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Original Article

Potential use of cassava bioethanol waste as ruminant feed in fermented total mixed ration: *In vitro* trial

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Abstract

This study identified the optimal inclusion levels of fresh cassava bioethanol waste (CBW) as well as the most effective additive types based on physical properties, chemical composition, and *in vitro* ruminal fermentation characteristics. The experiment used a 4x4 factorial arrangement in a CRD with four CBW levels (0, 5, 10, and 15% dry matter (DM)) as well as four additive treatments (none, dry yeast, probiotics, and non-starch polysaccharides (NSP) enzymes) in fermented total mixed rations (FTMR). FTMR was ensiled for 21 days before being evaluated. Interactions between CBW levels and additives significantly affected physical scores, chemical composition, and fermentation end-products ($P<0.05$), but did not influence cumulative gas yield or certain gas kinetic indices ($P>0.05$). The addition of dry yeast increased the gas produced from the immediately soluble fraction (b) and the potential extent of gas production (P). NSP enzyme increased cumulative gas volume at 72 and 96 hours after incubation as well as the gas produced from the immediately soluble fraction ($P<0.05$). Addition of probiotics increased cumulative gas volumes at 72 and 96 hours, DM degradability at 24 hours, and potential extent of gas production after 96 hours of incubation ($P<0.05$). The inclusion of 10% CBW with probiotics or NSP enzymes yielded the highest physical quality scores. While increasing CBW levels raised fiber content and initially shifted fermentation toward higher acetic acid levels at the expense of propionate, probiotics and NSP enzymes significantly enhanced cumulative gas production and NH_3-N concentrations. Remarkably, yeast and probiotics successfully redirected VFA profiles toward propionate at the 15% CBW inclusion level. Although higher CBW levels obviously reduced energy density, biological additives effectively mitigated this decline by facilitating greater fiber degradation. The results recommend inclusion of 10–15% CBW in FTMR with 10% being optimal when paired with probiotics or enzymes to improve nitrogen availability and physical quality. Further *in vivo* trials can validate these laboratory findings in ruminant performance.

Keywords: Cassava bioethanol waste, *Saccharomyces cerevisiae*, Probiotics, Fibrolytic enzymes, Ruminal fermentation

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Introduction

Cassava is a multipurpose crop that supports economic development, starch industries, animal production, and bioenergy in tropical regions. Cassava bioethanol waste (CBW) is generated during yeast fermentation of cassava root and ethanol distillation. In ASEAN countries, CBW is produced in large quantities with Thailand being the leading producer (about 1.5 million tons annually). Vietnam and Cambodia contribute moderately, and other countries show limited activity (Marx, 2019). Unlike corn grain-based dried distillers' grains with soluble (DDGS) that contains 25–30% crude protein and are widely used as animal feed (Buenavista et al., 2021), CBW has lower nutritional value, poor palatability, low protein, and high ash and fiber contents (Sriroth et al., 2012; Laorodphan et al., 2013). Variability in nutrient composition is common and depends on ethanol plant technologies and processing methods with crude protein, NDF, and ash ranging from 6.54–14.9%, 56.8–69.0%, and 10.1–19.5% DM, respectively (Yi, 2023). Although this property limits its direct use in animal feeds, several studies have demonstrated that CBW can be incorporated into ruminant diets—particularly when its nutritional value is improved through chemical treatment or bio-fermentation.

Dried CBW has previously been used at up to 15% in concentrates for Thai bulls (Laorodphan et al., 2013) and at 10% in total mixed rations for growing goats (Pornjantuek et al., 2015; Cherdthong et al., 2016) and lactating dairy cows (Wachirapakorn et al., 2016) without adverse effects. However, fresh CBW at 0.5% body weight reduced nutrient digestibility in cattle fed rice straw although average daily gain was unaffected (Pilajun et al., 2024). To overcome these nutritional limitations, CBW is often fermented using *Saccharomyces cerevisiae* with molasses and urea is added to improve palatability and nitrogen content (Sunato et al., 2013; Thungphoomrapeewong, 2015; Puramongkon, 2016; Cherdthong and Supapong, 2019). In dairy cattle, fermented CBW has shown neutral effects on intake and milk yield, while other studies reported increased milk fat concentration (Cherdthong and Supapong, 2019). Furthermore, Yi (2023) observed improved *in vitro* dry matter degradability and milk fat content of late-lactating dairy cows with combined live yeast and CBW supplementation. Besides these disadvantages, CBW has yeast residues that are abundant in beta-glucans, mannan-oligosaccharides, nucleotides, peptides, and amino acids. These compounds can enhance rumen fermentation, digestibility of nutrients, immunological responses, and mycotoxin binding in ruminants (Cherdthong et al., 2018; Xia et al., 2021; Baker et al., 2022; Gunun et al., 2022). Therefore, when appropriately processed and used strategically, these functional qualities promote CBW's potential as an economical and sustainable feed additive.

In terms of feed additives in ruminants, live yeast, particularly *Saccharomyces cerevisiae*, plays a crucial role in enhancing production performance. Research indicates that live yeast supplementation promotes sustainability and benefits animal welfare and farm profitability (Carpinelli et al., 2021) by improving ruminal fermentation, microbiota balance (Wang et al., 2022), digestibility, and immune responses (El Jeni et al., 2024; Zhang et al., 2024). This in turn leads to better health and higher product quality. Other probiotic supplements have shown beneficial effects in ruminants such as lactic acid bacteria (LAB), *Bacillus* spp., *Enterococcus*, and Bifidobacterium. These probiotics can support growth and feed efficiency in ruminants by improving the rumen microbial balance, thus raising the synthesis of volatile fatty acids, increasing fiber digestibility, and reducing the pathogen load (Kulkarni et al., 2022; Branco-Lopes et al., 2025). The effects are influenced by strain selection, dose, diet, animal age, and health status (Reuben et al., 2021). Multispecies probiotic blends, often including Bacillus and LAB, can also reduce methane emissions and improve fermentation efficiency, which further contribute to sustainable production systems (Saleem et al., 2025).

In contrast, exogenous non-starch polysaccharide (NSP) or fibrolytic enzyme supplementation has been widely investigated as a strategy to enhance fiber degradation and nutrient utilization in ruminants. These enzymes can be applied directly to feed, silage, or total mixed rations and increase feed intake, degradability of nutrients, and alter ruminal fermentation profiles (Anil et al., 2022; Yang et al., 2024). Meta-analyses and reviews indicate variable responses depending on enzyme formulation, diet composition, and application methods, but the overall evidence supports improvements in nutrient degradation and feed efficiency in both beef and dairy systems (Ramdani et al., 2025). In practical feeding trials, fibrolytic enzymes increased digestibility and intake in growing cattle and sheep with potential benefits for ruminal fermentation and volatile fatty acid production although the effects on performance metrics such as total tract digestibility can be inconsistent (Kondratovich et al., 2019; Bureenok et al., 2024; Ferreira et al., 2025). These tools are particularly valuable in diets based on high-fiber forages or low-quality roughages, thus contributing to more efficient nutrient utilization and potentially reducing environmental nitrogen losses.

This study's objective was to identify the proper level of CBW and additive type—such as yeast powder, probiotics, and NSP enzymes—for inclusion in fermented TMR for ruminants based on its physical properties, chemical composition, and *in vitro* ruminal fermentation characteristics and degradability.

