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Ammoniated sugarcane bagasse associated with cottonseed in sheep diets

P.P.P. Publio, A.J.V. Pires, I.C. Dutra*, M.P. Sousa, G.C. Figueiredo, H.O. Santos, G.R.S. Oliveira, E.P.L. Ferreira, A.F. Santos, P.H.S. Cardoso, F.A. Teixeira and M.L.P. Albuquerque

State University of Southwest of Bahia, BR45700-000 Itapetinga, Bahia, Brazil
*Correspondence: ingriidyduutra@gmail.com

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Abstract. The experiment was carried out in the goat farming sector and forage laboratory at UESB, with the objective of evaluating the association of ammoniated sugarcane bagasse with cottonseed in sheep diets. The experimental period was 21 days, with 17 days for animal adaptation and 4 days for data collection. The test was conducted in a double 4×4 Latin square, in a 2×2 factorial scheme, with the factors being ammoniated sugarcane bagasse at 30 or 50% and cottonseed at 0 or 20% inclusion, in the dry matter of the total diet. The digestibility test took place between the 18th and 21st days of each period. For this purpose, leftovers and feces from each animal were quantified and collected during this period. The interaction was not significant in any of the variables studied for sugarcane bagasse and cottonseed, nor was there any effect for the addition of cottonseed to the diets, for dry matter intake kg day^{-1}, %PC and g per $kg^{0.75}$. The nitrogen balance was positive, as the nitrogen ingested was greater than the nitrogen excreted in urine and feces, indicating that the animal retained protein, meeting its protein requirements. The inclusion of cottonseed resulted in lower digestibility averages. This reduction can be attributed to the lignin content present in the diets studied, since cottonseed has a higher lignin concentration than the other foods analyzed. The use of cottonseed and the increase in the inclusion level of sugarcane bagasse by 50% resulted in an increase in the time spent by animals in feeding, ruminating and chewing activities. Furthermore, a reduction in consumption and rumination efficiency was observed, which correlated with a decrease in dry matter intake. The level of 30% sugar cane bagasse associated with 20% cotton seed promoted greater consumption of dry matter.

Key words: ammonization, digestibility, intake, urea.

INTRODUCTION

Brazil is consolidating its prominent position in the global agricultural scenario, driven by significant production volumes (FAO, 2023). However, the industrialization of plant-based products generates a considerable volume of waste and by-products, estimated at approximately 35% of the original material (Wong et al., 2023). This scenario results in the production of millions of tons of material, many of which still lack fully explored applications and uses.

In this context, recent studies have investigated the potential of residues and by-products as viable alternatives to replace conventional bulky feeds or to act as sources of fiber in the diet of ruminants. The use of these materials may represent a promising strategy to reduce the environmental impact of agribusiness and optimize the use of natural resources (Van Soest, 1994; Detmann et al., 2012).

Although the use of plant by-products in animal feed represents a promising alternative, it is important to recognize that most of these materials present a significant challenge: their high fiber content. This characteristic, often accompanied by a high concentration of lignin, compromises the digestibility of carbohydrates and proteins, in addition to limiting the availability of certain minerals. The lignification of the cell wall, a process that gives rigidity and resistance to the plant structure, hinders the action of digestive enzymes, impairing the release and absorption of nutrients (Filho et al., 2016).

Sugarcane bagasse is a byproduct of sugar and ethanol production, and its availability is directly related to the amount of sugarcane processed in sugar and ethanol plants throughout the country. Overall, Brazil produces a significant amount of sugarcane bagasse each harvest. According to data from the Brazilian Institute of Geography and Statistics, sugarcane production in Brazil reached approximately 700 to 750 million tons per year in recent harvests (IBGE, 2021).

In its chemical and bromatological composition, sugarcane bagasse has as its main characteristics a high content of cell wall constituents, low digestibility and low crude protein content. Despite its nutritional limitations, it is an important source of fiber that can maintain ruminal health. Its low protein content leads to the need for nutritional corrections in diets based on sugarcane bagasse (Melati et al., 2017).

The use of physical, chemical, physicochemical or biological treatments tend to provide greater digestibility and use of the material (Brunerová et al., 2018). Aguilar et al. (2015) state that chemical treatment using urea or anhydrous ammonia is the most common, mainly because it increases the nitrogen content and reduces neutral detergent fiber, improving digestibility. Rocha et al. (2015) highlight that one of the effects of the action of ammonia on the material is the disarrangement of the cell wall components, being more expressive on the fraction of fiber insoluble in neutral detergent, by partially solubilizing the hemicellulose and causing the rupture of bonds between cell wall constituents and phenolic acids, thus occurring the partial depolymerization of lignin and subsequently greater action by ruminal microorganisms.

Some treatments provide satisfactory results for reducing fiber and subsequently improving digestibility. However, they require high implementation costs, making the method unfeasible. Physical and chemical treatments are the types of treatments that are most efficient and economically viable. The use of chemical treatment may promote an increase in the levels of dietary nitrogen in treated agro-industrial by-products, which may have effects on the intermediate metabolism of carbohydrates and proteins, as well as on the consumption and digestibility of nutrients, considering that chemical treatment will provide better availability of nutrients, particularly carbohydrates and proteins.

Unlike sugarcane bagasse, cottonseed is another byproduct resulting from industrialization that has a high protein and energy value. The use of oilseeds in the diet of ruminants has aroused great interest, mainly due to the ease with which these foods, their by-products or even parts of the plant can incorporate oils into the rations, increasing their energy density.

The combination of urea-ammoniated sugarcane bagasse with cottonseed in sheep diets is justified by the search for cost-effective, sustainable nutritional alternatives through the utilization of agro-industrial by-products. Ammoniation enhances the fiber digestibility of sugarcane bagasse by increasing nutrient availability, while cottonseed provides protein, energy, and effective fiber, promoting a more balanced nutrient profile. This association supports improved ruminal fermentation, animal performance, and overall sustainability of production systems, particularly in regions where these residues are readily available. Thus, the combination of ammoniated sugarcane bagasse and cottonseed was evaluated in the formulation of diets for sheep

MATERIALS AND METHODS

Ethics Committee

This study was conducted in strict accordance with the guidelines of Brazilian legislation regulating research involving the use of animals, established by the National Council for the Control of Animal Experimentation (CONCEA). The execution of the experiment was approved by the Ethics Committee on the Use of Animals (CEUA) of the Universidade Estadual do Sudoeste da Bahia, located in Itapetinga, Bahia, Brazil, under protocol number 219/2022.

Experimental description

The experiment was conducted in the Goat and Sheep Farming Department and the Forage and Pastures Laboratory at the State University of Southwest Bahia. Eight female Dorper-Santa Inês crossbred sheep with an average body weight and age of 20 kg ± 2.91 and 120 days ± 20, respectively, were used. The animals were identified, dewormed and confined in a covered shed and housed in individual suspended pens (1.20 m×1.00 m), with slatted floors, equipped with individual feeders and drinkers.

The experimental design adopted was a 4×4 double Latin square, with four diets and four experimental periods, in a 2×2 factorial scheme, with the factors being ammoniated sugarcane bagasse at 30 or 50% and cottonseed at 0 or 20% inclusion in the dry matter of the total diet.

