ammonia nitrogen (NH_3-N), an indicator of volatile nitrogen, was quantified using the micro-diffusion method of Conway and O'Malley (1942), while lactic acid concentrations were determined following the protocol by Barker and Summerson (1941). Silage quality, assessed based on pH and dry matter, was evaluated using the Flieg point calculation: Flieg point = 220 + (2 × % dry matter of silage − 15) − 40 × pH (Kilic, 1986). Total digestible nutrients (TDN) were estimated following the method of Jayanegara et al. (2019).

Nutrient content analysis

Nutrient analysis was performed according to AOAC International (2011) guidelines to ensure consistency and accuracy. Moisture content was determined by oven-drying the samples at 105 °C for 24 h. Crude ash content was obtained through incineration at 600 °C for 4 h, with subsequent calcium and total phosphorus analysis using gravimetric methods. Crude protein content was determined using the Kjeldahl method, crude fibre by sequential acid and alkali digestion, and ether extract by solvent extraction. Fibre analysis, including neutral detergent fibre (NDF) and acid detergent fibre (ADF) was analysed following the AOAC International (1990) protocols. Water-soluble carbohydrates (WSC), including sugars, were quantified using the anthrone method (Deriaz, 1961), and starch content was determined by acid hydrolysis following Pirt and Whelan (1951).

In vitro rumen fermentation assessment

Rumen fluid was collected approximately 15 min post-mortem from slaughtered animals at the IPB University slaughterhouse into thermally insulated bottles preheated to 40 °C to maintain fluid integrity (Ridla and Nahrowi, 2025). Sample collection was conducted in compliance with Indonesia's Government Regulation No. 41/2014 and Law No. 39/2021 on animal care standards.

The *in vitro* fermentation characteristics and diet degradability were evaluated according to the two-stage method described by Tilley and Terry (1963). Each feed sample (0.5 g) was first incubated with buffered rumen fluid at 39 °C for 48 h, followed by a second 48-h incubation in an acid-pepsin solution. The resulting residue was then dried to calculate *in vitro* dry matter degradability (IVDMD) and subsequently ashed to determine *in vitro* organic matter degradability (IVOMD).

Additionally, after 4 h of incubation, the samples were analysed for pH using a digital pH meter (Horiba-18), ammonia nitrogen (N-NH_3) levels using the Conway and O'Malley (1942) micro-diffusion technique, and total short-chain fatty acids (TSCFA) by steam distillation (Kromann et al., 1967).

Gas production, including methane, was assessed following the established protocol of Theodorou et al. (1994). Feed samples (0.75 g) were incubated with buffered rumen fluid in sealed vessels at 39 °C for 24 h. Gas production was monitored using a pressure transducer, with subsequent methane concentration determination performed by gas chromatographic analysis. This methodology enabled simultaneous assessment of fermentation kinetics and methane production characteristics.

Experimental design and data analysis

The study employed a completely randomised design (CRD) with five treatments and six replicates to evaluate the effects of experimental variants on TMR fermentation quality, nutrient composition (including proximate and fibre fractions), and water-soluble carbohydrate (WSC) content. The same CRD was used for *in vitro* assessment of rumen fermentation parameters (pH, ammonia, total short-chain fatty acids, gas and methane production, and degradability). Statistical analysis was performed using SPSS ver. 26, with significant differences ($P < 0.05$) determined by Tukey's post hoc test.

Results

TMR silage fermentation quality

The TMR silage fermentation quality results (Table 3) showed dry matter (DM) contents ranging from 31.28 to 32.39% across treatments, with no significant differences observed ($P > 0.05$). All formulations demonstrated high DM recovery rates (96.85–97.33%), exceeding the 95% threshold that indicates effective preservation with minimal DM losses during ensiling. These recovery values confirm the efficiency of the fermentation process in preserving the nutritional content of the silages.