Material and Methods

This experiment was conducted at the Office of the Experimental Farm and Central Laboratory, Faculty of Agriculture, Ubon Ratchathani University, Thailand. The experimental protocols and animal care procedures received approval from the ethics committee of Ubon Ratchathani University (Approval ID#10/2568/IACUC). This approval was granted in accordance with the Ethical Principles for the Use of Animals for Scientific Purposes established by the National Research Council of Thailand (NRCT).

Experimental design and treatments

The experiment followed a Completely Randomized Design (CRD) with a 4x4 factorial arrangement of treatments. Three replicates were performed for all treatment combinations. The fermented total mixed ration (FTMR) comprised four levels of fresh cassava bioethanol waste (CBW; 0, 5, 10, and 15% DM), and four types of feed additives including non-additive, dry yeast powder, probiotics, and non-starch polysaccharides enzymes at 1.0, 1.0, and 0.5 g/kg DM, respectively. Cassava bioethanol waste was collected from the Ubon Bioethanol plant located in the Na Yia district of Ubon Ratchathani Province. The waste contained 12.4% DM, 12.8% total ash, 15.1% crude protein (CP), 3.71% ether extract (EE), 54.7% neutral detergent fiber (NDF), and 42.5% acid detergent fiber (ADF), on a dry matter basis. Dry yeast powder contained *Saccharomyces cerevisiae* at 10^{10} CFU/g (Saf-instant®, France). Each kilogram of probiotics includes *Saccharomyces cerevisiae* at 10^9 CFU, *Bacillus subtilis* at 10^{11} CFU, and *Bacillus licheniformis* at 10^9 CFU (AST Co., Ltd., Thailand). The non-starch polysaccharide enzymes are composed of the following: xylanase at 316,000,000 U/kg, beta-glucanase at 4,800,000 U/kg, cellulase at 9,600,000 U/kg, mannanase at 49,000,000 U/kg, amylase at 164,000,000 U/kg, pectinase at 1,400,000 U/kg, and alpha-galactosidase at 800,000 U/kg (AST Co., Ltd., Thailand).

The feed formula of FTMR contained different levels of fresh CBW as shown in Table 1. The isonitrogenous content was calculated according to the nutrient component of feedstuffs, while palm oil was used to adjust the isocaloric content. Dry yeast, probiotics, and enzymes in powder form were well-dissolved in fresh water along with molasses before being mixed with other ingredients. To create a similar fermentation condition, fresh water was added to FTMR containing CBW with less than 15% DM for equal moisture content. After mixing thoroughly and removing air with a suction pump, 10 kg of FTMR was stored in a tightly tied black HDPE plastic bag and kept at room temperature.

Table-1. Feed ingredient of fermented total mixed ration containing cassava bioethanol waste.

Ingredients	**Cassava bioethanol waste, %DM**			
	0	**5**	**10**	**15**
Fresh Napier grass	50.0	50.0	50.0	50.0
Cassava chip	20.0	18.0	16.0	14.0
Rice bran	9.0	7.25	5.00	3.00
Soybean meal	10.0	9.75	9.50	9.25
Palm kernel meal	7.5	5.50	4.00	2.50
Cassava bioethanol waste	0.0	5.00	10.0	15.0
Palm oil	0.0	1.00	2.00	2.75
Molasses	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5
Premixed[1]	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0

[1] Ca 10%, P 5%, Mg 3%, Na 1%, Zn 4,000 mg/kg, Mn 4,000 mg/kg, Fe 10,000 mg/kg, Cu 1,000 mg/kg), I 70 mg/kg), Co 10 mg/kg), Se 20 mg/kg, Vit A 800,000 IU/kg, Vit D3 150,000 IU/kg, VitE 3,000 IU/kg.

Sample collection and physical characteristic assessment

After 21 days of fermentation, the physical properties of the FTMR including its color, odor, texture, and pH were assessed according to the standards outlined in the Good Quality Fermented Animal Feed Handbook (Division of Animal Feed, 2004). Five of the testers who have received explanations and have used test materials participated with a pH meter (HI-83141-1; RS Pro, Bremen, Germany). The details and scores of each characteristic are as follows:

Physical characteristic	Detail	Score
Color	Yellowish green or khaki	4
	Yellowish green or dark green	3
	Golden brown	2
	Dark brown or black	1
Odor	Smells like pickled fruit or vinegar	4
	Not fragrant, slightly pungent smell	3
	Very pungent and slightly unpleasant smell	2
	Foul-smelling or moldy	1
Texture	Firm, with some leaves and stems still intact	4
	Firm, with leaves and stems slightly decayed, slippery and slimy	3
	Firm, but the leaves and stems are very decayed	2
	Slime	1
pH	3.5-4.2	4
	4.3-4.7	3
	4.7-5.1	2
	> 5.1	1

In vitro gas production technique

Five male crossbred Thai-native and Lowline Angus cattle (200 ± 12.4 kg BW) were used as rumen fluid donors. The animals were fed clean, fresh water, and mineral blocks were offered *ad libitum*. The animals were fed an equal amount (DM basis) of fresh chopped Napier grass and a concentrate (14% CP, 2.5 Mcal/kg of ME, consisting of: 55% cassava chip, 10% rice bran meal, 20.5% soybean meal, 10% palm kernel meal, 1.0% palm oil, 2.0% molasses, 0.5% salt, 1.0% mineral premix). This was done *ad libitum* at 7:00 AM and 4:00 PM for 10 days prior to the collection of rumen fluid. Rumen fluid, about 300-350 ml, was collected from each animal using a stomach tube connected to a vacuum pump prior to morning feeding. Four layers of cheesecloth were used to filter the rumen fluid into pre-warmed insulated flasks. A strict anaerobic technique was used during the rumen fluid collection as described in Menke et al. (1979). Artificial saliva was prepared according to Menke and Steingass (1988). A mixture of artificial saliva and rumen fluid was blended in a 2:1 ratio to produce a rumen inoculation solution. Three bottles containing only rumen inoculation mixture were used as blanks. The net gas output was determined by subtracting the average gas production of the blank samples from each measurement. The glass bottles with 200 mg of FTMR treatment after 21 days of ensiling were pre-warmed in a 39°C water bath before filling with 35 ml of rumen inoculation mixture. The bottles were closed with rubber lids and aluminum caps and incubated at 39°C for *in vitro* gas tests. At 24, 48, and 96 h post incubation, a set of samples were collected to evaluate fermentation end-products and the degradability. The volume of gas production was recorded at 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, and 96 h of incubation.

Laboratory analysis

FTMR samples were ground through a 0.5-mm screen and analyzed for DM (method no. 930.15), total ash (method no. 923.03), crude protein (CP; method no. 976.06), and ether extract (EE; method no. 920.39) according to AOAC (1995). Neutral detergent fiber

(NDF) and acid detergent fiber (ADF) were measured according to Van Soest et al. (1991).

