



Research article

Cassava pulp and rain tree pods fermented with loog pang kao mark improve feed utilization, modulate rumen microbial populations and reduce methane production in beef cattle

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Abstract

Importance of the work: Sustainable ruminant production requires feed strategies that improve nutrient utilization while lowering enteric methane emissions. Cassava pulp and rain tree pods fermented with loog pang kao mark (CPRFLK) represent a promising feed supplement because they may enhance intake and digestibility, improve rumen fermentation, and reduce methane production.

Objectives: To evaluate the effects of cassava pulp and rain tree pods fermented with loog pang kao mark on feed intake, nutrient digestibility, rumen fermentation, methane production and microbial population in beef cattle.

Materials and Methods: A 4 × 4 Latin square design was used to randomly allocate four beef cattle to four dietary treatments. The treatments consisted of cassava pulp and rain tree pods fermented with loog pang kao mark (CPRFLK) at cassava pulp:rain tree pod ratios of 100:0 (T1), 60:40 (T2), 50:50 (T3) and 40:60 (T4). Loog pang kao mark was used as the fermentation starter in all CPRFLK formulations. Feeding rates for the diet were 1.5% body weight (concentrate 50%, CPRFLK 50%). The experiment was conducted over four periods, each lasting 26 d (21 d for adaptation followed by 5 d for data collection), during which each animal received its respective diet.

Results: Overall, rice straw consumption increased in the beef cattle fed CPRFLK at ratios of 60:40, 50:50, and 40:60, while DM intake increased at ratios of 50:50 and 40:60. In addition, these treatments enhanced DM and organic matter (OM) digestibility. The acetic acid concentration was reduced ($p < 0.05$), while the total VFA and propionic acid concentrations increased ($p < 0.05$), particularly in the cattle fed CPRFLK 40:60. Additionally, protozoal populations and CH₄ production decreased ($p < 0.05$) with CPRFLK 40:60 supplementation.

Main finding: Feeding concentrate along with CPRFLK 40:60 improved rumen fermentation by increasing total VFA, propionic acid, and total bacterial population, while reducing protozoal population and CH₄ production.

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Introduction

Thai farmers depend on raising cattle for a living, with the cost of feed being a major factor determining profitability, since farmers in tropical climates must rely heavily on agricultural by-products as feed alternatives because there is not enough roughage during the dry season (FAO, 2012). Cassava (*Manihot esculenta* Crantz) pulp is a major by-product of the starch industry. Amounts of approximately 300 t/yr are readily obtainable in Thailand and it is often utilized as cheap cow fodder (Office of Agricultural Economics, 2014; Khempaka et al., 2018). However, its limited digestibility and naturally low crude protein content (1.2–2.8% dry matter, DM) reduce its suitability (Yimmongkol, 2009).

Research has shown that adding urea and molasses improved the nutritional value of cassava pulp to some level (Norrapoke et al., 2018); However, there are still problems associated with imbalanced fermentation in the rumen, poor use of nitrogen in the rumen and insufficient benefits regarding microbial efficiency and methane reduction (Arya et al. 2024). Furthermore, traditional urea-molasses therapies do not improve rumen protozoal populations greatly, which are important for making methane and utilizing nitrogen (Dai et al., 2022). Consequently, more options are needed to enhance beef cattle fed diets based on cassava pulp, to make them more environment-friendly and to improve nutrient use and fermentation in the rumen.

The pods of the rain tree [*Samanea saman* (Jacq.) Merr.] are a common food source in the tropics, having a high crude protein content (around 18% DM), moderate amounts of structural carbohydrates and many easily fermentable carbohydrates (Gohl, 1981). Rain tree pods contain a lot of nutrients; however, they also contain many secondary plant chemicals such as condensed tannins, saponins, phenolic compounds and small amounts of alkaloids that when consumed in large amounts may serve as anti-nutritional factors (Uzoukwu et al. 2020). These bioactive substances have been reported to produce changes in microbial populations in the rumen, in fermentation pathways and in the efficiency of nutrient use. In particular, condensed tannins and saponins have been linked to reduced activity in protozoa, better energy efficiency and lower methane (CH₄) emissions from the gut (Mao, 2009). Evidence from other studies suggested that plant seed meals high in polyphenols could lower CH₄ generation while keeping or improving DM digestibility (Supamong et al., 2017a; 2017b). Similarly, Montoya-Flores et al. (2020) showed

that adding rain tree pods to cattle's diets lowered methane emissions.

The traditional Thai microbial inoculum 'loog pang kao mark' used in fermentation helps make feed easier to digest by changing the nutrients chemically and keeping them intact during ensiling (Owens, 2001). Lactic acid bacteria, especially *Lactobacillus* spp., produce organic acids during fermentation that reduce the pH, stop spoilage microorganisms and help to break down structural carbohydrate and protein-fiber complexes, making the nutrients more available (Zapasník et al. 2022).

