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In vitro Ruminal Fermentation Characteristics of Maize-Stover-Based Diet Supplemented with Concentrates Differing Level in Cassava Leaf Tuber Silage

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Abstract | Cassava is widely cultivated in many tropical regions and is valued for its carbohydrate-rich tubers and protein-rich leaves. While its tubers are already used in livestock diets because of their high energy content, its leaves and peels often remain underutilized byproducts. This study evaluated the effects of a maize stover-based diet supplemented with concentrates containing varying levels (0, 5, 10, 15, 20, 25, 30, and 35%) of cassava leaf-tuber silage (CLTS) on ruminal fermentation characteristics and microbial protein synthesis. In this study, eight treatments were analysed via an in vitro two-stage incubation procedure consisting of 48 hours of ruminal fermentation followed by 24 hours of acid–pepsin digestion. A randomized block design with three replicates per treatment was employed, and the data were analysed via ANOVA ($P < 0.05$). The results revealed that the level of cassava leaf-tuber silage significantly affected ($P < 0.05$) NH_3-N and EMPS. The addition of 15% CLTS to the concentrates resulted in optimal organic matter digestibility (60.47%) and EMPS (32–33 g N/kg FOM), whereas the addition of 30–35% CLTS decreased the NH_3-N value. These values remained above the 50 mg/L threshold necessary to support adequate microbial activity. It was concluded that CLTS at a level of 15% had optimal EMPS values, supported by a balanced volatile fatty acid and NH_3 profile.

Keywords | Cassava product, Silage, Fermentation, Microbial efficiency, Ruminant

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INTRODUCTION

Cassava (*Manihot esculenta,* Crantz) is a multifunctional plant renowned for its carbohydrate-rich tubers and protein-containing leaves, offering significant potential as an alternative livestock feed resource. However, its nutritional value is compromised by cyanogenic glycosides, which can release hydrogen cyanide and pose a risk of toxicity to livestock if not properly processed. Among the promising methods for mitigating these negative effects, ensiling has emerged as an effective technique. This process relies on microbial fermentation under anaerobic conditions to lower pH and inhibit spoilage, thereby increasing the safety, palatability, and overall feeding value of cassava byproducts (Du *et al.*, 2020; Unnawong *et al.*, 2023). Despite the exploration of various detoxification methods, there remains a critical need to optimize these techniques to safely incorporate cassava into livestock diets and enhance overall production efficiency. In addition to reducing cyanide content, ensiling improves nutrient stability, decreases storage costs, and can be more readily integrated into small-scale cattle production systems than can more sophisticated processing technologies (Baguma *et al*., 2022; Farinde, 2023). Cassava leaf-tuber silage (CLTS), in particular, combines energy-rich tubers with protein-rich leaves to provide a balanced feed source that meets multiple nutritional demands (Hasanah *et al*., 2023; Mulualem *et al*., 2020).

Recent studies have examined various cassava products, such as fermented mash, cassava peel silage, and cassava waste pulp, for their capacity to support ruminant production with lower feed costs (Kolapo *et al*., 2021; Korir *et al*., 2022; Onyango *et al*., 2021). According to previous studies, cassava silage has a DM content of 33.5%, OM content of 92%, and CP content of 20.5% (Ndaru *et al*., 2014), and tuber silage has a DM content of 33–42%, OM content of 98%, and CP content of 2.1% (Fanelli *et al*., 2023; Lukuyu *et al*., 2014). Wanapat *et al*. (2013) reported that the addition of cassava chips at 57, 80, and 84% yields dry matter degradation values ranging from 46.2--56.7%. Furthermore, the addition of cassava leaves to the feed formula at a level of 5–25% and 20% tuber skin in the ration resulted in a DMD of 68–80% and a DMO of 71–84% (Saputra *et al*., 2021). Some studies have shown that adequate processing significantly mitigates cyanide-risk (Qin *et al*., 2021), whereas others have reported improved diet palatability and reduced reliance on more expensive protein sources (Saputra *et al*., 2021; Kolapo *et al*., 2021).

