

Gliricidia sepium hay a potential replacement for cotton seed cake concentrate as a source of protein: Effects on serum, rumen metabolites and nutrient digestibility of young bunaji bulls

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Abstract

This study evaluated the potential of *Gliricidia sepium* (GS) hay to replace cottonseed cake (CSC) as a dietary protein source for growing Bunaji bulls by assessing blood metabolites, rumen fermentation characteristics, nutrient digestibility and nitrogen balance. Twenty growing Bunaji bulls (110.5 ± 2.0 kg) were randomly assigned to four dietary treatments in which GS replaced CSC at 0, 25, 50 and 75% of the protein source. The experiment lasted 56 days, comprising a 14-day adaptation period and a 42-day feeding trial, followed by a digestibility and nitrogen balance study. Blood samples were collected on days 0, 28 and 56, while rumen fluid was sampled before feeding and at 4 and 8 h post-feeding. *Gliricidia sepium* contained higher crude protein (26.38 vs. 21.75%) and ash but lower fibre fractions than CSC. Dietary inclusion of GS significantly ($P < 0.05$) improved packed cell volume, glucose and albumin concentrations, with the most favourable responses observed at the 50% replacement level. Total protein was maintained within normal physiological ranges, whereas blood urea nitrogen indicated efficient nitrogen utilisation during the feeding period. Rumen pH and ammonia nitrogen remained within normal physiological limits across treatments, while total volatile fatty acid concentrations were significantly influenced ($P < 0.05$) by GS inclusion. Most nutrient digestibility coefficients were unaffected by dietary treatment, although crude fibre digestibility declined slightly at higher GS inclusion levels. Nitrogen retention was greatest ($P < 0.05$) at the 50% replacement level, and all treatments maintained positive nitrogen balance. These findings demonstrate that replacing up to 50% of CSC with GS hay improves metabolic status, supports a favourable rumen environment, enhances nitrogen utilisation, and maintains nutrient digestibility without compromising animal health, making GS a sustainable alternative protein source for growing Bunaji bulls.

1. Introduction

Protein is an essential component of diets for animals, supporting tissue development, microbial protein synthesis, and overall animal productivity. However, the high cost and variable availability of conventional protein supplements can limit their use, particularly in tropical livestock production systems (Cherdthong, 2025). This has stimulated interest in alternative, locally available protein resources that can reduce dependence on conventional feed ingredients while maintaining adequate nutrient supply and animal performance. Among the potential alternatives, *Gliricidia sepium* has attracted considerable attention because of its adaptability, high biomass production, and potential nutritional value as a protein-rich forage.

Gliricidia sepium is a medium-sized leguminous tree that typically attains 10–15 m in height and 30–40 cm in stem diameter (Alamu et al., 2023). It is commonly propagated by seeds or stem cuttings and is widely utilised by smallholder farmers in cut-and-carry feeding systems. The foliage can be offered to livestock in fresh form or conserved as hay or silage, providing a potentially accessible source of nutrients for ruminants. However, the nutritive value and utilisation of *G. sepium* foliage may be influenced by the presence of secondary metabolites, including polyphenols, condensed tannins, alkaloids, flavonoids, and coumarins, which may affect voluntary feed intake and nutrient utilisation,

particularly when present at high concentrations. Processing of the foliage has therefore been suggested as a means of improving its utilisation and potentially reducing some of the adverse effects associated with these compounds (Rusdy et al., 2019; Suong et al., 2022).

Beyond its direct value as a livestock feed resource, *G. sepium* has important attributes that support its integration into sustainable livestock production systems. Its use in silvopastoral systems has increased because of its high biomass-producing capacity, potential for carbon sequestration, and ability to fix atmospheric nitrogen biologically, thereby contributing to soil fertility and potentially reducing dependence on inorganic nitrogen fertilisers (Rangel et al., 2019; Silva et al., 2022). These characteristics make *G. sepium* particularly attractive as a multipurpose forage resource in tropical production systems, where the availability of affordable and sustainable sources of dietary protein is an important consideration.

Nevertheless, the nutritional value and digestibility of *G. sepium* foliage can vary substantially. Such variation may be associated with management practices, harvesting intensity, stage of plant development, conservation method, and the concentration of secondary compounds in the foliage (Carvalho et al., 2017; Rusdy et al., 2019; Sobral et al., 2022). Furthermore, the process of conserving the foliage as hay or silage may result in nutrient losses and reductions in digestibility, partly through changes associated with acid detergent insoluble protein (ADIP), condensed tannins, and total phenolic compounds (Castro-Montoya & Dickhoefer, 2020). These factors suggest that the suitability of *G. sepium* as a protein source should be evaluated not only on the basis of its chemical composition but also in terms of its effects on nutrient utilisation and animal physiological responses.