The confinement and data collection period corresponded to four periods of 21 days each, consisting of the initial 17 days intended for the animals to adapt to the diet and the last four days for data collection.

Ammonization and experimental diets

The sugarcane bagasse came from a sugarcane brandy plant at Fazenda Bela Vista, in the municipality of Encruzilhada, BA. The material used was dried in the sun for five days, turned twice a day, and then processed in a forage machine (Nogueira EN-6700) and subsequently homogenized. At the time of ammoniation, the sugarcane bagasse was reconditioned to the original moisture content of 50%, using water together with 5% urea and 1% ground black-eyed peas (source of urease), on a dry matter basis. The urea was diluted in water, together with the homogenized black-eyed peas, and applied to the bulk. The storage was done in the field, in the form of a surface silo, covered with plastic tarpaulin on the bottom and top to prevent the loss of nitrogen from the urea to the soil and the environment, lasting 100 days.

The diets were calculated to meet the nutritional requirements of protein and energy of growing sheep with 20 ± 2.92 kg of body weight and an average daily gain of 200 g day^{-1}, according to NRC (2007).

The chemical and bromatological composition of the foods can be seen in Table 1, the percentage composition of the ingredients of the diets in Table 2, and the bromatological composition of the diets in Table 3.

Table 1. Chemical and bromatological composition of foods based on dry matter

Variable	Ground corn	Soybean meal	Cottonseed	Raw bagasse	Ammoniated bagasse
Dry matter (%)	79.9	83.0	91.3	44.4	45.7
Mineral matter [1]	1.0	6.2	4.1	1.7	3.7
ethereal extract [1]	6.4	1.9	20.4	1.5	3.6
Crude protein [1]	10.8	51.0	22.2	2.5	12.5
NDIP[2]	1.3	1.8	2.6	1.5	2.2
ADP[2]	0.6	1.4	2.0	1.0	0.6
CNFcp[1]	69.3	27.9	13.7	14.8	13.5
NDFcp[1]	12.5	12.9	39.7	79.5	66.8
ADF	2.9	8.3	33.5	59.4	54.3
Hemicellulose[1]	11.0	6.6	9.1	26.6	17.4
Cellulose[1]	0.4	6.7	24.9	46.0	47.1
lignin[1]	2.5	1.6	8.6	13.4	7.1
TDN[1, 3]	88.6	79.4	86.4	47.8	60.4
NDFi[1]	3.7	4.2	21.3	49.8	32.5

[1]Values in percentage of dry matter; [2]Percentage of total protein; NDIP = Neutral detergent insoluble protein; ADP = Acid detergent insoluble protein; CT = Total carbohydrate; CNFcp = non-fibrous carbohydrates corrected for ash and protein; NDFcp = Neutral detergent insoluble fiber corrected for ash and protein; ADF = Acid detergent insoluble fiber; TDN = Total digestible nutrients; NDFi = Neutral detergent indigestible fiber; 3Estimated by NRC (2001).

Table 2. Percentage composition of the diet

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
	30% Bagasse		50% Bagasse	
	0% CSEED	20% CSEED	0% CSEED	20% CSEED
Ammoniated bagasse	30.0	30.0	50.0	50.0
Cottonseed	0.0	20.0	0.0	20.0
Ground corn	52.8	39.7	32.8	19.7
Soybean meal	15.2	8.3	15.2	8.3
Mineral mix [1]	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0

[1]Guaranteed levels per 1,000 g of product: Calcium (Min.) 150.00 g (Max.) 210.00 g; Phosphorus (Min.) 75.00 g; Copper (Min.) 150.00 mg; Cobalt (Min.) 45.00 mg; Iodine (Min.) 1.00 mg; Manganese (Min.) 2.200.00 mg; Selenium (Min.) 3.00 mg; Zinc (Min.) 1.000.00 mg; Fluorine (Max.) 950.00 mg; Sodium (Min.) 110.00 g; Sulfur (Min.) 14.00 g.

Daily individual records of concentrate and roughage offered to the animals were collected, and leftovers after feeding were measured to calculate dry matter intake. The diets were provided ad libitum, twice a day, at 7:00 a.m. and 3:00 p.m., weighing the leftovers and adjusting the amount provided in order to maintain the leftovers at around 10% of the amount provided, with water permanently available to the animals.

Table 3. Bromatological composition of diets

Variable	Diet 1	Diet 2	Diet 3	Diet 4
	30% Bagasse		50% Bagasse	
	0% CSEED	20% CSEED	0% CSEED	20% CSEED
Dry matter (%)	68.5	70.6	61.7	63.8
Mineral matter [1]	4.3	4.6	4.9	5.1
ethereal extract [1]	4.7	7.8	4.2	7.3
Crude protein [1]	17.2	16.7	17.5	17.0
NDIP[2]	1.6	1.8	1.8	2.0
ADP[2]	0.7	0.9	0.7	0.9
CNFcp[1]	44.9	36.6	33.7	25.4
NDFcp[1]	32.4	37.8	45.8	51.2
ADF	19.1	24.8	29.3	35.1
Hemicellulose[1]	12.0	11.9	13.3	13.2
Cellulose[1]	15.3	19.8	24.7	29.2
lignin[1]	3.7	5.0	4.6	5.9
TDN[1, 3]	77.0	77.2	71.3	71.5
NDFi[1]	12.3	15.8	18.1	21.6

[1]Values in percentage of dry matter; [2]Percentage of total protein; NDIP = Neutral detergent insoluble protein; ADP = Acid detergent insoluble protein; CT = Total carbohydrate; CNFcp = non-fibrous carbohydrates corrected for ash and protein; NDFcp = Neutral detergent insoluble fiber corrected for ash and protein; ADF = Acid detergent insoluble fiber; TDN = Total digestible nutrients; NDFi = Neutral detergent indigestible fiber; 3Estimated by NRC (2001).

Chemical analysis

During the experimental period, daily morning samples of the feed offered, refusals, and feces were collected. The samples were placed in properly labeled plastic bags and stored in a freezer until further analysis. At the end of the experiment, a sample consisting of each animal per period was prepared. The samples were pre-dried in a forced ventilation oven at 55 °C for 72 hours. Subsequently, all samples were ground in a Wiley mill, passed through sieves with a 1 mm diameter mesh and examined for chemical composition in the Forage Laboratory of UESB – Itapetinga Campus.

Samples of the offered feed, food residues and feces were subjected to ruminal incubation in duplicate, using non-woven fabric (TNT) bags with 20 mg of dry matter per cm^2. The procedure was performed in the rumen of two crossbred steers, fed a mixed diet, for a period of 288 hours, following the methodology proposed by Valente et al. (2011). After incubation, the remaining material was processed by extraction with neutral detergent to quantify the levels of indigestible neutral detergent fiber (NDF), according to the guidelines of Detmann et al. (2012).

The determination of the chemical composition of the samples, including dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin, 1 was performed following the protocols described by Detmann et al. (2012). The neutral detergent fiber content corrected for ash and protein (NDFcp) was quantified in accordance with the recommendations of Licitra et al. (1996) and Mertens (2002). The analysis of non-protein nitrogen (NNP) was performed using the methodology suggested by Licitra et al. (1996).

