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TMR silage with sugarcane bagasse and urea: effects on intake, digestibility, and feeding behavior in dairy heifers

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Abstract. This study evaluated the effects of total mixed ration (TMR) silage containing two levels of sugarcane bagasse (40% and 50% of dry matter), with or without urea inclusion (2.5%), on intake, nutrient digestibility, nitrogen balance, microbial protein synthesis, and feeding behavior of dairy heifers. Eight crossbred heifers were assigned to two simultaneous 4×4 Latin squares in a 2×2 factorial arrangement. Urea inclusion reduced ($P < 0.05$) dry matter and neutral detergent fiber intake but improved dry matter and crude protein digestibility. Increasing bagasse level to 50% reduced ($P < 0.05$) intake and digestibility of nutrients, reflecting the higher fiber content and lower energy density of the diets. Nitrogen balance and microbial protein synthesis were not affected by treatments. Higher bagasse levels increased rumination time per unit of NDF and the number of ruminal boli, indicating greater physical demand for fiber processing. Overall, TMR silage containing 40% sugarcane bagasse without urea provided the best balance between intake and nutrient utilization. These results highlight the importance of optimizing fiber levels and nitrogen sources in TMR silage to improve efficiency in dairy heifer feeding systems under tropical conditions.

Key words: bagasse, digestibility, feeding behavior, nitrogen balance, TMR silage.

INTRODUCTION

Ensiling is the predominant forage conservation method in tropical regions, particularly in Brazil, due to its operational efficiency and suitability to climatic conditions. Crops such as corn, sorghum, and sugarcane are widely used in ruminant feeding systems. In recent years, total mixed ration (TMR) silage has gained attention as a strategy to improve feed management by allowing the storage of complete diets, reducing labor, and ensuring uniform nutrient intake (Pinto & Millen, 2018).

The use of TMR silage also facilitates the inclusion of agro-industrial by-products, contributing to cost reduction and sustainable waste utilization. Among these by-products, sugarcane bagasse stands out due to its wide availability in Brazil, the world's largest sugarcane producer (CONAB, 2020). Although bagasse is commonly used as a fiber source, its high lignin content and low digestibility may limit animal performance.

Urea is frequently used as a non-protein nitrogen source to enhance microbial protein synthesis and improve fiber degradation. However, its inclusion may negatively affect feed intake due to palatability issues and changes in fermentation patterns patterns (Ribeiro et al, 2026).

Despite the recognized potential of TMR silage and the availability of sugarcane bagasse, limited information exists on the combined effects of different bagasse levels and urea inclusion on intake, nutrient utilization, nitrogen metabolism, and feeding behavior in dairy heifers. Understanding these interactions is essential to optimize diet formulation and improve the efficiency of feeding systems under tropical conditions.

Therefore, this study aimed to evaluate the effects of TMR silage containing two levels of sugarcane bagasse (40% and 50% of dry matter), with or without urea inclusion, on intake, nutrient digestibility, nitrogen balance, microbial protein synthesis, and feeding behavior of dairy heifers.

MATERIALS AND METHODS

This research was conducted in accordance with the Ethics Committee on the Use of Animals (CEUA), under protocol number 235/2023, approved at the State University of Southwest Bahia - UESB. The experiment was carried out at Fazenda Bela Vista, in the municipality of Encruzilhada - BA, and the samples were analyzed in the Forage and Pasture, Animal Physiology, and Animal Nutrition Laboratories of UESB - Itapetinga Campus, Bahia.

The total mixed rations (TMRs) were produced at Fazenda Bela Vista. Fresh sugarcane bagasse was collected from a still located on the property. This was chopped in a stationary shredder to a particle size of 5 cm. After chopping, the concentrated components (soybean meal, ground corn, and mineral core), urea, and water were added to the bagasse in the proportions specified for each ration. The material was then manually homogenized to ensure it contained approximately 35% dry matter (DM). Subsequently, these mixtures were placed in 200-micron thick plastic bags with a capacity of 30 kg. The material was then manually compacted and sealed with nylon cable ties.

Eight crossbred Girolando heifers, with an average age of 12 months and an average initial body weight of 200 ± 10 kg, were used. The animals were identified at the beginning of the experimental period with plastic ear tags and dewormed (Abamectin-1% - Abmic® Microsules). After weighing, the animals were distributed into two simultaneous 4×4 Latin squares, in a 2×2 factorial design, with two proportions of sugarcane bagasse (40 or 50% dry matter basis) with and without the use of urea replacing soybean meal in the diet.

The animals were housed in individual 6×2 m (12 m^2) partially covered pens with concrete floors, equipped with individual feeders and waterers. The animals received ad libitum feed, divided into two daily meals (7 am and 1 pm) to allow for leftovers of approximately 10% of the feed provided. The diets were calculated to meet the nutritional requirements of crossbred Girolando heifers for a gain of 700 g day^{-1} (NRC 2001).

