RESEARCH ARTICLE

In vitro, *in situ* and *in vivo* comparison of two feeding regimes in water buffalo (*Bubalus bubalis*) under tropical conditions

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Abstract

The increasing demand for animal protein requires efficient production systems adapted to tropical conditions. Water buffalo farming is a variable option due to its hardiness and productive potential. This study compared the fermentative and productive performance of buffalo fed a commercial diet or a diet formulated with regional ingredients. Ten weaned male buffalo (230 ± 20.0 kg) were randomly assigned to the two diets for a 60-day fattening period. Dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR), and feed efficiency (FE) were recorded. And *in vitro* and *in situ* rumen fermentation assay were conducted. DMI and final weight did not differ between diets (P > 0.05). The formulated diet increased ADG by 30.4% with a trend towards significance (P = 0.09) and improved FCR and FE (P > 0.05). The commercial diet promoted greater *in vitro* gas production and nutrient degradations, and a higher soluble fraction *in situ*, whereas the formulated diet increased the potentially degradable fraction and lag time. Overall, the diet formulated with regional ingredients maintained comparable, and biologically superior, productive performance to the commercial diet in beef buffalo under tropical conditions, despite lower degradability *in vitro* and *in situ*.

Key words: Water buffalo, formulated diet, rumen fermentation, productive performance, regional ingredients.

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Introduction

The sustained increase in global demand for animal protein has driven the search for more efficient and environmentally responsible production systems. In this context, water buffalo farming, understood as the raising and productive use of the water buffalo (*Bubalus bubalis*), has gained relevance due to its capacity to sustain meat and milk production in environments were conventional cattle face nutritional, climatic, or health limitations (Batista *et al*., 2020; FAO, 2024). Water buffalo are described as hardy, long-lived, and versatile animals with good fertility and production capacity for both milk and meat, as well as productive and reproductive potential compared to other livestock species (Guerrero-Legarreta *et al.,* 2019; Bertoni *et al*., 2022).

From a zootechnical point of view, the water buffalo stands out for its biological efficiency in utilizing diets based on local resources of low nutritional quality, which is reflected in a favorable feed conversion and lower energy requirements per kilogram of gain (Mota-Rojas *et al.,* 2019; Rodríguez-González *et al.,* 2022). In breeding systems, buffalo reach weaning weights close to 240 kg with daily weight gains of around 0.5 kg, and carcass yields close to 58%, a value comparable to the carcass yield reported for cattle in tropical conditions (57 – 59%), which positions the species as attractive for meat production (Ramírez-Barboza *et al*., 2016; Guerrero-Legarreta *et al*., 2022). In addition, the relationship between feed intake, nitrogen use efficiency, and enteric methane (CH_4) production per unit of product has been described as favorable in the water buffalo compared to other ruminant species (Sheoran *et al*., 2023; Trapanese *et al*., 2024).

The physiological and nutritional adaptability of water buffalo to warm, humid climates makes this species a viable technical alternative for tropical and subtropical regions where the cost of concentrated feed limits the productivity of traditional beef cattle (Bertoni *et al.,* 2022; Minervino

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et al., 2020). However, achieving competitive productive performance in buffalo requires feeding programs that balance (a) the efficient use of commercial feeds with known formulations and high energy density, and (b) the utilization of locally available ingredients, which reduce costs and increase the producer's self-sufficiency. The choice between a commercial diet and a complete diet formulated with regional raw materials is not only a nutritional decision, but also an economic one and a technological adoption decision in emerging production units (Uzun *et al.,* 2018).

In this context, it is necessary to generate direct evidence on the productive performance of buffalo water under both feeding schemes. The objective of this study was to compare a commercial diet and a complete diet formulated with local ingredients for water buffalo, evaluating fermentation kinetics and substrate digestibility through *in vitro* assays, ruminal dry matter degradability through *in situ* assays, and the productive performance of growing animals through an *in vivo* assay.