The estimation of total carbohydrates (CT) was performed according to the methodology proposed by Sniffen et al. (1992), using the formula:

$$CT = 100 - (\% CP + \%EE + \%MM). \quad (1)$$

The calculation of the non-fibrous carbohydrate content (NFCp), corrected for ash and protein, was performed using the equation described by Weiss (1999):

$$NFCp = 100 - MM - EE - NDFp - CP. \quad (2)$$

For diets that included urea, the NFCp was determined using the equation suggested by Hall et al. (2000):

$$NFCp = 100 - [(\% CP - (\%PBurea + \%urea)) + \%NDFp + \%EE + \%MM] \quad (3)$$

where NDFp represents the crude protein from urea and NDFp represents the neutral detergent fiber corrected for ash and protein.

The calculation of total digestible nutrients (TDN) was performed following the method proposed by Weiss (1999), with the adaptation of the use of neutral detergent fiber (NDF) and non-fibrous carbohydrates (NFC) corrected for ash and protein. The estimation of the total digestible nutrient (TDN) content of the total feeds and diets was performed using the equations described by the NRC (2001), according to the formula:

$$TDN = CPD + 2.25 \text{ x } EED + DNFcpD + NFD \quad (4)$$

where CPD represents the digestible crude protein, EED the digestible ether extract, NDFcpD the digestible neutral detergent fiber and NFD the digestible non-fibrous carbohydrates.

Consumption and digestibility of dry matter and nutrients

The feed consumption of each animal (feed supplied - leftovers) was recorded daily to determine dry matter intake (DMI). To determine the apparent digestibility coefficient, feces were sampled from each animal from the 18^{th} to the 21^{st} day of each period, twice a day (morning and afternoon). The collection was made directly from the rectal ampoule of the animals, and then frozen in a freezer at -10 °C for later chemical composition analyses (Detmann et al., 2012).

The digestibility of the diet components was determined from the internal indicator indigestible neutral detergent fiber (NDFi), which was used to estimate fecal production and from this the digestibility coefficients were calculated. The feed samples (bagasse and concentrate), leftovers and feces were incubated in duplicate (20 mg DM cm^{-2}) for 288 hours in the rumen of an adult bovine. The bags were made of non-woven fabric (TNT), with dimensions of 4×5 cm (Casali et al., 2008). The amount of sample incubated was 1.0 g.

After the incubation period, the bags were removed, washed in running water, and the remaining material was taken to a forced ventilation oven at 55 °C for 72 hours. They were then removed from the oven, stored in a desiccator and weighed, obtaining the indigestible dry matter (DMi) levels from the residue. Next, the TNT bags containing MSi were placed in plastic jars with screw caps, 30 mL of neutral detergent were added per bag, and boiled for one hour using the autoclave. They were then washed with hot water and acetone, dried in an oven and weighed, according to the previous procedure, with the new residue being considered as indigestible neutral detergent fiber (NDFi).

The NDFi was used to determine the production of fecal dry matter (PDMF), using the following formula:

$$PDMF = \frac{(\text{quantity of NDFi consumed x } 100)}{\text{concentration of NDFi in feces}} \quad (5)$$

The digestibility coefficient (CD) of each nutrient was calculated by:

$$\mathrm{CD} = \frac{(\text{nutrient consumed } - \text{ nutrient excreted})}{\text{nutrient consumed x 100}} \quad (6)$$

Ingestive behavior

To assess ingestive behavior, animals were subjected to visual observations on days 18 and 19 of each experimental period, at 10-minute intervals, over a 24-hour period. Nocturnal observation was performed under artificial lighting, with a prior 3-day adaptation period. The variables recorded included feeding time, rumination, and idleness. Additionally, three observations per animal were performed in three different periods (morning, afternoon, and evening), recording the number of chews per ruminal bolus and the time spent ruminating each bolus, using digital stopwatches. Observations were conducted by trained observers, strategically positioned to minimize disturbance to the animals.

For the analysis of behavioral variables related to feed efficiency (g DM and NDF hour^{-1}), rumination efficiency (g DM and NDF bolus^{-1}) and average DM and NDF consumption per feeding period, the voluntary consumption recorded on the 18^{th} day of each experimental period was considered, from which a composite sample was prepared. Feeding and rumination efficiencies were calculated using the following equation:

$$\mathrm{EALDM} = \mathrm{CDM} \;/\; \mathrm{TAL}, \quad (7)$$

$$\mathrm{EALNDF} = \mathrm{CNDF} \;/\; \mathrm{TAL} \quad (8)$$

where EALDM (g DM consumed h^{-1}); EALNDF (g NDF consumed h^{-1}) = feed efficiency; CDM (g) = daily dry matter intake; CNDF (g) = daily NDF intake; TAL = time spent daily feeding.

$$\mathrm{EALNDF} = \mathrm{CNDF} \;/\; \mathrm{TAL}, \quad (9)$$

$$\mathrm{ERUDM} = \mathrm{CDM} \;/\; \mathrm{TRU} \quad (10)$$

where ERUDM (g DM ruminated h^{-1}); ERUNDF (g NDF ruminated h^{-1}) = rumination efficiency and TRU (h day^{-1}) = rumination time.

$$\mathrm{TMT} = \mathrm{TAL} + \mathrm{TRU} \quad (11)$$

where TMT (min day^{-1}) = total chewing time.

The number of boluses ruminated daily was obtained as follows: total rumination time (min) divided by the average time spent ruminating a bolus. The concentration of DM and NDFcp in each ruminated bolus (g) was obtained by dividing the amount of DM and NDFcp consumed (g day^{-1}) in 24 hours by the number of boluses ruminated daily.

The number of feeding, rumination and idle periods were counted by the number of activity sequences observed in the spreadsheet. The average daily duration of these activity periods was calculated by dividing the total duration of each activity (feeding, rumination and idle in min day^{-1}) by its respective number of periods.

During the evaluation of ingestive behavior, the average water consumption of each animal (WC) was quantified, which was obtained by the difference between the water offered and the excess. Evaporation was measured using buckets similar to those used to supply water, distributed within the experimental area, which was obtained by the difference in water volume over a 24-hour period. To calculate colloidal water intake (CWI), total water intake (TWA) and water intake efficiency (WIE), the following equations were used:

$$\text{CWI} = \frac{(\text{CDM kg x dietary water})}{(100 - \text{dietary water})} \quad (12)$$

$$\text{TWA} = \text{WC} + \text{CWI} \quad (13)$$

$$\text{WIE} = \text{IAT} / \text{CDM kg.} \quad (14)$$

Physiological parameters

On the 20th day of each period, spot urine collection was performed, through spontaneous urination of the animals, approximately four hours after providing the morning diet. The samples were collected through collectors prepared with plastic bags that were attached to the animals' prepuce. Immediately afterwards, the material obtained from each animal was filtered through gauze, where a 10 mL aliquot of the sample was separated and diluted in 40 mL of 0.036N H2SO4 according to Valadares et al. (1999) and stored in identified plastic bottles with screw caps. Dilution with the sulfuric acid solution occurred to maintain the pH below 3, with the aim of avoiding bacterial destruction of urinary purine derivatives. However, to determine uric acid, urine collection did not require the solution, a fact explained by the fact that its precipitation did not occur.