Yeasts, such as *Saccharomyces cerevisiae* and *Candida* spp., work together by using soluble carbohydrates, to boost the activity of lactic acid bacteria and keeping the fermentation environment stable by reducing nutrient loss, keeping fermentable substrates intact and raising the amount of easily digested parts in the fermented diet.

Conversion to silage is necessary to create anaerobic conditions that enable these microbial activities, stop oxidative destruction and give the enzymes and microbes enough time to change the feed components (Kung et al., 2018). In addition, the fermented product may affect rumen fermentation by promoting good bacterial populations and inhibiting rumen protozoa, which are strongly linked to methanogenesis (Morgavi et al. 2012). This would improve digestibility, with the combined effect providing a mechanistic explanation for the better feed consumption and rumen fermentation efficiency that were reported.

When used alone, fermented cassava pulp and rain tree pods promote rumen fermentation and minimize methane emissions (Li et al., 2018). However, not enough is known about what happens when they are used with loog pang kao mark, notably how they change the microbial communities in the rumen and how well beef cattle used feed. The current study hypothesized that feeding calves cassava pulp and rain tree pods that had been fermented with loog pang kao mark would help them utilize more nutrients in the feed, change the microbial communities in their rumens and lower methane emissions, which were known to be problems. Therefore, the current study aimed to assess the impact of this fermented feed on nutrient digestibility, rumen fermentation characteristics, microbial community dynamics and production performance in beef cattle.

Materials and Methods

The study was conducted at kalasin univerty (16.446668 N, 103.534802 W).

Ethical statement

All the procedures involving animals performed in this study were approved by the Animal Ethics and Care Committee Kalasin University, Kalasin, Thailand (REF: KSU-AE-022.).

Preparing and presenting feeds experimental feeds

Loog pang khao mak (LPKM), a traditional Thai solid-state fermentation starter, was purchased from local markets in Kalasin Province, Thailand. Typically, LPKM is based on rice or rice flour as a starter cake/ball containing a mixed consortium of amylolytic molds and fermentative yeasts and it may also include antibacterial herbs and spices, including galangal, garlic and pepper, depending on local formulations (Sivamaruthi et al., 2022). The LPKM was ground into a fine flour and stored at room temperature. The rain tree pods and cassava pulp were collected from Kalasin Province and sun-dried for 2–3 d (approximately 72 hr). The fermentation process used thick plastic bags, urea, molasses and LPKM. The rain tree pods and fermented cassava pulps were mixed

thoroughly with 4% urea, 4% molasses and 0.5% LPKM, maintaining a feedstuff-to-solution ratio of 1:1, as this ratio provided sufficient moisture to promote microbial growth and urea hydrolysis, while maintaining feed physical integrity during fermentation. The mixture was packed into 30 cm × 40 cm plastic bags and sealed tightly. The fermentation was conducted in an oxygen-free environment for 21 d, adhering to the protocol of Norrapoke et al. (2018).

Treatment, animals used in experiments and design

This experiment used four naturally born Thai beef cattle, each weighing 250 ± 50 kg at the start of the study. Each animal was housed in its own pen and given feed high in crude protein (CP) as 16% of their total diet. In addition, 1.5% of animal body weight was added as cassava pulp and rain tree pods that had been fermented with LPKM (CPRFLK). The feeding schedule involved a mix of equal parts of concentrate and CPRFLK for all treatments.

The fermentation ratios of cassava pulp to rain tree pod used to make CPRFLK were 100:0 (T1), 60:40 (T2), 50:50 (T3) and 40:60 (T4). Thus, even though the ratio of concentrate to CPRFLK stayed the same, the makeup of CPRFLK changed based on the fermentation ratios. Animals were provided with *ad libitum* access to water, mineral salt blocks and rice straw. The experiment utilized a 4×4 Latin square pattern. Table 1 shows the chemical makeup of the feeds used in the trial.

Table 1 Chemical composition of cassava pulp and rain tree pod fermented with loog pang kao mark (CPRFLK), concentrate and rice straw

Item	Concentrate (%)	CPRFLK (T1)	CPRFLK (T2)	CPRFLK (T3)	CPRFLK (T4)	Rice straw	Cassava pulp	Rain tree pod
Cassava chip	54.9							
Rice bran	8.2							
Coconut meal	20.9							
Palm kernel meal	6.7							
Urea	3	4	4	4	4			
Molasses	2.5	4	4	4	4			
Sulphur	0.5							
Tallow	2							
Salt	0.5							
Mineral premix*	1							
Loog pang kao mark		0.5	0.5	0.5	0.5			
Rain tree pod		91.5	54.9	45.75	36.6			
Cassava pulp		0	36.6	45.75	54.9			
Chemical composition								
DM (%)	95.26	49.26	56.05	54.92	52.83	96.73	89.33	91.50
				DM (%).....				
Ash	11.14	2.98	3.04	2.98	3.03	10.14	4.77	4.98
OM	88.86	97.02	96.96	97.02	96.97	89.86	95.23	95.02
CP	16.06	12.26	17.77	20.00	21.82	2.79	2.56	16.35