At 24, 48, and 96 h post incubation, fermentation inside the bottle was ended by freezing at -21°C before analysis. After thawing, the fermentation liquor was sampled to measure the pH (HI-83141-1; RS Pro, Bremen, Germany), NH_3-N (Kjeltech Auto 1030 Analyzer, Tecator, Hoganiis, Sweden) (method number 973.18; AOAC, 1995), and volatile fatty acid (VFA). Briefly, the amount of VFA was measured in the supernatant after adding 1 M H_2SO_4 to the fermentation liquor sample and centrifuging it for 15 minutes at 2,054×g. A 0.45-µm Millipore filter was used to filter the supernatant prior to its injection into the chromatographic apparatus. An Ultimate 3000 HPLC (Thermo Fisher Scientific, Waltham, MA, USA) was used and coupled with a C18 (4.6×250 mm) column (Chromaleon Dionex Corp) and UV-Vis detection at 210 nm. The mobile phase was 0.005 mol/L H_2SO_4 (de Sá et al., 2011).

The fermented content was filtered through clean and pre-weighed Gooch crucibles (40 mm of porosity), washed with hot distilled water (above 90°C), dried at 105°C for 16 h, and then residual DM was estimated. The dried FTMR sample and residue remaining were ash at 550 °C for determination of organic matter content (OM). The weight loss percentage was presented as *in vitro* DM and OM degradation (Tilley and Terry, 1963).

Calculations

Cumulative gas production data were fitted to the exponential equation using the Neway Excel curve-fitting program, as described by Ørskov and McDonald (1979) as follows:

$$y = a + b\,(1 - e^{-ct})$$

where ‘y’ is the gas produced at time ‘t’; ‘a’ is the gas production from the immediately soluble fraction; ‘b’ is the gas pro-duction from the insoluble fraction; ‘c’ is the gas production rate constant for the insoluble fraction (b); and ‘t’ is incubation time; and P (|a|+b) is the potential extent of gas production.

In vitro degradability calculations were made using the following equation:

$$\textbf{Degradability}\ (\%) = (WD-WC) - (WB-WC) / WS \times 100$$

Where WD = weight of the crucible and the residue after drying at 100 ºC, WB = weight of the crucible and the chemical reagent residue after drying at 100ºC (blank), WC = dry weight of crucible, and WS = dry weight of original sample (DM basis).

In addition, metabolizable energy (ME) content of FTMR was estimated based on the chemical contents, the volume of gas produced after incubation, and the degradability as follows:

$$\text{ME (MJ/kg DM)} = 1.06 + 0.157GP + 0.0084CP\ (\%) + 0.022EE\ (\%) - 0.0081Ash\ (\%)$$

(Menke and Steingass, 1988)

$$\text{ME (MJ/kg DM)} = 0.37 + 0.0142DOMD(g/kgDM) + 0.0077CP(g/kgDM)$$

(Givens et al., 1990)

Where GP: gas production at 24h incubation (ml/200mgDM); CP: crude protein; EE: ether extract; DOMD: *in vitro* OM degradability.

Statistical analysis

The recorded data were analyzed to the ANOVA of SAS OnDemand for Academics (SAS® Institute Inc., USA). The following model of 4x4 factorial arrangement in CRD was used:

$$Y_{ijk} = \mu + A_i + B_j + ABij + \varepsilon_{ijk}$$

where Y_{ijk} = observation from FTMR receiving treatment ij, replicate k

μ = the overall mean

A_i = the effects of the CBW level i

B_j = the effect on the additive j

AB_{ij} = the effect of interaction between the CBW level and the additive

ε_{ijk} = residual effects

The results were presented as mean values including the standard error of the mean. The differences between the CBW level, additive type, and treatment combination means were verified with Duncan's new multiple range test with a 95% confidence interval.

Results

Physical property

Physical property scores including color, odor, texture, and pH of FTMR containing CBW with various additives are shown in Table 2. There was an interaction effect between CBW level and additive type on odor, texture, pH, and total scores ($P<0.05$), but not color score or pH value—these were not different between CBW levels and additive type. The physical properties of FTMR were maximized upon inclusion of 10% DM of CBW with 1.0 g/kg DM of probiotics or 0.5 g/kg DM of NSP enzymes according to the high score for odor, texture, and pH. When CBW was included at 15% DM, supplementation with 1.0 g/kg DM of yeast powder improved the physical properties of FTMR comparable to the greatest treatment. In contrast, FTMR contained 15% DM of CBW, and NSP enzymes had the lowest total physical property score, i.e., the lowest odor, texture, and pH scores.

Table-2. Physical property scores of fermented total mixed rations containing cassava bioethanol waste with various additives.

CBW levels[1]	Additives[2]	Color	Odor	Texture	pH	pH score	Total[3]
0CBW	Non	3.09	2.91^{b}	3.27^{abc}	4.69	3.02^{bc}	12.3^{bc}
0CBW	Yeast	3.09	3.18^{ab}	3.18^{abc}	4.73	2.92^{bc}	12.4^{abc}
0CBW	Probiotics	3.18	2.82^{b}	3.27^{abc}	4.67	3.07^{b}	12.3^{abc}
0CBW	Enzymes	3.27	3.36^{ab}	3.45^{abc}	4.61	3.22^{ab}	13.3^{abc}
5CBW	Non	3.27	3.36^{ab}	3.27^{abc}	4.68	3.04^{bc}	12.9^{abc}
5CBW	Yeast	3.18	3.18^{ab}	3.00^{bcd}	4.78	2.81^{bc}	12.2^{abc}
5CBW	Probiotics	3.09	3.09^{ab}	2.82^{bc}	4.89	2.52^{c}	11.5^{c}
5CBW	Enzymes	3.45	3.45^{ab}	3.55^{ab}	4.58	3.29^{ab}	13.8^{abc}
10CBW	Non	3.27	3.27^{ab}	3.36^{abc}	4.66	3.11^{ab}	13.0^{abc}
10CBW	Yeast	3.00	2.82^{b}	3.36^{abc}	4.66	3.09^{b}	12.3^{abc}
10CBW	Probiotics	3.36	3.73^{a}	3.73^{a}	4.50	3.50^{ab}	14.3^{a}
10CBW	Enzymes	3.45	3.36^{ab}	3.73^{a}	4.51	3.48^{ab}	14.1^{ab}
15CBW	Non	3.18	3.45^{ab}	3.55^{ab}	4.56	3.34^{ab}	13.5^{abc}
15CBW	Yeast	3.55	2.91^{b}	3.91^{a}	4.43	3.68^{a}	14.1^{abc}
15CBW	Probiotics	3.27	3.27^{ab}	3.36^{abc}	4.65	3.12^{ab}	13.0^{abc}
15CBW	Enzymes	2.82	2.00^{c}	2.45^{d}	4.97	2.33^{c}	9.60^{d}
SEM		0.173	0.210	0.210	0.143	0.265	0.454
P-value							
CBW levels		0.715	0.032	0.056	0.511	0.081	0.118
Additives		0.980	0.301	0.929	0.755	0.774	0.881
Interaction		0.118	<0.001	<0.001	0.093	<0.001	<0.001

[1] Cassava bioethanol waste inclusion levels at 0, 5, 10, and 15 % DM.

[2] Additive inclusion including: Non, non-additive; Yeast powder 1.0 g/kg DM (10^{10} CFU/g *Saccharomyces scerivisiae*, Saf-instant®, France); Probiotics 1.0 g/kg DM (AST Co., Ltd., Thailand); Non-starch polysaccharides enzymes 0.5 g/kg DM (AST Co., Ltd., Thailand).

[3] Score of color + odor + texuture + pH..

$^{a-d}$Mean on the same column with different superscripts are significant different ($p<0.05$).

