JAPSI (Journal of Agriprecision and Social Impact)
Volume 2 Issue 2 July 2025
e-ISSN: 3032-7849; p-ISSN: 3046-5397, Page 220-239
DOI: https://doi.org/10.62793/japsi.v2i2.56
Available online at: https://journal.kdp.co.id/index.php/japsi

The Potential of Cassava Leaves as a Component of Complete Ruminant Feed Formulated with Odot Grass

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Abstract. *This study aimed to evaluate the effect of substituting cassava leaves (Manihot esculenta Crantz) into a complete feed based on odot grass (Pennisetum purpureum cv. Mott) on in vitro rumen fermentation characteristics, including gas production, dry matter degradation (DMD), organic matter degradation (OMD), ammonia (NH_3) concentration, and microbial protein synthesis. A laboratory experiment was conducted using a Completely Randomized Design (CRD) consisting of five dietary treatments (0%, 5%, 10%, 15%, and 20% cassava leaf inclusion) with four replications each. The feed samples were incubated for 48 hours using rumen fluid collected from slaughtered Limousin cattle, selected purposively from the Ruminant Abattoir in Malang City based on consistent diet and health status to minimize variability. Feed ingredients were sun-dried, ground, and mixed into complete feed formulations using standardized proportions. In vitro gas production was measured using a gas-tight syringe technique, while DMD and OMD were calculated by weighing residues after fermentation. NH_3 concentrations were analyzed using the Conway microdiffusion method, and microbial protein synthesis was estimated based on the weight of microbial biomass derived from fermentation residues. All variables were statistically analyzed using ANOVA followed by Duncan's Multiple Range Test at a 5% significance level. The results showed that a 20% substitution of cassava leaves significantly ($P<0.01$) improved the gas production rate, DMD, OMD, NH_3 concentration, and microbial protein synthesis, while decreasing gas production potential. These findings demonstrate the potential of cassava leaves as a sustainable protein-rich ingredient in ruminant complete feed formulations.*

Keywords: *Cassava leaves, Complete feed, Odot grass, In vitro fermentation, Microbial protein*

Received: February 17, 2025; Accepted: June 12, 2025; Published: July 5 2025
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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a tropical plant crucial to global food security, particularly in developing countries. It is one of the primary sources of carbohydrates and is widely cultivated in various tropical regions, including Indonesia. The average productivity of cassava in Indonesia reached 25.43 tons per hectare during the 2016–2020 period (Ministry of Agriculture, 2021). The high production of cassava provides benefits as a staple food. It generates large amounts of agricultural by-products, particularly cassava leaves, which are often underutilized despite their potential value in livestock feeding systems.

Cassava leaves especially the older ones, are frequently considered agricultural waste despite their high nutritional value (Jumare et al., 2024). In general, cassava leaves contain approximately 30–32% crude protein, indicating their potential as a high-quality feed ingredient for ruminants (Gundersen et al., 2022). Given their rich nutritional content, cassava leaves can be an economical and sustainable alternative protein source for livestock feed. Utilizing agricultural by-products such as cassava leaves in animal feed helps reduce organic waste and decreases dependence on conventional feed ingredients, which often experience price and availability fluctuations (Oghenejoboh et al., 2021). However, while previous studies have highlighted the nutritional potential of cassava leaves as animal feed, limited research has been conducted on their effectiveness when incorporated into complete feed formulations, particularly when combined with odot grass (*Pennisetum purpureum* cv. Mott). This study aims to address this research gap by evaluating cassava leaves as a complementary ingredient in complete feed and assessing their impact on rumen fermentation characteristics.

Despite their nutritional advantages, cassava leaves have some limitations as livestock feed. Cassava leaves contain antinutritional factors, particularly hydrocyanic acid (HCN), which can be harmful to livestock if consumed in large quantities (Fasae & Yusuf, 2022). Therefore, proper processing methods are required to extend the shelf life of cassava leaves while reducing HCN levels to ensure their safety as animal feed. Standard processing techniques include drying or fermentation in the form of silage. Drying has been proven to be an effective method for lowering cyanide content in cassava leaves, making them safer for livestock consumption (Junior et al., 2019).