The samples were frozen at -20 °C and subjected to analysis to quantify the urinary concentrations of urea, nitrogen, creatinine, allantoin, uric acid, xanthine and hypoxanthine.

Urine volume was estimated using the value of 20.37 mg kg^{-1} BW (Santos, 2017) as a reference for crossbred lambs, using the following formula:

$$\text{VU (L)} = \frac{20.37 \text{ mg kg}^{-1} \text{ x PC}}{\text{CCT (mg L}^{-1})} \quad (15)$$

where CCT = creatinine concentration (mg L^{-1}) in the urine sample (spot collection); VU = average urinary volume obtained on the days of urine collection; BW = animal body weight (kg).

Blood was collected from the jugular vein on the 21st day of the experimental period, approximately four hours after the morning meal was provided, using 5 mL tubes (VacutainerTM) with EDTA. The blood samples were then transferred to the laboratory, centrifuged at 3,500 rpm for 10 minutes, and the plasma was stored in eppendorf tubes and kept frozen (-20 °C) until the analyses were performed.

Urine creatinine and uric acid concentrations, as well as urinary and plasma urea, were determined using commercial kits from the Bioclin® brand. Urea values were converted to urea nitrogen by multiplying the values obtained by the conversion factor 0.4667. Urine allantoin, uric acid, xanthine and hypoxanthine levels were estimated by colorimetric methods, following the specifications described by Chen & Gomes (1992). Total urinary nitrogen was determined by the Kjeldhal method, as established by Silva & Queiroz (2002).

Nitrogen balance (N-retained, g day^{-1}) was calculated with:

$$\text{N} - \text{retained} = \text{N ingested (g)} - \text{N in feces (g)} - \text{N in urine (g)} \quad (16)$$

Total purine excretion (TP) was estimated by the sum of the amounts of allantoin, uric acid, xanthine and hypoxanthine excreted in urine. The amount of microbial purines absorbed (mmol day^{-1}) was calculated from the total purine excretion (mmol day^{-1}), using the equations proposed by Chen & Gomes (1992), specific for sheep:

$$\text{TP (mmol day}^{-1}) = 0.84\text{PA} + (0.150 \text{ x PV}^{0.75}\text{x}^{e-0.25} \text{ PA}). \quad (17)$$

where TP corresponds to total purines (mmol day^{-1}) and PA are the absorbed purines (mmol day^{-1}).

The intestinal microbial nitrogen flow (g NM day^{-1}) was estimated from the amount of absorbed purines (mmol day^{-1}), according to the equation by (Chen & Gomes, 1992):

$$\text{NM (g day}^{-1}) = \frac{70 \text{ x PA}}{0.83 \text{ x } 0.116 \text{ x } 1{,}000} \quad (18)$$

Assuming a value of 70 for the nitrogen content in purines (mg mmol^{-1}); 0.83 for the intestinal digestibility of microbial purines; and 0.116 for the NPURINE:NTOTAL ratio in bacteria.

Statistical analysis

The data were analyzed using the SAS OnDemand for Academics statistical software (SAS Institute Inc., 2023). An analysis of variance (ANOVA) was performed, considering as sources of variation the levels of sugarcane bagasse inclusion, the presence of cottonseed, and their interaction. Treatment means were compared using the *F*-test at a 5% significance level, according to the statistical model:

$$Y_{ij(k)} = m + L_i + Cj + t_k + E_{ij(k)} \quad (19)$$

where $Y_{ij(k)}$ = the observed value of the variable; m = the mean of all experimental units for the variable under study; L_i = effect of row i; C_j = effect of column j; t_k = effect of treatment k; and $E_{ij(k)}$ = experimental error (residue).

RESULTS AND DISCUSSION

The interaction between hydrolyzed sugarcane bagasse (HCB) and cottonseed was not significant ($p > 0.05$) for any of the variables studied in (Table 4). However, the inclusion level of HCB in 30% of the diet showed a significant effect ($p < 0.05$), which favored greater intake of dry matter (DMI) (kg day^{-1}, % BW, g kg^{-1} BW 0.75), crude protein (kg day^{-1}, g kg^{-1} BW 0.75), ether extract (kg day^{-1}) and total digestible nutrients (kg day^{-1}). On the other hand, there was a lower intake of NDFcp (kg day^{-1}, % BW), a fact that can be attributed to the lower fiber content of the treatments mentioned.

Dry matter intake in relation to body weight (% BW) averaged 3.37% in the case of the 30% BCH level. These results are in accordance with the estimates provided by the NRC (2007) for lambs with an average daily gain of 200 g, indicating an average dry matter intake of 0.910 kg per day, which represents 3.3% of body weight. It is relevant to note that in tropical environments, dry matter intake by sheep is normally in the range of 30 to 50 g per kg of body weight, as reported by (Pereira et al., 2014).

When using a higher level of BCH (50%) and including cottonseed in the diets, an increase in the NDFcp levels in the diets was observed, as evidenced in (Table 3). However, no statistically significant effects ($p > 0.05$) were observed in the NDFcp intake (kg day^{-1}) related to BCH or cottonseed.

It is important to highlight that dry matter intake (DMI) is predominantly influenced by physical filling in diets with high NDF content, while in diets with high energy density, chemostatic mechanisms play a more prominent role (Mertens, 1994). In the context of this study, the low nutritional quality of the material and the high fiber content in the diet containing 50% sugarcane bagasse (BCH) may have limited feed intake, likely due to physical factors such as rumen fill. The elevated proportion of indigestible fiber increases rumen retention time, reducing the rate of passage and triggering satiety signals, which limit voluntary intake in ruminants. On the other hand, the lower level of inclusion of BCH in the diet, with 30% and 70% concentrate, may have favored consumption due to acceptability by the animals and the greater energy density of the diet, since concentrated foods provide a significant amount of nutrients to meet the metabolic demands of the animals. As highlighted by Pazdiora et al. (2020), a diet with a lower amount of indigestible fiber can be advantageous for sheep consumption in several situations, due to greater energy density, better use of nutrients, stimulation of consumption and less rumen filling.

CP intake averaged 0.155 (kg day^{-1}) and 13.22 (g kg^{-1} $BW^{0.75}$) (Table 4). The similar DM intake between the diets allowed for similar CP intake. The values obtained in this study for this variable were above those recommended by the NRC (2007), with CP intake of 0.148 kg day^{-1}. This difference can be justified by the selection of animals for cottonseed and concentrate, observed during feeding. These foods have more than twice the protein value of ammoniated sugarcane bagasse (Table 1), thus allowing for high CP intake by the animals.

