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Article

Gliricidia sepium as a Potential Replacement for Cottonseed Cake as a Source of Protein: Effects on Serum, Rumen Metabolites and Nutrient Digestibility of Young Bunaji Bulls

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Simple Summary

The need for cheaper alternative protein sources to solve the challenges of scarcity and high cost of coneventional feed ingredients cannot be overemphasized. This study has shown that partial replacement of cottonseed cake with Gliricidia sepium foliage can address the challenge of scarcity and high cost of conventional protein concentrates. The sudy showed that GS inclusion in the diet of growing bumaji bulls improved dietary protein quality, reduced fibre fractions, maintained normal rumen functions and supported normal blood and biochemical parameters in experimental animals. Based on the findings, inclusion levels of up to 50% replacement of cottonseed cake with Gliricidia sepium can be recommended without negative effects on animal health. The use of Gliricidia sepium therefore represents a sustainable protein source that can contribute to improved ruminant productivity, particularly in tropical production systems where conventional protein supplements are expensive or scarce.

Abstract

This study evaluated the potential of *Gliricidia sepium* foliage as a protein source to replace cottonseed cake (CSC) in the diets of young Bunaji bulls, with emphasis on nutrient digestibility, rumen fermentation characteristics and serum metabolites. Animals were allocated to four dietary treatments in which *G. sepium* replaced CSC at 0, 25, 50 and 75% levels. The basal diet was Brachiaria *decumbens* hay, while concentrates were offered at 2% of body weight. Blood samples were collected at the start, mid-point and end of the 44 day feeding trial to determine packed cell volume (PCV), total protein (TP) and blood urea nitrogen (BUN). Rumen pH, ammonia nitrogen and total volatile fatty acids were measured before and after feeding. Metabolic study was conducted at the end of the feeding period. Results showed that PCV, TP and BUN were within normal physiological ranges. Rumen fermentation characteristics were comparable across treatments. Nutrient digestibility was generally unaffected, except for crude fibre, while all diets resulted in positive nitrogen balance, with higher nitrogen retention at 50% inclusion levels. In conclusion, *Gliricidia sepium* foliage can effectively replace cottonseed cake up to 50 % in diets of Bunaji bulls without compromising health status, rumen function or nutrient utilization.

Keywords: gliricidia**;** bunaji**;** rumen**;** protein**;** digestibility

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1. Introduction

Gliricidia sepium is a medium-sized leguminous tree, typically attaining 10–15 m in height and 30–40 cm in stem diameter [1]. It is commonly propagated by seed or stem cuttings and is widely adopted by smallholder farmers in cut-and-carry feeding systems. Under these systems, Gliricidia foliage may be offered fresh or conserved as hay or silage. Conservation is often recommended to enhance palatability and reduce the potential negative effects of secondary metabolite such as polyphenols, condensed tannins, alkaloids, flavonoids and coumarins which can limit voluntary intake and nutrient utilization, particularly in cattle [2,3].

Beyond its value as a forage resource, *G. sepium* plays a strategic role in sustainable livestock production systems. Its integration into silvopastoral systems has increased due to its high biomass yield, effective carbon sequestration capacity and biological nitrogen fixation, which contributes to soil fertility and reduces dependence on inorganic nitrogen fertilizers [4,5]. However, substantial variability in the nutritional value and digestibility of Gliricidia foliage has been reported. This variability is largely attributed to differences in management practices, harvesting intensity, conservation methods and concentrations of secondary compounds [2,6,7]. Additionally, nutrient losses and reductions in digestibility may occur during haymaking and ensiling, partly due to increased levels of acid detergent insoluble protein (ADIP), condensed tannins and total phenolics [8].

Gliricidia has been evaluated extensively as a protein supplement in ruminant feeding systems, including its use as dehydrated foliage [9,10], and its nutritional potential has been addressed in earlier reviews [4]. Nevertheless, information remains limited regarding its use as a direct substitute for conventional protein supplements such as cottonseed cake in beef cattle diets. This gap is particularly evident in relation to its effects on serum biochemical indices, rumen fermentation characteristics and nutrient digestibility in indigenous cattle breeds, including Bunaji cattle.

