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Development and Evaluation of Complete Diet using *in vitro* Gas Production Technique in Semi-Arid Borno State.

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Abstract

The study was conducted to formulate and evaluate the nutrient composition of a complete diet in semi-arid Borno State. Locally available feed resources were used to formulate five diets, and were subjected to chemical analysis and in vitro gas production. For the in vitro gas production, 200 mg of the samples were each incubated in a 100 ml glass syringes for 3, 6, 12 and 24 hours. The result of the chemical composition revealed that the dry matter content ranged from 92.35–94.30%, crude protein from 11-12%, crude fibre 30.75–39.50% and metabolisable energy from of 8.74 to 10.57 MJ. The result showed that dry matter, crude protein, ether extract, ash and metabolisable energy were not significantly (P>0.05) different. The in vitro gas production revealed that gas production at 24 h was significantly (P<0.05) different with the highest values recorded in T4 (20.000 ml) and T5 (19.000 ml), followed by T3 (15.667 ml) and T1 (16.333 ml). The higher gas production in T4 and T5 could indicate that these formulations contained substrates that were more efficiently fermented over the longer incubation period. It was concluded that T4 and T5 that have a balanced fibre content and higher levels of fermentable carbohydrates exhibited significantly higher gas production at 24 hours, suggesting more efficient microbial fermentation. This shows that optimizing fibre and energy sources will enhance ruminal fermentation in ruminants.

Keywords: Supplementary, *in vitro*, semi-arid, fermentable carbohydrate, fibre.

Description of problem

Dairy production in semi-arid regions, such as Borno State in north eastern Nigeria, is faced with several challenges, including erratic rainfall patterns, prolonged dry seasons, and limited availability of high-quality forages. According to (1), the primary challenge faced by dairy farmers in semi-arid regions is the scarcity and high cost of conventional feed resources, particularly during the dry season when natural forage availability declines sharply. The high cost and seasonal variability of conventional feed ingredients further exacerbate the problem, making it difficult for smallholder farmers to maintain consistent milk yields throughout the year (2). These conditions often lead to significant fluctuations in feed resources, resulting in inadequate nutrition for dairy cows, which in turn affects milk production and overall herd health (3). Moreover, (4) reports that the shortage leads to undernourished cows, which suffer from decreased milk production, poor reproductive performance, and increased susceptibility to diseases. Given the limited financial capacity of smallholder farmers to purchase expensive commercial feeds, there is a pressing need to explore alternative, locally available feed resources that can supplement the diets of dairy cows.

Furthermore, the underutilization of agricultural by-products, such as crop residues and fruit and vegetable waste, represents a missed opportunity for providing affordable and sustainable nutrition to livestock. These resources, which are often discarded or used inefficiently, could be harnessed to create complete diets that meet the nutritional needs of dairy cows, thereby reducing feed costs and improving milk yield (5). The use of alternative feed ingredients such as agricultural by-products, tuber peels, and browse leaves is gaining attention due to their availability, low cost, and nutritional potential. Browse plants are also known for their high protein content and ability to thrive in arid and semi-arid regions, making them ideal for formulating total mixed rations for livestock. Similarly, tuber peels, including cassava and yam peels, are abundant agro-industrial wastes that can serve as viable energy sources (6). By incorporating these alternative feed ingredients, it may be possible to meet the nutritional needs of lactating cows at a lower cost, while also promoting sustainability and reducing environmental waste.

Incorporating agricultural by-products such as soybean waste, cabbage waste, and *Adansonia digitata* leaves as protein sources, and energy-rich alternatives like yam peels, sweet potato peels, and tigernut milk residue, could provide an affordable and sustainable approach to supplementary feeding. These ingredients are often underutilized despite their nutritional potential, and integrating them into formulated rations for lactating cows may improve feed efficiency while reducing waste from crop production.

The study, therefore, seeks to address the problem of feed scarcity and high costs in semi-arid Borno State by developing and evaluating complete diets for dairy cows using alternative feed resources. The study aims to assess the effectiveness of these diets in improving milk production, cow health, and overall farm productivity, providing a viable solution to the challenges faced by dairy farmers in the region.

