Research Article

Steam Conditioning Improves Physical Quality and Rumen Fermentation of Corn Stover–Cassava Pellets

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Abstract | Open-field burning of corn stover contributes substantially to $PM_{2.5}$ and greenhouse gas emissions in tropical farming systems. Converting this abundant agricultural residue into durable, transportable ruminant feed provides an opportunity to simultaneously reduce residue burning and improve feed resource utilization. This study evaluated the effects of steam conditioning on the physical quality, chemical composition, ruminal fermentation kinetics, and in vitro degradability of corn stover–cassava meal pellets. A 2 × 4 factorial arrangement in a completely randomized design compared two processing methods (non-steamed and steam-conditioned) and four corn stover: cassava meal ratios (45:50, 55:40, 65:30, and 75:20), with all formulations containing 2% urea and 3% molasses. The results showed that steam conditioning reduced fines (5.60 vs. 4.93%) and increased modified pellet durability index (92.81 vs. 94.43%), bulk density (331.93 vs. 339.60 g/L), and pellet hardness (23.06 vs. 25.33 N) ($P < 0.05$), whereas pellet durability index was unaffected. Increasing corn stover inclusion progressively increased crude protein (10.42–12.02% Dry mater: DM), neutral detergent fiber (35.65–46.40% DM), and acid detergent fiber (23.64–33.74% DM), but reduced pellet quality. Steam conditioning also increased the slowly fermentable gas fraction (71.74 vs. 75.92 mL), fractional gas production rate (0.046 vs. 0.055 mL/h), effective gas production (64.52 vs. 68.94 mL), cumulative gas production at 96 h (63.64 vs. 68.44 mL), and estimated metabolizable energy (1.80 vs. 2.09 Mcal/kg DM) ($P < 0.05$), without affecting in vitro dry matter or organic matter digestibility. These findings demonstrate that steam conditioning improved the functional value of corn stover-based pelleted roughage by enhancing ruminal fermentation efficiency without increasing overall nutrient digestibility, providing a practical processing strategy for upgrading agricultural residues into value-added ruminant feed.

Keywords | Corn stover; Steam conditioning; Pellet quality; Gas production kinetics; Ruminal fermentation; In vitro degradability.

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INTRODUCTION

In tropical cropping systems, agricultural residues are frequently disposed of by open-field burning, generating $PM_{2.5}$ and greenhouse gas emissions while forfeiting an abundant source of lignocellulosic biomass with potential value as ruminant feed (Taweesan *et al*., 2025; Blasi *et al*., 2023). In Thailand, large quantities of rice straw, corn stover, sugarcane tops, bagasse, and cassava by-products are produced annually but remain largely underutilized. Valorizing these residues as ruminant feed offers a practical approach to reducing biomass waste, improving feed resource efficiency, and supporting more efficient utilization of agricultural resources within the Bio-Circular-Green (BCG) economy framework (Jusakulvijit *et al*., 2021).

Among these agricultural residues, corn stover represents an abundant lignocellulosic biomass and a readily Available, low-cost roughage resource for ruminants. However, its feeding value is constrained by variable but generally low crude protein content and high concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, and silica, which restrict microbial degradation, voluntary intake, and nutrient digestibility (Yi *et al*., 2026; Cook *et al*., 2016). Previous studies have shown that corn stover and corn silage can successfully replace conventional roughage sources in beef cattle diets when supported by appropriate supplementation and processing strategies (Chea *et al*., 2015; Zhang *et al*., 2022). Biological treatments, such as microbial fermentation, can partially disrupt lignocellulosic structures and improve fiber degradability and ruminal fermentation (Wang *et al*., 2021). However, these approaches often require extended processing periods and additional management inputs, limiting their practical application. Consequently, simple and cost-effective physical processing technologies are needed to improve the feeding value and practical utilization of corn stover.

One promising physical processing strategy is feed pelleting, which converts low-density agricultural residues into compact, durable roughage with improved handling, storage, and transportation efficiency (Jaelani *et al*., 2024). By increasing bulk density while reducing dust generation, feed segregation, and handling losses, pelleting improves feed management in commercial ruminant production systems (Truelock *et al*., 2020). These advantages are particularly important during long-distance cattle transportation, where pelleted roughage provides a convenient source of physically effective fiber to maintain rumen function, stimulate chewing activity and saliva secretion, reduce the risk of ruminal acidosis associated with concentrate feeding, and alleviate transportation-related stress (Flint *et al*., 2014; Earley *et al*., 2017; Scilacci *et al*., 2024). However, pellet quality depends not only on the pelleting process but also on feed formulation and the physicochemical properties of binding ingredients. Therefore, selecting inexpensive ingredients with strong binding capacity is essential for producing durable pelleted roughage while maximizing the value of agricultural residues.

Cassava meal is an attractive natural binder for pelleted feeds because it is widely available in tropical regions, cost-effective, and rich in readily gelatinized starch. Under adequate moisture conditions, cassava starch begins to gelatinize at approximately 60–70°C, absorbing water, swelling, and forming adhesive bridges that strengthen interparticle bonding within the pellet matrix (Muramatsu *et al*., 2014). Partial starch gelatinization enhances pellet integrity by increasing mechanical strength and resistance to breakage during handling and transportation. In addition to promoting starch gelatinization, steam conditioning supplies heat and moisture that facilitate partial softening of plant cell walls, increase fiber plasticity, and improve the accessibility of structural carbohydrates to ruminal microorganisms (Boltz *et al*., 2020). The effectiveness of steam conditioning depends primarily on steam quality, moisture content, particle size, starch characteristics, and conditioner retention time, which collectively determine the extent of starch gelatinization and the resulting pellet durability and hardness (Vukmirović *et al*., 2017).

Although previous studies have investigated corn stover utilization, pelleting technology, biological treatments, and steam conditioning separately, limited information is available on how steam conditioning interacts with corn stover–cassava meal formulations, particularly in pelleted roughage intended for ruminant feeding, to influence pellet physical quality, chemical composition, ruminal fermentation kinetics, estimated metabolizable energy, and in vitro degradability within a single processing system. In particular, the relationship between steam-induced microstructural changes and subsequent fermentation responses has not been clearly established. We hypothesized that steam conditioning would enhance starch gelatinization and interparticle bonding, thereby improving pellet integrity and microbial accessibility to fermentable substrates, resulting in improved ruminal fermentation characteristics without compromising nutrient digestibility. Therefore, this study evaluated the interactive effects of steam conditioning and corn stover-to-cassava meal ratio on pellet physical properties, chemical composition, gas production kinetics, estimated metabolizable energy, and in vitro rumen degradability. The findings provide practical evidence for optimizing corn stover pellet processing to improve feed manufacturing and support the efficient utilization of agricultural residues in ruminant feeding systems.