Overall, these studies indicate that the strategic supplementation of properly processed cassava byproducts, particularly when processed by ensiling, can increase feed safety and nutrient availability, thereby optimizing key rumen fermentation parameters such as the NH_3-N concentration, VFA production, and the efficiency of microbial protein synthesis, which ultimately leads to improved ruminant productivity. Ruminants rely on microbial fermentation in the rumen to break down fibrous plant materials and synthesize microbial protein, ultimately influencing growth, milk yield, and overall performance (Blummel *et al*., 1997). The key indicators of rumen function include the ammonia (NH_3-N) concentration, volatile fatty acid (VFA) production, and the efficiency of microbial protein synthesis (EMPS) (Blummel *et al*., 1997; McDonald *et al*., 2022). A balanced rumen environment is critical: while sufficient NH_3-N ensures that the microbial population can thrive, excessive protein levels can be wasteful and lead to elevated nitrogen excretion (Chuzaemi *et al*., 2020; Korir *et al*., 2022). Volatile fatty acids, including acetate, propionate, and butyrate, are the main energy substrates derived from carbohydrate fermentation and play pivotal roles in animal productivity (Filipek and Dvorak, 2009). Because rumen microbes transform dietary protein into microbial cells, the ability of a diet to maximize EMPS enhances nitrogen use efficiency and reduces feed costs (Blummel *et al*., 1997; Saputra *et al*., 2021). Previous studies have indicated that balanced diets combining fibrous crop residues with protein-rich feed supplements can maintain efficient rumen fermentation (Korir *et al*., 2022; Retnaningrum *et al*., 2020), but optimal proportions remain underresearched, particularly for cassava leaf–tuber mixtures.

Accordingly, this study aimed to evaluate the effects of a maize stover-based diet supplemented with concentrates containing varying levels (0, 5, 10, 15, 20, 25, 30, and 35%) of CLTS on ruminal fermentation characteristics, including the NH3-N concentration, VFA profile, digestibility, and EMPS. Notably, the cassava leaves used in this experiment were derived from bitter varieties of cassava (*Manihot esculenta,* Crantz), which are not highly competitive with humans. Furthermore, this study identified an optimal CLTS level that sustains microbial efficiency in the rumen. Consequently, the findings are important for contributing to scientific discourse and practical applications, particularly in supporting the use of cassava products in beef cattle diets.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

Ethical approval was granted by the Animal Ethics Committee of Universitas Brawijaya under protocol number 131-KEP-UB-2021, ensuring compliance with national guidelines on animal welfare.

This research was conducted at the Feed and Animal Nutrition Laboratory, Faculty of Animal Science, Universitas Brawijaya, Malang, Indonesia, for proximate analysis, in vitro digestibility, and evaluation of rumen fermentation products. Moreover, the production of volatile fatty acids (VFAs) was analysed at the Laboratory of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia. A randomized block design (RBD) comprising eight dietary treatments and three replicates of each was used. This design was selected to control for potential variability in the rumen fluid source and experimental conditions, aligning with recommendations to minimize confounding factors in small-sample experiments (Amalnerkar *et al*., 2020; Nikitina *et al*., 2019; Konietschke *et al*., 2020).

The primary diet consisted of a basal substrate of maize stover (MS) at 20% dry matter (DM) supplemented with differing levels of CLTS to achieve 80% DM for the concentrate portion. CLTS inclusion levels ranged from 0% to 35%. The decision to use CLTS was predicated on evidence suggesting that ensiling reduces cyanogenic glycosides and enhances nutrient stability (Ndaru *et al*., 2014; Retnaningrum *et al*., 2020; Kusmartono *et al*., 2022). In preparation for silage, fresh cassava leaves and tubers were mixed at a ratio of 1:10 (leaf-to-tuber ratio, w/w) and ensiled with 3% molasses for 21 days under anaerobic conditions. The feed formulation treatments applied on a dry matter basis are presented in Table 1.

Table 1: Feed formulation treatments.