The potential of *G. sepium* as a protein supplement for ruminants has been investigated in different feeding systems, including its utilisation as dehydrated foliage (Asaolu et al., 2012; Winarti et al., 2022), while its broader nutritional and agronomic potential has also been discussed in previous reviews (Rangel et al., 2019). However, despite these studies, information remains limited regarding the use of *G. sepium* as a direct replacement for conventional protein supplements such as cottonseed cake in diets for growing beef cattle. In particular, there is insufficient information on how graded replacement of cottonseed cake with *G. sepium* influences nutrient digestibility, rumen fermentation metabolites, and serum biochemical indices in Nigerian indigenous cattle breeds. This information is important because the replacement of a conventional protein source with a forage-based alternative may alter the availability and utilisation of dietary nutrients, ruminal fermentation processes, and systemic metabolic responses.

Bunaji cattle are an important indigenous cattle breed in the tropical production environment and constitute a valuable genetic resource for beef and livestock production. However, their productivity in these systems may be constrained by limitations in the availability and affordability of high-quality protein supplements (Madziga et al., 2026). The identification of locally available protein resources capable of partially or substantially replacing conventional ingredients such as cottonseed cake could therefore contribute to the development of more sustainable feeding strategies for growing Bunaji bulls.

Assessing the effects of such replacement on nutrient digestibility, rumen metabolites, and serum biochemical indices provides a comprehensive basis for determining the nutritional suitability of *G. sepium* as an alternative protein source.

Therefore, this study evaluated the potential of *Gliricidia sepium* as a replacement for cottonseed cake in the diets of young Bunaji bulls. Specifically, the study assessed the effects of graded replacement of cottonseed cake with *G. sepium* on serum biochemical indices, rumen fermentation metabolites, and nutrient digestibility. It was hypothesised that *G. sepium* could replace cottonseed cake at appropriate levels without adversely affecting nutrient utilisation, ruminal fermentation, or the physiological status of young Bunaji bulls.

2. Materials and Methods

2.1. Study site

The study was conducted at the experimental pens of the Beef Research Programme, National Animal Production Research Institute (NAPRI), Shika (11°12'N, 7°33'E), located at an elevation of 640 m above sea level. The study area falls within the Northern Guinea Savannah ecological zone, with an average annual rainfall of approximately 1,100 mm, occurring mainly from late April/early May to late September/early October. The experimental protocol was reviewed and approved by the Ahmadu Bello University Animal Care and Use Committee (Approval No. ABUCAUC/2022/016). The experiment lasted 56 days, comprising a 14-day adaptation period followed by a 42-day experimental feeding period.

2.2. Forage and concentrate feed collection

Brachiaria decumbens hay was sourced from NAPRI. Cottonseed cake (CSC) was purchased from ABJ Ginnery, Katsina, Nigeria. *Gliricidia sepium* (GS) forage was harvested around Bomo village, Samaru-Zaria, Nigeria. The leaves and succulent portions of the GS forage were chopped to approximately 4 cm in length and air-dried for three days before incorporation into the experimental diets after the antinutritional factors and chemical composition (Table 1) were determined.

Table 1

Chemical composition and anti-nutritional factors of *Gliricidia sepium* leaves and cottonseed cake

Parameter	GS	CSC	ANF	Fresh GS	Dry GS
Dry matter (DM, %)	93.56	97.91	Tannin (mg/100 g)	0.28	0.42
Ash (%)	7.62	4.37	Oxalates (mg/100 g)	1.04	1.46
Ether extract (EE, %)	5.12	8.15	Phytates (mg/100 g)	0.46	0.48
Crude fibre (CF, %)	8.52	17.62	Saponin (mg/100 g)	1.08	1.64
Neutral detergent fibre (NDF, %)	43.19	34.21	Trypsin inhibitor (mg/100 g)	0.24	0.40
Acid detergent fibre (ADF, %)	26.38	21.75			
Crude protein (CP, %)	26.38	25.75			
GS = <i>Gliricidia sepium</i> ; CSC = cottonseed cake; DM = dry matter; EE = ether extract; CF = crude fibre; NDF = neutral detergent fibre; ADF = acid detergent fibre; ANF=Antinutritional factor					

2.3. Animals and their management

Twenty growing Bunaji bulls, approximately 15 months old and weighing between 109 and 113 kg (mean live weight: 110.5 kg), were used for the study. The animals originated from the same research herd, with only one male calf selected from each dam to reduce the likelihood of close genetic relationships among the experimental animals. All bulls had been raised under similar farm conditions and subjected to comparable feeding, health, and husbandry practices before the study.

