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Productive and feeding performance of dairy cows fed sugarcane bagasse ammoniated with urea and cottonseed

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Abstract. The objective was to evaluate the use of ammoniated sugarcane bagasse (ASB) and cottonseed (CS) in the diet for lactating cows and their effects on intake, nutrient digestibility, dairy performance, microbial synthesis and ingestive behavior. The experiment was carried out at Bela Vista Farm (Encruzilhada-BA) and at the Forage and Pasture Laboratory of UESB (Itapetinga-BA), using eight multiparous ½ Holstein/Zebu crossbred cows with an average lactation of 20 kg day^{-1} and body weight of 454.7 ± 23.6 kg. The cows were distributed in two 4×4 Latin squares, in a 2×2 factorial scheme, evaluating ammoniated sugarcane bagasse (30% or 40%) associated or not with cottonseed (0% or 18%). The experiment had four periods of 21 days, 17 of adaptation and 4 of collection, totaling 84 days. The interaction between the proportions of ASB and CS was not significant for any of the variables evaluated. The inclusion of 30% sugarcane bagasse obtained from sugarcane bagasse resulted in higher intakes of dry matter (DM), crude protein (CP), ether extract (EE), non-fibrous carbohydrates (NFC), and total digestible nutrients (TDN), without influencing the consumption of neutral detergent fiber corrected for ash and protein. Higher intake ($P < 0.05$) of DM, CP, NFC, and TDN was observed in the diet without sugarcane bagasse, and in the diet with sugarcane bagasse, EE intake was higher ($P < 0.05$). The ASB content and the inclusion of CS influenced digestibility at 40% ASB, and with CS, they provided lower digestibility. Furthermore, individual factors did not affect these variables ($P < 0.05$). Feed efficiency and percentage of total solids in milk were higher for diets with 40% ASB. The use of 40% ASB with CS is recommended.

Key words: feeding behaviour, forage alternatives, milk composition, rumen fermentation, sustainability.

INTRODUCTION

Dairy farming plays an essential role in food security by providing high-value nutritional products. However, in the Brazilian semiarid region, this activity is mostly carried out by small-scale farms that face significant challenges due to irregular rainfall and high temperatures. These factors reduce forage availability throughout the year, compromise herd performance, and increase the need for low-cost feed alternatives (Lima et al., 2021).

Sugarcane bagasse is one of the main by-products of the sugar and alcohol agroindustry, with Brazil being the largest producer of sugarcane in the world, Producing an average of 678.7 million tons per year (Silva et al., 2025). Although it is a roughage with low nutritional value, characterized by high fiber and low protein content, its wide availability and low cost make it a strategic option for supplementation. Ammoniation with urea has been used to improve its nutritional value by increasing fiber digestibility and voluntary intake by ruminants (Publio et al., 2025).

Another relevant by-product is cottonseed, widely available in Brazil. This feedstuff has high protein and energy content and does not require prior processing, which allows it to partially replace conventional ingredients in the diets of dairy cows. Its inclusion may contribute to the nutritional balance of diets and enhance productive efficiency in systems with limited resources (Costa et al., 2011).

The use of by-products such as sugarcane bagasse enriched with urea and cottonseed is a strategic alternative to mitigate costs, utilize agro-industrial residues, and improve feeding efficiency in dairy cows in semiarid regions. However, it is necessary to evaluate the effects of this association on intake, digestibility, milk production, and feeding behavior of the animals (Sun et al., 2022).

This experiment was designed to evaluate the productive and feeding performance of dairy cows fed sugarcane bagasse ammoniated with urea, either with or without cottonseed.

MATERIALS AND METHODS

The experiment was carried out in two stages: the field trial at Fazenda Bela Vista, in Encruzilhada-BA, and the laboratory analyses at the Forage and Pasture Laboratory of the State University of Southwest Bahia (UESB), Itapetinga-BA campus. Eight multiparous crossbred ½ Holstein × Zebu cows, averaging 20 kg of milk day^{-1}, 454.7 ± 23.6 kg live weight, in mid-lactation were used.

