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Sugarcane total mixed ration silage ensiling with chitosan and homolactic microbial inoculant: characteristics of silage and animal digestion

Silagem de dieta total de cana de açúcar ensilada com quitosana e inoculante microbiano homoláctico: características da silagem e digestão animal

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ABSTRACT

This study aimed to evaluate total mixed ration silages with sugarcane and the additives microbial inoculant and chitosan. Thirty mini-silos were used in a completely randomized design, with three treatments and ten replications. Silages were composed of sugarcane mixed with corn bran, whole soybean, urea, and mineral mixtures at a 50:50 roughage to concentrate ratio. Treatments consisted of control silage, microbial additive (*Lactobacillus plantarum* + *Pediococcus acidilactici*, 4 g/t of KeraSil, Kera Nutrição Animal), and chitosan (10 g/kg of natural matter). Silages were evaluated for fermentation and microbiological profile, fermentation losses, aerobic stability, chemical-bromatological composition, intake, and digestibility. Fermentation profile showed no significant difference between treatments for pH values, with a mean value of 4.79. Production of acetic and propionic acids showed no difference between treatments, with mean values of 7.34 and 0.053 mmol/kg DM, respectively. Dry matter, organic matter, and crude protein intake of the total mixed ration silage differed statistically from the other treatments ($P<0.05$), but fresh sugarcane and sugarcane silage intake did not differ from each other ($P>0.05$). Digestibility values of DM, OM, and NDF were higher in the

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total mixed ration silage (P<0.05), while sugarcane silage and fresh sugarcane showed no difference from each other (P>0.05). Total mixed ration silage increased nutrient intake and digestibility, with a better fermentation pattern when added with the microbial inoculant.
Keywords: digestibility, ruminants, silage, inoculants, total mixed ration

RESUMO

O objetivo foi avaliar as silagens de ração total com cana de açúcar e aditivos inoculante microbiano e quitosana. Foram utilizados trinta mini-silos utilizando o delineamento inteiramente casualizado, com três tratamentos e dez repetições. As silagens foram compostas por cana-de-açúcar misturada com farelo de milho, soja integral, uréia e misturas minerais na proporção de 50:50. Os tratamentos foram: silagem controle, aditivo microbiano (*Lactobacillus plantarum* + *Pediococcus acidilactici*, 4 g/t de KeraSil, Kera Nutrição Animal) e quitosana (10 g/kg da matéria natural). As silagens foram avaliadas quanto à fermentação e perfil microbiológico, perdas de fermentação, estabilidade aeróbica, composição químico-bromatológica, consumo e digestibilidade. O perfil de fermentação não mostrou diferença significativa entre tratamentos para os valores de pH, com um valor médio de 4,79. A produção de ácidos acéticos e propiônicos não mostrou diferença entre os tratamentos, com valores médios de 7,34 e 0,053 mmol/kg DM, respectivamente. A matéria seca, a matéria orgânica e a ingestão de proteína bruta da mistura total de ração silagem diferiram estatisticamente dos outros tratamentos (P<0,05), mas a ingestão de cana-de-açúcar fresca e de silagem de cana-de-açúcar não diferiram uma da outra (P>0,05). Os valores de digestibilidade de DM, OM e NDF foram maiores na silagem de ração mista total (P<0,05), enquanto a silagem de cana de açúcar e a cana de açúcar fresca não mostraram diferença entre si (P>0,05). A silagem de ração mista total aumentou a ingestão de nutrientes e a digestibilidade, com um melhor padrão de fermentação quando adicionado o inoculante microbiano.
Palavras-chave: digestibilidade, ruminantes, silagem, inoculantes, ração mista total

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the main crops grown in tropical regions. Its favorable agronomic characteristics in Brazil, such as high dry matter production per hectare, maturation during low forage production, and low production cost, have led farmers to use it extensively in cattle feed.

Sugarcane is mainly used fresh, which increases demand for labor and limits its use in large properties. It can also be ensiled but its high concentration of soluble carbohydrates and low percentage of dry matter at ensilage process causes significant losses in its nutritional value, in addition to reducing its acceptability by animals (Barbosa et al. 2014).

In this context, total mixed ration silage appears as a feasible alternative to reduce labor and losses during Sugarcane ensiling, once its main objective is to produce a full, balanced feed to meet requirements of a certain animal category. It contains minerals, vitamins, and additives, besides allowing the use of its by-products with low acceptability by animals (Yuan et al. 2015).