Chemical composition

The chemical composition of FTMR was affected by CBW level and additive type interaction ($P<0.05$) except for OM or total ash proportions (Table 3). Dry matter left after 21 days of ensiling of FTMR containing CBWs was lower than the control. The inclusion of 15% CBW with addition of NSP enzymes to FTMR presented the lowest DM content (22.0%) even though it is not different from FTMR containing

5% CBW with probiotics, 10% CBW with non-additive or with enzymes, and 15% CBW with non-additive. Although the crude protein content was formulated to be similar, the ensiled FTMR showed significantly different results after 21 days. FTMR without CBW but with added probiotics had the highest crude protein content (15.2%), but this was not different from FTMR containing 5% CBW, 5% CBW with probiotics, and 10% and 15% CBW with non-additive. In terms of fiber fractions, the inclusion of CBW to FTMR precisely increased NDF and ADF proportions. The highest NDF content was presented in 10% CBW with NSP enzyme (50.2%) as well as 15% CBW with non-additive (49.9%) inclusions. The highest ADF content was both 15% CBW without (28.6%) and with NSP enzymes (28.4) inclusions.

Table-3. Chemical composition of fermented total mixed ration containing cassava bioethanol waste with various additives.

CBW levels[1]	Additives[2]	DM, %	OM	CP	EE	NDF	ADF	Ash
			% DM					
0CBW	Non	31.4^{a}	91.2	12.7^{c}	3.22^{de}	47.1^{ab}	20.6^{c}	8.78
0CBW	Yeast	26.4^{b}	93.2	13.0^{bc}	3.94^{d}	44.0^{bc}	22.8^{bc}	6.77
0CBW	Probiotics	25.4^{bc}	90.5	15.2^{a}	4.53^{cd}	37.6^{bc}	19.9^{cd}	9.54
0CBW	Enzymes	27.9^{ab}	92.5	11.8^{c}	3.20^{e}	36.6^{c}	16.7^{d}	7.54
5CBW	Non	25.1^{bc}	91.0	14.5^{ab}	5.42^{bc}	43.4^{bc}	23.3^{bc}	9.02
5CBW	Yeast	26.0^{bc}	90.8	12.3^{c}	4.76^{cd}	45.0^{b}	25.7^{ab}	9.19
5CBW	Probiotics	23.0^{c}	91.1	14.8^{ab}	5.59^{bc}	38.2^{bc}	19.3^{cd}	8.86
5CBW	Enzymes	23.5^{bc}	89.8	13.9^{b}	5.54^{bc}	46.4^{ab}	26.6^{ab}	10.2
10CBW	Non	23.0^{c}	91.0	14.4^{ab}	6.62^{a}	44.6^{bc}	25.1^{ab}	9.00
10CBW	Yeast	25.3^{bc}	91.6	12.2^{c}	5.26^{bc}	50.0^{a}	22.6^{bc}	8.36
10CBW	Probiotics	24.9^{bc}	90.4	12.4^{c}	5.04^{c}	43.5^{bc}	24.9^{b}	9.61
10CBW	Enzymes	22.8^{c}	90.8	13.3^{bc}	5.78^{b}	50.2^{a}	24.4^{bc}	9.18
15CBW	Non	22.8^{c}	90.0	14.7^{ab}	6.85^{a}	49.9^{a}	28.6^{a}	9.98
15CBW	Yeast	24.5^{bc}	91.9	12.8^{bc}	4.79^{cd}	44.8^{b}	22.6^{bc}	8.15
15CBW	Probiotics	27.1^{ab}	91.3	12.1^{c}	5.02^{c}	43.4^{bc}	24.7^{bc}	8.74
15CBW	Enzymes	22.0^{c}	90.7	13.1^{bc}	6.43^{ab}	46.5^{ab}	28.4^{a}	9.26
SEM		1.32	1.28	0.44	0.24	1.58	1.03	1.28
P-value		3.93			0.72	4.74	3.09	
CBW levels		<.001	0.125	<.001	<.001	<.001	<.001	0.126
Additives		<.001	0.114	<.001	<.001	<.001	<.001	0.112
Interaction		<.001	0.379	<.001	<.001	<.001	<.001	0.341

[1] Cassava bioethanol waste inclusion levels at 0, 5, 10, and 15 % DM.

[2] Additive inclusion including: Non, non-additive; Yeast powder 1.0 g/kg DM (10^{10} CFU/g *Saccharomyces scerivisiae*, Saf-instant®, France); Probiotics 1.0 g/kg DM (AST Co., Ltd., Thailand); Non-starch polysaccharides enzymes 0.5 g/kg DM (AST Co., Ltd., Thailand).

[a-d] Mean on the same column with different superscripts are significant different ($p<0.05$).

SEM, standard error of the mean.

In vitro gas production

Interactions between the CBW level and the additive type in FTMR did impact the kinetics gas production including the gas production from the immediately soluble fraction (a) and the gas production rate constant (c) ($P<0.05$). The gas production from the insoluble fraction (b) and the potential extent of gas production (P) were not impacted nor were the cumulative gas volumes in the *in vitro* trial (Table 4). The cumulative gas and kinetic gas production were not affected by CBW levels; however, the 72 h and 96 h cumulative gas, the gas production from the insoluble fraction, and the potential extent of gas production did differ between additive types ($P < 0.05$). Treating FTMR with probiotics or NSP enzymes increased the 72 h and 96 h cumulative gas volume, but yeast powder did not (relative to the non-treated FTMR). Gas production from the insoluble fraction was increased in both FTMR treated with yeast, probiotics, and enzymes; however, the potential extent of gas production was not increased with addition versus controls. Gas production from the immediately soluble fraction was highest when 15% CBW was included. In comparison, the gas production rate constant was highest when 10% CBW was included (without an additive) (Table 6).

Table-4. Cumulative gas and kinetic of gas production of fermented total mixed ration containing cassava bioethanol waste with various additives in *in vitro* trial.

	Cumulative gas, ml/0.5g				**ME[3]**	**Kinetic of gas production**			
Factor	**24 hr**	**48 hr**	**72 hr**	**96 hr**		**a**	**b**	**c**	**P**
CBW levels[1]									
0CBW	54.2	71.4	78.5	82.7	7.81	-2.46	89.0	0.038	92.3
5CBW	51.9	73.2	80.7	84.5	7.74	-1.91	93.1	0.034	97.1
10CBW	47.9	64.1	70.0	73.0	7.63	-1.88	85.0	0.041	88.7
15CBW	49.3	67.3	74.7	78.0	7.26	-1.99	84.5	0.036	90.4
Additives[2]									
Non	45.5	60.0	65.1^{b}	67.6^{b}	6.94	2.40^{a}	69.6^{b}	0.040	73.6^{b}
Yeast	50.1	69.5	77.3^{ab}	81.4^{ab}	7.88	-5.61^{c}	98.5^{a}	0.035	104.8^{a}
Probiotics	54.5	74.6	82.4^{a}	86.9^{a}	7.87	-3.39^{bc}	96.2^{a}	0.034	99.6^{a}
Enzymes	53.3	71.9	79.1^{a}	82.3^{a}	7.76	-1.79^{b}	88.4^{a}	0.039	91.8^{ab}
SEM	5.31	8.27	9.13	9.63	0.67	1.55	12.0	0.004	12.5
P-value									
CBW levels	0.543	0.719	0.731	0.675	0.568	0.971	0.727	0.164	0.798
Additives	0.119	0.057	0.038	0.032	0.128	<.001	0.008	0.300	0.008
Interaction	0.300	0.544	0.548	0.561	0.304	<.001	0.598	0.047	0.526

[1] Cassava bioethanol waste inclusion levels at 0, 5, 10, and 15 % DM.

