Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v26e-82271E

e-ISSN 1809-6891
Animal science | Research article

Cashew nutshell liquid as an additive on rumen fermentation dynamics and nitrogen utilization in pasture-supplemented steers

Líquido da casca da castanha de caju como aditivo na dinâmica da fermentação ruminal e na utilização de nitrogênio em novilhos suplementados em pasto

Douglas Gabriel Anschau[1], Rafael Henrique Tonissi Buschinelli de Goes*[1], Jefferson Rodrigues Gandra[2], Nayara Gonçalves da Silva[1], Sullyvan Silva Oliveira[1], Luis Carlos Vinhas Ítavo[3], Jaqueline Luiza Royer[1], Luiz Miguel Anschau[1], Carolina Marques Costa Araújo[1]

1 Universidade Federal da Grande Dourados (UFGD), Dourados, Mato Grosso do Sul, Brazil
2 Universidade Federal do Sul e Sudeste do Pará (Unifesspa), Xinguara, Pará, Brazil
3 Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brazil
*corresponding author: rafaelgoes@ufgd.edu.br

Received: April 09, 2025. Accepted: September 26, 2025. Published: October 09, 2025. Editor: Rondineli P. Barbero

Abstract: The objective was to evaluate the effects of supplementing technical cashew nutshell liquid (CNSLt) on rumen fermentation and nitrogen utilization in grazing steers. Five crossbred rumen-cannulated steers were evaluated. The pasture used was *Urochloa brizantha*, cv. Marandu. The treatments were: supplementation without CNSLt infusion (control), supplementation + 300 mg/kg DM, supplementation + 600 mg/kg DM, supplementation + 900 mg/kg DM, and supplementation + 1,200 mg/kg DM of CNSLt. The inclusion of CNSLt in the steers' diet did not alter (P>0.05) intake or apparent digestibility. Among the fermentation parameters, only valeric fatty acid differed between treatments (P=0.040). The inclusion of 600 mg/kg DM CNSLt resulted in the highest valeric acid values (1.06). Higher inclusion of CNSLt led to lower fecal N excretion (P=0.029). The other N utilization variables, purine derivatives, and urea and creatinine metabolism were not affected by the treatment (P>0.05). In conclusion, the addition of CNSLt to the diet of grazing steers does not alter pasture intake or digestibility. The addition of 600 mg/kg DM CNSLt to the diet increased valeric acid production, improving rumen fermentation. The linear decrease in fecal N excretion suggests better N utilization with increasing CNSLt in the diet.

Keywords: additive; *Anacardium occidentale*; grazing cattle; nitrogen utilization; rumen fermentation.

Resumo: O objetivo foi avaliar os efeitos da suplementação líquida da casca de castanha de caju (CNSLt) sobre a fermentação ruminal e a utilização de nitrogênio em novilhos em pastejo. Foram avaliados cinco novilhos mestiços canulados no rúmen. A pastagem utilizada foi a *Urochloa brizantha*, cv. Marandu. Os tratamentos foram: suplementação sem infusão de CNSLt (controle), suplementação + 0,300 mg/kg MS, suplementação + 0,600 mg/kg MS, suplementação + 0,900 mg/kg MS e suplementação + 1.200 mg/kg MS de CNSLt. A inclusão de CNSLt na dieta dos novilhos não alterou (P> 0,05) o consumo e a digestibilidade aparente. Entre os parâmetros de fermentação, apenas o ácido graxo valérico diferiu entre os tratamentos (P=0,040). A inclusão de 600 mg/kg MS de CNSLt apresentou os maiores valores de ácido valérico (1,06). Quanto maior a inclusão de CNSLt, menor a excreção fecal de N (P=0,029). As outras variáveis de utilização de N, os derivados de purina e o metabolismo da ureia e da creatinina não foram afetados pelo tratamento (P>0,05). Pode-se concluir que a adição de CNSLt à dieta de novilhos em pastejo não altera a ingestão e a

digestibilidade do pasto. A adição de 600 mg/kg MS de CNSLt à dieta promoveu um aumento na produção de ácido valérico, o que melhorou a fermentação ruminal. A diminuição linear da excreção fecal de N sugere uma melhor utilização do N com o aumento do CNSLt na dieta.

Palavras-chave: aditivo; *Anacardium occidentale*; gado em pasto; utilização de nitrogênio; fermentação ruminal.

1. Introduction

The use of alternative feed resources in animal production has gained considerable attention in recent years, particularly in the context of sustainable livestock farming. Among these alternatives, technical cashew nutshell liquid (CNSLt), a by-product of the cashew (*Anacardium occidentale*) processing industry, has emerged as a promising candidate due to its rich composition of bioactive compounds, including anacardic acid and cardanol, which have demonstrated potential benefits for animal health and productivity [1,2].

In this regard, anacardic acid, a secondary compound found in cashew nutshell liquid, has been shown to act as a surfactant on the membranes of Archaea and gram-positive bacteria [3,4], influencing ruminal fermentation [2, 5-6] and the efficiency of nutrient absorption in ruminants [2]. Previous studies have indicated that the inclusion of CNSLt in ruminant diets can lead to increased rumen fermentation efficiency, resulting in increased volatile fatty acid production and improved nitrogen retention [2,7].

In addition, the effects of CNSLt on rumen metabolism are particularly relevant in the context of mitigating methane emissions under in vivo and in vitro conditions [8-9], which is a significant concern in beef production systems [7]. Therefore, the manipulation of rumen fermentation by including CNSLt in grazing cattle diets can enhance nutrient utilization, promote sustainable pasture use, and reduce greenhouse gas emissions from livestock production [10,7].

This research aimed to evaluate the effects of CNSLt supplementation on nutrient intake and digestibility, rumen fermentation, and nitrogen utilization in grazing steers. This study sought to provide insights into the potential of CNSLt as a functional feed additive that can increase the efficiency and sustainability of beef production systems.

2. Material and methods

2.1. Experimental design and animals

This study followed the ethical principles established by the Animal Use Ethics Committee/CEUA/UFGD (Protocol 023/2015); it was conducted at the Ruminant Nutrition and Production Sector (NERU/UFGD), the Agroenergy and Environmental Conservation Research Laboratory Center (LAPAC/FINEP), and the Animal Nutrition Laboratory (LANA/UFGD), located in the municipality of Dourados, state of Mato Grosso do Sul, situated at 22°11'43. 49" south latitude and 54°55'77" west latitude. The experiment was carried out during the dry-water transition period, from September to November 2018.

