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SUGARCANE BAGASSE SILAGE TREATED WITH ANYDRUM AMMONIA AS EXCLUSIVE FORAGE IN SHEEP DIET

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ABSTRACT

The effects of adding 0; 2; 3 and 4% of anhydrous ammonia (NH_3) to sugarcane bagasse (based on dry matter) were studied in order to evaluate the chemical composition, consumption and apparent digestibility of nutrients in sheep. Four sheep were used, kept in total confinement, with an average live weight of 40.7 kg, for 72 days, distributed in a 4 x 4 Latin square experimental design. The use of anhydrous ammonia did not influence the levels of crude protein, hemicellulose, cellulose and lignin, but there was a linear reduction ($P<0.05$) of the fiber content in acid detergent and a quadratic effect ($P<0.05$) for the fiber content in neutral detergent, with increasing doses of anhydrous ammonia in sugarcane bagasse. There was no effect of ammoniated sugarcane bagasse ($P>0.05$) on the intake and digestibility of dry matter, fiber in neutral detergent, fiber in acid detergent, hemicellulose, cellulose and lignin. It is concluded that sugarcane bagasse ammoniated with up to 4% NH_3 should not be used as exclusive forage in the diet of sheep in confinement.

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INTRODUCTION

The cultivation of sugarcane in Brazil had a great impulse after the 70's with the creation of the National Alcohol Program (Proálcool) and byproducts were generated as a result of this program, mainly sugarcane bagasse (SCB) that has been used as a single roughage or as part of the fibrous fraction of the ruminant diet, especially in times of forage shortage and in animal husbandry systems in confinement (Conab, 2018). The national estimate for the 2021/22 sugarcane crop is 628.1 million tons, with SCB representing the largest fraction of the residues produced by the Brazilian agribusiness, which, on average, is 250 kg for each ton of ground cane sugar, that is, 157 million tons. SCB can be used in the composition of the animal diet in the form of silage or "in natura" (Costa; Saliba; Santana, 2014). In sugar-alcohol producing regions, SCB can accumulate in the plants at the end of the harvest and present advantageous prices, arousing the interest of ranchers due to the opportunity to reduce the cost of food and the possibility of increasing the profitability of the production system (Siqueira et al., 2019).

However, SCB has chemical and physical characteristics that reduce its use and voluntary consumption in animal feed, being considered of low nutritional value, as it has a high cell wall content (above 60%), high fiber content in acid detergent (above 40%) and reduced crude protein contents (below 6%), in addition to low dry matter digestibility (40 to 50%) (Kermani; Bahrololoum; Koohzadi, 2019). When offered as the sole component of the diet, SCB does not meet the animal's maintenance needs. Thus, if the objective is to achieve maintenance or weight gains, SCB needs to be supplemented and the improvement of the nutritional value of the forage can be achieved with the addition of chemical compounds such as anhydrous ammonia (NH_3) which has a high nitrogen content through the process known as ammonization (Soares et al., 2015). The remarkable effect of ammonization on SCB is the increase in the content of nitrogen compounds, which is generally low, limiting the growth of rumen microorganisms, making it difficult for them to access the point where it is possible to break the cellulosic polymer, usually resulting in a decrease in the rate and extent of fiber digestion (Cruz and Silva, 2016). However, Missio (2016) considers that, regardless of the chemical treatment used for SCB in ruminant feed, before supplying it, a concentrated feed should be added that makes it possible to complement the nutrients in the diet and meet the nutritional requirements of the animals. Chemical treatments are the most used in the treatment of SCB, and in general they have limitations, since sodium hydroxide reduces the efficiency of fiber digestion; ammonization presents difficulty in handling and risk of intoxication and the effects of using urea on cell wall constituents have been contradictory (Manju et al., 2019), reinforcing the need for further studies. In this context, the objective of this work was to evaluate the effect of the addition of anhydrous ammonia on the chemical composition, consumption and digestibility of nutrients of SCB used as exclusive roughage in diets for sheep kept in confinement.

MATERIAL AND METHODS

All management practices involving the animals were approved by the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais, certificate 153/2009. The experiment was conducted at the Veterinary School of the Federal University of Minas Gerais, in Belo Horizonte (MG) from June to August 2010. Sugarcane bagasse (SCB) from the milling process for the manufacture of brandy (aguardente) was used. The chemical-nutritional composition and in vitro dry matter digestibility (IVDMD) of SCB are shown in Table 1. Four adult male, castrated sheep, with no defined breed pattern (NDBP), with mean body weight of 40.75 kg were used, housed in individual cages of 1.50 m in length and 0.80 m in width, provided with individual troughs and drinkers.