Ingredients	Treatments (% DM)							
	A	B	C	D	E	F	G	H
Maize Stover	20	20	20	20	20	20	20	20
Palm Kernel Meal	20	20	20	20	20	20	20	20
Copra Meal	50	45	40	35	30	25	20	15
Coffee Husk	10	10	10	10	10	10	10	10
Cassava Leaf-Tuber Silage	0	5	10	15	20	25	30	35

Each dietary treatment was formulated to meet the baseline nutrient requirements for beef cattle, with particular attention given to the crude protein (CP) and crude fibre (CF) contents. The chemical composition of each treatment was analysed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and crude fibre (CF) following standard methods recommended by the AOAC (2005).

Sample Preparation and Analytical Procedures

Prior to in vitro incubation, the dietary mixtures were ground to pass through a 1 mm screen. Rumen fluid was collected from a local slaughter facility, ensuring that donor cattle had access to typical concentrate-roughage diets so that microbial populations would be similar to those in smallholder settings (Foster *et al*., 2023). The rumen fluid was rapidly transferred into prewarmed, thermally insulated flasks and transported to the laboratory under anaerobic conditions to preserve microbial conditions (Filipek and Dvorak, 2009).

In vitro Digestibility

A two-stage in vitro digestion method (Tilley and Terry, 1963) was employed to simulate ruminal and subsequent gastric digestion (Foster *et al*., 2023). First, each dietary sample (approximately 0.5 g on a DM basis) was incubated at 39 °C for 48 h in a buffered rumen fluid medium. McDougall's buffer solution was used to maintain pH and osmotic stability (McDonald *et al*., 2022). The samples were placed in 100 mL glass syringes or culture tubes with rubber stoppers to ensure anaerobic conditions. After 48 h, the supernatant was separated for analysis, and the residue was subjected to an acidic pepsin stage at 39 °C for 24 h to simulate abomasal digestion. Dry matter digestibility (DMD) and organic matter digestibility (OMD) were calculated by comparing pre- and postincubation DM and OM.

Rumen Fermentation Parameters

Immediately following 48 h of ruminal incubation, subsamples of rumen fluid were collected for ammonia (NH_3-N) and VFA analysis. NH_3-N was determined via Conway's microdiffusion technique (Conway, 1962), a sensitive colorimetric approach used to quantify ammonia release (Dong *et al*., 2021). For VFA production, the steam distillation method described by Filipek and Dvorak (2009) was employed, and the resulting distillate was analysed by gas chromatography. The total VFA production and individual VFA proportions (acetate, propionate, and butyrate) are reported in mMol/L.

Microbial Protein Synthesis Efficiency

The efficiency of microbial protein synthesis (EMPS) was estimated by relating nitrogen (N) incorporation into the microbial mass to the quantity of FOM, following Blummel *et al*. (1997). EMPS was expressed as grams of nitrogen per kilogram of fermented organic matter (g N/kg FOM). This parameter acts as an indicator of how effectively rumen microbes convert dietary protein into microbial cells rather than losing it as waste nitrogen (Dong *et al*., 2021).

Analyses of Data

One-way analysis of variance (ANOVA) was conducted to determine differences among the eight dietary treatments, using the statistical model for randomized block designs outlined by Steel and Torrie (1980). Duncan's multiple range test was applied for post hoc comparisons of treatment means at a significance level of $p < 0.05$. Where pertinent, the study accounted for small sample size limitations by examining data distributions and outliers; a bootstrap-based approach was considered for sensitive parameters, which is consistent with best practices in small-sample research (Nikitina *et al*., 2019; Amalnerkar *et al*., 2020; Konietschke *et al*., 2020). Data analysis and coding were performed via ANOVA with SAS 9.4 software.

RESULTS AND DISCUSSION

Chemical Composition and Nutrient Profile

The feed ingredients used in this study were maize stover, palm kernel meal, copra meal, coffee husk, and cassava leaf-tuber silage (Table 2). All the ingredients had nutrient contents within the range of values reported in a previous study by Kusmartono *et al*. (2022). The nutritional content of maize stover in this study was greater than that reported by Reddy *et al*. (2016), who reported DM content of 90.5%, OM content of 88.9%, CP content of 5.52%, EE content of 2.39%, and CF content of 30.99%. This difference could be affected by variety, harvest age, and soil conditions (Sardans *et al*., 2011). The crude protein content of the palm kernel cake in this study aligns with the findings of Hasanah *et al*. (2023), who reported a crude protein content of 15.5%.