Added to the benefits of total mixed

ration silages, additives can be used in ensiling of different foods, acting broadly to influence lactic acid production, reducing pH, avoiding *Clostridium* fermentation, reducing yeast populations to make silages stable when exposed to air, and hence improving animal performance (Muck et al. 2018). Chitosan is a feasible alternative as additive to limit silage losses, once it can restrict growth of bacteria and of other undesirable microorganisms due to its antimicrobial activity (Gandra et al. 2016).

Based on the above, this study aimed to evaluate total diet mixed ration silages with Sugarcane and different additives.

MATERIAL AND METHODS

Trial 1

Ensilage process and treatments

The study was carried out at the Department of Animal Science of the School of Agricultural Sciences, Federal University of Grande Dourados, Dourados city, Mato Grosso do Sul State, Brazil. It is located at 22°14′ S and 54°49′ W, with an altitude of 450 m. Thirty mini-silos were used in a completely randomized design, with three treatments and 10 replications. Silage was composed of sugarcane mixed with corn meal, whole raw soybean, urea, and mineral mixtures at a 50:50 roughage to concentrate ratio with different additives, formulated according to Small Ruminant Nutrition System (SRNS), aiming to meet average day gain of 200 g/day for feedlot lambs.

Treatments consisted 1- CON (control silage, without additive) 2- INO (microbial additive (*Pediococcus acidilactici*: 3.9×10^{10} CFU/g + *Propionibacterium acidipropionici*, 4 g/t fresh matter of KeraSil, Kera Nutrição Animal), and 3- CHI (chitosan 10 g/kg of fresh matter).

Silages were placed in plastic buckets (30 cm in height and 30 cm in diameter) equipped with Bunsen valves to prevent gas penetration and allow gas to escape. Sand (2 kg) was placed at the bottom of buckets, separated from rehydrated corn by a nylon mesh (500 µm). Silage was packed to a density of 950 kg/m^3, sealed, weighed, and stored at ambient temperature (28.5 ± 2.3 °C, mean ± SD) for 60 days. The silos were weighed before opening to determine gas losses. The top layer of silos (5 cm) was discarded at opening and samples were collected and homogenized for chemical (500 g), fermentation profile (300 g), and microbiological profile analysis (100 g).

Fermentation and microbiological profile

Silage juice was extracted by pressure, and its pH was immediately measured by electrodes (MB-10, Marte, Santa Rita do Sapucaí, Brazil). A silage sample (3 kg) was placed back to buckets and maintained at ambient temperature (26.3 ± 2.21 °C, mean ± SD). The samples were collected every 24 h for pH, DM, and mold and yeast evaluations. A subsample was diluted in distilled water (1:10 w: v), processed in a mixture for 30 s, and silage pH was measured using a potentiometer (LUCA-210, Lucadema, São José do Rio Preto, Brazil).

Silage samples (10 g) were diluted in a chloride solution (9 g/L, 90 mL) for microbial analysis. Microorganisms were counted in triplicate through a series of decimal dilution in plates with MRS agar (De Man, Rogosa, and Sharpe) for LAB medium (Briceño and Martínez 1995), nutrient agar for aerobic and anaerobic bacteria medium (incubation for 48 h at 30 °C), and yeast and mold counting according to Rabie et al. (1997), using potato dextrose agar and

incubation for 120 h at 26 °C. Absolute values were obtained from CFU, being then transformed. The concentration of ammoniacal nitrogen in silage juice was determined using a colorimetric method (Foldager 1977). Volatile fatty acids and ethanol concentrations were determined using a gas chromatograph (Focus GC, Thermo Fisher Scientific Inc., Waltham, MA) equipped with an automatic sample injector (AS-3000, Thermo Fisher Scientific Inc.), a glass column (2.0 m × 1/5″, 80/120 Carbopack® BDA/ 4% Carbowax® 20M phase), and a flame ionization detector at 270 °C. Chromatography oven and injector temperature were adjusted to 190 and 220 °C, respectively. Hydrogen was used as carrier gas with a flow rate of 30 mL/min. Lactic acid concentration was measured by high-performance liquid chromatography (HPLC, Shimadzu LC-10ADVP, Shimadzu Inc., Kyoto, Japan), according to Ding et al. (1995).