Table 1. Chemical-bromatological composition and in vitro dry matter digestibility (IVDMD) of sugarcane bagasse in percentage of dry matter

Components	Composition (%)
Dry matter	50.0
Crude Protein	1.60
Neutral detergent fiber	93.3
Fiber in acid detergent	62.7
Hemicellulose	30.6
Cellulose	45.3
Lignin	16.5
IVDMD	32.8

The experimental period lasted 72 days and the trial was subdivided into four periods of 18 days each, with 10 days of adaptation and eight days of data collection. The animals were distributed in a 4 X 4 Latin square design, and the treatments consisted of different levels (0; 2; 3 and 4%) of anhydrous ammonia (NH_3) in diets with SCB as roughage; the proportion of ingredients in the experimental diets, in percentage of dry matter, is presented in Table 2. Three surface silos were made with about one ton of SCB, using plastic canvas; later, a container with a capacity of 50 kg of NH_3 was used for the application of NH_3, and the necessary amount to be added to the silo was obtained by difference of weight, then NH_3 was applied at two opposite points of each silo, using ½ inch PVC pipes perforated every 20 cm, with a diameter of 0.5 cm. The BAC was stored in the silos for 10 months before being open to supply to the animals.

Table 2. Proportion of ingredients in experimental diets, in percentage of dry matter (DM)

Ingredients	Concentration (NH_3) % DM			
	0	2	3	4
Sugarcane bagasse	90.4	88.4	87.4	86.4
Anhydrous ammonia (NH_3)	0.0	2.0	3.0	4.0
Urea	6.0	6.0	6.0	6.0
Limestone	2.8	2.8	2.8	2.8
Dicalcium phosphate	0.4	0.4	0.4	0.4
Common salt	0.3	0.3	0.3	0.3
Premix	0.1	0.1	0.1	0.1
Total	100	100	100	100

Equal proportions of urea were added to the diets, thus leveling the protein content (PC) in relation to the treatment with a concentration of 4% NH_3, and the calculation was performed based on the protein equivalent of urea. In each treatment, the PC analyses were performed in advance for the aforementioned adjustment. Sulphur flower was added as a source of Sulphur 96%, in a 9:1 ratio to aid the formation of sulphur amino acids. The diets were provided twice a day, in the morning and in the afternoon, with an interval of eight hours between the periods. DM intake was *ad libitum*, with leftovers removed and weighed daily to determine daily consumption, allowing an average of 10% leftovers. The animals had free access to water and mineral mixture. For the calculation of the supplied amounts of the diet, the consumption of 1.5 to 2% of the live weight was considered. To estimate the apparent digestibility of nutrients, total collection of feces from the animals was performed from the 12th to the 18th day of each experimental period. The collection carried out in each period, per animal, was made with the aid of bags made of curvim, fixed to the animals through leather harnesses. The feces were weighed in the morning, removing approximately 10% of the total, and frozen in a freezer at -10^{o}C for further analysis. For the determination of the individual voluntary consumption of nutrients, during 7 days, the offered samples of the diet and leftovers were taken daily at 6:30 h, weighed and placed in plastic bags. Later on, the samples were stored at -20°C, and after thawing, a composite sample per animal was prepared. The samples of the offered diets, leftovers and feces were pre-dried in a forced ventilation oven at a temperature of 55°C for 72 hours, ground in a Willey mill with a 1 mm mesh sieve. To calculate the consumption in metabolic weight unit ($kg^{0.75}$) the animals were weighed, and the average weights between the beginning and the end of each experimental phase were recorded.

For the measurement and estimation of the consumption and digestibility of nutrients and characterization of the diets, in the samples of the offered diets, leftovers and feces, the contents of dry matter (DM) and crude protein (CP) were analyzed, and the CP was obtained by multiplication of N by the factor 6.25 according to the methodology of Silva and Queiroz (2009). For the quantification of fiber in neutral detergent (NDF), fiber in acid detergent (ADF), cellulose (CEL), hemicelluloses (HEM) and lignin (LIG), the methodology described by Van Soest et al. (1991) was adopted, and the digestibility of the diets was determined by the “in vitro” method described by Tilley and Terry (1963). The experimental design was in 4x4 Latin square, the results were interpreted by regression analysis, at a 5% probability level, according to the mathematical model: $Yijk= \mu + \tau i + \theta j + \lambda k + \varepsilon ijk$ where, Y = Response variable, μ = Mean value of Y, τi= Effect of treatment i (i = 0 to 4), θj= Effect of animal j (j = 0 to 4), λk = effect of period k (k = 0 to 4) and εijk = Experimental error. Analyses of variances were performed using the statistical program (Sas, 2011).