MATERIALS AND METHODS

Animal ethics

All experimental procedures were conducted in accordance with the institutional guidelines for animal care and use and were approved by the Animal Ethics Committee of Rajamangala University of Technology Lanna under approval no. RMUTL-IACUC 021/2025.

Experimental design

A 2 × 4 factorial arrangement in a completely randomized design (CRD) was used to evaluate the effects of steam conditioning and dietary formulation on the characteristics of corn stover pellets. Factor A comprised two pelleting conditions: non-steam-conditioned and steam-conditioned. Factor B consisted of four corn stover (CS) to cassava meal (CM) ratios: 45:50, 55:40, 65:30, and 75:20. All experimental formulations consisted of corn stover, cassava meal, 2% urea, and 3% molasses, resulting in a total formulation of 100% on an as-fed basis (Table 1). The analyzed chemical composition of the major feed ingredients is presented in Table 1. Corn stover contained 7.41% crude protein, 55.39% neutral detergent fiber (NDF), 38.15% acid detergent fiber (ADF), and 3,802.43 kcal/kg gross energy, reflecting its fibrous nature as the primary roughage source. Cassava meal contained 2.53% crude protein, 12.52% NDF, 6.51% ADF, and 3,305.80 kcal/kg gross energy and served as a starch-rich ingredient to facilitate particle binding during steam conditioning. Consequently, eight dietary treatments were established. Each treatment was manufactured as three independent pelleting batches, with each batch considered an independent experimental unit. All experimental diets were formulated to contain at least 10% crude protein to provide adequate nitrogen availability for ruminal microbial activity.

Diets and pelleting process

The pelleting procedure was adapted from Teixeira Netto et al. (2019). Sundried corn stover was ground using a hammer mill equipped with a 2-mm screen, whereas cassava meal was sieved to obtain a 0.5-mm particle size before formulation. The ingredients were weighed according to the dietary compositions shown in Table 1. Urea (2%) and molasses (3%) were dissolved in 500 mL of water before mixing with the remaining ingredients to ensure uniform distribution throughout the diet. The mixed mash was subsequently processed under either non-steam-conditioned or steam-conditioned pelleting according to the assigned treatment. The prepared mixture was subsequently divided into two portions according to processing treatment. The first portion was processed without steam conditioning, whereas the second portion was subjected to steam conditioning using a laboratory-scale steam chamber under atmospheric pressure for 1 min after steam generation. This procedure was modified from Khongphetsak *et al.* (2019). The moisture content of the mash before and after steam conditioning was 12.5 and 18.0%, respectively. Both processed mixtures were then pelleted using a pelletizer equipped with a 6-mm die (die length-to-diameter ratio = 6:1) to produce pellets with an approximate length of 25 mm. The pelleting production rate was approximately 50 kg/h. The extended drying period (72 h) was applied to obtain stable dry matter values for accurate determination of chemical composition, which is a standard practice in feed analysis laboratories (AOAC, 1995), gas production kinetics, and in vitro degradability.

Physical property and chemical composition analysis

After drying, pellet morphology and particle binding characteristics were examined using a stereo microscope at 1× and 4× magnifications. Physical pellet quality was evaluated according to Fahrenholz (2012). Fine percentage was determined by separating particles passing through a 2.0-mm sieve and calculated as follows: Fine percentage (%) = (weight of fine / weight of pelleted feed) × 100. Pellet hardness was determined using a texture analyzer (TA. XT. Plus, Stable Micro Systems, Surrey, UK) fitted with an SMS P/36R compression probe at a test speed of 10 mm/min. The maximum force (N) required to fracture each pellet was recorded as pellet hardness. Pellet durability was determined using a Holmen NHP100 pellet tester (TekPro Ltd., Norfolk, UK). Approximately 500 g of pellets after removal of fines were subjected to the durability test for 60 s. After tumbling, the pellets were sieved to remove generated fines and reweighed. Pellet durability index (PDI) was calculated as: PDI (%) = (weight of pellets after tumbling / weight of pellets before tumbling) × 100 (American Society of Agricultural Engineers, 1997). The modified pellet durability index (MPDI) was determined using the same procedure as the PDI test, except that five hexagonal bolts were added to the 500-g sample during the 2-min tumbling period to increase impact forces and provide a more rigorous evaluation of pellet durability (Cutlip *et al.*, 2008). The MPDI test was applied as an additional impact resistance evaluation to assess pellet stability under increased mechanical stress. MPDI was calculated as: MPDI (%) = (weight of pellets after tumbling / weight of pellets before tumbling) × 100 (American Society of Agricultural Engineers, 1997). Bulk density was determined according to Nurkowski (2025) using the following equation: Bulk density (d) = mass (g) / volume (L). In addition, pellet samples remaining after physical quality evaluation, including both intact pellets and fines, were ground to pass through a 0.5-mm screen prior to chemical analysis. Dry matter (DM), ash, ether extract (EE), and crude protein (CP) contents were determined according to AOAC (1995) procedures. Fiber fractions, including neutral detergent fiber (NDF) and acid detergent

Table 1: Ingredient composition and calculated crude protein of pelleted diets under different steam treatments and corn stover to cassava meal ratios.

Factor A (Heat treated process)	Non-treated				Steam-treated			
Factor B (CS:CM ratio) (%)	Corn stover (CS):Cassava meal (CM)				Corn stover (CS):Cassava meal (CM)			
	45:50	55:40	65:30	75:20	45:50	55:40	65:30	75:20
Interaction A x B	T1	T2	T3	T4	T5	T6	T7	T8
Ingredient composition (%)								
Corn stover	45.00	55.00	65.00	75.00	45.00	55.00	65.00	75.00
Cassava meal	50.00	40.00	30.00	20.00	50.00	40.00	30.00	20.00
Urea	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Molasses	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Estimated feed cost (USD/kg)	0.151	0.137	0.124	0.111	0.151	0.137	0.124	0.111
Calculated chemical value (%DM)								
Dry matter (%)	89.72	89.71	89.70	89.69	89.72	89.71	89.70	89.69
Organic matter	93.18	92.72	92.27	91.81	93.18	92.72	92.27	91.81
Crude protein	10.49	10.97	11.46	11.95	10.49	10.97	11.46	11.95
Ether extract	1.58	1.77	1.95	2.13	1.58	1.77	1.95	2.13
Ash	4.82	5.28	5.73	6.19	4.82	5.28	5.73	6.19
Crude fiber	15.59	18.40	21.21	24.02	15.59	18.40	21.21	24.02
NDF	31.19	35.48	39.76	44.05	31.19	35.48	39.76	44.05
ADF	20.42	23.59	26.75	29.91	20.42	23.59	26.75	29.91
ADL	3.57	4.01	4.45	4.89	3.57	4.01	4.45	4.89
ME, (Mcal/kg)	2.57	2.42	2.28	2.13	2.57	2.42	2.28	2.13

*TDN=Total digestible nutrients, NDF = Neutral detergent fiber, ADF - Acid detergent fiber, ADL - Acid detergent lignin, and ME = Metabolizable energy.

fiber (ADF), were analyzed following the method of Van Soest *et al*. (1991).