The animals were assigned to two 4×4 Latin squares in a 2×2 factorial arrangement, consisting of ammoniated sugarcane bagasse at two inclusion levels (30 and 40%) associated or not with cottonseed (18% of the diet). Cows were housed in individual pens (8 m^2), covered, with concrete floors, and equipped with individual feeders and waterers. The study was conducted over 84 days, with four 21-day experimental periods, comprising 17 days for adaptation and 4 days for data collection, conducted from February to May 2023.

The experiment was designed in a 2×2 factorial scheme and limited to a restricted number of animals (n = 8), reflecting budgetary limitations for acquiring infrastructure and herds, given the restrictions on government resources allocated to research. However, the use of two 4×4 Latin squares was required to maximize statistical power and improve the use of available resources, allowing each animal to receive all four diets throughout the four experimental periods.

Diets were formulated according to NRC (2001) to meet the nutritional requirements of cows producing 20 kg of milk day^{-1}. The forage-to-concentrate ratio was 30:70 for diets with 30% sugarcane bagasse and 40:60 for diets with 40%, and all diets were isonitrogenous. Diets were offered ad libitum twice daily (07:00 and 15:00 h), allowing approximately 10% orts.

Table 1. Chemical analysis of the ingredients in the experimental diets

Item	Ingredients				
	Raw sugarcane bagasse	Ammoniated bagasse (8% urea)	Ground corn	Soybean meal	Cottonseed
Dry matter (%)	59.4	42.5	80.6	84.1	88.2
Crude protein[1]	1.3	20.0	9.9	52.5	28.9
Ether extract[1]	1.5	3.4	6.7	1.5	20.2
NDFap[1]	80.2	62.7	19.4	23.8	49.4
NDFi[1]	59.5	40.8	3.9	0.9	20.1
Gray [1]	1.7	4.6	2.1	6.2	4.2
Lignin[1]	22.9	10.4	1.4	0.7	7.4
NFC[1]	16.7	9.2	73.2	30.1	7.9
TDN[2]	37.0	45.7	84.5	83.3	81.5

[1]Values in percentage of dry matter; NDFcp – neutral detergent fiber corrected for ash and protein; NDFi – indigestible neutral detergent fiber; NFC – non-fiber carbohydrates; TDN – total digestible nutrients; [2]estimated according to NRC (2001).

Offered feed, residual feed, and fecal samples were collected throughout the sampling periods for chemical analysis, as described by Detmann et al. (2022). Dry matter (DM), ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), neutral detergent fiber corrected for ash and protein (NDFap), acid detergent fiber (ADF), lignin, and indigestible NDF (iNDF) were determined. Total carbohydrates (Sniffen et al., 1992), non-fiber carbohydrates (Detmann et al., 2012), and total digestible nutrients (NRC, 2001) were calculated using specific equations. Nutrient intake was estimated from chemical composition, and fecal excretion was determined using iNDF as an internal marker.

Table 2. Percentage composition of experimental diets

Ingredients	Diets 1	Diets 2	Diets 3	Diets 4
	30% Ammoniated bagasse		40% Ammoniated bagasse	
	0% CS	18% CS	0% CS	18% CS
Ammoniated bagasse	30.00	30.00	40.00	40.00
Cottonseed	0.00	18.00	0.00	18.00
Corn	56.00	43.00	46.00	33.00
Soybean	11.00	6.00	11.00	6.00
Mineral mixture	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00
Chemical composition of diets				
Dry matter (%)	74.28	75.11	70.57	71.03
Crude protein[1]	14.18	14.99	14.68	14.85
Ether extract[1]	4.26	5.98	3.66	5.23
NDF_{ap}[1]	23.93	27.94	27.99	31.20
NDFi[1]	13.14	15.25	16.94	18.85
Gray [1]	5.75	5.68	6.48	6.29
Lignin[1]	3.45	4.15	4.37	4.96
NFC[1]	51.87	45.42	47.20	42.01
TDN[2]	65.53	66.93	64.66	63.14

[1]Values in percentage of dry matter; 2estimated according to NRC (2001).