Materials and methods

Experimental Site

The experiment was conducted at the Department of Animal Science Laboratory, University of Maiduguri. Borno State is geo-located on Latitude 11˚5'N, Longitude 13°09' E and at altitude of 320 meters above sea level. The area falls within the Sahelian Region (semi-arid zone) of West Africa which is noted for great climatic and seasonal variations. It is characterized by short duration of erratic rainfall of about 3-4 months with 203 mm as annual rainfall and a very long dry season of about 8-9 months. The mean ambient temperature is low in December to January ranging from 15-19 °C and high in March to June, ranging from 33-44 °C. The relative humidity is usually 45 % in August and drops to about 5 % in December and January. Day length varies from 11-12 hours (7).

Sources of Feed and Preparation

The samples of the feed ingredients were sourced locally within the three geo-political zone of the state based on availability. Fresh feed samples were properly sundried for a period of 7 days. All samples were ground to pass through 1- and 2-mm sieve for chemical analysis and degradability studies, respectively and stored in labelled polyethene bags for the study. The feed ingredients used for the study were cowpea husk and haulms, groundnut haulms, sorghum stover, maize bran, soybean milk residue, cottonseed cake, copra meal, wheat offal and *Faidherbia albida pods*.

Treatments/Experimental diets

Five (5) different experimental diets were compounded using the selected feed ingredients as shown in Table 1.

Chemical Analysis

Samples of the formulations were analysed for Dry matter (DM), Crude protein (CP), Crude fibre (CF), Ether extract (EE), Nitrogen-free Extract (NFE) and ash, using the methods of (8). Acid detergent fibre (ADF) and Neutral detergent fibre (NDF) were determined according to the procedure of (9) and hemicellulose was determined as the difference between NDF and ADF. The metabolisable energy (ME) content was estimated using the formular of (10).

Table 1: Percentage Composition of the Formulations

Feed ingredients	Formulations (%)				
	T1	T2	T3	T4	T5
Cowpea husk	17	17	0	0	25
Groundnut haulms	40	0	20	0	0
Sorghum stover	0	40	20	37	25
Cowpea haulms	0	0	20	20	0
Maize bran	20	10	10	10	15
Wheat offal	0	0	0	0	15
Cottonseed cake	20	20	0	20	17
Copra meal	0	10	0	0	0
Soybean milk residue	0	0	10	0	0
Faitherbia albida pods	0	0	17	10	0
Bone meal	2	2	2	2	2
Salt	1	1	1	1	1

***In vitro* Gas Production Procedure**

Management, feeding and collection of rumen fluid

Rumen fluid was collected from rumen of two fistulated donor cows under the same feeding regime (gamba grass hay, and a total of 2.4kg complete diet given in two meals daily). The rumen fluid was taken before the morning feeding. The rumen liquor was filtered through two layers of cheese cloth into a warm flask (kept in a bucket of water at 37–38°C) and flush with carbon dioxide (11).

Mineral salt lick and water were provided *ad libitum* throughout the study period.

Buffer and Inoculum

Buffer and mineral solution (35.0 g $NaHCO_3$ + 4.0 g $(NH_4)HCO_3$ + 5.7 g $(Na_2)HPO_4$ + 0.57 g KCl + 0.47 g NaCl + 0.6 g $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$ per 1000 ml) were prepared and placed in a water bath at 39 ˚C. The mixture and CO_2 flushed rumen fluid was added to the buffered mineral solution in a ratio of 1:2 v/v, and maintained at 39˚C in a water bath.

***In vitro* Procedure**

In vitro gas production was conducted using the method described by (12). Approximately 200 mg of feed was weighed and placed into a 100 ml graduated glass syringe fitted with a piston. Buffered rumen fluid (30 ml) was pipetted into each syringe containing feed samples. Pistons were lubricated with Vaseline and inserted into the syringes. The syringes were immediately placed into water bath maintained at 39 °C for the incubation (12). Three syringes with only buffered rumen fluid were incubated and considered as the blank incubation to correct for gas production in the absence of a substrate. The syringes were shaken by hand, twice in the first hour and four times daily at later times. Gas production was recorded at 3, 6, 12, 24 and 48 h. The model for estimating *in vitro* gas production is based on the equation modified by (12). The equation is: $P = a + b(1 - e^{(-ct)})$.

Where:

P = cumulative gas production (mL/g DM) at time t, a = gas production from the immediately soluble fraction (mL/g DM), b = gas production from the slowly fermentable fraction (mL/g DM), c = rate constant for gas production from the slowly fermentable fraction (/h), t = incubation time (h).