[2] Additive inclusion including: Non, non-additive; Yeast powder 1.0 g/kg DM (10^{10} CFU/g *Saccharomyces scerivisiae*, Saf-instant®, France); Probiotics 1.0 g/kg DM (AST Co., Ltd., Thailand); Non-starch polysaccharides enzymes 0.5 g/kg DM (AST Co., Ltd., Thailand).

[3] ME (MJ/kg DM) = 1.06 + 0.157GP(ml/200mgDM) + 0.0084CP(%) + 0.022EE(%) – 0.0081Ash(%) (Menke and Steingass, 1988).

[a-b] Mean on the same column with different superscripts are significant different ($p<0.05$).

SEM, standard error of the mean.

Ruminal degradability

The dry matter degradability of FTMR was different between additive types, but organic matter degradability was different between CBW levels ($P<0.05$) at 24 h of *in vitro* incubation. The addition of probiotics resulted in higher DM degradability than other additives. In contrast, the inclusion of CBW significantly decreased OM degradability versus controls. The degradability of FTMR after 48 h and 96 h of *in vitro* incubation was influenced by CBW level and additive type interaction ($P<0.05$; Table 5). The inclusion of 15% CBW with added probiotics had the lowest DM degradability after 48 h of *in vitro* incubation. Moreover, FTMR treated with probiotics resulted in the lowest DM degradability after 96 h incubation and OM degradability after 48 h and 96 h incubation; these samples did not have CBW inclusions (Table 6).

Table-5. *In vitro* ruminal degradability of fermented total mixed ration containing cassava bioethanol waste with various additives.

Factor	**DM degradability, %**			**OM degradability, %**			**ME[3]**
	24 hr	**48 hr**	**96 hr**	**24 hr**	**48 hr**	**96 hr**	
CBW levels[1]							
0CBW	62.0	69.0	73.8	60.9^{a}	67.6	77.7	9.87^{a}
5CBW	61.2	65.9	73.8	55.6^{b}	64.2	75.1	9.34^{b}
10CBW	67.0	65.0	72.6	55.7^{b}	63.8	74.5	9.28^{b}
15CBW	61.1	62.8	70.0	50.7^{b}	61.8	73.2	8.53^{c}
Additives[2]							
Non	56.1^{b}	66.8	75.1	53.4	65.1^{a}	77.7	9.04
Yeast	61.0^{b}	66.9	70.7	55.5	66.0^{a}	72.8	9.09
Probiotics	73.2^{a}	61.2	71.4	56.3	58.4^{b}	74.8	9.38
Enzymes	61.8^{b}	67.4	72.6	56.9	66.5^{a}	74.9	9.44
SEM	5.00	3.55	2.84	3.27	3.46	3.25	0.32
P-value							
CBW levels	0.279	0.151	0.163	0.004	0.449	0.853	<.001
Additives	0.001	0.093	0.022	0.475	0.012	0.154	0.192
Interaction	0.150	0.015	<.001	0.134	0.002	0.029	0.003

[1] Cassava bioethanol waste inclusion levels at 0, 5, 10, and 15 % DM.

[2] Additive inclusion including: Non, non-additive; Yeast powder 1.0 g/kg DM (10^{10} CFU/g *Saccharomyces scerivisiae*, Saf-instant®, France); Probiotics 1.0 g/kg DM (AST Co., Ltd., Thailand); Non-starch polysaccharides enzymes 0.5 g/kg DM (AST Co., Ltd., Thailand).

[3] ME (MJ/kg DM) = 0.37 + 0.0142DOMD(g/kgDM) + 0.0077CP(g/kgDM) (Givens et al., 1990).

[a-b] Mean on the same column with different superscripts are significant different ($p<0.05$).

SEM, standard error of the mean.

Metabolizable energy

The metabolizable energy (ME) was calculated from *in vitro* cumulative gas volume and chemical contents of FTMR and was similar between CBW levels and additive types ($P>0.05$; Table 4). However, ME calculated using OM degradability was influenced by CBW level and additive type interactions ($P<0.05$; Table 5). The ME of FTMR decreased with CBW inclusion level, but it increased with addition of live yeast, probiotics, or NSP enzymes (Table 6).

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Table-6. *In vitro* kinetic of gas production and degradability of fermented total mixed ration as affected by cassava bioethanol waste x additives interaction.

CBW levels[1]	Additives[2]	Kinetic of gas production		DM degradability, %		OM degradability, %		ME[3]
		a	c	48 hr	96 hr	48 hr	96 hr	
0CBW	Non	-1.89^{bcde}	0.033^{b}	71.0^{abc}	81.4^{a}	68.5^{abc}	83.6^{a}	9.46^{bc}
0CBW	Yeast	-2.16^{cdef}	0.035^{b}	67.2^{abcd}	68.8^{bcd}	67.7^{abcd}	73.3^{abc}	9.47^{bc}
0CBW	Probiotics	-2.63^{cdefg}	0.042^{ab}	60.1^{cd}	51.4^{e}	49.9^{e}	62.4^{c}	9.52^{bc}
0CBW	Enzymes	-3.17^{cdefg}	0.041^{ab}	77.2^{a}	76.7^{abc}	78.6^{a}	80.1^{ab}	10.85^{a}
5CBW	Non	0.31^{bcd}	0.039^{ab}	71.2^{abc}	78.4^{ab}	69.7^{abc}	79.9^{ab}	9.68^{bc}
5CBW	Yeast	-4.78^{defg}	0.034^{b}	61.9^{cd}	69.1^{bcd}	60.6^{cde}	69.0^{bc}	8.91^{cd}
5CBW	Probiotics	-5.57^{efg}	0.034^{b}	66.5^{abcd}	74.6^{abcd}	64.5^{bcd}	77.5^{ab}	9.91^{bc}
5CBW	Enzymes	2.38^{bc}	0.029^{b}	65.7^{abcd}	72.1^{abcd}	63.7^{bcd}	74.0^{abc}	8.86^{cd}
10CBW	Non	3.42^{ab}	0.053^{a}	65.0^{abcd}	75.0^{abcd}	64.7^{bcd}	76.5^{ab}	8.99^{c}
10CBW	Yeast	-7.81^{g}	0.034^{b}	63.5^{bcd}	67.9^{bcd}	62.2^{bcde}	70.1^{bc}	8.80^{cd}
10CBW	Probiotics	-2.13^{cdef}	0.031^{b}	64.6^{bcd}	73.0^{abcd}	63.1^{bcd}	75.5^{ab}	9.87^{bc}
10CBW	Enzymes	-1.03^{bcde}	0.043^{ab}	66.9^{abcd}	75.1^{abcd}	65.1^{bcd}	76.6^{ab}	9.47^{bc}
15CBW	Non	7.75^{a}	0.033^{b}	61.5^{cd}	65.4^{d}	59.0^{cde}	70.6^{bc}	8.03^{d}
15CBW	Yeast	-7.68^{fg}	0.036^{b}	75.3^{ab}	76.3^{abc}	74.7^{ab}	78.8^{ab}	9.18^{bc}
15CBW	Probiotics	-2.68^{cdefg}	0.035^{b}	54.7^{d}	71.5^{abcd}	54.8^{de}	74.4^{ab}	8.30^{cd}
15CBW	Enzymes	-5.36^{efg}	0.040^{ab}	59.8^{cd}	66.6^{cd}	58.6^{cde}	69.1^{bc}	8.60^{cd}
SEM		1.55	0.004	3.55	2.84	3.46	3.25	0.32
P-value		<0.001	0.039	0.011	0.001	0.002	0.044	<0.001

[1] Cassava bioethanol waste inclusion levels at 0, 5, 10, and 15 % DM.

