Amylolytic Enzyme in Rehydrated Corn and Sorghum Grain Silage in the Diet of Ears

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Abstract

Aimed of this work was to evaluate rehydrated corn and sorghum grains silages, with and without α-AMYLASE, on fermentation profile, nutritional value, digestion and metabolism on diets for sheep. Two experiments were conducted. In the first experiment 28 silos were divided into: 1- RSGS (rehydrated sorghum grain silage); 2- RSGS+A (rehydrated sorghum grain silage + amylase,); 3- RCGS (rehydrated corn grain silage); 4 RCGS+A (rehydrated corn kernel silage + amylase). In experiment II, 12 lambs were distributed in: RSGS; RSGS+A; RCGS; RCGS+A. In experiment I, there was an effect of grain x enzyme interaction for GL MN. The enzyme reduced the NFC content. In experiment II intake of DM kg/day was not affected by the starch content in the diet, with an average of 1.2 kg/day meaning that the type of grain and the enzyme had no influence on the intake of animals. There was an interaction for the intake of starch (kg/day), blood urea and N-NH_3. Lambs fed with RCGS+A had a higher concentration of ruminal ammonia. The use of enzymes improved the fermentation profile of the silages. RSGS can replace RCGS in sheep feed without modifying nutrient intake and digestibility.

Introduction

The high starch content in corn and sorghum grains encourages the use of these foods in ruminant diets, as they are a source of energy for the growth of ruminal microorganisms, which are responsible for producing volatile fatty acids (VFAs), responsible for the supply animal energy (Faustino et al., 2018).

However, the digestibility of starch granules can be affected by hydrophobic matrix, which prevents the fixation of microorganisms in rumen, reducing starch digestibility (Ferraretto et al., 2015).

The hydrophobic effect of prolamins is reduced when the grains undergo mechanical, chemical and enzymatic processing that is capable of breaking the hydrogen bonds, releasing starch granules (Silva et al., 2020).

The reconstitution and silage process of the grain makes starch more available to be used in the rumen fermentation process (Ferraretto et al., 2015). The addition of exogenous enzymes in the silage of rehydrated grains helps the starch digestive process (Oliveira et al., 2019).

Considering the effect of rehydration and the use of α-AMYLASE, the hypotheses raised in this work were that rehydrated sorghum silage without and with enzyme has a nutritional value similar to rehydrated corn silage, digestion and metabolism and that will not be influenced by the type of grain and/or enzyme utilization, rehydrated sorghum grain silage can replace rehydrated corn grain silage without loss of animal performance.

The aimed of this research was to evaluate the rehydrated corn and sorghum grains silages, without and with α-AMYLASE, on fermentation profile, nutritional value, digestion and metabolism on diets for sheep.

Material And Methods

Experiment I

The corn and sorghum grains used in this experiment were harvested during the 2018/2019 harvest. After harvesting, the corn and sorghum grains were ground in knife mills (4 mm), hydrated until reaching a dry matter content between 50 and 55% and homogenized. The experimental silos were made in polyethylene tubes 40 cm in height and 30 cm in diameter. At the bottom of the silos, dry sand (2 kg) was placed, separated from the forage by a nylon cloth (50 mesh – porosity) to quantify the effluent produced. The material was compacted with a density of 930kg/m^3. The experimental silos were sealed with adhesive tape, weighed and stored. The chemical composition of the material before ensiled is shown in Table 1.

Table 1
Chemical composition of grains before ensiling (percentage based on DM)

Nutrients %	Sorghum	Sorghum + Enzyme	Corn	Corn + Enzyme
Dry matter	58.30	57.80	59.30	56.60
Organic matter	98.40	98.30	98.30	98.50
Ashes	1.59	1.63	1.64	1.50
Ethereal extract	1.71	1.18	4.22	3.43
Crude protein	12.55	11.40	11.00	10.90
Neutral detergent fiber	14.01	13.90	12.82	11.80
Acid detergent fiber	4.90	4.50	4.01	3.90

Twenty-eight experimental silos were used, distributed in a 2 X 2 factorial scheme, with two levels of enzyme and two cereals, where the treatments were: Treatment 1: rehydrated corn silage without the addition of α-AMYLASE enzyme; Treatment 2: rehydrated corn silage with α-AMYLASE (amylase, Kerazyme 3035, enzymatic activity 300 U mL^{-1}); Treatment 3: rehydrated sorghum silage without the addition of α-AMYLASE enzyme; Treatment 4: rehydrated sorghum silage with α-AMYLASE (amylase, Kerazyme 3035, enzymatic activity 300 U mL^{-1}).