RESULTS AND DISCUSSION

The CP content of the NH_3-treated SCB did not differ (P>0.05) from the untreated SCB (0%) possibly due to the low CP content of the SCB, as the diets were isoproteic, containing 6% urea (Table 2) and the mechanism of action of chemical agents, since they act on the fibrous fraction of foods, would not have a direct influence on CP levels. (Table 3). The CP content of the ruminant diet for the supply of nitrogen necessary for ruminal microbial activities, aiming at not compromising consumption and digestibility, should be at least 6% to 8%. In this study, except for the diet with 4% NH3 (7.9% of CP), all were below this limit. According to Silva et al. (2015) on average, for sheep in the finishing phase, the CP content in the diet should be 16%, indicating that SCB needs a protein correction. There was a significant effect (P<0.05) of the treatment with NH_3 for the levels of FND (P=0.05) and FAD (P=0.01), which presented lower values, when compared to the treatment without NH_3, in the treatments with 3% and 4% NH3, respectively (Table 3). Similar behaviors were obtained by Pires et al. (2004) when evaluating SCB treated with NH_3 in ruminant feed, and observed a reduction in FND and smaller variations in FAD values.

Table 3. Chemical composition of experimental diets, based on dry matter

Items	Concentration (NH_3) % DM							p-value	
	0	2	3	4	AE	R^2	[1]EPM	Linear	Quadratic
CP	2.1	3.5	3.9	7.9	-	-	1.23	0.13	0.26
FND	91.1	89.4	89.1	89.5	$Y=91,16-1,39X+0,24X^2$	99.7	0.46	0.16	<0.05*
FAD	61.7	60.2	59.9	58.9	Y=61,71-0,06717X	97.8	0.58	<0.01*	0.15
HEM	32.6	31.4	31.5	32.6	-	-	0.34	0.84	0.12
CEL	44.8	44.3	40.8	42.2	-	-	0.93	0.22	0.63
LIG	15.2	17.9	13.5	14.6	-	-	0.93	0.69	0.82

CP = crude protein; FND= fiber in neutral detergent; FAD= fiber in acid detergent; HEM= hemicellulose; CEL=cellulose; LIG= lignin; AE=Adjusted equation; R^2=determination coefficient; [1]EPM = Standard error of the mean; *Significant at the 5% level (P<0.05).

Table 4. Nutrient consumption in percentage of metabolic weight ($g/kg^{0.75}$) in sheep fed with sugarcane bagasse treated with different levels of anhydrous ammonia (NH_3) in the diet in percentage of dry matter

Items		Concentration (NH_3) % DM			EPM1	*p-value	
	0	2	3	4		Linear	Quadratic
DM	26.3	28.9	34.6	30.3	1.75	0.29	0.62
FND	17.6	19.2	26.1	21.0	1.84	0.37	0.72
FAD	10.7	13.1	17.2	14.7	1.35	0.2	0.52
HEM	6.10	6.30	8.60	7.00	0.56	0.43	0.79
CEL	8.90	10.9	14.5	12.2	1.17	0.21	0.53
LIG	1.70	2.10	2.70	2.20	0.21	0.29	0.59

DM= dry matter; FND= fiber in neutral detergent; FAD= fiber in acid detergent; HEM= hemicellulose; CEL=cellulose; LIG= lignin; [1]EPM = Standard error of the mean; *Significant at the 5% level (P<0.05).

Table 5. Apparent digestibility of nutrients in sheep fed with sugarcane bagasse treated with different levels of anhydrous ammonia (NH_3) in the diet as a percentage of dry matter

Items	Concentration (NH_3) % DM				[1]EPM	p-value*	
	0	2	3	4		Linear	Quadratic
DM	47.2	50.6	64.0	54.1	3.62	0.36	0.71
FND	50.9	51.1	58.3	52.1	1.76	0.57	0.85
FAD	50.9	53	59.5	54.5	1.83	0.35	0.68
HEM	45.1	47.4	55.7	51.5	2.34	0.22	0.61
CEL	56.3	57.6	64.6	59.9	1.82	0.34	0.72

DM= dry matter; FND= fiber in neutral detergent; FAD= fiber in acid detergent; HEM= hemicellulose; CEL=cellulose;[1]EPM = Standard error of the mean; *Significant at the 5% level (P<0.05).