Data obtained for chemical composition and *in vitro* gas production were subjected to analysis of variance (ANOVA) in a Completely Randomized Design (CRD) using Statistix (10.0) package. Significant differences between means were separated using Duncan`s Multiple Range Test at 5% level.

$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$

Where;

Y_{ij}= observation on the i^{th} treatment of the j^{th} random error,

μ = overall mean,

I_i = fixed effect of dietary treatment,

ε_{ij} = random error

Results and Discussion

Chemical Composition of the Formulated Diets

Chemical composition of the various formulations (T1, T2, T3, T4 and T5(control)) is shown in Table 2. The dry matter DM, crude protein CP, ether extract EE, ash and metabolizable energy ME contents were not significantly ($p > 0.05$) different while crude fibre CF, nitrogen free extract NFE, neutral detergent fibre NDF and acid detergent fibre ADF contents were significantly ($p < 0.05$) different among the diets. Significant differences are observed in CF content, with T2 having the highest (39.50%) and T1, T4, and control having similar lower values (around 31-32%). The ash content ranges from 3.50% (T1) to 5.75% (T2). NFE is highest in T1 (50.25%) and lowest in T2 (39.96%). ADF and NDF values were highest in T2 (45.46 and 54.88% respectively) and lowest in T1 (37.48 and 49.13% respectively). T1 recorded the highest ME value (10.57 MJ) while T2 had the least (8.74 MJ).

Table 2: Chemical Composition of Formulations for Lactating Dairy Cows

FORMS	DM	CP	EE	CF	ASH	NFE	ADF	NDF	MJ
T1	92.35	11.75	3.75	30.75[c]	3.50	50.25[a]	37.48[d]	49.13[c]	10.57
T2	93.75	12.04	2.75	39.50[a]	5.75	39.96[c]	45.46[a]	54.88[a]	8.74
T3	92.75	11.86	1.75	35.50[b]	4.00	46.89[b]	41.81[b]	52.25[ab]	9.41
T4	92.60	12.20	2.75	31.75[c]	4.25	49.05[ab]	38.39[c]	49.78[bc]	10.12
T5(control)	94.30	11.00	1.90	32.00[c]	4.00	51.10[a]	38.62[c]	48.1[c]	9.95
SEM	0.393[NS]	0.357[NS]	0.348[NS]	0.927*	0.396[NS]	1.122*	0.857*	0.736*	0.378[NS]

Key: Means with different superscripts within a column differ significantly ($p < 0.05$). SEM = Standard Error of the Mean; [a, b, c,....] means in the column bearing different superscripts are significantly different ($p<0.05$); NS = not significant.

The dry matter content varied from 92.35% (T1) to 94.30% (T5). High DM indicates the low moisture content of the feed, which is essential for preserving feed quality and ensuring consistency in nutrient intake. The CP content of the formulated diet varied from 12.20% (T4) to 11.00% (T5) and are not statistically significant. The higher CP content recorded in T4 was due to the source of proteins (cotton seed cake and *Faidherbia albida* pods) used in the rations which had a high content of crude protein. The CP content in T2 and T4 fell within the 12-18% range recommended for lactating cows (21). Also, (13) recommend a CP level of around 12-18% for high-producing dairy cows. Meanwhile, T1, T3, and the control had CP levels above the critical 7% threshold necessary in forages to improve voluntary intake and digestibility, below which feed intake in ruminants would decrease (22). According to (13) adequate protein intake is essential for milk production and maintaining cow health. The CP levels in all formulations fall within an acceptable range for lactating dairy cows.

Ether Extract (EE) recorded the highest in T1 (3.75%) and lowest in T3 (1.75%). These variations are also not statistically significant. Adequate fat levels are important for energy provision and metabolic functions (14). The differences in EE content can be attributed to the characteristics and types of feed used in the diet formulations, and may influence the energy density and palatability of the diets. Crude fibre content ranged from 39.50% (T2) to 30.75% (T1). Previous studies (15) reports CF range of 29.07 – 37.16% for formulations with fibrous feeds. According to (16) crude fibre is essential for rumen function and digestion. Higher fibre in T2 suggest a richer mineral profile and thus may benefit rumen health but could reduce energy density. The ash content, indicating mineral content, ranges from 3.50% (T1) to 5.75% (T2) and are not significantly different. Previous authors (17) recommended range of 6.68 - 9.48 % for feeding ruminants. Ash content is important for providing essential minerals required for various physiological functions (18). Nitrogen-Free Extract (NFE) content is highest in T1 (50.25%) and lowest in T2 (39.96%). Carbohydrates are crucial for energy (13). Higher NFE in T1 and the control suggests these formulations may be more energy-dense. The Acid detergent fibre and neutral detergent fibre content ranged from 45.46 and 54.88% to 37.48 and 49.13% respectively. ADF and NDF value range of 35.94 and 53.34% to 43.32 and 53.34%, respectively were reported for formulations with fibrous feeds for ruminants (15). Acid detergent fibre is an indication of cell wall components less digestible by ruminants. Lower ADF values generally indicate higher digestibility, while higher NDF limits intake due to fill effect but is essential for rumen health (16). Thus, T1 might be more digestible compared to T2. High CF and NDF levels can limit intake and reduce the energy density of the diet (16). Previous research emphasizes the importance of balancing fibre content to ensure adequate intake and nutrient absorption (19).

