

Effect of different study factors on the *in situ* ruminal degradability of ammonified sugarcane bagasse

Effecto de los diferentes factores de estudio en la degradabilidad ruminal *in situ* del bagazo de caña amonificado

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Abstract: This study evaluated the *in situ* degradation of ammonified sugarcane bagasse silage as cattle feed during the dry season. Samples of fresh sugar cane bagasse (*Saccharum* sp) were randomly taken from the "Puro Bravo" farm, located in the "La Soledad" site, Junin canton, province of Manabi. A Completely Randomized Design with 3x2 Factorial Arrangement was used, where Factor A was represented by the different levels of urea inclusion (0, 3 and 5 %) and Factor B, fermentation times of sugarcane bagasse (30 and 44 days), each combination of these factors consisted of three replications. The best treatment is 5% urea, day 30 of fermentation, both in bromatology and degradability, and the degree of association of degradability at different times was related to the nutritional value of the ammonified bagasse, which is related to degradability and fiber. Ammonification with urea improved nutritional values, characterized by increasing crude protein and decreasing NDF, FDA and LDA as urea levels increased. The differences were highly significant ($P < .0001$), indicating that the results were highly significant for each of the variables studied, which allowed relating the degree of association of degradability at different times with the nutritional value of the ammonified bagasse silage.

Keywords: Agribusiness, degradability, nutrition, dry season.

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Resumen: Mediante este estudio se pudo evaluar la degradación *in situ* del ensilaje del bagazo de caña de azúcar amonificado, como alimento del ganado bovino en época seca. Se tomaron aleatoriamente muestras de bagazo fresco de caña de azúcar (*Saccharum sp*) de la Hacienda "Puro Bravo", ubicada en el sitio "La Soledad", cantón Junín, provincia de Manabí. Se utilizó un Diseño Completamente Aleatorizado con Arreglo Factorial 3x2, donde el Factor A se representó por los diferentes niveles de inclusión de urea (0, 3 y 5 %) y el Factor B, tiempos de fermentación del bagazo de caña (30 y 44 días), cada combinación de estos factores constó con tres repeticiones. El mejor tratamiento es el de urea al 5%, día 30 de fermentación, tanto en bromatología como en degradabilidad, además se relacionó el grado de asociación de la degradabilidad en distintos tiempos con el valor nutricional del bagazo de caña amonificado, el mismo que sí se relaciona con la degradabilidad y con la fibra. La amonificación con urea mejora los valores nutricionales, caracterizado por el aumentando de la proteína cruda y disminución de, así como la FDN, FDA y LDA en la medida de que se aumentan niveles de urea. Las diferencias fueron altamente significativas ($P < .0001$). Lo expuesto indica que los resultados fueron altamente significativos para cada una de las variables estudiadas, con lo cual se pudo relacionar el grado de asociación de la degradabilidad en distintos tiempos con el valor nutricional del ensilaje de bagazo de caña amonificado.

Palabras clave: Agroindustria, degradabilidad, nutrición, temporada seca.

1. Introduction

Effect of urea level (0.3 and 5%) and different fermentation times (30 and 44 days) on the chemical composition of sugarcane bagasse. The experiment was conducted at the Hacienda "Puro Bravo", located in the site "La Soledad", canton Junín, province of Manabí; geographically located between the coordinates 0° 56" 8" South latitude, 80° 11" 0" West longitude and an altitude of 15 meters above sea level (masl)(PDOT .2014).

The present experiment lasted three months, from the execution of the project until the bromatological analyses were obtained.

FACTORS UNDER STUDY

FACTOR A: Urea levels (0, 3 and 5 %)

FACTOR B: Bagasse fermentation times (30 and 44 days)

EXPERIMENTAL DESIGN

A Completely Randomized Design with 3x2 Factorial Arrangement was used, where Factor A was represented by the different levels of urea inclusion (0, 3 and 5 %) and Factor B, cane bagasse fermentation times (30 and 44 days). Each combination of these factors consisted of three replicates.