Fermentation losses and aerobic stability

Silos were weighed after ensiling and at silo opening to determine fermentation losses. Gas losses (GL), effluent losses (EL), and dry matter recovery (DMR) were calculated according to Jobim et al. (2007), as follows:

$$Gl\left(\frac{g}{kg}DM\right) = \frac{SWE\ (g) - SWO(g)}{DME\ (kg)}$$

where SWE is the silo weight at ensilage, SWO is the silo weight at the opening, and DME is total DM ensiled.

$$EP\left(\frac{g}{kg}DM\right) = \frac{WSAO(g) - WSAE(g)}{DME\ (kg)}$$

where WSAO is the weight of the silo after opening (g) and WSAE is the weight of the silo before ensilage (g).

$$DMR\left(\frac{g}{kg}DM\right) = \frac{DMO(g)}{DME\ (kg)}$$

where DMO is total DM after opening the silos (kg) and DME is total DM before ensilage (kg).

Aerobic stability was calculated by placing back silage samples (3 kg) from each treatment in the buckets, which was maintained at ambient temperature (26.3 ± 2.21 °C, mean ± SD). The samples were collected every 24 h for pH, DM, and mold and yeast evaluation. A subsample was diluted in distilled water (1:10 w: v), processed in a mixture for 30 s, and silage pH was measured using a potentiometer (LUCA-210, Lucadema, São José do Rio Preto, Brazil).

Nutrive value

Silage samples were dried at 60 °C for 72 h, ground on a 1-mm sieve Wiley mill (MA580, Marconi, Piracicaba, Brazil), and analyzed for DM (method 950.15) and crude protein (CP, N × 6.25; Kjeldahl 984.13 method), according to AOAC (2000). Neutral detergent fiber (NDF, without sodium sulfite) was analyzed according to Van Soest et al. (1991). Starch content of samples was determined by degradation enzymes, with readings performed on a spectrophotometer, as described by Bach Knudsen (1997). Other chemical analyses included ash (method 942.05), ether extract (EE, method 920.39), acid detergent fiber (ADF), and lignin content (method 973.18), according to AOAC (2000).

Statistical analysis

Data were subjected to SAS (version 9.1.3, SAS Institute, Cary, NC 2004), verifying the normality of residuals and homogeneity of variances using PROC UNIVARIATE.

The statistical analysis was performed using the PROC MIXED from SAS (SAS Institute Inc. 2011) (Littell et al.

2006). The data from the silo experiment was analyzed using the following model:

$$Yi = \mu + Ti + ei,$$

in which $T_{j:i} \approx N(0, \sigma^2)$, $e_{ijkl} \approx N(0, \sigma^2)$, where Yij is the observed value, μ is the overall average, Ti is the fixed effect of the treatment (i = 1, 2, and 3), eij is the random residual error, N is the Gaussian deviation, and σe2 is the error variance. The effects of treatments were analyzed by adjust TUKEY TEST for PROC MIXED, 5% significance level was considered. The reference data and microbiological counts were transformed to log10

Trial 2

Intake and digestibility

Nine castrated lambs (25.4 ± 4.57 kg of body weight and 6.0 ± 0.4 months) were used in a 3 × 3 Latin square design, consisting of periods of 19 days, with the last 5 days for data recording and sampling. Diet was formulated for a mean daily gain of 200 g using the Small Ruminant Nutrition System (SRNS).

After the trial 1, sugarcane total mixed ration added with microbial inoculant was ensiled in 200-L barrels for digestion and metabolism experiment with lambs. In this trial, the experimental treatments were: 1- CON (fresh sugarcane + concentrate), 2- SC (sugarcane silage + concentrate), 3- STMR (sugarcane total mixed ration, same used in trial 1). The roughage: concentrate ratio was 50:50, the same used for making the total mixed ration sugarcane silos, as well as the concentrate ingredients.

The animals were housed in metabolic cages and fed twice a day between 7:00 am and 13:00 h, aiming at leftovers between 10 and 15%. Samples of animal feed and leftovers were collected daily during the sampling period to form a composite sample for subsequent chemical analysis.

The total fecal collections were performed on days 16–18 of each experimental period using a device from the metabolic cage that separates urine from feces. Feces were weighed every 24 hours of collection and a 10% aliquot of each collection day was used to analyze DM, CP, NDF, and starch digestibility.