Five crossbred, rumen-cannulated steers, averaging 300 kg in weight and 22 months in age, were assigned to a 5×5 Latin square design. Each experimental period lasted 16 days: seven for adaptation and nine for data collection. The animals were kept in individual 0.2-hectare paddocks of *Urochloa brizantha* cv. Marandu (syn. *Brachiaria*).

2.2. Diets and supplementation

Technical cashew nutshell liquid (CNSLt; Usibras Company, Aquiraz, Ceará, Brazil) was administered directly into the rumen at the following levels of 0, 300, 600, 900, and 1,200 mg/kg DM. CNSLt composition: 10.03 mg/g anacardic acid, 540.77 mg/g cardanol, 102.34 mg/g cardol, and 19.17 mg/g 2-methylcardol.

The chemical analysis of the CNSLt was performed by High-Performance Liquid Chromatography (Varian 210 model) with a Diode Array Detector (DAD), and software Star WS (workstation 2.0). The column used was a C18 reverse-phase column (25 cm × 4.6 mm × 5 µm) (Phenomenex). The elution system consisted of an acetonitrile/water/acetic acid gradient (66/33/2 v:v:v) (A) and tetrahydrofuran (B). The elution started with 10% B and reached 100% B within 40 minutes. The pump flow rate was 1 mL/min, and the injection volume was 20 µL. The analysis was conducted at 22 °C for both the preparation of the analytical curve and the product analysis. Injections were performed in triplicate. The product was solubilized in an acetonitrile/water solution (66:35 v/v), providing a final concentration of 1000 µg/mL. The external standard curves employed to quantify anacardic acid, cardanol, 2-methylcardol, and cardol in the CNSLt product were prepared using compounds of 97 % purity at concentrations of 10–100 µg/mL. The results were expressed in mg/g sample obtained from an external standardization curve with a correlation coefficient of 0.9992 for all analyzed compounds.

The supplement was balanced according to the NRC [11], containing 19.66 % crude protein, consisting of 35 % corn, 15 % soybean meal, 30 % wheat bran, 5.5 % protected urea, 6 % NaCl, and 8.5 % commercial mix. The animals received 0.8 % of their body weight in supplement per day, in the morning (08:00). On the first day of each experimental period, the steers were weighed to adjust the supplement supply.

2.3. Sample collection, laboratory analysis, and calculations

2.3.1 Forage

On the first day of each experimental period, forage samples were collected to determine the mass and morphological composition of the forage. Forage mass was determined by taking ten ground-level frames (0.5 × 0.5 m) per paddock. The collected samples were subsampled. One subsample was dried in a forced ventilation oven at 60 °C and processed in a knife mill (1 mm) for subsequent analysis and determination of the chemical composition. Another subsample was separated into leaf, stem + sheath, and dead material to quantify the morphological composition (Table 1).

Table 1. Forage mass, morphological, and chemical characteristics of *Urochloa brizantha* cv. Marandu during the experimental period.

	CNSLt[1] (mg/kg DM)					
	0	300	600	900	1,200	Mean
Green dry matter (ton/ha)	1.62	1.27	1.48	1.28	1.63	1.46
Stem (%)	37.94	29.49	29.99	33.52	33.71	32.93
Leave (%)	33.55	35.60	29.68	27.74	35.39	32.39
Senescent material (%)	28.52	34.91	40.34	38.75	30.90	34.68
Chemical composition (%)						
Dry matter	37.65	41.65	39.30	42.53	39.29	40.08
Ash	8.84	8.21	8.51	7.15	9.27	8.40
Organic matter	82.88	82.70	82.40	83.00	90.74	84.34
Crude protein	4.91	4.98	5.14	5.72	6.05	5.36
Neutral detergent fiber	72.60	72.20	70.69	73.59	69.57	71.73
Acid detergent fiber	57.96	57.56	57.41	57.86	55.96	57.35
Total digestible nutrients	53.51	53.68	54.31	53.10	54.77	53.87
TDN:CP [2]	10.90	10.78	10.57	9.28	9.05	10.05

[1]CNSLt = technical cashew nutshell liquid; [2]Total digestible nutrient: crude protein ratio.

2.3.2 Nutrient intake and total apparent digestibility

Samples of feces, supplement, and forage obtained by ruminal emptying and cutting close to the ground were evaluated for dry matter (DM), crude protein (CP) obtained by determining total N using the micro Kjeldahl technique (Nx6.25), mineral matter or ash [12], and organic matter (OM = 100-Ash). The acid detergent fiber (ADF) content was determined as described by Van Soest and Robertson [13], and the lignin content was obtained through oxidation with potassium permanganate [14]. Neutral detergent fiber (NDF) analyses were carried out according to Mertens [15]. Total digestible nutrient (TDN) content of the forage was calculated based on the ADF content, according to the equation: %TDN = 83.79 - 0.4171*NDF.

DM intake was estimated based on the total fecal DM excretion and the indigestible NDF (NDFi) content in feces, supplement, and forage. To determine the daily fecal excretion of DM, titanium dioxide (TiO_2) was supplied to the rumen via cannula for ten consecutive days (5 days of adaptation and 5 days of collection) [16]. The TiO_2 indicator was packaged in paper cartridges and was supplied starting on the second day of each experimental period at a rate of 10 g/day at 08:00.

TiO_2 concentrations were analyzed by UV/Vis spectrophotometry, according to the methodology described by Myers *et al.* [17]. The following formula was used to estimate fecal DM output: DMp = TiO_2 ingested (g) / TiO_2 concentration in feces (g/g DM). Feces were collected from the rectal ampulla of cattle starting on day 7. The samples were placed in plastic bags, identified, and taken to the laboratory for evaluation of the chemical composition.

To determine the NDFi, 0.5 g of the samples was placed in TNT bags made of polypropylene (cut and sealed to a size of 5 × 5 cm with a pore size of 100 microns). The bags were previously dried, weighed, and incubated in the rumen (in situ) for 288 h [18]. Pasture DM intake was estimated according to Dias *et al.* [19] using the following equation: DMi (kg/day) = {[FE*CIFZ] - IS} / CIFO + DMIS; where: DMi = dry matter intake (kg/day); FE = fecal excretion (kg/day); CIFZ = concentration of the indicator in feces (kg); IS = indicator present in the supplement (kg/day); CIFO = concentration of the indicator in the forage (kg); and DMIS = dry matter intake of the supplement (kg/day).