At 45 days of fermentation, the silos were weighed again to determine gas losses and then opened, samples were collected and taken to pre-drying in a forced ventilation oven at 55º C for 72h and after this period they were ground. in a Willey type mill at 1 mm, and dried for 16h in an oven at 105º C to determine dry matter (DM, method 950.15), ash (method 942.05), organic matter (MO, 1000-ash), crude protein (CP, N × 6.25, Kjeldahl method 984.13) ether extract (EE, method 920.39), acid detergent fiber (ADF) and lignin (method 973.18), (AOAC, 2000). (NDF) (Van Soest et al., 1991)

The starch content was determined according to the methodology described by Hendrix (1993). The concentrations of non-fibrous carbohydrates (NFC) were obtained from the equation:

NFC = 100 – (%CP + %EE + %ashes + %NFC).

Microbiological analyzes were performed according to Silva et al. (1997).

For ruminal kinetics, three animals cannulated in the rumen were used, distributed in a randomized block design. The bags were then deposited in the ventral sac region of the rumen by incubation time in reverse order (0, 3, 6, 9, 12, 24, 36 and 48 hours) to be removed all at the same time, at the end of the period, and in this way, promote uniform washing of the material when the rumen is removed. The remaining residues were quantified for DM contents (950.15) (AOAC, 2000).

Experiment II

Twelve whole lambs (27.3 ± 7.5 kg of body weight and 6.4 ± 0.3 months), were distributed in three 4 × 3 Latin squares, consisting of periods of 14 days. Diets were formulated with an average daily gain of 200 g, using the Small Ruminants Nutritional System (Table 2). The animals were housed in metabolic cages, fed twice a day.

Lambs were distributed according to the following experimental diets: SGUS; SGUS+E; SGUM; SGUM+E. The diet provided contained a forage: concentrate ratio of 12:88, where the forage was Cynodon spp. (12%), and the concentrate composed of rehydrated grain silage (68%), a mineral protein mixture (20%) consisting of ground soybean grain (85%) and mineral mixture (15%).

To assess intake, leftovers were weighed daily and the supply adjusted for *ad libitum* intake with leftovers calculated at 10 to 15%.

To estimate the total apparent digestibility of dry matter and nutrients, total feces were collected from the 15th to the 20th day of each experimental period. The samples obtained were homogenized to compose a sample composite of each animal in each period. The stool samples collected were pre-dried in an oven with forced ventilation (60°C/72 hours) and processed in a knife mill with 1 mm porosity sieves. Subsequently, these samples were analyzed for DM, MO, PB, EE, NDF and starch according to the methodology previously described for food analysis.

Table 2
Proximate and nutritional composition of experimental diets

Ingredients	Experimental diets (g/kg) [1]			
	RSGS	RSGS+A	RCGS	RCGS+A
Tifton hay	300	300	300	300
RCGS	400	-	-	-
RCGS+E	-	400	-	-
RSGS	-	-	400	-
RSGS+E	-	-	-	400
Ground soybean	250	250	250	250
Mineral mix	50	50	50	50
Nutritional composition (g/kg DM)				
Dry matter	703.6	663.3	684.0	637.7
Organic matter	936.0	936.3	975.0	984.1
Crude protein	142.0	161.0	159.9	162.3
Ethereal extract	64.0	63.0	65.3	65.2
Starch	429.8	446.8	450.3	436.6
Neutral detergent fiber	142.0	161.0	159.9	162.3
Acid detergent fiber	74.0	77.2	75.3	76.2
lignin	30.1	28.5	28.0	25.4
Non-fibrous carbohydrate	480.0	429.8	481.6	496.1
Ashes	63.9	63.6	62.7	63.2
Total Digestible Nutrients	836.6	834.5	835.9	835.2
Net Gain Energy	1.89	1.90	1.90	1.90

RSGS: sorghum rehydrate grain silage; RSGS+E: sorghum silage rehydrated with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+E: corn silage rehydrated with α-AMYLASE.

At 12th and 13th day total urine collection was performed to quantify the urinary volume. The samples were stored for analysis of allantoin, uric acid, xanthine and hypoxanthine (Chen and Gomes 1992).