During the preliminary management period, the animals received albendazole at 1 mL/10 kg body weight for the control of internal parasites, while ectoparasite infestation was managed with Triatix® administered at 1 mL/4 L of water. The bulls were provided with the experimental concentrate at a level equivalent to 1% of their body weight, whereas *Brachiaria hay*, drinking water, and mineral salt lick were made available *ad libitum* (Table 2).

Table 2
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,					

The animals were subsequently distributed randomly among four dietary treatments, comprising five bulls per treatment, according to a completely randomised design, with each bull serving as an experimental unit. The animals were maintained individually in open concrete stalls throughout the feeding period. Four experimental concentrates (Table 3) were formulated by replacing cottonseed cake (CSC) with *Gliricidia sepium* (GS) foliage at 0, 25, 50, and 75% replacement levels. The remaining concentrate components comprised maize offal, poultry litter obtained from a layer deep-litter poultry house, rice offal, bone meal, and salt. During the feeding phase, concentrate was supplied daily at 2% of individual body weight, while *Brachiaria* hay, fresh drinking water, and mineral salt lick were offered *ad libitum*. The experimental feeding period lasted 42 days, giving a total experimental duration of 56 days, including the 14-day adaptation period. The bulls were housed in individual open concrete stalls under uniform management conditions.

Table 3
Ingredient and nutrient composition of experimental diets (%)

Feed stuff	Level of <i>Gliricidia sepium</i> inclusion (%)			
	0	25	50	75
<i>Ingredient composition</i>				
Cotton seed cake	10	7.5	5.0	2.5
<i>G. sepium</i>	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
<i>Nutrients Composition</i>				
DM	95.74	94.45	98.84	98.84
ASH	11.91	12.51	11.97	13.01
EE	18.33	19.59	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				

2.4. Blood collection and analysis

Blood samples were collected from each animal at the beginning (Day 0), midpoint (Day 28), and end (Day 56) of the experimental feeding period. Approximately 7 mL of blood was collected from the jugular vein of each animal using a sterile disposable syringe fitted with an 18-gauge needle. Of the blood collected, 2 mL was transferred into ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes for haematological analyses. Packed cell volume (PCV) was determined using the microhaematocrit

method, while total protein (TP) was measured using a refractometer, following standard laboratory procedures described by Jain (1986). These analyses were conducted at the Clinical Laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The remaining 5 mL of blood was transferred into plain, EDTA-free tubes and allowed to clot at room temperature. The samples were subsequently centrifuged at 2,500 rpm for 5 min to separate the serum.

The resulting serum was carefully harvested, stored at -20°C until analysis, and subsequently analysed for blood urea nitrogen (BUN), glucose, and albumin at the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The biochemical parameters were determined using commercial diagnostic kits (Randox Laboratories Ltd., UK) according to the manufacturer's instructions. BUN was determined following the method described by Ogunsanmi et al. (2002).

2.5. Feed intake and rumen fluid evaluation

Daily feed intake was determined as the difference between the quantity of feed offered and the correspondingorts. Rumen fluid was collected during the final experimental sampling period at 0 h (immediately before feeding), and at 4 and 8 h after feeding, using a stomach tube. The tube, approximately 90 cm in length and fitted with a metal filter at one end, was guided through a mouthpiece into the rumen, while a suction pump connected to the other end was used to withdraw rumen fluid.

Rumen pH was measured immediately, within one minute of sample collection, using a Philips digital pH meter (model 9409). The rumen fluid was subsequently strained through muslin cloth. A 20 mL aliquot of the filtrate was mixed with an equal volume of 1 N H_2SO_4 saturated with MgSO_4 to acidify the sample, precipitate proteins, and inhibit microbial activity. The mixture was centrifuged at 3,000 rpm and allowed to stand undisturbed for 10 min. Thereafter, 20 mL of the supernatant was transferred into labelled plastic bottles and stored at -20°C until analysis for rumen ammonia nitrogen ($\text{NH}_3\text{-N}$).