Table 1 Continued

Item	Concentrate (%)	CPRFLK (T1)	CPRFLK (T2)	CPRFLK (T3)	CPRFLK (T4)	Rice straw	Cassava pulp	Rain tree pod
AIA	1.54	0.92	0.70	0.81	0.88	5.77	0.95	0.22
NDF	45.52	31.40	31.37	30.85	30.73	70.42	48.01	29.22
ADF	21.62	25.10	25.42	24.50	26.54	47.26	34.23	24.66

CC = concentrate; CPRFLK = cassava pulp and rain tree pods fermented with loog pang kao mark; Treatments (cassava pulp-to-rain tree pod ratio): T1= CPRFLK (100:0); T2= CPRFLK (60:40); T3= CPRFLK (50:50); T4= CPRFLK (40:60); RS = rice straw; DM=dry matter; OM=organic matter; CP=crude protein; AIA= acid insoluble ash; NDF= neutral detergent fiber; ADF= acid detergent fiber.

High urea inclusion reflects experimental formulation and does not represent recommended practical feeding levels.

Characteristics of fermentation

Cassava pulp and rain tree pods were combined in specified ratios and fermented with LPKM under anaerobic conditions at room temperature. While specific fermentation parameters (lactic acid concentration, $\text{NH}_3\text{-N}$ and microbial counts) were not directly assessed in this study, prior research on cassava pulp-based and lactic acid-driven fermentation systems utilizing traditional Thai starter cultures suggested that the final pH is generally in the range 3.8–4.5, with lactic acid as the predominant organic acid and $\text{NH}_3\text{-N}$ levels remaining comparatively low, indicative of effective preservation and protein safeguarding during fermentation. These attributes were deemed appropriate for the preservation of ruminant feed and the modulation of rumen fermentation.

Microbial attributes of loog pang kao mark

LPKM is a traditional Thai fermentation starter recognized for its diverse microbial communities, predominantly comprising lactic acid bacteria, yeasts and molds. Other research indicated that often lactic acid bacteria exist at concentrations of roughly 1×10^6 – 1×10^8 colony-forming units (CFU)/g, whereas yeasts are generally found in the range 1×10^4 – 1×10^6 CFU/g (Halim et al., 2017). These microbes are essential for carbohydrate fermentation and lactic acid generation; consequently improving feed preservation and fermentative quality. In the current investigation, LPKM was applied uniformly across all treatments to guarantee consistent inoculum efficacy.

Scientific justification of cassava pulp rain tree pod fermented loog pang khao mak ratios

The CPRFLK ratios (100:0, 60:40, 50:50, 40:60) were chosen to illustrate a spectrum of escalating rain tree pod incorporation. Rain tree pods are recognized for their

condensed tannin content, which, at modest concentrations, has been shown to inhibit rumen protozoa and methane emissions while preserving nutrient intake and digestibility (Anantasook et al. 2014). The chosen ratios were intended to assess dose-dependent effects of tannin-rich substrates on rumen fermentation traits and methane reduction, informed by other research and the initial findings.

The experiment took place across four periods, each lasting 26 d. The first 21 d were used for acclimatization to the new environment, while the last 5 d were used to collect data. The animals were fed twice a day between 0800 hours and 1600 hours. The concentrate and CPRFLK were given individually based on the dietary therapies applicable to each trail group.

Data collection and analysis

The body weight (BW) of the animals was recorded at the beginning and end of each experimental period to determine the BW change. Daily feed intake was calculated by subtracting the amount of feed refused from the total amount offered, according to Equation 1:

$$\text{Dry matter intake (DMI, kg/d)} = \text{DM offered (kg/d)} - \text{DM refused (kg/d)} \quad (1)$$

where DMI = dry matter intake, DM = dry matter and all parameters are presented in kilograms per day.

Feed refusals were collected and weighed daily prior to the morning feeding and all remaining feed from the previous day was discarded. Samples of concentrate (CPRFLK) and rice straw were collected during the final 7 d of each experimental period for chemical analysis.

Urine samples were collected using spot sampling, while fecal samples were obtained via rectal sampling. Digestibility coefficients were estimated using the acid insoluble ash (AIA) technique as an internal marker. Apparent nutrient digestibility as a percentage was calculated using the Equation 2:

$$\text{Apparent digestibility} = 100 \times [1 - (\text{AIA-feed} \times \text{Nutrient feces}) / (\text{AIA feces} \times \text{Nutrient feed})] \quad (2)$$

All feed, fecal and urine samples were oven-dried at 72 °C until constant weight and subsequently ground to pass through a 1 mm screen using a Cyclotech Mill (Tecator; Höganäs, Sweden). Chemical composition analyses were conducted following the guidelines of the Association of Official Analytical Chemists (2012) for dry matter (DM; ID 967.03), ash (ID 942.05), ether extract (EE; ID 920.39) and crude protein (CP; ID 984.13).