In vitro gas production kinetics and digestibility analysis

In vitro gas production kinetics and digestibility were conducted according to the method of Makkar *et al.* (1995). Rumen fluid was collected from three slaughtered beef steers within 5 min postmortem. The fluid from each animal was filtered through four layers of cheesecloth, pooled in equal volumes, and maintained at 39°C under anaerobic conditions during transport to the laboratory. The pooled fluid was then used as the microbial inoculum. Artificial saliva solution was prepared following Menke and Steingass (1988) by mixing distilled water, buffer solution, macro-mineral solution, micro-mineral solution, and resazurin indicator in a 5-L flask under continuous CO_2 flushing to maintain anaerobic conditions. The solution was stirred continuously at 39°C using a magnetic stirrer, followed by the addition of a reducing solution until the indicator changed from blue to pink, indicating anaerobic conditions. Fresh rumen fluid was subsequently mixed with the artificial saliva solution under continuous CO_2 flushing and used for in vitro gas production kinetics and digestibility analyses.

Following inoculation, fermentation bottles were incubated in a hot air oven at 39°C, and cumulative gas production was recorded every 1 h during the first 12 h, every 3 h from 12 to 24 h, and every 6 h thereafter until 96 h of incubation. Gas production data were fitted to the nonlinear model of Ørskov and McDonald (1979) using the FRCURVE nonlinear regression program implemented in Microsoft Excel (Chen *et al*., 2004) to estimate gas production kinetics parameters according to the following equation: $Y=a+b(1-e^{-ct})$ where Y = cumulative gas production at time t, a = gas production associated with the soluble fraction or initial fermentation phase (mL), b = gas production from the insoluble but fermentable fraction (mL), c = rate constant of gas production (mL/h), and t = incubation time (h). Because negative values were obtained in some treatments, these values were interpreted as indicating an initial fermentation lag phase rather than a reduction in soluble substrate availability. Potential gas production (d) was subsequently estimated as: $d=|a|+b$. Effective gas production (EP) was calculated according to Menke and Steingass (1988). In addition, in vitro dry matter degradability (IVDMD) and in vitro organic matter degradability (IVOMD) were determined at 24 and 48 h of incubation. Metabolizable energy (ME) was estimated rather than directly measured using the modified equation of Menke and Steingass (1988): ME (Mcal/kg DM) = [0.157(G24h) + 0.0084(CP) + 0.022(EE) – 0.0081(CC) + 1.06] / 4.184 where G24h is gas production at 24 h incubation, CP is crude protein, EE is ether extract, and CC is crude ash content.

Statistical analysis

Data were analyzed using a two-way analysis of variance (ANOVA) based on a 2 × 4 factorial arrangement in a completely randomized design (CRD), with steam conditioning and corn stover-to-cassava meal ratio as fixed effects. Prior to ANOVA, data were evaluated for normality and homogeneity of variance. The statistical model used for data analysis was: $Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$ where Y_{ijk} = observed value, μ = overall mean, A_i = effect of steam-conditioning treatment (i=1–2), B_j = effect of corn stover –cassava meal ratio (j=1–4), $(AB)_{ij}$ = interaction effect between factors A and B, and e_{ijk} = experimental error. When significant differences were detected, treatment means were compared using Tukey's honestly significant difference test (Tukey's HSD) at P < 0.05 according to Steel and Torrie (1980). Statistical analyses were performed using the PROC GLM procedure of SAS University Edition (SAS Institute Inc., Cary, NC, USA).

RESULT

Physical property and chemical composition

Steam conditioning (Factor A) and the corn stover meal (CS) ratio (Factor B) significantly affected pellet physical characteristics (Table 2). Significant interactions were detected for fines content (P = 0.021) and pellet hardness (P = 0.042), whereas PDI, MPDI, and bulk density were unaffected by the interaction (P > 0.05). Steam conditioning reduced fines (4.93 vs. 5.60%; P < 0.001) and increased MPDI (94.43 vs. 92.81%; P < 0.001), bulk density (339.60 vs. 331.93 g/L; P = 0.011), and pellet hardness (25.33 vs. 23.06 N; P < 0.001), while PDI remained unchanged (P = 0.816). Pellet hardness was highest in the 45 and 55% corn stover inclusion treatments (Figure 1). Increasing corn stover from 45 to 75% increased fines (1.52–9.15%) and bulk density (265.15–381.98 g/L) but reduced PDI, MPDI, and pellet hardness (P < 0.001). The stereomicroscopic observations (Figure 2) were consistent with these results. Non-steamed pellets exhibited rough surfaces, exposed fibers, and visible void spaces, whereas steam-conditioned pellets showed smoother surfaces and fewer visible voids, particularly in the 45CS:50CM and 55CS:40CM formulations.

Steam conditioning and the CS ratio significantly affected dietary chemical composition (Table 3). Significant interactions were observed for DM, CP, NDF, ADF, and ash (P < 0.001), whereas EE was unaffected (P = 0.228). Moreover, steam conditioning slightly increased the apparent concentrations of CP (11.42 vs. 11.14% DM), NDF (43.02 vs. 40.01% DM), and ADF (30.45 vs. 27.06% DM) (P < 0.001), with no effect on DM, ash, or EE (P > 0.05). Increasing corn stover inclusion altered the nutrient profile of the pellets, resulting in decreased DM (95.34–93.44% DM) and ash (11.97–9.86% DM), while increasing CP (10.42–12.02% DM), NDF (35.65–46.40% DM), ADF (23.64–33.74% DM), and EE (2.15–2.78% DM) (P < 0.001). The highest CP, NDF, and ADF concentrations were observed in the steam-conditioned 75CS:20CM diet (Figure 3).

In vitro gas production kinetics and digestibility

Steam conditioning and the CS:CM ratio affected in vitro gas production kinetics differently (Table 4). A significant interaction was detected only for gas production from the rapidly fermentable fraction (a; P = 0.008), whereas no interactions were observed for the slowly fermentable fraction (b), fractional rate of gas production (c), potential gas production (d), effective gas production, or cumulative gas production at 96 h (P > 0.05). Steam conditioning significantly increased b (75.92 vs. 71.74 mL; P = 0.047), c (0.055 vs. 0.046 mL/h; P = 0.006), effective gas production (68.94 vs. 64.52 mL; P = 0.025), and cumulative gas production at 96 h (68.44 vs. 63.64 mL; P = 0.021), whereas a and d were unaffected (P > 0.05). Increasing corn stover inclusion significantly decreased a from −5.07 to −9.27 mL (P < 0.001), resulting in more negative a value that may indicate an extended initial fermentation lag phase, while b, c, d, effective gas production, and cumulative gas production remained unchanged (P > 0.05). Consistent with these results, cumulative gas production curves (Figure 4) showed that steam-conditioned diets produced greater gas volumes throughout incubation than non-steamed diets, with the highest cumulative gas production observed for the steam-conditioned 75CS:20CM diet during the 96-h incubation period.