[2] Additive inclusion including: Non, non-additive; Yeast powder 1.0 g/kg DM (10^{10} CFU/g *Saccharomyces scerivisiae*, Saf-instant®, France); Probiotics 1.0 g/kg DM (AST Co., Ltd., Thailand); Non-starch polysaccharides enzymes 0.5 g/kg DM (AST Co., Ltd., Thailand).

[3] ME (MJ/kg DM) = 0.37 + 0.0142DOMD(g/kgDM) + 0.0077CP(g/kgDM) (Givens et al., 1990).

[a-g] Mean on the same column with different superscripts are significant different ($p<0.05$).

SEM, standard error of the mean.

In vitro fermentation end-products

The fermentation end-products of FTMR including ammonia nitrogen (NH3-N) and volatile fatty acid (VFA) concentrations after 24 h and 48 h of *in vitro* incubation were influenced by CBW level and additive type interactions ($P<0.05$; Table 7 and Table 8). The inclusion of CBW decreased while the addition of probiotics or NSP enzymes increased NH3-N concentration. Acetic acid (C2) and butyric acid concentrations (C4) were increased, while propionic acid (C3) concentration was decreased with CBW inclusion levels. The non-CBW FTMR had the highest total VFA concentration and C3 proportion, thus resulting in the lowest C2:C3 ratio—especially when NSP enzymes were added. However, when 15% CBW was included, these VFA concentrations were switched with yeast or probiotics added to FTMR. The proportion of C3 and the C2:C3 ratios were inverted and NH3-N and total VFA concentrations were increased with time of *in vitro* fermentation (24 h vs 48 h).

Table-7. Ruminal fermentation end-products of fermented total mixed ration containing cassava bioethanol waste with various additives at 24h incubation in *in vitro* trial.

CBW levels[1]	**Additives**[2]	**NH$_3$-N, mg/dL**	**Volatile fatty acids, mmol/dL**				**Volatile fatty acids, %TVFA**			
			TVFA[3]	**C2**	**C3**	**C4**	**C2**	**C3**	**C4**	**C2:C3**
0CBW	Non	10.1^{bc}	45.5^{abcde}	27.7^{bcd}	12.7^{bcd}	2.07^{cde}	60.9^{bcde}	27.9^{b}	11.2^{cdefgh}	2.19^{efg}
0CBW	Yeast	9.57^{bcd}	42.6^{defg}	26.5^{cde}	10.6^{efg}	5.60^{bcd}	62.1^{abc}	24.8^{de}	13.1^{cdef}	2.51^{abc}
0CBW	Probiotics	11.4^{ab}	44.6^{abcdef}	27.6^{bcd}	12.5^{bcd}	4.50^{de}	61.9^{abc}	28.0^{b}	10.1^{efgh}	2.22^{ef}
0CBW	Enzymes	12.2^{a}	48.7^{a}	29.3^{ab}	14.6^{a}	4.75^{de}	60.3^{bcde}	30.0^{a}	9.67^{fgh}	2.01^{g}
5CBW	Non	9.64^{bcd}	47.4^{abc}	30.0^{a}	12.9^{bc}	4.41^{de}	63.5^{ab}	27.2^{bc}	9.30^{gh}	2.33^{bcdef}
5CBW	Yeast	9.71^{bcd}	43.7^{cdef}	25.9^{def}	10.5^{efg}	7.27^{ab}	59.2^{cde}	24.1^{e}	16.6^{ab}	2.46^{abcd}
5CBW	Probiotics	10.3^{bc}	43.7^{cdef}	27.1^{cde}	11.7^{bcdef}	4.90^{cde}	62.0^{abc}	26.8^{bc}	11.2^{cdefgh}	2.31^{bcdef}
5CBW	Enzymes	10.8^{ab}	41.1^{fg}	25.7^{ef}	10.4^{efg}	4.99^{cde}	62.5^{abc}	25.4^{cde}	12.2^{cdefg}	2.47^{abcd}
10CBW	Non	8.76^{cd}	42.2^{efg}	27.2^{cde}	11.4^{cdef}	3.59^{e}	64.6^{a}	27.0^{bc}	8.42^{h}	2.39^{bcde}
10CBW	Yeast	9.02^{bcd}	45.9^{abcde}	27.8^{bcd}	11.9^{bcde}	6.13^{bcd}	60.6^{bcde}	26.0^{bcde}	13.4^{bcde}	2.33^{bcdef}
10CBW	Probiotics	10.6^{ab}	42.6^{defg}	25.4^{ef}	11.1^{defg}	6.12^{bcd}	59.7^{cde}	26.1^{bcde}	14.2^{bc}	2.29^{def}
10CBW	Enzymes	10.2^{abc}	44.5^{bcdef}	27.2^{cde}	11.8^{bcde}	5.50^{bcd}	61.1^{bcd}	26.5^{bcd}	12.4^{cdefg}	2.30^{cdef}
15CBW	Non	8.34^{d}	39.1^{g}	24.3^{f}	9.64^{g}	5.14^{cde}	62.2^{abc}	24.7^{de}	13.2^{edef}	2.52^{ab}
15CBW	Yeast	8.56^{cd}	48.3^{ab}	28.4^{abc}	13.2^{ab}	6.74^{abc}	58.7^{de}	27.4^{b}	13.9^{bcd}	2.14^{efg}
15CBW	Probiotics	9.76^{bcd}	46.6^{abcd}	27.0^{cde}	11.5^{cdef}	8.17^{a}	57.9^{e}	24.6^{de}	17.5^{a}	2.35^{bcdef}
15CBW	Enzymes	9.83^{bcd}	41.1^{fg}	26.7^{cde}	10.1^{fg}	4.28^{de}	65.1^{a}	24.5^{e}	10.4^{defgh}	2.66^{a}
SEM		0.524	1.075	0.485	0.432	0.464	0.811	0.506	0.903	0.054
P-value										
CBW levels		<0.001	0.220	0.035	0.002	0.046	0.633	<0.001	0.009	0.001
Additives		<0.001	0.345	0.498	0.977	<0.001	<0.001	0.075	<0.001	0.338
Interaction		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1] Cassava bioethanol waste inclusion levels at 0, 5, 10, and 15 % DM.

[2] Additive inclusion including: Non, non-additive; Yeast powder 1.0 g/kg DM (10^{10} CFU/g *Saccharomyces scerivisiae*, Saf-instant®, France); Probiotics 1.0 g/kg DM (AST Co., Ltd., Thailand); Non-starch polysaccharides enzymes 0.5 g/kg DM (AST Co., Ltd., Thailand).

[3] TVFA, total VFA which C2+C3+C4; C2, acetic acid; C3, propionic acid; C4, butyric acid.

$^{a-g}$Mean on the same column with different superscripts are significant different ($p<0.05$).

SEM, standard error of the mean.