Additionally, the crude protein contents of the copra cake and coffee husk in this study were 22.8% and 9.35%, respectively. These values fall within the ranges reported by Icahyaningrum *et al.* (2024) . However, the cassava silage in this study contained a higher crude protein (CP) content (2.82%) than the 2.04% reported by Khota *et al.* (2023). This difference is attributed to the use of a combination of leaves and tubers in the current study, with the leaves having a relatively high CP content of 22% (Ndaru *et al.*, 2014).

Table 2: Chemical analysis of feed ingredients.

Ingredients	DM (Oven 105°C)	OM	CP	EE	CF
	(%)	(% DM)			
Maize Stover	94.02	88.93	9.20	1.09	33.21
Palm Kernel Meal	89.50	95.99	15.52	8.66	20.79
Copra Meal	88.93	90.11	22.8	1.84	20.40
Coffee Husk	90.23	82.29	9.35	1.45	31.89
Cassava Leaf-Tuber Silage	92.75	95.58	2.82	0.74	5.17

DM: dry matter; **OM:** organic matter; **CP:** crude protein; **EE:** extract ether; **CF:** crude fibre.

This nutrient profile aligns with previous studies indicating that the ensiling of cassava byproducts can maintain or improve their nutritive value while reducing cyanogenic glycosides (Hawashi *et al.*, 2019; Terefe *et al.*, 2022). In particular, the presence of leaves can increase dietary fibre, potentially affecting fermentation kinetics in the rumen (Retnaningrum *et al.*, 2020). According to Putri *et al.* (2019), balancing degradable and undegradable protein sources is crucial for optimizing microbial growth. Consequently, careful attention was given to adjusting the protein content to maintain adequate rumen-degradable protein (RDP) without causing excessive ammonia loss.

Although CLTS-based diets had lower CP contents than the control diet did (see suggested Table 3), the chemical composition remained within acceptable ranges to support rumen microbial activity when combined with maize stover (McDonald *et al.*, 2022; Putri *et al.*, 2021). Similar observations were reported by Khejornsart *et al.* (2022), who reported that adding cassava pulp to fermented total mixed rations improved the rumen ecology and microbial protein synthesis despite moderate CP levels. The percentage of CP in the diets used in this study ranged from 10.23–17.34%, which is appropriate for beef cattle. In accordance with the recommendations of Rayburn (2009) and NRC (2000), the CP requirement for growing beef cattle ranges from 9.60% to 12.90% DM.

Dry Matter and Organic Matter Digestibility

As shown in Table 4, the dry matter digestibility (DMD) across the treatments ranged from approximately 56.49% to 61.92%. Although there was no statistically significant difference ($p > 0.05$) among the treatments, a slight decrease was observed in the diets with the highest CLTS content (30–35%). This observation corresponds with previous findings by Saputra *et al.* (2021), who reported that cassava-based diets could exhibit moderate reductions in DMD when inclusion rates surpass certain thresholds. However, the DMD values recorded here generally remained above 55%, indicating that CLTS did not markedly impede rumen microbial degradation (Foster *et al.*, 2023).

The ensiling process contributes to preserving the degradability of cassava materials by reducing antinutritional compounds (Hawashi *et al.*, 2019; Terefe *et al.*, 2022). Moreover, ensiling facilitates lactic acid bacterial activity, which breaks down fibrous structures and partially hydrolyses complex carbohydrates (Du *et al.*, 2020). Consequently, the reduced presence of cyanogenic glycosides minimizes toxicity and enhances microbial access to fermentable substrates

Table 3: Chemical composition and formulation of the experimental diets.