Table 3. Fermentation quality of total mixed rations (TMR)

Parameter	TMR-1	TMR-2	TMR-3	TMR-4	TMR-5	SEM	*P*-value
DM, %	31.28	32.39	31.91	31.27	32.23	0.46	0.241
Dry matter recovery, %	97.33	96.87	96.95	97.15	96.85	0.73	0.323
pH	4.06	4.11	4.12	4.18	4.12	0.01	0.314
Lactic acid, % DM	2.25	2.13	2.04	1.89	1.92	0.01	0.112
N-ammonia, mM	1.05	1.29	1.05	1.53	1.21	0.04	0.124
WSC, % DM	6.63^{a}	6.11^{a}	5.62^{ab}	4.58^{b}	4.34^{b}	0.93	0.042
Fleig point	105.16	105.38	104.02	100.23	104.66	2.02	0.423

TMR-1 – pineapple peel to maize husk ratio: 40:0, TMR-2 – pineapple peel to maize husk ratio: 30:10, TMR-3 – pineapple peel to maize husk ratio: 20:20, TMR-4 – pineapple peel to maize husk ratio: 10:30, TMR-5 – pineapple peel to maize husk ratio: 0:40%; DM – dry matter, WSC – water soluble carbohydrate, SEM – standard error of the means; ^{ab} means in the same row without a common superscript are significantly different at $P < 0.05$

The silage pH values were consistent for all samples in the range of 4.06 to 4.18, indicating optimal fermentation conditions limiting the development of spoilage organisms. Lactic acid content, a critical marker of silage quality, ranged from 1.89 to 2.25% DM, with no significant differences between treatments, confirming stable pH results.

Volatile nitrogen (N-NH_3) concentrations, used to gauge protein preservation, ranged from 1.05 to 1.53 mM, which was within acceptable levels indicating limited protein degradation and efficient nitrogen retention across all treatments.

Residual water-soluble carbohydrate (WSC) levels showed significant variation between treatments ($P < 0.05$), with TMR-1 and TMR-2 retaining higher concentrations (6.63 and 6.11% DM, respectively) ($P < 0.05$) compared to TMR-4 and TMR-5 (4.58 and 4.34% DM) *($P < 0.05$)*, indicating better sugar preservation in the former silages.

Fleig points, an index for evaluating silage quality, ranged from 100.23 to 105.38, confirming excellent preservation in all TMRs, with no statistically significant differences between treatments.

Nutritional composition of ensiled TMR

The nutritional composition of the ensiled total TMRs revealed significant differences in key parameters (Table 4). Crude ash content varied significantly *($P < 0.05$)*, with TMR-1 containing the lowest level (4.45% DM) and TMR-5 the highest (6.57% DM), reflecting distinct mineral compositions.

Table 4. Nutritional composition of ensiled total mixed rations (TMR) following the 40-day ensiling period

Parameter	TMR-1	TMR-2	TMR-3	TMR-4	TMR-5	SEM	*P*-value
DM, %	31.28	32.39	31.91	31.27	32.23	0.46	0.321
Crude ash, % DM	4.45^{a}	5.53^{ab}	5.89^{ab}	6.45^{a}	6.57^{a}	0.82	0.032
Crude protein, % DM	14.02	14.86	14.94	14.52	15.27	0.51	0.241
Ether extract, % DM	4.54	4.35	4.67	4.78	4.87	0.21	0.132
Crude fibre, % DM	19.23	19.98	20.34	20.05	20.22	0.40	0.231
N-free extract, % DM	57.76^{a}	55.28^{ab}	54.16^{b}	54.2^{b}	53.07^{b}	1.91	0.026
NDF, % DM	46.06^{b}	46.36^{b}	46.07^{b}	47.98^{ab}	48.98^{a}	1.60	0.032
ADF, % DM	38.56	38.32	38.29	39.42	39.94	0.97	0.145
Calcium, % DM	0.24	0.28	0.31	0.33	0.35	0.01	0.067
Total phosphorus, % DM	0.38	0.45	0.54	0.49	0.53	0.01	0.081
TDN, % (predicted)	69.46	68.69	68.66	68.88	68.94	0.45	0.212

TMR-1 – pineapple peel to maize husk ratio: 40:0, TMR-2 – pineapple peel to maize husk ratio: 30:10, TMR-3 – pineapple peel to maize husk ratio: 20:20, TMR-4 – pineapple peel to maize husk ratio: 10:30, TMR-5 – pineapple peel to maize husk ratio: 0:40%; DM – dry matter, NDF – neutral detergent fibre, ADF – acid detergent fibre, TDN – total digestible nutrients, SEM – standard error of the means; ^{ab} means in the same row without a common superscript are significantly different at $P < 0.05$

CP content differed slightly between TMRs, ranging from 14.02 to 15.27% DM, i.e. levels appropriate for ruminant dietary requirements, providing adequate support for microbial protein synthesis and basic metabolic demands. For carbohydrate composition, nitrogen-free extract (NFE) differed significantly *($P < 0.05$)*, with TMR-1 showing the highest NFE (57.76% DM) and TMR-5 the lowest (53.07% DM), which could influence the overall energy intake in ruminants consuming these TMRs.