The sample size ($n = 8$) was defined based on similar studies using Latin square designs in ruminant nutrition, which allow efficient control of animal and period variability and are widely accepted for metabolism and intake trials (Detmann et al., 2012).

The chemical composition of the ingredients and experimental diets can be seen in Tables 1 and 2.

Parameters evaluated

Samples of the supplied ingredients, feces, and leftovers were analyzed for the following contents: DM (method G-001/1), crude ash (method M-001/1), CP (method N-001/1), EE (method G-004/1), NDF (method F-002/1), NDFap (methods N-004/1 and N-002/1), ADF (method F-004/1), lignin (method F-005/1), and NDFi (method F-009/1), as described in Detmann et al. (2012).

Total carbohydrate (TC) content was calculated using the equation proposed by Sniffen et al. (1992):

$$\mathrm{TC} = 100 - (\mathrm{CP\%} + \mathrm{EE\%} + \mathrm{ash\%}) \tag{1}$$

where CP = crude protein, EE = ether extract, and ash = % of the sample. Non-fibrous carbohydrates (NFC) of the samples were calculated according to the formula of Detmann et al. (2012):

$$\mathrm{NFC} = 100 - (\mathrm{CP\%} + \mathrm{EE\%} + \mathrm{MM\%} + \mathrm{NDFap}) \tag{2}$$

where EE = ether extract, MM = ash, and NDFap = neutral detergent fiber. Total digestible nutrients (TDN) were calculated according to NRC (2001):

$$\mathrm{TDN} = \mathrm{DPP} + (\mathrm{EDE} \cdot 2.25) + \mathrm{NDF} + \mathrm{NDFap}) \tag{3}$$

where DPP = digestible crude protein; EED = digestible ether extract; NDF = digestible neutral detergent fiber; NDFap = digestible non-fibrous carbohydrates.

Table 1. Chemical composition of the ingredients used in the experimental diets

Composition	Ingredients		
	Raw sugarcane bagasse	Ground corn	Soybean meal
Dry matter (%)	59.4	80.6	84.1
Crude protein[1]	1.3	9.9	52.5
Ether extract[1]	1.5	6.7	1.5
NDFap[1]	80.2	19.4	23.8
NDFi[1]	59.5	3.9	0.9
Ash[1]	1.7	2.1	6.2
Lignin[1]	22.9	1.4	0.7
CNF[1]	16.7	73.2	30.1
NDT[2]	37.0	84.5	83.3

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

Table 2. Ratio of ingredients and chemical composition of experimental diets after ensiling

Item	40% bagasse		50% bagasse	
	0% Urea	2.5% Urea	0% Urea	2.5% Urea
Ingredient ratios (%)				
Raw sugarcane bagasse	40.0	40.0	50.0	50.0
Ground corn	39.6	50.0	28.0	38.3
Soybean meal	17.5	4.5	19.0	6.2
Urea	-	2.5	-	2.5
Mineral Mixture[1]	3.0	3.0	3.0	3.0
Chemical Composition (%)				
Dry matter	32.8	32.0	29.9	31.2
Crude protein	13.2	13.7	13.4	13.6
Ether extract	2.9	3.1	3.1	3.7
NDFap	38.1	37.9	44.4	44.7
NDFi	15.5	16.5	20.6	21.6
Ash	6.1	5.4	6.3	5.3
Lignin	6.5	6.9	8.8	9.0
CNF	37.5	37.1	29.8	30.2
TDN[2]	66.4	69.7	61.5	63.0

[1] Guaranteed levels (per kg of active elements): calcium – 187 g; phosphorus – 85 g; magnesium – 15 g; sodium – 90 g; sulfur – 18 g; copper – 1,350 mg; cobalt – 80 mg; iron – 1,450 mg; iodine – 90 mg; manganese – 1,700 mg; selenium – 22 mg; zinc – 5,800 mg; fluorine maximum – 850 mg; phosphorus (P) solubility in 2% citric acid – 95% (minimum). [2] Estimated according to NRC (2001).

Dry matter intake and nutrient digestibility

To determine dry matter intake, the amount of feed offered and leftovers for each animal was recorded daily throughout the experimental period. From the 17th to the 21st day of each period, samples of diets, concentrate, and leftovers were collected and stored in plastic bags, identified, and frozen at -10 °C for chemical-bromatological analyses.

The animals were weighed at the beginning and end of each experimental period to estimate nutrient intake. For the purpose of quantifying and evaluating voluntary intake, the feed provided between the 17th and 21st day of each experimental period was considered, with leftovers counted between the 18th and 22nd day. Subsequently, the samples were dried in a forced-air oven at 60 °C for 72 hours, then ground in a knife mill equipped with a 1 mm mesh sieve. The samples were then stored in hermetically sealed and labeled vials for compositional analysis.