Rumen ammonia nitrogen concentration was determined by steam distillation into boric acid, followed by titration with 0.01 N hydrochloric acid, according to the procedure described by Whitehead et al. (1976). Volatile fatty acids (VFA) were determined from appropriately preserved rumen fluid samples using the laboratory procedure adopted for the study.

2.6. Digestibility study

A digestibility and nitrogen balance study were conducted during the final 10 days of the experimental feeding period. This period comprised a 3-day adaptation period followed by a 7-day total collection period. During the digestibility trial, each bull remained on its assigned experimental diet corresponding to 0, 25, 50, or 75% replacement of cottonseed cake with *Gliricidia sepium*.

All the animals were housed individually in metabolism crates and fed their respective experimental rations. During the digestibility trial, each animal received concentrate and *Brachiaria* hay at 1% of body weight each, while clean drinking water and mineral salt lick were provided ad libitum.

Representative samples of the feed offered, including hay and concentrate, were collected daily. Total faecal output from each bull was collected quantitatively every 24 h during the 7-day collection period. The entire faecal output was weighed, thoroughly mixed, and a 10% representative subsample was collected immediately after weighing. The subsamples were oven-dried at 65°C for 48 h, ground to pass through a 1-mm sieve, and stored in airtight, properly labelled containers until chemical analysis.

Urine was collected quantitatively over 24 h for seven consecutive days using collection buckets fitted with funnels and containing 50 mL of 10% sulfuric acid to minimise nitrogen volatilisation. The total daily urine volume was recorded, and a 10% aliquot was collected, transferred into labelled screw-cap bottles, and stored at – 20°C until nitrogen determination by the Kjeldahl method.

Nitrogen balance components were calculated as follows:

Nitrogen intake (NI) = CP intake / 6.25

Nitrogen retention (NR) = NI – (Faecal N + Urinary N)

Nitrogen retention (%) = (NR / NI) × 100

2.7. Chemical analysis

Samples of feed ingredients, experimental diets, *Brachiaria* hay, and faeces were analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fibre (NDF), and acid detergent fibre (ADF) using standard procedures described by AOAC (2019). Urinary nitrogen was determined by the Kjeldahl method (AOAC, 2000). Neutral detergent fibre and acid detergent fibre were determined according to the procedure of Van Soest (1991).

2.8. Statistical analysis

Data on feed intake, nutrient digestibility, nitrogen balance, rumen fermentation characteristics, haematological parameters, and serum biochemical variables were analysed using one-way analysis of variance (ANOVA) in SAS software (Version 9.4; SAS Institute Inc., Cary, NC, USA). The statistical model used for each response variable was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} represents the observation from the j^{th} bull receiving the i^{th} dietary treatment, μ is the overall mean, T_i is the fixed effect of *Gliricidia sepium* substitution level (0, 25, 50, and 75%), and e_{ij} is the random residual error. Individual bulls were considered the experimental units and were treated as random subjects within dietary treatments.

For variables measured repeatedly over time, including blood parameters collected at the beginning, midpoint, and end of the study, the effect of sampling time and its interaction with dietary treatment should be evaluated using an appropriate repeated-measures analysis rather than a simple one-way

ANOVA. The assumptions of normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Where necessary, data that did not satisfy these assumptions were log- or square-root-transformed before 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.

Result of anti-nutritional factors in GS shows that the concentration of tannins, oxylate, phytates, saponin and trypsin inhibitors are higher in the dried than in the fresh leaves which may be due to moisture loss and concentration in dry matter.

The nutrient composition of experimental diets is presented in Table 2. 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 3). 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.

3.3. Effect of experimental diets on blood parameters

Packed cell volume (PCV) did not differ significantly among dietary treatments at the beginning of the experiment ($P > 0.05$; Table 4). However, significant differences were observed at the middle and final sampling periods ($P < 0.05$). At the middle of the experiment, animals fed the diet containing 50% *Gliricidia sepium* (GS) recorded the highest PCV value (40.40%), which was significantly higher than that of animals fed the 0% inclusion diet (28.80%). The PCV values of animals fed the 25% (34.40%) and 75% (34.60%) inclusion levels were statistically similar. At the end of the experiment, the 50% GS inclusion level maintained the highest PCV value (44.20%), followed by the 75% (38.80%) and 25% (36.20%) inclusion levels, while the lowest value was recorded in animals fed the control diet (27.60%). Thus, PCV increased from the middle to the final sampling in animals receiving 25, 50, and 75% GS, whereas a slight decline was observed in animals fed the control diet.