From the 15th to the 19th of each experimental period, total urine collections were performed to quantify the urinary volume. Spot samples were collected during spontaneous urination, at 11:00 am, that is, four

hours after the provision of the meal at 7:00 am. A 10 mL aliquot of urine was diluted in 40 mL of 0.036 N sulfuric acid.

In this process, the pH was adjusted, if necessary, to values below 3, with droplets of concentrated sulfuric acid, in order to prevent bacterial destruction of the purine derivatives and precipitation of uric acid. The samples were stored at –18 ºC for further analysis of purine derivatives allantoin, uric acid, xanthine and hypoxanthine (CHEN and GOMES 1992).

For the nitrogen balance, the quantification of nitrogen content in urine and feces was performed according to (AOAC, 2000). The calculation was performed according to the following formulas:

N absorbed = N consumed (N stool)

N retained= N consumed (N feces + N urine)

On the 21st day, 4 hours after feeding, ruminal fluid was collected through an esophageal tube (Ortolani et al., 1981). After collection, pH was measured. Ammonia was determined by the methodology of Broderick and Kang (1980), and SCFAs by Erwin et al. (1961).

Statistical Analysis

Ruminal Kinetics

Data were fitted to a nonlinear regression using the SAS (Sas Institute, NC, Cary) (Orskov and Mcdonald, 1979):

$Y=a+b\ (1-e^{-ct})$,

Y = accumulated degradation, after time t; a = intercept of the degradation curve when t = 0; b = potential for degradation of the water-insoluble fraction; a+b = potential degradation when time is not a limiting factor; c = rate of degradation by the fermentative action of b; t = incubation time.

Experiment I and II

The data obtained were submitted to SAS, verifying the normality of the residues and homogeneity of the variances. The data were analyzed by PROC MIXED adopting a significance of 5%, according to the model:

$Y_i = \mu + S_i + E_j + S_i * E_j + e_{ij}$

Y i = dependent variable, μ = overall mean, S i = silage fixed effect (i = 1 to 2); E j = enzyme effect and S i * E j = interaction effect and e ij = error. The degrees of freedom were corrected by DDFM=kr.

$Y_{ijkl} = \mu + A_i + P_j + C_k + D_l + e_{ijkl}$

Y ijyk = dependent variable, μ = overall mean, A i = animal effect (j = 1 to 12), P j = period effect (y = 1 to 3), C k = squared effect (k =1 to3), D l = diet effect (l = 1 to 3) and e ijkl = error. The random effect of the model was characterized by: A i and P j.

Results

Experiment I

The type of grain, enzyme and interaction of these factors (P =0.452 and P =0.317) did not affect effluent losses in kg/ton or %DM. The enzyme increased losses by gas losses in natural (GL MN; P=0.026), with the effect of grain x enzyme interaction.

RCGS had higher gas losses in dry matter (GL DM; P=0.037) and total dry matter loss (TDML; P=0.049), resulting in a lower dry matter recovery (REC DM; P=0.004), compared to RSGS. Enzyme α-AMYLASE utilization reduced (P=0.003) by 10% the REC DM (Table 3).

Table 3. Fermentation losses according to experimental treatments

Item	EXPERIMENTAL TREATMENTS				SEM	P value		
	RSGS	RSGS+A	RCGS	RCGS+A		SILAGE	ENZ	INT
EL NM	4.99[b]	8.82[a]	4:48[b]	11.70[a]	0.664	0.112	0.0001	0.026
GL DM	2.78	2.89	2.94	2.93	0.022	0.037	0.247	0.201
EL (kg/ton)	37.38	37.60	36.80	41.44	1,405	0.578	0.409	0.452
PE DM	1.45	1.43	1.44	1.65	0.056	0.348	0.389	0.317
TDML	4.23	4.33	4.38	4.59	0.054	0.049	0.155	0.581
REC DM	87.41	82.30	83.54	78.62	0.839	0.004	0.003	0.936

RSGS: rehydrated sorghum grain silage; RSGS+A: rehydrated sorghum silage with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+A: rehydrated corn silage with α-AMYLASE; SEM: standard error of the mean. different letters on the same line differ at 5% on Tukey's test. GL NM: gas losses in natural matter; EL: effluent loss; LG DM: loss by gas in dry matter; TDML: total dry matter loss; REC DM: dry matter recovery;

RCGS had a higher count of lactic acid bacteria (LAB) (P =0.021), fungi (P=0.024) and a higher pH value (P=0.001) compared to RSGS. However, RSGS had a higher yeast count (P=0.011).