For fecal production estimation, indigestible neutral detergent fiber (iNDF) was used as an internal indicator, obtained after ruminal incubation of 0.5 g corresponding to each sample of feed, leftovers, and feces, packaged in bags made of non-woven fabric (TNT) with a grammage of 100 g m^{-2}, 5×5 cm for 288 hours. The residue was considered indigestible (Detmann et al., 2012). The apparent digestibility of nutrients (D) was determined by the formula described by Silva & Leão (1979):

$$\text{D} = [(\text{kg nutrient ingested} - \text{kg nutrient excreted}) \ / \ \text{kg nutrient ingested}] \cdot 100. \quad (4)$$

Balance of nitrogen compounds and microbial synthesis

On the 21st day of each experimental period, spot urine samples were collected from spontaneous urination of the animals, approximately four hours after the morning feeding. The samples were filtered through gauze, and a 10 mL aliquot was diluted with 40 mL of sulfuric acid (0.036 N) (Valadares et al., 1999) for quantification of urinary concentrations of urea, total nitrogen, creatinine, allantoin, and uric acid.

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

Urine creatinine and uric acid concentrations, and urine and plasma urea concentrations, were determined using commercial kits (Bioclin). Urea values were converted to urea nitrogen by multiplying the obtained values by a factor of 0.4667. Urinary allantoin and uric acid levels were determined using a colorimetric method, according to Chen & Gomes (1992) specifications, and total nitrogen content was estimated using the Kjeldahl method.

Nitrogen balance (N-retained, g day^{-1}) was calculated as: N-retained = N ingested (g) – N in feces (g) – N in urine (g).

Creatinine excretion (mg kg^{-1} BW) used to estimate urine volume from spot samples was obtained for each animal according to the equation described by Chizzotti (2004):

$$\text{EC} = 32.27 - 0.01093 \cdot \text{BW} \quad (5)$$

where EC = daily creatinine excretion (mg kg^{-1} BW); and BW = body weight (kg). However, urine volume was estimated from the ratio between daily creatinine excretion (mg kg^{-1} BW) obtained from the previous equation and the average creatinine concentration (mg L^{-1}) in the spot urine samples, multiplied by the animal's respective BW.

Total purine derivative excretion (TPD) was obtained by summing the amounts of allantoin and uric acid excreted in the urine. The amount of microbial purines absorbed (mmol/day) was estimated from the total purine derivative excretion (mol/day) using the equation proposed by Verbic et al., (1990):

$$\mathrm{PA} = (\mathrm{TPD} - 0.385 \cdot \mathrm{PV}\ 0.75)/0.85 \qquad (6)$$

where PA is the microbial purines absorbed (mmol day^{-1}); TD corresponds to the total purines (mmol day^{-1}); 0.85 = recovery of absorbed purines as purine derivatives in the urine and 0.385 = endogenous excretion of purine derivatives in the urine (mol) per unit of metabolic size.

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

$$\mathrm{NM}\ (\mathrm{g\ day^{-1}}) = (70 \cdot \mathrm{PA})/(0.83 \cdot 0.116 \cdot 1{,}000), \qquad (7)$$

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

Ingestive behavior

For the evaluation of ingestive behavior, the eight steers were visually observed for 24 hours on the 20th day of each period, and observations were recorded at 10-minute intervals, including feeding, rumination, and resting time (Mezzalira et al., 2011). On the same day, three observations were made for each animal: in the morning, at noon, and in the evening. Data were collected by trained observers using digital stopwatches. During the nighttime observation, the environment was kept under artificial lighting, and the animals underwent an adaptation period. The dietary variables (feeding, rumination, and resting) were obtained using equations adapted from Bürger et al. (2000).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in a 2×2 factorial arrangement within a Latin square design. The model included the fixed effects of bagasse level, urea inclusion, and their interaction, while animal and period were considered random effects. Means were compared using the *F*-test, and significance was declared at $P < 0.05$.

A complete list of abbreviations used is provided in Table 3.

Table 3. List of abbreviations

ADF – Acid Detergent Fiber;
BW – Body Weight;
CP – Crude Protein;
DM – Dry Matter;
EE – Ether Extract;
iNDF – Indigestible Neutral Detergent Fiber;
NDF – Neutral Detergent Fiber;
NDFap – Neutral Detergent Fiber corrected for ash and protein;
NDFapI – NDF corrected for ash and protein Intake;
NFC – Non-Fiber Carbohydrates;
NM – Microbial Nitrogen;
NPN – Non-protein nitrogen;
NRC – National Research Council;
PA – Purines Absorbed;
SB – Sugarcane Bagasse;
SEM – Standard Error of the Mean;
TDN – Total Digestible Nutrients;
TMR – Total Mixed Ration;
TPD – Total purine derivatives;
UR – Urea;
VFAs – volatile fatty acids.

RESULTS AND DISCUSSION

There was no significant interaction ($P > 0.05$) between bagasse proportion and urea inclusion for the evaluated intake variables (Table 4). Urea inclusion resulted in lower dry matter intake and lower NDFap intake ($P < 0.05$). The reduction in dry matter intake observed with urea inclusion suggests a negative effect on palatability, likely associated with its bitter taste and odor, which may induce aversive responses in ruminants (Detmann et al., 2007). Additionally, elevated ammonia concentrations in silage may contribute to reduced intake (Huhtanen et al., 2002).