The levels of HEM, CEL and LIG of SCB were not influenced (P>0.05) by the addition of NH_3 (Table 3), indicating that the treatment with NH_3 has no effect on these variables and is possibly justified by the absence of elements in NH_3 capable of altering these fractions of the food. These results were similar to those obtained by Pimentel et al. (2015) who did not identify changes in the chemical composition of SCB treated with calcium oxide (CaO). The effect of ammonization on the chemical composition of FND and FAD of SCB, obtained in this study, are contrary to those of Rezende et al. (2013) when stating that there is no interference of chemical agents on the solubilization of fibrous fractions; and corroborate those by Oliveira et al. (2011) when they stated that ammonizating agents solubilize the fibrous fractions and improve digestion, due to the breakdown of lignocellulosic bonds, favoring microbial action. Analysis of variance showed no difference (P>0.05) for consumption in percentage of metabolic weight g/kg 0.75 of DM, FDN, FDA, HEM, CEL and LIG (Table 4) of the animals, which shows the inexistence of benefits regarding the consumption of these nutrients when NH_3 is used to improve the nutritional value of SCB up to 4% inclusion in DM. There was no effect (P>0.05) of the addition of NH_3 in SCB on DM consumption, indicating that the reduction in FAD levels at all inclusion levels and FND up to 3% inclusion (Table 4) of NH_3 was not enough to influence the DM consumption of the evaluated animals. These results do not agree with those obtained by Gunun et al. (2016) when stating that SCB, due to its lower nutritional value and high levels of FND, FAD and LIG, lead to lower DM consumption. Values higher than those in this study for DM consumption were obtained by Castrillo et al. (1995) who, in sheep, observed DM consumption of roughage treated with 3% NH_3, as a function of the metabolic weight of 50.6 $g/kg^{0.75}$, and Murta et al. (2011) evaluating SCB treated with calcium oxide obtained values for DM consumption of 80.7 $g/kg^{0.75}$. There was no effect (P>0.05) of the addition of NH_3 to SCB on FND and FAD consumption (Table 4).

The values for FND and FAD consumption ranged from 17.6 to 21.0; 10.7 to 14.7 $g/kg^{0.75}$, respectively, with the addition of NH_3 in SCB (0 to 4%). It was expected that the reduction of FND contents (up to 3%) and FAD of the diets with the addition of NH_3 (Table 3), would reduce the consumption of these nutrients, however, this behavior was not observed, and the measured consumptions were not altered. Although not significant, when considering absolute values, it is believed that the treatment with 3% NH_3 provided the animals with a more digestible diet and with a higher rate of passage. Regarding DM digestibility, there was no significant effect (P>0.05) of the addition of NH_3 in SCB, and the values of DM digestibility ranged from 47.2 to 54.1% with the addition of NH_3 in SCB (0 to 4%). Possibly, the lack of response to digestibility is related to the FAD, which in this study, decreases with ammonization, but was not enough to change the digestibility of DM. The absolute values for DM digestibility observed in the treatment with 4% NH_3 (54.1%) were lower (72.9%) than those obtained by Murta et al. (2011) studying the effects of adding calcium oxide to SCB in the feeding of crossbred Santa Inês sheep. There was no effect (P>0.05) of NH_3 on the apparent digestibility coefficient of FND, whose values ranged from 50.9% to 52.1% (Table 5). It is believed that these results may have been influenced by the proportions of cell wall components, HEM, CEL and LIG in the experimental diets, which did not vary significantly. FAD digestibility ranged from 50.9 to 54.5% when the levels of inclusion of NH_3 in SCB ranged from 0 to 4%. No effect (P>0.05) was detected on the FAD digestibility, it is believed that the lower numerical value referring to the LIG of the diet with 3% NH_3 (13.5%) may have been responsible for increasing the coefficient of FAD digestibility. The digestibility of FND and FAD observed in this study for the treatment with 4% NH_3 (52.1% and 54.5%) respectively, were lower (60.2% and 61.2%) than those obtained by Cândido et al. (1999) and higher (44.6% and 33.4%) than those obtained by Oliveira et al. (2008) when evaluating SCB and sugarcane, respectively, treated with calcium oxide in sheep feed. The values obtained in this study for the digestibility of fibrous fractions are in agreement with Freitas et al. (2018) reported that SCB is a lignocellulosic material with low nutritional value and low digestibility, and its composition is recognized for limiting the digestion of fibrous polysaccharides in the rumen, which are not always accessed by ruminal microorganisms due to the presence of phenolic compounds, which form a physical barrier to microbial action. There was no effect (P>005) of the hydrolysis process promoted by NH_3 on the digestibility of HEM and CEL, and it was not possible in this study to verify the possible benefit of NH_3 in promoting the disruption of the complex bonds of lignin with HEM and CEL, leading to, consequently, to the increase of digestibility. However, values contrary to those obtained in this study can be explained by the different ways of processing sugarcane that can bring differences in the digestibility of its byproducts.

CONCLUSIONS

The use of anhydrous ammonia up to 3% reduced the levels of fiber in neutral detergent and acid detergent in sugarcane bagasse. Anhydrous ammonia was not efficient to improve the consumption potential and digestibility of sugarcane bagasse in feeding confined sheep.

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