Table 4. Dry matter and nutrient intake of sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	*Value - p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
Consumption (kg day^{-1})								
DM	0.910	0.699	0.834	0.773	0.030	< 0.0001	0.1372	0.7500
CP[1]	0.172	0.140	0.161	0.150	0.004	0.0002	0.1337	0.9865
EE[1]	0.038	0.032	0.026	0.044	0.002	0.0042	< 0.0001	0.4296
NDFcp[1]	0.171	0.192	0.172	0.197	0.008	0.0787	0.0894	0.5525
CNF	0.484	0.285	0.426	0.342	0.023	< 0.0001	0.0014	0.8936
TDN[1]	0.670	0.481	0.604	0.547	0.024	< 0.0001	0.0695	0.5518
Consumption (% body weight)								
DM	3.37	2.62	3.07	2.92	0.092	< 0.0001	0.3010	0.1951
NDFcp	0.63	0.74	0.63	0.74	0.025	0.0239	0.0297	0.2276
Consumption (g kg^{-1} $BW^{0.75}$)								
DM	76.70	59.51	69.99	66.21	0.008	< 0.0001	0.2470	0.2829
CP	14.51	11.94	13.49	12.95	0.001	< 0.0001	0.2996	0.3418

1% of dry matter; DM – dry matter; CP – crude protein; EE – ether extract; NDFcp – neutral detergent fiber corrected for ash and protein; CNFcp – non-fibrous carbohydrates corrected for ash and protein; TDN – total digestible nutrients; SEM = standard error of the mean. $P > 0.05$ not significant by *F*-test.

The consumption of ether extract (EEC), expressed in kilograms per day (kg day^{-1}), showed a significant effect ($p < 0.05$) in relation to the use of BCH and the use of cottonseed. The lower inclusion of BCH, corresponding to 30% of the dry matter (DM) of the diet, resulted in a higher consumption of dry matter, directly reflecting in a higher consumption of EE. This increase in EE consumption is correlated with a lower intake of neutral detergent fiber (NDF), since sugarcane bagasse has a high concentration of fibers. In addition, it was observed that the consumption of EE was higher in the treatments in which cottonseed was included. This result can be explained by the bromatological composition and energy density of cottonseed (Table 1), which allowed a higher content of EE in the diets to which it was added (Table 3).

Limitations in dry matter intake were not due to the lipid content of the diet, since there was no significant effect ($p > 0.05$) on dry matter intake (kg day^{-1}) under the use of cottonseed. The inclusion of cottonseed led to higher average ether extract (EE) concentrations in the diets, exceeding the upper limit of the recommended dietary EE range (5 to 7%). This range is known to be adequate for sheep and will not result in a reduction in intake, either due to regulatory mechanisms that control feed intake or the limited capacity of ruminants to oxidize fatty acids (Palmquist & Mattos, 2011).

A study conducted by Ferro (2014) to evaluate the use of common bean residue in sheep feeding demonstrated lower EE intake by sheep fed the highest levels of common bean residue inclusion, and this reduction in EE intake was attributed to the lower concentration of this nutrient in common bean residue when it replaced cottonseed meal, as included in diets for feedlot sheep. Correia et al. (2014) did not observe the influence of diets with different lipid levels on intake in sheep fed canola grains, canola meal and canola meal, which presented, respectively, levels of 8.40%, 5.85% and 4.25% of ether extract (EE) in the diets. On the other hand, Fiorentini et al. (2013) stated that the mechanisms by which dietary lipid levels affect dry matter (DM) intake, although unclear, may include the effect of fat on ruminal fermentation, intestinal motility, diet acceptability, fat oxidation in the liver, chain length, and degree of unsaturation.

The consumption of non-fibrous carbohydrates (NFC) showed an average value of 0.384 kg day^{-1} (Table 4). A significant effect ($p < 0.05$) related to the higher level of BCH and inclusion of cottonseed was observed in the consumption of this nutrient (kg day^{-1}) among the diets, which can be justified by the levels of neutral detergent fiber corrected for protein (NDFcp) present in the diets (Table 3).

The intake of total digestible nutrients (TDN) showed an average of 0.575 kg per day as detailed in (Table 4). The similar intake of dry matter (DM) among the different diets contributed to the uniformity in CP intake. Notably, the crude protein (CP) intake values obtained in this study were close to the recommendations of the NRC (2007), which establish a daily CP intake of 0.148 kg. This similarity can be attributed to the animals' preference for cottonseed and/or concentrate, evidenced during the feeding period. These foods have a protein content twice as high as that of BCH, as shown in (Table 1), which allowed an adequate CP intake by the animals.

Regarding the digestibility of DM and nutrients, no significant effect ($p > 0.05$) was observed for the interaction between BCH and cottonseed. The use of BCH provided a significant effect ($p < 0.05$) on the digestibility coefficient of neutral detergent insoluble fiber (CDNDFcp), non-fibrous carbohydrates (CDCNFcp) and total digestible nutrients (CDNDT), with means of 52.48; 80.22 and 71.47, respectively. Cottonseed promoted a significant effect ($p < 0.05$) on the digestibility coefficient of dry matter (CDDM), neutral detergent insoluble fiber (CDNDFcp), non-fibrous carbohydrates (CDCNFcp) and ether extract (CDEE), with means of 69.41; 52.48; 80.22 and 68.14, respectively (Table 5).

Table 5. Nutrient digestibility coefficient (CD) and total digestible nutrients (TDN) in sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	*Value - p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
CDDM	71.3	67.4	72.0	66.7	1.133	0.0511	0.0102	0.2239
CDCP	68.9	69.6	70.9	67.7	1.698	0.8377	0.3609	0.2526
CDNDFcp	48.2	56.7	55.7	49.2	1.850	0.0078	0.0339	0.4983
CDNFCcp	84.3	76.1	82.5	77.9	1.361	0.0010	0.0390	0.1011
CDEE	68.0	68.2	60.7	75.5	2.595	0.9361	0.0004	0.6170
$NDT_{observado}$	74.3	68.6	72.1	70.8	1.224	0.0146	0.5498	0.3106

DM – Dry matter; CP – crude protein; EE – ether extract; NDFcp – neutral detergent fiber corrected for ash and protein; NFCcp – non-fibrous carbohydrates corrected for ash and protein; SEM = standard error of the mean. $P > 0.05$ not significant by F-test.

The use of cottonseed demonstrated a significant effect ($p < 0.05$) on the CDMS. Therefore, the inclusion of cottonseed resulted in lower digestibility averages. This reduction can be attributed to the lignin content present in the diets studied, since cottonseed has a higher lignin concentration compared to the other foods analyzed (Table 1). As pointed out by Van Soest (1994), lignin is the main isolated factor responsible for the reduction of the degradability of a food. Filho (1985) states that soluble carbohydrates have digestibility close to 90% and structural carbohydrates of approximately 50%. Thus, the increase in fiber in the diet may have caused the reduction in CDDM. Thus, the substrates from diets containing cottonseed were partially degraded by the ruminal microbiota, resulting in part of the dry matter becoming unavailable for fermentation.

The inclusion of sugarcane bagasse and/or cottonseed did not demonstrate a significant influence on the crude protein digestibility coefficient (CDCP) ($p > 0.05$), with an average of 69.3%. This result indicates that the protein fraction present in these foods did not affect ruminal fermentation by microorganisms. When the supply of this nutrient is limited, efficient synchronization between energy and protein does not occur, resulting in energy loss and reduced nutrient degradation due to the decrease in microbial flora.