Fibre content, particularly neutral detergent fibre (NDF), showed significant differences between TMRs *($P < 0.05$)*. TMR-1 and TMR-2 had lower NDF levels (46.06 and 46.36% DM, respectively) compared to TMR-5 (48.98% DM), which could influence degradability and fermentation products. On the other hand, total digestible nutrients (TDN) were similar across treatments, suggesting comparable energy availability. While moisture, protein, and energy content showed minimal variation between treatments, the differences in fibre and nitrogen-free extract (NFE) levels could partially explain variations in nutrient utilisation and digestive efficiency. However, nutrient digestion, absorption, and utilisation in ruminants are complex processes influenced by multiple factors, including feed composition, animal physiology, and environmental conditions. Therefore, although NDF and NFE provide some information, they alone cannot fully explain the variations observed in nutrient metabolism and digestive efficiency.

Rumen fermentation dynamics

The rumen fermentation (Table 5) showed stable pH values (6.81–6.85), with no significant differences observed between individual TMRs. These values remained within the optimal range (6.0–7.0) for rumen microbial function, demonstrating that each formulation provided adequate buffering capacity and fermentable substrates to maintain a conducive fermentation environment. Ammonia-nitrogen ($N\text{-}NH_3$) concentrations also showed no significant variation, ranging from 9.38 to 10.47 mM, indicating uniform protein degradation rates and stable nitrogen supply for microbial growth in all experimental variants. Total short-chain fatty acid (TSCFA) concentrations were significantly higher *($P < 0.05$)* in TMR-1 and TMR-2 (131.61–132.34 mM) compared to other formulations. This increased TSCFA production indicates more intense fermentation activity, as TSCFA are key by-products of microbial fermentation and reflect the efficiency of substrate degradation and microbial metabolism in the rumen.

Table 5. Total mixed rations (TMR) *in vitro* rumen fermentation dynamics, methane emission, and degradability

Parameter	TMR-1	TMR-2	TMR-3	TMR-4	TMR-5	SEM	*P*-value
pH	6.82	6.81	6.83	6.85	6.84	0.01	NS
NH_3, mM	10.47	10.13	9.78	10.21	9.82	0.28	0.084
TSCFA, mM	131.61^{a}	132.34^{a}	116.54^{b}	112.67^{bc}	106.78^{c}	10.13	0.034
Total gas, ml/g	158.34^{a}	156.34^{a}	147.34^{ab}	149.18^{b}	148.76^{b}	4.49	0.015
Methane, % TSCFA	15.98^{b}	15.87^{b}	15.79^{b}	17.85^{a}	17.98^{a}	0.89	0.023
IVDMD, %	67.11^{a}	67.21^{a}	66.13^{ab}	65.34^{b}	65.35^{b}	0.76	0.043
IVOMD, %	66.25^{a}	66.13^{a}	65.96^{ab}	65.96^{b}	64.48^{b}	0.72	0.036

TMR-1 – pineapple peel to maize husk ratio: 40:0, TMR-2 – pineapple peel to maize husk ratio: 30:10, TMR-3 – pineapple peel to maize husk ratio: 20:20, TMR-4 – pineapple peel to maize husk ratio: 10:30, TMR-5 – pineapple peel to maize husk ratio: 0:40%; IVDMD – *in vitro* dry matter degradability, IVOMD – *in vitro* organic matter degradability, NH_3 – ammonia, TSCFA – total short-chain fatty acids, SEM – standard error of the means; ^{abc} means in the same row without a common superscript are significantly different at $P < 0.05$

Gas production followed a similar trend, as TMR-1 and TMR-2 generated higher *(P* < 0.05) total gas volumes (158.34 and 156.34 mg/g, respectively) than TMR-4 and TMR-5 (149.18 and 148.76 mg/g), suggesting that TMR-1 and TMR-2 contained more fermentable substrates. Methane production, expressed as a percentage of TSCFA, was significantly lower *(P* < 0.05) in TMR-1 (15.98%) and TMR-2 (15.87%) compared to TMR-4 (17.85%) and TMR5 (17.98%) *(P* < 0.05), reflecting more efficient fermentation in TMR-1 and TMR-2. Additionally, TMR-1 and TMR-2 had significantly higher *(P* < 0.05) *in vitro* dry matter degradability (IVDMD; 67.11% and 67.21%, respectively) and *in vitro* organic matter degradability (IVOMD; 66.25% and 66.13%, respectively) compared to TMR-4 and TMR-5.