Chemical analysis, calculation, and statistical analysis

Samples of silages, food ingredients, leftovers, and feces were analyzed for DM (method 950.15) and crude protein (CP, N × 6.25; Kjeldahl method 984.13), according to AOAC (2000). Nutrient digestibility (NuD) was estimated as:

$$NuD\left(\frac{g}{kg}\right)^{n} = \frac{Nufecal\ (g)}{Nuintake} + \frac{Nufecal\ (g)}{(kg)}$$

where Nuintake is the nutrient intake and Nufecal is the excreted fecal nutrient.

Data were subjected to SAS (version 9.1.3, SAS Institute, Cary, NC 2004), verifying the normality of residuals and homogeneity of variances using PROC UNIVARIATE.

The statistical analysis was performed using the PROC MIXED from SAS (SAS Institute Inc. 2011) (Littell et al. 2006).The data from experiment 2 were analyzed according to the following model:

$$Yijkl = \mu + Si + aj: i + T_k + P_l + e_{ijkl},$$

in which aj: $i \approx N(0, \sigma a2)$, $e_{ijkl} \approx N(0, \sigma e2)$, where Yijkl is the value of the dependent variable, μ is the overall average, Si is the fixed effect of the Latin square (i = 1, 2, and 3), aj: i is the random effect of the j-th animal on the i-th Latin square (j = 1 to 9), T_k is the fixed effect of the treatment (k = 1, 2, and 3), P_l is the fixed effect of the experimental period (l = 1, 2, and 3), eijkl is the random

experimental error, N is the Gaussian deviation, σa2 is the variation of animals, and σe2 is the error variation. The effect of the treatment was analyzed as orthogonal contrasts: (1) treatments (INO + CHI) vs CON and (2) INO vs CHI. A 5% significance level was considered for all statistical analyses.

RESULTS

Fermentation and microbiological profile

No significant difference (P=0.76) was observed between treatments for pH values, which had a mean value of 4.79. Production of acetate and propionate acids did not differ between treatments, with mean values of 7.34 and 0.053 mmol/kg DM, respectively (Table 1). A significant difference (P<0.05) was observed between treatments for ammoniacal nitrogen, ethanol, butyrate, lactic acid bacteria, fungi, and molds.

Table 1. Fermentative and microbiological profile of sugarcane total mix ration silage with different additives

Item	Treatments[1]			SEM	P-value
	CON	INO	CHI		
pH	4.71	4.81	4.86	0.14	0.77
N-NH_3(mg/dL)	34.60^{a}	11.60^{c}	20.80^{b}	3.28	0.01
mmol/kgMS					
Ethanol	4.91^{a}	3.84^{b}	4.40^{ab}	0.48	0.01
Acetate	7.08	7.26	7.69	0.32	0.74
Propionate	0.051	0.065	0.044	0.01	0.07
Butyrate	0.292^{b}	0.502^{a}	0.242^{b}	0.04	0.03
Microbiology (log_{10})					
Latic bacteria	6.54^{ab}	7.03^{a}	6.23^{b}	0.32	<0.01
Fungi and molds	8.02^{a}	6.45^{b}	4.04^{c}	0.231	<0.01

[1]CON (total mix ration silage with no additive added). INO (4 g/ton *Pediococcus acidilactici*: 3.9x10^{10} cfu/g + *Propionibacterium acidipropionici*: 3.75x10^{10} cfu/g) CHI (inclusion of chitosan 10 g/kg of natural matter). [2]SEM (standard error of the mean).$^{a-c}$ Different letters on the same line differ in the TUKEY test set by the SAS PROC MIXED

Fermentation losses and aerobic stability

Gas losses in fresh matter showed a statistical difference (P<0.05) between the control treatment and additives, but no difference was observed between additives (Table 2). Gas losses based on DM differed statistically (P<0.05) for the treatments and control. Effluent losses (kg/t) differed (P<0.05) between the evaluated additives, with higher values in TMR with the additive INO (P<0.05). Aerobic stability showed a statistical difference between the treatments and control for sum of °C, temperature in °C, and time in hours (P<0.05), but no difference was observed between the additives INO and CHI.