Treatments	Chemical Composition (%)					Formulation (%)				
	DM (%)	OM	CP	EE	CF	MS	PKM	CM	CH	CLTS
	(% DM)					(%)				
A	90.15	91.28	17.34	3.15	22.97	20.00	20	50	10	0
B	90.34	91.59	16.32	3.11	22.22	20.00	20	45	10	5
C	90.53	91.90	15.31	3.07	21.46	20.00	20	40	10	10
D	90.72	92.21	14.29	3.04	20.70	20.00	20	35	10	15
E	90.91	92.52	13.28	3.00	19.95	20.00	20	30	10	20
F	91.10	92.83	12.26	2.96	19.19	20.00	20	25	10	25
G	91.29	93.14	11.25	2.93	18.44	20.00	20	20	10	30
H	91.49	93.45	10.23	2.89	17.68	20.00	20	15	10	35

Treatments included diets with 0% (A), 5% (B), 10% (C), 15% (D), 20% (E), 25% (F), 30% (G), and 35% (H) CLTS. **DM:** dry matter; **OM:** organic matter; **CP:** crude protein; **EE:** extract ether; **CF:** crude fibre; **MZ:** maize stover; **PKM:** palm kernel meal; **CM:** copra meal; **CH:** coffee husk; **CLTS:** cassava leaf-tuber silage.

Table 4: Nutrient digestibility, NH_3-N, VFA concentrations and the efficiency of microbial protein synthesis (EMPS).

Parameters	A	B	C	D	E	F	G	H	SEM	p value
Nutrient digestibility										
DMD (%)	56.90±5.71	56.49±3.78	57.99±4.90	60.99±5.43	59.98±6.16	59.29±7.28	57.71±3.23	61.92±7.85	1.049	ns
OMD (%)	57.57±2.50	57.49±1.71	58.28±2.14	62.47±3.05	60.47±3.46	60.28±4.52	60.91±2.36	61.71±5.94	0.697	ns
NH_3-N (mg N-NH_3/l)	151.30 ± 2.55^{d}	147.48 ± 2.13^{cd}	141.53 ± 2.96^{bcd}	139.40 ± 4.25^{bcd}	136.43 ± 6.38^{bc}	134.73 ± 5.53^{bc}	130.05 ± 6.80^{b}	113.48 ± 6.38^{a}	2.420	**
VFA concentrations (mMol/l)										
Total	159.59± 5.40	167.82 ± 17.97	144.27 ± 15.39	159.58 ± 13.75	175.43 ± 16.28	163.02 ± 10.90	171.68 ± 19.02	145.72 ± 11.24	3.288	ns
Acetate	89.48± 4.23	92.38±5.96	80.93± 4.84	91.50±8.30	96.67±10.04	89.02±8.54	90.98±10.5	81.38±10.54	1.751	ns
Propionate	35.02± 4.99	40.73±6.08	33.13 ± 5.30	37.63±5.08	41.80 ± 2.90	38.45±6.12	39.10± 2.91	31.43 ± 5.42	1.109	ns
Butyric	35.10± 7.05	34.70±6.19	30.20± 5.44	30.45±4.65	36.97 ± 5.94	35.55±2.20	41.60± 6.70	32.90 ± 5.46	1.198	ns
C_2/C_3 ratio	2.18 ± 0.32	2.36 ± 0.12	2.30 ± 0.11	2.36 ± 0.18	2.52 ± 0.29	2.46 ± 0.34	2.47 ± 0.18	2.61 ± 0.23	1.661	ns
EMPS (g N/kg FOM)	28.96 ± 1.19^{ab}	27.39 ± 1.21^{a}	31.06 ± 2.86^{bc}	32.50 ± 1.56^{c}	31.51 ± 2.59^{bc}	30.27 ± 1.42^{abc}	30.51 ± 0.59^{abc}	27.52 ± 0.63^{a}	0.462	*

***a-c** Means within rows with different superscripts are significantly different (P<0.05); ****a-d** Means within rows with different superscripts are significantly different (P<0.01); **ns:** not significant.

(Gomez *et al*., 2023). These correlation effects may explain why diets containing CLTS did not result in a steep decline in dry matter digestibility (DMD). Similarly, the organic matter digestibility (OMD) values ranged between approximately 57.49% and 62.47%. Furthermore, diets with 15–25% CLTS presented slightly greater organic matter digestibility (OMD) than did the other treatments, with either no CLTS or very high levels. Hasanah *et al*. (2023) reported a comparable pattern, attributing improved OMD to fermentation-induced breakdown of plant cell walls, particularly in cassava silage.