Discussion

Vastolo et al. (2022) recommend maintaining DM content in TMR silage between 30–35% for optimal fermentation. This range ensures a balanced moisture-to-substrate ratio, which is critical for efficient microbial activity while minimising spoilage risks. The current study's DM recovery rates (96.85–97.33%) align with the quality benchmarks established by Kung et al. (2018), who reported that well-preserved silage typically maintained ≥95% DM recovery, indicating effective preservation with minimal nutrient losses during storage.

The silage pH values (4.06–4.18) observed in this study were below the 4.2 threshold, indicating effective suppression of undesirable microbial activity (Dentinho et al., 2023). These optimal pH levels were supported by lactic acid concentrations ranging from 1.89 to 2.25% DM, demonstrating successful fermentation. As the dominant acid driving pH reduction, the presence of lactic acid at these levels confirms proper preservation conditions and stability during storage (Okoye et al., 2023).

The ammonia nitrogen (N-NH_3) concentrations (1.05–1.53 mM) measured in this study indicated limited proteolysis during ensiling, i.e. effective protein preservation (Muck et al., 2018). The observed values confirm efficient nitrogen retention, with ammonia-nitrogen (N-NH_3) concentrations serving as a reliable indicator of optimal fermentation quality. These results demonstrate that the fermentation process effectively preserved protein, thereby contributing to the overall quality of the silage for livestock feed (Kung et al., 2018).

The high water-soluble carbohydrate (WSC) content in TMR-1 and TMR-2 suggests improved fermentation potential and nutritional value for ruminants. All treatments achieved Fleig point scores within the 81–100 range, indicating excellent silage quality (Kilic, 1986). While Fleig points remained consistent across treatments, the higher residual WSC levels in TMR-1 and TMR-2 demonstrated better preservation of fermentable substrates, which likely contributed to their superior fermentation characteristics (Table 3).

The observed differences in ash content between treatments reflect differences in the mineral composition of the TMR formulations, primarily influenced by the varying proportions of ingredients such as maize husk, which contributes substantially to the inorganic fraction. Since crude ash content represents the mineral component of the feed, these variations are nutritionally relevant for livestock metabolism and growth. Additionally, the ensiling process may further concentrate mineral content through organic matter losses during fermentation (Ernawati and Abdullah, 2021). These treatment-specific mineral profiles have direct implications for animal nutrition, as they affect dietary mineral availability – a critical factor for livestock health and productivity (Ernawati and Abdullah, 2021).

The nutritional composition of agricultural by-products can vary considerably depending on factors such as geographic origin, processing methods, and seasonal availability. Therefore, while the findings of this study provide valuable information, their broader applicability requires careful consideration given the specific by-products and experimental conditions employed. The crude protein (CP) range of 14.02–15.27% DM is consistent with protein requirements for beef cattle nutrition at different production phases. TMR-5, with the highest CP content (15.27%), may be suitable for cattle with elevated protein demands, such as lactating cows or growing-finishing animals (Liu et al., 2019).

The NDF content observed in TMR formulations showed important variation, with TMR-1 (46.06% DM) and TMR-2 (46.36% DM) containing lower levels compared to TMR-4 and TMR-5 (approaching 49% DM). Considering that NDF levels above 40–50% can negatively impact intake due to increased bulk density (Shi et al., 2023), the values obtained for the first two TMRs seem more beneficial. The acid detergent fibre (ADF) content remained relatively consistent across the TMR formulations, ranging from 38.29 to 39.94%. TMR-5 had the highest ADF content (39.94%), indicating a potentially reduced degradability compared to the lower ADF values in TMR-1 and TMR-2 (38.29 and 38.32%, respectively). While these fibre characteristics may affect feed utilization and energy availability (Khurshid et al., 2023), additional research is needed to fully characterize these relationships.