The excretion of total purine derivatives (TPD) was estimated by the sum of the amounts of allantoin and uric acid excreted in the urine and allantoin in the milk. The amount of absorbed microbial purines ($mmol^{-1}$) was estimated from the excretion of total purines ($mmol^{-1}$), using the equation proposed by Verbic et al. (1990): AP = (TPD – 0.385× BW0.75) / 0.85, where: AP = absorbed purines (mmol day^{-1}) TPD = total purine derivatives (mmol day^{-1}) 0.85 = recovery of absorbed purines as DP in the urine 0.385 = endogenous excretion of DP in the urine (mmol) per unit of metabolicsize. Microbial nitrogen (g NM day^{-1}) was estimated from the amount of purines absorbed (mmol day^{-1}) according to the equation by Chen & Gomes (1992): NM (g day^{-1}) = (70×PA)/ (0.83×0.116 ×1,000). Where: 70 = purine nitrogen content (mg $mmol^{-1}$) 0.83 = intestinal digestibility of microbial purines 0.116 = purine-N:total-N ratio in bacteria.

Microbial synthesis efficiency was calculated as follows: ESPBmic = [(NM× 6.25)]/ITDN.

Milk yield was recorded daily from the 17th to the 21st day of each experimental period. Individual milk samples were collected for analysis of protein, fat, lactose, total solids, solids-not-fat, casein, somatic cell count (SCC), and milk urea nitrogen (MUN) at the Dairy Cattle Laboratory (ESALQ/USP). Composite samples were also stored for allantoin determination, according to Broderick & Clayton (1997).

Table 3. List of abbreviations

ASB	Ammoniated Sugarcane Bagasse
CS	Ammoniated Sugarcane Bagasse
DMI	Dry Matter Intake
DM	Dry Matter
ITDN	Intake Total Digestible Nutrients
CP	Crude Protein
EE	Ether Extract
NDF	Neutral Detergent Fiber
NDFap	Neutral Detergent Fiber corrected for ash and protein
ADF	Acid Detergent Fiber
iNDF	Indigestible Neutral Detergent Fiber
NFC	Non-Fiber Carbohydrates
TDN	Total Digestible Nutrients
BW	Body Weight
MW	Metabolic Weight
SEM	Standard Error of the Mean
TPD	Total Purine Derivatives
AP	Absorbed Purines
NM	Microbial Nitrogen
ESPBmic	Microbial Protein Synthesis Efficiency
SCC	Somatic Cell Count
MUN	Milk Urea Nitrogen
MP	Milk Production
PFCM	Production of Fat-Corrected Milk
DDE	Defatted Dry Extract
FE	Feed Efficiency
NRC	National Research Council
DMI	Dry Matter Intake
CPI	Crude Protein Intake
EEI	Ether Extract Intake
NDFapI	NDF corrected for ash and protein Intake
NFCI	Non-Fiber Carbohydrates Intake
TDNI	Total Digestible Nutrients Intake

Feeding behavior was evaluated on the 21st day of each period by continuous visual observation over 24 hours at 10-minute intervals, recording feeding, rumination, and idling activities (Mezzalira et al., 2011). The efficiencies of feeding and rumination were assessed based on Bürger et al. (2000).

Data were subjected to analysis of variance using the *F*-test at a 5% probability level with the SAS® Studio software (2024).

Table 3 presents the abbreviations used in the results and discussion.

RESULTS AND DISCUSSION

No statistically significant interaction was found between the proportions of ammoniated sugarcane bagasse (ASB) and cottonseed (CS) on nutrient intake ($P > 0.05$) (Table 4). The inclusion of 30% ASB resulted in higher intakes of DMI, CPI, EEI, NFCI, and TDNI ($P < 0.05$). The NDF ap was not influenced ($P > 0.05$).

Higher intakes of DMI, CPI, NFCI, and TDNI were observed in the diet without CS compared to the diet with CS. Ether extract intake was higher in the diet with CS inclusion ($P < 0.05$).

For the intake variables related to body weight (BW%) and metabolic weight (MW%), the inclusion of 30% ASB and the diet without CS resulted in higher dry matter intake. NDF ap intake (BW%) was influenced by ASB, with the diet with 30% ASB showing higher intake.