Materials and Methods

Location

The chemical analysis of the diets and the *in vitro* and *in situ* assays were performed at the Animal Nutrition Laboratory of the Faculty of Veterinary Medicine and Animal Science No. 2, located in Cuajinicuilapa, Guerrero, Mexico. The *in vivo* trial was conducted from May to July 2024 at the "Los Laureles" Ranch, also located in Cuajinicuilapa, Guerrero, Mexico (16° 28′ 19′′ N, 98° 25′ 00′′ W, 50 m above sea level). The region has a warm sub-humid climate with summer rains, average annual rainfall, and an average temperature of 1100–1300 mm and 24–28 °C (INEGI, 2022).

In vitro assay

Culture medium

The culture medium included the following components: 45.9% (1009.8 mL) of distilled water; 5% (110 mL) of mineral solution I [6 g of KH_2PO_4 (Sigma-Aldrich®) dissolved in 1000 mL of distilled water]; 5% (110 mL) of mineral solution II [6 g of KH_2PO_4 (Sigma-Aldrich®), 6 g of $(NH_4)2SO_4$ (Merck®), 12 g of NaCl (Sigma-Aldrich®), 2.45 g of $MgSO_4$ (SIGMA-ALDRICH®) and 1.6 g of $CaCl_2$ $2H_2O$ (SIGMA-ALDRICH®), dissolved in 1000 mL of distilled water]; 0.1% (2.2 mL) of resazurin (Sigma-Aldrich®), 5% (110 mL) of buffer solution; 4% (88 mL) of reducing solution; and 35% (770 mL) of fresh ruminal fluid, previously filtered through a fine mesh cloth to remove macroparticles of organic matter.

Biodigesters and incubation

In a 120 mL serological vial, 0.5 g of sample with a particle size of 1 mm at constant weight and 40 mL of culture medium were added. All vials were kept under continuous CO_2 flow to maintain anaerobic conditions, sealed with a neoprene stopper (20 mm Ø) and an aluminum ring with a removable center, and each vial was considered a biodigester. The biodigesters were placed in a water bath at 39 °C and inoculated with 10 mL of fresh rumen fluid (pH 6.4 and 4.9 x 10^9 bacterial cells mL^{-1}), which was considered time zero. The ruminal fluid was obtained from a *Bos taurus* x *Bos indicus* crossbred bovine of 400 + 30 kg PV equipped with a ruminal cannula, fed on pastures with pangola grass and with commercial supplement, and previously the ruminal fluid was centrifuged at 1137 x g for 3 min to precipitate the food particles and protozoa.

Biogas production

Total biogas production was determined by plunger displacement in a 50 mL glass syringe (BD Yale®, Brazil), recording the volume generated at 12, 24, 48, and 72 h of incubation (Hernández-Morales *et al.,* 2018). Methane (CH_4) production was quantified using a Taygon® tubing (2.38 mm internal diameter and 45 cm length) with hypodermic needles (20 G x 32 mm) at the ends. The needles were used to connect a biodigester to a trap vial. The trap vials were filled with a modified 2N NaOH solution [80 g NaOH (Merck®) in 1000 mL of distilled water] from the methodology of Stolaroff *et al.* (2008). CH_4 production was considered as the mL displaced from the NaOH (2N) solution at 12, 24, 48, and 72 h of incubation. The *in vitro* dry matter degradation (DMSiv) and neutral detergent fiber at 72 h was calculated using the formula described by Hernández-Morales *et al.* (2018).

In situ assay

Sample preparation and incubation in the rumen

Five-gram samples of the commercial and formulated diets were prepared and placed in poly-silk bags (10 x 20 cm). Each treatment was replicated three times for each of the incubation times (0, 2, 4, 8, 12, 18, 24, 32, 48, 60, and 72 hours). The samples had a particle size of 1 mm, and the bags were sealed with plastic ties (100 x 2.5 mm).

Prior to inserting into the cow's rumen, the bags were immersed in water at 39 °C for 10 minutes. Subsequently, the bags were attached to a galvanized iron chain (1.5 x 100 cm) that was connected to the rumen cannula plug. The bags were inserted into the rumen in reverse order of the incubation time to allow for simultaneous collection of samples at the end of the incubation period. After the incubation period, the bags were rinsed with cold running water until the rinse water ran clear. The bags corresponding to 0 hours were not incubated in the rumen; instead, they underwent the same rinsing protocol as the incubated bags. Finally, the bag residues were dried at 55 °C for 72 hours and weighed to determine digestibility by weight difference.