In traditional ruminant farming systems, forage alone is often insufficient to meet optimal nutritional requirements, necessitating supplementation with concentrates as an additional energy source to maintain a balanced diet (Sulfiar et al., 2022; Susanti et al., 2024). However, providing forage and concentrate separately can lead to selective feeding behavior and inefficient nutrient utilization. One strategy to overcome this issue and improve feeding efficiency is to formulate complete feed, which consists of a uniform mixture of forage and concentrate. Complete feed ensures that livestock consume a balanced diet in every bite, optimizing nutrient intake and supporting growth and productivity (Berthel et al., 2022). Given the need for high-quality, cost-effective forage sources, odot grass (*Pennisetum purpureum cv. Mott*) has gained attention due to its high yield, good palatability, and fiber content that meets ruminant dietary requirements.

This study evaluates cassava leaves as a component in the formulation of complete feed based on odot grass. By combining protein-rich cassava leaves with odot grass as a fiber source, this complete feed formulation is expected to enhance feed efficiency and support sustainable livestock production. Specifically, this research examines the effects of cassava leaf inclusion on *in vitro* gas production, dry matter degradation (DMD), organic matter degradation (OMD), NH_3 concentration, and microbial protein synthesis. Furthermore, the study aims to determine the optimal level of cassava leaf inclusion in odot grass-based complete feed while considering the impact of processing methods on its nutritional value and safety. The findings of this research will provide valuable insights into the feasibility of using cassava leaves in complete feed formulations, contributing to more efficient and sustainable livestock feeding strategies.

LITERATURE REVIEW

Feed is a crucial factor in livestock farming as it determines the productivity level of livestock and the income of farmers. The continuous availability of quality feed throughout the year is essential for sustainable livestock production. A balanced ration is a feed formulation containing nutrients in appropriate amounts to meet the physiological, reproductive, and production needs of livestock when provided in the right quantity. A mixture of forage and concentrate is referred to as complete feed, which undergoes physical treatment and supplementation to enhance its effectiveness and facilitate storage (Gustiani & Permadi, 2015). Complete feed is designed to provide sufficient nutrients for livestock and can serve as the sole feed source, except for water. Compared to conventional feeding methods, where farmers provide forage and concentrate separately, complete feed helps prevent fluctuations in rumen pH and microbial composition, reducing the risk of digestive disturbances. Additionally, it offers longer shelf life, ease of handling, and greater efficiency in transportation, making it a more practical choice for farmers.

One of the major challenges in ruminant nutrition is ensuring a continuous supply of high-quality forage (Ndaru et al., 2020; Huda et al., 2020). Forage alone is often insufficient to meet the optimal nutritional requirements of livestock, necessitating supplementation with protein- and energy-rich concentrates. However, providing forage and concentrate separately may lead to selective feeding behavior, where animals consume only preferred components, reducing feed efficiency. To overcome this issue, complete feed formulations integrate forage and concentrate into a uniform mixture, ensuring livestock consume a nutritionally balanced diet in every bite.

Among the various forages available, Odot grass (*Pennisetum purpureum* cv. Mott) has been identified as a high-yield, high-quality option for ruminant feed (Huda et al., 2020). Odot grass can produce up to 92.4 tons/ha/year in fresh condition or equal to 20.77 tons of dry matter/ha/year, with 60 days of harvesting (Zaini et al., 2021). However, despite its advantages, odot grass alone cannot adequately meet the protein requirements of ruminants, particularly high-producing animals. Thus, it is necessary to supplement with alternative protein sources, such as cassava leaves, to enhance the overall nutritional value of complete feed.

Cassava leaves are widely available by-products of cassava (*Manihot esculenta* Crantz) cultivation. Unlike cassava roots, which are primarily used for human consumption, cassava leaves are often discarded despite their significant nutritional value. They contain 23.36% dry matter, 19.41% crude fiber, 29% crude protein, 34.08% nitrogen-free extract (NFE), 9.41% fat, and 8.83% ash (Ndaru et al., 2014). Their high protein content makes them an attractive alternative to conventional protein sources such as soybean meal. Additionally, cassava leaves contain tannins, which have been reported to function as anti-parasitic agents, potentially improving livestock health. However, excessive tannin intake may reduce protein digestibility by binding with proteins and inhibiting enzyme activity (Chuzaemi et al., 2022). Thus, optimizing the inclusion level of cassava leaves in complete feed is necessary to balance their nutritional benefits with potential anti-nutritional effects.