Balancing the protein and energy ratio in the diet is critical for optimal microbial fermentation (Blummel *et al*., 1997). In this study, the partial substitution of CM with CLTS provided a mixture of readily fermentable carbohydrates from cassava tubers and moderate protein from cassava leaves, thus supporting ruminal microbes. Similarly, a study by Gerimipour *et al*. (2019) revealed that improving energy and protein balance can increase digestibility in Holstein calves. In the present context, moderate levels of CLTS likely sustained microbial populations, leading to effective OM breakdown.

Dry matter digestibility (DMD) and organic matter digestibility (OMD) remained stable or exhibited modest increases in most diets containing CLTS. This finding confirms earlier research showing that ensiling can increase nutrient availability by reducing structural polysaccharides (Hasanah *et al*., 2023) and toxic elements such as cyanogenic glycosides (Qin *et al*., 2021; Onua and Musa, 2023). Even at higher CLTS levels (30–35%), DMD and OMD did not decline significantly, suggesting that cassava leaves and tubers are effectively utilized by rumen microbes (Tilley and Terry, 1963; Foster *et al*., 2023).

Rumen Fermentation Characteristics

Ammonia (NH_3-N) concentration: Table 4 shows that the treatments had a significant effect (p < 0.01) on the NH_3-N concentration, which ranged from approximately 151.30 mg/L in the control diet (0% CLTS) to approximately 113.48 mg/L in the diet with 35% CLTS. Although this decline was statistically significant, the NH_3-N levels remained above the critical threshold of 50 mg/L recommended by McDonald *et al*. (2022) for sustaining adequate microbial growth. This trend suggests that a decrease in dietary crude protein content reduces ammonia release during ruminal protein breakdown (Abbasi *et al*., 2019). A more pronounced decrease at higher CLTS levels further reinforces the idea that the dietary protein profile directly influences ruminal NH_3-N concentrations (Korir *et al*., 2022; Parra *et al*., 2022). Moreover, this reduction in N-NH_3 can be attributed to the lower protein composition of the diet, yet the recorded concentrations remained sufficiently high to support microbial activity (Retnaningrum *et al*., 2020). In addition, ensiling also stabilizes protein fractions, potentially limiting rapid degradation (Iommelli *et al*., 2022). A balanced NH_3-N profile is advantageous, as excessive ammonia not only signifies inefficiency but can also increase nitrogen excretion into the environment (Abbasi *et al*., 2019).

A significant result of this study is the stability of the rumen ammonia (NH_3-N) levels when CLTS was included at 15–20% in the treatment. Although NH_3-N concentrations decrease as CLTS levels increase, values remain above the threshold (~50 mg/L) necessary for adequate microbial proliferation (McDonald *et al*., 2022). Furthermore, the partial hydrolysis and detoxification achieved through the ensiling of cassava leaves and tubers (Hawashi *et al*., 2019; Terefe *et al*., 2022) may facilitate better nitrogen utilization, thereby supporting microbial cell proliferation.

Volatile fatty acids (VFAs): The total VFA concentration did not significantly differ across the treatments ($p > 0.05$), ranging from approximately 144.27 to 175.43 mMol/L (Table 4). This range falls within values commonly observed for diets supporting active rumen fermentation (Filipek and Dvorak, 2009; Dong *et al*., 2021). The normal range of total VFA production required for ruminant viability is approximately 70–160 mM (Tillman *et al*., 1998; Nopitasari *et al*., 2013). Thus, the VFA concentration in this study remained within the normal range.

Similarly, the molar proportions of acetate, propionate, and butyrate were relatively stable, reflecting a balanced carbohydrate–protein ratio across the varying CLTS levels. A slight increase in propionate was noted in diets with approximately 15–20% CLTS, which could indicate better fermentable carbohydrate availability from cassava products (Unnawong *et al*., 2023). Enhanced propionate production is often associated with more efficient glucose metabolism and improved energy utilization by the host animal (Putri *et al*., 2019). However, the lack of significant differences supplemented with CLTS had a minimal negative impact on the overall fermentation pattern. Furthermore, Khejornsart *et al*. (2022) reported that adding cassava pulp to total mixed rations preserved VFA profiles conducive to healthy rumen activity.