Higher bagasse inclusion (50%) reduced intake, which can be attributed to increased dietary fiber concentration. Diets with higher neutral detergent fiber (NDF) levels tend to limit intake due to rumen fill, as fibrous components have slower degradation rates and occupy ruminal space for longer periods (Detmann et al., 2012).

The higher intake of non-fiber carbohydrates and total digestible nutrients observed in diets containing 40% bagasse reflects their greater energy density, reinforcing the importance of balancing fiber and energy sources in TMR silage formulations.

Table 4. Dry matter and nutrient intake of the diet by dairy heifers fed total mixed ration silage containing sugarcane bagasse with or without urea

Item	SB		Urea		SEM[1]	*P*-value		
	40%	50%	0%	2.5%		SB	UR	SB × UR
Consumption (kg day^{-1})								
Dry matter (DM)	5.03	4.66	5.06	4.63	0.1	0.02	0.01	0.06
Crude protein	1.00	0.97	1.01	0.96	0.0	0.52	0.24	0.97
Ether extract	0.17	0.15	0.16	0.16	0.0	0.21	0.59	0.05
NDFap[2]	1.70	1.86	1.88	1.73	0.0	0.04	0.04	0.10
NFC[3]	1.88	1.39	1.71	1.55	0.0	0.00	0.09	0.16
TDN[4]	3.44	2.94	3.09	2.99	0.0	0.00	0.08	0.12
Consumption (% of body weight)								
DM	2.43	2.22	2.43	2.21	0.08	0.04	0.04	0.13
NDFap	0.83	0.89	0.89	0.83	0.03	0.08	0.08	0.11
Consumption (% metabolic weight)								
DM	92.28	85.06	92.39	84.94	2.84	0.04	0.03	0.11

[1]SEM: standard error of the mean; [2]Neutral Detergent Fiber corrected for ash and protein; [3]Non-fiber carbohydrates; [4]Total digestible nutrients; SB = sugarcane bagasse; UR = urea.

And the protein and energy levels expressed as crude protein (CP) and total digestible nutrients (TDN) are within the recommendations of the NRC (2001), thus there was no imbalance in the protein/energy ratio, which could lead to reduced consumption due to low microbial growth (Calsamiglia et al., 2010).

The average dry matter intake (DM) of 4.85 kg day^{-1} for heifers weighing 220 kg was below the estimate of 5.50 kg day^{-1} by the NRC (2001). One factor that may have led to this lower DM intake compared to the NRC (2001) recommendation is the quality of the forage (sugarcane bagasse) and, consequently, higher levels of neutral detergent fiber. This occurred in this study, as the average NDF (neutral detergent fiber) content of the diet was 41.3%, while the value recommended by the NRC (2001) is 28%. Thus, the high fiber content may have limited intake due to filling, with diets with higher bagasse content (50%) resulting in lower DM intake.

Feed restriction due to filling, or physical intake limitation, occurs when the rumen's physical capacity is reached, regardless of whether the animal has satisfied its energy or nutrient needs. This limitation is common in high-fiber, bulky diets, such as low-quality forages like sugarcane bagasse, which have low energy density and occupy a lot of space in the rumen. In these cases, even if the animal wants to consume more to meet its nutritional needs, it cannot due to the limited space in the rumen (Detmann et al., 2012).

Another factor that may have influenced dry matter intake is the urea content, as diets containing urea showed lower dry matter intake compared to diets without urea. According to Detmann et al. (2007), urea emerges as an effective regulator of supplement intake among grazing cattle, thanks to its association with aversive sensations in the animals. The sensory properties of urea, notably its bitter taste and distinctive odor, also exert control over supplement intake.

Some authors have reported lower dry matter intake in cattle fed silages containing high levels of NH3-N (Rook & Gill, 1990; Cushnahan et al., 1995; Huhtanen et al., 2002).

Lazzari et al. (2021) evaluated the intake of beef cattle fed TMR silage with different protein sources, observing lower intake for silage with urea when compared to silage with protein sources from soybean meal and soybean grain, obtaining a negative variation in intake of 10 percentage points. Thus, these data corroborate the data obtained in this work, since diets with urea also obtained lower dry matter intake.

The influence of higher bagasse content (50%) and the inclusion of urea on limiting intake can be confirmed when analyzing DM intake as a percentage of body weight and as a percentage of metabolic weight, where diets containing higher bagasse content and diets with urea inclusion resulted in lower DM intake for these indices.

The level of bagasse influenced NDFap consumption, which can be explained by the fiber content of the diet, since the diet with 50% bagasse has higher levels of NDFap, NDFi and Lignin (Table 2), having 6.55; 5.1; 2.2 percentage points more respectively when compared with diets containing 40% bagasse, consequently diets with 50% bagasse tend to have higher fiber consumption.