The total apparent digestibility coefficients of the nutrients (DM, CP and NDF) were calculated using the following equation: Apparent digestibility = (DM ingested - DM excreted) / DM ingested; where: DM ingested = amount of dry matter consumed (kg/day); DM excreted = amount of dry matter excreted in feces (kg/day); CP = crude protein; and NDF = neutral detergent fiber.

2.3.3 Rumen fluid

On the 12th day of each period, the animals were restrained, and fluid samples were collected via rumen cannula. Samples were collected at 0, 2, 4, 6, and 8 hours after the supplement was administered. The rumen fluid samples were collected from different parts of the rumen (dorsal and ventral sacs) and filtered through four layers of gauze.

The pH was determined immediately using a portable digital pH meter. Aliquots (10–20 mL) of the samples were centrifuged at 3500 rpm for 5 minutes, and 1800 µL supernatant was collected and mixed with 100 µL of a 20% orthophosphoric acid solution. All samples were frozen for short-chain fatty acid analysis. To determine the concentration of ammonia nitrogen ($N\text{-}NH_3$), an aliquot of 40 µL was fixed with 1 µL of 1:1 HCl, separated, and frozen at −18 °C for later analysis. The $N\text{-}NH_3$ content was determined according to the INCT-CA method N-007/1, as described by Detmann *et al.* [18].

The ammonia concentration in rumen fluid was estimated using the micro-Kjeldahl system without acid digestion. Potassium hydroxide (2N) was used as the distillation base after the sample was centrifuged at 1,000 x g for 15 minutes.

The water-soluble fatty acid (WSFA-2) concentrations were determined using the methodology described by Del Valle *et al.*[20] through gas chromatography using a Shimadzu© GC-2010 Plus (Shimadzu, Barueri, Brazil) chromatograph equipped with an AOC-20i automatic injector, a Stabilwax-DATM capillary column (30 m, 0.25 mm ID, 0.25 µm df, Restek©), and a flame ionization detector (FID). First, the samples were acidified with 1 M orthophosphoric acid (p.a., Ref. 100573, Merck©), and then fortified with a mixture of free volatile acids (Ref. 46975, Supelco©). A 1µL aliquot of each sample was injected at a split ratio of 40:1 using helium as the carrier gas at a linear velocity of 42 cm/s, achieving the separation of analytes in a chromatographic run of 11.5 minutes. The injector and detector temperatures were 250°C and 300°C, respectively, and the initial column temperature was 40°C. The column temperature ramp began with a gradient from 40 to 120°C at a rate of 40°C/min, followed by gradients from 120 to 180°C and from 180 to 240°C at rates of 10°C/min and 120°C/min, respectively. The temperature remained at 240°C for an additional 3 minutes.

To quantify the analytes, the method was calibrated using dilutions of the WSFA-2 standard (Ref. 47056, Supelco©) and glacial acetic acid (Ref. 33209, Sigma-Aldrich©), which were analyzed under the conditions described above. The peaks were determined and integrated using GCsolution software v. 2.42.00 (Shimadzu©).

Determining the WSFA-2 concentration made it possible to estimate the enteric methane production of the cattle used, using the methodology of Moss *et al.* [21], which calculates the generation of CH4 based on the proportions of acetic acid (C2), propionic acid (C3) and butyric acid (C4), using the following equation: CH4= 0.45 (C2) - 0.275 (C3) + 0.4 (C4).

2.3.4 Urine

On the 13th day, spot urine samples were collected from each period, four hours after the supplement was given, during spontaneous urination [22]. To determine the concentrations of creatinine, urea, uric acid, and allantoin, 10 mL of urine was diluted with 40 mL of sulfuric acid (0.036 N) to prevent the degradation of purine derivatives and the precipitation of uric acid. A second aliquot of 100 mL was stored in 1 mL of sulfuric acid (36 N) and analyzed to determine the total urinary N concentration. All samples were identified and frozen. The samples were identified and immediately frozen at -18°C for later analysis.

Allantoin was determined using the colorimetric method according to the technique of Fujihara *et al.* [23] as described by Chen and Gomes [24]. Commercial kits (Labtest, Lagoa Santa, Brazil; Gold Analisa Diagnostica Ltda, Belo Horizonte, Brazil) were used to determine the concentration of creatinine and uric acid.

The sum of the amounts of allantoin and uric acid excreted in the urine, expressed in mmol/day, was used to calculate total purine derivative (PD) excretion. The absorbed microbial purines (Pabs, mmol/day) were calculated from the excretion of PD in the urine (mmol/day), using the equation:

PD = 0.85*Pabs + 0.385*BW0.75, where 0.85 is the recovery of absorbed purines as urinary purine derivatives and 0.385*BW0.75 is the endogenous contribution to purine excretion [25].

The total urinary volume was determined by calculating the ratio of creatinine concentration in urine to creatinine excretion per unit body weight (BW), using the standard value of 27.36 mg/kg BW [26]. Daily urea-N and creatinine-N excretion was obtained by multiplying the respective concentrations by the 24-hour urinary volume and then multiplying that product by 0.466 or 0.3715, which correspond to the N content of urea and creatinine, respectively. Based on the average daily creatinine excretion obtained during the experiment (in mg/kg CP/day) and the creatinine concentration (in mg/L) of the spot urine sample, the daily urine volume (UrV) was estimated. UrV (L/day) = (27.36 × CP)/[creatinine], where 27.36 represents the average daily creatinine excretion value in ppm CP obtained by Rennó *et al.* [26] in crossbred and Zebu steers; BW is the animal's body weight; and [creatinine] is the creatinine concentration in mg/L found in the spot urine sample of the animals.

Quantification of microbial biomass in rumen samples was carried out using purine bases as indicators. The unit g microbial DM/kg carbohydrates degraded in the rumen (CHODR) was used as the basic reference for measuring the efficiency of microbial protein synthesis.

The balance of N compounds (NB) was obtained by subtracting the total N ingested from the total N excreted in urine and feces. N concentrations in feces and urine samples were determined using the micro-Kjeldahl system. Based on these values, retained nitrogen (NRet) was quantified by subtracting the estimated value of the basal endogenous nitrogen (BEN) requirement from the NB. BEN considers tissue endogenous N and dermal N losses as 0.35 and 0.018 of the metabolic weight, respectively.