Digestibility kinetics

The *in situ* digestibility kinetics estimates of DM were calculated using a nonlinear regression procedure, using

the PROC NLIN procedure of SAS Institute Inc (2011) using the equation described by McDonald (1981):

P = a + b(1 − e^(−ct)).

Where: P = ruminal digestibility at time t (%); a = rapidly soluble digestible fraction; b = slow or potentially digestible fraction; c = the rate at which b is digested; t = incubation time (h) in the rumen.

In vivo assay

Animals and handling

Ten weaned male buffalo (*Bubalus bubalis*, 230 ± 20.0 kg BW), 280+20 days old, were used. They received prophylactic treatment with antiparasitic (Cydectin® NF, Moxidectin, 0.2 mg kg^{-1} BW, via SC), bacterin (Bobact® 8, 5.0 mL $animal^{-1}$, via IM), metabolic stimulant (Catosal™, butafosfan + B12, 10 mL $animal^{-1}$, via IM) and vitamins ADE (Vigantol® ADE, 4 mL $animal^{-1}$, via IM) and were housed in pens grouped by treatment, provided with a feeder and drinker. The handling of the buffaloes was governed by the Regulation for the use and care of animals intended for research specified in NOM-062-ZOO-1999 "Technical specifications for the production, care and use of laboratory animals".

Treatments

The diet was formulated according to the nutritional requirements for cattle, with 1.0 Mcal NEg kg^{-1} DM, for a gain of 1000 g d^{-1} (NRC, 2001). The treatments (T) were T1: Formulated diet; T2: Commercial diet [(Bovimás Engorda Óptima 12 MP® (Agromás, Mexico)].

The chemical composition of the diets is shown in Table 1. Samples for analysis were processed in a Thomas-Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) with a 1 mm diameter sieve. Chemical analysis was performed according to AOAC (2005) methods: dry matter (method 930.15), ash and organic matter (method 942.05), and crude protein (CP; method 984.13). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were evaluated using an ANKOM analyzer (Ankom Technology Corp. A200, Fairport, NY, USA).

Table 1: Chemical composition of the diets evaluated in the feeding of buffalo

Ingredients	Commercial	Formulated
Composition on a dry basis (%)		
Corn kernel	-	38.5
Sugarcane molasses	-	7.0
Soybean oil	-	2.0
Soybean meal	-	20.0
Corn stover	30.0	30.0
Mineral blend	-	2.5
Agromás®	70.0	
Chemical composition		
Dry matter (%)	90.94	95.17
Crude protein (%)	13.93	16.80
Organic matter (%)	92.45	92.59
Neutral detergent fiber (%)	48.79	63.88
Acid detergent fiber (%)	29.39	35.09
Ash (%)	7.55	7.41
Metabolizable energy (Mcal/kg)	1.86	2.3

Bovimas Engorda 12 MP Agromás® = Ground and/or rolled grains and cereals, cereal and/or oilseed by-products, oilseed meals, coconut palm by-products, malt by-products, cane molasses, vegetable and/or animal bypass fat, NPN, calcium carbonate, monocalcium phosphate, sodium bicarbonate, sodium chloride, vitamins (ADE), minerals (oxides and/or sulfates and/or chelated minerals of copper, zinc, iron, manganese, cobalt, sodium selenite, EDDI, manganese oxide, mycotoxin absorbent, propionic acid, Saccharomyces cerevisiae yeast culture, antioxidant (BHT/ETQ) and rumen function modifier of vegetable origin. Guaranteed Analysis: Crude Protein 12%, Crude Fat 3%, Crude Fiber 15%, Ash 8% Humidity 12%, N.E.N. 50%.

Productive variables

The buffalo adapted to the diets (Table 1) for 15 days and the evaluation period was 60 days. The feed was offered at 08:00 (75% of ration) and 16:00 (25% of ration) and water was available *ad libitum*. Dry matter intake (DM, kg d^{-1}) was calculated by the difference between the feed offered and rejected each day. Rejected feed was collected for each treatment between 25 and 30 days and between 55 and 60 days; the samples were homogenized, and a composite sample was taken to determine dry matter content. Results are shown by week and as an overall average.