Table 5. Dry matter and nutrient digestibility coefficient of the diet of dairy heifers fed total mixed ration silage containing sugarcane bagasse with or without urea

Digestibility coefficient	Bagasse		Urea		SEM[1]	*P*-value		
	40%	50%	0%	2.5%		SB	UR	SB × UR
DM[2]	63.7	53.5	57.7	59.6	1.1	0.00	0.14	0.03
CP[3]	67.8	64.1	63.2	68.6	1.0	0.01	0.00	0.05
EE[4]	73.1	72.7	73.3	72.5	0.8	0.66	0.39	0.57
NDFap[5]	53.9	46.5	50.4	49.9	1.8	0.00	0.79	0.27
NFC[6]	83.9	80.7	82.4	82.2	0.6	0.00	0.77	0.62
TDN[7]	68.5	63.1	64.7	67.0	0.6	0.00	0.01	0.29

[1]SEM: standard error of the mean; [2]Crude protein; [3]Ether extract; [4]Neutral Detergent Fiber corrected for ash and protein; [5]Non-fiber carbohydrates; [6]Total digestible nutrients; SB = sugarcane bagasse; UR = urea.

There was a significant interaction ($P < 0.05$) between the proportion of bagasse and the inclusion of urea for dry matter digestibility (Table 5), with the DM digestibility being higher in the diets with both bagasse levels, 40% and 50%, when urea was included (Table 6). The proportion of bagasse and the inclusion of urea influenced CP digestibility, with higher digestibility observed in the diet containing 40% bagasse and with the inclusion of urea ($P < 0.05$).

The proportion of bagasse influenced the digestibility of CP, NDFap, NFC, and TDN ($P < 0.05$), with diets containing 40% bagasse showing higher digestibility. For CP and TDN digestibility, in addition to the proportion of bagasse, the inclusion of urea also had an influence, with urea inclusion providing higher digestibility ($P < 0.05$).

The improvement in digestibility with urea inclusion is likely associated with its role as a non-protein nitrogen source, stimulating the growth of ruminal microorganisms, particularly fibrolytic bacteria. This effect enhances fiber degradation and increases nutrient availability. Conversely, the reduction in digestibility observed at higher bagasse levels reflects the increased lignin and indigestible fiber content, which limits microbial access to cell wall components. These results highlight the negative impact of excessive low-quality fiber on nutrient utilization.

Additionally, the ensiling process may enhance starch availability through proteolysis, particularly in diets rich in concentrate, contributing to improved digestibility and feed efficiency, providing greater digestibility of CP, NDFap, NFC, and thus an increase in TDN.

Miyaji et al. (2017) reported an increase in ruminal starch degradation in TMR silage containing flaked corn. An increase in starch digestibility is expected for dry ground or dry rolled corn (or sorghum) when ensiled as part of an TMR if stored for at least two months (based on high-moisture corn silage data). Such an increase in starch digestibility has been associated with greater feed efficiency in animals fed TMR silages (Lazzari et al., 2021).

In their research, Xu et al. (2007) partially replaced cattail hay and alfalfa hay with moist coffee husks in a silage of TMR (0, 100, or 200 g per kg DM) and reported a linear increase in NDF and ADF content and a linear decrease in apparent NDF and ADF digestibility with the inclusion of coffee husks. Consequently, cell wall hydrolysis and changes in fiber content and digestibility during ensiling are primarily dependent on the quality of the ingredients included in the TMR.

The inclusion of urea resulted in higher TDN (Total Digestible Nutrients) and CP (Crude Protein) digestibility. This can be explained by the growth-promoting action of urea on proteolytic bacteria during the ensiling process of the total diet. In addition, the diets contained a significant amount of starch-rich concentrates (60% and 50% concentrate for the diets with 40% and 50% bagasse, respectively), with the majority of this concentrate consisting of ground corn. The corn used was flinted corn, i.e., hard corn coated with prolamins; thus, the action of proteolytic bacteria also increases the availability of starch and proteins in the rumen environment.

The inclusion of urea in conventional forage silage can be detrimental due to its buffering capacity, leading to difficulties in pH reduction during the ensiling process, and because it contributes to proteolysis in forages with high crude protein content (e.g., legumes, temperate grasses), it becomes undesirable and leads to lower nitrogen use efficiency (Huhtanen et al., 2008; Hymes-Fecht et al., 2013).

However, in corn and sorghum silages (plant or grain silages), proteolysis has been positively associated with starch digestibility due to the degradation of hydrophobic proteins (prolamins) surrounding the starch granules (Hoffman et al., 2011).

In TMR silages, which are mixtures of ingredients, we found no report that considered the contribution of microorganisms and plant proteases to proteolysis. Meanwhile, enterobacteria, clostridia, and bacilli are the main agents involved in protein degradation during silage storage (McDonald et al., 1991; Pahlow et al., 2003).

Higher DM digestibility (Table 6) was observed in diets with urea inclusion and a lower proportion of bagasse. This is due to the lower proportion of fibrous material in the diet, and these diets showed lower levels of NDFap, NDFi, and lignin when compared to diets with 50% bagasse.