2.3.5 Blood

On the 14th day, 4 hours after the supplement was administered, blood was collected by puncturing the tail vein and using heparin as an anticoagulant. After collection, the tubes were centrifuged (5,000 rpm for 15 min), and the serum was stored in 2-mL polypropylene conical tubes. All samples were refrigerated at -18°C. Plasma urea and creatinine levels were determined using a commercial kit (Gold Analisa®, Diagnostica Ltda).

2.3.6 Extrusa

On the 15th day, the forage consumed by the animals (extrusa) was collected by manually emptying the rumen. After this, the animals were taken to their respective paddocks, where they grazed for approximately 40 minutes until the next rumen emptying. After collecting the extrusa, the rumen contents were exchanged between the animals [28–29]. This procedure was performed to reduce the adaptation period to the diets. All samples were homogenized, packed in labeled plastic bags, frozen at -18°C, and transported to the Animal Nutrition Laboratory at UFGD for further analysis.

2.4 Statistical analysis

All statistical analyses were carried out using SAS ® 9.2 [30]. The following model was adopted for the effects of diet evaluation:

$$Yijl = \mu + Ai + Pj + Dl + erijl;$$

Where Yijl = dependent variable, μ = overall mean, Ai = animal effect (i = 1 to 5), Pj = period effect (j = 1 to 5), Dl = diet effect, and eijl = experimental error.

Rumen fermentation data were analyzed using the PROC MIXED REPEATED command to evaluate repeated measures over time according to the following model:

$$Yijk = \mu + Ai + Pj + Dk + Ty + Ty\ (Dk)\ eijk;$$

Where: Yijyk = dependent variable, μ = overall mean, Ai = animal effect (i = 1 to 5), Pj = period effect (j = 1 to 5), Dk = treatment effect (k =1 to 5), Tk = time effect (k =1 to 5), Ty (Dk) = interaction between diet and time and eijk = experimental error.

The data were submitted to an analysis of variance using PROC MIXED with LSMEANS and simple polynomial regression, and were submitted to a Tukey test at a 5% significance level.

3. Results

The forage mass, morphological fractions, and chemical composition of the pasture are listed in Table 1. On average, the treatments had 1.46 t/ha of green matter, of which 32.9% was leaves, 32.4% was stems, and 34.7% was senescent material. The average values for dry matter, crude protein, and total digestible nutrients were 40.08, 5.36, and 53.87, respectively (Table 1).

The chemical composition of the extrusa is described in Table 2. The average contents of dry matter, crude protein, and total digestible nutrients were 17.21, 7.42, and 60.11, respectively (Table 2).

Table 2. Chemical composition of extrusa from steers supplemented with different levels of CNSLt.

	CNSLt[1] (mg/kg DM)					
	0	300	600	900	1,200	Mean
Dry matter	18.08	18.49	16.71	16.45	16.35	17.21
Organic matter	88.10	89.41	89.53	88.73	88.93	88.94
Crude protein	7.47	7.29	6.56	7.64	8.16	7.42
Neutral detergent fiber	55.44	56.46	57.87	58.15	55.90	56.76
Acid detergent fiber	37.69	40.54	41.33	40.28	39.69	39.90
Ash	11.90	10.59	10.47	11.27	11.07	11.06
Total digestible nutrients	60.67	60.24	59.65	59.54	60.47	60.11
TDN:CP [2]	8.12	8.26	9.09	7.79	7.41	8.13

[1]CNSLt = technical cashew nutshell liquid; [2]Total digestible nutrient: crude protein ratio.

The inclusion of CNSLt in the diet of steers did not alter (P>0.05) the intake of pasture and supplement (Table 3). Similarly, the intake of other nutrients and apparent digestibility were similar (P>0.05) between the diets (Table 3).

Table 3. Nutrient intake (DM basis) and apparent digestibility of steers supplemented with different levels of CNSLt.

	CNSLt (mg/kg DM)					SEM[1]	P- value[2]		
	0	300	600	900	1,200		Treat	L	Q
Nutrient intake (kg/day)									
Pasture	6.62	6.21	5.53	5.35	6.51	0.37	0.684	0.696	0.265
Supplement	2.22	2.22	2.41	2.07	2.19	0.13	0.823	0.812	0.802
Total dry matter	8.85	8.44	7.94	7.42	8.71	0.46	0.702	0.704	0.411
Organic matter	7.01	7.26	6.83	6.22	7.43	0.37	0.938	0.935	0.599
Crude protein	0.90	0.85	0.79	0.74	0.85	0.04	0.512	0.542	0.412
Neutral detergent fiber	5.15	5.09	4.70	4.47	5.22	0.27	0.812	0.810	0.456
Apparent digestibility									
Dry matter	0.40	0.48	0.42	0.47	0.48	0.01	0.269	0.207	0.922
Organic matter	0.44	0.53	0.47	0.52	0.52	0.01	0.305	0.265	0.706
Crude protein	0.45	0.51	0.48	0.47	0.58	0.03	0.251	0.290	0.673
Neutral detergent fiber	0.44	0.54	0.42	0.53	0.48	0.01	0.627	0.556	0.706

[1]SEM = standard error of the mean. [2]Probability of linear (L) or quadratic (Q) supplementation effect (Treat). CNSLt = technical cashew nutshell liquid.

Of the fermentation parameters, only the valeric fatty acid content differed between treatments (P=0.040). The inclusion of 600 mg/kg DM of CNSLt resulted in the highest values of valeric acid (1.06; Table 4).

Table 4. Ruminal fermentation parameters of steers supplemented with different levels of CNSLt.