Formulation 1 (T1) recorded the highest ME value (10.57 MJ) while T2 had the least (8.74 MJ). The ME values in the current formulations are comparable to those reported in other studies, which typically range from 9 to 11 MJ/kg DM (20). ME is crucial for milk production and maintenance (13). T2 has the lowest energy content, possibly due to its higher fibre content, whereas T4 provides the highest energy, potentially supporting better production outcomes. Ensuring adequate energy intake is critical for supporting milk production and maintaining body condition.

The results of the *in vitro* gas production study presented in Table 3 suggest that the ratio of ingredients in total mixed rations formulated for dairy cows can significantly affect gas production during *in vitro* fermentation. Gas production at 3 h for all treatments (T1 to T5) was recorded as 0.000 ml. This indicates that there is a delay in microbial fermentation, with little to no gas production observed in the early stages of incubation. According to (23), it might be due to factors such as the nature of the feed ingredients used in the total mixed ration or slow microbial adaptation to the substrate.

Table 3: ***Invitro* gas production** of total mixed ratio formulated for dairy cows

TREATMENT	3	6	12	24
T1	0.000	1.1000	10.000	16.333^{ab}
T2	0.000	0.8333	12.000	14.333^{a}
T3	0.000	0.9667	12.667	15.667^{ab}
T4	0.000	1.0667	13.667	20.000^{a}
T5	0.000	0.9333	14.667	19.000^{ab}
SEM	0.000^{NS}	0.2039^{NS}	1.2111^{NS}	1.1547*

Means with different superscripts within a column differ significantly (p < 0.05). SEM = Standard Error of the Mean; $^{a, b, c}$,.... means in the column bearing different superscripts are significantly different (p<0.05), ns = not significant. *= Significant (P<0.05)

Gas production begins after 6 h of incubation, with values ranging from 0.8333 ml in T2 to 1.1000 ml in T1. There were no significant differences between treatments (P>0.05), indicating that the formulations had a similar effect on fermentation at this time point. At 12 h, gas production increased significantly across treatments. T5 recorded the highest gas production at 14.667 ml, which was significantly higher (P < 0.05) than that of T1 (10.000 ml). At 24 h *in vitro* gas production further increased, with the highest values recorded in T4 (20.000 ml) and T5 (19.000 ml), followed closely by T3 (15.667 ml) and T1 (16.333 ml). T2 recorded the lowest gas production at 14.333 ml. There was a significant difference between treatments at this stage ($P < 0.05$). The higher gas production in T4 and T5 could indicate that these formulations contained substrates that were more efficiently fermented over the longer incubation period. This aligns with findings from previous studies that reported higher gas production in diets with easily fermentable energy and protein sources (24).

The *in vitro* gas production data shows that the formulations in T4 and T5 could have greater fermentability, making them potentially better options for improving rumen fermentation and energy availability in dairy cows. This would be beneficial for lactating cows, as higher gas production is often associated with higher microbial activity and better feed digestibility. However, it is important to balance this with potential issues related to ruminal acidosis if the gas production is too rapid.

Conclusion

The *invitro* gas production patterns indicate that treatments with a balanced fibre content and higher levels of fermentable carbohydrates (like T4 and T5) exhibited significantly higher gas production at 24 hours, suggesting more efficient microbial fermentation. Treatments with higher crude fibre (T2) or less fermentable components (T3) resulted in lower gas production. These findings highlight the importance of optimizing fibre and energy sources to enhance ruminal fermentation in dairy cows.

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