The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to Van Soest et al. (1991), using heat-stable amylase and without sodium sulfite. Residual ash and acid insoluble ash (AIA) were determined during the NDF and ADF procedures, following Schneider and Flatt (1975).

Blood samples (approximately 10 mL each) were drawn from the jugular vein at 0 hr, 3 hr and 6 hr post feeding on the final day of the data collection period. Anesthesia was not used before collecting blood samples. Blood samples were placed in separate tubes with 12 mg of ethylenediaminetetraacetic acid (EDTA) and spun in a table-top centrifuge (PLC-02; Gemmy Industrial Corp., New Taipei City, Taiwan) at 5,000×g for 10 min to separate the plasma. The plasma samples were kept at −20°C until they were tested for hematocrit (Hct) and blood urea nitrogen (BUN), using the procedures described by Crocker (1967) and Kaneko et al. (1997), respectively. On the last day of the experiment, samples of rumen fluid were taken at 0 hr and 4 hr after eating using a method that collected the stomach contents through the mouth. A 15 mL perforated plastic conical tube (rumen sieve) coupled to a 90 cm polyvinyl chloride orogastric tube and an electric vacuum pump were used to remove about 50 mL of rumen fluid from the central rumen region and immediately, a portable pH/temperature meter (HI-8424; Hanna Instruments; Woonsocket, Hanna Instruments, Woonsocket, RI., USA) was used to record the pH and temperature of the rumen. The rumen fluid was filtered through four layers of cheesecloth and 45 mL of the filtered fluid was combined with 5 mL of 1 M sulfuric acid to inhibit the fermentation of microbes.

A table-top centrifuge (PLC-02; Gemmy Industrial Corp., Taiwan) was used to spin the acidified samples at 16,000×g for 15 min. High-performance liquid chromatography (HPLC; Agilent 1260 Infinity; Agilent Technologies; Santa Clara, CA., USA) to find the concentration of volatile fatty acid (VFA) according to the method of Samuel et al. (1997).

An Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad; Hercules, CA, USA) with 0.005 M sulfuric acid as the mobile phase and a flow rate of 0.6 mL/min were used to separate the sample, keeping the column at 60°C for 30 min. External standard solutions were used with known amounts of acetate, propionate and butyrate to identify and measure individual VFAs. Five standard concentrations were used to make calibration curves and the peak regions exhibited linearity with coefficients of determination of more than 0.99.

The colorimetric phenol-hypochlorite method, as described by Broderick and Kang (1980), was applied to determine the concentration of ammonia nitrogen (NH₃-N) in the supernatant. The absorbance was measured at 630 nm using an ultraviolet-visible spectrum spectrophotometer (UV-1800; Shimadzu; Kyoto, Japan). Standard solutions of ammonium sulfate were used to make calibration curves.

For microbial counting, 1 mL of rumen fluid was kept in 9 mL of formalin solution. A hemocytometer (Boeco; Hamburg, Germany) was used to count the number of bacteria, protozoa and fungi. Target DNA was quantified using 10-fold serial dilutions of previously quantified DNA standards, from 1 × 10³ to 1 × 10⁹ copies.

Quantification of microbial population

The method was used of Yu and Morrison (2004) for repeated bead beating with a plus column (RBB+C) to get community DNA from 0.5 g of rumen fluid and digesta. This made the DNA yields much higher. In total, 16 samples were collected, each from the four distinct time intervals, four distinct treatments and two rumen fluid sampling intervals (0 hr and 6 hr post feeding). Spectrophotometry and agarose gel electrophoresis were used to evaluate the amount and quality of these DNA samples. A quantitative test was constructed based on amplifying the target 16S rDNA of each species using specific primers (Table 2), with the purified DNA quantified using the spectrophotometer and several dilutions. The amount of target DNA was measured based on 10-fold dilutions of the DNA standards that had already been measured. After that, a Chromo 4™ system (BioRad; Hercules, CA., USA) was used for real-time polymerase chain reaction (PCR) amplification and detection.