	CNSLt[1] (mg/kg DM)					SEM[2]	P- value[3]		
	0	300	600	900	1,200		Treat	L	Q
pH	6.64	6.76	6.73	6.67	6.69	0.02	0.540	0.991	0.281
N-NH_3	21.86	23.45	21.86	19.76	26.02	1.14	0.164	0.102	0.308
Fatty acids									
Acetic[A](%)	69.44	70.38	70.88	70.64	70.00	0.34	0.742	0.592	0.188
Propionic[B](%)	17.38	17.31	17.60	18.07	17.81	0.19	0.741	0.239	0.902
Isobutyric[C](%)	0.87	0.78	0.88	0.75	0.76	0.03	0.606	0.276	0.881
Butyric[D] (%)	10.51	9.74	8.31	8.64	9.44	0.41	0.499	0.282	0.161
Isovaleric (%)	0.94	0.97	1.25	0.93	1,03	0.05	0.444	0.723	0.381
Valeric (%)	0.84ab	0.79b	1.06a	0.95ab	0.94ab	0.03	0.040	0.094	0.237
Total[E](mmol/L)	70.70	70.80	46.20	67.00	78.70	5.58	0.454	0.762	0.154
C2:C3	4.01	4.07	4.04	3.91	3.93	0.05	0.833	0.357	0.706
Methane[F]	21.67	21.89	14.11	20.24	24.02	1.73	0.463	0.809	0.158

[1]CNSLt = technical cashew nutshell liquid.[2]SEM = standard error of the mean. [3]Probability of linear (L) or quadratic (Q) supplementation effect (Treat) [4]Methane = 0.45(C2) – 0.275(C3) + 0.4(C4) according to Moss *et al.*[21] (A)Y= 38.457+14.691X-0.0794X^2; r2= 0.34; (B)Y= 17.65 + 24.657X – 0.01657X2;r2= 0.35; (C)Y=0.724+-0.0485X; r2= 0.54; (D)Y= 4.095+ 4.258X- 0.00801X^2; r2= 0.32; (E)Y= 49.316+28.338X-0.05155X^2; r2= 0.44; (F)Y= 22.28 + 24.367X – 0.02650X2. Different letters indicate 5% significance by Tukey's test.

The inclusion of CNSLt linearly influenced fecal N excretion (P=0.029); the higher the inclusion of the additive, the lower the fecal N excretion. The other N utilization variables and purine derivatives were not affected by the treatment (Table 5).

Table 5. Microbial protein synthesis and nitrogen utilization in steers supplemented with different levels of CNSLt.

	CNSLt[1] (mg/kg DM)					SEM[2]	P-value		
	0	300	600	900	1,200		Treat	L	Q
Purine derivatives (mmol/L)									
Allantoin	65.97	73.99	31.67	46.26	48.93	3.42	0.492	0.289	0.497
Uric acid	33.92	66.85	18.72	22.85	28.77	4.78	0.499	0.413	0.981
Total purines	99.89	140.85	50.39	69.11	77.71	5.72	0.513	0.345	0.755
Absorbed purines	102.76	151.75	43.65	66.32	76.47	6.73	0.505	0.341	0.754
Microbial N (g/day)	74.71	110.33	31.73	48.22	55.59	3.45	0.505	0.341	0.754
Microbial protein	466.94	689.56	198.36	301.38	347.49	5.67	0.505	0.341	0.754
Nitrogen utilization (g/day)									
N intake	144.97	136.66	126.63	118.29	136.89	7.78	0.838	0.546	0.415
Fecal N [(a)]	75.58	62.95	64.49	59.65	57.84	2.46	0.167	0.022	0.426
Urinary N	2.96	2.15	1.62	2.33	1.67	0.20	0.266	0.144	0.426
Absorbed N	66.41	71.54	60.50	56.34	77.37	8.90	0.916	0.950	0.649
Retained N	66.38	71.51	60.48	56.32	77.35	2.91	0.916	0.674	0.238

[1]CNSLt = technical cashew nutshell liquid.[2]SEM = standard error of the mean. [3]Probability of linear (L) or quadratic (Q) supplementation effect (Treat); (a) Y= 71.865 - 0.01295X; r2= 0.21.

The inclusion of CNSLt did not affect (P>0.05) the metabolism of urea and creatinine in steers (Table 6).

Table 6. Urea and creatinine metabolism of steers supplemented with different levels of CNSLt.

	CNSLt[1] (mg/kg DM)					SEM[2]	P-value[3]		
	0	300	600	900	1,200		Treat	L	Q
Urine (mg/dL)									
Urea	821.22	803.34	634.42	800.35	623.49	32.56	0.122	0.102	0.950
Creatinine	1.50	2.27	2.70	1.72	2.43	0.12	0.089	0.419	0.135
Urea-N	382.69	374.35	295.64	372.97	290.55	8.98	0.172	0.102	0.950
Creatinine-N	0.559	0.846	1.065	0.641	0.901	0.00	0.432	0.321	0.555
Blood (mg/dL)									
Urea	22.75	30.79	25.59	27.22	26.08	2.45	0.297	0.664	0.226
Creatinine	3.76	3.57	4.00	3.31	3.92	0.87	0.552	0.944	0.684
Urea-N	10.60	14.35	11.92	12.88	12.15	1.67	0.297	0.664	0.226
Creatinine-N	1.39	1.32	1.48	1.23	1.46	0.45	0.552	0.944	0.684
Excretion (mg/kg BW)									
Urea	987.58	920.64	553.50	1118.52	669.22	18.89	0.561	0.166	0.678
Creatinine	28.47	28.54	28.41	28.53	28.51	4.56	0.794	0.893	0.848
Clearance 24-h period (mg/mL)									
Urea	48.45	32.85	22.00	38.96	26.00	3.67	0.712	0.821	0.832
Creatinine	6.95	7.07	6.46	7.58	6.44	1.67	0.731	0.836	0.824
Fractional excretion (%)									
Urea	79.95	87.78	56.23	78.23	87.18	5.67	0.821	0.804	0.731

[1]CNSLt = technical cashew nutshell liquid.[2]SEM = standard error of the mean. [3]Probability of linear (L) or quadratic (Q) supplementation effect (Treat).

4. Discussion

According to Minson [31], the minimum forage limit in tropical grass pastures should be 2,000 kg/ha of total dry matter to avoid limiting animal intake. However, Euclides [32] states that during periods of large seasonal accumulation of dead material, such as the dry-water transition, forage consumption is not correlated with the total forage available but rather with the masses of green and leaf blade dry matter. This author states that leaf blade dry matter should always exceed 1,100 kg/ha, a value that corroborates the results found here (Table 1). Therefore, forage availability was not a limiting factor for animal intake.

The average DM content of the forage (40.08%) was within the desirable range to ensure adequate nutrient intake [33]. However, the CP content of 5.36% suggests that the pasture may be deficient in protein, since the recommended crude protein intake for ruminants is usually greater than 12% [34], which confirms the importance of using supplementation in this experiment, since low protein concentrations can limit animal performance [35]. A TDN content above 55% is generally considered adequate for the maintenance and production of ruminants [36] (Table 1).