Average daily weight gain (ADG, g d^{-1}) was determined by the difference between final and initial weight, divided by the number of days of evaluation. Feed conversion ratio (FCR) was calculated as the ratio of dry matter intake (DMI) to ADG. Feed efficiency (FE) was calculated as the ratio of average daily gain (ADG) to dry matter intake (DMI).

Experimental design and statistical analysis

For *in vivo* assay, the experimental design was completely randomized with two treatments and five replicates. Data were analyzed using PROC GLM (SAS Institute, Inc., 2012), and treatment means were compared using Tukey's test ($P \leq 0.05$). *In vitro* degradation and *in situ* digestibility variables were analyzed in a completely randomized design using InfoStat software (Di Rienzo *et al.,* 2020). Mean comparisons were performed using Tukey's test ($P \leq 0.05$).

Results and Discussion

In vitro assay

In vitro degradation allowed for the characterization of the fermentative behavior of diets based on biogas and methane production, dry matter degradability, and neutral detergent fiber (Table 2; Posada and Noguera, 2005; Bashar *et al.,* 2024). The commercial diet generated higher biogas volumes than the formulated diet at all incubation stages ($P \leq 0.05$). At 12 and 48 h, biogas production was 573% and 325% greater than that of the formulated diet, respectively; at 48 and 72 h, the differences decreased, although they remained 68% and

48% higher than the formulated diet. This pattern indicates a high availability of rapidly fermentable carbohydrates in the commercial diet; these results are consistent with those reported by Foster *et al*. (2023) who mention that in *in vitro* tests, diets with a high proportion of non-structural carbohydrates generate greater biogas production in the early stages of fermentation.

Methane (CH_4) production followed a similar response (Table 2). The commercial feed produced significantly higher volumes of CH_4 at all time points evaluated ($P \leq 0.05$), with increases of 808% and 200% at 12 and 24 h, respectively, compared to the formulated diet. At 48 and 72 h, the differences remained around 51% and 21%. These variations reflect more intense methanogenic activity in the commercial diet associated with an increase in hydrogen availability during fermentation, which served as a substrate for methanogenic archaea (Pepeta *et al.,* 2024). Conversely, the lower biogas and CH_4 production observed with the formulated diet is consistent with a moderate fermentative profile, linked to a higher proportion of structural fractions (Table 1), which reduces the accessibility of cell wall polysaccharides to the ruminal microbiota (Patra *et al*., 2017; Martins *et al.,* 2023).

The differences in gas production were accompanied by markers in the degradation of dry matter (DM) and neutral detergent fiber (NDF) at 72 h of incubation (Table 2). The commercial diet reached degradations of 59.6% and 143.2%, respectively, compared to the formulated diet ($P < 0.0001$ in both cases). These differences reinforce the hypothesis of a greater availability of fermentable nutrients in the commercial diet, favored by the lower proportion of structural compounds and the inclusion of byproducts present in the Agromas® feed. This behavior is consistent with that reported by Rojas-González *et al*. (2023), who indicated that diets with lower structural fiber content exhibit greater degradability under *in vitro* conditions. In contrast, the formulated diet, containing fibrous sources (corn stover) and vegetable oil, showed lower degradability; this combination may have modified the hydrolysis capacity of the rumen microorganisms (Ferreira and Thiex, 2023). This behavior corresponds to the greater relative weight of the structural fractions observed later in the *in situ* kinetics.

In situ assay

The *in situ* trial allowed for the description of dry matter (DM) degradation kinetics in the rumen by incubating the diets at different times and fitting them to a first-order degradation model (Table 3; Goulart *et al.,* 2020). In the initial time points (0–18 h), the commercial diet showed higher DM digestibility than the formulated diet ($P < 0.0001$ at all time points). At 0 h, the soluble fraction of the commercial diet was 141.8% higher than that of the formulated diet, and at 18 h the difference reached 190.7%. This behavior confirms a soluble and rapidly degradable fraction in the commercial feed, consistent with the higher gas production observed in the *in vitro* trials.