Dry matter and nutrient intake decreased with increasing ASB percentage in diets to 40% bagasse, which is possibly related to the increase in iNDF and lignin in diets containing 40% (Table 4).

Table 4. Dry matter intake and nutrients in the diet of crossbred cows fed ammoniated sugarcane bagasse (ASB) with or without cottonseed (CS)

Item	ASB		CS		SEM[1]	*P*-value		
	30	40	0	18		ASB	CS	ASB×CS
Intake (kg day^{-1})								
Dry matter	18.38	16.94	18.21	16.41	0.65	0.001	0.008	0.360
Crude protein	2.96	2.53	2.87	2.63	0.08	0.002	0.035	0.868
Ether extract	1.04	0.75	0.80	0.99	0.07	0.007	0.043	0.830
NDFap[2]	5.21	4.95	5.08	5.07	0.16	0.147	0.998	0.634
NFC[3]	9.98	7.62	9.94	7.66	0.36	0.001	0.001	0.302
TDN[4]	13.47	10.86	12.96	11.36	0.39	0.001	0.007	0.965
Intake (% body weight)								
DM[6]	3.96	3.32	3.93	3.45	0.08	0.001	0.001	0.998
NDFap	1.04	0.99	1.01	1.01	0.02	0.021	0.975	0.151
Intake (% metabolic weight)								
DM	0.192	0.157	0.186	0.163	4.36	0.001	0.002	0.872

[1]SEM: standard error of the mean; [2]Neutral Detergent Fiber corrected for ash and protein; [3]Non-fiber carbohydrates; [4]Total digestible nutrients; [6]Dry matter; ASB = sugarcane bagasse; CS = cottonseed.

Recent research in Brazil has shown greater intake of crossbred cows compared to the NRC (Da Silva et al. 2024; Jesus et al., 2020), since the NRC uses data only from Holstein cows, raised in different conditions to the animals in this study, which are ½ Holstein/Zebu crossbred cows. This difference can be attributed to several factors, such as the breed of animals, climatic conditions and food management, which can influence intake in isolation or in combination.

In studies, Almeida et al. (2018), evaluating non-ammonized sugarcane bagasse as a roughage in lactating cows with four different levels of sugarcane bagasse (45, 50, 55 and 60%) and a control diet, reported that DM intakes (14.6, 13.9, 12.9, 11.3) respectively, decreased linearly as a function of bagasse inclusion.

The inclusion of CS affected ($P < 0.05$) the intake of crude protein (CP) due to the DM intake and the diets being isonitrogenous. Animals that received diets without CS showed higher CP intake (Table 4).

EE intake was higher for cows fed CS ($P < 0.05$) than for cows fed the diet without CS. As in the present study, Nogueira et al. (2019), evaluating dry matter intake, nutrient digestibility, and feeding behavior of cows fed CS (30% DM of the diet) and vitamin E, observed that the addition of cottonseed to the diets, regardless of the inclusion of vitamin E, increased EE intake by 306%. In the present study, the EE concentration in the experimental diet increased with the inclusion of CS (18% DM of the diet), increasing EE intake by 123%.

This increase in EE concentration occurs due to the chemical composition of CS, which presents a high amount of this component (20.2%) (Table 1), in diets with inclusion of CS as a replacement, mainly for soybean meal, a food that has quantities of EE (1.5%) (Table 1), favored the increase in the energy density of the diet. This also influenced dry matter intake, in which cows fed a diet with CS of higher energy density obtained lower intake (DMI = 17.41 kg day^{-1}) compared to cows fed a diet without CS (DMI = 19.91 kg day^{-1} (Table 4).

ASB was expected to limit intake due to its NDF content and potential filling effect. It is well established that ruminant DMI is limited by physical distension of the gastrointestinal tract (Molavian et al., 2020). The filling effect of the diet may be influenced by the quantity and degradation kinetics of NDFap, as reported by Grant & Cotanch (2012).