Total protein (TP) concentration was not significantly affected by dietary treatment at the beginning of the experiment ($P > 0.05$). Significant differences among dietary treatments were observed at the middle

and final sampling periods ($P < 0.05$). At the middle of the experiment, animals fed the 0% (70.24 g/L), 25% (70.00 g/L), and 50% (70.04 g/L) inclusion levels recorded statistically comparable TP concentrations, whereas animals receiving the 75% GS diet had the lowest value (60.52 g/L), which differed significantly from the 0% inclusion treatment. At the end of the experiment, animals fed the 25% (60.82 g/L), 50% (70.08 g/L), and 75% (60.94 g/L) GS diets recorded statistically similar TP concentrations, while the 0% inclusion treatment had a significantly lower value (60.22 g/L) than the 25, 50, and 75% GS treatments.

Blood urea nitrogen (BUN) concentration differed significantly among dietary treatments at the beginning of the experiment ($P < 0.05$). Animals fed the 50% GS inclusion diet recorded the highest BUN concentration (3.04 mmol/L), which was significantly higher than the value observed in animals fed the 0% inclusion diet (1.52 mmol/L), while the 25% (2.14 mmol/L) and 75% (2.06 mmol/L) inclusion levels were intermediate and statistically comparable with the other treatments. At the middle of the experiment, no significant difference was observed in BUN concentration among dietary treatments ($P > 0.05$), although the highest numerical value was recorded in animals fed the control diet (3.39 mmol/L), while the lowest value was observed at the 25% GS inclusion level (2.23 mmol/L). At the end of the experiment, significant differences were observed among treatments ($P < 0.05$). The highest BUN concentration was recorded in animals fed the 50% GS diet (1.02 mmol/L), whereas the lowest value was observed in animals fed the 25% inclusion diet (0.74 mmol/L). The BUN values recorded for the 0% (0.87 mmol/L) and 75% (0.93 mmol/L) inclusion levels were intermediate and statistically comparable with the other treatments.

Blood glucose concentration was significantly influenced by dietary treatment at the initial, middle, and final sampling periods ($P < 0.05$). At the beginning of the experiment, animals fed the 50% GS inclusion diet recorded the highest glucose concentration (90.3 mg/dL), which was statistically similar to that of animals fed the 25% inclusion diet (89.1 mg/dL). The lowest glucose concentrations were recorded in animals fed the 0% (85.2 mg/dL) and 75% (86.8 mg/dL) inclusion diets, which were statistically comparable. At the middle of the experiment, glucose concentration increased across all dietary treatments, with animals receiving 50% GS recording the highest value (98.5 mg/dL), statistically comparable to that of animals fed 25% GS (96.8 mg/dL). The 0% (91.6 mg/dL) and 75% (93.4 mg/dL) treatments recorded significantly lower but statistically similar glucose concentrations. At the end of the experiment, glucose concentration increased further across all treatments. Animals fed 50% GS recorded the highest value (105.8 mg/dL), followed by those fed 25% GS (103.6 mg/dL), while the 0% (97.3 mg/dL) and 75% (99.5 mg/dL) treatments recorded significantly lower and statistically comparable values.

Albumin concentration was also significantly affected by dietary treatment at all sampling periods ($P < 0.05$). At the beginning of the experiment, animals fed the 50% GS inclusion diet had the highest albumin concentration (3.7 g/dL), which was statistically comparable to that of animals receiving 25% GS (3.6 g/dL). The lowest values were recorded in animals fed the 0% (3.4 g/dL) and 75% (3.5 g/dL) inclusion diets. At the middle of the experiment, albumin concentration increased across all dietary treatments,

with the highest value observed in animals fed 50% GS (3.9 g/dL), followed by those fed 25% GS (3.8 g/dL). Animals receiving the 0% (3.6 g/dL) and 75% (3.7 g/dL) inclusion diets had lower but statistically comparable values. At the end of the experiment, albumin concentration increased further in all treatments, with animals fed 50% GS recording the highest value (4.1 g/dL), followed by the 25% inclusion level (4.0 g/dL). The lowest values were recorded in animals fed the 0% (3.8 g/dL) and 75% (3.9 g/dL) inclusion diets. Overall, the results indicate that inclusion of GS, particularly at the 50% level, was associated with higher glucose and albumin concentrations throughout the experimental period.

