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A Comprehensive Meta-Analysis of Cassava Addition in a Buffalo Diet: *In Vivo* Investigations on Performance and Rumen Health

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ABSTRACT

This meta-analysis compiles data on buffalo consumption of cassava as a feed ingredient to evaluate its impact on *in vivo* rumen fermentation, feed intake, nutrient intake, growth performance, digestibility, nitrogen metabolism, haematology, microbiology, and milk yield. A systematic search of Scopus and Web of Science identified 19 *in vivo* experiments. Cassava varieties were categorized as by-products, foliage, and roots, while buffaloes were stratified based on management system, breed, and sex. A linear mixed model was applied to estimate the effects of cassava inclusion. The findings indicated feed and nutrient intake, particularly crude protein intake and nitrogen retention, increased significantly (p<0.05), while crude protein digestibility showed no significant difference. Microbiological parameters, including total bacterial and fungal counts, also increased significantly (p<0.05), whereas methane production after 24 hours declined significantly (p<0.05). Although production parameters such as body weight, feed conversion, and milk yield were not significantly affected, a trend toward improvement was observed, except for feed conversion. Cassava root and foliage exhibited the highest digestibility and nitrogen retention compared to by-product (p<0.05). A restricted feeding system resulted in higher ammonia (NH_3-N) concentrations, protozoa count, and proteolytic and cellulolytic microbial populations compared to *ad libitum* feeding and an extensive system (p<0.05). Murrah buffaloes showed greater feed intake, while male buffaloes demonstrated higher digestibility (p<0.05). In conclusion, dietary cassava, approximately 1.5% to 20.5% DM, potentially stimulates rumen fermentation, nutrient intake, digestibility, and microbiology but has only a modest effect on production parameters. High cassava inclusion may reduce feed acceptability, thereby decreasing feed efficiency.

Keywords: *feed utilisation; methane production; microbiological parameters; Murrah buffalo; nitrogen metabolism*

INTRODUCTION

Raising growing buffaloes in tropical regions, where it is characterized as high humidity and temperature, can significantly impact their performance and productivity (Pertiwi *et al*., 2019). The daily nutritional requirements of buffaloes depend on factors such as body weight and the intended purpose of rearing, whether for meat or milk production (Felini *et al*., 2024; Paengkoum *et al*., 2021). Feed intake plays a crucial role, as buffaloes must efficiently utilize nutrients during the growth phase to enhance both production quantity and quality (Mohd Azmi *et al*., 2021). Addressing slow growth rates and ensuring high-quality beef production necessitate nutrient-rich feed, including energy and protein content, which can sometimes be costly. To mitigate this challenge, it is essential to explore affordable feed alternatives that maintain high nutritional value while evaluating their impact on buffalo performance and rumen fermentation characteristics. Cassava (*Manihot esculenta*), a widely available and cost-effective root crop, presents a viable solution (Vastolo *et al*., 2024; Koakoski *et al*., 2024; Palmonari *et al*., 2021). It is extensively cultivated in tropical and subtropical regions, particularly in Africa, Asia, and South America, where it serves as both a staple food and an important economic crop. In regions where buffalo farming is common, such as Southeast Asia and parts of South America, cassava's year-round availability and affordability make it a valuable feed resource. Its versatility allows for utilization in various forms, including roots, foliage (leaves), and by-products, each offering distinct nutritional benefits.

Cassava leaves contain a high protein concentration, ranging from approximately 21.5% to 30.3%, while the roots are rich in total carbohydrates (65.9%-71.5%) but contain low protein levels (1.89%-2.21%) (Fanelli *et al*., 2023; Gundersen *et al*., 2022; Morgan & Choct, 2016). Additionally, cassava peels contain 5%-8% protein and 40%-50% carbohydrates, whereas cassava pulp has a relatively high carbohydrate content (60%-70%) but a low protein content (1%-2%) (Morgan & Choct, 2016; Stupak *et al*., 2006; Bhuiyan & Iji, 2015; Jamil & Bujang, 2016; Falade & Akingbala, 2010). The nutritional quality of cassava is assessed based on its effects on growth performance, milk production, and overall health (Pertiwi *et al*., 2019; Wanapat *et al*., 2000a; Wanapat *et al*., 2000b). Previous studies have indicated that incorporating cassava into buffalo diets can positively influence growth performance (Bata *et al*., 2020). Buffaloes fed with cassava-based diets exhibited greater average daily weight gains compared to those receiving conventional feed (Foiklang *et al*., 2011; Wanapat *et al*., 2013). When balanced with protein-rich supplements, cassava enhances nutrient utilization (Falade & Akingbala, 2010). For instance, research has demonstrated that supplementing cassava with protein sources improved nitrogen retention and overall feed efficiency in buffaloes (Dagaew *et al*., 2021). The impact of cassava supplementation on milk production in buffaloes has been inconsistent (Supapong & Cherdthong, 2020). Some studies have reported increased milk yield due to the high energy content of cassava (Dagaew *et al*., 2021; Lunsin *et al*., 2012; Srisaikham *et al*., 2018), while others had observed no significant differences or even reductions in milk production likely due to insufficient protein intake (Hong *et al*., 2014; Roza *et al*., 2021a). Therefore, a well-balanced diet with cassava and an appropriate protein source is essential for maintaining or enhancing meat and milk production (Bell *et al*., 2023).

In recent years, simulation models have become increasingly valuable for optimizing buffalo production, particularly in diverse environments. These models facilitate the prediction and evaluation of buffalo performance under varying conditions, including different climates, resource availability, and geographic locations. By integrating environmental factors and resource constraints, simulation models assist in identifying optimal feeding strategies and management practices tailored to specific regions. However, the application of these models in buffalo production remains underexplored, necessitating further research to fully understand their potential and limitations.

Recent studies partially supported the beneficial effects of cassava on growth performance and rumen fermentation, indicating its potential as a feed source for buffaloes. However, inconsistencies in empirical studies may introduce bias and weaken the conclusions. To ensure that the findings are robust and statistically sound, a quantitative and objective analytical approach is required.

This meta-analysis aimed to consolidate *in vivo* studies on cassava-based feed for buffaloes, considering the key parameters such as rumen fermentation characteristics, nutrient intake and digestibility, growth performance, nitrogen balance, purine derivatives, hematology, microbiology, and milk production. It was hypothesized that the inclusion of cassava in buffalo diets, particularly in the form of roots and foliage, will significantly enhance rumen fermentation parameters, nutrient digestibility, and overall growth performance without adversely affecting feed intake or milk production. Furthermore, optimal levels of cassava inclusion were anticipated to improve nitrogen metabolism and microbiological profiles in the rumen, leading to enhanced feed efficiency and buffalo health. Moreover, this study uniquely evaluated the effects of various forms of cassava on buffalo performance, thereby filling a critical gap in the literature and providing new insights into effective feeding strategies that can enhance buffalo productivity and health.

MATERIALS AND METHODS

PICO and Article Search Strategy

PICO is a framework for structuring a meta-analysis consisting of population (P), intervention (I), comparison (C), and outcome (O). The breakdown is as follows: P= buffalo, I= cassava added to the diet, C= comparison between the control group and the treatment group (cassava), and O= results of the observed parameters. Buffaloes studied were *Bubalus bubalis*, which include both meat and milk producers. The type of cassava was not specified, meaning it could refer to roots, leaves, peels, or other by-products.

In the initial phase, a search on Scopus and Web of Science yielded 314 results through a search strategy for published articles. This strategy incorporated MeSH terms such as "buffalo", "cassava", and other relevant terms related to "*in vivo*" studies.

Eligibility Criteria and Dataset Development

To ensure a rigorous selection process, the inclusion criteria were carefully formulated within a comprehensive framework encompassing the population, intervention, comparators, outcomes, and study design. The primary focus was on buffalo and cassava, specifically evaluating growth performance. Additionally, only English-language, peer-reviewed articles were included. A preliminary dataset was compiled following a thorough review of cassava-related research, which incorporated *in vivo* studies based on these criteria. In line with the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (Figure 1), relevant papers were systematically selected for analysis. The organization and referencing of published materials were managed using the Mendeley reference system, ensuring the documentation of author names, publication years, study details (original research), cassava varieties used, and reported outcomes.