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Table-8. Ruminal fermentation end-products of fermented total mixed ration containing cassava bioethanol waste with various additives at 48h incubation in *in vitro* trial.

CBW levels[1]	Additives[2]	NH_3-N, mg/dL	Volatile fatty acids, mmol/dL				Volatile fatty acids, %TVFA			
			TVFA[3]	C2	C3	C4	C2	C3	C4	C2:C3
0CBW	Non	12.3^{abc}	50.8^{b}	33.1^{b}	13.9^{cd}	3.85^{b}	65.1^{b}	27.3^{defg}	7.59^{bc}	2.38^{bcd}
0CBW	Yeast	12.6^{abc}	46.4^{cd}	31.9^{bcde}	12.1^{hi}	2.40^{bc}	68.8^{a}	26.0^{gh}	5.15^{bc}	2.65^{a}
0CBW	Probiotics	13.3^{ab}	49.1^{bc}	33.0^{b}	14.7^{b}	1.39^{c}	67.2^{ab}	30.0^{ab}	2.82^{c}	2.25^{ef}
0CBW	Enzymes	13.7^{a}	54.5^{a}	35.5^{a}	16.5^{a}	2.57^{bc}	65.1^{b}	30.2^{a}	4.69^{bc}	2.15^{f}
5CBW	Non	10.5^{bc}	45.4^{d}	29.9^{fg}	13.0^{defg}	2.50^{bc}	65.9^{ab}	28.6^{abcd}	5.50^{bc}	2.30^{bcde}
5CBW	Yeast	10.8^{bc}	44.8^{d}	28.9^{gh}	12.1^{ghi}	3.81^{b}	64.5^{b}	27.0^{defg}	8.50^{b}	2.39^{bcd}
5CBW	Probiotics	12.3^{abc}	46.6^{cd}	31.1^{def}	13.4^{de}	2.20^{bc}	66.6^{ab}	28.7^{abcd}	4.75^{bc}	2.32^{bcde}
5CBW	Enzymes	13.1^{ab}	45.3^{d}	29.3^{gh}	12.3^{fghi}	3.75^{bc}	64.6^{b}	27.2^{defg}	8.23^{b}	2.37^{bcd}
10CBW	Non	9.84^{c}	47.0^{cd}	30.5^{efg}	13.3^{de}	3.12^{bc}	65.0^{b}	28.4^{bcde}	6.64^{bc}	2.29^{cde}
10CBW	Yeast	10.3^{bc}	50.3^{b}	32.8^{bc}	14.5^{bc}	3.05^{bc}	65.2^{b}	28.8^{abcd}	6.08^{bc}	2.27^{def}
10CBW	Probiotics	12.6^{abc}	46.0^{d}	29.6^{fg}	12.2^{ghi}	4.20^{b}	64.4^{b}	26.5^{fgh}	9.10^{b}	2.43^{b}
10CBW	Enzymes	12.7^{abc}	47.2^{cd}	30.4^{efg}	13.7^{cde}	3.12^{bc}	64.3^{b}	29.1^{abc}	6.61^{bc}	2.21^{ef}
15CBW	Non	10.3^{bc}	46.0^{d}	27.7^{h}	11.5^{i}	6.76^{a}	60.4^{c}	25.1^{h}	14.5^{a}	2.41^{bc}
15CBW	Yeast	10.6^{bc}	51.3^{b}	32.7^{bcd}	14.7^{b}	3.86^{b}	63.8^{b}	28.7^{abcd}	7.50^{bc}	2.23^{ef}
15CBW	Probiotics	11.8^{b}	49.0^{bc}	31.3^{cdef}	13.1^{def}	4.56^{b}	64.0^{b}	26.7^{efg}	9.29^{b}	2.40^{bc}
15CBW	Enzymes	12.3^{abc}	45.8^{d}	29.8^{fg}	12.9^{efgh}	3.06^{bc}	65.2^{b}	28.2^{cdef}	6.62^{bc}	2.31^{bcde}
SEM		0.641	1.075	0.485	0.432	0.464	0.811	0.506	0.903	0.054
P-value										
CBW levels		<0.001	<0.001	<0.001	<0.001	0.005	0.002	0.020	0.002	0.198
Additives		<0.001	0.378	0.012	<0.001	0.167	0.165	0.005	0.117	0.001
Interaction		<0.001	<0.001	<0.001	<0.001	0.030	0.046	<0.001	0.013	<0.001

[1] Cassava bioethanol waste inclusion levels at 0, 5, 10, and 15 % DM.

[2] Additive inclusion including: Non, non-additive; Yeast powder 1.0 g/kg DM (10^{10} CFU/g *Saccharomyces scerivisiae*, Saf-instant®, France); Probiotics 1.0 g/kg DM (AST Co., Ltd., Thailand); Non-starch polysaccharides enzymes 0.5 g/kg DM (AST Co., Ltd., Thailand).

[3] TVFA, total VFA which C2+C3+C4; C2, acetic acid; C3, propionic acid; C4, butyric acid.

$^{a\text{-}i}$Mean on the same column with different superscripts are significant different ($p<0.05$).

SEM, standard error of the mean.

Discussion

The significant interaction between CBW inclusion levels and types of additives suggests that the CBW significantly affects fermentation pathways based on the microbiological or enzymatic context. Optimal physical trait scores at 10% CBW supplemented with probiotics or NSP enzymes indicate that these additives improve structural integrity, buffering capacity, and required acidification patterns. Probiotic microorganisms such as *Bacillus* spp. and LAB are known to improve sensory profiles by accelerating lactic acid production, lowering pH early in fermentation, and competitively excluding spoilage microbes (Okoye et al., 2023; Branco-Lopes et al., 2025). Although yeast derivatives are recognized for supporting ruminal fermentation, their use at 15% CBW may have stabilized the high-moisture residue and reduced proteolytic spoilage, thus maintaining quality comparable to the most effective treatments. In contrast, over-degradation of the high fiber fractions may be the cause of the poor physical characteristics of FTMR with NSP enzymes at 15% CBW, which results in excessive firmness, a slippery texture, and undesirable odors (Thungphoomrapeewong, 2015;

Anil et al., 2022; Ramdani et al., 2025). These findings indicate that—due to CBW's low palatability and high fiber content—its successful integration into FTMR requires careful selection and optimization of additives to ensure fermentation stability, physical quality, and subsequent feed utilization.

The chemical stability and nutrient composition of FTMR are strongly influenced by the fibrous nature of CBW and the mode of action of microbial or enzymatic additives. The consistent reduction in DM after 21 days of ensiling across CBW treatments imitates the intrinsic challenges of preserving high-moisture agro-industrial residues where extensive fermentation and effluent losses can contribute to DM reduction (Muck et al., 2018). The low DM observed in the 15% CBW with NSP enzyme treatment (22.0%) may be attributed to enhanced hydrolysis of structural carbohydrates thus increasing soluble substrates for microbial fermentation and accordingly increasing fermentative losses. The progressive increase in NDF and ADF with higher CBW inclusion is consistent with its lignocellulosic profile—often exceeding 60% NDF depending on processing conditions (Yi, 2023). The elevated NDF in the 10% CBW + NSP enzyme treatment suggests that enzyme efficiency is inhibited by fiber resistance and lignin relations, which limit complete hydrolysis (Ramdani et al., 2025). In contrast, the relatively stable CP concentration in probiotic-supplemented treatments indicates that LAB can reduce proteolysis and ammonia-N accumulation during ensiling, thus preserving true protein fractions (Muck et al., 2018; Okoye et al., 2023).