Therefore, the objective of this study was to evaluate *Gliricidia sepium* as a replacement for cottonseed cake as a protein source in the diets of young Bunaji bulls by assessing its effects on serum biochemical indices, rumen fermentation metabolites and nutrient digestibility

2. Materials and Methods

2.1. Study Site

The study was conducted at the experimental pens of the Beef Research Programme at the National Animal Production Research Institute (NAPRI), Shika (11°12′N, 7°33′E), with an elevation of 640 m above sea level. The area falls within the Northern Guinea Savannah Zone having an average annual rainfall of 1100 mm which starts from late April/early May and ends in late September/early October. The study was carried out for a period of ninety (90) days.

2.2. Forage and Concentrate Feed Collection

Brachiaria decumbens hay was sourced at NAPRI. Cotton Seed Cake was purchased from ABJ Ginnery in Katsina. Gliricidia sepium forage was harvested around Bomo village, Samaru-Zaria. The leaves and succulent portions were chopped to a length of 4 cm and air dried for 3 days.

2.3. Animals and Their Management

Prior to the Metabolism Trial, animals were adjusted to confinement for fourteen days. During this period, albendazole (1 ml/10 kg b.w.) and Triatix (1 ml to 4 litres) were administered against endo and ecto-parasites, respectively. During this adjustment period, the animals were fed the concentrate at 1% body weight while Brachiaria hay, water and mineral salt lick were provided ad libitum. After the 14 days adjustement period, they were fed at 2% of their body weight the experimemtal diet for a period of 30 days..

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2.4. Blood Sampling

Blood samples were taken from animals at the start (day zero), middle (day 15) and termination (day 30) of the feeding trial. Seven (7) ml of blood was taken from the jugular vein of each animal using disposable syringe and an 18G needle. 2ml was put into vacutainer tubes containing Ethylene diaminetetraacetic acid (EDTA) as the anticoagulant. The sample was analyzed at the Clinical Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, for Packed Cell Volume (PCV) using micro haematocrit and Total Protein (TP) using refractometer as described in the routine laboratory procedures of [11]. The balance of 5ml was introduced into EDTA-free bottles and analyzed for Blood Urea Nitrogen (BUN) according to the method described by [12] at the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital, Zaria.

2.5. Feed Intake and Rumen Fluid Evaluation

Feed intake was determined by the difference between quantity offered and the orts. Rumen fluid was collected from the animals at zero hour before feeding, at 4 and 8 hours after the introduction of feed using a stomach tube. This tube, approximately 90 cm long with a metal filter on one end, was guided through a mouthpiece into the bull's rumen, while a suction pump connected to the other end extracted the rumen fluid. The pH of the fluid was immediately measured within one minute of collection using a Philips digital pH meter (model 9409). Following this, the rumen fluid was strained through a muslin cloth and a 20 mL sample of the filtrate was mixed with an equal volume of 1 N H_2SO_4 saturated with $MgSO_4$ to acidify, deproteinize and inhibit bacterial activity. The mixture was then centrifuged at 3000 rpm and left undisturbed for 10 minutes. A 20 mL portion of the supernatant was transferred into plastic bottles and frozen at −20 °C until analyzed for rumen ammonia nitrogen (NH_3-N). Ammonia concentration in the rumen was measured using steam distillation into boric acid, followed by titration with 0.01 N hydrochloric acid, as outlined by [13].

2.6. Metabolism Study

The animals were housed in individual metabolism crates and fed rations at 0, 25, 50 and 75% GS replacing CSC. An adjustment period of fourteen days was allowed before a 7-day collection period of total urine and faeces [14]. Each animal was fed 1% body weight of concentrate and 1% body weight of hay. The daily feacal outputs were dried for initial determination of dry matter (DM). Water and mineral lick were provided *ad libitum*.

Representative fed samples (hay and concentrate) and total daily fecal output from each bull was collected once every 24 hours. The entire fecal mass was weighed, and a 10% representative sub-sample was taken immediately after weighing. Each sub-sample was oven-dried at 65 °C for 48 hours, ground to pass a 1-mm sieve, and stored in airtight, properly labeled containers for subsequent nutrient analysis.

Urine was collected over a 24-hour period for seven days using collection buckets fitted with funnels and containing 50 mL of 10% sulfuric acid to prevent nitrogen volatilization. The total daily urine volume was measured, and a 10% aliquot was taken, transferred into labeled screw-cap bottles, and stored at –20 °C until nitrogen determination using the Kjeldahl method.