Despite their high nutritional value, the utilization of cassava leaves in ruminant diets is limited due to the presence of antinutritional compounds, primarily hydrocyanic acid (HCN). Cassava plants naturally produce cyanogenic glycosides, which can release toxic hydrogen cyanide when consumed in large quantities. If improperly processed, high HCN levels can cause toxicity in livestock (Fasae & Yusuf, 2022). Therefore, proper processing methods are required before cassava leaves can be safely used in feed formulations.

Several processing methods have been studied to reduce cyanide levels in cassava leaves, including sun-drying, oven-drying, ensiling, fermentation and adding chemical reagent (Ojiambo et al., 2023; Narwati & Setiawan, 2024; Junior et al., 2019; Devi & Diarra, 2021). Among these, drying is considered the most effective and practical approach. Studies have shown that sun-drying cassava leaves for y 3-4 days sun drying after soaking can significantly lower HCN content, making them safer for livestock consumption (Devi & Diarra, 2021). Ensiling, which involves fermentation with beneficial microbes, has also been reported to reduce cyanide content while improving palatability and digestibility (Terefe et al., 2022). However, more research is needed to determine the most effective and economically feasible processing method for large-scale livestock production.

Previous studies have explored the individual benefits of odot grass and cassava leaves, but limited research has examined their combined effects in complete feed formulations. The inclusion of cassava leaves in complete feed is expected to improve crude protein digestibility and fiber utilization, leading to enhanced feed efficiency. However, the optimum level of cassava leaf inclusion in odot grass-based complete feed remains unclear, particularly in terms of rumen fermentation characteristics and nutrient availability.

To evaluate the nutritional and fermentation effects of cassava leaves in odot grass-based complete feed, several parameters must be considered rumen gas production, which reflects the feed's potential as an energy source; dry matter degradation (DMD) and organic matter degradation (OMD), which indicate digestibility levels; NH_3 concentration, which represents nitrogen availability for rumen microbes; microbial protein synthesis, which serves as an indicator of microbial efficiency in utilizing feed components *in vitro*.

By assessing these parameters, this study aims to determine the optimal substitution level of cassava leaves in complete feed while ensuring safety, digestibility, and nutrient efficiency. The findings will provide valuable insights into alternative feeding strategies that enhance livestock productivity while utilizing agricultural by-products in a sustainable manner.

RESEARCH METHODS

The research and formulation of complete feed were conducted at the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Universitas Brawijaya, Malang. The collection of rumen fluid was carried out at the Ruminant Abattoir in Malang City. This study employed a Completely Randomized Design (CRD) with five dietary treatments and four replications per treatment (Sudarwati et al., 2019). The treatments consisted of odot grass-based complete feed substituted with varying levels of cassava leaves, namely T0: 100% complete feed (50% odot grass + 50% concentrate); T1: 95% complete feed + 5% cassava leaves; T2: 90% complete feed + 10% cassava leaves; T3: 85% complete feed + 15% cassava leaves; T4: 80% complete feed + 20% cassava leaves. The study aimed to evaluate the effects of cassava leaf substitution on rumen fermentation parameters, including gas production, dry matter degradation (DMD), organic matter degradation (OMD), NH_3 concentration, and Efficiency of Microbial Protein Synthesis (EMPS).

Feed Preparation

The complete feed was formulated uasing concentrate, sourced from Pujon Dairy Cooperative, odot grass (*Pennisetum purpureum* cv. Mott), obtained from the Sumber Sekar Field Laboratory, and cassava leaves (*Manihot esculenta* Crantz), collected from cassava plantations in Kepoh District, Bojonegoro Regency. Since cassava leaves contain hydrocyanic acid (HCN), a pre-treatment process was conducted to ensure feed safety. The leaves were sun-dried for 48 hours to reduce HCN levels naturally, then the leaves were grounded to a uniform particle size (approximately 1 mm) to facilitate mixing and uniform feed composition. These processing steps were undertaken to minimize cyanide toxicity risks and enhance nutrient availability for ruminants. The results of the proximate analysis obtained for the nutrient composition of cassava leaf feedstuff and the complete feed fed during the experimental period are presented in Table 1.