Table 2. Fermentation losses and aerobic stability of sugarcane total mix ration silage with different additives

Item	Treatments[1]			SEM	P-value
	CON	INO	CHI		
Losses					
Gases (fresh matter)	2.22[b]	3.30[a]	3.78[a]	0.22	<0.001
Gases (dry matter)	8.92[b]	6.42[c]	10.26[a]	0.48	<0.001
Effluent (kg/ton)	8.87[b]	7.60[c]	15.73[a]	1.37	0.003
Effluent (dry matter)	0.887[b]	0.760[b]	1.57[a]	0.14	0.003
Total (dry matter)	9.81[b]	7.18[c]	11.83[a]	0.54	<0.001
Recovery (dry matter)	90.18[b]	92.81[c]	88.16[a]	0.54	<0.001
Aerobic stability					
Sum (ºC)	219.86[a]	217.36[b]	217.08[b]	0.29	<0.001
Temperature (ºC)	27.06[a]	26.50[b]	26.62[b]	0.08	<0.001
Times (hours)	64.80[b]	93.60[a]	98.40[a]	3.43	<0.001

[1]CON (total mix ration silage with no additive added). INO (4 g/ton *Pediococcus acidilactici*: $3.9x10^{10}$ cfu/g + *Propionibacterium acidipropionici*: $3.75x10^{10}$ cfu/g) CHI (inclusion of chitosan 10 g/kg of natural matter). [2]SEM (standard error of the mean). [a-c] Different letters on the same line differ in the TUKEY test set by the SAS PROC MIXED

Chemical-bromatological composition

Chemical-bromatological components showed no statistically significant difference in the use of additives in TMR silage for contents of DM, OM, NDF, ADF, EE, TDN, ashes, and net energy for lactation (P>0.05). Crude protein and non-fibrous carbohydrates differed statistically between treatments (P<0.05), but no difference was observed for non-fibrous carbohydrates between additives (Table 3).

Table 3. Chemical composition of sugarcane total mix ration silage with different additives

Item	Treatments[1]			SEM[2]	P-value
	CON	INO	CHI		
Dry matter (% fresh matter)	64.98	66.78	64.80	0.49	0.19
Organic matter	94.51	94.72	94.43	0.05	0.06
Crude protein	15.00[c]	17.70[a]	16.36[b]	0.49	0.05
Neutral detergente fiber	40.59	36.85	37.77	2.58	0.06
Acid detergente fiber	17.45	16.56	17.09	0.39	0.54
Non-fiber carbohydrate	32.04[b]	37.22[a]	36.38[a]	2.71	0.04
Fat	5.50	4.93	5.26	0.43	0.21
Ash	5.48	5.27	5.56	0.05	0.06
Total digestible nutrients[3]	66.85	69.58	68.03	1.08	0.06
Net lactation energy[3] (Mcal/kg)	1.59	1.76	1.62	0.03	0.08

[1]CON (total mix ration silage with no additive added). INO (4 g/ton *Pediococcus acidilactici*: 3.9x10[10] cfu/g + *Propionibacterium acidipropionici*: 3.75x10[10] cfu/g) CHI (inclusion of chitosan 10 g/kg of natural matter). [2]SEM (standard error of the mean).[3]Calculated according to NRC, 2001. [a-c] Different letters on the same line differ in the TUKEY test set by the SAS PROC MIXED

Intake and digestibility

The NDF intake values of TMR showed no statistical difference (P>0.05) between treatments (Table 4), with means of 0.221, 0.207, and 0.240 kg/day for sugarcane silage, TMR, and fresh sugarcane, respectively. DM, OM, and CP intake of total mixed ration silage differed statistically from the other treatments (P<0.05), but fresh sugarcane and sugarcane silage intake did not differ from each other (P>0.05). Digestibility of DM, OM, and NDF were higher in total mixed ration silage (P<0.05), while sugarcane silage and fresh sugarcane did not differ from each other (P>0.05).

Table 4. Intake and digestibility of experimental diets

Item	Experimental diets			SEM[1]	P-value
	Fresh sugarcane	Sugarcane silage	Sugarcane total mix ration silage		
Intake (kg/dia)					
Dry matter	0.581[b]	0.506[b]	0.853[a]	0.06	<0.01
Organic matter	0.535[b]	0.489[b]	0.781[a]	0.06	<0.01
Crude protein	0.102[b]	0.081[b]	0.130[a]	<0.01	0.04
NDF	0.207	0.221	0.240	0.02	0.47
Digestibilility (g/kg)					
Dry matter	658.08[b]	653.98[b]	787.71[a]	2.05	<0.01
Organic matter	682.04[b]	677.45[b]	807.03[a]	1.95	<0.01
Crude protein	860.67	850.38	895.52	1.43	0.24
NDF	488.47[b]	479.25[b]	534.93[a]	5.22	0.02