Supapong *et al*. (2019) reported that volatile fatty acids (VFAs) are the end products of carbohydrate metabolism by ruminal microbes and serve as a significant energy source, accounting for approximately 80% of the energy available to ruminants (Mayulu *et al*., 2020). The VFA concentration observed in this study depends on several factors, including nutrient digestibility (dry matter and organic matter), the rate of VFA absorption, the activity of the ruminal microbial community, incubation time, particle size, and fibre digestibility (Tilahun *et al*., 2022; Patra and Yu, 2013). As shown in Table 4, the high VFA concentrations among the treatments indicate increased microbial activity resulting from the effective fermentation of the organic matter present in the diet. While an optimal increase in volatile fatty acids (VFA) reflects an effective fermentation process, excessively high levels can disrupt the ruminal balance (Mayulu *et al*., 2019). Therefore, on the basis of the above explanation, supplementing the diet with CLTS is recommended to achieve a balanced dry matter composition and optimize VFA production.

Microbial protein synthesis efficiency: The efficiency of microbial protein synthesis (EMPS), measured in grams of nitrogen per kilogram of fermented organic matter (g N/kg FOM), revealed notable patterns among the treatments. Diets containing 10–20% CLTS produced the highest EMPS (Table 4), reaching approximately 32–33 g N/kg FOM, whereas those with either no CLTS or very high CLTS (30–35%) presented slightly lower EMPS values, approximately 28–30 g N/kg FOM. This outcome provides information about the optimal CLTS inclusion level, which is hypothesized to balance protein and energy supplies for microbial proliferation (Blummel *et al*., 1997; Parra *et al*., 2022).

Studies by Putri *et al*. (2021, 2024) emphasize the importance of matching rumen degradable protein with available fermentable substrates to maximize microbial protein synthesis. In the present study, moderate CLTS supplementation likely provided sufficient fermentable carbohydrates from cassava tubers, facilitating microbial cell growth while preventing protein wasting. Moreover, the amino acids and peptides derived from cassava leaves may have additional building blocks for microbial protein assembly (Ndaru *et al*., 2014). This synergy concurs with Abbasi *et al*. (2019), who reported that adjusting dietary protein sources can substantially affect microbial population dynamics and thus EMPS.

The efficiency of microbial protein synthesis (EMPS) was highest in diets supplemented with 15% CLTS. This observation aligns with reports that balancing fermentable carbohydrates and protein can optimize EMPS (Blummel *et al*., 1997; Putri *et al*., 2021). When cassava tubers supply readily fermentable carbohydrates, the rumen ecosystem can more effectively incorporate amino acids and peptides from cassava leaves into microbial biomass (Lu *et al*., 2019; Suhartanto *et al*., 2022). Similar synergistic effects have been observed with other fibrous feed additives, where co-fermentation of carbohydrate-rich and protein-rich substrates supports microbial growth (Direkvandi *et al*., 2020; Khejornsart *et al*., 2022). Taken together, these results indicate that ensiled cassava byproducts strategically balanced with maize stover can maintain or even enhance certain aspects of rumen fermentation compared with traditional protein feeds, provided that antinutritional factors are sufficiently reduced (Qin *et al*., 2021; Baguma *et al*., 2022).

CONCLUSIONS AND RECOMMENDATIONS

In summary, supplementation with cassava leaf-tuber silage (CLTS) at a 15% level in concentrates based on maize stover is necessary for optimizing the efficiency of microbial protein synthesis (EMPS). This supplementation level is recommended to balance protein and energy supplies for microbial protein synthesis.

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NOVELTY STATEMENT

The findings are expected to contribute meaningfully to scientific discourse and practical applications of cassava-tuber silage for low-cost strategies for cattle production.

AUTHOR'S CONTRIBUTIONS

Poespitasari Hazanah Ndaru: Played a role in data curation, formal analysis, methodology, and writing the original draft of the manuscript.
Kusmartono, Mashudi, and Ifar Subagiyo: Contributed to the conceptualization, investigation, supervision, validation, and writing and editing of the manuscript.
All the authors have read and approved the final version of the manuscript submitted to the journal.

Conflict of Interest

No potential conflicts of interest relevant to this article are reported.

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