However, this was not what occurred in the present study, since ammoniated bagasse was used, in which ammoniation with urea probably caused the breaking of the esterified bonds within the cell wall, as proven by the reduction of iNDF from 59.5 to 40.8% DM, of lignin from 22.9 to 10.4% DM and an increase in TDN from 37.0 to 45.7 (Table 1). Providing higher intake than expected for the two percentages of bagasse.

The average intake of NDF accp was 1.01% of BW, a value within the limit suggested by Mertens (1997), of 1.2% of BW for lactating cows, since, above this value, it would be limiting for DM intake, when its voluntary intake is highly related to the NDF content in the diets.

In research, Da Silva et al. (2024), evaluating the intake of dairy cows fed 40% ASB in the diet, found a maximum NDF intake of 1.30% BW, above that recommended by Mertens (1997) to avoid limiting intake due to fiber content, and a dry matter intake of 3.9% BW. The authors reported that even with the high level of fiber intake, there was no limitation on DM intake. In the present study, levels of 30 and 40% ASB were used, which demonstrated that these percentages did not limit the animals' intake.

Table 5 presents the values referring to the digestibility coefficients, there was no significant interaction between the proportions of ASB and CS ($P > 0.05$). The ASB content and the inclusion of CS influenced the digestibility of crude protein in which the percentage of 40% sugarcane bagasse and with CS provided lower digestibility ($P < 0.05$).

This probably occurred because the protein in whole cottonseed is located mainly inside the seed, surrounded by the husk and mixed with fat, which delays its release into the rumen (Mullenix et al., 2022), and the diet with 40% bagasse has a higher portion of roughage and a lower portion of concentrate, with sugarcane bagasse having a low protein content and relatively low quality.

In the 30% ASB diet, there is a lower roughage to higher concentrate ratio, which contributes to greater protein digestibility. In diets without CS, the protein source used is soybean meal, which has greater digestibility than the CS protein, contributing to its increase.

Table 5. Digestibility coefficient of dry matter and nutrients in the diet of crossbred cows fed ammoniated sugarcane bagasse (ASB) associated or not with cottonseed (CS)

Item	ASB		CS		SEM[1]	*P*-value		
	30	40	0	18		ASB	CS	ASB×CS
Dry matter	45.87	46.1	45.65	46.32	0.92	0.816	0.489	0.673
Crude protein	64.91	60.34	66.84	58.42	0.82	0.001	0.001	0.266
Ether extract	63.15	61.63	59.73	65.05	0.66	0.062	0.001	0.369
NDFap[3]	34.18	37.89	36.59	35.48	3.08	0.272	0.731	0.331
NFC[4]	83.38	83.29	81.03	85.63	0.40	0.832	0.001	0.631
TDN[5]	66.23	63.9	65.09	65.04	0.70	0.016	0.936	0.081

[1]SEM: standard error of the mean; [2]Probability; [3]Neutral Detergent Fiber corrected for ash and protein; [4]Non-fiber carbohydrates; [5]Total digestible nutrients; ASB = sugarcane bagasse, CS = cottonseed.

It was observed that the inclusion of CS provided higher digestibility coefficients for the ether extract and non-fibrous carbohydrates, this occurred due to the bromatological characteristics of CS, which has 20% EE with high digestibility.

The diet with 30% ASB inclusion had a higher total digestible nutrient (TDN) content. This was likely because ASB has a high NDF value, and at 40% inclusion, there is a higher roughage:concentrate ratio, which contributes to lower dietary digestibility, compared to the diet with 30% ASB inclusion, which has a lower amount of dietary fiber and a higher concentrate ratio, which has greater digestibility.

Nogueira et al. (2019) reported that the inclusion of 30% CS in the diet of Holstein cows improved the EE digestibility coefficients by 14% and decreased the NFC digestibility coefficients by 9.0%. For the dry matter and NDF digestibility coefficients, the ASB percentage and CS inclusion factors did not influence the digestibility of the diets.

Table 6 presents the values of microbial protein synthesis and microbial efficiency. There was no significant interaction between the ASB proportion and CS inclusion factors for the microbial production and microbial efficiency variables ($P > 0.05$). The factors did not individually influence the evaluated variables.