The inclusion criteria included that the research must be published in English, indexed in Scopus, and possess a DOI. Research must utilize random sampling, incorporate at least three repetitions, present numerical results with suitable units, and disclose essential factors, including performance, feed intake,

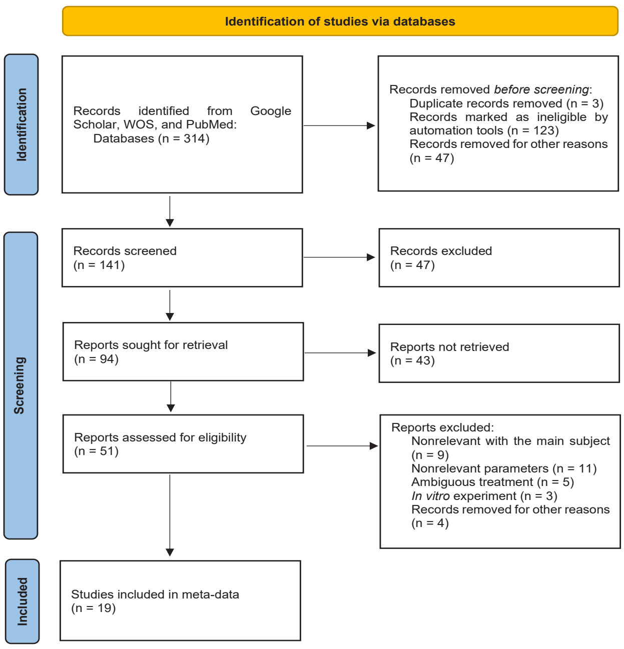

Figure 1. Summary of the procedure of article selection and assessment in accordance with the PRISMA protocol.

nutrient digestibility, rumen characteristics, nitrogen metabolism, hematology, microbiology, and milk production.

To ensure the integrity of the database, a systematic selection process based on abstracts and full-text contents was conducted according to the inclusion criteria, resulting in the identification of 19 papers containing a total of 56 experimental units, as presented in Table 1. This selection procedure involved assessing titles, abstracts, and full-text materials, considering information relevant to the study objective.

The details of the authors, number of replications, study design, treatment duration, sources of variability, and response variables were recorded using spreadsheet software (Microsoft Excel 2019). The assembled database encompassed cassava inclusion levels ranging from 0% (control) to 20.5% DM. Cassava is sourced from by-products (residues from cassava food processing, such as peels and pulp), foliage (green waste or fresh cassava leaves), and roots (tubers of the cassava plant). The studies identified a diverse range of buffalo breeds, including Murrah, River, and Swamp buffaloes. The reported rearing systems were either extensive (grazing-based) or intensive (Table 1). Only two references have been reported on milk production (Roza *et al*., 2021a; Roza *et al*., 2021b).

All those sources of heterogeneity were tested for their effects on the models. All possible variables identified in the studies were included in the datasets, including feed intake, nutrient intake, growth performance, nutrient digestibility, nitrogen balance, purine derivatives, rumen characteristics, hematology, microbiology, and milk production for *in vivo* studies.

In detail, the parameters included were feed intake, total intake (TLI), concentrate intake (CSI), and forage intake (FRI). Nutrient intake consisted of organic matter intake (IOM), crude protein intake (ICP), neutral detergent fiber intake (INDF), and acid detergent fiber intake (IADF). Both feed and nutrient intakes were quantified through conversion with metabolic body weight (g/kg $BW^{0.75}$/d DM). Growth performance encompasses final body weight (FBW; kg), live weight change (LWC; kg), average daily gain (kg/d), and the feed conversion ratio (FCR). Nutrient digestibility parameters included dry matter digestibility (DMD; %), organic matter digestibility (OMD; %), crude protein digestibility (CPD; %), NDF digestibility (NDFD; %), and ADF digestibility (ADFD; %). The parameters related to nitrogen balance and purine derivatives included absorbed nitrogen (ABN), retained nitrogen (RTN), purine derivative excretion (PDE; mmol/d), and purine derivative absorption (PDA; mmol/d). The rumen characteristics included pH (4 hours), temperature (Temp.; °C), ammonia (NH_3-N; 2 hours), volatile fatty acids (VFA; mol/100 mol), acetate (C2; mol/100 mol), propionate (C3; mol/100 mol), butyrate (C4; mol/100 mol), the ratio of C2:C3, methane production (CH_4; 24 hours, mL/100 mL), and total gas production (TGP; 0-6 hours, 6-12 hours, 12-24 hours; mL). Hematology included blood urea nitrogen (BUN; mg/dL). The microbiological parameters included bacteria (0 hours and 4 hours; log10 CFU/mL), protozoa

Table 1. Studies included in the meta-analysis were experimental trial

Cassava part	Rearing	Cassava (% DM)	Total animal	Experimental design	Breed	Sex	Age (months)	Reared period (days)	Ref.
Root	Intensive (ad libitum)	0-10.3	4	LSD	Swamp	Male	30	21	Chanthakhoun *et al*., 2011
Root	Intensive	0-18.9	16	LSD	Swamp	Male	36	-	Chanthakhoun *et al*., 2012
Foliage	Extensive	0-6.5	9	CRD	Murrah	Female	48	-	Roza *et al*., 2021b
Foliage	Extensive	0-6.5	4	LSD	Swamp	Female	54	-	Roza *et al*., 2015
Foliage	Extensive	0-6.5	16	LSD	-	Female	60	-	Roza *et al*., 2021a
Foliage	-	0-1.5	4	LSD	Swamp	Male	-	21	Foiklang *et al*., 2011
Foliage	Extensive	0-18.4	6	CRD	Swamp	Female	-	90	Granum *et al*., 2007
Root	Extensive	0-17.7	4	-	Murrah	Female	-		Hasanah *et al*., 2020
Root	Intensive	0-9.2	30	CRD	River type	-	15	10	Huang *et al*., 2020
Root	Intensive	0-20.5	16	LSD	Swamp	Male	-	21	Hung *et al*., 2013
Root	Intensive	0-7.2	16	LSD	Swamp	Male	54	21	Kang *et al*., 2012
Foliage and root	Extensive	0-16.7	6	RBD	Swamp	Male	12	42	Khampa *et al*., 2009
Root	-	0-8.1	4	LSD	Swamp	-	-	21	Khejornsart *et al*., 2011
Root	Intensive	0-20.3	16	LSD	Swamp	Male	-	21	Khy *et al*., 2012
By-product	Intensive	0-10	4	LSD	-	-	-		Maeda *et al*., 2007
-	Extensive	0-20.5	4	CRD	-	-	-	120	Uriyapongson *et al*., 2007
Root	-	0-12.9	4	LSD	Swamp	-	-	21	Vinh *et al*., 2011
Root	Intensive	0-16.7	16	LSD	Swamp	Male	48	21	Wanapat *et al*., 2012
Root	Intensive	0-17.7	16	LSD	Swamp	Male	48	21	Wanapat *et al*., 2013

Note: By-product = residues from cassava food processing such as peel and pulp, CRD = completely randomized design, Extensive = grazing system, Foliage = green waste or fresh leaves from cassava, Grazing = buffalo husbandry system involving timed pasture feeding, Intensive = buffalo husbandry within enclosures with full provision of feed by the farmer, LSD = Latin-square design, RBD = randomized block design, River Type = river buffalo, Root = tuber of the cassava plant, Swamp = marsh buffalo.

(0 hours and 4 hours; log10 CFU/mL), fungi (0 hours and 4 hours; log10 CFU/mL), the amylolytic group (log10 CFU/mL), the proteolytic group (log10 CFU/mL), the cellulolytic group (log10 CFU/mL), *Ruminococcus albus* (log10 CFU/mL), and *Fibrobacter succinogenes* (log10 CFU/mL). Finally, milk production adjusted for a 7% fat correction factor was considered (FCM 7%; kg/d). The parameters representing the use of cassava as feed for buffaloes in milk production are LWC (kg) and milk production (FCM 7%; kg/d). These parameters align with those outlined in Roza's research (Roza *et al*., 2021a; Roza *et al*., 2021b), which explicitly mentions both parameters.

A summary of the descriptive statistical data for the observed variables is presented in Table 2. These tables provide details on the number of studies (n), mean values (mean), standard deviation (sd), maximum values (max), and minimum values (min) for cassava treatments.

Data Analysis and Validation

The data were analyzed via mixed effects models. The substitution levels of cassava are continuous data and were considered fixed effects within the model, as previously described (Sauvant *et al*., 2008). The type of cassava product, rearing system, buffalo breed, and buffalo sex were considered discrete variables and were tested against the variables. The general model used to examine the effects of inclusion levels was as follows:

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X^2_{ij} + S_i + b_iX_{ij} + e_{ij} \quad (1)$$

$$Y_{ij} = \mu + \beta_a + (\beta_a \times \beta_b)\, Xij + s\beta_{ij} + S_i + e_{ij} \quad (2)$$

In the first equation, Y_{ij} represented the anticipated outcome of the dependent variable Y at level j of the continuous variable X. The overarching intercept for all studies, denoted as B_0, was a fixed effect. B_1 and B_2, also fixed effects, corresponded to the overall linear (L) and quadratic (Q) regression coefficients of Y on X across all studies. Within this study-i, X_{ij} signifies the mean value at level j of the continuous variable X. S_i is the random effect attributed to study-i, whereas b_i pertains to the random effect influencing the regression coefficient of Y on X within study-i. The residual error is represented by e_{ij}. In the second equation, Y_{ij} is the estimated means of response variable Y of the j_{th} observation in the ith experiment, μ is the overall mean, β_a is the fixed effect of categorical data (cassava products, rearing system, buffalo breed, and buffalo sex), β_b is fixed effect of covariates, $\beta_a \times \beta_b$ is interaction terms between categorical data and covariates of the j_{th} observation in the ith experiment, $s\beta_{ij}$ is random interaction between the i experiment and the j treatment group of factors β, S_i is random effect of the experiment, and e_{ij} is residual error. Statistical analysis employed models that incorporated P values, root mean square errors (RMSE), and the coefficient of determination (R^2) between the observed and estimated values. The results were considered statistically significant when the P value was less than or equal to 0.05. Data analysis was performed using the "lme4" library (R version 4.3.1) (R Core Team, 2022).