The lower DM content (17.21 %) of the extrudate reflects the effect of the extrusion process on the material's physical properties to improve starch gelatinization and protein denaturation [37]. The CP content was 7.42 %, which can still be considered below the ideal level for ruminants. Insufficient protein supplementation compromises nutrient utilization efficiency and can cause an imbalance between energy and protein in the rumen, which affects rumen fermentation and consequently feed intake [38]. When the TDN: CP ratio exceeds 7 (Table 2), there is a protein deficiency relative to the energy content

of the forage [39], which can reduce intake. However, supplementing up to 0.8 % of body weight had no significant impact on DM forage intake, or digestibility in steers on pasture (Table 3). This demonstrates that CNSLt does not impair nutrient use and supports its viability as a dietary additive.

Consistent results have been reported in other ruminant species and production systems, such as Thai cattle and swamp buffalo [40], young bulls [41], feedlot-finished bulls [42], lactating dairy cows [43], dry cows [3], and sheep [44] fed diets supplemented with CNSLt. Intake and digestibility are important parameters when considering the application of new candidate additives in relation to animal growth and production performance and the modulation of rumen fermentation. Therefore, the absence of negative effects on intake and digestibility is key to the acceptance of CNSLt in ruminant feed [40].

Ruminal fermentation parameters also support the absence of detrimental effects. Ruminal pH values remained above 6.2, which is essential for cellulolytic microbial activity and fiber degradation [45]. Ammonia concentrations (26.02 mg/dL) were within the optimal range for microbial protein synthesis [46], supporting adequate nitrogen availability for rumen microbes (Table 4). The increase in valeric acid concentrations is particularly noteworthy because it indicates increased microbial fermentation of amino acids [33, 34, 47]. This suggests that CNSLt may stimulate proteolytic microbial populations, thereby improving nitrogen turnover and potentially enhancing protein availability for the host animal.

Similar to other volatile fatty acids, valeric acid is fundamental in the energy metabolism of ruminants, accounting for approximately 70% of their total energy requirements [47]. Valeric acid levels are related to the breakdown of proteins and amino acids in the rumen. Microbial fermentation of proteins produces short-chain fatty acids, including valeric acid, as end products [33]. An increase in valeric acid concentration suggests greater microbial activity in protein fermentation, particularly in amino acid-rich diets [34]. Therefore, the inclusion of CNSLt may favor rumen microbiota that break down proteins, which could improve fermentation efficiency and nutrient availability (Table 4).

The inclusion of CNSLt in the diet modifies not only rumen fermentation but also intestinal and fecal fermentation [8]. A significant portion of the nitrogen ingested by ruminants is excreted, either through feces or urine, instead of being converted into animal protein. Therefore, reducing fecal N excretion by increasing CNSLt in the mixture indicates more efficient use of dietary protein (Table 5).

In addition, N excretion in the feces is directly related to the suppression of methane generation, meaning that including CNSLt in the diet can mitigate the environmental impact of keeping ruminant cattle on pasture [8, 48].

Finally, the absence of changes in urea and creatinine metabolism across treatments (Table 6) suggests that CNSLt supplementation did not affect systemic nitrogen metabolism. As Harmeyer and Martens [49] reported, these metabolites reflect protein intake and the energy: protein ratio of the diet. This finding reinforces the idea that the additive did not compromise metabolic homeostasis.

5. Conclusion

Supplementing technical cashew nutshell liquid (CNSLt) in the diet of grazing steers did not affect pasture intake or digestibility. Adding 600 mg/kg DM of CNSLt to the diet increased valeric acid production and improved rumen fermentation. The linear reduction in fecal nitrogen excretion suggests improved nitrogen utilization with an increase in CNSLt in the diet.

Conflicts of interest statement

The authors declare no conflicts of interest.

Data availability statement

The full dataset supporting the results of this study was published in the article itself.

Author contributions

Conceptualization: Buschinelli de Goes, R. H. T. and Anschau, D. G.; Data curation: Anschau, D. G., Gandra, J. R., and Ítavo, L. C. V.; Formal analysis: Silva, N. G. and Oliveira, S. S.; Funding acquisition: Buschinelli de Goes, R. H. T.; Investigation: Anschau, D. G.; Methodology: Anschau, D. G., Royer, J. L., and Anschau, L. M.; Project administration: Buschinelli de Goes, R. H. T.; Resources: Buschinelli de Goes, R. H. T.; Software: Gandra, J. R.; Supervision: Ítavo, L. C. V.; Validation: Buschinelli de Goes, R. H. T. and Araújo, C. M. C.; Visualization: Araújo, C. M. C.; Writing of the original draft: Anschau, D. G.; and Writing, proofreading, and editing: Araújo, C. M. C.

Acknowledgments

The authors thank Claudia Andrea Lima Cardoso of the State University of Mato Grosso do Sul (UEMS) for her support in the chromatographic analysis of the liquid from the cashew nutshell liquid. In addition, the authors express appreciation to Antonio Ferriani Branco for providing the technical cashew nutshell liquid. Financial support for the project was provided by the National Council for Scientific and Technological Development (Conselho Nacional de Pesquisa e Desenvolvimento Científico e Tecnológico, CNPq), Process: 310837/2020-0 / 309888/2023-9, Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES. Process: 88887.964847/2024-00), Brasília, Brazil; and the Foundation for Support and Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT), Processes: 71/032.380/2022 and 83/035.027/2024.

References

1. Morais S, Silva K, Araujo H, Vieira Í, Alves D, Fontenelle R, Silva A. Anacardic acid constituents from cashew nut shell liquid: NMR characterization and the effect of unsaturation on its biological activities. Pharmaceuticals. 2017; 10:31. https://doi.org/10.3390/ph10010031

2. Díaz TG, Branco AF, Ítavo LCV, dos Santos GT, Carvalho ST, Teodoro AL, Oliveira RL. In vitro gas production kinetics and digestibility in ruminant diets with different levels of cashew nut shell liquid. Revista Semina: Ciências Agrárias. 2018; 39:1669-1682. https://doi.org/10.5433/1679-0359.2018v39n4p1669

3. Shinkai T, Enishi O, Mitsumori M, Higuchi K, Kobayashi Y, Takenaka A, Mochizuki M. Mitigation of methane production from cattle by feeding cashew nut shell liquid. Journal of Dairy Science. 2012; 95:5308-5316. https://doi.org/10.3168/jds.2012-5554