Table 6. Microbial protein synthesis and microbial efficiency of crossbred cows fed ammoniated sugarcane bagasse (ASB) associated or not with cottonseed (CS)

Item	ASB		CS		SEM[1]	*P*-value		
	30	40	0	18		ASB	CS	ASB×CS
Microbial production (g day^{-1})								
Microbial CP	1,548.7	1,456.1	1,396.3	1,608.5	225.74	0.696	0.383	0.945
Microbial efficiency								
gCP kg^{-1} TDN	115.6	133.1	110.8	137.8	18.36	0.381	0.195	0.903

[1] SEM: standard error of the mean; ASB = sugarcane bagasse; CS = cottonseed.

Microbial protein synthesis averaged 1,502.4 g day^{-1} and was not influenced by the diets ($P > 0.05$). Because the diets were calculated to be isonitrogenous and maintained the same NFC proportion, they likely contributed to this result. This NFC balance, together with the release of ammonia into the ruminal fluid resulting from the degradation of nitrogen compounds when animals consumed diets with an average of 14.7% crude protein (Table 2), possibly suggests that the diets promoted the synchronization of protein and energy degradation and possibly provided ammonia nitrogen for use by the ruminal microbiota in microbial synthesis, since the synthesis of microbial protein in ruminants relies directly on the availability of carbohydrates and nitrogen in the rumen (NRC, 2001).

According to Valadares Filho et al. (2010), the efficiency of microbial synthesis is 120 gCP kg^{-1} TDN as a reference for tropical conditions. In this present study, the diets provided promoted an average microbial efficiency of 124.3 gCP kg^{-1} TDN, a value close to that recommended by Valadares Filho et al. (2010).

According to Bünemann et al. (2020), a low ruminal pH can reduce fiber digestion and, therefore, decrease microbial efficiency, which is defined as microbial protein synthesized by fermented organic matter. A decrease in fiber digestion can prevent a uniform energy supply throughout the day, which is necessary for optimal microbial growth. This did not occur because the fiber content used likely provided ideal conditions for the rumen and its microbial population.

The addition of CS did not influence ($P > 0.05$) the microbial efficiency (gCP kg^{-1} TDN), despite CS being rich in ether extract that can generally interfere with the synthesis of microbial protein due to a possible toxic effect on microorganisms (Doreau & Ferlay, 1995). However, intake of this EE likely did not affect microbial growth in the rumen. The exact mechanism of lipid toxicity on these microorganisms is still unclear, but it is suggested that it may be related to the barrier that lipids create around feed particles, impairing microbial colonization and degradation (Van Soest, 1994).

Table 7 presents the values related to the production and composition of milk from crossbred cows fed ammoniated ASB associated or not with CS. There was no significant interaction between the factors percentage of ASB and inclusion of CS for the milk production and composition variables ($P > 0.05$).

The percentage of ASB influenced the variables of total solids and feed efficiency, in which the proportion of 40% of ASB provided higher averages with 13.14 and 0.99, respectively. For feed efficiency, there was an influence of the inclusion of CS ($P < 0.05$), where cows fed diets with cottonseed obtained greater feed efficiency with a value of 0.96.

It was expected that diets with 30% ASB would obtain greater feed efficiency, because it had a higher roughage: concentrate ratio and would provide a greater quantity of nutrients than the diet with 40% bagasse. However, the diet with a concentrate: roughage ratio of 60:40 was sufficient to meet the maintenance and production needs of the cows, since they are crossbred cows with an average production of 20 kg of milk, their genetics did not allow greater production even with a diet with better quality nutrients. Visibly, the cows began to gain weight and increase their body condition score as the experimental period progressed, so these nutrients, in addition to meeting maintenance and production requirements, also contributed to the increase in body weight.

Dry matter intake (DMI) is the starting point for nutrient intake, particularly protein and energy, and is therefore crucial for animal performance. Although cows consumed less DM and nutrients in the 40% diets, this did not affect milk production, and milk intake was higher than estimated (DMI = 14.4 kg day^{-1}).

There was no influence of the diets on milk production, corrected milk production and fat content ($P > 0.05$), possibly indicating that the supply of nutrients to the animal, and especially to the mammary gland, was not limited in any of the diets provided.