RCGS+A had the highest LAB count, influencing a reduction of 0.78% in pH and 23% in the production of N-NH_3 (%NT). The enzyme reduced the fungus count by 2.52% (Table 4). There was an effect of the enzyme on LAB (P=0.022), fungi (P=0.004), pH (P=0.021) and (N-NH_3 (%NT)) (P=0.001).

Table 4. Microbiological profile according to experimental treatments

Item	EXPERIMENTAL TREATMENTS				SEM	P value		
	RSGS	RSGS+A	RCGS	RCGS+A		SILAGE	ENZ	INT
Bacteria (log10)								
Lactics	6.86	5.65	7.51	6.48	0.112	0.021	0.022	0.558
totals	6.04	5.11	7.67	5.95	0.141	0.214	0.547	0.654
aerobics	6.34	6.85	7.28	5.70	0.222	0.324	0.332	0.214
(log10)								
Fungi	6.04	6.18	8.15	6.34	0.224	0.024	0.004	0.654
Yeasts	5.65	5.90	4.56	5.92	0.321	0.011	0.554	0.221
Fermentation								
pH	3.84	3.83	4.58	3.80	0.022	0.001	0.021	0.547
N-NH_3 (NT%)	32.03	23.97	43.67	18.45	2,547	0.554	0.001	0.478

RSGS: rehydrated sorghum grain silage; RSGS+A: rehydrated sorghum silage with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+A: rehydrated corn silage with α-AMYLASE; SEM: standard error of the mean.

The enzyme reduced the NFC (P=0.032), increased (P=0.017) the NDF content, reducing the starch content (P=0.047). RCGS had higher levels of OM, EE, starch, TND and NEg compared to RSGS (Table 5).

Table 5. Nutritional value of rehydrated grain silages without and with α-AMYLASE

Items	EXPERIMENTAL TREATMENTS				SEM	P-value		
	RSGS	RSGS+A	RCGS	RCGS+A		SILAGE	ENZ	INT
Dry matter	59.80	58.65	58.60	59.72	0.213	0.932	0.982	0.172
Organic matter	98.73	98.55	98.72	98.80	0.245	0.009	0.270	0.105
crude protein	11.41	10.63	10.68	10.18	0.001	0.121	0.094	0.710
Insoluble Nitrogen in Neutral Detergent	0.65	0.611	0.64	0.658	0.001	0.135	0.541	0.542
Insoluble Nitrogen in Acid Detergent	0.29	0.27	0.28	0.27	0.002	0.245	0.145	0.235
Ethereal extract	3.04	3.58	4.09	4.56	0.004	0.007	0.161	0.918
Fiber in neutral detergent	11.55	14.07	10.88	12.54	0.354	0.172	0.017	0.057
Starch	61.82	59.76	70.07	66.82	0.847	0.040	0.047	0.128
Non-Fibrous Carbohydrates	72.72^{b}	70.26^{c}	75.06^{a}	73.56^{ab}	0.987	0.078	0.011	0.032
Total Digestible Nutrients	85.22	85.23	87.06	87.87	1,234	0.005	0.215	0.215
Net energy of gain	1.96	1.98	2.01	2.05	0.547	0.012	0.454	0.554

RSGS: rehydrated sorghum grain silage; RSGS+A: rehydrated sorghum silage with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+A: rehydrated corn silage with α-AMYLASE; SEM: standard error of the mean. different letters on the same line differ at 5% on Tukey's test.

RSGS+A (P=0.001) had higher fraction A, however RCGS+A had higher values (P=0.002) of fraction B and DP (P=0.050). RSGS presented higher values of fraction A (P=0.002), C (P=0.001) and indegradable fraction (P=0.002), resulting in lower values (P=0.001) of fraction B and potential degradability (Table 6).

Table 6. DM Degradability of Experimental Treatments

Item	EXPERIMENTAL TREATMENTS				SEM	P value		
	RSGS	RSGS+A	RCGS	RCGS+A		SILAGE	ENZ	INT
Fraction A	49.21	54.09	37.61	46.95	2,652	0.002	0.001	0.564
Fraction B	30.94	21.99	45.18	39.25	1,871	0.001	0.002	0.678
Fraction C	0.07	0.09	0.02	0.03	0.001	0.001	0.548	0.632
Potential degradability	80.15	76.08	82.79	86.20	5,581	0.001	0.050	0.324
Indegradable fraction	19.85	23.92	17.21	13.80	2,887	0.002	0.547	0.412

RSGS: rehydrated sorghum grain silage; RSGS+A: rehydrated sorghum silage with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+A: rehydrated corn silage with α-AMYLASE; SEM: standard error of the mean.