Table 2 Effect of cassava pulp and rain tree pod fermented with loog pang kao mark on feed intake and intake distribution in beef cattle

Item	T1	T2	T3	T4	SEM	<i>p</i> - value
Total DM intake						
(kg/day)	7.57±0.36 ^b	7.89±0.36 ^{ab}	8.46±0.36 ^a	8.39±0.36 ^a	0.18	0.04
(%BW)	2.08±0.14	2.18±0.14	2.32±0.14	2.22±0.14	0.07	0.11
(g/kgBW ^{0.75})	90.74±5.24	95.11±5.24	101.22±5.24	97.88±5.24	2.62	0.09
Rice straw intake						
(kg/day)	2.92±0.12 ^c	3.01±0.12 ^{bc}	3.18±0.12 ^b	3.40±0.12 ^a	0.06	0.01
(%BW)	0.81±0.04 ^c	0.83±0.04 ^{bc}	0.87±0.04 ^{ab}	0.91±0.04 ^a	0.02	0.01
(g/kgBW ^{0.75})	35.12±1.32 ^c	36.31±1.32 ^{bc}	38.05±1.32 ^{ab}	39.86±1.32 ^a	0.66	0.01
Concentrate and CPRFLK intake						
(kg/day)	4.64±0.38	4.88±0.38	5.28±0.38	4.99±0.38	0.19	0.23
(%BW)	1.27±0.14	1.35±0.14	1.45±0.14	1.32±0.14	0.07	0.26
(g/kgBW ^{0.75})	55.62±5.56	58.80±5.56	63.17±5.56	58.02±5.56	2.78	0.25

BW = body weight; CPRFLK = cassava pulp and rain tree pods fermented with loog pang kao mark; Treatments (cassava pulp-to-rain tree pod ratio): T1= CPRFLK (100:0); T2= CPRFLK (60:40); T3= CPRFLK (50:50); T4= CPRFLK (40:60); DM=dry matter; ADG= average daily gain; SEM = standard error of mean.

Mean±SE within the same row followed by different lowercase superscript letters are significantly different ($p < 0.05$).

For *F. succinogenes*, the standard PCR conditions were: a total of 48 cycles of denaturation (each 30 s at 94°C), annealing (30 s at 60°C) and extension (30 s at 72°C). There was a 9 min denaturation in the first cycle and a 10 min extension in the last cycle. The 16S rRNA amplification of the other two species was performed in a similar manner, but using an annealing temperature of 55°C. This study followed the protocol described by Denman and McSweeney (2006) to quantify the total bacterial population using specific primers and optimized PCR conditions. Using the community DNA treatment pool set, four standards were created from the samples. Every real-time PCR test involved creating sample-derived DNA standards using standard PCR. Next, the PCR product was measured using the spectrophotometer and purified using a QIAquick PCR Purification Kit (QIAGEN Inc.; Valencia, CA, USA). After that, the copy number concentration was computed for each standard from the sample using the length of the PCR product and the mass concentration. Prior to real-time PCR, 10-fold serial dilutions were prepared in Tri-EDTA [26]. Then, four real-time PCR standards were created. The same parameters applied to the conventional PCR previously described were applied to the real-time PCR assays of the target genes. The Biotools QuantiMix Easy Syg Kit (B&M Labs S.A.; Madrid, Spain) for amplification of real-time PCR. Every PCR was run in duplicate.

Statistical analyses

The data were analyzed using PROC GLM/PROC MIX procedures in the SAS software SAS Institute (2013) and a 4×4 Latin square design. The model $Y_{ijk} = \mu + M_i + A_j + P_k + \epsilon_{ijk}$

was utilized to examine the data. In this model, μ is the overall mean, M_i is the treatment effect ($i = 1-4$), A_j is the animal effect ($j = 1-4$), P_k is the period effect ($k = 1-4$); and ϵ_{ijk} is the residual effect. Duncan's new multiple range test was used to identify differences across treatment means (Steel and Torrie, 1985), where $p < 0.05$ was used to test mean differences for significance.

Results

Table 1 presents the concentration, roughage and chemical composition of CPRFLK used in the study. The crude protein concentrations for the concentrate, CPRFLK, RS, rain tree pod and cassava pulp were 16.06%, 12.26%, 2.79%, 16.35% and 2.56% DM, respectively, while the NDF concentrations were 45.52 % DM, 31.40 % DM, 70.42 % DM, 29.22% DM and 48.01% DM, respectively, and the ADF concentrations were 21.62%, 25.10%, 47.26%, 24.66% and 34.23% DM, respectively.

Table 2 presents the effects of feeding concentrates containing CPRFLK on feed intake and production-related responses. The Average daily gain was not statistically analyzed due to the short experimental periods inherent in the Latin square design. Total DM intake was higher in the beef cattle fed CPRFLK at 50:50 and 40:60 than in those fed 100:0. Increasing the inclusion level of rain tree pods increased the total feed intake and rice straw intake, while no significant differences were observed in the concentrate and CPRFLK intake among treatments. BW gain was calculated using (Final body weight – Initial body weight) / 26 d.

However, the 4×4 Latin square design was intended primarily for digestion and rumen fermentation evaluation and changes in BW over the short 26 d experimental period in a crossover design provide only limited evidence of long-term production performance. Nevertheless, the cattle fed CPRFLK containing fermented cassava pulp alone had lowest rice straw intake.