The statistical model used was as follows:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} : k-th observation of the i-th level of factor A and j-th level of factor B

μ General average.

α_i Effect of the i-th level of factor A (inclusion of urea) $i=1,2$ and 3

β_j Effect of the j-th level of factor B (Days of fermentation) $j= 1$ and 2

$\alpha\beta_{ij}$ First-order interaction effect of the i-th level of factor A with the j-th level of factor B.

ε_{ijk} Random effect or experimental error with zero mean and common variance.

MANAGEMENT OF THE EXPERIMENTS

CHARACTERISTICS OF THE SUGARCANE BAGASSE SILAGE PROCESSING SITE

The study was conducted between the months of January and May 2019 (harvest time), a shed was available that met the conditions and

characteristics to develop the Ammonification process such as: protected from direct solar radiation, rodents and with a dry environment that favored the integral maintenance of the study material; close to the milling equipment to develop the process of sugar cane bagasse.

OBTAINING BAGASSE FROM SUGAR CANE

The sugarcane crop was selected with an age of six (6) months, material that was harvested, collected and transported to the sugarcane mill for the extraction of sugarcane juice; as a result of this process, sugarcane bagasse was obtained and used as vegetative material.

AMMONIFICATION PROCESS WITH UREA

The nitrogen source used was agricultural urea (46% N), the ammonification procedure consisted of filling the 20 kg containers with the bagasse, then compacting it and moistening it in the urea-water solution until the containers were full, the containers were covered and hermetically sealed with packing tape to guarantee an anaerobic environment.

The arrangement of the treatments, resulting from the combination of the study factors, is as follows:

Percentage of Urea	Fermentation times (Days)
Urea 0% Urea	30
	44
Urea 3%.	30
	44
Urea 5%.	30
	44

After the first 15 days, the containers were opened with their respective treatments and days of fermentation, which allowed the ammoniated material to be ventilated, guaranteeing the excessive elimination of NH₃.

The samples were obtained with their respective doses of urea and fermentation times, and the drying and milling process was carried out.

One kg of ammonified sugarcane bagasse, per treatment, was

dehydrated for 72 hours, at temperatures between 45 and 60°, by means of an artisanal machine belonging to the Production Department of the Faculty of Veterinary Sciences. Once the aforementioned process was completed, the weights of each sample were obtained to determine the moisture value.

Materials and methods

After drying the sample, it was ground by means of a special blade mill with sieves of 1 mm diameter for bromatological analysis and 2 mm for degradation. Subsequently, the ground samples were placed in plastic tetra pack bags duly labeled with date, quantity and characteristics of the sample.

Once the ground treatments were obtained with their respective treatments with the fermentation days, 1 kg of each of them were weighed for the respective bromatological analysis.

Effect of urea level and different fermentation times on in situ ruminal degradability of sugarcane bagasse dry matter.

The present experiment was carried out in the Department of Animal Production of the Faculty of Veterinary Science of the Technical University of Manabí, located in the Santa Ana canton, parish of Lodana, geographically located in the east center of the province of Manabí, at 1° 12' latitude South and 80° 22" longitude West. Its altitude is 50 meters above sea level and its highest point reaches a height of 400 meters above sea level.

In Santa Ana, the rainy season is very hot, oppressive and cloudy and the dry season is hot, sultry and partly cloudy. During the course of the year, the temperature generally varies from 20 °C to 30 °C and rarely drops below 19 °C or rises above 32 °C (ECURED, 2019).

The average temperature is 20.2 °C, with an average annual precipitation of 4222.7 mm, with the months of August y September the driest and June y July the rainiest (ECURED, 2019).

The time taken for this research was about three (3) months in the field (February - April).

The research was carried out at the animal production department of the

Technical University of Manabí, Faculty of Veterinary Sciences, located in the Santa Ana canton, in the Lodana parish.