Nitrogen balance components were calculated as follows:

$$\text{Nitrogen Intake (NI)} = \text{CP intake} / 6.25 \quad (1)$$

$$\text{Nitrogen Retention (NR)} = \text{NI} - (\text{Faecal N} + \text{Urinary N}) \quad (2)$$

$$\text{Nitrogen Retention (\%)} = (\text{NR} / \text{NI}) \times 100 \quad (3)$$

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2.7. Chemical Analyses

Feed ingredients, mixed diets, hay and faeces were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF) and acid detergent fiber (ADF) using [15] standard methods. Urinary nitrogen was determined by the Kjeldahl method [16]. Neutral Detergent Fibre and ADF were determined according to the procedure of [17].

2.8. Statistical Analysis

All experimental data including feed intake, growth performance, nutrient digestibility, serum metabolites, carcass characteristics and economic variables were analyzed using one-way analysis of variance (ANOVA) in SAS software (Version 9.4; SAS Institute Inc., Cary, NC, USA). The statistical model applied for each response variable was:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (4)$$

where Y_{ij} represents the observation from the jth bull in the ith dietary treatment group, μ is the overall mean, T_i is the fixed effect of the *GS* substitution level (0, 25, 50 and 75%), and e_{ij} is the random residual error term. Individual bulls served as the experimental units and were treated as random subjects within treatments.

Assumptions of ANOVA including normality of residuals and homogeneity of variance were evaluated using the Shapiro Wilk and Levene's tests, respectively. Data violating these assumptions were log- or square-root–transformed prior to reanalysis. Treatment means were separated using Duncan's Multiple Range Test, and statistical significance was declared at $P < 0.05$.

3. Results

3.1. Chemical Composition and Anti-Nutritional Factors

Table 1 shows the result of chemical analysis for GS and CSC. The values obtained for CP (26.38%) and Ash (7.62%) for GS was higher than that of CSC (21.75% and 4.32% respectively). Gliricidia sepium had lower values for CF (8.52%) and NDF (27.40%) as compared to CSC with a CF and NDF contents of 17.62% and 49.08% respectively. These values from proximate analysis result indicate a higher nutrient profile in Gliricidia as compared to CSC.

Table 1. Chemical composition (%) of CSC and *Gliricidia sepium* leaves.

	DM	ASH	EE	CF	NDF	ADF	CP
GS	93.56	7.62	8.52	8.52	43.19	26.38	26.38
CSC	97.91	4.37	16.15	17.62	34.21	21.75	21.75

GS = *Gliricidia sepium*, CSC = Cotton seed cake, DM = Dry matter, EE = Ether extract, CF = Crude fibre, NDF = Neutral detergent fibre, ADF = Acid detergent fibre.

Result of anti-nutritional factors in GS is presented in Table 2. The levels of tannins, oxylate, phytates, saponin and trypsin inhibitors showed a higher concentration of these factors in the dried samples than in the fresh leaves may be due to moisture loss and concentration in dry matter.

Table 2. Levels of anti-nutritive factors in *Gliricidia sepium* (mg/100g).

Anti-nutritive factor	Fresh sample	Dry sample
Tannin	0.28	0.42
Oxalates	1.04	1.46
Phytates	0.46	0.48
Saponin	1.08	1.64
Trypsin inhibitor	0.24	0.40

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3.2. Nutrient Composition of Experimental Diets

Proximate analysis of individual feed ingredients was carried out before use (Table 3). The composition of experimental diets is shown in Table 4 while the nutrient composition of experimental diets is presented in Table 5. Dry matter content of diets containing 50% and 75% GS replacement levels were the same while 0% and 25% GS replacement levels had DM values of 95.74% and 94.45%, respectively (Table 4). Although diets were formulated to be isonitrogenous (12%), proximate analysis revealed that the diets had CP of 13%. The CF and EE values ranged between 17.58 and 19.59. The mean ash content was highest in 75% while the lowest value was in 0%. The NDF values of 0, 25 and 50% were higher than that of 75%. The 0% had the highest ADF value while 75% had the lowest.

Table 3. Proximate analysis of feed ingredients and *Brachiaria decumbens* hay.