Table 3 presents the effects of feeding a concentrate diet with CPRFLK on nutritional digestibility. There were significant variations in the digestibility of dry matter and organic matter between the treatments. The improved digestibility of beef cattle could be attributed to the increased proportion of condensed tannin (CT) in the rain tree pods. Additionally, the increase in microorganisms that followed the increase in degradability may have contributed to this improvement. There were no significant differences between the treatments for CP, NDF and ADF.

The results of the effects of feeding concentrate with CPRFLK on BUN and Hct levels in the beef cattle are shown in Table 4. There were no significant difference in the BUN levels across the treatments, with values in the range 10.51–10.96 mg/dL.

Table 5 illustrates the impact of CPRFLK on CH_4 production and rumen fermentation in beef cattle. In response to CPRFLK, there were no significant changes in temperature, butyric acid, $\text{NH}_3\text{-N}$ or ruminal pH. The addition of cassava pulp and rain tree pods fermented with loog pang kao mark resulted in a significant reduction in the acetic acid content. Beef cattle treated with CPRFLK had significantly highest levels of propionic acid and total VFA, particularly in the 40:60 treatment.. This increase could be explained by the inclusion of CPRFLK in a diet containing readily available energy sources, such as molasses and starch sugar (which are rapidly fermented in the rumen and promote propionate production). CH_4 production was significantly reduced with the CPRFLK supplementation, likely due to the presence of CT in the rain tree pods. The reduction in methane may be linked to CT's ability to decrease protozoal numbers. However, it was concluded that feeding CPRFLK to beef cattle did not negatively impact rumen fermentation. Different procedures for calculating methane production based on VFA concentrations would yield varying results.

Table 3 Effect of cassava pulp and rain tree pod fermented with loog pang kao mark on nutrient digestibility in beef cattle.

Item	T1	T2	T3	T4	SEM	<i>p</i> value
Apparent digestibility (%)						
DM	50.08±2.20 ^b	53.93±2.20 ^a	54.13±2.20 ^a	54.97±2.20 ^a	1.10	0.04
OM	52.17±2.32 ^b	56.29±2.32 ^a	56.47±2.32 ^a	57.29±2.32 ^a	1.16	0.04
CP	51.84±3.46	54.79±3.46	55.80±3.46	58.93±3.46	1.73	0.13
NDF	45.52±3.32	48.95±3.32	49.77±3.32	50.47±3.32	1.66	0.26
ADF	27.98±3.02	29.72±3.02	31.00±3.02	34.15±3.02	1.51	0.11

DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; SEM = standard error of mean; CPRFLK = cassava pulp and rain tree pods fermented with loog pang kao mark; Treatments (cassava pulp-to-rain tree pod ratio): T1= CPRFLK (100:0); T2= CPRFLK (60:40); T3= CPRFLK (50:50); T4= CPRFLK (40:60); SEM = standard error of mean.

Mean±SE within the same row followed by different lowercase superscript letters are significantly different ($p < 0.05$).

Table 4 Effect of cassava pulp and rain tree pod fermented with loog pang kao mark on Blood urea nitrogen (BUN) and hematocrit (HCT) in beef cattle

Item	T1	T2	T3	T4	SEM	<i>p</i> value
BUN (mg/dL)						
0 hr post feeding	10.05±0.70	10.45±0.70	10.08±0.70	10.35±0.70	0.35	0.81
3 hr post feeding	10.30±0.88	10.90±0.88	10.38±0.88	10.53±0.88	0.44	0.78
6 hr post feeding	11.30±0.86	11.53±0.86	11.08±0.86	11.15±0.86	0.43	0.88
Mean	10.55±0.78	10.96±0.78	10.51±0.78	10.68±0.78	0.39	0.85
HCT (%)						
0 hr post feeding	29.50±5.98	30.38±5.98	31.88±5.98	31.75±5.98	2.99	0.93
3 hr post feeding	30.00±2.90	32.75±2.90	31.75±2.90	29.88±2.90	1.45	0.48
6 hr post feeding	29.50±3.18	32.13±3.18	28.88±3.18	28.50±3.18	1.59	0.43
Mean	29.67±3.78	31.75±3.78	30.83±3.78	30.04±3.78	1.89	0.87

CPRFLK = cassava pulp and rain tree pods fermented with loog pang kao mark; Treatments (cassava pulp-to-rain tree pod ratio): T1= CPRFLK (100:0); T2= CPRFLK (60:40); T3= CPRFLK (50:50); T4= CPRFLK (40:60); SEM = standard error of mean.

Table 5 Effect of cassava pulp and rain tree pod fermented with loog pang kao mark on rumen fermentation and CH₄ production in beef cattle.