After 24 h, the differences between diets decreased. At 24 and 30 h, dry matter digestibility was similar between treatments ($P > 0.05$), indicating that the potentially degradable fraction of the formulated diet can approximate that of the commercial feed when available during an intermediate fermentation time (Rupp *et al*., 2021). However, in later stages (36, 48, and 72 h), the commercial treatment again showed a significant difference ($P \leq 0.05$), with relative advantages ranging from 6.5% to 9.7%. Overall, the temporal kinetics showed that the commercial diet consistently maintained higher dry matter degradation, although the most pronounced gap was concentrated in the first few hours of fermentation.

Table 2: *In vitro* fermentative characteristics of a commercial diet and one made with regional ingredients

Variable	Treatment	Incubation time, h			
		12	24	48	72
Biogas production (mL g^{-1} DM)					
	Commercial	59.31	126.48	167.28	181.4
	Formulated	8.82	29.74	99.32	127.2
	P value	<0.0001	<0.0001	<0.0001	0.0004
	SEM	7.69	15.03	10.6	9.25
Methane production (mL g^{-1} DM)					
	Commercial	8.17	20.25	38.15	43.01
	Formulated	0.9	6.74	25.33	35.52
	P value	0.0249	0.0355	0.0007	0.0383
	SEM	1.69	3.32	2.23	1.86
Degradations (%)					
		DM (72 h)	NDF (72 h)		
	Commercial	70.54	57.07		
	Formulated	43.93	23.46		
	P value	<0.0001	<0.0001		
	SEM	3.71	4.69		

DM = dry matter; NDF = neutral detergent fiber; SEM = standard error of the mean

The analysis of kinetic parameters complements this interpretation (Table 3). The soluble fraction (a) was higher in the commercial diet compared to the formulated diet (266.8%; P < 0.0001), confirming an immediate release of nutrients at the start of ruminal fermentation. In contrast, the potentially degradable fraction (b) was 84.4% higher in the formulated diet (P < 0.0001) compared to the commercial diet, suggesting a higher proportion of structural components degradable over time, as observed in *in situ* and *in vitro* degradation kinetics with fibrous substrates (Van Soest *et al.,* 1991; Foster *et al.,* 2023).

The rate at which this fraction (c) is digested showed no difference between diets (P = 0.1638), suggesting that, once fraction b is available, the digestion rate is similar in both substrates. Finally, the lag time (k) was 7.6 times longer in the formulated diet than in the commercial diet (P < 0.0001). This delay in the effective onset of structural fraction degradation is associated with the need for a longer time for the hydrolysis of lignocellulolytic bonds and the adaptation of the rumen microbiota (Pu *et al.,* 2022).

Taken together, the *in situ* results describe two contrasting digestion patterns. The commercial feed provides an abundant soluble fraction, a rapid onset of degradation, and high and sustained dry matter digestibility over time, while the formulated diet is characterized by a reduced soluble fraction, a larger potentially degradable structural fraction, and a prolonged delay before degradation becomes effective. These findings complement the *in vitro* observations, showing that the lower overall degradability of the formulated diet is not due to the absence of degradable fractions, but rather to slower kinetics conditioned by its structural characteristics.

Table 3: *In situ* digestibility of dry matter of a commercial diet and one made with regional ingredients

	Diet			
Time	**Commercial**	**Formulated**	**P value**	**SEM**
0	48.14	19.91	<0.0001	6.34
2	53.2	22.34	<0.0001	6.92
4	51.81	23.15	<0.0001	6.44
8	53.42	25.76	0.0001	6.24
12	57.76	21.79	0.0001	8.11
18	65.28	22.46	0.0001	9.52
24	67.63	67.12	0.78	0.77
30	77.57	71.08	0.4431	3.71
36	74.89	70.29	0.0002	1.04
48	79.46	72.44	<0.0001	1.58
72	83.5	77.58	0.0005	1.35
a	48.23	13.15	<0.0001	6.634
b	44.09	81.29	<0.0001	7.14
c	0.025	0.029	0.1638	0.001
k	0.36	2.75	<0.0001	0.464

a = rapidly soluble digestible fraction; b = slow or potentially digestible fraction; c = rate at which b is digested; k = Lag time; SEM = standard error of the mean.