Total digestible nutrient (TDN) values between 65–70% are considered optimal for finishing beef cattle, supporting efficient growth and weight gain. While TDN values were similar between individual variants, TMR-1, showed its highest proportion (69.46%), suggesting superior energy density, potentially enhancing cattle growth performance (Ahn et al., 2019). The pineapple-based TMR-1 demonstrated particularly favourable nutritional characteristics, combining moderate protein (14.02%), lower fibre content (NDF 46.06%, ADF 38.29%), and higher energy availability (TDN 69.46%). This profile makes it especially suitable for growth and finishing phases in beef cattle production. In contrast, the maize husk-based TMRs (TMR-4 and TMR-5) formulations contained higher fibre levels (NDF approaching 49%, ADF up to 39.94%), which could moderately restrict intake and energy utilization while still meeting basic protein and energy requirements for maintenance and growth (Fraval et al., 2024). However, it should be noted that all formulations lacked specific vitamin and mineral fortification, an important consideration for meeting complete nutritional needs, particularly during demanding production stages with higher micronutrient demands.

All TMR formulations maintained rumen pH within the optimal range (6.0–7.0), supporting efficient microbial fermentation and fibre digestion. These stable rumen pH values indicated an appropriate dietary balance of fermentable carbohydrates and buffering capacity, effectively preventing ruminal acidosis (Perez et al., 2024).

Ammonia nitrogen levels of 9.38–10.47 mM reflect adequate nitrogen availability, supporting microbial growth and nutrient absorption in the rumen. The significantly higher TSCFA production in TMR-1 (131.61 mM) and TMR-2 (132.34 mM) demonstrated increased fermentative activity compared to other formulations. These elevated TSCFA levels provide a greater energy supply for ruminants, potentially improving metabolic performance (Liu et al., 2022; Rosani et al., 2024).

The increased gas production in TMR-1 (158.34 mg/g) and TMR-2 (156.34 mg/g) versus TMR-4 (149.18 mg/g) and TMR-5 (148.76 mg/g) (P < 0.05) was indicative of more intense fermentation processes, reflecting greater substrate availability for rumen microbes (Hernández Ruiz et al., 2024). While increased gas output generally reflects more intensive microbial digestion, it does not automatically guarantee improved fermentation efficiency, as it may also correlate with higher methanogenesis (Elghandour et al., 2023; Pangesti et al., 2024). Importantly, TMR-1 (15.98% of TSCFA) and TMR-2 (15.87%) demonstrated significantly lower methane production than TMR-4 (17.85%) and TMR-5 (17.98%) (P < 0.05), indicating both greater fermentation intensity and reduced energy loss in the form of methane (Hernández Ruiz et al., 2024; Pangesti et al., 2024).

The higher *in vitro* degradability of TMR-1 (IVDMD 67.11%, IVOMD 66.25%) and TMR-2 (IVDMD 67.21%, IVOMD 66.13%) compared to other formulations suggested improved nutrient degradability potential. These outcomes aligned with their balanced crude protein (14.02–14.86%) and moderate NDF content (46.06–46.36%), which facilitated microbial fermentation (Tresia et al., 2024). Conversely, the elevated NDF levels in TMR-4 and TMR-5 (47.98–48.98%) were associated with reduced degradability and increased methane production, reflecting less efficient fermentation and energy yield. While these *in vitro* results demonstrate clear advantages of TMR-1 and TMR-2 in terms of degradability and fermentation efficiency, further *in vivo* validation is necessary to assess actual nutrient absorption and feed efficiency in live animals (Irawan et al., 2024).

Conclusions

This study demonstrates that total mixed rations (TMR) formulations with balanced crude protein levels (14.02–14.86%) and moderate neutral detergent fibre content (NDF 46.06–46.36%), particularly TMR-1 (40:0 pineapple peel:maize husk) and TMR-2 (30:10 pineapple peel:maize husk), improve fermentation efficiency, and degradability, while reducing methane emissions compared to TMRs with higher fibre levels. These findings position pineapple peel-based TMRs as nutritionally advantageous options for beef cattle, particularly during the growth and finishing phases. However, the absence of vitamin and mineral supplementation, and the exclusive use of *in vitro* methods precludes full assessment of animal performance. Future research should incorporate vitamin/mineral fortification and *in vivo* trials to validate these findings and optimise TMR formulations for practical application.

Conflict of interest

The Authors declare that there is no conflict of interest.

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