Item	T1	T2	T3	T4	SEM	p value
Ruminal pH	6.85±0.10	6.88±0.10	6.86±0.10	6.83±0.10	0.05	0.76
Ruminal temperature (°C)	38.17±0.78	38.03±0.78	38.01±0.78	38.07±0.78	0.39	0.99
NH ₃ -N (mg/dL)	18.29±2.48	19.26±2.48	21.40±2.48	21.60±2.48	1.24	0.26
Total VFA (mmol/L)	107.67 ^b ±1.38	108.91 ^b ±1.38	109.13 ^{ab} ±1.38	111.41 ^a ±1.38	0.69	0.04
VFA profile (mol/100 mol)						
Acetic acid	64.47±2.14 ^a	59.28±2.14 ^b	59.54±2.14 ^b	56.85±2.14 ^b	1.07	0.01
Propionic acid	25.80±2.26 ^b	29.67±2.26 ^{ab}	29.07±2.26 ^{ab}	32.82±2.26 ^a	1.13	0.03
Butyric acid	9.73±1.22	11.05±1.22	11.39±1.22	10.34±1.22	0.61	0.31
CH ₄ production ^A , (mol/100mol TVFA)	25.81±1.62 ^a	22.94±1.62 ^{ab}	23.35±1.62 ^{ab}	20.69±1.62 ^b	0.81	0.02

VFA = volatile fatty acid; TVFA = total volatile fatty acid; CPRFLK = cassava pulp and rain tree pods fermented with loog pang kao mark; Treatments (cassava pulp-to-rain tree pod ratio): T1= CPRFLK (100:0); T2= CPRFLK (60:40); T3= CPRFLK (50:50); T4= CPRFLK (40:60); SEM = standard error of mean.

Mean±SE within the same row followed by different lowercase superscript letters are significantly different ($p < 0.05$).

^A = calculated according to Moss et al. (2000); CH₄ production = 0.45(acetate) – 0.275(propionate) + 0.4(butyrate).

Table 6 shows no significant changes in the total direct count of bacteria or fungal zoospores in beef cattle treated with CPRFLK. However, there was a significant decrease in the protozoal population in beef calves fed CPRFLK, particularly in the CPRFLK (40:60) group. The presence of condensed tannin in the rain tree pods may have been responsible for this reduction.

Table 7 presents the estimated microbial population based on the real-time PCR data. The fermentation of the rain tree pods and cassava pulp with LPKM did not significantly alter the amounts of *R. albus*, *R. flavefaciens* and *F. succinogenes*. However, in the beef cattle fed T3 and T4, the total bacterial population was significantly higher than in the control group.

Discussion

The higher total dry matter intake observed in the 50:50 and 40:60 treatments, together with the increased rice straw intake in the CPRFLK-fed cattle, indicated that the combination of fermented cassava pulp and rain tree pods improved the acceptability of the diet and the effectiveness of rumen fermentation, rather than reducing intake. Anantasook et al. (2014) found that adding rain tree pods to the diet did not change how much cassava pulp and rain tree pods were eaten. This suggested that the fermentation process with LPKM may have changed the physical and chemical properties of the

Table 6 Effect of cassava pulp and rain tree pod fermented with loog pang kao mark on microbial population in beef cattle.

Item	T1	T2	T3	T4	SEM	p value
Total direct count (cells/mL)						
Protozoa ($\times 10^5$)	5.26±0.14 ^a	5.11±0.14 ^a	5.09±0.14 ^a	4.84±0.14 ^b	0.07	0.03
Anaerobic fungi ($\times 10^7$)	5.90±0.06	5.88±0.06	5.88±0.06	5.87±0.06	0.03	0.90
Bacteria ($\times 10^{11}$)	6.19±0.08	6.17±0.08	6.20±0.08	6.18±0.08	0.04	0.92

CPRFLK = cassava pulp and rain tree pods fermented with loog pang kao mark; Treatments (cassava pulp-to-rain tree pod ratio): T1= CPRFLK (100:0); T2= CPRFLK (60:40); T3= CPRFLK (50:50); T4= CPRFLK (40:60); SEM = standard error of mean.

Mean±SE within the same row followed by different lowercase superscript letters are significantly different ($p < 0.05$).

Table 7 Effect of cassava pulp and rain tree pod fermented with loog pang kao mark on microbial population.