4. Oh S, Shintani R, Koike S, Kobayashi Y. Ginkgo fruit extract as an additive to 831 modify rumen microbiota and fermentation and to mitigate methane production. Journal of Dairy Science. 2017; 100:1923-1934. https://doi.org/10.3168/jds.2016-11928

5. Mitsumori M, Enishi O, Shinkai T, Higuchi K, Kobayashi Y, Takenaka A, Nagashima K, Mochizuki M, Kobayashi Y. Effect of cashew nut shell liquid on metabolic hydrogen flow on bovine rumen fermentation. Animal Science Journal. 2014; 85:227-232. https://doi.org/10.1111/asj.12133

6. Vieira ERDQ, Goes RHTBG, Diaz TG, Osmari MP, Seno LO, Ítavo LCV, Jacaúna AG. Chitosan combined with technical cashew nut shell liquid improves in vitro ruminal parameters and gas production kinetics. Revista Brasileira de Zootecnia. 2022; 51:e20200186. https://doi.org/10.37496/rbz5120200186

7. Adetunji A, Aderinboye R, Adebayo K, Ojo V, Idowu P, Mtileni B. Effect of cashew nut shell liquid at varying inclusion levels on rumen fermentation and methane production in vitro. Journal of Animal Behaviour and Biometeorology. 2020; 8:82-87. https://doi.org/10.31893/jabb.20011

8. Tamori K, Matsunaga B, Boonsaen P, Khongpradit A, Sawanon S, Nagashima K, Kobayashi Y. Feeding cashew nut shell liquid decreases methane production from feces by altering fecal bacterial and archaeal communities in Thai local ruminants. Animal Science Journal. 2021; 92:e13569. https://doi.org/10.1111/asj.13569

9. Cuervo W, Gomez C, Tarnonsky F, Fernandez-Marenchino I, Podversich F, Maderal A, DiLorenzo N. Effects of cashew nutshell extract inclusion into a high-grain finishing diet on methane emissions, nutrient digestibility, and ruminal fermentation in beef steers. Journal of Animal Science. 2025; 103:skae359. https://doi.org/10.1093/jas/skae359

10. Pereira D, Goes R, Martinez A, Gandra J, Presendo E, Santos M, Alvez J. In vitro evaluation of the association of chitosan and cashew nut shell liquid as additives for ruminants. Revista Brasileira de Saúde e Produção Animal. 2019; 20: e05102019. https://doi.org/10.1590/s1519-994005102019

11. National Research Council (NRC). Nutrient requirements of dairy cattle. 8th rev. ed. Washington (DC): National Academies Press; 2016.

12. AOAC. Official Methods of Analysis. 15th ed. Arlington (VA): AOAC International; 1990.

13. Van Soest PJ, Robertson JB. Analysis of forages and fibrous foods: a laboratory manual for animal science. Ithaca (NY): Cornell University; 1985.

14. Van Soest PJ, Wine RH. Determination of Lignin and Cellulose in Acid-Detergent Fiber with Permanganate. Journal AOAC International. 1968; 51:780-785. https://doi.org/10.1093/jaoac/51.4.780

15. Mertens DR. Gravimetric determination of amylase treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal AOAC International. 2002; 85(5):1212-40.

16. Ferreira MA, Valadares Filho SC, Marcondes MI, Paixão MI, Paulino MF, Valadares RFD. Avaliação de indicadores em estudos com ruminantes: digestibilidade. Revista Brasileira de Zootecnia. 2009; 38:1568-1573. https://doi.org/10.1590/S1516-35982009000800022

17. Myers WD, Ludden PA, Nayigihugu V, Hess BW. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. Journal of Animal Science. 2004; 82:179-183. http://dx.doi.org/10.2527/2004.821179x

18. Detmann E, Souza MA, Valadares Filho SC, Queiroz AC, Berchielli TT, Saliba EOS, Cabral LS, Pina DS, Ladeira MM, Azevedo JAG. Métodos para análise de alimentos. Visconde do Rio Branco: Suprema; 2012. 214 p.

19. Dias AOC, Goes RHTB, Gandra JR, Takiya CS, Branco AF, Jacaúna AG, Oliveira RT, Vaz MSM. Increasing doses of chitosan to grazing beef steers: Nutrient intake and digestibility, ruminal fermentation, and nitrogen utilization. Animal Feed Science and Technology. 2017; 225:73-80. https://doi.org/10.1016/j.anifeedsci.2017.01.015

20. Del Valle TA, Zenatti TF, Antonio G, Campana M, Gandra JR, Zilio EMC, Mattos LFA, Morais JGP. Effect of chitosan on the preservation quality of sugarcane silage. Grass and Forage Science. 2018; 73:630-638. https://doi.org/10.1111/gfs.12356

21. Moss AR, Jouany JP, Newbold J. Methane production by ruminants: its contribution to global warming. Annales de Zootechnie. 2000; 49:231-253. https://doi.org/10.1051/animres:2000119

22. Chizzotti ML, Valadares Filho SC, Valadares RFD, Chizzotti FHM, Campos JMS, Marcondes MI, Fonseca MA. Intake, digestibility and urinary excretion of urea and purine derivatives in heifers with different body weights. Revista Brasileira de Zootecnia. 2006; 35:1813–1821. https://doi.org/10.1590/S1516-35982006000600032

23. Fujihara T, Orskov ER, Reeds PJ, Kyle DJ. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. The Journal of Agricultural Science. 1987; 109:7-12. https://doi.org/10.1017/S0021859600080916

24. Chen XB, Gomes MJ. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives. Aberdeen: Rowett Research Institute; 1992. 22p.