Nutrients	Maize offal	Rice offal	Poultry litter	CSC	Brachiaria hay
DM	97.01	97.20	97.39	97.91	98.86
ASH	5.16	16.29	23.96	4.37	6.41
EE	12.21	11.74	15.39	16.15	11.20
CF	18.21	17.96	11.48	17.62	46.68
NDF	32.68	51.68	53.73	49.08	71.65
ADF	17.00	32.06	29.27	34.21	48.94
CP	9.88	5.33	20.88	21.75	2.88

DM = Dry matter, EE = Ether extract, CF = Crude fibre, NDF = Neutral detergent fibre, ADF = Acid detergent fibre,. CP = Crude protein, CSC = Cotton seed cake.

Table 4. Ingredient composition of experimental diets.

	Level of *Gliricidia sepium* inclusion (%)			
Feed stuff	0	25	50	75
Cotton seed cake	10	7.5	5.0	2.5
G. sepium	0	2.5	5.0	7.5
Maize offal	40	40	40	40
Poultry litter	17	19	22	25
Rice offal	30	28	25	22
Bone meal	2	2	2	2
Salt	1	1	1	1
Total (kg)	100	100	100	100
Calculated analysis:				
Crude protein	12.36	12.22	12.21	12.20

Table 5. Nutrient composition of experimental diets.

	Level of *Gliricidia sepium* inclusion (%)			
Nutrients (%)	0	25	50	75
DM	95.74	94.45	98.84	98.84
ASH	11.91	12.51	11.97	13.01
EE	18.33	1959	17.97	18.66
CF	19.69	20.08	19.88	17.58
NDF	40.15	42.41	40.79	35.02
ADF	23.75	22.83	20.77	21.53
CP	13.18	13.26	13.31	13.30

DM = Dry matter, EE = Ether extract, CF = Crude fibre, NDF = Neutral detergent fibre, ADF = Acid detergent fibre, CP = Crude protein.

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3.3. Effect of Experimental Diets on Blood Parameters

Packed cell volume did not differ significantly among dietary treatments at the start of the experiment (P > 0.05; Table 6). At the mid-experimental sampling, animals receiving 25 and 75% Gliricidia sepium exhibited comparable PCV values, whereas those fed the 50% inclusion level recorded the highest PCV (40.40%), which was significantly higher (P < 0.05) than the value observed in animals fed the 0% inclusion diet, which had the lowest PCV (28.80%). At the end of the experiment, a similar statistical pattern to that observed at mid-trial was maintained. However, PCV values in animals fed 25, 50 and 75% GS increased further, while a continued decline was observed in animals on the 0% inclusion (control) diet.

Total protein concentrations were not significantly affected by dietary treatment at the start of the experiment (P > 0.05; Table 6). By the mid-experimental period, differences among treatments became evident, with animals fed the 0% (7.24 g/dL) and 75% (6.52 g/dL) inclusion levels showing significantly different TP values (P < 0.05), whereas those receiving 25 and 50% GS had comparable concentrations. At the termination of the experiment, only animals maintained on the 0% inclusion diet exhibited TP values that were significantly different (P < 0.05) from those of animals fed diets containing GS, while no differences were observed among the 25, 50 and 75% inclusion levels.

At the start of the experiment, blood urea nitrogen concentrations in animals fed the 25 and 75% GS diets did not differ significantly (P > 0.05). Animals receiving the 50% inclusion level recorded the highest BUN value (3.04 mg/dL), which was significantly higher (P < 0.05) than that observed in animals fed the 0% inclusion diet (1.52 mg/dL). By the mid-experimental period, no significant differences in BUN were detected among treatments (P > 0.05), although animals on the 0% inclusion diet exhibited the highest numerical value (3.39 mg/dL), while those receiving 25% GS recorded the lowest value (2.23 mg/dL). At the end of the experiment, BUN concentrations declined markedly across all treatments. No significant difference (P > 0.05) was observed between animals fed the 0 and 75% inclusion diets; however, animals receiving 25 and 50% GS differed significantly (P < 0.05), with the 50% inclusion level maintaining the highest blood urea concentration.

Table 6. Effect of dietary substitution level on haematological parameters of experimental animals.