Therefore, lower fiber content and higher concentrate content lead to increased degradation and passage rates because diets with higher concentrate content are more easily degraded in the rumen environment than diets rich in fiber, which are rich in starch and have a higher degradation and passage rate. Diets with higher fiber content, on the other hand, have slower degradation and, due to their composition, present high levels of components with low degradability such as NDFap and components with low or zero degradability such as NDFi and Lignin, consequently reducing their passage rate and digestibility.

Table 6. Dry matter digestibility coefficient of dairy heifers fed total mixed ration silage containing sugarcane bagasse with or without urea

Proportion of bagasse	0% urea	2.5% urea	Average	SEM[1]
	DM digestibility			
40 (%)	63.2 Aa	64.3 Aa	63.7	1,119
50 (%)	51.1 Bb	55.9 Ba	53.5	
Average	57.12	60.10		

[1] SEM: standard error of the mean, means followed by the same lowercase letter in the row and the same uppercase letter in the column do not differ from each other by the *F* test ($P < 0.05$).

The increase in digestibility with urea inclusion may also be associated with ammoniation effects during ensiling, which can partially disrupt lignocellulosic bonds and improve fiber accessibility to ruminal microorganisms.

Although urea inclusion improved nutrient digestibility, its practical application should be carefully considered, as the associated reduction in dry matter intake may limit overall animal performance depending on production goals.

These results reinforce the importance of balancing nitrogen supplementation and intake regulation in diet formulation.

This trade-off highlights the importance of evaluating both intake and digestibility responses when using non-protein nitrogen sources in TMR silage systems.

According to Oliveira et al. (2007), the main purpose of ammoniation is to enrich the nutritional value of forage by providing non-protein nitrogen and improving its digestibility. In this way, ammoniation of forages results in greater degradation of the cell wall, including cellulose and hemicellulose, due to the extension of its molecules, the breaking of hydrogen bonds, and the increased hydration of the fiber.

Urea doses have demonstrated the ability to reduce neutral detergent fiber and acid detergent fiber in sugarcane bagasse. Ammoniation improves the digestibility of sugarcane bagasse as a result of changes in cell wall constituents, since it causes partial solubilization of hemicellulose and breaking of the bond between lignin and carbohydrates in the cell wall, favoring the action of rumen microorganisms (Oliveira et al., 2011), in addition to improving its nutritional value with an increase in crude protein through the availability of NPN (non-protein nitrogen).

In addition to improving fiber solubility, the addition of a non-protein nitrogen (NPN) source, such as urea, increases nitrogen availability in the rumen. This stimulates the growth and activity of ruminal microorganisms, especially fibrolytic bacteria, responsible for the degradation of plant fiber present in fibrous feeds consumed by ruminants. As a result, ruminal kinetics are accelerated, promoting greater degradation of plant fiber and increased production of volatile fatty acids (VFAs), such as acetate, propionate, and butyrate. These VFAs are absorbed by the rumen wall and provide energy for the host animal.

The results obtained in this work corroborate those found by Lazzari et al. (2021), who analyzed digestibility under the effect of protein sources in silages of TMRs, evaluating the inclusion of urea, soybean meal, and soybean grain, and the silages with urea showed greater DM digestibility compared to the true protein sources.

The proportions of sugarcane bagasse and the inclusion of urea did not influence ($P > 0.05$) urinary volume, microbial CP production and microbial efficiency, with average values of 10.83 L day^{-1}, 562.82 g day^{-1} and 177.92 g CP kg^{-1} TDN, respectively (Table 7).

Microbial protein production in ruminants is strongly influenced by the availability of carbohydrates and nitrogen in the rumen (NRC, 2001). Adequate energy supply is crucial, as it is the main limiting factor for microbial growth. The primary function of this growth is to generate ATP, which is essential for the utilization of ammonia in amino acid synthesis and microorganism growth (Possenti et al., 2008).

In other studies, urinary volume was also not influenced by the use of TMR silage with different by-products, (Miyaji et al., 2017) and (Lazzari et al., 2021), being evaluated in lactating cows and Nellore heifers, respectively.

Table 7. Urinary volume, microbial protein synthesis, and microbial efficiency of dairy heifers fed total mixed ration silage containing sugarcane bagasse with or without urea

Item	Bagasse		Urea		SEM[1]	*P*-value		
	40	50	Sem	Com		SB	UR	SB × UR
Urine volume (L day^{-1})	9.8	11.8	10.3	11.4	1.5	0.25	0.50	0.65
Microbial production (g day^{-1})								
Microbial CP	539.4	586.2	556.2	569.4	51.9	0.53	0.85	0.13
Microbial efficiency								
G CP per kg TDN	156.1	198.1	173.2	182.5	24.9	0.14	0.71	0.12

[1]SEM: standard error of the mean; SB = sugarcane bagasse; UR = urea.