1.2 5.2 Experiment II

Starch intake (P=0.047) showed a grain x enzyme interaction effect. RCGS+A had 8% higher than the other treatments (Table 7).

Table 7. Intake and digestibility of DM and nutrients

ITEM	EXPERIMENTAL TREATMENTS				SEM	VALUE OF P		
	RSGS	RSGS+A	RCGS	RCGS+A		SILAGE	ENZ	INT
Intake g/day								
Nitrogen	30.47	28.96	29.25	32.76	1,123	0.357	0.476	0.080
Excretion g/day								
Feces	7.35	7.67	7.43	10.15	0.499	0.106	0.056	0.128
Urine	7.43	8.27	7.84	9.38	0.630	0.413	0.202	0.707
Balance g/day								
Absorbed	23.11	21.28	21.82	22.61	0.901	0.987	0.685	0.310
Withheld	15.68	13.00	13.97	13.22	0.986	0.659	0.311	0.567
Microbial nitrogen synthesis (g/day)								
Nitrogen	16.07	15.08	15.23	14,076	1,130	0.639	0.584	0.966
Protein	100.45	94.25	95.21	87.97	7,064	0.639	0.584	0.966
Urea (mg/dL)								
Urine	243.45	244.67	249.48	216.56	10,302	0.459	0.290	0.255
Blood	81.91	78.86	67.60	88.20	6803	0.668	0.137	0.048

RSGS: rehydrated sorghum grain silage; RSGS+A: rehydrated sorghum silage with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+A: rehydrated corn silage with α-AMYLASE; SEM: standard error of the mean.

Lambs fed with RCGS+A had a higher concentration of ruminal ammonia compared to the other treatments. There was interaction of the type of grain with the enzyme on blood urea and N-NH_3.

Table 8. Nitrogen balance, microbial protein synthesis and ruminal fermentation parameters

ITEM	EXPERIMENTAL TREATMENTS				SEM	P value		
	RSGS	RSGS+A	RCGS	RCGS+A		Silage	ENZ	INT
Intake kg/day								
Dry matter	1,230	1,180	1,140	1,280	0.032	0.966	0.397	0.072
Organic matter	1,140	1,090	1,060	1,200	0.030	0.773	0.392	0.065
Crude protein	0.190	0.181	0.182	0.204	0.007	0.357	0.476	0.080
Ethereal extract	0.108	0.102	0.108	0.120	0.005	0.197	0.656	0.217
Fiber in Neutral Detergent	0.301	0.280	0.268	0.302	0.008	0.695	0.659	0.060
Starch	0536 b	0.509 bc	0.495 c	0.562 a	0.014	0.806	0.386	0.047
Digestibility (%)								
Dry matter	80.38	78.83	78.64	77.93	0.872	0.377	0.450	0.778
Organic matter	82.91	79.48	81.68	77.75	0.852	0.299	0.013	0.859
Crude protein	75.61	73.22	74.10	69.08	1,140	0.148	0.031	0.493
Ethereal extract	86.32	89.01	89.60	86.28	0.961	0.867	0.847	0.073
Fiber in Neutral Detergent	64.82	56.71	56.67	56.62	2,263	0.371	0.376	0.383
Starch	92.30	91.76	91.05	91.20	0.475	0.240	0.921	0.651

RSGS: rehydrated sorghum grain silage; RSGS+A: rehydrated sorghum silage with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+A: rehydrated corn silage with α-AMYLASE; SEM: standard error of the mean. different letters on the same line differ at 5% on Tukey's test.

RCGS+A had the highest production of N-NH_3. There was an effect of the interaction of grain x enzymes on the fermentative profile for N-NH_3. The animals fed with silage-containing enzymes showed higher (P=0.021, P=0.012 and P=0.044) production of ammonia, propionate and methane compared to animals that did not receive enzyme silage. Lambs fed with RCGS had higher production of butyrate and total acids of 30.65% and 3.8% compared to those fed RSGS (Table 9).