Table 4
Effect of dietary substitution level on haematological and serum biochemical parameters of experimental animals

Parameter	Time	Level of <i>G. sepium</i> inclusion				SEM	P-value
		0%	25%	50%	75%		
PCV (%)	Initial	38.80	39.40	44.00	40.00	2.98	NS
	Middle	28.80 ^a	34.40 ^b	40.40 ^b	34.60 ^b	1.82	*
	Final	27.60 ^a	36.20 ^b	44.20 ^b	38.80 ^b	1.03	*
TP (g/L)	Initial	70.66	70.28	70.60	70.08	0.31	NS
	Middle	70.24 ^a	70.00 ^{ab}	70.04 ^{ab}	60.52 ^a	0.21	*
	Final	60.22 ^a	60.82 ^a	70.08 ^b	60.94 ^a	0.12	*
BUN (mmol/L)	Initial	1.52 ^a	2.14 ^b	3.04 ^b	2.06 ^{ab}	0.32	*
	Middle	3.39	2.23	3.07	2.57	0.44	NS
	Final	0.87 ^{ab}	0.74 ^a	1.02 ^a	0.93 ^{ab}	0.07	*
Glucose (mg/dL)	Initial	85.2 ^a	89.1 ^a	90.3 ^a	86.8 ^a	1.05	*
	Middle	91.6 ^a	96.8 ^a	98.5 ^a	93.4 ^a	1.12	*
	Final	97.3 ^a	103.6 ^a	105.8 ^a	99.5 ^a	1.18	*
Albumin (g/dL)	Initial	3.4 ^a	3.6 ^a	3.7 ^a	3.5 ^a	0.08	*
	Middle	3.6 ^a	3.8 ^a	3.9 ^a	3.7 ^a	0.07	*
	Final	3.8 ^a	4.0 ^a	4.1 ^a	3.9 ^a	0.07	*

^a, ^b, ^{ab} = 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 5) 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 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 5), 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 5
Intake and ruminal parameters of young Bunaji bulls fed varied levels of GS inclusion

Parameter	Level of <i>G. sepium</i> inclusion						P-value
	Time	0%	25%	50%	75%	SEM	
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	14.50 ^a	15.75 ^a	10.25 ^b	7.50 ^c	1.19	*
	4 h	9.88 ^b	15.50 ^a	15.50 ^a	10.63 ^b	2.67	*
	8 h	6.00 ^{bc}	8.75 ^b	12.00 ^a	8.50 ^b	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 6 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 6
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						

4. Discussion

4.1. Chemical composition and anti-nutritional factors

The chemical composition of GIS 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 (Tahuk et al., 2022; Aguilar et al., 2024). 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 (Gonçalves Junior et al., 2025).

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 Silva et al. (2024) and Schroeder (2010). 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 (Molina-Botero et al., 2019; Silva et al., 2024). Although GS has been associated with growth depression and toxicity in non-ruminant species such as poultry and rabbits (Edoh et al., 2019), 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 Rira et al. (2022). It is also in agreement with the findings reported by Guerreiro et al. (2021) 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 substrate led to a moderate decrease in VFA production and to a very pronounced depression of odd 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 (Silva et al., 2024). Although palatability issues related to volatile compounds on the leaf surface have been documented (Molina-Botero et al., 2020), 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 (Rutherglen, 1995; NRC, 1996). Consequently, dietary protein was unlikely to be a limiting factor for growth performance.

The higher ash content observed in GS-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 (Rusdy et al., 2021). 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 Cruz-Matías et al. (2026) who used tropical legume tree and coffee pulp to reduce enteric methane emission by 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 CH₄ 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

The absence of significant treatment effects on packed cell volume (PCV) and total protein at the beginning of the experiment indicates that the animals were physiologically comparable before the introduction of the dietary treatments. Therefore, the changes observed during the feeding period were likely associated with differences in nutrient supply and utilisation resulting from the replacement of cottonseed cake with GS.

The PCV values recorded throughout the experimental period remained within the normal physiological range reported for healthy cattle, suggesting that dietary GS inclusion did not adversely affect erythrocyte status or compromise animal health. The improvement observed in PCV among GS-fed animals, particularly at moderate inclusion levels, may be associated with the contribution of GS to dietary protein and mineral supply required for erythropoiesis. Similar observations have been reported in ruminants fed tropical forage legumes, where moderate inclusion of protein-rich browse species maintained normal haematological profiles while supporting animal productivity (Oyibo et al., 2020; Torres-Chablé et al., 2024). However, PCV is influenced by several physiological factors, including hydration status, age, nutritional condition, and disease status, and should therefore be interpreted alongside other indicators of animal health.