Tabel 1. Nutrient content of complete feed

Nutrient content (%DM)	Composition of ingredients (%)		
	Odot grass	Cassava leaves	Concentrate
DM	94.1	94.22	94.4
Fresh DM	9.3	20.22	94.4
OM	83.84	90.59	91.62
CP	9.21	36.19	19.91
CF	34.12	25.46	14.71
EE	1.72	6.19	3.61
Ash	16.16	8.38	9.41
NFE	38.8	23.79	52.37
TDN	56.43	57.78	71.45
NDF	57.35	42.70	26.77
ADF	35.41	28.39	7.45

Information: Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fiber (CF), Ether extract (EE), Nitrogen-Free Extract (NFE), Total Digestible Nutrients (TDN), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF).

Rumen Fluid Collection and Handling

Rumen fluid was obtained from freshly slaughtered Limousin cattle at Gadang Abattoir, Malang City. To preserve microbial viability, the fluid was collected immediately post-slaughter in pre-warmed anaerobic flasks, then filtered it through four layers of nylon cloth to remove large particles and fiber residues. The rumen fluid was mixed with a pre-warmed buffer solution and maintained at 39°C under anaerobic conditions before use. The buffer solution consists of NH_4HCO_3, $NaHCO_3$, Macro minerals ($Na_2HPO_4 \cdot 2H_2O$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, NaCl), Micro minerals ($CaCl_2 \cdot 2H_2O$, $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, $FeCl_2 \cdot 6H_2O$) and reducing solution (NaOH, $Na_2S \cdot 9H_2O$), resazurin solution.

***In Vitro* Incubation and Gas Production Measurements Procedure**

The *in vitro* gas production and degradation measurements were conducted following the method of Blümmel et al. (1997). Each sample (500 mg of dried feed) was incubated in 100 mL of rumen-buffer solution under anaerobic conditions at 39°C for 48 hours. Gas production was measured at 2, 4, 8, 16, 24, 36, and 48 hours post-incubation using a gas-tight syringe. The rate and total volume of gas produced were recorded to determine feed fermentability and microbial activity. Gas production is a parameter used to measure the activity of rumen microbes in the synthesis of energy and protein derived from these microbes (Hariyani and Chuzaemi, 2019). The use of the in vitro gas production model can serve as a method to measure and predict the effects of feed on fermentation in the rumen, rumen microbial growth, and feed digestibility. Gas production is calculated using the following equation (1) :

$$Vblanko = Vblanko\ \text{t} - Vo, \quad \text{Gas production} = (V1 - Vo - Vblanko). \qquad (1)$$

Gas Production Kinetics (Cahyaningtyas et al., 2018). The measurement of gas production potential and rate can be performed using the following equation (2) :

$$\text{Yt} = \text{b}\ (1 - e^{-CT}) \qquad (2)$$

Where, Y represents the gas production at a specific time (t) measured in ml per 500 mg of dry matter (DM). The parameter b indicates the gas production potential (ml/500 mg DM) at time t, while c represents the rate of gas production expressed in ml per hour. The variable T refers to the incubation time in hours, and E denotes the exponential constant used in the equation.

Dry Matter Degradation (DMD) and Organic Matter Degradation (OMD)

At the end of the incubation period, residual feed was filtered, dried, and weighed to calculate DMD and OMD percentages, indicating the extent of feed digestibility. OMD refers to the amount of nutrients in feed ingredients that can be digested by the body, such as proteins, carbohydrates, fats, and vitamins. DMD of Gas Production Residue in vitro (Setiawan and Chuzaemi, 2020*)* is an important parameter to measure the percentage of dry matter in feed that is degraded during fermentation. The measurement of DMD and OMD in vitro potential and rate can be performed using the following equation (3) and (4), respectively based on Tilley & Terry (1963):

$$\text{DMD (\%)} = \frac{DM\ Sample - (DM\ Residue - DM\ Blank)}{DM\ Sample} \qquad (3)$$

$$\text{OMD (\%)} = \frac{OM\ Sample - (OM\ Residue - OM\ Blank)}{OM\ Sample} \qquad (4)$$

In this formula, DMD (%) represents the percentage of dry matter degradability. DM sample refers to the dry matter content of the sample (mg), DM residue is the dry matter content of the residue after fermentation (mg), and DM blank denotes the dry matter content of the blank residue (mg). This method provides insights into the digestibility of feed ingredients and their efficiency in supporting microbial activity in the rumen.