		Level of *G. sepium* inclusion					
Parameter	**Time**	**0%**	**25%**	**50%**	**75%**	**SEM**	**P-value**
P C V (%)	Initial	38.80	39.40	44.00	40.00	2.98	NS
	Middle	28.80c	34.40b	40.40a	34.60b	1.82	*
	Final	27.60c	36.20b	44.20a	38.80b	1.03	*
T P (g/dL)	Initial	70.66	70.28	70.60	70.08	0.31	NS
	Middle	70.24a	70.00ab	70.04ab	60.52b	0.21	*
	Final	60.22b	60.82a	70.08a	60.94a	0.12	*
BUN (mmol/L)	Initial	1.52b	2.14ab	3.04a	2.06ab	0.32	*
	Middle	3.39	2.23	3.07	2.57	0.44	NS
	Final	0.87ab	0.74b	1.02a	0.93ab	0.07	*

a,b,c = means within the same row with different superscripts differ significantly (P < 0.05) SEM = standard error of means; NS = not significant (P > 0.05); * = P < 0.05.

3.4. Effect of Experimental Diets on Rumen pH and Metabolites

Only crude fibre digestion showed significant (P < 0.05) difference. The 0% inclusion level diet gave the best digestibility while 50% had the least digestibility. The rumen pH of experimental animals at zero hours before feeding (Table 7) ranged between 7.06 and 7.36. The pH of animals on dietary treatment 0% (7.36) was higher and significantly (P < 0.05) different from animals on 75% (7.06). Animals on 25 and 50% had similar pH values. At 4 and 8 hours after feeding no statistical difference was observed across the treatments. Results obtained for rumen ammonia showed no

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significance (P > 0.05) difference across the treatments at zero hours before feeding and at 4 and 8 hours after feeding.

For total volatile fatty acids (Table 7), a range of 7.50–15.75 was observed at zero hours before feeding across the treatments. The values of TVFAs for animals on the control diet and 25% were both higher and significantly (P < 0.05) different from animals on diets containing 50 and 75%. No significant (P > 0.05) differences were observed at 4 and 8 hours after feeding across the treatments.

Table 7. Intake and ruminal parameters of young Bunaji bulls fed varied levels of GS inclusion.

		Level of G. sepium inclusion					
Parameter	**Time**	**0%**	**25%**	**50%**	**75%**	**SEM**	**P-value**
Rumen pH	0 h	7.36a	7.23ab	7.13ab	7.06b	0.08	*
	4 h	7.65	7.25	7.28	6.92	0.33	NS
	8 h	6.76	7.26	7.16	7.23	0.30	NS
RAN (g/100 g)	0 h	5.44	4.59	3.91	3.36	0.69	NS
	4 h	5.49	9.31	7.58	8.59	2.42	NS
	8 h	2.76	3.06	3.60	2.91	0.69	NS
TVFA (µmol)	0 h	14.50a	15.75a	10.25b	7.50c	1.19	*
	4 h	9.88b	15.50a	15.50a	10.63b	2.67	*
	8 h	6.00bc	8.75b	12.00a	8.50b	2.66	*

a, b, c = means within the same row with different superscripts differ significantly (P < 0.05), DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre, SEM = standard error of means, NS = not significant (P > 0.05); * = P < 0.05.

3.5. Nutrient Digestibility and Nitrogen Balance

Table 8 shows the result of the metabolism study. Only crude fibre digestion showed significant (P<0.05) difference. The 0 % inclusion level diet gave the best digestibility while 50% had the least digestibility. Nitrogen retention was highest and similar with 50 and 0% diets but lower and similar in 25 and 75% diets. All treatments had positive nitrogen balance.

Table 8. Nutrient digestibility and nitrogen balance of cattle fed varied levels of *GS.*

	Level of *GS* inclusion (%)					
	0	25	50	75	SEM	LOS
Nutrient						
DM	98.43	98.27	98.31	98.23	0.20	NS
CP	7.98	8.13	8.40	8.68	0.28	NS
ASH	21.40	22.76	21.58	24.36	1.11	NS
EE	9.79	9.20	11.72	11.31	1.18	NS
CF	37.83^{a}	34.80^{ab}	32.31^{b}	35.22^{ab}	1.60	*
NDF	66.48	64.20	67.39	66.88	2.20	NS
ADF	51.05	52.26	50.14	51.12	1.59	NS
Nitrogen balance						
Nitrogen intake (g/day)	208	195	213	208	0.14	NS
Faecal nitrogen (g/day)	128	130	134	139	0.05	NS
Urinary nitrogen (g/day)	30	29	26	35	0.07	NS
Nitrogen retained (g/day)	50^{b}	36^{c}	53^{a}	34^{d}	0.12	*

a, b, c, d: Means within the same row with different superscripts differ significantly (P<0.05), DM = Dry matter, CP = Crude protein, EE = Ether extract, CF = Crude fibre, NDF = Neutral detergent fibre, * = P<0.05 ADF = Acid detergent fibre, SEM = Standard error of means, LOS = Level of significance.