Serum total protein and albumin are important indicators of protein nutritional status and hepatic function in ruminants. The treatment differences that emerged during the feeding period suggest that dietary GS influenced protein metabolism after adaptation to the experimental diets. The slightly higher concentrations observed at moderate GS inclusion may reflect improved availability and utilisation of absorbed amino acids, resulting from the contribution of GS protein to the diet. A recent systematic review reported that GS foliage possesses considerable nutritional value as a protein supplement for ruminants and can replace conventional protein sources when incorporated at appropriate levels; however, excessive inclusion may reduce nutritional benefits because of increased fibre concentration and secondary metabolites (Silva et al., 2024). This observation agrees with the present findings, where moderate GS inclusion produced more favourable responses than complete replacement.

Blood urea nitrogen (BUN) provides an indication of nitrogen metabolism and reflects the relationship between dietary protein degradation, ruminal ammonia production, microbial protein synthesis, and hepatic urea formation. The reduction in BUN observed towards the end of the feeding period may indicate improved efficiency of nitrogen utilisation as the animals adapted to the experimental diets. Efficient synchronisation between ruminal nitrogen release and fermentable energy availability enhances microbial protein synthesis and reduces unnecessary nitrogen losses, thereby influencing circulating urea concentrations (Tan et al., 2021; Hailemariam et al., 2021). Nevertheless, BUN concentrations should be interpreted together with dietary crude protein intake, ruminal ammonia concentration, and nitrogen balance because they are influenced by several nutritional and physiological factors. The decline in circulating urea may also partly reflect the timing of blood collection relative to feeding, as blood urea concentration can decrease as ruminal ammonia absorption declines after peak post-feeding fermentation (Gomes et al., 2016, Madziga et al., 2025).

The progressive increase in blood glucose concentration during the feeding period suggests improved energy metabolism as animals adapted to the experimental diets. In ruminants, glucose is primarily derived through hepatic gluconeogenesis, with propionate produced during ruminal fermentation serving as a major precursor. Consequently, variations in dietary composition and ruminal fermentation resulting from GS inclusion may have influenced glucose availability. Recent studies have emphasised the close relationship between rumen microbial activity, fermentation end-products, and host energy metabolism (Mizrahi et al., 2021; Liu et al., 2021). However, the absence of a consistent linear response among inclusion levels suggests that moderate replacement of cottonseed cake with GS may provide a more favourable balance between protein supply, energy availability, and fibre concentration than higher inclusion levels.

The blood profile indicates that GS inclusion supported normal physiological function and positively influenced indicators of protein and energy metabolism without compromising animal health. The more favourable responses observed at moderate inclusion levels suggest that the nutritional value of GS depends on achieving an appropriate balance between its high protein content and the potential limitations associated with fibre and secondary metabolites.

4.4. Effect of experimental diets on rumen metabolites

Rumen pH is a critical indicator of ruminal health because it affects microbial activity, fibre degradation, and fermentation efficiency. In the present study, rumen pH remained within the optimal physiological range before and after feeding across all dietary treatments, indicating that replacement of cottonseed cake with GS did not negatively affect the ruminal environment. Similar findings have been reported in ruminants receiving tropical forage legumes, where tree fodder inclusion-maintained rumen pH within the range required for cellulolytic microbial activity (Hossain, 2020; Humer et al., 2018). The stability of rumen pH suggests that the experimental diets provided an appropriate balance between fermentable nutrients and structural fibre, supporting normal rumen fermentation.

Ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration reflects the availability of nitrogen for microbial growth and microbial protein synthesis. In the present study, $\text{NH}_3\text{-N}$ concentrations after feeding were sufficient to support microbial activity across all treatments. The relatively higher $\text{NH}_3\text{-N}$ concentration maintained by animals receiving the 50% GS diet suggests improved synchronisation between nitrogen release from dietary protein and the availability of fermentable carbohydrates. Such synchronisation enhances microbial protein synthesis and improves nitrogen utilisation efficiency (Tan et al., 2021; Hailemariam et al., 2021). Although microbial protein synthesis was not directly measured, the observed ammonia pattern provides an indication of improved nitrogen availability within the rumen. Similar improvements in ruminal nitrogen metabolism following supplementation with tropical forage resources have been reported by de Almeida Rufino et al. (2025).