Microbial protein synthesis plays a crucial role in ruminant nutrition, providing proteins in the quantity and quality essential for their well-being. Recent studies (Nguyen et al., 2017) have found that this synthesis represents between 50% and 80% of the total protein absorbed by the animal. The microbial efficiency ratio recommended as a minimum ideal by Valadares Filho et al. (2016) is 120 g microbial protein per kg of total digestible nutrients (TDN) as a reference for microbial synthesis efficiency under tropical conditions. In this study, a measured value of 177.92 g microbial protein per kg of TDN was obtained, falling within the premise recommended by Valadares Filho et al. (2016).

This factor is particularly important for the category used in this study, where heifers require a higher protein intake for their growth and muscle development, but with low fat deposition, as this can hinder mammary gland development (Campos & Assis, 2005).

The results obtained for nitrogen balance and urea excretion are presented in (Table 8). Regarding the inclusion of urea, treatments without urea resulted in greater N excretion in feces ($P < 0.05$). The proportion of bagasse influenced the N balance, with the 40% proportion showing the highest N balance ($P < 0.05$). The proportions of sugarcane bagasse and the inclusion of urea did not influence ($P > 0.05$) plasma urea nitrogen, urinary urea, and urinary urea nitrogen.

Table 8. Nitrogen compound balance of dairy heifers fed total mixed ration silage containing sugarcane bagasse with or without urea

Item	Bagasse		Urea		SEM[1]	*P*-value		
	40%	50%	0%	2.5%		SB	UR	SB × UR
N g^{-1} day balance								
N ingested	160.4	156.1	162.4	156.1	6.4	0.52	0.24	0.97
N in feces	52.0	54.7	58.0	50.8	2.6	0.33	0.01	0.15
N in urine	22.2	23.5	22.9	22.9	3.3	0.69	0.99	0.86
N retained	86.2	77.8	81.5	82.5	4.0	0.08	0.81	0.41
Nitrogen balance	53.6	49.3	49.6	53.2	1.6	0.03	0.06	0.13
Percentage of N								
Plasma urea N	2.0	2.6	2.2	2.4	0.3	0.174	0.702	0.678
Urea urine	126.8	129.2	127.6	128.5	27.9	0.931	0.975	0.420
Urine urea N	5.91	6.02	5.99	5.94	1.3	0.933	0.977	0.422

[1] SEM: standard error of the mean; N = Nitrogen; SB = sugarcane bagasse; UR = urea.

When it comes to the balance of nitrogen compounds, it is essential to consider both nitrogen intake and excretion by ruminants. The quantity and quality of nitrogen in the diet affect the efficiency of microbial protein synthesis in the rumen, as well as nitrogen excretion in urine and feces. This is particularly important due to the potential environmental impact of excess nitrogen excreted by animals, such as contributing to the eutrophication of water bodies.

Research indicates that the composition of TMR silage can influence the nitrogen balance in ruminants. For example, Bueno et al. (2020) observed that the inclusion of certain ingredients in TMR silage can affect the rate of digestion and utilization of nitrogen by ruminal microorganisms. Furthermore, the protein-to-energy ratio in the diet can influence the efficiency of microbial synthesis and, consequently, the nitrogen balance in the animal.

Another aspect to consider is the impact of TMR silage fermentation on nitrogen metabolism. The anaerobic fermentation that occurs during the ensiling process can affect the availability and form of nitrogen in the diet, potentially influencing the digestibility and utilization of nitrogen by ruminants.

The results obtained for feeding behavior are presented in Table 9. There was no significant interaction ($P > 0.05$) between the level of bagasse and the inclusion of urea for the variables analyzed. When analyzed separately, the inclusion of urea significantly increased ($P < 0.05$) the rumination and chewing time of DM in min g^{-1} of DM. The 50% bagasse proportion increased the rumination time of NDFap in min g^{-1} of NDFap.

Table 9. Feeding behavior of dairy heifers fed total mixed ration silage containing sugarcane bagasse with or without urea

Item	Bagasse		Urea		SEM[1]	*P*-value		
	40%	50%	0%	2.5%		SB	UR	SB × UR
Feeding								
Min day^{-1}	306.3	345.0	320.0	331.3	28.9	0.22	0.71	0.18
Min kg^{-1} DM	62.1	74.4	64.6	71.9	6.9	0.12	0.33	0.29
Min kg^{-1} NDF ap^{-1}	182.0	186.1	174.9	194.1	20.3	0.87	0.37	0.41
% per day	21.3	24.0	22.2	23.0	2.0	0.22	0.71	0.18
Rumination								
Min day^{-1}	460.0	502.5	467.5	495.0	25.7	0.14	0.32	0.32
Min kg^{-1} DM	99.0	102.4	93.5	107.9	5.4	0.55	0.03	0.08
Min kg^{-1} NDF ap^{-1}	247.8	301.1	253.3	295.7	15.9	0.01	0.37	0.05
% per day	31.9	34.9	32.5	34.4	1.8	0.14	0.32	0.32
Leisure								
Min day^{-1}	654.0	609.3	652.5	613.8	34.1	0.91	0.30	0.65
% day^{-1}	46.8	41.1	45.3	42.6	2.4	0.91	0.30	0.65
Chew								
N per cake	51.5	54.3	56.1	49.7	3.1	0.41	0.08	0.92
s per cake	46.6	49.5	50.8	45.3	3.21	0.40	0.13	0.90
n per day	33,238.2	30,274.3	30,995.1	32,517.5	1,377.4	0.07	0.31	0.27
Min day^{-1}	808.8	805.0	787.5	826.3	34.1	0.91	0.30	0.65
Min kg^{-1} DM	164.5	173.4	158.0	179.9	8.1	0.31	0.03	0.69
Min kg^{-1} NDF ap^{-1}	483.9	434.0	428.1	489.8	25.4	0.09	0.05	0.46