NH_3 Concentration Analysis

NH_3 concentration was measured using the Conway microdiffusion technique to evaluate nitrogen availability for microbial protein synthesis as had been done by Kozloski et al. (2006). For ammonia analysis, the supernatant of rumen fluid after harvesting was collected and adding boric acid solution (H_3BO_3), Na_2CO_3 and being incubated overnight using Conway dish. The NH_3 content reflects feed fermentation in the rumen, protein digestibility, and the activity and role of microbes in supporting livestock body functions. The measurement of NH_3 content, using samples from the supernatant of *in vitro* gas production incubated for 48 hours, was performed using the Conway microdiffusion technique. The NH_3 concentration was measured at the end of the incubation, as determined by the following equation (5):

$$NH_3 \text{ Concentration (mM)} = (\text{vol. } H_2SO_4 \times N\ H_2SO_4 \times 1000) \quad (5)$$

In this formula, NH_3 Concentration (mM) represents the ammonia concentration in the sample. vol. H_2SO_4 refers to the volume of H_2SO_4 used for titration (ml), and N H_2SO_4 denotes the normality of H_2SO_4 (0.005 N). This method is used to determine the concentration of ammonia as an indicator of protein fermentation and microbial activity in the rumen.

Efficiency of Microbial Protein Synthesis

Microbial protein synthesis was evaluated using the supernatant obtained from 48-hour in vitro gas production incubation. This parameter serves as an indicator of microbial efficiency in converting feed nutrients into biomass. The synthesis process is influenced by factors such as nitrogen degradation rate, ammonia (NH_3) availability, amino acid assimilation, microbial nitrogen requirements, and the nature of rumen fermentation determined by feed composition. Microbial biomass was estimated as ESPM (g N/kg

Digestible Organic Matter in the Rumen (DOMR)) in this following equation (6) based on Blümmel et al.(1997).

EMPS (g N/kg DOMR) = (Microbial Biomass (g) × Microbial N (%) × 1000) / Digestible Organic Matter (kg) (6)

Here, DOM refers to the digestible organic matter of the substrate, which is computed by multiplying the organic matter content of the feed sample with the organic matter digestibility coefficient. This approach allows for a quantitative assessment of microbial nitrogen production relative to the availability of fermentable organic substrates.

Statistical Analysis

All data were analyzed using Analysis of Variance to determine statistical significance (Sudarwati et al., 2019). Differences among treatments were further examined using Duncan's Multiple Range Test (DMRT) at $P<0.05$ to identify significant effects of cassava leaf substitution levels. This study was conducted in compliance with the ethical guidelines of the Faculty of Animal Science, Universitas Brawijaya. The collection and handling of rumen fluid followed standard animal welfare protocols, ensuring that no live animals were subjected to unnecessary stress or harm.

RESULTS AND DISCUSSION

In Vitro Total Gas Production

The results of gas production analysis of complete feed based on odot grass (*Pennisetum purpureum* cv. Mott) substituted with cassava leaves (*Manihot esculenta* Crantz) after 48 hours of in vitro incubation are presented in Table 2.

Table 2. Total gas production values at different incubation times.

Treatments	Incubation Time (hours)						
	2 (ml/500 mg DM)	4 (ml/500 mg DM)	8 (ml/500 mg DM)	16 (ml/500 mg DM)	24 (ml/500 mg DM)	36 (ml/500 mg DM)	48 (ml/500 mg DM)
T0	4.17± 0.02	4.17± 0.02	20.07 ± 0.56	32.58 ± 3.08	59.51 ± 2.87	76.88 ± 2.91	86.01 ± 4.17
T1	4.71 ± 0.59	10,48 ±0.86	20.95 ± 1.02	35.36 ± 1.26	61.90 ± 1.66	78.05 ± 1.18	87.48 ± 1.16
T2	4.97 ± 1.02	9.68 ± 1.05	20.14 ± 1.37	37.41 ± 1.42	62.87 ± 1.91	79.26 ± 2.05	87.90 ± 1.95
T3	4.97 ± 1.32	10.98 ± 0.58	21.70 ± 1.00	38.17 ± 0.54	65.19 ± 0.70	80.78 ± 0.70	89.41 ± 0.99
T4	5.98 ± 0.53	11.45 ± 0,85	21.85 ± 0.61	40.59 ± 0.91	66.69 ± 1.04	81.95 ± 0.65	90.28 ± 0.43