[1]SEM (standard error of the mean). [a-c] Different letters on the same line differ in the TUKEY test set by the SAS PROC MIXED

DISCUSSION

Fermentation and microbiological profile

Among the evaluated treatments, TMR added with INO showed a lower value of ammoniacal nitrogen (11.6%), close to 10% of the recommended for silage with a good fermentation pattern (Costa et al. 2016). According to Pires et al. (2013), the N–NH_3 contents in silage can demonstrate whether the ensiled protein content is being degraded. In the present study, an increase in the N–NH_3 values higher than the recommended may be a result of ureolysis of urea present in the TMR formulation (Chenost & Kayouli 1997).

Ethanol values decreased with inoculants compared to the control silage, but with no statistical difference between microbial inoculant and chitosan. Ethanol is the main alcohol produced in the fermentation process,

being produced by different types of microorganisms (Kung et al. 2018). High concentrations of soluble sugars in sugarcane works as a substrate for yeasts to produce ethanol, increasing losses. The reduction in ethanol production in the additive INO can be explained by the acetic acid production by the *Pediococcus acidilactici* present in its composition. Acetic acid has an antifungal action with the ability to control the development of fungi and yeasts that degrade soluble sugars (Kung et al. 2018). Moreover, the additive CHI may have reduced ethanol production through its antimicrobial effect on fungi. The lowest value of butyrate was observed in TMR added with INO.

The presence of butyric acid silage may indicate the activity of clostridia, which can lead to dry matter loss and low energy recovery (Pahlow et al. 2003). The development of lactic bacteria increased in TMR when added with microbial inoculant due to the presence of the lactic acid bacteria *Pediococcus acidilactici*.

The development of fungi and molds in TMR decreased with the use of additives compared to the control silage, where chitosan provided a lower development of these microorganisms possibly due to its action on the development of fungi (Roller & Covill 1999). The control of fungi and molds in silage is one of the main points to be observed to produce good quality silage, as the development of these microorganisms can affect the production of ethanol during the fermentation process and decrease the aerobic stability of silage after opening the silo.

Fermentation losses and aerobic stability

Gas losses in the TMR added with inoculant were lower compared to chitosan and the control silage. These losses were 28 and 37% lower than the control silage and the silage added with chitosan, respectively. The additive INO is formed by homofermentative bacteria and has a higher production of lactic acid, which is the main responsible for reducing the pH and controlling the development of unwanted microorganisms, especially those of the genus *Clostridium* sp., which break down soluble carbohydrates producing CO_2 and water.

The amount of effluent losses showed low values. Sugarcane silages usually show high effluent losses. These results differed from those found by Araki et al. (2017), who evaluated the use of microbial inoculants (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*) associated with NaCl, CaO, and urea and found effluent losses of 27.8, 21.3, 21.2, and 21.9 g/kg respectively. The difference found in the studies can be attributed to the use of ingredients with higher dry matter content, increasing the final dry matter content of silage, and avoiding effluent losses, which can lead to nutrient losses through leaching.

The total losses were lower in the silage added with INO, directly interfering with DM recovery, which was also the lowest in this treatment. Gusmão et al. (2018) found values of dry matter losses of 129, 78.5, 86.5, 48.9, and 40.7 g/kg in the total mixed ration silage based on elephant grass with different ingredients (corn bran and soybean meal; corn bran, soybean meal, and molasses; citrus pulp and soybean meal; citrus pulp, and soybean meal, and molasses, respectively).

The parameters of aerobic stability, temperature (°C), and time (hours) were

similar between treatments. The evaluation of aerobic stability indicates the behavior of the ensiled material after air contact over time. The increase in aerobic stability with the evaluated additives can occur in different ways. The additive INO has heterofermentative bacteria in its composition and produces acetic acid. Unlike lactic acid, which can be oxidized by yeasts after opening the silo, acetic acid acts on microorganisms under aerobic conditions, thus preventing the degradation of silage nutrients and a decrease in animal production (Carvalho et al. 2013; Pahlow et al. 2003). Moreover, the increase in aerobic stability using the additive CHI can be due to its antifungal action (Roller & Covill 1999). Gandra et al. (2016) evaluated the chitosan effect on the aerobic stability of sugarcane silage and observed that its incorporation led to a decrease in the temperature of stability in mini-silos and prolonged the period of aerobic stability of the sugarcane silage.