Item	T1	T2	T3	T4	SEM	p value
Real-time polymerase chain reaction technique (copies/mL of rumen content)						
Total bacteria ($\times 10^9$)	3.44±0.04 ^b	3.75±0.04 ^{ab}	3.88 ±0.04 ^a	4.07±0.04 ^a	0.02	0.03
Protozoa ($\times 10^6$)	5.30±0.26 ^a	4.75±0.26 ^a	4.40±0.26 ^{ab}	3.03±0.26 ^b	0.13	0.02
<i>F. succinogenes</i> ($\times 10^6$)	2.62±0.02	2.95±0.02	2.78±0.02	2.92±0.02	0.01	0.09
<i>R. flavefaciens</i> ($\times 10^6$)	1.96±0.08	2.05±0.08	2.13±0.08	2.07±0.08	0.04	0.87
<i>R. albus</i> ($\times 10^6$)	1.72±0.02	1.82±0.02	1.90±0.02	1.72±0.02	0.01	0.32

CPRFLK = cassava pulp and rain tree pods fermented with loog pang kao mark; Treatments (cassava pulp-to-rain tree pod ratio): T1= CPRFLK (100:0); T2= CPRFLK (60:40); T3= CPRFLK (50:50); T4= CPRFLK (40:60); SEM = standard error of mean.

Mean±SE within the same row followed by different lowercase superscript letters are significantly different ($p < 0.05$).

cassava pulp and rain tree pods, making them more acceptable and available for nutrients. The lack of changes in concentrate intake further substantiated that CPRFLK did not adversely impact voluntary feed consumption, aligning with the findings of Norrapoke et al. (2022) on the supplementation of fermented cassava pulp with urea and molasses.

While CPRFLK did not consistently influence total nutrient digestibility, the noted enhancements in DM and OM digestibility could be attributed to the moderate concentrations of CTs found in the rain tree pods, even if the decrease in disposable energy due to the decrease in cassava pulp resulted in a decrease in digestibility (Iommelli et al., 2022). CT can lower the breakdown of too much protein in the rumen at low-to-moderate levels, making it easier for microbes to use nitrogen for energy and for nitrogen to be released at the same time for microbial growth. This method may elucidate the constancy of crude protein, NDF and ADF digestibility, as excessive tannin-fiber binding seemed improbable at the inclusion levels used in the current investigation. Sommai et al. (2020) made similar observations, finding no consistent changes in fiber degradability when moderate amounts of tannin-containing diets were added.

The absence of treatment effects on BUN indicated that CPRFLK supplementation did not interfere with ruminal nitrogen equilibrium or protein metabolism. The BUN levels stayed in the best physiological range (Hammond, 1983), which meant that the ruminal ammonia was used well without losing too much nitrogen. Hct readings were unchanged, further validating that CPRFLK did not induce metabolic or health-related stress in the animals. These results corroborated other research indicating that fermented cassava-based feeds did not negatively impact hematological parameters in beef cattle (Norrapoke et al., 2022).

Changes in how the rumen ferments provide more information about how CPRFLK works. The decrease in acetate levels and the rise in propionate and total VFA showed that hydrogen was being used more to make propionate. Often, this change is linked to less methane being made, since hydrogen is being used elsewhere instead of for methanogenesis. The abundance of easily fermentable carbohydrates from cassava pulp and molasses probably made the propionogenic pathways stronger. On the other hand, CT from the rain tree pods may have preferentially stopped microorganisms that make hydrogen. This mechanism corresponds with the hydrogen balance theory posited by Moss et al. (2000) and elucidates the notable decrease in CH₄ production seen in the calves administered CPRFLK, especially at the 40:60 ratio.

Even though there was little change in the total number of bacteria and fungi, the drop in protozoal counts implied that the CT had a targeted effect rather than a general effect on rumen microbiota. Protozoa are known to host methanogens and facilitate interspecies hydrogen transfer; hence, their decline is directly correlated with diminished methane emissions (Morgavi et al. 2012). Studies utilizing rain tree, *Lotus pedunculatus* and other CT-rich forages have shown that tannin-containing feeds can also kill protozoa (Woodward et al., 2001; Bhattacharya et al., 2009). Furthermore, the lack of alterations in important cellulolytic bacteria (*R. albus*, *R. flavefaciens* and *F. succinogenes*) shows that CPRFLK did not hurt the ability to break down fiber, which supports its use as a sustainable feed additive.

In general, based on the current results, CPRFLK improved feed intake, DM and OM digestibility, and rumen fermentation efficiency, mainly via changes in microbial ecology and fermentation pathways. The combination of fermentable energy and modest quantities of CT seemed to boost propionate production and lower methane emissions without harming the animal's health or ability to eat.

Conclusion

Feeding concentrate containing CPRFLK at 50:50 and 40:60 increased total DM intake, while CPRFLK at 60:40, 50:50 and 40:60 increased rice straw intake and improved DM and OM digestibility. In addition, protozoal population and CH₄ production were reduced, particularly with CPRFLK 40:60. Consequently, feeding ruminants with CPRFLK as feedstock may be an alternative with potential to increase the efficiency of rumen fermentation and lessen the negative effects ruminants have on the environment, resulting in improved sustainability in animal husbandry. However, more investigation is needed into how CPRFLK affects the variety of methanogenic bacteria.

Conflict of Interest

The authors have no potential conflicts of interest relevant to this article.

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