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4. Discussion

4.1. Chemical Composition and Anti-Nutritional Factors

The chemical composition of *GS* observed in the present study confirms its classification as a high-quality tropical legume forage suitable for ruminant nutrition. The crude protein (CP) concentration recorded aligns with earlier reports describing GS as a valuable protein supplement for cattle consuming low-quality basal diets [18,19]. The comparatively higher ash content further indicates a superior mineral contribution relative to cottonseed cake (CSC), supporting previous findings that GS can enhance dietary mineral supply in ruminant production systems [20].

The relatively low neutral detergent fibre (NDF) concentration of GS suggests improved rumen degradability and potential for increased voluntary intake, as reduced cell wall fractions are commonly associated with faster rumen passage rates and enhanced feed intake. Conversely, the moderate acid detergent fibre (ADF) concentration may impose some limitation on fibre digestibility, particularly of cellulose-bound fractions, as previously reported by [21] and [22] Nonetheless, the overall fibre profile of GS reflects a favourable balance between intake stimulation and maintenance of rumen function.

The anti-nutritional factors detected in GS leaves were present at concentrations comparable to those previously reported in tropical legumes [21,23]. Although GS has been associated with growth depression and toxicity in non-ruminant species such as poultry and rabbits [24], ruminants possess effective ruminal microbial systems capable of detoxifying many secondary plant metabolites. Notably, the tannin levels observed in this study were considerably lower than concentrations reported to impair ruminal fermentation. This finding agrees with report of [25]. It is also in agreement with the findings reported by [26] who evaluated the effect of increasing doses of CT extract from C. ladanifer on rumen fermentation and BH when an oil-supplemented high-concentrate substrate was used and discovered that increasing doses of C. ladanifer CT extract (0, 25, 50, 75 and 100 mg/g kg DM) using oil-supplemented high-concentrate subtract led to a moderate decrease in VFA production and to a very pronounced depression of old and branched- chain fatty acids (OBCFA) and dimethyl acetals (DMA) production, without affecting the biohydrogenation (BH) or the BH products yield. Our findings therefore, suggest that the levels observed are unlikely to compromise nutrient utilization.

Under practical feeding conditions, GS has consistently been reported as non-toxic to ruminants [21]. Although palatability issues related to volatile compounds on the leaf surface have been documented [27], the absence of depressed intake in the present study indicates adequate animal adaptation to *GS*-based diets, even at higher inclusion levels.

4.2. Nutrient Composition of Experimental Diets

The progressive increase in dietary dry matter content with increasing *GS* inclusion indicates enhanced nutrient density of the experimental diets. Although the diets were formulated to be isonitrogenous, the slightly higher analysed CP concentrations across treatments ensured that protein supply exceeded the minimum requirement for growing cattle, estimated at approximately 12% CP [28,29]. Consequently, dietary protein was unlikely to be a limiting factor for growth performance.

The higher ash content observed in *Gliricidia Sepium* -based diets reflects the superior mineral profile of the legume compared with CSC. This characteristic may reduce the need for additional mineral supplementation, particularly during periods of forage scarcity such as the dry season [30]. Ether extract concentrations remained relatively consistent across treatments, indicating comparable dietary energy contributions among diets.

A reduction in both NDF and ADF contents was observed with increasing GS inclusion, reflecting partial replacement of CSC with a less fibrous protein source. This reduction in structural carbohydrate concentration is expected to improve rumen fermentation efficiency and nutrient availability, thereby contributing to improved animal performance. This finding is in conformity with that of [31] who used tropical legume tree and coffee pulp to reduce enteric methane emission by

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cattle fed a low-quality forage diet and concluded that supplementation with G. sepium, alone or in combination with COP, could be used as part of a strategy to reduce enteric CH_4 production in tropical cattle production systems in tropical regions of southern Mexico, where both additives are available.