Furthermore, the interrelationship of each variable, namely, the factors and output parameters, was examined via principal component analysis (PCA) (Greenacre *et al*., 2022; Kassambara, 2017). The

Table 2. Descriptive statistics of cassava meta-data from *in vivo* experiments

Response variables	n	mean	sd	max	min
Cassava (% DM)	64	9.19	7.39	20.5	0
Feed intake					
Total intake (g/kg $BW^{0.75}$/d DM)	48	80.7	18.1	155	44.5
Concentrate					
Concentrate (g/kg $BW^{0.75}$/d DM)	38	19.8	17.5	110	4
Cassava by-product and root (g/kg $BW^{0.75}$/d DM)	34	9.9	7.87	27.4	1.1
Other (g/kg $BW^{0.75}$/d DM)	19	11.5	6.36	24.9	3.1
Forage					
Forage (g/kg $BW^{0.75}$/d DM)	34	68.1	13.1	113	49.4
Cassava foliage (g/kg $BW^{0.75}$/d DM)	6	21.7	5.1	25.8	12.6
Other (g/kg $BW^{0.75}$/d DM)	34	66.7	10.2	87.6	52.6
Nutrient intake					
Organic matter (g/kg $BW^{0.75}$/d DM)	24	48.4	14.4	75.9	26.8
Crude protein (g/kg $BW^{0.75}$/d DM)	30	4.3	2.3	8.9	1.7
NDF (g/kg $BW^{0.75}$/d DM)	24	37.8	18.5	66	3.5
ADF (g/kg $BW^{0.75}$/d DM)	20	26.3	16	52.3	2.6
Growth performance					
Final body weight (kg)	9	393	19.7	416	360
Live weight change (kg)	9	61.8	36.3	110	9
Average daily gain (kg/d)	12	0.77	0.34	1.1	0.1
Feed conversion	12	13.5	14.8	57	4.9
Nutrient digestibility					
DM digestibility (%)	32	59.7	9.4	72.5	27.2
OM digestibility (%)	31	64.7	5.7	74.6	53.2
CP digestibility (%)	36	58.2	8.6	77.6	47.5
NDF digestibility (%)	34	56.8	11.3	78.4	21.8
ADF digestibility (%)	30	47.7	12.5	63.2	22.4
Nitrogen balance and purine derivate					
Purine derivate excretion (mmol/d)	8	72.9	24.7	107	48.7
Purine derivate absorption (mmol/d)	8	78.1	12.2	101	68.3
Absorbed N	16	40.3	9.7	57.6	25
Retained N	16	25.1	9.12	42.1	12.9
Rumen characteristics					
pH 4 hours	43	6.7	0.21	7.08	6.2
Temperature (°C)	20	38.9	0.37	39.5	38.3
NH_3-N (mg/100 mL)	40	13.8	3.4	21.3	8.8
Volatile fatty acid (mol/100 mol)	32	96.1	12.7	116	60.1
Acetate (mol/100 mol)	32	67.5	5.8	77.4	54.5
Propionate (mol/100 mol)	32	20.3	4.7	28.3	11.4
Butyrate (mol/100 mol)	31	8.7	2.7	14.3	2.3
Acetate/Propionate	32	3.5	0.96	5.91	2.1
Methane 24 hours (mL/100 mL)	5	24.2	2.4	28.1	22.5
Haematology					
Blood urea nitrogen (mg/dL)	20	11.9	3.3	19.4	3.6
Microbiology					
Bacteria 0 hours (Log_{10} CFU/mL)	23	9.4	0.73	10.9	8.3
Bacteria 4 hours (Log_{10} CFU/mL)	19	10	1.4	12.1	8.5
Protozoa 0 hours (Log_{10} CFU/mL)	19	6.4	1.5	8.8	4.4
Protozoa 4 hours (Log_{10} CFU/mL)	19	7	1.9	8.9	4.6
Fungi 0 hours (Log_{10} CFU/mL)	23	6.5	1.4	8.4	4.3
Fungi 4 hours (Log_{10} CFU/mL)	15	6.9	1.7	8.6	4.4
Amylolytic group (Log_{10} CFU/mL)	28	8.1	0.66	9.3	7
Proteolytic group (Log_{10} CFU/mL)	28	7.7	0.95	9.1	6.3
Cellulolytic group (Log_{10} CFU/mL)	28	8.7	0.74	10.1	7.4
Ruminococcus albus (Log10 CFU/mL)	8	7.7	1.2	8.9	6.3
Fibrobacter succinogenes (Log_{10} CFU/mL)	8	8.7	0.57	9.5	8.2
Milk					
Milk production (kg/d at7% FCM)	5	3.6	2	7.2	2.2

Note: ADF = acid detergent fiber, CFU = colony forming unit, CP = crude protein, DM = dry matter, max = maximum, min = minimum, n = number of data points, NDF = neutral detergent fiber, OM = organic matter, R^2 = coefficient of determination, sd = standard deviation.

factors included differences in studies (Experiment), the duration of treatment periods (Treatment), the type of rearing system (Rearing), the buffalo breed (Breed), the buffalo age at the start of treatment (Age), and the buffalo sex (Sex). Moreover, the output parameters included in the PCA analysis were *in vivo* parameters, as presented in Table 5. The PCA analysis and corresponding graph were generated using the "factoextra" package in R (Kassambara, 2017; Adli *et al*., 2023). The relative contribution (cos^2 value) evaluated the extent to which a variable was represented in PCA. A value of ≥0.7 indicated strong representation, while a range of 0.5-0.7 suggested moderate significance. If cos^2 was less than 0.5, the variable was poorly represented and inadequately explained within the model.

Response surface methodology (RSM) was used to determine the optimal level of cassava supplementation (Design Expert 13). The determination of the optimal dose was based on output parameters, which included OM and CP digestibility, NH_3-N production, acetate, and propionate. The factors used were the amount of cassava supplementation and the composition of DM, OM, CP, NDF, and ADF in the total feed mixture, all expressed as %DM. The desirability values ranged from 0 to 1, where 0 indicated an undesirable outcome and 1 indicated a highly desirable or optimal result (Sholikin *et al*., 2019; Njoku and Otisi, 2023; Mang *et al*., 2015; Adli *et al*., 2024).

RESULTS

In vivo Digestibility, Fermentation Characteristics, and Microbiology

The *in vivo* nutrient digestibility of OM, CP, NDF, and ADF remained unchanged with the addition of cassava to the buffalo diet (Table 3). However, there was a significant increase in DMD with the cassava treatment (p=0.01). Among the different types of cassava, the roots exhibited the highest digestibility of DM, NDF, and ADF (p<0.01), followed by the foliage, the control diet, and the by-products (Table 4). The type of rearing and the buffalo breed did not significantly affect nutrient digestibility (Tables 5-6). Nevertheless, male buffalo demonstrated greater DMD compared to females (p<0.01; Table 7).

The characteristics of the rumen fermentation are presented in Table 3. Many parameters show no noticeable effects from cassava supplementation. However, methane gas production is significantly decreased (Q; p<0.01). Moreover, the most substantial differences in acetate production among the cassava parts are observed in the roots, followed by the control and foliage parts, with values of 68.8, 62.3, and 56.4 mol/100 mol, respectively (p<0.01; Table 4). Ammonia production (after 2 hours) is higher in intensively reared buffalo fed with a controlled diet (p<0.001; Table 5).

The total bacterial count (0 h) increased significantly after the buffaloes were fed with cassava, following a quadratic pattern (p<0.05; Table 6). The fungal population (4 h) also increases significantly in response to cassava inclusion (p<0.01). The amylolytic group shows a significant linear decrease (p<0.01). The population of *R. albus* bacteria increases significantly with the addition of cassava (p<0.01). Cassava foliage and roots have a significantly higher fungal population at 4 h (p<0.05).

Regarding the management system and sex of the buffaloes, the following results are observed: protozoa (0 h), the proteolytic group, and the cellulolytic group are more prevalent under intensive restricted management (p<0.05; Table 6). However, there is no significant difference in the microbiological profiles between female and male cattle (Table 7).

Feed Intake and Nutrient Intake

The total intake (g/kg $BW^{0.75}$/d DM) exhibits a quadratic increase (p<0.01; Table 3), which is consistent with the observed patterns for cassava intake (both by-product and root), which has also increased following a quadratic pattern (p<0.01). However, total forage intake significantly and linearly increases (p<0.01). Specifically, cassava foliage intake is significantly increased (p<0.01), but other parts of cassava, such as the by-products and roots (Table 4), do not differ significantly, resulting in no significant effect on overall feed intake. Compared to intensive management, extensive management resulted in a greater intake of cassava concentrate (by-products and roots) (p<0.05; Table 5). Additionally, the Murrah buffalo shows greater concentrate intake (total concentrate, cassava, and others) than the Swamp buffalo did (p<0.01; Table 6). Compared to male livestock, female livestock consume more concentrate (total concentrate and cassava) (p<0.01; Table 7).