25. Verbic J, Chen XB, Macleod NA, Ørskov ER. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. The Journal of Agricultural Science. 1990; 114:243-248. https://doi.org/10.1017/S0021859600072610

26. Rennó LN, Valadares RF, Valadares Filho SC, Leão MI, Silva JFC, Cecon PR, Gonçalves LC, Dias HLC, Linhares RS. Concentração plasmática de uréia e excreções de uréia e creatinina em novilhos. Revista Brasileira de Zootecnia. 2000; 29:1235-1243. https://doi.org/10.1590/S1516-35982000000400038

27. Cappelle ER, Valadares Filho SDC, Silva JFCD, Cecon PR. Estimativas do valor energético a partir de características químicas e bromatológicas dos alimentos. Revista Brasileira de Zootecnia. 2001; 30:1837-1856. https://doi.org/10.1590/S1516-35982001000700022

28. Kim DH, Mcleod KR, Klotz JL, Koontz AF, Foote AP, Harmon DL. Evaluation of a rapid determination of fasting heat production and respiratory quotient in Holstein steers using the washed rumen technique. Journal of Animal Science. 2013; 91:4267-4276. https://doi.org/10.2527/jas.2012-5595

29. Kim DH, Mcleod KR, Koontz AF, Foote AP, Klotz JL, Harmon DL. Effect of intake on fasting heat production, respiratory quotient and plasma metabolites measured using the washed rumen technique. Animal. 2015; 9:58-66. https://doi.org/10.1017/S1751731114002183

30. SAS Institute Inc. SAS/STAT® 9.2 user's guide. Cary (NC): SAS Institute Inc.; 2009. 1042 p.

31. Minson DJ. Forage in ruminant nutrition. San Diego: Academic Press; 1990.

32. Euclides VPB. Alternativas para intensificação da produção de carne bovina em pastagem. Campo Grande, MS: Embrapa Gado de Corte; 2000.

33. Rajtar P, Górka P, Śliwiński B, Wieczorek J, Boros D, Micek P. Effect of rye grain derived from different cultivars or maize grain use in the diet on ruminal fermentation parameters and nutrient digestibility in sheep. Annals of Animal Science. 2021; 21:959-976. https://doi.org/10.2478/aoas-2020-0118

34. Liu J, Bai Y, Liu F, Kohn RA, Tadesse DA, Sarria S, Song J. Rumen microbial predictors for short-chain fatty acid levels and the grass-fed regimen in Angus cattle. Animals. 2022; 12:2995. https://doi.org/10.3390/ani12212995

35. Mikulová K, Petrič D, Komáromyová M, Batťányi D, Kozłowska M, Cieslak A, Váradyová Z. Growth performance and ruminal fermentation in lambs with endoparasites and in vitro effect of medicinal plants. Agriculture. 2023; 3:1826. https://doi.org/10.3390/agriculture13091826

36. Resende JCJ, Pratti Daniel JL, Barreto-Vianna ARDC, Vogas Peixoto J, Correia Guimarães G, Fátima Costa SD, Cruz Meirelles FD. Determination of volatile fatty acids clearance in intact ruminal digesta. CES Medicina Veterinaria y Zootecnia. 2019; 14: 8-17.

37. Vargas JE, Andrés S, López-Ferreras L, Snelling TJ, Yáñez-Ruíz DR, García-Estrada C, López S. Dietary supplemental plant oils reduce methanogenesis from anaerobic microbial fermentation in the rumen. Scientific Reports. 2020; 10:1613. https://doi.org/10.1038/s41598-020-58401-z

38. Marques LT, Fischer V, Zanela MB, Ribeiro MER, Stumpf W, Manzke NE. Fornecimento de suplementos com diferentes níveis de energia e proteína para vacas jersey e seus efeitos sobre a instabilidade do leite. Revista Brasileira de Zootecnia. 2010; 39:2724-2730. https://doi.org/10.1590/s1516-35982010001200024

39. Moore JE, Brant MH, Kunkle WE, Hopkins DI. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. Journal of Animal Science. 1999; 77:122–135. https://doi.org/10.2527/1999.77suppl_2122x

40. Konda S, Onodera R, Kanchanasatit E, Boonsaen P, Sawanon S, Nagashima K, Suzuki Y, Koike S, Kobayashi Y. Effect of cashew nut shell liquid feeding on fermentation and microbiota in the rumen of Thai native cattle and swamp buffaloes. Livestock Science. 2019; 226:99-106. https://doi.org/10.1016/j.livsci.2019.06.011

41. Valero MV, Farias MS, Zawadzki F, Prado RM, Fugita CA, Rivaroli DC, Prado IN. Feeding propolis or essential oils (cashew and castor) to bulls: performance, digestibility, and blood cell counts. Revista Colombiana de Ciencias Pecuarias. 2016; 29:33-42. https://doi.org/10.17533/udea.rccp.v29n1a04

42. Carvalho VM, Ávila VAD, Bonin E, Matos AM, do Prado RM, Castilho RA, do Prado IN. Effect of extracts from baccharis, tamarind, cashew nut shell liquid and clove on animal performance, feed efficiency, digestibility, rumen fermentation and feeding behavior of bulls finished in feedlot. Livestock Science. 2021; 244:104361. https://doi.org/10.1016/j.livsci.2020.104361

43. Coutinho DA, Branco AF, Santos GTD, Osmari MP, Teodoro AL, Diaz TG. Intake, digestibility of nutrients, milk production and composition in dairy cows fed on diets containing cashew nut shell liquid. Acta Scientiarum Animal Sciences. 2014; 36:311-316. https://doi.org/10.4025/actascianimsci.v36i3.23512

44. Kang S, Suzuki R, Suzuki Y, Koike S, Nagashima K, Kobayashi Y. Rumen responses to dietary supplementation with cashew nut shell liquid and its cessation in sheep. Animal Science Journal. 2018; 89:1549-1555. https://doi.org/10.1111/asj.13100

45. Russel JB, Wilson DB. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? Journal of Dairy Science. 1996; 79:1503-1509. https://doi.org/10.3168/jds.S0022-0302(96)76510-4

46. Owens FN, Bergen WG. Nitrogen metabolism of ruminant animal: historical perspective; current understanding and future implications. Journal of Animal Science. 1983; 57:498-518. https://doi.org/10.2527/animalsci1983.57Supplement_2498x

47. Li Q, Tu Y, Ma T, Cui K, Zhang J, Diao Q, Bi Y. Effects of two feeding patterns on growth performance, rumen fermentation parameters, and bacterial community composition in Yak calves. Microorganisms. 2023; 11: 576. https://doi.org/10.3390/microorganisms11030576

48. Narabe C, Kamiyama S, Saito M, Boonsaen P, Khongpradit A, Sawanon S, Kobayashi Y. Cashew nut shell liquid potentially mitigates methane emission from the feces of Thai native ruminant livestock by modifying fecal microbiota. Animal Science Journal. 2021; 92:e13614. https://doi.org/10.1111/asj.13614

49. Harmeyer J, Martens H. Aspects of urea metabolism with reference to the goat. Journal of Dairy Science. 1980; 63:1707-1728. https://doi.org/10.3168/jds.S0022-0302(80)83132-8