In vivo assay

The buffalo in both treatments began the experimental period with equivalent live weights (231.7 kg; P = 0.9488), confirming a homogeneous allocation of the animals (Table 4). After 60 days of evaluation, final live weight showed no difference between diets (P = 0.2969), with an average weight of 304.4 kg and an approximate total weight gain of 72.7 kg during the period. Daily dry matter intake was similar between diets (P = 0.1145), indicating that the differences in productive response were not explained by changes in total feed intake.

Average daily gain (ADG) showed biologically relevant responses favoring the formulated diet. Buffaloes fed the formulated diet had a 30.4% higher ADG than those fed the commercial diet (P = 0.098), indicating a trend toward greater daily growth with similar dry matter intake. This response was reflected in more favorable feed utilization indicators with the formulated diet.

Table 4: Productive variables of buffalo fed a commercial diet and a formulated diet

	Diet		P	
Variable	**Commercial**	**Formulated**	**value**	**SEM**
Initial live weight (kg)	231.4	232.0	0.9488	4.27
Final live weight (kg)	294.8	314.0	0.2969	8.72
Dry matter intake (kg d^{-1})	9.12	9.33	0.1145	0.07
Daily weight gain (kg d^{-1})	1.05	1.37	0.0986	0.09
Feed conversion ratio	9.26	7.00	0.1609	0.79
Feed efficiency	0.12	0.14	0.1434	0.01

SEM = standard error of the mean

Feed conversion was 24.4% lower with the formulated diet, and feed efficiency was 16.6% higher, although in both cases the differences did not reach statistical significance (P > 0.05). Even so, these numerical differences, along with the trend in average daily gain (ADG), suggest efficient utilization of the formulated ration in terms of weight gain per unit of dry matter consumed.

Analysis of weekly dry matter intake provides additional elements for interpreting these results (Table 5). In weeks 1, 2, 6, and 7, no differences in dry matter intake were observed (P > 0.05), although in week 2 a significant trend toward higher dry matter intake was recorded in the group fed the formulated diet (P = 0.0527). In the intermediate phase of the trial (weeks 3, 4, and 5), dry matter intake with the formulated diet was 6.62%, 4.92%, and 5.76% higher than that of the commercial diet, with statistically significant differences in each week (P = 0.0002, 0.0016, and 0.0095, respectively). In weeks 8 and 9, the pattern reversed, with intakes 1.36% and 1.26% higher for the commercial diet (P = 0.0351 and 0.0064).

Table 5: Weekly dry matter intake of buffalo fed a commercial diet and a formulated diet

	Diet			
Week	**Commercial**	**Formulated**	**P value**	**SEM**
1	7.77	8.04	0.3917	0.15
2	8.48	8.96	0.0527	0.13
3	8.76	9.34	0.0002	0.10
4	8.93	9.37	0.0016	0.08
5	8.86	9.37	0.0095	0.11
6	9.73	9.70	0.8683	0.10
7	10.38	10.36	0.9514	0.15
8	9.56	9.43	0.0351	0.03
9	9.52	9.40	0.0064	0.02

SEM = standard error of the mean

Taking together, these patterns indicate that the formulated diet showed a slightly higher acceptance, especially in the intermediate fattening phase, while towards the end of the period the animals with commercial diets marginally increased their consumption to approach a similar productive performance.

The *in vivo* results, considered together with the *in vitro* and *in situ* trials, show that the formulated diet, based on regional ingredients and with a higher content of structural fiber, sustained competitive and even biologically superior daily growth rates to those observed with the commercial diet, despite having lower ruminal degradability and gas production in the *in vitro* and *in situ* systems (Dixit *et al.,* 2023; Foster *et al*., 2023).

These results contrast with previous reports, such as those by Hernández-Sánchez *et al*. (2022), who recorded lower average daily gain (ADG) (0.92 kg d^{-1}) in buffalo fed a high-fiber diet (44% NDF) with regular crude protein (CP) content (12%). Similarly, Borghese *et al*. (2010) documented lower ADG values (0.86 kg d^{-1}) than in the present study in buffalo fed a conventional diet based on conserved forage (60.6% corn silage, 13.6% hay, and 25.8% concentrate). Therefore, the results of this study suggest that the use of appropriately formulated regional ingredients represents a viable and efficient strategy for optimizing the productive performance of buffalo under tropical conditions and in resource-constrained systems.