No interaction between steam conditioning and the CS:CM ratio was detected for IVDMD, IVOMD, or metabolizable energy (ME) (Table 5; P > 0.05). Steam conditioning did not affect IVDMD or IVOMD at either incubation time or their mean values (P > 0.05), but significantly increased estimated ME from 1.80 to 2.09 Mcal/kg DM (P = 0.007). Increasing corn stover inclusion significantly reduced IVDMD at 24 h (69.52 to 55.96%; P < 0.001), mean IVDMD (69.83 to 60.38%; P = 0.009), IVOMD at 24 h (72.51 to 59.47%; P < 0.001), and mean IVOMD (74.73 to 64.18%; P = 0.006). However, neither

Table 2: Effects of steam treatment and corn stover to cassava meal ratios on physical quality characteristics of pelleted diets.

Items	Fines (%)	PDI (%)	MPDI (%)	Bulk Density (g/L)	Pellet hardness (N)
Interaction A x B (Heat treated process × Corn stover (CS): Cassava meal (CM) ratio)					
Non-steamed × 45CS: 50CM	1.692^{d}	97.641	96.199	264.150	25.595^{b}
Non-steamed × 55CS: 40CM	2.648^{c}	96.685	95.767	338.500	24.340^{b}
Non-steamed × 65CS: 30CM	8.762^{a}	91.238	90.973	349.650	21.690^{cd}
Non-steamed × 75CS: 20CM	9.307^{a}	90.360	88.279	375.400	20.605^{d}
Steamed × 45CS: 50CM	1.348^{d}	98.652	97.883	266.148	28.730^{a}
Steamed × 55CS: 40CM	1.870^{d}	98.130	97.443	346.124	25.745^{b}
Steamed × 65CS: 30CM	7.530^{b}	92.470	92.561	357.578	24.515^{b}
Steamed × 75CS: 20CM	8.990^{a}	91.010	89.822	388.556	22.310^{c}
Factor A (Heat treated)					
Non-steamed	5.603^{A}	93.981	92.805^{B}	331.925^{b}	23.058^{B}
Steam-Treated	4.934^{B}	95.066	94.427^{A}	339.601^{a}	25.325^{A}
Factor B (CS:CM ratio) (%)					
45CS: 50CM	1.520^{D}	98.146^{A}	97.041^{A}	265.149^{D}	27.163^{A}
55CS: 40CM	2.259^{C}	97.408^{A}	96.605^{A}	342.312^{C}	25.043^{B}
65CS: 30CM	8.146^{B}	91.854^{B}	91.767^{B}	353.614^{B}	23.103^{C}
75CS: 20CM	9.148^{A}	90.685^{B}	89.051^{C}	381.978^{A}	21.458^{D}
SEM	0.716	0.702	0.731	9.133	0.518
P-value					
Factor A (Heat treated process)	<0.001	0.816	<0.001	0.011	<0.001
Factor B (CS: CM ratio)	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction A x B	0.021	0.944	0.998	0.554	0.042

PDI = Standard pellet durability index, MPDI = Modified pellet durability index $^{A-D}$ Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test (P<0.01), $^{a-d}$; Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test (P<0.05), SEM; standard error of the mean.

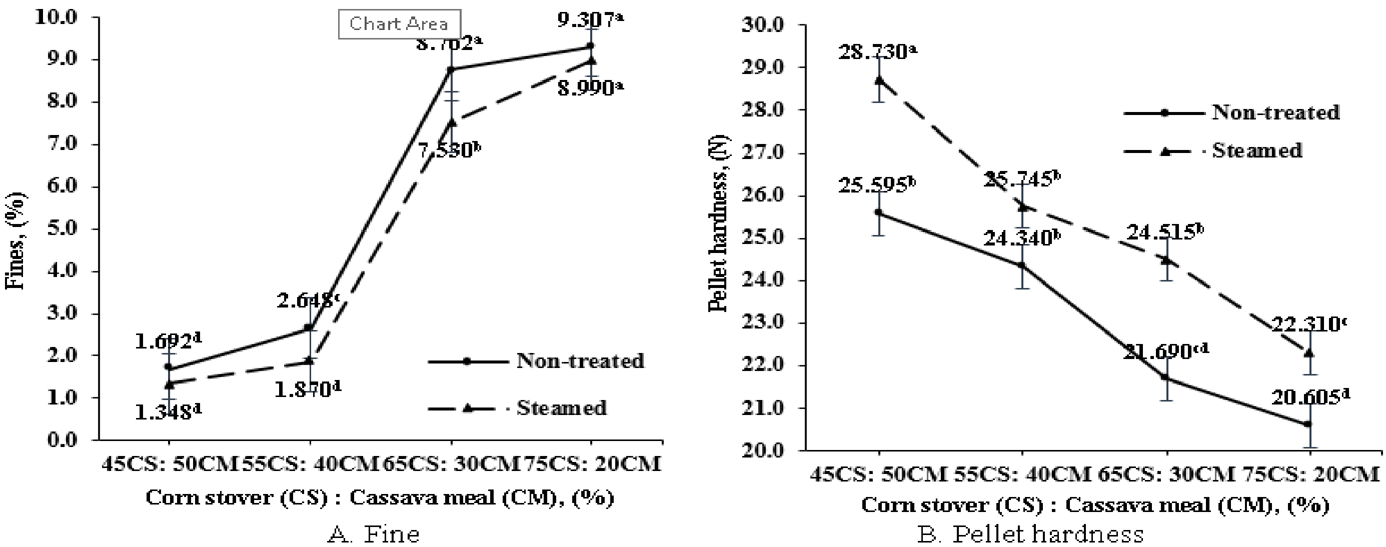

Figure 1: Interactive effects of steam conditioning and corn stover to cassava meal ratios on fine percentage (A) and hardness of pelleted diets (B). $^{A-D}$ Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test (P<0.01).