In vitro gas production kinetics explain the fermentability and potential energy availability of FTMR-containing CBW. The significant interaction between CBW inclusion level and additive type on the immediately soluble fraction (*a*) and the fractional rate constant (*c*) indicates that fermentation dynamics are highly dependent on both substrate composition and additive-mediated biological activity. As reported, CBW is characterized by elevated fiber and considerable ash content—these factors impact microbial accessibility and buffering capacity (Laorodphan et al., 2013; Fathima et al., 2022; Pilajun et al., 2024). The increased gas production from the insoluble fraction (b) in probiotic-, yeast-, or NSP-enzyme-treated FTMR suggests enhanced degradation of lignocellulosic components, thus improving substrate availability for ruminal microbes. The greater cumulative gas production at 72 and 96 h in probiotic and NSP enzyme treatments is consistent with evidence that fibrolytic enzymes and beneficial microbes promote sustained hydrolysis of structural carbohydrates (Pilajun and Wanapat, 2018; Branco-Lopes et al., 2025; Ramdani et al., 2025). The elevated soluble fraction (*a*) at 15% CBW inclusion indicates the contribution of residual soluble components and yeast cell contents from ethanol processing, which supports rapid initial fermentation. However, the highest gas production rate constant (*c*) observed at 10% CBW without additives suggests a balance between fermentable substrate and structural constraints, beyond which excessive fiber and ash may restrict microbial colonization and fermentation rates. Similar findings indicate that, although additives can enhance total fermentative productivity, substrate physicochemical properties essentially regulate early microbial attachment and gas kinetics (Rabee et al., 2022).

The 24 h DM degradability results demonstrate that probiotics may stimulate early-stage fermentation by stabilizing the pH and providing growth factors that enable rapid microbial attachment to the feed particles. However, the subsequent reduction in DM and OM degradability at 48 h and 96 h—particularly at 15% CBW inclusion with probiotics—presents a complex metabolic shift. This result may be attributed to the probiotic inconsistency, where a rapid initial burst of microbial activity leads to accumulation of fermentation byproducts such as lactic acid that can temporarily suppress the activity of more specialized cellulolytic bacteria during the later and more critical phases of fiber breakdown (Reuben et al., 2021; Yang et al., 2025). Furthermore, the significant decline in OM degradability is related to higher CBW levels and is a direct consequence of the byproduct's complex matrix. CBW with high concentrations of NDF and lignin-carbohydrate complexes act as physical barriers to enzymatic penetration (Sriroth et al., 2012; Zhang et al., 2016; Yi, 2023). The high ash—and especially sand content—often found in cassava residues also serve as non-fermentable diluents that further reduce the potential for organic matter disappearance (Puramongkon, 2016; Vuong et al., 2021). These results align with previous findings in which the efficacy of biological additives in high-fiber rations is frequently limited by the slow degradation rates of insoluble fractions, thus requiring a more synchronized approach between microbial activity and the structural complexity of the byproduct being used (Markowiak and Śliżewska, 2018; Chowdhury et al., 2025).

The inconsistency between gas-based and OM-degradability-based ME calculations indicates that while the total volume of fermentation gases may remain stable, the actual disappearance of the organic matter is highly delicate to the ration's fiber and ash levels. The observed decline in ME at higher inclusion levels of CBW is likely a dilution effect caused by its high NDF and ash content. However, the significant interaction results reveal that the addition of live yeast, probiotics, or NSP enzymes can effectively mitigate this energy decline. These additives enhance ME by facilitating the degradation of recalcitrant lignin-carbohydrate complexes, thus unlocking fermentable energy sources (Cherdthong et al., 2018; Baker et al., 2022; Gunun et al., 2022; Su et al., 2024). Probiotics and yeast further optimize energy availability by stabilizing rumen pH and potentially shifting fermentation toward more efficient VFA profiles, which reduce energy loss through methane (Vohra et al., 2016; Saleem et al., 2025). Consequently, strategic supplementation allows CBW to produce energy in ruminant diets (Wang et al., 2022).

The interaction between CBW levels and additive types significantly affects the ruminal fermentation profile—particularly the distribution of VFAs and nitrogen metabolism. The decline in NH_3-N observed with increasing CBW levels may be due to the byproduct's lignocellulosic structure and low soluble protein content, which limit microbial proteolytic activity (Pornjantuek et al., 2015; Gharechahi et al., 2023). Conversely, the elevation of NH_3-N by probiotics and NSP enzymes suggests enhanced degradation of the ration's protein fraction or increased microbial turnover. The shift toward higher C2 and C4 proportions at the expense of C3 as CBW levels increase is consistent with the fermentation of high-fiber substrates. As the main glucogenic precursor, a decrease in C3 indicates reduced energy efficiency, which is typically associated with residues that do not contain starch (Nichols et al., 2019). However, the converting effect observed at 15% CBW when complemented with yeast or probiotics is a critical finding. This finding indicates that these additives effectively alter the fermentation pathway by stimulating propionate-producing bacteria or stabilizing the rumen environment, which promotes more efficient energy extraction from fibrous materials (Cherdthong et al., 2018; Chowdhury et al., 2025). The variation of the C2:C3 ratio over time (24 h vs. 48 h) indicates microbial community succession and the decrease of readily fermentable carbohydrates, thus shifting towards the more resistant fiber fractions of the CBW. Probiotics and NSP enzymes can be used strategically to lower the high C2:C3 ratios found in diets rich in byproducts (Mousa et al., 2022; Bureenok et al., 2024; Ferreira et al., 2025), thus enhancing the overall metabolic efficiency of the FTMR.

Conclusions

The inclusion of 10–15% CBW in FTMR is technically feasible. The optimal results are achieved with a 10% inclusion rate when combined with probiotics or NSP enzymes, which improve physical property and nitrogen availability. At a 15% inclusion rate, the addition of live yeast is crucial for stabilizing sensory properties and promoting the production of VFAs towards propionate. Although CBW's high fiber and ash content reduces energy density, biological additives can effectively counteract these declines by enhancing microbial fiber degradation. It is important to prioritize pre-treatment methods that reduce ash content and limit soil and sand contamination in fresh CBW. The results are based on in vitro trials that cannot fully simulate long-term animal physiological adaptations, selective intake, or palatability; therefore, future research needs to focus on long-term *in vivo* trials to assess how these FTMR formulations affect feeding behavior and efficiency of conversion into meat and milk yield and quality.

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Ethical Approval Statement

The study was conducted at Ruminant Farm and laboratory of the Faculty of Agriculture, Ubon Ratchathani University, Thailand, which approved by the Institutional Animal Care and Use Committee of Ubon Ratchathani University (ID#10/2568/IACUC).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Pilajun R, upon reasonable request.

Contribution of Authors

Pilajun R & Yeanpet C: Project administration, conceptualization, methodology, investigation, formal analysis, visualization, validation, data curation, writing – original draft & editing.

Lunpha A & Jitchati R: Conceptualization, visualization, validation, data curation, writing – review & editing.

Lunsin R & Chung ELT: Conceptualization, visualization, writing – review & editing.

Kaewluan W: Methodology, investigation & formal analysis.

All authors read and approved the final draft of the manuscript.

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