Fistulated crossbred cattle were used for a period of 15 consecutive days with a rest of one day for the inclusion of the samples.

In an analytical balance, 2.5 g of sample were weighed, ground to 2 mm, and each one was placed in a nylon bag previously labeled, which were sealed with plastic ties, of each treatment to be analyzed, with three repetitions, for its location in the rumen. Subsequently, they were extracted from the rumen at 0, 3, 6, 12, 24, 48 and 72 hours, respectively, with the different samples taken. Then, they were placed in a cooler with ice, the cannula was closed, and the surroundings of the cannula were washed with an iodine solution and with applications of silver spray (bactrovet) to avoid contamination.

The samples were taken to the freezer and incubated. After 72 hours, they were removed from the freezer and washed several times in a washing machine until the residues were eliminated and the water was colorless; this process was carried out for each of the hours and treatments to be evaluated.

All the bags for the zero hour (0) were not introduced in the animal, they were only included at the moment of washing the samples. Subsequently, the samples were centrifuged, the moorings were removed and they were taken to the oven for 24 hours at 105 °C. After 24 hours, the samples were weighed, then it was verified how much they degraded in each time.

For the processing of the samples, the analysis used by (Orskov and McDonald 1979) was used. The calculation of degradability was carried out using the following formula:

$$\text{Degradabilidad} = \frac{M_{\text{pre}} - M_{\text{post}}}{M_{\text{pre}}} \times 100$$

Where:

%DISMS: Percentage of *in situ* DM degradation.

Mpre: Pre-incubated matter

Mpost: Post-incubated matter

The data were analyzed through an Analysis of Variance. Differences between the study and interaction factors were observed through Tukey's Test at 5% probability, using the SAS statistical package (2013). Descriptive statistics were also performed, taking into account the mean (central tendency), standard deviation and coefficient of variation (dispersion measures). The results were represented in tables and graphs, bearing in mind the interest they reflect, according to the objectives set.

3. Result

In the analysis of variance (Annex 6), a highly significant difference was observed for the main factor urea ($P < .0001$), giving the best soluble fraction value for the ammonification of sugarcane bagasse at 5% urea (Table 4.3), which was 49.93%. The results obtained are similar to those found in the study by Castellano et al. (2017) who conclude that the average DIVMS values present a highly significant difference ($P < 0.01$) between urea levels. In addition, an increase of 11.17% of DIVMS was observed when applying 6% urea (66.59%). In contrast, differences were found for the effect of the origin of the panca. In another study by Fuentes et al. (2001) reported values of 66.05, 71.50 and 71.94% DIVMS when treating ground, chopped and whole corn stover, respectively, with 4% ammonia over a period of 4 weeks in relation to untreated stover (64.67% DIVMS). This is also corroborated with the results of the study Tesfaye et al. (2006) observed that treatment with 5% urea increased DIVMS by 7.7%, in proportion to the untreated panca.

In Figure 4.3, a significant direction change interaction ($P > 0.0453$) is highlighted. The improvement in digestibility of highly fibrous materials treated with urea can be attributed to the effect of ammonia on the fiber cell walls. The results found in the study by Araiza et al. (2013) in which it is indicated that the chemical action of ammonia allows breaking the bonds of the structural complexes of cellulose and hemicellulose, which favors greater quantity and availability of soluble carbohydrates for ruminal microorganisms; as well as Reyes (2018) found that the soluble or rapidly degradable fraction (A) of ammonified peanut shells achieved values ranging between 50.68 and 53.19%. The exposed results differ from reported by Araiza et al.

(2013) with higher values in corn and apple silages that were in the order of 39.49 % and 42.53 %. These results show that the interaction is significant because the simple factor urea is affecting the study variable.

Table 2 Effect of different levels of urea 3 and 5% on *in situ* ruminal degradability (%) of dry matter (DM) of ammonified sugarcane bagasse.