Figure 2 : Stereomicroscope micrographs of pelleted diets prepared with varying roughage-to-concentrate ratios under non-steamed and Steamed conditions (CS= corn stover and CM=cassava meal). Microstructural features of pellet surface morphology, including particle aggregation, porosity, and the extent of structural modification, are presented at two magnification levels (left: 1X; right: 4X)

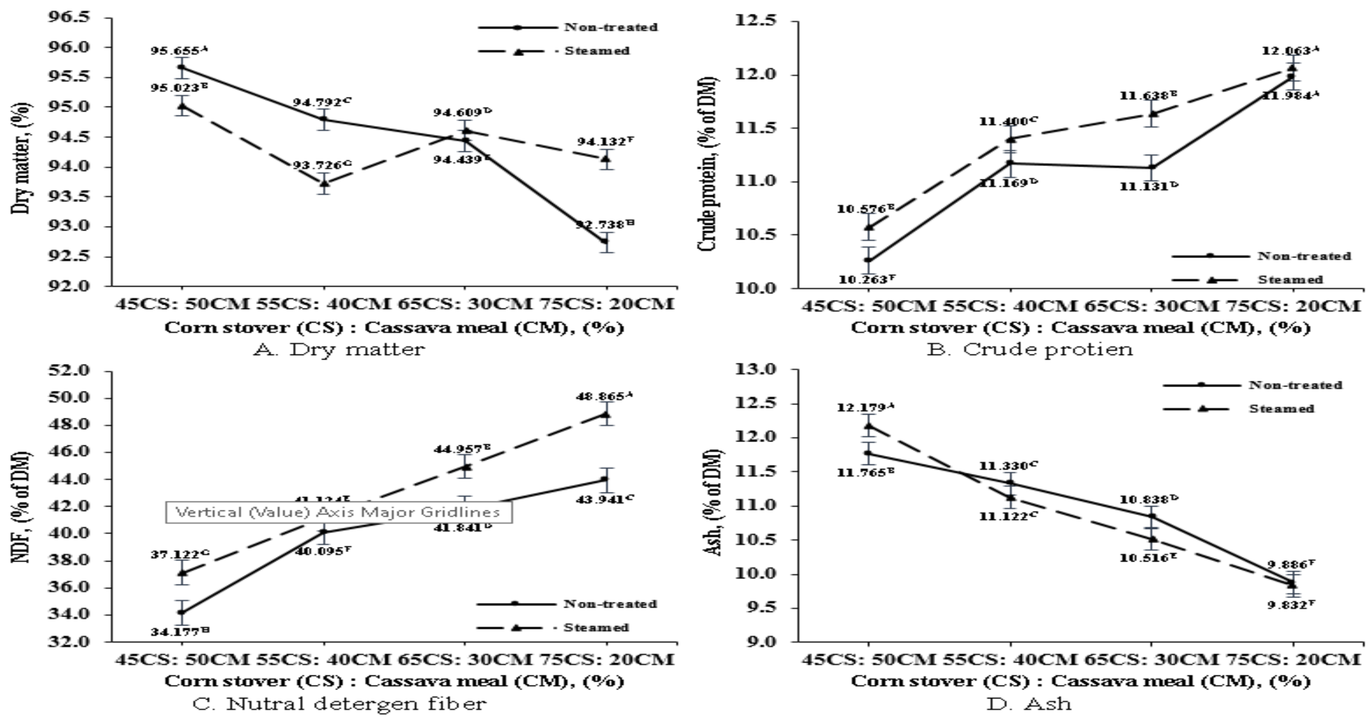

Figure 3: Interactive effects of steam conditioning and corn stover to cassava meal ratios on the chemical composition of pelleted diets. A-H Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test (P < 0.01).

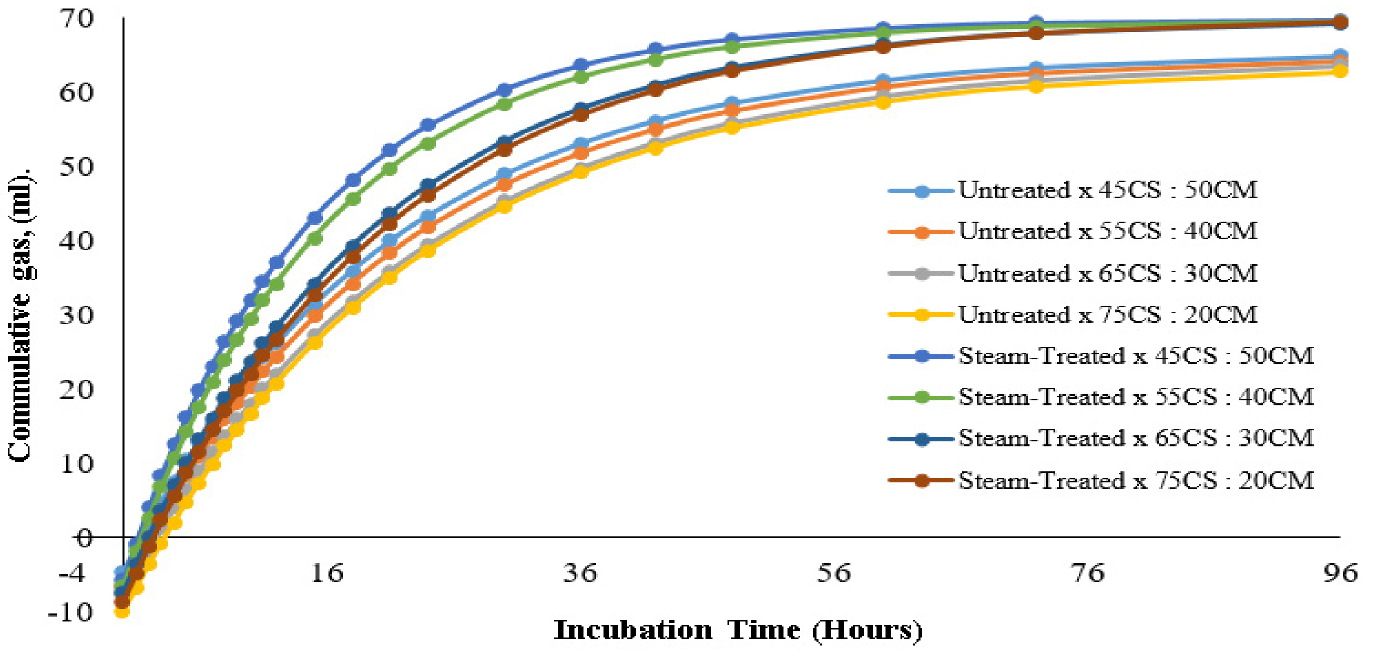

Figure 4: Cumulative gas production profiles of pelleted diets containing different corn stover to cassava meal ratios with and without steam treatment during in vitro incubation

Table 3: Interactive effects of steam treatment and corn stover to cassava meal ratios on dry matter and chemical composition of pelleted diets.