Meta-analysis revealed curvilinear models of DMI (kg/d and kg/$BW^{0.75}$) in response to increasing cassava inclusion in buffalo diets (p<0.01; Table 3). Significant, positive quadratic patterns (p<0.01) are also found for OM, CP, NDF, and ADF intake with increasing dietary cassava levels. Table 4 compares the effects of different types of cassava products on buffaloes. There is no difference between the cassava products and the control group in terms of DMI, OM, and NDF intake. However, adding cassava foliage to the diet resulted in the highest crude protein intake (p<0.01).

Growth Performance

The growth performance of buffaloes fed with a cassava-based diet significantly resulted in improved FCR (p<0.05; Table 3). However, FBW, LWC, and ADG do not show significant differences. Likewise, the types of rearing and buffalo breeds exhibit no significant variation (Tables 4 and 5).

Hematology and Milk Production

The concentration of BUN is unaffected in buffaloes fed with cassava, as indicated by the meta-analysis (p=0.75; Table 3). Similarly, variations in cassava (including cassava by-products, foliage, and roots) have no significant effect on serum BUN levels (p=0.86; Table 4). Table 3 shows that milk production in buffaloes fed

Table 3. Regression equation for cassava (% DM) on feed intake, nutrient intake, growth performance, nutrient digestibility, nitrogen balance, purine derivatives, rumen characteristics, hematology, microbiology, and milk production in buffalo

Response variables	M	N	Variable estimates				Model estimates		
			Intercept		Slope		p-value	RMSE	R^2
			Value	SE	Value	SE			
Feed intake									
Total intake (g/kg $BW^{0.75}$/d DM)	L	48	2.2	0.661	2.2	0.661	0.003	4.7	0.95
	Q				-0.128	0.035	0.001		
Concentrate									
Concentrate (g/kg $BW^{0.75}$/d DM)	L	38	23.1	8.35	0.093	0.193	0.63	2.3	0.99
Cassava by-product and root (g/kg $BW^{0.75}$/d DM)	L	34	-3.7	2.43	2	0.236	<0.001	0.55	0.99
	Q				-0.0517	0.0087	<0.001		
Other (g/kg $BW^{0.75}$/d DM)	L	19	23.1	4.01	-0.74	0.195	<0.001	1.7	0.9
Forage									
Forage (g/kg $BW^{0.75}$/d DM)	L	34	66.8	5.78	-0.146	0.027	<0.001	2.7	0.96
	Q				-0.146	0.026	<0.001		
Cassava foliage (g/kg $BW^{0.75}$/d DM)	L	6	-5.7	3.31	1.3	0.052	<0.01	0.03	1
Other (g/kg $BW^{0.75}$/d DM)	L	34	63.9	3.77	2.3	0.52	0.0002	2.7	0.92
	Q				-0.137	0.025	<0.001		
Nutrient intake									
Organic matter (g/kg $BW^{0.75}$/d DM)	L	24	43.9	7.14	1.8	0.459	0.002	2.1	0.98
	Q				-0.104	0.0207	<0.001		
Crude protein (g/kg $BW^{0.75}$/d DM)	L	30	3.6	0.812	0.151	0.0478	0.005	0.24	0.98
	Q				-0.0053	0.0023	0.034		
NDF (g/kg $BW^{0.75}$/d DM)	L	24	39.2	6.98	1.2	0.436	0.016	2	0.98
	Q				-0.0812	0.0197	0.001		
ADF (g/kg $BW^{0.75}$/d DM)	L	20	27.5	7.78	0.775	0.298	0.022	1.3	0.99
	Q				-0.0517	0.013	0.002		
Growth performance									
Final body weight (kg)	L	9	385	15.1	0.353	0.647	0.6	7.9	0.83
Live weight change (kg)	L	9	54.9	25.8	-0.198	0.603	0.78	6.9	0.96
Average daily gain (kg/d)	L	12	0.74	0.21	-0.002	0.01	0.86	0.13	0.84
Feed conversion	L	12	7.4	10	1.1	0.363	0.02	4.7	0.92
Nutrient digestibility									
DM digestibility (%)	L	32	55.1	3.5	0.427	0.154	0.01	2.9	0.89
OM digestibility (%)	L	31	63.1	2.2	0.199	0.144	0.18	2.4	0.76
CP digestibility (%)	L	36	57.6	3	0.201	0.192	0.3	4.1	0.71
NDF digestibility (%)	L	34	58.9	4.1	-0.021	0.25	0.93	4.2	0.81
ADF digestibility (%)	L	30	45.6	4.6	0.255	0.308	0.41	4.1	0.82
Nitrogen balance and purine derivate									
Purine derivate excretion (mmol/d)	L	8	104	58	-6.17	4.2	0.19	5.5	0.99
Purine derivate absorption (mmol/d)	L	8	109	48.3	-6.18	4.6	0.24	6.8	0.98
Absorbed nitrogen	L	16	26.5	13.2	4.73	2.3	0.068	5.7	0.88
	Q				-0.248	0.093	0.019		
Retained nitrogen	L	16	13	3.1	3.1	0.753	0.001	5.5	-
	Q				-0.122	0.036	0.005		
Rumen characteristics									
pH 4 hours	L	43	6.8	0.07	-0.006	0.004	0.21	0.08	0.85
Temperature (°C)	L	20	39.1	0.17	-0.022	0.014	0.19	0.25	0.43
NH3-N (mg/100 mL)	L	40	15	1.2	-0.115	0.103	0.28	2.7	0.32
Volatile fatty acid (mol/100 mol)	L	32	94.7	5.2	0.111	0.377	0.77	5.8	0.74
Acetate (mol/100 mol)	L	32	67	2.3	0.043	0.192	0.83	3.5	0.54
Propionate (mol/100 mol)	L	32	21.1	1.5	-0.099	0.053	0.08	0.68	0.97
Butyrate (mol/100 mol)	L	31	9	1.1	-0.016	0.089	0.86	1.4	0.64
Ratio of C2/C3	L	32	3.3	0.33	0.019	0.018	0.3	0.25	0.9
Methane 24 hours (mL/100 mL)	L	5	152	132	-55.6	2.9	0.033	0.02	1
	Q				3	0.15	0.032		
Hematology									
Blood urea nitrogen (mg/dL)	L	20	12.3	1.4	-0.034	0.126	0.79	3.2	-

Response variables	M	N	Variable estimates				Model estimates		
			Intercept		Slope		p-value	RMSE	R^2
			Value	SE	Value	SE			
Microbiology									
Bacteria 0 hours (Log_{10} CFU/mL)	Q	23	9.1	0.31	0.0758	0.0218	0.004	0.07	0.99
Bacteria 4 hours (Log_{10} CFU/mL)					-0.0034	0.001	0.005		
Protozoa 0 hours (Log_{10} CFU/mL)	L	19	9.6	0.51	0.004	0.005	0.478	0.08	0.99
Protozoa 4 hours (Log_{10} CFU/mL)	L	19	6.5	0.72	-0.004	0.01	0.72	0.11	0.99
Fungi 0 hours (Log_{10} CFU/mL)	L	19	7.3	0.75	0.001	0.003	0.99	0.04	1
Fungi 4 hours (Log_{10} CFU/mL)	L	23	6.4	0.52	-0.003	0.013	0.83	0.14	0.98
Amylolytic group (Log_{10} CFU/mL)	Q	15	6.6	0.68	0.0779	0.0167	0.002	0.05	1
Proteolytic group (Log_{10} CFU/mL)					-0.0029	0.008	0.007		
Cellulolytic group (Log_{10} CFU/mL)	L	28	8.3	0.24	-0.016	0.007	0.03	0.08	0.98
Bacteria 0 hours (Log_{10} CFU/mL)	L	28	8	0.35	-0.016	0.005	0.08	0.06	0.99
Bacteria 4 hours (Log_{10} CFU/mL)	L	28	8.8	0.27	-0.003	0.008	0.75	0.1	0.97
Ruminococcus albus (Log_{10} CFU/mL)	L	8	8.3	0.67	0.089	0.0188	0.017	0.06	0.99
	Q				-0.0061	0.001	0.008		
Fibrobacter succinogenes (Log_{10} CFU/mL)	L	8	9.1	0.53	-0.04	0.017	0.07	0.11	0.97
Milk									
Milk production (kg/d at7% FCM)	L	5	4	1.66	0.095	0.063	0.25	0.25	0.97

Note: ADF = acid detergent fiber, CFU = colony forming unit, CP = crude protein, DM = dry matter, L = linear, M = model, Max = maximum, Min = minimum, N = number of data points, NDF = neutral detergent fiber, OM = organic matter, Q = quadratic, R^2 = coefficient of determination, RMSE = root mean square error, SE = standard error.

with cassava as a feed source ranges from 2.17 to 7.16 kg/d. As a key performance indicator for buffaloes, milk production is not significantly influenced by cassava feeding.