Information: T0 = 100% co.plete feed (50% odot grass and 50% concentrate); T1 = 95% complete feed + 5% cassava leaves; T2 = 90% complete feed + 10% cassava leaves; T3 = 85% complete feed + 15% cassava leaves; T4 = 80% complete feed + 20% cassava leaves.

Treatment T4 exhibited the highest total gas production (90.28 ± 0.43 ml/500 mg DM), while the lowest was observed in Treatment T0 (86.01 ± 4.17 ml/500 mg DM). Variations in gas production are influenced by differences in the utilization of fermentation products in the rumen, with lower values often linked to high-fiber, low-quality rations (Nurjannah et al., 2016). The gradual increase from T0 to T4 is influenced by the inclusion of cassava leaves, which stimulate rumen microbial activity and enhance fermentation performance. The total gas production in this study (86.01–90.28 ml/500 mg DM) was lower than the 121.71–127.46 ml/500 mg DM reported by Khoriyah et al. (2016), likely due to differences in feed composition and microbial populations.

Gas production significantly increased between the 2nd and 48th hours of incubation, with a more pronounced rise in the first 24 hours, reflecting substrate availability. The slowdown in production signals a reduction in volatile fatty acid (VFA) production, thereby decreasing energy availability for livestock (Puspitasari et al., 2015; Hariyani & Chuzaemi, 2019).

Potential Gas Production and Gas Production Rate

The potential gas production value reflects the extent to which organic materials in the rumen can be digested, while the gas production rate represents the speed of gas formation during the incubation period from 0 to 48 hours (Khoriyah et al. 2016). The results of the analysis of potential gas production and gas production rate are presented in Table 3.

Table 3. Gas Production Potential and Gas Production Rate

Treatments	Potential Gas Production *(ml/500 mg DM)*	Gas Production Rate (ml/hour)
T0	143.48^{d} ± 7.24	0.021^{a} ± 0.0006
T1	136.53^{c} ± 3.79	0.023^{b} ± 0.0012
T2	134.60^{c} ± 2.23	0.024^{c} ± 0.0013
T3	132.22^{b} ± 2.58	0.026^{d} ± 0.0017
T4	129.32^{d} ± 3.04	0.027^{e} ± 0.0011

Information: T0 = 100% complete feed (50% odot grass and 50% concentrate); T1 = 95% complete feed + 5% cassava leaves; T2 = 90% complete feed + 10% cassava leaves; T3 = 85% complete feed + 15% cassava leaves; T4 = 80% complete feed + 20% cassava leaves.

The gas production potential value relative to the total gas production after 48 hours of in vitro incubation, as shown in Table 4, indicated significant differences (P<0.01). The treatment T0 had the highest potential gas production value at 143.48 ml/500 mg DM, followed by T1 with 136.53 ml/500 mg DM, T2 with 134.60 ml/500 mg DM, T3 with 132.22 ml/500 mg DM, and the lowest value was observed in T4 at 129.32 ml/500 mg DM. Treatment T0 had the highest gas production potential, while T4 had the lowest. The decrease in gas production potential does not align with the increase in total gas production, which increases with the substitution of cassava leaves. This is because total gas production is related to easily fermentable feed, while gas production potential refers to feed materials that are difficult to ferment but are still potentially useful. Muqier et. al. (2017) stated higher fiber content and lower water-soluble carbohydrates and crude protein result in slower fermentation, producing different gas and fermentation products.