Items	**Chemical composition (% of DM)**					
	Dry matter (%)	Crude protein	NDF	ADF	Ash	Ether extract
Interaction A x B (Heat treated process × Corn stover (CS): Cassava meal (CM) ratio) (%)						
Non-steamed × 45CS: 50CM	95.655^{A}	10.263^{F}	34.177^{H}	22.555^{F}	11.765^{B}	2.157
Non-steamed × 55CS: 40CM	94.792^{C}	11.169^{D}	40.095^{F}	25.361^{E}	11.330^{C}	2.228
Non-steamed × 65CS: 30CM	94.439^{E}	11.131^{D}	41.841^{D}	29.795^{C}	10.838^{D}	2.248
Non-steamed × 75CS: 20CM	92.738^{H}	11.984^{A}	43.941^{C}	30.536^{C}	9.886^{F}	2.803
Steamed × 45CS: 50CM	95.023^{B}	10.576^{E}	37.122^{G}	24.722^{E}	12.179^{A}	2.146
Steamed × 55CS: 40CM	93.726^{G}	11.400^{C}	41.124^{E}	27.198^{D}	11.122^{C}	2.125
Steamed × 65CS: 30CM	94.609^{D}	11.638^{B}	44.957^{B}	32.922^{B}	10.516^{E}	2.660
Steamed × 75CS: 20CM	94.132^{F}	12.063^{A}	48.865^{A}	36.944^{A}	9.832^{F}	2.761
Factor A (Heat treated)						
Non-steamed	94.406	11.137^{B}	40.014^{B}	27.062^{B}	10.955	2.359
Steam-Treated	94.373	11.419^{A}	43.017^{A}	30.447^{A}	10.912	2.423
Factor B (CS:CM ratio) (%)						
45CS: 50CM	95.339^{A}	10.419^{C}	35.650^{D}	23.638^{D}	11.972^{A}	2.151^{B}
55CS: 40CM	94.259^{C}	11.284^{B}	40.609^{C}	26.280^{C}	11.226^{B}	2.176^{B}
65CS: 30CM	94.524^{B}	11.384^{B}	43.399^{B}	31.359^{B}	10.677^{C}	2.454^{AB}
75CS: 20CM	93.435^{D}	12.023^{A}	46.403^{A}	33.740^{A}	9.859^{D}	2.782^{A}
SEM	0.172	0.124	0.895	0.927	0.165	0.069
P-value						
Factor A (Heat treated process)	0.094	<0.001	<0.001	<0.001	0.311	0.504
Factor B (CS: CM ratio)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction A x B	<0.001	0.001	<0.001	<0.001	<0.001	0.228

A-H Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test ($P < 0.01$). SEM = standard error of the mean; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Table 4: Effects of steam treatment and corn stover to cassava meal ratios on in vitro gas production kinetics, effective gas production, and cumulative gas production.

Items	Kinetics of gas production				Effective gas production	Cumulative gas 96 h (ml)
	a (ml)	b (ml)	c(ml/h)	d(ml)		
Interaction A x B (Heat treated process × Corn stover (CS): Cassava meal (CM) ratio) (%)						
Non-steamed × 45CS: 50CM	-4.690^{A}	70.180	0.048	74.870	65.490	64.783
Non-steamed × 55CS: 40CM	-6.490^{BC}	70.980	0.047	77.470	64.490	63.733
Non-steamed × 65CS: 30CM	-7.770^{CD}	72.280	0.044	80.050	64.510	63.440
Non-steamed × 75CS: 20CM	-9.940^{E}	73.530	0.045	83.470	63.590	62.607
Steamed × 45CS: 50CM	-5.445^{AB}	73.769	0.062	79.214	68.325	67.960
Steamed × 55CS: 40CM	-6.559^{BC}	74.137	0.059	80.695	67.578	67.181
Steamed × 65CS: 30CM	-7.311^{C}	77.049	0.052	84.360	69.738	69.182
Steamed × 75CS: 20CM	-8.605^{D}	78.738	0.049	87.343	70.133	69.445
Factor A (Heat treated)						
Non-steamed	-7.223	71.743^{b}	0.046^{B}	78.965	64.520^{b}	63.641^{b}
Heat treated	-6.980	75.923^{a}	0.055^{A}	82.903	68.943^{a}	68.442^{a}
Factor B (CS:CM ratio) (%)						
45CS: 50CM	-5.067^{A}	71.975	0.055	77.042	66.907	66.372
55CS: 40CM	-6.524^{B}	72.558	0.053	79.083	66.034	65.457
65CS: 30CM	-7.541^{C}	74.665	0.048	82.205	67.124	66.311
75CS: 20CM	-9.273^{D}	76.134	0.047	85.407	66.861	66.026
SEM	0.339	0.988	0.002	1.176	0.894	0.947
P-value						
Factor A (Heat treated process)	0.210	0.047	0.006	0.081	0.025	0.021
Factor B (CS: CM ratio)	<0.001	0.429	0.199	0.061	0.974	0.985
Interaction A x B	0.008	0.979	0.671	0.997	0.862	0.878

$^{A-D}$ Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test (P < 0.01), ^{a,b} Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test (P < 0.05), SEM = standard error of the mean, a = the intercept and ideally reflects the fermentation of the soluble fraction (ml); b = the fermentation of the insoluble but fermentable fraction (ml); c = rate of gas production ((mL/h); d = |a| + b = potential gas production (mL).

48-h digestibility nor ME differed among CS:CM ratios (P > 0.05). Overall, steam conditioning increased gas production characteristics and estimated ME values without affecting IVDMD or IVOMD, whereas increasing corn stover inclusion reduced short-term in vitro digestibility responses.

DISCUSSION

Steam conditioning markedly improved the physical quality of corn stover–cassava meal pellets, as evidenced by higher MPDI, pellet hardness, and bulk density together with reduced fines generation, indicating stronger interparticle cohesion during pelleting. Although starch gelatinization was not directly quantified, these responses are consistent with the established effects of hydrothermal conditioning, whereby heat and moisture increase starch plasticization and improve the binding capacity of starch-rich ingredients such as cassava meal, resulting in stronger particle bonding and greater pellet durability (Ma *et al*., 2022; Teixeira Netto *et al*., 2019). Under adequate moisture conditions, cassava starch begins to gelatinize at approximately 60–70°C, promoting partial starch gelatinization and the formation of adhesive bridges between adjacent particles, thereby strengthening pellet structure (Muramatsu *et al*., 2014). Hydrothermal conditioning can increase fiber plasticity and particle deformability by modifying moisture distribution and cell-wall physical properties, thereby improving particle packing during compression without necessarily altering lignin concentration. Consequently, the improvements observed in the present study are more likely attributable to enhanced physical cohesion within the pellet matrix than to alterations in fiber chemistry. These findings are consistent with previous reports demonstrating that steam conditioning enhances pellet durability by strengthening interparticle bonding and reducing pellet breakage during manufacture (Cutlip *et al*., 2008; Raji *et al*., 2008).

Table 5: Effects of steam treatment and corn stover to cassava meal ratios on in vitro dry matter digestibility, organic matter digestibility, and metabolizable energy of pelleted diets.