PCA Results and Optimum Conditions for The *In vivo* Experiment

Based on the PCA results, several output parameters influence buffalo production when fed with a cassava-based diet. These parameters include feed intake (concentrate and forage), nutrient intake (IADF, IOM, and INDF), nutrient digestibility (DMD, CPD, and ADFD), fermentation characteristics (C2, C3, and the C2:C3 ratio), nitrogen retention (PDA and PDE), and bacterial populations (amylolytic, proteolytic, and protozoa). The influential parameters had a threshold > 0.2, derived from a minimum correlation cutoff of |r| > 0.8, which identified the primary associations illustrated in Figure 2. Feed intake components based on cassava (OM intake and CP intake) increased propionate production and enhanced protein digestibility. In contrast, the acetate-to-propionate ratio showed an opposing trend. Interestingly, the rise in CP intake did not correspond with an increase in proteolytic activity.

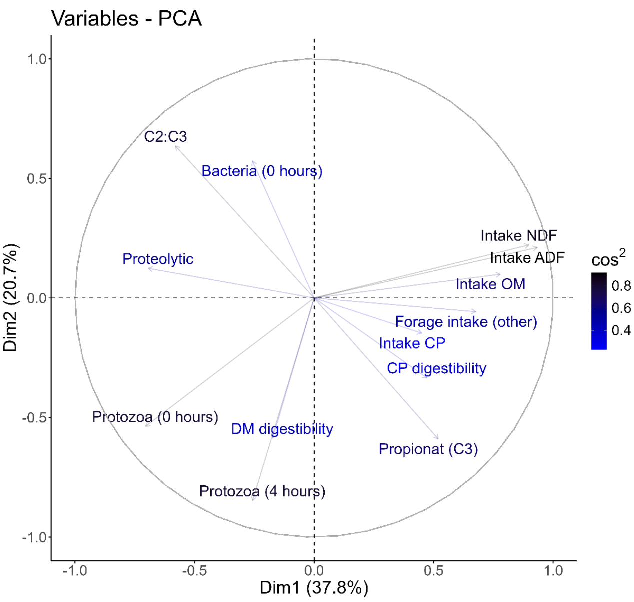

Figure 2. Principal component analysis graph illustrating the relationships between parameters and factors, including cassava type, buffalo age, breed, and sex. Parameters and factors that significantly contributed to dimensions 1 and 2.

The reciprocal relationships among various factors influencing cassava inclusion, such as DM, OM, CP, NDF, and ADF, in the total mixed rations for buffalo were analyzed using RSM. The findings encompassed OMD, CPD, and the concentrations of NH_3-N, acetate, and propionate (Figure 3). The analysis of these parameters indicates that the optimal level of cassava inclusion was 12.1% DM. This should be combined with 61% DM, 49.3% OM, 7.7% CP, 59.3% NDF, and 44.7% ADF. This combination yielded favorable production outcomes, including an OMD of 71.1%, CPD of 68%, NH_3-N at 21.2 mg/100 mL, acetate at 54.4 mol/100 mol, and propionate at 28.3 mol/100 mol (Figure 4).

DISCUSSION

Rumen Fermentation Characteristics and Microbiology

Rumen fermentation is a crucial aspect of nutrient utilisation in ruminants and significantly influences overall digestive efficiency. Observations on the effects of cassava on buffalo rumen fermentation have highlighted its potential as a valuable feed resource for buffaloes. Cassava promotes an increase in amylolytic and cellulolytic species, such as *R. albus*.

Table 4. Effect of cassava type (by-product, foliage, and root) on feed intake, nutrient intake, growth performance, nutrient digestibility, nitrogen balance, purine derivatives, rumen characteristics, hematology, microbiology, and milk production of buffaloes in an *in vivo* experiment

Response variables	Control	By-product	Foliage	Root	SEM	*P*-value
Feed intake						
Total intake (g/kg $BW^{0.75}$/d DM)	82	81.3	85	86.3	1.2	0.93
Concentrate						
Concentrate (g/kg $BW^{0.75}$/d DM)	4.1	-	4.6	25.3	6.9	0.8
Cassava by-product and root (g/kg $BW^{0.75}$/d DM)	-	-	1.12	9.79	2.6	0.31
Other (g/kg $BW^{0.75}$/d DM)	-	-	6	13	1.8	0.4
Forage						
Forage (g/kg $BW^{0.75}$/d DM)	83.4	-	87.6	68.9	3.9	0.56
Other (g/kg $BW^{0.75}$/d DM)	70.9	-	71.8	66	1.3	0.79
Nutrient intake						
OM (g/kg $BW^{0.75}$/d DM)	73	-	75.9	41.1	2.1	0.05
CP (g/kg $BW^{0.75}$/d DM)	5.6 ^{b}	-	6.7 ^{c}	3 ^{a}	0.1	0.002
NDF (g/kg $BW^{0.75}$/d DM)	63.8	-	66	34.2	3.1	0.23
ADF (g/kg $BW^{0.75}$/d DM)	50.7	-	52.3	20	2	0.17
Growth performance						
Final body weight (kg)	379	-	381	390	0.28	0.42
Live weight change (kg)	32.5	-	22.6	36.4	0.27	0.45
Average daily gain (kg/d)	0.69	-	0.65	0.81	0.03	0.9
Feed conversion	15.5	-	30.9	12.9	1.7	0.42
Digestibility						
DM digestibility (%)	53.7 ^{a}	53.4 ^{ab}	67.3 ^{b}	60 ^{ab}	0.46	0.01
OM digestibility (%)	65.9	64.8	69.2	64.5	0.52	0.48
CP digestibility (%)	56.8	52.7	64.3	59.8	0.78	0.23
NDF digestibility (%)	51.6 ^{ab}	37.7 ^{a}	60.6 ^{b}	61.2 ^{b}	0.72	0.01
ADF digestibility (%)	41.1 ^{ab}	30.3 ^{a}	51.6 ^{b}	50.5 ^{b}	0.59	0.01
Nitrogen balance and purine derivate						
Purine derivate excretion (mmol/d)	51.5	-	48.7	95	0.18	0.8
Purine derivate absorption (mmol/d)	70.8	-	68.3	86.1	0.98	0.62
Absorbed nitrogen	44.5	-	50.3	38.4	1.8	0.39
Retained nitrogen	14.2 ^{a}	-	19.4 ^{ab}	28.3 ^{b}	1.2	0.03
Rumen characteristics						
pH 4 hours	6.7	6.5	6.6	6.7	0.02	0.34
Temperature (°C)	38.9	-	39	38.9	0.08	0.95
Ammonia 2 hours (mg/100 mL)	13.1	11.1	12.5	14.1	0.67	0.82
Volatile fatty acid (mol/100 mol)	99.6	-	109	94.6	1.6	0.33
Acetate (mol/100 mol)	62.3 ^{ab}	-	56.4 ^{a}	68.8 ^{b}	1	0.02
Propionate (mol/100 mol)	21.3	-	19.2	20	0.13	0.07
Butyrate (mol/100 mol)	9.3	-	8.1	8.8	0.41	0.77
Ratio of C2/C3	3.1	-	3.1	3.6	0.07	0.37
Hematology						
Blood urea nitrogen (mg/dL)	12.6	-	13.3	11.7	0.75	0.86
Microbiology						
Bacteria 0 hours (Log_{10} CFU/mL)	9.1	-	9.1	9.4	0.07	0.83
Bacteria 4 hours (Log_{10} CFU/mL)	9.7	-	10	9.6	0.29	0.06
Protozoa 0 hours (Log_{10} CFU/mL)	4.9	-	4.9	7.2	0.1	0.21
Protozoa 4 hours (Log_{10} CFU/mL)	4.6	-	4.6	7.7	0.35	0.26
Fungi 0 hours (Log_{10} CFU/mL)	6.1	-	6.1	6.5	0.13	0.94
Fungi 4 hours (Log_{10} CFU/mL)	6.1 ^{a}	-	6.6 ^{b}	7.1 ^{ab}	0.34	0.04
Amylolytic group (Log_{10} CFU/mL)	7.7	-	7.7	8.3	0.14	0.73
Proteolytic group (Log_{10} CFU/mL)	6.4	-	6.3	8	0.17	0.19
Cellulolytic group (Log_{10} CFU/mL)	7.8	-	8	8.9	0.14	0.25
Ruminococcus albus (Log_{10} CFU/mL)	7.8	-		8.1	0.02	0.47
Fibrobacter succinogenes (Log_{10} CFU/mL)	8.8	-		8.5	0.02	0.27
Milk						
Milk production, kg/d at 7% FCM	2.6	-	3.1	7.2	0.02	0.07

Note: $^{a, b, c}$ Values within a row with different superscripts indicate significant differences (p<0.05). ADF = acid detergent fiber, CFU = colony forming unit, CP = crude protein, DM = dry matter, NDF = neutral detergent fiber, OM = organic matter, SEM = standard error of the mean.