Studies involving the inclusion of cottonseed or sugarcane bagasse in the diet of sheep and dairy cows did not demonstrate significant changes in CDPB, with values ranging from 69.7% to 87.2% (Murta et al., 2011; Soares, 2017; Jesus, 2020).

The digestibility coefficient of neutral detergent insoluble fiber corrected for ash and protein (CDNDFcp) showed a significant value ($p < 0.05$), with lower averages observed in diets that included cottonseed. This behavior in relation to this variable can be justified by the same considerations previously discussed in relation to the coefficient of dry matter digestibility (DCDM). In addition, the presence of the indigestible neutral detergent fiber (NDF) fraction may have contributed, since its concentration is inversely related to digestibility. This fraction is eliminated from the rumen only through passage, and is expected to have a significant impact on ruminal repletion capacity (Oliveira et al., 2011). Therefore, as the iNDF content increases (as indicated in Table 1), fiber digestibility decreases, which justifies the lower digestibility of NDFcp present in diets with higher levels of sugarcane bagasse (50%) and inclusion of cottonseed (20%).

The NFC digestibility coefficient was significantly ($p < 0.05$) by BCH and cottonseed in the diet, with an average of 80.2%. The digestion of non-fibrous carbohydrates (NFC) in the rumen is highly desirable, considering the evolutionary adaptation of ruminants for the efficient use of these substrates as the main source of energy for ruminal microorganisms. The fermentation of NFC generates volatile fatty acids, which are essential for the animal's energy metabolism, and promotes the synthesis of microbial protein, which, when digested in the small intestine, represents a fundamental source of amino acids for the animal. This process optimizes the conversion of ingested nutrients, contributing to feed efficiency and productive performance of ruminants, provided there is an adequate balance between the fermentation rate and the maintenance of ruminal homeostasis. In diets containing 30% sugarcane bagasse, CDNFCcp showed higher averages compared to the other treatments. This value can be explained by the higher fraction of corn present. Corn contains a high percentage of NFC and when it is used in large quantities, this constituent increases, since its fractions A (sugars and organic acids) and B1 (pectin and starch) are rapidly degraded in the rumen.

Due to the high content of ether extract present in cottonseed and its high digestibility, a significant effect ($p < 0.05$) on the consumption of ether extract (EE) and a higher digestibility coefficient of this nutrient were observed in diets in which cottonseed was used. It is important to emphasize that EE does not constitute a nutritionally homogeneous fraction, which results in a variation in the digestibility of this component between different foods (NRC, 2007).

The observed TDN showed a significant difference ($p < 0.05$) between the BCH levels, when the 30% level was used, which showed greater digestion in relation to the 50% level in the diet. This result is justified by the greater participation of corn in this diet, since this food has the highest TDN content compared to bagasse (Table 1) and, therefore, presented a high value in the composition of the experimental diet (74.3%), which possibly favored its greater digestibility. However, the observed TDN presented values close to the estimated TDN of the diets, according to the NRC (2007).

In the evaluation of water consumption, a significant effect ($p < 0.05$) was observed only for the variable colloidal water intake (IWC) (Table 6).

Table 6. Water intake of sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	*Value - p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
IWD	2.28	2.15	2.18	2.25	0.137	0.5508	0.7409	0.2188
IWC	0.45	0.51	0.50	0.47	0.032	0.0233	0.2049	0.2428
IWT	2.74	2.67	2.68	2.72	0.140	0.7446	0.8525	0.1763
IA%PC	10.43	10.03	9.89	10.58	0.626	0.6417	0.4248	0.0686

IWD – drinking water intake; IWC – colloidal water intake; IWT – total water intake SEM = standard error of the mean. $P > 0.05$ not significant by *F*-test.

The relationship between water intake and food intake is influenced by a variety of factors, including food composition, environmental conditions such as temperature, and production requirements. According to Neto et al. (2016), colloidal water intake is directly influenced by moisture content as well as nutrients present in the food.

Castro et al. (2020), studying the inclusion of different levels of cottonseed (0, 8, 16 and 24%) in the diet of confined sheep, concluded that the voluntary water intake of sheep was not influenced by the different levels of cottonseed. According to Forbes (1968), water intake is two to three times greater than DM intake, increasing when animals are fed diets rich in protein and/or mineral; in contrast, foods with low DM content tend to reduce water intake. The use of cottonseed did not affect water intake, a result possibly explained by the similarity of the chemical composition of the experimental diets, especially in terms of DM and CP (Table 3). Furthermore, the lack of effect of the use of cottonseed on the intake of DM and other nutrients may have contributed to the water intake results.

The interaction between sugarcane bagasse and cottonseed was not significant ($p > 0.05$) for any of the variables studied under the ingestive behavior of the animals. However, significant differences ($p < 0.05$) were observed for the use of cottonseed under the times allocated to feeding (ALM), rumination (RUM) and idleness (OCIO) activities and a significant effect ($p < 0.05$) between the BCH levels for rumination times (RUM) (Table 7). Result explained by higher energy density and fiber content of the diets, corroborating the results obtained in the consumption and digestibility coefficients of the nutrients studied.

Table 7. Total time spent on feeding, rumination and idleness activities of sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	Value - *p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
feeding	235.00	227.50	211.25	251.25	11.31	0.6843	0.0408	0.4997
rumination	409.38	466.25	403.13	472.50	17.74	0.0477	0.0184	0.2317
idleness	795.63	746.25	825.63	716.25	24.90	0.2251	0.0123	0.2609

ALM, RUM and OCIO in minutes per animal day^{-1}. SEM = standard error of the mean. $P > 0.05$ not significant by F-test.

Rumination time is a variable of ingestive behavior influenced by the nature of the diet, since the higher the fiber content, the longer the time spent ruminating. Rumination time is highly correlated with NDF consumption. According to Van Soest (1994), an increase in NDF content promotes an increase in rumination time due to the greater need for fiber processing.

As shown in (Table 7), the period dedicated to idle activity was longer compared to other activities. This observation is justified by the fact that the diets tested presented a significant proportion of concentrate, which contains digestible nutrients capable of readily meeting the maintenance and meat production needs of the animals. As a result of this state of satiety provided by the diets, the sheep demonstrated a longer idle time, possibly due to the inhibition of the hunger center, which, in turn, culminated in a reduction in the periods of feeding and rumination.

The rumination period was in an intermediate position among the other activities. This occurs because fiber consumption is directly related to the time dedicated to rumination, since fiber requires greater processing in the rumen (Missio et al., 2010). Almeida et al. (2018), working with levels of inclusion of sugarcane bagasse in the DM of the diet of dairy cows (0, 45, 50, 55 and 60%), concluded that rumination time and feeding and rumination efficiency decreased, while idle time increased linearly with the inclusion of sugarcane bagasse in the diets, which corroborates the results found in the present study, demonstrating the physical effect (filling) exerted by the NDF content in the diet.

Total chewing time showed a significant effect ($p < 0.05$) between cottonseed levels, while the number of chews per cud showed a significant effect ($p < 0.05$) between sugarcane bagasse levels (Table 8).