Items	IVDMD (%)			IVOMD (%)			ME, Mcal/ kg DM
	24 h	48 h	mean	24 h	48 h	mean	
Interaction A x B (Heat treated process × Corn stover (CS): Cassava meal (CM) ratio) (%)							
Non-steamed × 45CS: 50CM	69.150	70.028	69.761	70.388	76.419	73.403	1.888
Non-steamed × 55CS: 40CM	62.030	68.120	65.370	66.660	73.390	70.120	1.831
Non-steamed × 65CS: 30CM	61.251	66.365	63.808	64.340	71.080	67.848	1.745
Non-steamed × 75CS: 20CM	54.460	62.663	60.057	58.340	67.296	63.945	1.723
Steamed × 45CS: 50CM	69.892	70.640	69.890	74.640	77.480	76.060	2.189
Steamed × 55CS: 40CM	63.987	68.278	66.132	69.224	73.560	71.307	2.114
Steamed × 65CS: 30CM	61.260	67.370	64.310	64.616	71.660	68.000	2.049
Steamed × 75CS: 20CM	57.451	66.970	60.710	60.593	70.480	64.410	2.004
Factor A (Heat treated)							
Non-steamed	61.723	66.794	64.749	64.932	72.046	68.829	1.797^{B}
Steam-Treated	63.148	68.315	65.261	67.268	73.295	69.944	2.089^{A}
Factor B (CS:CM ratio) (%)							
45CS: 50CM	69.521^{A}	70.334	69.826^{A}	72.514^{A}	76.950	74.732^{A}	2.039
55CS: 40CM	63.009^{B}	68.199	65.75^{AB}	67.94^{AB}	73.475	70.71^{AB}	1.972
65CS: 30CM	61.256^{BC}	66.868	64.05^{AB}	64.47^{BC}	71.370	67.92^{AB}	1.897
75CS: 20CM	55.956^{C}	64.817	60.384^{B}	59.467^{C}	68.888	64.178^{B}	1.863
SEM	1.226	0.890	1.001	1.254	1.025	1.111	0.052
P-value							
Factor A (Heat treated process)	0.387	0.411	0.767	0.187	0.524	0.544	0.007
Factor B (CS: CM ratio)	<0.001	0.216	0.009	<0.001	0.053	0.006	0.566
Interaction A x B	0.916	0.842	0.999	0.874	0.946	0.961	0.999

$^{A-C}$ Means within the same column with different superscript letters differ significantly according to Tukey's honestly significant difference (HSD) test ($P < 0.01$). SEM = standard error of the mean; IVDMD = in vitro dry matter digestibility; IVOMD = in vitro organic matter digestibility; ME = metabolizable energy.

The effectiveness of steam conditioning was strongly dependent on feed formulation. Increasing corn stover in clusion from 45 to 75% progressively increased fines while reducing pellet durability, hardness, and bulk density, indicating that the beneficial effects of conditioning diminished as the proportion of fibrous material increased. Replacing cassava meal with corn stover reduced the availability of thermoplastic starch required for binding bridge formation during compression (Muramatsu *et al*., 2014), thereby limiting starch gelatinization and reducing the number of adhesive bridges formed between particles. Whereas the lignocellulosic fibers exhibited greater elastic recovery ("spring-back"), producing a less cohesive pellet matrix with increased internal porosity and susceptibility to fragmentation. Consistent with this interpretation, cassava-based pellets have been reported to exhibit superior durability because of the high amylopectin content of cassava starch, and adequate moisture availability during conditioning can promote starch-mediated binding and improve pellet durability. Stereomicroscopic observations further supported these physical responses: steam-conditioned pellets exhibited smoother and more compact surfaces, whereas diets containing 65–75% corn stover displayed pronounced fiber tearing, irregular particle organization, and numerous internal voids, which may contribute to reduced structural cohesion and greater susceptibility to fragmentation. Similar relationships between pellet microstructure and mechanical stability have been reported previously (Tumuluru *et al*., 2020). Collectively, these findings demonstrate that pellet quality depends on the interaction between hydrothermal processing and ingredient composition, where adequate starch availability is essential for steam conditioning to maximize pellet durability while increasing the utilization of agricultural residues.

The chemical composition of the pelleted diets was governed primarily by ingredient composition, whereas steam conditioning exerted comparatively smaller effects. The progressive increase in crude protein with increasing corn stover inclusion primarily reflected differences in the inherent chemical composition of the feed ingredients rather than any direct effect of steam conditioning. Although corn stover is generally considered a low-protein roughage, its crude protein concentration can vary considerably (approximately 5.3–12.1% DM) depending on cultivar, maturity stage, fertilization, and harvesting conditions (Tuturoong *et al*., 2020). In the present study, the analyzed corn stover contained a higher crude protein concentration than cassava meal, thereby explaining the observed increase in dietary crude protein as the proportion of corn stover increased. Likewise, the higher NDF and ADF concentrations observed after steam conditioning should be interpreted as a concentration effect rather than an actual increase in structural carbohydrates. During hydrothermal conditioning, moisture addition and subsequent drying may reduce the proportion of soluble constituents or redistribute nutrients on a dry matter basis, thereby increasing the relative proportion of insoluble fiber fractions without increasing the absolute fiber content (He *et al*., 2015). Similar responses have been reported by Liu *et al*. (2019), who showed that conventional steam treatment increased the proportional fiber fraction, whereas more intensive steam-explosion processing produced greater structural modification of lignocellulosic biomass. Therefore, analytical increases in NDF and ADF do not necessarily indicate lower nutritional value. Instead, steam conditioning may partially disrupt hydrogen bonding within the lignocellulosic matrix, increase cell-wall porosity, and improve microbial access to structural carbohydrates, thereby enhancing fermentability despite minimal changes in chemically determined fiber concentrations (Wang *et al*., 2020).

Steam conditioning combined with increasing corn stover inclusion significantly influenced ruminal fermentation characteristics. The unchanged rapidly fermentable fraction (a), together with the significant increases in the slowly fermentable fraction (b), fractional rate of gas production (c), effective gas production, and cumulative gas production, indicate that hydrothermal conditioning primarily improved the accessibility of structural carbohydrates rather than increasing the amount of readily fermentable substrate. Previous studies have shown that heat and moisture collectively modify lignocellulosic structures, alter starch physicochemical properties, and increase microbial accessibility to structural carbohydrates (Ma *et al*., 2022; Srakaew *et al*., 2021). Rather than acting through a single mechanism, these hydrothermal modifications likely acted synergistically to accelerate microbial fermentation, explaining the greater gas production kinetics observed in the present study. Despite these improvements, the absence of significant differences in the rapidly fermentable fraction (a) indicates that steam conditioning did not substantially increase the availability of soluble substrates, but instead enhanced the utilization of slowly fermentable structural carbohydrates. Although fermentation parameters (b), (c), and (d) were not significantly affected by increasing corn stover inclusion, their numerical increases suggest a gradual transition toward slower and more sustained fermentation. Such fermentation patterns are typical of lignocellulosic substrates, in which microbial colonization and enzymatic hydrolysis of cellulose and hemicellulose occur progressively over time, providing a continuous supply of fermentable substrates for fibrolytic microorganisms rather than a rapid release of soluble carbohydrates (Wang *et al*., 2020). Consequently, improvements in fermentation characteristics were more closely associated with enhanced substrate accessibility than with extensive structural degradation of the fiber matrix. This interpretation indicates that steam conditioning affected fermentation-related responses rather than directly demonstrating improved fermentation efficiency.