Table 5. Impact of buffalo rearing on feed intake, nutrient intake, growth performance, nutrient digestibility, nitrogen balance, rumen characteristics, and microbiology in *in vivo* experiments

Response variables	Intensive		Extensive	SEM	*P*-value
	Restricted	*Ad libitum*			
Feed intake					
Total intake (g/kg $BW^{0.75}$/d DM)	81	73.4	97.8	8.8	0.55
Concentrate					
Concentrate (g/kg $BW^{0.75}$/d DM)	16.4	17.9	53.8	15	0.21
Cassava by-product and root (g/kg $BW^{0.75}$/d DM)	7.3 [a]	7.05 [ab]	22.5 [b]	6.2	0.02
Other (g/kg $BW^{0.75}$/d DM)	13	-	6.1	4.9	0.23
Forage					
Forage (g/kg $BW^{0.75}$/d DM)	67.2	58.9	81.1	7.9	0.601
Other (g/kg $BW^{0.75}$/d DM)	66.9	58.9	57	3.7	0.47
Growth performance					
Final body weight (kg)	401	-	382	12.9	0.7
Live weight change (kg)	47.7	-	55.2	5.3	0.93
Average daily gain (kg/d)	1.1	-	0.6	0.32	0.42
Feed conversion	7.9	-	18.9	7.8	0.66
Digestibility					
DM digestibility (%)	62.3	51.4	51.6	4.4	0.31
OM digestibility (%)	65	56.1	71.2	5.4	0.12
CP digestibility (%)	57.4	52.2	67.8	5.6	0.23
NDF digestibility (%)	58.3	55.6	63	2.7	0.92
ADF digestibility (%)	47.6	37.3	53.2	5.7	0.68
Nitrogen balance					
Absorbed nitrogen	37.9	39.5	-	1.1	0.83
Retained nitrogen	26.2	32.5	-	4.45	0.22
Rumen characteristics					
pH 4 hours	6.7	6.8	-	0.09	0.654
Temperature (°C)	38.9	39.2	-	0.22	0.493
Ammonia 2 hours (mg/100 mL)	14.4 [b]	9 [a]	-	3.8	<0.001
Volatile fatty acid (mol/100 mol)	92.4	93	-	0.43	0.97
Acetate (mol/100 mol)	67.7	72.7	-	3.6	0.06
Propionate (mol/100 mol)	19.4	18.3	-	0.78	0.84
Butyrate (mol/100 mol)	8.3	9	-	0.52	0.82
Ratio of C_2/C_3	3.7	4	-	0.21	0.79
Microbiology					
Bacteria 0 hours (Log_{10} CFU/mL)	9.3	9.6	9.6	0.12	0.97
Bacteria 4 hours (Log_{10} CFU/mL)	9.6	-	9.8	0.2	0.87
Protozoa 0 hours (Log_{10} CFU/mL)	8.5 [b]	6.1 [a]	4.4 [a]	1.5	0.02
Protozoa 4 hours (Log_{10} CFU/mL)	7.7	-	4.6	2.2	0.16
Fungi 0 hours (Log_{10} CFU/mL)	7	5.7	6.1	0.47	0.84
Fungi 4 hours (Log_{10} CFU/mL)	7.1	-	6.3	0.56	0.72
Amylolytic group (Log_{10} CFU/mL)	8.4	7.6	-	0.58	0.37
Proteolytic group (Log_{10} CFU/mL)	8.4 b	6.5 [a]	-	1.3	0.04
Cellulolytic group (Log_{10} CFU/mL)	9.1 b	7.6 [a]	-	1.1	0.05

Note: [a, b, c] Values within a row with different superscripts indicate significant differences (p<0.05). A hyphen (-) indicated the absence of data related to the comparative categorization in the meta-analysis. ADF = acid detergent fiber, CFU = colony forming unit, CP = crude protein, DM = dry matter, NDF = neutral detergent fiber, OM = organic matter, SEM = standard error of the mean.

This enhancement indicates improved carbohydrate degradation in the rumen, which contributes to greater nutrient availability for the animal (Wang & McAllister, 2002). The rise in rumen microbial populations, along with the positive impact on carbohydrate-degrading bacteria, suggests a favorable environment for efficient fermentation and nutrient utilization.

The significant increase in the rumen fungal population following the addition of various types of cassava introduces an interesting dimension to buffalo rumen fermentation. Fungi play a crucial role in fiber degradation within the rumen, contributing to the breakdown of complex plant materials (Joomjantha & Wanapat, 2008). This increase in the fungal population suggests a potential role of cassava in enhancing fiber digestion, which is essential for overall nutrient absorption and utilization by buffaloes. Previous studies have shown that the bacterial population in the rumen fluid of cassava-fed animals tends to be higher than that in the rumen fluid of exclusively grazed animals,

Table 6. Influence of buffalo breed on feed intake and digestibility

Response variables	Murrah	River	Swamp	SEM	*p*-value
Feed intake					
Total intake (g/kg $BW^{0.75}$/d DM)	155 [b]	102 [ab]	79.8 [a]	22.3	0.004
Concentrate					
Concentrate (g/kg $BW^{0.75}$/d DM)	110 [b]	-	105 [a]	47.4	<0.001
Cassava by-product and root (g/kg $BW^{0.75}$/d DM)	27.4 [b]	-	7.3 [a]	10.1	0.004
Other (g/kg $BW^{0.75}$/d DM)	110 [b]	-	74.8 [a]	47.4	<0.001
Forage					
Forage (g/kg $BW^{0.75}$/d DM)	49.4	-	72.3	11.4	0.22
Digestibility					
DM digestibility (%)	-	69.3	58.7	5.3	0.37
CP digestibility (%)	-	67.5	59.5	4	0.37

Note: [a, b, c] Values within a row with different superscripts indicate significant differences (p<0.05). CP = crude protein, DM = dry matter, River Type = river buffalo, SEM = standard error of the mean.

Table 7. Comparative study of feed intake, nutrient intake, digestibility, and microbiology in female and male buffalo

Response variables	Female	Male	SEM	p-value
Feed intake				
Total intake (g/kg $BW^{0.75}$/d DM)	109	81.7	13.7	0.202
Concentrate				
Concentrate (g/kg $BW^{0.75}$/d DM)	110 [b]	106 [a]	47.1	<0.001
Cassava by-product and root (g/kg $BW^{0.75}$/d DM)	27.4 [b]	7.4 [a]	10	0.007
Forage				
Forage (g/kg $BW^{0.75}$/d DM)	49.4	72.9	11.8	0.24
Other (g/kg $BW^{0.75}$/d DM)	57	68.2	5.64	0.34
Digestibility				
DM digestibility (%)	34.8 [a]	61.7 [b]	13.4	0.01
CP digestibility (%)	58.2	59.6	0.72	0.88
Microbiology				
Bacteria 0 hours (Log_{10} CFU/mL)	9.6	9.2	0.2	0.72
Bacteria 4 hours (Log_{10} CFU/mL)	9.8	9.6	0.14	0.87
Protozoa 0 hours (Log_{10} CFU/mL)	4.4	7.1	1.4	0.24
Protozoa 4 hours (Log_{10} CFU/mL)	4.6	7.7	1.6	0.16
Fungi 0 hours (Log_{10} CFU/mL)	6.1	6.4	0.15	0.84
Fungi 4 hours (Log_{10} CFU/mL)	6.3	7.1	0.39	0.71

Note: [a, b, c] Values within a row with different superscripts indicate significant differences (p<0.05). CFU = colony forming unit, CP = crude protein, DM = dry matter, SEM = standard error of the mean.

particularly after four hours of post-feeding (Granum *et al*., 2007). Moreover, the number of protozoa tended to decrease in all animals fed cassava. These trends align with earlier observations highlighting the influence of feeding strategy, pH, and available substrates in the rumen on microbial ecology and growth (Bell *et al*., 2023; Roza *et al*., 2021). Cassava hay may provide the rumen with adequate fiber and cassava-protein complexes, potentially stimulating bacterial growth and subsequent fermentation in the rumen.

A previous study demonstrated the effects of different levels of cassava leaf flour on the characteristics of the rumen mixture, including NH_3-N production, rumen microbial activities, VFA production, and dry matter digestibility (Roza *et al*., 2013). The results revealed that 30% cassava leaf mixture significantly influenced key aspects of rumen function, resulting in the lowest NH_3-N concentration, whereas 10% cassava leaf mixture resulted in notable bacterial counts and VFA production. These findings align with an earlier assertion that NH_3-N levels in the rumen serve as indicators of both degradation processes and protein synthesis by rumen microbes (Franzolin & Alves, 2010). Ammonia in the rumen is essential for the growth and development of microbes and the synthesis of microbial proteins, with approximately 82% of microbial species utilizing ammonia as a nitrogen source.

The strategic addition of cassava hay has been shown to influence the rumen of buffaloes by increasing pH levels, optimizing the rumen ecology, and enhancing the utilization of bypass proteins, such as tannin–protein complexes (Chanjula *et al*., 2004). Moreover, feeding cassava hay can strategically increase the consumption of low-quality forages, leading to improved productivity in ruminants in terms of both milk yield and weight gain (Bell *et al*., 2023; Roza *et al*., 2021b).