Mohamed et al. (1988) reported that supplementation with whole oilseeds, such as cottonseed, can maintain or increase milk fat percentage. This is because, despite containing high levels of oil, the fat present in these seeds is encapsulated. This encapsulation allows for slow release in the rumen, and some of the fat escapes to be utilized in the intestine. This means that the fat from oilseeds is released gradually, which may contribute to maintaining or increasing fat levels in the animals' milk.

The results of the physicochemical analyses of the milk, as presented in Table 7, met the requirements established by Normative Instruction No. 77 (BRASIL, 2018). This regulation establishes the minimum levels of fat 3.0%, protein 2.9%, lactose 4.3% and defatted dry extract in the milk 8.4%. This suggests that the milk produced by cows receiving diets with ASB and CS inclusion, under the same conditions, does not present significant changes in its physicochemical characteristics.

Table 7. Milk production and composition of crossbred cows fed ammoniated sugarcane bagasse (ASB) with or without cottonseed (CS)

Item	ASB		CS		SEM[1]	*P*-value		
	30	40	0	18		ASB	CS	ASB×CS
MP(kg day^{-1})	19.01	19.31	18.99	19.32	0.48	0.553	0.553	0.366
PFCM (kg day^{-1})	18.07	18.88	18.62	18.35	0.64	0.250	0.673	0.892
Fat	3.58	3.81	3.83	3.55	0.12	0.110	0.063	0.258
Protein	3.69	3.75	3.71	3.73	0.07	0.397	0.868	0.061
Lactose	4.47	4.54	4.45	4.55	0.03	0.114	0.073	0.069
Total Solids	12.71	13.14	13.03	12.83	0.07	0.001	0.057	0.081
DDE[3]	9.16	9.33	9.19	9.29	0.07	0.065	0.229	0.084
FE[4]	0.78	0.99	0.82	0.92	0.04	0.004	0.040	0.092

[1]Standard error of the mean; [2]Probability; [1]in percentage; MP = milk production; PFCM = production of fat-corrected milk; [3]DDE= defatted dry extract; [4]FE= feed efficiency (kg of milk kg^{-1} of dry matter consumed). ASB = sugarcane bagasse, CS = cottonseed.

In this study, the average values for fat, protein, lactose, and defatted dry extract were 3.7, 3.7, 4.5, and 9.2, respectively. Therefore, this milk is considered within the normal standards established by law and can be used by dairy industries to produce various dairy products. This result indicates that CS supplementation does not compromise the quality of the milk produced.

Costa (2017), evaluating the different levels of inclusion of whole cottonseed (0, 6, 12, 18 and 24%) in the total diet of dairy cows, found similar results for milk composition, reporting that there was no difference in milk composition; however, milk production was affected by the inclusion of CS, presenting a quadratic effect.

Table 8 presents the values relating to the analysis of feeding behavior of crossbred cows, of crossbred cows fed with ASB associated or not with CS.

There was no significant interaction between the ASB percentage and CS inclusion factors (*P* < 0.05). However, the factors increased the time spent on the chewing variable in minutes g^{-1} of DM and minutes g^{-1} of NDF in diets with a 40% ASB inclusion percentage, with averages of 48.93 minutes g^{-1} of DM and 163.57 minutes g^{-1} of NDF. And for CS inclusion, there was a greater time spent in diets with CS, with 47.46 minutes g^{-1} of DM.

According to Mendes et al. (2010), one of the main elements that directly influences behavioral activities is the levels of neutral detergent fiber present in the diet, especially rumination. According to Van Soest (1994), this behavior is affected by the physical and chemical properties of the diet and is proportional to the cell wall content of the roughage. Despite the high amount of ASB used, the percentages of 30 and 40% did not influence feeding, rumination, or idleness (*P* > 0.05).