Steam conditioning did not significantly affect IVDMD or IVOMD at either 24 or 48 h, indicating that the mild hydrothermal treatment applied in the present study was insufficient to extensively disrupt the lignin–carbohydrate complex, which remains the principal barrier limiting microbial degradation of lignocellulosic biomass (Zhang *et al*., 2022; Jami *et al*., 2014). Although hydrothermal conditioning partially modified the lignocellulosic matrix and improved physical accessibility, it did not generate the extensive cell-wall disruption or porous "honeycomb-like" structure produced by more severe pretreatments such as steam explosion (Ma *et al*., 2022). Consequently, microbial access was improved without substantially increasing the overall extent of fiber degradation. Despite the similar IVDMD and IVOMD values, steam conditioning significantly increased gas production kinetics and estimated metabolizable energy (ME), indicating that hydrothermal treatment altered the rate and efficiency of ruminal fermentation rather than the total extent of substrate degradation. Partial starch gelatinization together with structural loosening of the feed matrix likely reduced physical barriers to microbial colonization, enabling ruminal microorganisms to access and ferment the readily digestible fraction more rapidly without substantially increasing degradation of structural carbohydrates (He *et al*., 2015; Ma *et al*., 2022). Consequently, microbial fermentation proceeded more rapidly despite similar total substrate disappearance. This indicates that steam conditioning primarily shortened the lag phase and accelerated microbial colonization and utilization of accessible substrates rather than increasing the ultimate extent of fiber degradation. Because the Menke and Steingass equation predicts ME primarily from gas production, the enhanced fermentation kinetics resulted in higher estimated ME values despite unchanged IVDMD and IVOMD. These findings therefore indicate that steam conditioning improved fermentation efficiency without increasing the overall extent of substrate degradation. Similar findings have been reported for hydrothermal processing, which can improve microbial accessibility and fermentation characteristics of lignocellulosic substrates without necessarily increasing total digestibility (Wang *et al*., 2020). By contrast, more intensive pretreatments, including steam explosion and alkaline treatment, extensively disrupt lignocellulosic architecture and produce much larger improvements in fiber digestibility (Chang *et al*., 2012; Huang *et al*., 2017). Accordingly, conventional steam conditioning should be regarded as a practical processing strategy for improving pellet quality and fermentation efficiency rather than as a pretreatment designed to maximize fiber degradation. The observed increase in gas production kinetics suggests that steam conditioning accelerated microbial fermentation of the digestible substrate. Although volatile fatty acid (VFA) production, methane emissions, and rumen microbial populations were not determined in the present study, the enhanced gas production kinetics observed after steam conditioning are consistent with previous reports demonstrating that hydrothermal pretreatment increases substrate accessibility through partial disruption of the lignocellulosic matrix and partial starch gelatinization, thereby facilitating earlier microbial colonization and more rapid fermentation (He *et al*., 2015; Ma *et al*., 2022). Previous studies have further shown that improved substrate accessibility may shift ruminal fermentation toward greater propionate production, a lower acetate-to-propionate ratio, reduced hydrogen availability for methanogenesis, and the enrichment of fibrolytic and carbohydrate-utilizing microorganisms, including *Fibrobacter, Ruminococcus,* and *Prevotella* (He *et al*., 2015; Wang *et al*., 2020; Xu *et al*., 2025). Because these variables were not evaluated in the present study, these mechanisms should be regarded as biologically plausible explanations supported by previous literature rather than direct evidence from our experiment.

Under the conditions of the present study, the 45CS:50CM formulation provided the highest pellet quality and favorable fermentation characteristics; however, higher corn stover inclusion levels remain relevant for maximizing agricultural residue utilization when feed density and durability requirements are optimized. Although steam conditioning increases processing energy requirements, the resulting improvements in pellet durability, reduced fines generation, and greater bulk density may partially offset these costs through improved feed handling and transportation efficiency. However, future techno-economic analyses are required to determine the commercial feasibility of this processing strategy. Future research should investigate the underlying mechanisms by which steam conditioning improves ruminal fermentation efficiency, including physicochemical characterization of starch gelatinization, volatile fatty acid (VFA) profiles, methane production, microbial colonization, and rumen microbial community composition. In addition, in vivo validation is warranted to confirm the practical applicability of these findings under commercial feeding conditions.

CONCLUSION

Steam conditioning enhanced the physical quality of corn stover–cassava meal pellets by reducing fines generation and increasing modified pellet durability, bulk density, and pellet hardness. Although increasing corn stover inclusion altered nutrient composition by increasing crude protein and structural fiber concentrations, it progressively compromised pellet quality and early-stage in vitro digestibility, which was associated with the greater proportion of lignocellulosic material. Steam conditioning increased gas production characteristics and estimated metabolizable energy through improvements in the slowly fermentable fraction, gas production rate, cumulative gas production, and estimated metabolizable energy without altering overall in vitro dry or organic matter digestibility, suggesting improved accessibility of fermentable substrates rather than extensive disruption of the lignocellulosic matrix. Among the evaluated formulations, the 45CS:50CM formulation showed the highest pellet quality and favorable fermentation characteristics under the experimental conditions. Collectively, these findings demonstrate that steam conditioning is a practical hydrothermal processing approach for improving pellet physical quality and fermentation-related characteristics while supporting the utilization of agricultural residues as pelleted roughage for ruminants. Further in vivo validation and techno-economic evaluation are warranted before large-scale commercial application.

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NOVELTY STATEMENT

This study contributes new insight into the use of steam conditioning for corn stover–cassava meal pellets by linking improvements in pellet physical quality with changes in ruminal fermentation characteristics. The results suggest that the benefits of steam conditioning are associated with improved accessibility of fermentable substrates rather than extensive modification of the lignocellulosic matrix, providing a practical basis for enhancing the utilization of agricultural residues in sustainable ruminant feeding systems.

AUTHOR'S CONTRIBUTIONS

All authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

Atichat Thongnum: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Writing – original draft, and Project administration; **Manatsanun Nopparatmaitree:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing, and Project administration; **Kattareeya Taja:** Data curation, Formal analysis, Resources, and Software; **Soranot Chotnipat:** Data curation, Validation, and Writing – review & editing.

Chalong Wachirapakorn: Conceptualization, Resources, Supervision, and Validation.

Generative AI and AI assisted technology statement

The authors declare that generative AI was used solely for English language editing to improve grammar, spelling, and readability. The AI-assisted tool was not used to generate scientific content, analyze or interpret data, draw conclusions, or make scientific decisions. The authors take full responsibility for the accuracy, integrity, and originality of the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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