Moreover, the dietary inclusion of cassava was found to enhance the ecology and promote protein synthesis in the rumen microbes of buffaloes (Wanapat

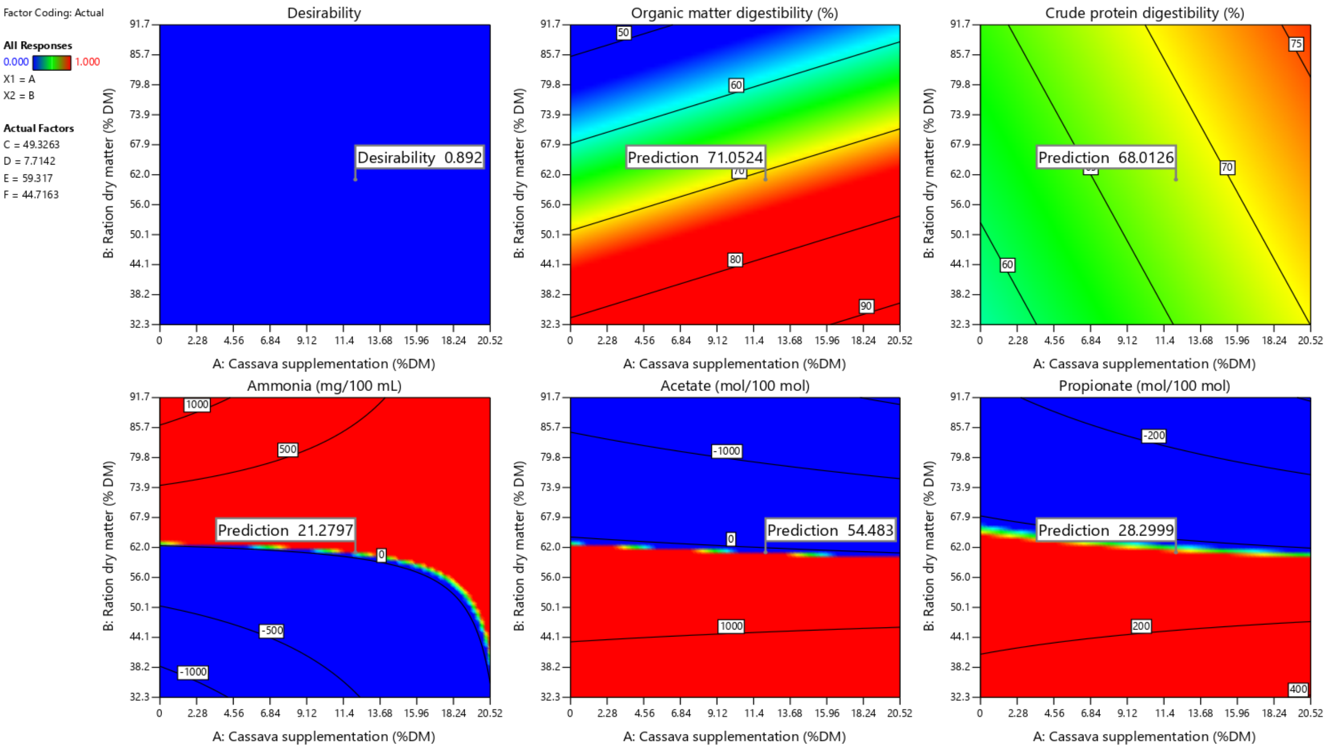

Figure 3. Various factors, including organic matter digestibility, crude protein digestibility, ammonia, acetate, and propionate, were analysed after cassava was added to the buffalo diet. A = cassava supplementation level, B = ration dry matter, C= ration organic matter, D = ration crude protein, E = ration neutral detergent fiber, and F = ration acid detergent fiber.

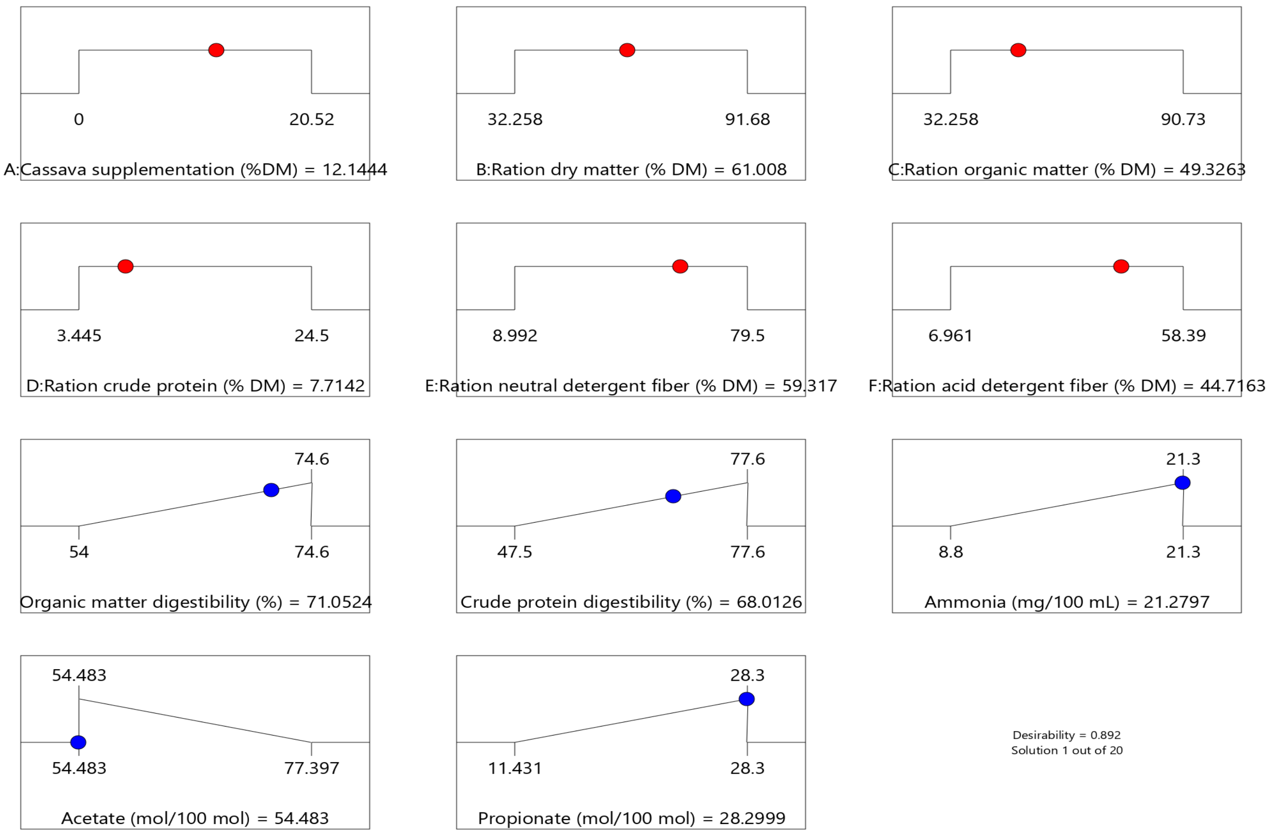

Figure 4. The optimal inclusion level of cassava in the buffalo diet. Under ideal conditions, supplementing with 12.1% DM of cassava could result in an estimated organic matter digestibility of 71.1%, crude protein digestibility of 68%, ammonia concentration of 21.3 mg/100 mL, acetate concentration of 54.5 mol/100 mol, and propionate concentration of 28.3 mol/100 mol. Red dots represented the independent parameters (cassava supplementation level, ration dry matter, ration organic matter, ration crude protein, ration neutral detergent fiber, and ration acid detergent fiber), while blue dots indicated the dependent parameters (organic matter digestibility, crude protein digestibility, ammonia, acetate, and propionate).

et al., 2012). A comparative study reported that the total bacteria content was between 13.2 × 10^8 mL^{-1} in cows and 16.2 × 10^8 mL^{-1} in buffaloes, and the number of bacteria is greater in the rumen fluid of buffaloes (Wanapat *et al*., 2012). Notably, the cellulolytic effect was 2-3 times greater in buffaloes than in cows. The percentage of cellulolytic bacteria in cattle was 19.5%, and in buffalo, it was 42.3% of the total bacteria. In the rumen of buffalo, there are efficient crude fiber-digesting bacteria that are not found in cows. Hence, the digestibility of feed in buffalo is better than that in cows. This is thought to be caused by the high population of cellulolytic microbes in odorous livestock (Wanapat *et al*., 2012). Buffalo is generally reared with low-quality feed because buffalo rumen bacteria have adapted well to forage feed and agricultural waste, which are usually of low quality with high lignocellulose (Wanapat *et al*., 2012; Pandya *et al*., 2010). The increased activity of bacteria in the rumen of buffaloes is indicated by the faster and higher production rate of VFA in buffaloes than in cows (National Research Council, 2002). The inoculum from buffalo rumen fluid is the best at degrading fiber in feed derived from agricultural waste. Buffaloes are able to utilize low-quality feed because it is supported by the large volume of the buffalo rumen, high salivary secretion, slow rate of feed leaving the rumen, cellulolytic activity and high microbial population.