The gas production rate in this study also showed significant differences (P<0.01), with treatment T4 having the highest rate at 0.027 ml/hour, followed by T3 at 0.026 ml/hour, T2 at 0.024 ml/hour, T1 at 0.023 ml/hour, and the lowest gas production rate was observed in T0 at 0.021 ml/hour. Complete feed based on odot grass substituted with cassava leaves showed an increasing trend in the gas production rate with each treatment. Treatment T0, which did not include cassava leaves, produced the lowest gas production rate, as the organic matter degraded less compared to the other treatments. Overall, factors such as feed composition, substrate availability, and protein content influence the rate and pattern of gas production in *in vitro* rumen fermentation (Elghandour et al., 2016).

Dry Matter Degradation (DMD) and Organic Matter Degradation (OMD) *In Vitro*

The average DMD and OMD in complete feed based on odot grass substituted with cassava leaves can be seen in Table 4.

Table 4. DMD and OMD values of complete feed

Treatments	Potential Gas Production *(ml/500 mg DM)*	Gas Production Rate (ml/hour)
T0	78.63[a] ± 0.27	76.52[a] ± 0.39
T1	79.61[bc] ± 0.28	77.31[bc] ± 0.58
T2	79.98[c] ± 0.12	77.81[c] ± 0.36
T3	80.68[d] ± 0.29	78.98[d] ± 0.14
T4	82.23[e] ± 0.12	79.86[e] ± 0.38

Information: T0 = 100% complete feed (50% odot grass and 50% concentrate); T1 = 95% complete feed + 5% cassava leaves; T2 = 90% complete feed + 10% cassava leaves; T3 = 85% complete feed + 15% cassava leaves; T4 = 80% complete feed + 20% cassava leaves.

The substitution of odot grass-based complete feed with cassava leaves significantly impacted ($P<0.01$) the dry matter degradability (DMD). DMD values increased progressively with higher cassava leaf levels, ranging from T0 (78.63%) to T4 (82.23%), indicating improved rumen degradability. This enhancement in degradability is influenced by factors such as microbial activity in the rumen, feed composition, feeding level, and animal-specific characteristics (Dewi et al., 2015). The lignin content also plays a critical role in feed quality and its degradation process (Rachman, 2018).

Similarly, organic matter degradability (OMD), involving the breakdown of carbohydrates, proteins, fats, and vitamins, was significantly influenced ($P<0.01$) by cassava leaf substitution. The trend in OMD paralleled the DMD pattern, as organic matter forms a major part of dry matter. Higher DMD values corresponded to increased OMD values due to their close relationship. Interestingly, DMD values were consistently higher than OMD values, attributed to the exclusion of ash content in organic matter. Zahra (2018) highlighted that feed with similar nutrient compositions shows proportional patterns in the degradability of dry matter and organic matter.

NH_3 Concentration *In Vitro*

The in vitro analysis of NH_3 concentration in complete feed based on odot grass substituted with varying levels of cassava leaves is presented in Table 5.

Table 5. In vitro NH_3 concentration values

Treatments	NH_3 concentration (mM)
T0	$2.59^{a}\pm0.06$
T1	$3.31^{bc}\pm0.11$
T2	$4.73^{d}\pm0.08$
T3	$3.33^{c}\pm0.07$
T4	$5.33^{e}\pm0.10$

Information: T0 = 100% complete feed (50% odot grass and 50% concentrate); T1 = 95% complete feed + 5% cassava leaves; T2 = 90% complete feed + 10% cassava leaves; T3 = 85% complete feed + 15% cassava leaves; T4 = 80% complete feed + 20% cassava leaves.

The analysis of variance in Table 6 shows that substituting odot grass-based complete feed with cassava leaves significantly affects NH_3 concentration ($P<0.01$). The highest NH_3 concentration was recorded in T4, consisting of 80% complete feed and 20% cassava leaves, while the lowest was observed in T0, with 100% complete feed (50% concentrate and 50% odot grass). NH_3 concentration in rumen fermentation is influenced by nutrient content, especially crude protein, which varies depending on the type of forage consumed by livestock. Low NH_3 levels may result from the low solubility of feed, particularly protein. Less soluble protein tends to escape rumen degradation more easily (Hambakodu et al., 2021).