Chemical-bromatological composition

The highest CP and NFC values were found in the silage added with INO. The highest concentration of these nutrients in this treatment may be related to a higher amount of lactic acid bacteria producing a higher amount of lactic acid, reducing the pH, and controlling the development of microorganisms that cause undesirable fermentation and nutrient losses. The low variation between components of the chemical-bromatological composition of silage in the present study was similar to that found by Chen et al. (2014). These authors evaluated the effect of the application of molasses, lactic acid bacteria, and propionic acid, as well as the association of molasses/propionic acid and lactic acid bacteria/propionic acid on the production of the total mixed ration silage. They found a difference only for NDF values (317, 300, 334, 330, and 302 g/kg DM, respectively). The low difference in the composition of the evaluated silages can be due to the low variation in their composition.

Intake and digestibility

Total mixed ration silage increased dry matter intake compared to fresh sugarcane and sugarcane silage, with an increase of 31.88 and 40.67%, respectively. The increase in dry matter intake led to an increase in organic matter and crude protein intake in TMR. The increased DM intake by animals can be due to an increase in the passage rate. The interaction between concentrate and roughage may have positively affected the ruminal parameters, increasing the digestibility and intake of animals (Van Soest 1994).

The digestibility of DM, OM, and NDF was higher in the TMR silage. The low digestibility of fresh sugarcane and sugarcane silage is related to the low quality of sugarcane fiber. In addition, the presence of concentrate foods in the total mixed ration silage favors the development of ruminal microorganisms, increasing nutrient digestibility. According to Geron et al. (2013), the inclusion of concentrate in the diet of sheep favored the increased intake and digestibility of DM and nutrients due to their higher digestibility in comparison to roughage foods.

CONCLUSIONS

Total mixed ration silage increased nutrient intake and digestibility, with a better fermentation patter when added with microbial inoculant.

REFERENCES

AOAC. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Arlington, VA, USA, 2000.

ARAKI, H. M. C.; OLIVEIRA, E. R.; GANDRA, J. R.; GOES, R. H. T. B.; TAKIYA, C. S.; JACAÚNA, A. G.; OLIVEIRA, K.M.P.; VASQUES, D.N.; CÔNSOLO, N.R.; DEL VALLE, T.A., and DUAN ORBACH, N. 2017. Association of Biological and Chemical Additives on Nutrient Composition, Total Losses, Microbiological and Fermentative Profile of Sugarcane Silage. **Iranian Journal of Applied Animal Science**, v.7, n.4, p.577-584.

BACH KNUDSEN, K.E., 1997. Carbohydrate and lignin contents of plant material used in animal feeding. **Animal Feed Science and Technology**. v.67, n4, p.319–338.

CARVALHO, B. F.; ÁVILA, C. L. S.; MIGUEL, M. G. C. P.; PINTO, J. C.; SANTOS, M. C., and SCHWAN, R. F. 2014. Aerobic stability of sugar-cane silage inoculated with tropical strains of lactic acid bacteria. **Grass and Forage Science**, v.70, n.4, p.308-323. https://doi.org/10.1111/gfs.12117.

CHEN, L.; GUO, G.; YUAN, X.; ZHANG, J.; LI, J., and SHAO, T. 2016. Effects of applying molasses, lactic acid bacteria and propionic acid on fermentation quality, aerobic stability and in vitro gas production of total mixed ration silage prepared with oat–common vetch intercrop on the Tibetan Plateau. **Journal of the Science of Food and Agriculture**. v.96, n.4, p.1678-1685. https://doi.org/10.1002/jsfa.7271.

COSTA, R.F.; PIRES, D.A.D.A.; MOURA, M.M.A.; SALES, E.C.J.D.; RODRIGUES, J.A.S., and RIGUEIRA, J.P.S. 2016. Agronomic characteristics of sorghum genotypes and nutritional values of silage. Acta Scientiarum. **Animal Sciences**. V.38, n.2., p.127-133. http://dx.doi.org/10.4025/actascianimsci.v38i2.29567

CHENOST, M.; C. KAYOULI. 1997. Roughage utilization in warm climates, FAO Animal Production and Health Paper 135. **Food and Agriculture Organization**, Rome, p. 226.