Feed Intake and Nutrient Intake

The meta-analysis results indicate that the average substitution of cassava is approximately 10.3% DM, or even up to 100% DM. The feed intake significantly increased due to the inclusion of cassava. The results of feed intake after the addition of cassava suggest that cassava is a viable feed ingredient for buffaloes, as the animals have no issues with its acceptability. Several previous studies have shown that adding various cassava products, such as hay, cassava residue, and cassava roots, at levels between 9% and 40% DM did not negatively affect feed intake in buffaloes or cattle (Foiklang *et al*., 2011; Dagaew *et al*., 2021; Supapong & Cherdthong, 2020; Huang *et al*., 2020). A recent study reported that fresh cassava roots commonly found in Thailand contain between 2.3% and 2.8% CP (Dagaew *et al*., 2021; Supapong & Cherdthong, 2020). The increase in CP intake due to cassava foliage is expected because the foliage would have increased the CP content of the diets offered. Earlier studies also reported that the incorporation of cassava hay had increased the feed intake (kg/d) compared to that of other feeds, such as rice bran, *Phaseolus calcaratus*, and mulberry hay (Foiklang *et al*., 2011). However, this study revealed a lower nutrient intake when buffaloes were given cassava roots. This is understandable, as cassava roots contain a low CP content, which is less than 5%.

Nutrient Digestibility

The data reveals a significant increase in DM digestibility as a result of cassava. These findings suggest that cassava has a beneficial effect on the ability of buffaloes to digest dry matter. In fact, there have been reports of the positive impact of cassava on DM digestibility in ruminants since cassava is known for its high energy content, which increases the digestibility of dry matter (Huang *et al*., 2020), particularly in livestock with efficient rumen fermentation (Wanapat *et al*., 2012; Iqbal *et al*., 2018). Research has also revealed that various segments of cassava plants have diverse impacts on the digestibility of nutrients. Among these, cassava roots presented the greatest digestibility levels for DM, NDF, and ADF. In addition to cassava roots, cassava foliage presented the next highest digestibility, followed by the control diet and cassava by-product. These findings are in agreement with the literature that emphasizes the importance of considering specific parts of cassava to be used in ruminant diets (Wanapat *et al*., 2012). Thus, cassava root is often preferred over other parts, such as cassava by-products, because of its high energy content (Inthapanya & Preston, 2014; Huang *et al*., 2020; Hung *et al*., 2013).

Growth Performance

The data show no significant impact of cassava supplementation on buffalo growth performance based on parameters such as FBW, LWC, ADG, and FCR. While cassava is often used as an alternative energy source to replace concentrate in ruminants, these findings suggest that it can serve as a viable substitute without negatively affecting final body weight (379-390 kg), which falls within the normal range for buffaloes (Wanapat *et al*., 2012). The absence of adverse effects on final weight supports the potential of cassava as an alternative feed ingredient in buffalo diets. However, its use requires appropriate processing to minimize antinutritional compounds that may be harmful to livestock (Wanapat *et al*., 2012).

Additionally, cassava supplementation did not influence buffalo's live weight, which ranged from 22.6 kg to 36.4 kg. This is likely due to cassava's role in the biohydrogenation process during metabolism, facilitating the conversion of nutrients into propionic acid, a key precursor for glucose synthesis (Wanapat *et al*., 2012). Consequently, cassava inclusion did not affect ADG, which ranged from 0.65 kg/day to 0.81 kg/day, nor did it negatively impact FCR, which varied between 12.9 and 30.9. Interestingly, cassava was found to enhance rumen microbial ecology and promote microbial protein synthesis, contributing to improved digestive efficiency in buffaloes (Wanapat *et al*., 2012).

Hematology and Milk Production

The BUN levels remained unchanged alongside those of cassava, indicating that cassava has no adverse effect on the serum BUN concentration of buffalo. This is attributed to efficient nitrogen (N) utilization during the catabolism process (Wanapat *et al*., 2012), which reflects the beneficial effect of cassava.

However, excessive amounts of cassava (up to 50%) can negatively affect the pH of the rumen and subsequent fermentation products due to the high starch content of cassava (Wanapat *et al*., 2012). Such high starch levels can lead to ruminal acidosis, a condition characterized by a decrease in rumen pH, which can disrupt the normal fermentation processes. This may result in reduced feed intake, impaired nutrient absorption, and even health issues such as laminitis in buffalo. Therefore, while cassava can be a valuable dietary component, its inclusion must be carefully managed to ensure it remains within optimal levels for the health and productivity of the animals.

The key performance indicator analyzed in this study was milk production. The findings indicate that cassava supplementation does not alter buffalo milk yield, suggesting that cassava has no negative impact. This aligns with the physiological characteristics of buffaloes, which are more efficient than cattle in nitrogen recycling and fiber digestion. Buffaloes also maintain stable ruminal NH_3-N levels, supporting fermentation and feed utilization efficiency (National Research Council, 2002). Furthermore, buffaloes can effectively utilize high-fiber feed, particularly agricultural by-products, due to the diverse microbial population in their rumen, which provides essential energy and protein for productivity (Wanapat *et al*., 2012).

However, these findings suggest that cassava's effect on milk production is not significant, which may be attributed to several factors. One possible reason is inconsistency in feed formulation, which could affect nutrient balance (Pradhan, 1994). The interaction between cassava and other feed components in the diet may also influence nutrient availability. Additionally, variations in cassava quality, such as starch and fiber content, may lead to inconsistent results.

Another factor to consider is the presence of anti-nutritional compounds, such as cyanogenic glycosides, which can form toxic cyanides with the help of the enzyme β-glucosidase (Cressey & Reeve, 2019). Although no reports specifically address the toxicity threshold of cyanide in buffaloes, signs of toxicity have been widely documented (Suharti *et al*., 2021; Moses *et al*., 2024). A toxic level is reached when cyanide accumulation in the blood exceeds 1 mg/liter in both poultry and ruminants (Kennedy *et al*., 2021). Cyanide poisoning in cattle is characterized by restlessness, respiratory distress, tachycardia, and cyanosis of the mucous membranes (Kennedy *et al*., 2021). Death may occur within two hours of high-dose exposure, accompanied by seizures and coma. Cyanide inhibits cellular respiration by binding iron in the cytochrome complex, leading to cell death due to anoxia (Sonobe *et al*., 2021; Mao *et al*., 2023). Low-dose exposure can be detoxified into thiocyanate, but high doses increase the risk of mortality (Kennedy, 2021). Although studies in this meta-analysis did not confirm cyanide toxicity effects, some findings reported performance decline (Huang *et al*., 2020; Roza *et al*., 2021a). Moreover, differences in study design might contribute to inconsistent results. Variations in management systems, feed formulations, milk production measurement methods, and study duration may influence result interpretation. Therefore, a more comprehensive meta-analysis with standardized methodological approaches is necessary to establish stronger conclusions.

Overall, this meta-analysis indicates that cassava supplementation in buffalo feed enhances rumen fermentation, nutrient intake, and nitrogen retention, although it does not significantly affect weight gain or milk production. Cassava, particularly its roots and leaves, has the potential to serve as an alternative feed source in sustainable livestock systems. However, the main challenge lies in its palatability, which may influence feed intake and efficiency. Additionally, attention should be given to potential toxin exposure, such as cyanogenic compounds. Although this meta-analysis cannot further confirm the toxicity effects of cyanogenic compounds, the primary parameters, such as performance, only show a decline, albeit insignificant, and there are no reports concerning buffalo mortality. Another limitation of our meta-analysis is the variability in data caused by the diverse types of buffalo (breed and sex) and cassava (cassava part). Furthermore, we have clarified this by conducting a subgroup meta-analysis that outlines the differences between buffalo types and cassava varieties based on categorization.

In disseminating these findings, the use of social media platforms (such as Twitter, Facebook, and Instagram), as examined by Lamanna *et al*. (2024), is a strategic approach in delivering scientific information interactively to farmers and industry professionals. This method can enhance understanding and adoption of more efficient and environmentally friendly feeding practices. Therefore, further research is needed to optimize cassava-based feed formulations and evaluate effective communication strategies to support their implementation in the livestock industry.

CONCLUSION

The meta-analysis findings indicate that the incorporation of cassava into buffalo diets is beneficial, as it has no adverse impact on feed intake, rumen fermentation, overall buffalo performance, or milk production. The optimal inclusion level, determined through response surface methodology, was identified as 12.1% DM of the diets. Furthermore, the findings indicated that the fermentation characteristics of the rumen and the microbiological profile were not negatively affected by the cassava-based feed, showing an increase in populations of amylolytic bacteria and protozoa. Therefore, the use of cassava, particularly its root of approximately 1.5% to 20.5% DM, can be regarded as an effective feeding strategy to enhance the performance and health of buffalo.

CONFLICT OF INTEREST

A. Jayanegara serves as an editor of the Tropical Animal Science Journal but has no role in the decision to publish this article. The writer asserts that there are no conflicts of interest or funding concerns that arose during the formulation of this meta-analysis manuscript.

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