Conclusion

In vitro, in situ, and *in vivo* trials together indicate that a diet formulated with regional ingredients can sustain a productive performance comparable to that of a commercial diet in beef buffalo under tropical conditions, constituting a viable nutritional alternative in systems with limited availability of commercial concentrates.

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Conflict of Interest

The authors declare that there is no economic, personal or academic conflict of interest that could have inappropriately influenced the conduct of this research or the preparation of the manuscript.

References

Bashar MK, Haese E, Sultana N, Rodehutscord M. 2024. *In vitro* ruminal fermentation, methane emissions, and nutritional value of different tropical feedstuffs for ruminants. *J Adv Vet Anim Res* 11(4): 924–935. DOI: https://doi.org/10.5455/javar.2024.k842

Batista JN, Pereira FB, Pereira Filho JM, de Lima Junior V, dos Santos VLF, Araújo MJ, et al. 2020. Replacing corn bran and soybean meal in the diet with spineless cactus and cottonseed affects ingestive behaviour, performance, carcass characteristics and meat quality of Murrah water buffalo. *Anim Prod Sci* 60(7): 877–886. DOI: https://doi.org/10.1071/AN19260

Bertoni A, Álvarez-Macías A, Mota-Rojas D, Dávalos JL, Minervino AHH. 2022. Description of four dual-purpose river buffalo (*Bubalus bubalis*) farms in tropical wetlands in Mexico. Part 1: Social aspects, herd distribution, feeding, reproductive, and genetic management. *J Buffalo Sci* 11: 8–18.

Borghese A, GM T, M M, M R, Sabia E, Corrado P. 2010. Fattening of buffalo young bulls with different diets. *Rev Vet* 21: 511–516.

Dixit S, Kumar S, Sharma R, Banakar PS, Deb R, Tyagi AK. 2023. Rumen microbial diversity, enteric methane emission and nutrient utilization of crossbred Karan-Fries cattle (*Bos taurus*) and Murrah buffalo (*Bubalus bubalis*) consuming varied roughage concentrate ratio. *Anim Biotechnol* 34(6): 1857–1875. DOI: https://doi.org/10.1080/10495398.2022.2053696

FAO. 2024. Gateway to dairy production and products. Food and Agriculture Organization of the United Nations. Disponible en: https://www.fao.org

Ferreira G, Thiex N. 2023. Symposium review: Fiber and *in vitro* methods, analytical variation, and contributions to feed analysis. *J Dairy Sci* 106(6): 4464–4469. DOI: https://doi.org/10.3168/jds.2022-22407

Foster JL, Smith WB, Rouquette FM, Tedeschi LO. 2023. Forages and pastures symposium: An update on *in vitro* and *in situ* experimental techniques for approximation of ruminal fiber degradation. *J Anim Sci* 101: skad097. DOI: https://doi.org/10.1093/jas/skad097

Goulart RS, Vieira RAM, Daniel JLP, Amaral RC, Santos VP, Toledo Filho SG, et al. 2020. Effects of source and concentration of neutral detergent fiber from roughage in beef cattle diets on feed intake, ingestive behavior, and ruminal kinetics. *J Anim Sci* 98(5): skaa107. DOI: https://doi.org/10.1093/jas/skaa107

Hernández-Sánchez D, Rodríguez-Florentino R, Ramírez-Bribiesca E, Crosby Galván MM, Mata-Espinosa MÁ, Pinto-Ruiz R. 2022. Comportamiento productivo de búfalos (*Bubalus bubalis L.*) en dos sistemas de producción y dos pesos al sacrificio. *Agro-Divulgación* 2(5): 1–12. Disponible en: https://agrodivulgacioncolpos.org/index.php/1agrodivulgacion1/article/view/104

Iannuzzi A, Parma P, Iannuzzi L. 2021. The cytogenetics of the water buffalo: A review. *Animals (Basel)* 11(11): 1–17. DOI: https://doi.org/10.3390/ani11113109

Martins LF, Cueva SF, Lage CFA, Ramin M, Silvestre T, Tricarico J, et al. 2023. A meta-analysis of methane mitigation potential of feed additives evaluated *in vitro*. *J Dairy Sci* (online ahead of print). DOI: https://doi.org/10.3168/jds.2023-23419