Parameters	UREA EFFECT			Pr > F
	0%	3%	5%	
FS (%)	13,22c±3,13	44,57b±3,13	49,93a±3,13	<.0001
FI (%)	37,90a±1,36	26,04b±1,46	26,59b±0,71	<0.0045
TD (%)	0,04a±1,32	0,04a±1,08	0,03a±4,19	<0.7757
DE 2%.	3,93c±3,93	61,61b±5,56	65,17a±7,01	<.0001
DE 5%.	30,48c±4,82	55,95b±1,44	59,76a±0,98	<.0001
DE 8%.	26,28c±4,87	53,15b±1,54	57,25a±0,45	<.0001

FS: Soluble fraction; FI: Insoluble but potentially degradable fraction; TD: Degradability rate; DE: Effective degradability.

a,b,c Different letters in the column differ statistically at 5 % (Tukey).

Table 3. Effect of different ammonification times (30 and 44) on *in situ* ruminal degradability (%) of dry matter (DM) of ammonified sugarcane bagasse.

Parameters	EFFECT OF AMMONIFICATION TIME			Pr > F
	30	44		
FS (%)	35,11a ± 6.18	36,71a ± 5.39		0.1963
FI (%)	29,04a ± 2.93	31,32a ± 2.66		0.3999
TD (%)	0,04a ± 2.57	0,031b ± 2.80		0.0051
DE 2%.	55,73a ± 4.14	54,74b ± 6.42		0.0507
DE 5%.	49,38a ± 5.12	48,08b ± 4.68		0.0162
DE 8%.	46,06a ± 4.80	45,05a ± 6.73		0.1118

FS: Soluble fraction; FI: Insoluble but potentially degradable fraction; TD: Degradability rate; DE: Effective degradability.

a,b,c Different letters in the column differ statistically at 5 % (Tukey).

The insoluble but potentially degradable fraction was highly significant in the analysis of variance (P 0.0045) only for the simple factor urea (Annex 7). Urea at 3% and 5% were statistically equal with values of 26.04 and 26.59, respectively, demonstrating a numerical difference (Table 4.3).

In the analysis of variance, a highly significant difference was observed for the study factor time (Annex 8), with the lowest degradability rate (day 44) with a value of 0.031% (Table 4.3). These results contrast with those of Reyes (2018) who corroborates that degradation rates lower than 0.02 h^{-1} are characteristic of low quality feeds that need more time in the rumen for degradation. Another study that differs from the results obtained are those of Araiza et al. (2013) which shows that degradation rates are higher than those reported for corn silage with 0.039 h^{-1} , except in treatment four with 9% urea, which obtained 0.02 h^{-1} .

Effective degradability at 2, 5 and 8%.

Effective degradability at 2%.

The analysis of variance (Annex 9) for this variable was highly significant for the simple urea effect (P 0.0001), as was the interaction with (P 0.0001). The analysis of variance (Annex 11) for this variable was highly significant for the simple urea effect (P 0.0001), as was the interaction with (P 0.0002).

These results are similar to those reported by Luna. (2017) for whom chemical treatment with different levels of urea improved digestibility using 4 % ammonia in corn stover and wheat bran. Similarly, Saadullah et al. (1980) and Fondevila et al. (1994)(1994) corroborate the above, reporting that the treatment of straw with $3 \pm 5 \%$ urea increased dry matter digestibility by $11 \pm 15 \%$, which could be attributed to the decrease in neutral detergent fiber content.

4. Conclusions

Based on the literature consulted and the results obtained in the present bromatological study, concerning the operational aspects to evaluate the

in situ degradation of ammonified sugarcane bagasse silage to supplement the diet of cattle in times of drought, the following conclusions can be drawn:

The rumen degradation rate remained constant at all urea inclusion levels, which is very important in ruminant feeding, taking into consideration that high degradability rates can cause digestive problems.

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