[1] SEM: standard error of the mean; N = Nitrogen; SB = sugarcane bagasse; UR = urea.

The increase in rumination time and number of ruminal boli observed with higher bagasse inclusion reflects the greater physical demand required to process fibrous diets. High NDF content stimulates chewing activity, which is essential to reduce particle size and facilitate microbial digestion.

Urea inclusion increased rumination time per unit of dry matter, possibly due to enhanced microbial activity and greater fiber degradation, which may alter ruminal dynamics and chewing behavior.

These findings reinforce the relationship between dietary fiber characteristics and ingestive behavior, highlighting the importance of fiber quality in TMR silage systems, with fiber effectiveness being a primary factor in stimulating chewing (Grant & Albright, 1995).

According to Oliveira et al. (2007), diets with urea provided greater rumination and dry matter chewing time; ruminant diets that include urea can increase dry matter chewing and rumination time. This occurs because urea is a source of non-protein nitrogen that can be used by rumen microorganisms to synthesize microbial protein. When urea is provided in the diet, rumen microorganisms become more active in the degradation of dietary fiber, which can lead to an increase in the time spent by animals in chewing and rumination activities.

There was no significant interaction ($P > 0.05$) between bagasse level and urea inclusion for the number of periods and average time spent per period on activities (Table 10). When analyzed separately, the 50% bagasse proportion significantly increased ($P < 0.05$) the NDFap rumination time expressed in g NDFap per hour and increased the number of boluses per day (cakes per day). Urea inclusion significantly increased ($P < 0.05$) the number of boluses per day (cakes per day) and reduced the amount of DM rumination in (g DM per hour).

The higher number of ruminal boli observed in diets with 50% bagasse is consistent with increased fiber content, which stimulates rumination as a mechanism to enhance particle breakdown and digestion.

Table 10. Number of periods and average time spent per period on feeding, rumination, and resting activities of dairy heifers fed a total mixed ration silage containing sugarcane bagasse with or without urea

Item	Bagasse		Urea		SEM[1]	*P*-value		
	40%	50%	0%	2.5%		SB	UR	SB × UR
Periods (Number per day)								
Feeding	14.9	15.9	15.5	15.3	1.8	0.59	0.89	0.98
Rumination	14.3	14.8	14.3	14.8	0.8	0.56	0.56	0.12
Idleness	22.8	23.0	22.3	23.5	1.4	0.86	0.39	0.60
Time spent per period (min)								
Feeding	20.6	22.1	20.8	22.0	1.8	0.43	0.53	0.18
Rumination	36.4	31.5	33.3	34.7	2.1	0.05	0.52	0.52
Idleness	27.9	28.4	29.7	26.6	2.9	0.86	0.32	0.36
Feeding								
g DM per hour	1013.0	830.8	975.1	868.9	85.5	0.07	0.26	0.63
g NDF ap per hour	344.3	331.1	358.4	317.8	32.8	0.72	0.26	0.67
Rumination								
Cakes per day	562.9	668.5	566.3	665.2	30.4	0.013	0.017	0.36
g MS per hour	611.2	611.6	655.3	567.5	33.8	0.989	0.047	0.11
g NDFap per hour	207.4	244.6	241.6	210.3	13.4	0.031	0.057	0.12

[1]SEM: standard error of the mean; N = Nitrogen; SB = sugarcane bagasse; UR = urea.

The same relationship occurs for the inclusion of urea, where diets with urea provided a longer rumination time of dry matter (Table 9), consequently leading to an increase in the number of boluses ruminated per day, being used as a strategy to increase fiber degradation and digestion.

CONCLUSIONS

TMR silage containing 40% sugarcane bagasse without urea provided the best balance between intake and nutrient utilization in dairy heifers. Although urea improved digestibility, its negative impact on intake may limit its practical application.

Increasing bagasse levels to 50% negatively affected intake and digestibility, emphasizing the importance of controlling fiber inclusion in TMR silage. Overall, optimizing the combination of fiber sources and nitrogen supplementation is essential to improve feeding efficiency in tropical ruminant production systems.

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