T4 exhibited the highest NH_3 concentration compared to T0, T1, T2, and T3. According to Harahap et al. (2017), the optimal NH_3 concentration for rumen microbial growth ranges between 6.0–17.65 mM. However, in this study, the NH_3 concentration did not reach the optimum range, with the highest concentration being 5.33 mM. Factors such as feed intake, feed solubility, incubation time, carbohydrate content, and rumen pH also influence NH_3 concentration (Rahayu et al., 2018).

Microbial Protein Synthesis (MPS)

The results of microbial protein synthesis in complete feed based on odot grass substituted with cassava leaves after 48 hours of incubation are presented in Table 7.

Table 7. In vitro microbial protein synthesis values after 48 hours of incubation

Treatments	ESPM (g N/kg DOMR)
T0	30.5^{a}±0.35
T1	32.34^{bc}±0.38
T2	32.64^{c}±0.92
T3	33.99^{d}±0.14
T4	34.98^{e}±0.23

Information: T0 = 100% complete feed (50% odot grass and 50% concentrate); T1 = 95% complete feed + 5% cassava leaves; T2 = 90% complete feed + 10% cassava leaves; T3 = 85% complete feed + 15% cassava leaves; T4 = 80% complete feed + 20% cassava leaves.

Substitution of complete feed based on odot grass with cassava leaves significantly affected microbial protein synthesis ($P<0.01$).The highest microbial protein synthesis was observed in T4 (20% cassava leaf substitution) with an average of 34.98 g N/kg DOMR. This was followed by T3 (15% cassava leaf substitution) at 33.99 g N/kg DOMR, T2 (10% cassava leaf substitution) at 32.64 g N/kg DOMR, T1 (5% cassava leaf substitution) at 32.34 g N/kg DOMR, and the lowest in T0 (no cassava leaf substitution) at 30.5 g N/kg DOMR.

According to Barlian, et al.(2020), factors influencing microbial protein synthesis include the balance between nitrogen (N) and energy (ATP) availability in the rumen. The T4 treatment achieved a balanced supply of NH_3 and energy, as indicated by its highest NH_3 concentration. Cassava leaves contain Branched-chain amino acids (BCAA), which enhance microbial capacity to digest feed materials. Branched-chain amino acids serve as a carbon source for the synthesis of cellulolytic microbial proteins. Crude protein (CP) is a crucial component for microbial protein synthesis, as it indicates the availability of nitrogen for rumen microbes (Zhang et al., 2022). However, this is only valid as long as nitrogen concentration is sufficient and protein is not utilized as an energy source.

Microbial protein synthesis from the substitution of complete feed based on odot grass with cassava leaves ranged from 30.5 to 34.98 g N/kg DOMR**.** Sairullah et al. (2016), explained that the microbial protein synthesis required for rumen microbial growth has an average microbial protein value for all feed materials fermented in the rumen, ranging from 15–45 g N/kg DOMR, with an average of 30 g N/kg DOMR. Based on these results, the substitution of complete feed based on odot grass with cassava leaves can be considered an alternative feed for ruminants, as it meets the standard requirements for microbial protein synthesis. Based on the research results, it can be concluded that substituting complete feed based on odot grass (*Pennisetum purpureum* cv. Mott) with cassava leaves (*Manihot esculenta* Crantz) at different levels can improve total gas production, gas production rate, dry matter degradation (DMD), organic matter degradation (OMD), NH_3 concentration, and microbial protein synthesis. The best treatment was found in 20% substitution of odot grass-based complete feed with cassava leaves, showing significant improvements in total gas production, gas production rate, DMD, OMD, NH_3 concentration, and microbial protein synthesis.

CONCLUSION

The use of cassava leaves (*Manihot esculenta* Crantz) in ruminant complete feed has shown significant potential for improving feed efficiency and rumen fermentation characteristics. Research indicates that substituting odot grass (*Pennisetum purpureum* cv. Mott) with cassava leaves at different levels can enhance total gas production, gas production rate, dry matter degradation (DMD), organic matter degradation (OMD), ammonia (NH_3) concentration, and microbial protein synthesis. The best results were achieved with a 20% substitution level, which led to the greatest improvements in these parameters. This suggests better nutrient utilization and increased microbial activity in the rumen. Overall, cassava leaves represent a promising alternative protein source in complete feed formulations, contributing to more sustainable and efficient livestock feeding practices.

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