Table 8. Total chewing time (TCT), number of chews per cud (NCC), time per cud (TFC), number of cuds per day (NCCD) of sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	Value - *p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
TCT	644.38	693.75	614.38	723.75	24.90	0.2251	0.0123	0.2609
NCC	45.07	48.46	46.19	47.34	1.19	0.0616	0.5063	0.0587
TFC	63.00	71.24	65.06	69.18	1.62	0.0012	0.0710	0.3238
NCCD	550.18	598.23	538.17	610.24	26.76	0.2217	0.0738	0.9934

TCT in minutes, NCC in seconds per animal day^{-1}, TFC and NCCD in numbers. SEM = standard error of the mean. $P > 0.05$ not significant by *F*-test.

The higher TMT can be explained by the selection of animals when feeding and the higher NDF content of diets containing cottonseed, which corroborates the results found in (Table 7).

MBN is directly influenced by dry matter intake and fiber content in the diet. Diets containing higher amounts of concentrate tend to reduce NMB due to the rapid degradation of soluble carbohydrates in the ruminal environment, as indicated by (Mendes et al., 2010). Thus, the significant effect on NMB ($p < 0.05$) can be explained by the difference in concentrate in the diets, since diets with 30% sugarcane bagasse presented lower NDF levels and higher amounts of NFC and TDN (Table 3).

Regarding the number of periods (Table 9), the number of feeding (NFP), rumination (NPR) and idle (NPI) periods showed a difference ($p < 0.05$) for the use of sugarcane bagasse.

Table 9. Number of periods of feeding (NFP), rumination (NPR) and idle (NPI) and time per period of feeding (TFP), rumination (TPR) and idle (TPI) of sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	Value - *p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
NFP	10.19	8.19	9.19	9.19	0.463	0.0251	1.0000	0.8803
NPR	17.38	14.56	15.88	16.06	0.547	0.0157	0.8608	0.5226
NPI	24.31	21.06	22.94	22.44	0.563	0.0056	0.6343	0.4082
TFA	23.58	28.50	23.47	28.61	1.381	0.0620	0.0523	0.5234
TPR	24.14	32.09	25.78	30.45	1.347	0.0007	0.0268	0.3610
TPI	32.92	37.23	38.02	32.13	2.113	0.3078	0.1684	0.2025

NFA, NPR and NPI: number of periods; TPA, TPR and TPI: time per period, in minutes per period. SEM = standard error of the mean. $P > 0.05$ not significant by *F*-test.

The number of periods was influenced by sugarcane bagasse. This fact is explained by the composition of the diet (Table 3) and selection of animals, since diets with lower bagasse content (30%) promoted higher DMI, therefore higher passage rate, which favored a greater number of periods for the variables studied. A result also found by Pereira (2015) and Públio (2018), where cattle on pasture that received a greater supply of nutrients via supplement presented a greater number of feeding, rumination and idle periods.

The time per rumination period showed a significant effect ($p < 0.05$) on the use of sugarcane bagasse and cottonseed. The NDF content is possibly directly related to this result, where treatments with a lower level of bagasse (30%) showed a reduction in NDF consumption and a higher consumption of NFC, as can be seen in (Table 4). This result corroborates those reported by Mertens (1994), where, as the percentage of concentrate in the diet increases, there is a reduction in the effectiveness of dietary fiber, as a result of the particle size of the diet consumed. A diet with smaller particle size reduces the time per rumination period as a result of the reduced need for rumination.

The interaction between sugarcane bagasse and cottonseed was not significant ($p > 0.05$) for any of the variables studied on feed and rumination efficiency (Table 10).

Table 10. Feeding efficiencies of dry matter (EFDM), neutral detergent fiber (EFNDF), crude protein (EFCP), ether extract (EFEE) and non-fibrous carbohydrates (EFCNF) and rumination efficiencies of dry matter (ERDM), neutral detergent fiber (ERNDF), crude protein (ERCP), ether extract (EREE) and non-fibrous carbohydrates (ERCNF) of sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	Value - *p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
EFDM	0.245	0.211	0.260	0.196	0.016	0.2703	0.0465	0.3268
EFNDF	0.046	0.059	0.055	0.050	0.004	0.1286	0.5173	0.2384
EFCP	0.046	0.042	0.050	0.038	0.003	0.4847	0.0506	0.3338
EFEE	0.010	0.009	0.008	0.011	< 0.001	0.4535	0.0552	0.5737
EFCNF	0.130	0.087	0.131	0.086	0.008	0.0068	0.0059	0.4188
ERDM	0.136	0.099	0.135	0.101	0.007	0.0091	0.0166	0.3828
ERNDF	0.026	0.028	0.028	0.025	0.001	0.5779	0.4856	0.2126
ERCP	0.026	0.020	0.026	0.020	0.001	0.0252	0.0173	0.4187
EREE	0.006	0.004	0.004	0.006	< 0.001	0.0239	0.0076	0.3250
ERCNF	0.073	0.041	0.069	0.045	0.004	< 0.001	0.0010	0.5940

EFDM, EFNDF, EFCP, EAEE and EFCNF: in kg MS hour^{-1}, kg DNF hour^{-1}, kg PB hour^{-1}, kg EE hour^{-1} and kg CNF hour^{-1} respectively; ERDM, ERNDF, ERCP, EREE and ERCNF: in kg MS hour^{-1}, kg DNF hour^{-1}, kg PB hour^{-1}, kg EE hour^{-1} and kg CNF hour^{-1}, respectively. SEM = standard error of the mean. $P > 0.05$ not significant by *F*-test.

Feeding time (Table 7) varied between cottonseed levels and DM intake was reduced with higher bagasse levels (Table 4), resulting in lower feed efficiency kilogram of dry matter per hour (Table 10), with a significant effect ($p < 0.05$) for the use of cottonseed, showing lower efficiency at higher levels of sugarcane bagasse use. This response is related to the composition of the diet; mainly the composition and NDF content of the cottonseed and its acceptability by the animals. The bagasse had long particles which caused difficulty in grasping and low acceptability, causing the animals to spend time selecting and consuming the diet.

The non-fibrous carbohydrate feed efficiency (NFFE) showed a significant effect ($p < 0.05$) in relation to the two factors analyzed, sugarcane bagasse and cottonseed. This observation can be justified by the composition of the diets, since the diets containing 30% sugarcane bagasse presented a greater inclusion of concentrated feeds. This resulted in an increase in the concentration of non-fibrous carbohydrates in the diet, since concentrates are notably richer in non-fibrous carbohydrates when compared to the other feeds in the diet, such as cottonseed and sugarcane bagasse.

When these concentrates are added in larger quantities to the diet of animals, the total amount of non-fibrous carbohydrates in the feed increases significantly. These non-fibrous carbohydrates are more easily digested by the animals, providing a readily available source of energy. This can improve feed efficiency and promote greater weight gain in animals such as cattle, sheep or goats, depending on the context in which this diet is used. Therefore, the use of concentrates in larger quantities in the animal diet can result in an increase in the content of non-fibrous carbohydrates, which can be beneficial in situations where a greater energy supply is desired, such as in meat, dairy or other animal production systems.