Table 8. Feeding behavior of crossbred cows fed ammoniated sugarcane bagasse associated or not with cottonseed

Item	ASB		CS		SEM[1]	*P*-value		
	30	40	0	18		ASB	CS	ASB×CS
Food								
min day^{-1}	323.7	358.7	342.5	340.0	33.6	0.33	0.94	0.33
min kg^{-1} DM	16.2	23.7	18.2	21.7	3.1	0.54	0.32	0.43
min kg^{-1} NDF cp^{-1}	62.6	78.7	69.5	71.8	9.5	0.14	0.81	0.36
%/day	22.4	24.9	23.7	23.6	2.3	0.33	0.94	0.33
Rumination								
min day^{-1}	425.0	416.2	398.7	442.5	44.7	0.85	0.36	0.57
min kg^{-1} DM	21.6	25.1	21.0	25.7	2.9	0.27	0.16	0.62
min kg^{-1} NDF cp^{-1}	82.6	84.7	80.1	87.2	9.6	0.83	0.49	0.64
% day^{-1}	29.5	28.9	27.6	30.7	3.1	0.85	0.36	0.57
Leisure								
min day^{-1}	691.2	665.0	698.7	657.5	35.9	0.49	0.29	0.81
% day^{-1}	48.0	46.1	48.5	45.6	2.4	0.49	0.29	0.81
Chew								
n $cake^{-1}$	48.1	44.8	47.3	45.5	3.2	0.34	0.60	0.26
s $cake^{-1}$	55.0	51.5	55.1	51.4	3.2	0.33	0.30	0.26
N day^{-1}	22203.0	21847.6	20463.9	23586.6	2474.8	0.89	0.25	0.56
min day^{-1}	748.7	775.0	741.2	782.5	35.9	0.49	0.29	0.81
min kg^{-1}DM	37.8	48.9	39.3	47.4	1.4	0.00	0.00	0.48
min kg^{-1}NDF cp^{-1}	145.2	163.5	149.7	159.0	4.4	0.00	0.08	0.33

[1] Standard error of the mean, ASB = sugarcane bagasse, CS = cottonseed.

The ASB percentage influenced the time spent chewing, with cows fed 40% ASB in the diets spending 48.93 min g^{-1} DM and 163.57 min g^{-1} NDF/pwt. This was expected due to the amount of NDF in this diet. The same occurred when cows were fed diets with CS for the chewing variable, where cows spent more time with 47.46 min g^{-1} DM.

These results can be explained by the similarity in the nutritional composition of the diets and in the composition of the ammoniated bagasse, which due to its chemical characteristics, mainly in relation to the fibrous fraction that had a significant reduction in the content of NDFap, NDFi and lignin, causing changes in the behavioral parameters evaluated, presenting averages of 341.2 (min day^{-1}) for feeding and 420.6 (min day^{-1}) for rumination.

The lack of differences in the variables evaluated indicates that ASB, when ammoniated, had improved digestibility, resulting in shorter rumination times compared to conventional feeding and idle periods. Consequently, this improvement in digestibility did not cause significant differences in the cattle's chewing activity.

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The variables evaluated indicate that ASB, when ammoniated, had improved digestibility, resulting in shorter rumination times compared to conventional feeding and idle periods. Consequently, this improved digestibility did not cause significant differences in the cattle's chewing activity.

Mendes et al. (2010) reported that one of the main factors influencing cattle behavioral activities is related to the percentage of neutral detergent fiber (NDF) present in their diets. Specifically, they highlight the impact of NDF content on the animals' rumination, a behavior fundamental for efficient fiber degradation.

As noted by Van Soest (1994), rumination is influenced by the physical and chemical properties of the diet, being proportional to the cell wall content of the roughage present in it. This means that diets with a higher NDF content require more chewing time for their degradation, thus stimulating rumination. Therefore, the composition of diets, especially fiber content, plays a crucial role not only in cattle nutrition but also in their natural behaviors, directly influencing their well-being and overall health.

CONCLUSIONS

The use of diets containing 40% ammoniated sugarcane bagasse (ASB) with the inclusion of cottonseed (CS) is recommended for lactating Holstein × Zebu crossbred cows, compared to the 30% ASB diet. Although the study was limited to a 2×2 design and a restricted number of animals due to resource constraints, the combination of 40% ASB with CS optimized feed efficiency and milk quality.

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