FOLDAGER, J., 1977. Protein Requirement and Non-protein Nitrogen for High Producing Cow in Early Lactation. PhD Diss. Michigan State University, East Lasing, MI.

GANDRA, J.R.; OLIVEIRA, E.R.; TAKIYA, C.S.; GOES, R.H.T.B.; PAIVA, P.G.; OLIVEIRA, K.M.P. GANDRA, E.R.S.; ORBACH, N.D.; and HARAKI, H.M.C. 2016. Chitosan improves the chemical composition, microbiological quality, and aerobic stability of sugarcane silage. **Animal Feed Science and Technology**. v.214, p.44-52. https://doi.org/10.1016/j.anifeedsci.2016.02.020.

GERON, L. J. V.; MEXIA, A. A.; CRISTO, R. L.; GARCIA, J.; DA SILVA CABRAL, L.; TRAUTMANN, R. J., and ZEOULA, L. M. 2013. Consumo, digestibilidade dos nutrientes e características ruminais de cordeiros alimentados com níveis crescentes de concentrado em ambiente tropical no Vale do Alto Guaporé-MT. Semina: **Ciências Agrárias**, v.34, n.5, p.2497-

2510. DOI: 10.5433/1679-0359.2013v34n5p2497.

GUSMÃO, J. O.; DANÉS, M. A. C.; CASAGRANDE, D. R.; and BERNARDES, T. F. 2018. Total mixed ration silage containing elephant grass for small-scale dairy farms. **Grass and Forage Science**. v.73, n.3, p.717-726. DOI: 10.1111/gfs.12357.

JOBIM, C.C.; NUSSIO, L.G.; REIS, R.A.; AND SCHMIDT, P. 2007. Methodological advances in evaluation of preserved forage quality. **Revista Brasileira de Zootecnia**. V.36, p.101–119.

MUCK, R. E.; NADEAU, E. M. G.; MCALLISTER, T. A.; CONTRERAS-GOVEA, F. E.; SANTOS, M. C., amd KUNG JR, L. 2018. Silage review: recent advances and future uses of silage additives. **Journal of dairy science**, v.101, n.5, p.3980-4000. https://doi.org/10.3168/jds.2017-13839.
National Research Council (NRC). Nutrient Requirements of Dairy Cattle, seventh ed. National Academic Press, Washington, D.C, 2001.

LITTELL, R. C., MILLIKEN, G. A., STROUP, W. W., WOLFINGER, R. D., AND SCHABENBERGER, O. 2006. SAS® for Mixed Models, Second Edition, Cary, NC: SAS Institute Inc.

PAHLOW G.; MUCK R.E.; DRIEH U.I.S.F.; EL FERINK S.J.W.H.O.; and SPOELSTRA S.F. 2003 Microbiology of ensiling. In: Buxton D.R., Muck R.E. and Harrison J.H. (eds) **Silage science and technology**, v.42, p. 31–94. Madison,WI, USA: ASA, CSSA, SSSA.

RABIE, C.J.; LIIBBEN, A.; MARAIS, G.J., and JANSEN VAN VUUREN, H., 1997. Enumeration of fungi in barley. **International Journal of Food Microbiology**, v.35, n.2, p.117–127.

ROLLER, S.; COVILL, N. 1992. The antifungal properties of chitosan in laboratory media and apple juice. **International Journal of Food Microbiology**. v.47, n.1-2, p.67-77.

ROCHA, W.J.B.; ROCHA JÚNIOR, V.R.; SILVA, G.W.V.; REIS, S.T.; RUAS, J.R.M.; SOARES, C.; MENEZES, J.C., and BORGES, L.D.A. 2014. Fermentative characteristics of sugar cane silages with additives. **Revista Brasileira de Saúde Produção Animal**, v.15, n.4, p.801-814.

VAN SOEST, P.J.; ROBERTSON, J.B.; and LEWIS, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**. v.74, n.10, 3583–3597.

VAN SOEST, P. J. 1994. Nutritional ecology of the ruminant. 2. ed. London: Constock Publishing Associates. 476 p.

YUAN, X.; GUO, G.; WEN, A.; DESTA, S. T.; WANG, J.; WANG, Y.; and SHAO, T. (2015). The effect of different additives on the fermentation quality, in vitro digestibility and aerobic stability of a total mixed ration silage. **Animal Feed Science and Technology**, v.207, p.41-50. https://doi.org/10.1016/j.anifeedsci.2015.06.001.

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