The rumination efficiency of dry matter (ERDM), crude protein (ERCP), ether extract (EREE) and non-fibrous carbohydrates (ERCNF) showed a significant effect ($p < 0.05$). Rumination efficiency is an important factor to control the use of low-digestible feeds. Generally, these variables are influenced by DM and NDF intake. Almeida et al. (2018) observed greater rumination efficiency when animals consumed larger amounts of these nutrients, a behavior similar to that observed in this study.

The use of cottonseed and the increase in the inclusion level of sugarcane bagasse by 50% resulted in an increase in the time spent by the animals in feeding, ruminating and chewing activities, expressed in minutes per kilogram of dry matter (min kg^{-1} DM). In addition, a reduction in the efficiency of consumption and rumination was observed, expressed in kilograms of dry matter per hour (kg DM $hour^{-1}$), which was correlated with the decrease in dry matter intake, as indicated in (Table 4). This behavior is related to the greater selectivity of the diet and the increase in the times of feeding and ruminating activities (Table 7). These changes can be attributed to the introduction of whole cottonseed, which has the ability to increase the content of physically effective neutral detergent insoluble fiber in the diet, resulting in a greater time dedicated to chewing, as suggested by (Pires et al., 1997). Filho et al. (2016) using sugarcane bagasse ammoniated with urea obtained a significant effect on the amount of NDF and an improvement in the NDF profile, an effect similar to that found in this study.

None of the variables studied (Table 11) showed a significant effect ($p < 0.05$) for the interaction studied. No significant effect ($p > 0.05$) was found for fecal-N, urinary-N and urine urea nitrogen (NUU) between the inclusion levels of sugarcane bagasse and cottonseed.

Table 11. Apparent nitrogen balance, urinary urea nitrogen excretion (NUU), plasma urea nitrogen concentration (NUP) and plasma creatinine (CCP) in sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagasse		CSEED		SEM	Valor - *p*		
	30%	50%	0%	20%		Bagasse	CSEED	Bagasse ×CSEED
g day^{-1}								
N-ingested	27.68	22.57	25.98	24.27	0.767	0.0002	0.1337	0.9865
N-fecal	8.68	6.76	7.57	7.86	0.547	0.0581	0.7651	0.3966
N- urinary	1.58	1.50	1.44	1.64	0.143	0.7886	0.4955	0.6136
N- retained	17.43	14.31	16.97	14.77	0.621	0.0140	0.0703	0.3991
NUU	7.46	7.51	7.54	7.43	0.533	0.8635	0.9081	0.0575
mg dL^{-1}								
NUP	20.67	24.80	23.39	22.09	0.726	0.0076	0.3559	0.7033
CCP	0.57	0.66	0.65	0.57	0.022	0.0150	0.0300	0.4425

SEM = standard error of the mean. $P > 0.05$ not significant by *F*-test.

The biochemical composition of blood corresponds to the balance between the input, output and metabolism of nutrients in animal tissues. This balance is regulated by complex metabolic-hormonal mechanisms that play a fundamental role in maintaining homeostasis. The absence of homeostatic balance can result in a reduction in zootechnical performance and even in diseases associated with production, depending on the severity of the imbalance (González et al., 2000). Therefore, biochemical analyses of blood serum play a crucial role in evaluating the functioning of various systems of the organism (Russell & Roussel, 2007). They are essential tools for assessing the nutritional condition of animals and determining the appropriate clinical intervention in cases of disorders (Lima et al., 2012).

Plasma urea nitrogen (NUP), ingested nitrogen and nitrogen balance showed a significant effect ($p < 0.05$), with mean values of 22.74 mg dL^{-1}, 25.13 and 15.87 g day^{-1}, respectively. This result was justified by dry matter consumption (Table 4), which showed a similar effect to those obtained for the variable ingested nitrogen. The nitrogen balance was positive, since the ingested nitrogen was greater than the nitrogen excreted in urine and feces, indicating that the animal retained protein, meeting its protein requirements as reported by Bach et al. (2005), indicating a balance between protein and energy in the diet.

Plasma creatinine (CCP) showed a significant effect ($p < 0.05$) between the levels of sugarcane bagasse and cottonseed inclusion.

The mean creatinine results were below the reference range (1.2–1.9) established for sheep, as described by (Kaneko et al., 2008). It is likely that the age of the animals is associated with a high metabolic rate of muscle deposition, consequently resulting in lower serum creatinine concentrations than those indicated by the reference. Creatinine is a non-protein nitrogen metabolite that is formed from the muscle metabolism of creatine and phosphocreatine. According to Kaneko et al. (2008), the circulating creatinine concentration is not directly influenced by diet or protein catabolism. However, it is related to the amount of protein in the diet and may serve as a late indicator in the diagnosis of renal failure.

Creatinine is a product of muscle metabolism and its production and excretion is directly related to the metabolism of this tissue (Niekerk et al., 1963). Thus, animals with different body conditions and different proportions of muscle and fat can excrete different amounts of creatinine per unit of live weight.

Table 12. Microbial nitrogen, microbial crude protein and microbial efficiency in sheep fed diets containing ammoniated sugarcane bagasse associated with cottonseed

Variable	Bagaço		Caroço		EPM	Valor - *p*		
	30%	50%	0%	20%		Bagaço	Caroço	Bagaço ×Caroço
g day^{-1}								
Nmicrobial	6.63	4.74	5.46	5.91	0.2987	0.0004	0.3149	0.5621
CPmicrobial	41.45	29.61	34.10	36.95	1.8673	0.0004	0.3054	0.5538
g CP kg NDT^{-1}								
EM	59.72	56.06	54.97	60.81	2.0583	0.2979	0.1042	0.3293

SEM = standard error of the mean. $P > 0.05$ not significant by *F*-test. Nmicrobial – Microbial nitrogen; Microbial CP – Microbial crude protein; EM – Microbial efficiency.

Microbial nitrogen and microbial crude protein (Table 12) showed a significant effect ($p < 0.05$) for the bagasse factor. The synthesis of microbial nitrogen and microbial crude protein in ruminants are favored by the balanced supply of fermentable carbohydrates and nitrogen sources, ensuring synchronization between energy and nitrogen in the rumen (Nichols et al, 2022). An adequate pH (6.0–6.8), moderate passage rate, and the presence of essential minerals (sulfur, cobalt, phosphorus) are essential to optimize microbial growth. In addition, quality fibers improve ruminal fermentation and feed conversion efficiency, maximizing the production of microbial protein, essential for the productive performance of ruminants (Santos et al, 2021). The higher fraction of concentrate in the diet with 30% BCH promoted the maximization of microbial nitrogen production, consequently greater supply of microbial crude protein. However, there was no difference in microbial efficiency as a result of the composition of the feed used, especially the high fiber and lignin content, implying less use of nutrients and low ruminal efficiency, a result that corroborates those discussed in (Table 5).

CONCLUSIONS

The inclusion of 30% ammoniated sugarcane bagasse in the total diet, combined with 20% cottonseed, resulted in improved nutrient intake and digestibility in sheep. This suggests that such dietary combination contributes to a more favorable ruminal environment, promoting better fiber degradation and overall metabolic efficiency. These findings highlight the potential of utilizing agro-industrial by-products as economically viable and nutritionally viable feed alternatives in small ruminant production systems. This is particularly relevant in semiarid regions, where the availability and quality of conventional forages are often limited.

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