Total volatile fatty acid (TVFA) concentrations increased after feeding across treatments, reflecting increased microbial fermentation of dietary substrates. Since volatile fatty acids represent the primary source of metabolizable energy for ruminants, higher post-feeding concentrations indicate active microbial degradation and fermentation of feed components (Mizrahi et al., 2021; Liu et al., 2021). The comparatively greater TVFA concentrations observed with moderate GS inclusion suggest enhanced fermentability of the diets and improved microbial utilisation of available nutrients. These findings agree with de Almeida Rufino et al. (2025), who reported improved ruminal fermentation responses following supplementation with tropical forage resources. Conversely, Sossou et al. (2025) reported no significant effects on ruminal short-chain fatty acid concentrations in goats fed cowpea silage-based diets, demonstrating that responses in rumen fermentation depend on forage type, dietary composition, animal species, and feeding management.

Collectively, the rumen metabolite responses indicate that GS replacement of cottonseed cake maintained a favourable ruminal environment and supported efficient microbial fermentation. The apparent advantage of moderate GS inclusion suggests improved synchronisation between nitrogen availability and energy supply; however, further studies involving microbial population analysis and direct measurement of microbial protein synthesis are required to confirm these mechanisms.

4.5. Effect of experimental diets on nutrient digestibility and nitrogen balance

The inclusion of GS did not significantly affect the digestibility of most nutrients, indicating that GS successfully replaced cottonseed cake without adversely affecting nutrient utilisation. This suggests that the protein supplied by GS was effectively utilised by the animals and that the experimental diets maintained adequate nutrient balance for normal digestive function. Previous research has demonstrated that GS foliage has potential as an alternative protein source for ruminants because of its high crude protein content, favourable nutrient profile, and acceptable digestibility when included at appropriate levels (Silva et al., 2024). Similarly, Molina-Botero et al. (2019) reported improved nutrient utilisation in crossbred heifers supplemented with GS foliage without detrimental effects on rumen function.

The reduction in crude fibre digestibility observed at higher GS inclusion levels may be related to differences in fibre composition and the presence of plant secondary metabolites, particularly condensed tannins and phenolic compounds. At moderate concentrations, these compounds may improve nitrogen utilisation by reducing excessive ruminal protein degradation; however, higher concentrations may interfere with microbial attachment to plant cell walls and reduce fibre degradation (Joch et al., 2018; Kelln et al., 2021). This may explain the slight decline in fibre digestibility at greater GS inclusion despite the absence of significant effects on other nutrient digestibility parameters.

Nitrogen balance provides an integrated assessment of dietary protein utilisation and the efficiency with which absorbed nitrogen is retained for growth and tissue synthesis. The positive nitrogen balance observed across all treatments indicates that the experimental diets supplied sufficient nitrogen to meet the requirements of the growing bulls. The numerically higher nitrogen retention observed at moderate GS inclusion suggests improved nitrogen use efficiency, potentially due to better synchronisation between ruminal nitrogen release and fermentable energy availability. Improving nitrogen utilisation efficiency is important not only for animal productivity but also for reducing nitrogen losses into the environment (Tan et al., 2021; Hailemariam et al., 2021).

Above all, the digestibility and nitrogen balance results demonstrate that GS can effectively replace cottonseed cake as a protein source in diets of growing Bunaji bulls without compromising nutrient utilisation. The favourable responses observed at moderate inclusion levels support previous findings that GS is a valuable tropical forage resource capable of reducing dependence on conventional protein supplements while maintaining digestive efficiency and nitrogen utilisation (Molina-Botero et al., 2019; Silva et al., 2024). However, the slight reduction in fibre digestibility at the highest inclusion level suggests that excessive replacement may provide limited additional benefits, highlighting the importance of identifying an optimal dietary inclusion level.

5. Conclusion

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 GS 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 cost-effective and sustainable protein source that can contribute to improved ruminant productivity, particularly in tropical production systems where conventional protein supplements are expensive or scarce.

Declarations

CRedit authorship contribution statement

Abisoye Adeola Adesote: Writing - original draft, Conceptualization, Writing- review and editing, Resources, Project administration, Methodology, Investigation, Conceptualization. Immanuel Ishaku Madziga: Writing – review & editing, Visualization, Investigation, methodology. Oluwagbemiga A Dada: Writing – review & editing, Formal analysis, data curation. Grace Jokthan: Supervision, Methodology

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work no AI was used.

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Declaration of Competing Interest

All authors declare no conflicting interest.

Data availability

Not applicable

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