Minervino AHH, Zava M, Vecchio D, Borghese A. 2020. *Bubalus bubalis*: A short story. *Front Vet Sci* 7: 570413. DOI: https://doi.org/10.3389/fvets.2020.570413

Mohd Azmi AF, Ahmad H, Mohd Nor N, Goh YM, Zamri-Saad M, Abu Bakar MZ, et al. 2021. The impact of feed supplementations on Asian buffaloes: A review. *Animals (Basel)* 11(7): 1–25. DOI: https://doi.org/10.3390/ani11072033

Mor P, Bals B, Tyagi AK, Teymouri F, Tyagi N, Kumar S, et al. 2018. Effect of ammonia fiber expansion on the available energy content of wheat straw fed to lactating cattle and buffalo in India. *J Dairy Sci* 101(9): 7990–8003. DOI: https://doi.org/10.3168/jds.2018-14584

NRC. 2001. Nutrient requirements of dairy cattle. 7th ed. Washington (DC): National Academy Press; p. 43–104.

Patra A, Park T, Kim M, Yu Z. 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. *J Anim Sci Biotechnol* 8: 13. DOI: https://doi.org/10.1186/s40104-017-0145-9

Pepeta BN, Hassen A, Tesfamariam EH. 2024. Quantifying the impact of different dietary rumen modulating strategies on enteric methane emission and productivity in ruminant livestock: A meta-analysis. *Animals (Basel)* 14(5): 1–22. DOI: https://doi.org/10.3390/ani14050763

Posada SL, Noguera RR. 2005. Técnica *in vitro* de producción de gases: una herramienta para la evaluación de alimentos para rumiantes. *Livest Res Rural Dev* 17(4): 36. Disponible en: http://lrrd.cipav.org.co/lrrd17/4/posa17036.htm

Pu XX, Zhang XM, Li QS, Wang R, Zhang M, Zhang SZ, et al. 2022. Comparison of *in situ* ruminal straw fiber degradation and bacterial community between buffalo and Holstein fed with high-roughage diet. *Front Microbiol* 13: 1079056. DOI: https://doi.org/10.3389/fmicb.2022.1079056

Rojas-González AJ, Arriaga-Jordán CM, Sánchez-Torres JE, Mejía-Uribe LA, Rayas-Amor AA, Morales-Almaráz E. 2023. *In vitro* assessment of ruminal biohydrogenation of polyunsaturated fatty acids in diets with different types and levels of protected fat and diverse sources of fibre. *Trop Anim Health Prod* 56(1): 28. DOI: https://doi.org/10.1007/s11250-023-03859-y

Rupp C, Westreicher-Kristen E, Susenbeth A. 2021. *In situ* and *in vitro* determination of the protein value of feeds for ruminants. *Arch Anim Nutr* 75(5): 329–344. DOI: https://doi.org/10.1080/1745039X.2021.1962149

Sheoran S, Dey A, Sindhu S. 2023. Reduction of methane and nitrogen emission and improvement of feed efficiency, rumen fermentation, and milk production through strategic supplementation of eucalyptus (*Eucalyptus citriodora*) leaf meal in the diet of lactating buffalo (*Bubalus bubalis*). *Environ Sci Pollut Res Int* 30(60): 125510–125525. DOI: https://doi.org/10.1007/s11356-023-31089-0

Trapanese L, Petrocchi Jasinski F, Bifulco G, Pasquino N, Bernabucci U, Salzano A. 2024. Buffalo welfare: A literature review from 1992 to 2023 with a text mining and topic analysis approach. *Ital J Anim Sci* 23(1): 570–584. DOI: https://doi.org/10.1080/1828051X.2024.2333813

Uzun P, Masucci F, Serrapica ML, Varricchio C, Pacelli S, Claps, Francia AD. 2018. Use of mycorrhizal inoculum under low fertilizer application: Effects on forage yield, milk production, and energetic and economic efficiency. *J Agric Sci* 156: 127–135. DOI: https://doi.org/10.1017/S0021859618000072

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74(10): 3583–3597. DOI: https://doi.org/10.3168/jds.S0022-0302(91)78551-2