4.3. Effect of Experimental Diets on Blood Parameters

Packed cell volume values remained within the physiological reference range for healthy cattle throughout the experimental period [32], indicating that *GS* inclusion did not adversely affect animal health. The observed mid-experimental decline followed by recovery likely reflects dietary adaptation rather than pathological stress.

Total serum protein concentrations were within normal limits reported for cattle [33], suggesting adequate dietary protein supply and effective microbial protein synthesis. Blood urea nitrogen concentrations were generally lower than standard reference values reported by [34], particularly toward the end of the trial. This pattern is likely associated with the once-daily feeding regime, as blood urea concentrations decline with time after feeding due to reduced ruminal ammonia absorption [35].

4.4. Effect of Experimental Diets on Rumen Metabolites

Pre-feeding rumen pH values were neutral to slightly alkaline across treatments, likely reflecting anticipatory salivation associated with conditioned feeding behaviour. Following feeding, animals receiving *GS*-based diets exhibited a more rapid return to optimal rumen pH, suggesting improved buffering capacity and greater ruminal stability.

The minimum rumen ammonia nitrogen concentration required to sustain optimal microbial growth has been achieved. While this threshold was attained primarily by animals fed CSC-based diets prior to feeding, all treatments achieved adequate concentrations four hours post-feeding. The decline in rumen ammonia concentration observed at eight hours post-feeding across treatments is likely attributable to the single daily feeding strategy. Notably, animals receiving the 50% GS diet maintained relatively higher ammonia concentrations for longer durations, indicating improved synchronization of nitrogen and fermentable energy supply. The findings obtained in our study are in line with the work of [36].

Total volatile fatty acid concentrations increased post-feeding in animals receiving moderate to high levels of GS, reflecting enhanced fermentative activity. Given that volatile fatty acids constitute the primary source of metabolizable energy for ruminants, this response suggests superior fermentability of GS relative to CSC. The finding in our study is contrary to that of [37] who stated that lack of significant differences in ruminal fluid SCFAs among the groups may be related to the improved OM fermentability of the experimental diets owing to the positive correlation between the total SCFA concentration and OM fermentability when they studied Nutrient Utilization, ruminal fermentation, and Health responses of maintenance goats to cowpea (*Vigna unguiculata var. sesquipedalis*) silage as a sustainable alternative to commercial feed.

4.5. Nutrient Digestibility and Nitrogen Balance

The inclusion of GS did not significantly affect the digestibility of most nutrients, with the exception of crude fibre. The reduction in crude fibre digestibility at higher GS inclusion levels may reflect variations in fibre composition and ruminal degradation characteristics. Nevertheless, nitrogen retention remained positive across all treatments, indicating adequate protein utilization. Improved nitrogen retention observed in animals receiving moderate GS inclusion suggests enhanced efficiency of nitrogen capture and reduced urinary nitrogen losses. In contrast, lower nitrogen retention in CSC-based diets may be associated with reduced ruminal synchrony and higher fibre-induced limitations on nutrient utilization. The results of our study are in accordance with that of [22] who concluded that the incorporation of 15% of *E. cyclocarpum* mixed with *G. sepium* into the

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diet of crossbred heifers for 80 d improved production by reducing methane emissions (g/kg DMI, g/kg DDMI, g/kg DCP, energy loss as CH_4, kg/kg ADG/year) when they tested the effects of long-term diet supplementation with *Gliricidia sepium* foliage mixed with *Enterolobium cyclocarpum* pods on enteric methane, apparent digestibility, and rumen microbial population in crossbred heifers.

5. Conclusions

The results of this study demonstrate that *Gliricidia sepium* can be safely and effectively used as a partial replacement for cottonseed cake in ruminant diets. Inclusion of *Gliricidia sepium* improved dietary protein quality, reduced fibre fractions, maintained stable rumen fermentation characteristics and supported normal haematological and biochemical parameters in experimental animals. Despite the presence of anti-nutritional factors, their concentrations remained within acceptable limits and did not exert adverse physiological effects.

Based on the findings, inclusion levels of up to 50% replacement of cottonseed cake with *Gliricidia sepium* can be recommended without negative effects on rumen function or animal health. The use of *Gliricidia sepium* therefore represents a sustainable protein source that can contribute to improved ruminant productivity, particularly in tropical production systems where conventional protein supplements are expensive or scarce.

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