Table 9. Ruminal fermentation profile

Item	Experimental treatments				SEM	P value		
	RSGS	RSGS+A	RCGS	RCGS+A		SILAGE	ENZ	INT
pH	5.87	5.79	5.82	5.88	0.021	0.871	0.687	0.547
$N\text{-}NH_3$	25.80 b	26.29 b	23.59 c	29.48 a	2,062	0.547	0.021	0.021
mmol/L								
Acetate	35.27	35.93	35.69	35.12	1,166	0.228	0.325	0.665
Propionate	18.11	19.29	18.58	23.67	1,118	0.554	0.012	0.579
Butyrate	7.08	8.87	10.21	8.12	0.608	0.025	0.259	0.841
Isobutyrate	0.23	0.27	0.22	0.27	0.024	0.225	0.816	0.658
Valerate	1.23	1.40	1.70	2.26	0.195	0.558	0.258	0.385
Isovalerate	1.01	1.51	1.05	1.19	0.119	0.567	0.459	0.288
Total	62.84	65.28	66.11	70.62	2,142	0.002	0.589	0.844
branched-chain	2.47	3.18	2.97	3.71	0.209	0.335	0.594	0.459
Acetate/propionate	2.19	2.22	2.23	1.81	0.145	0.468	0.514	0.555
Methane	13.45	11.69	15.03	12.54	0.145	0.641	0.044	0.547

RSGS: rehydrated sorghum grain silage; RSGS+A: rehydrated sorghum silage with α-AMYLASE; RCGS: rehydrated corn grain silage; RCGS+A: rehydrated corn silage with α-AMYLASE; SEM: standard error of the mean. different letters on the same line differ at 5% on Tukey's test.

Discussion

The lower REC DM for silages with α-AMYLASE is effect of the higher LG in this material, an effect caused by the enzyme that provided a greater amount of substrates, causing a greater amount of aerobic bacteria to occur, competing with LAB, for sugars and consuming the organic acids, influencing for an extension of the fermentation, producing CO_2, generating greater losses of DM (Oliveira et al., 2019).

The higher LG in silages containing enzymes is linked to secondary fermentation, carried out by yeasts, enterobacteria, and aerobic bacteria that grow at a pH close to 4, using fermentable carbohydrates, found in greater quantities in silages containing enzyme, due to the action of α -AMYLASE that degrades starch into smaller molecules, making them available for use by microorganisms, resulting in an increase in LG and reducing REC DM, (Gizotto et al., 2020).

The pH of silages containing α-AMYLASE was not negatively influenced by the lower amount of LAB, with a pH close to 3.8 which reduced the amount of fungi in the silage, influencing the nutritional value of the

silage, as it was not found a significant difference in chemical composition compared to other treatments.

Losses of DM during the fermentation process result in increased concentrations of more than 11% NDF, due to the effect of concentration, as it is an insoluble fraction, which is not used by microorganisms as a substrate during the period that the material was ensiled (Oliveira et al., 2019).

The 2.67% reduction in NFC is due to the action of α-AMYLASE, which solubilized part of the starch in sugars that can be fermented by LAB, reducing their concentrations in the ensiled material. The reduction in NFC concentrations was expected due to the rehydration of grains that potentiate the activity of endogenous and exogenous amylolytic enzymes, increasing starch degradation, increasing the concentration of propionate in the rumen, influencing a 16.5% reduction in the production of methane (Mombach et al., 2019).

With the exception of RCGS, all silages had acceptable pH values (3.6 to 4.2) (Oliveira et al., 2019). This finding may be related to the greater amount of ammonia that neutralized the desirable acids resulting in a final pH increasing even with a greater amount of LAB (SILVA et al., 2020), corroborating the high presence of fungi, total and aerobic bacteria.

RCGS was the treatment with the lowest fraction “a” (37.61%), this is due to the greater microbial activity observed, which consumed highly fermentable carbohydrates, reducing the amount of carbohydrates in the “a” fraction (Oliveira et al., 2019).

The treatments containing α-AMYLASE had a higher soluble fraction, and RSGS+E had a higher fraction “a” (54.09), due to the effect of α-AMYLASE that hydrolyzed starch into smaller molecules, intensifying the amount of fermentable carbohydrates, facilitating the action of bacteria in the rumen environment (Oliveira et al., 2019), however, was the treatment that presented the highest fraction C (0.09%) and FI (23.9%).

The intake of DM kg/day was not affected by the starch content in the diet, with an average Intake of 1.2 kg/day meaning that the type of grain and the enzyme had no influence on the intake of animals, being influenced by the concentration of propionate and lactate in the rumen. Due to the hypophagic effect of propionate, potentiated by increasing its concentration in the rumen, reducing Intake by increasing the amount of glucose in the bloodstream, stimulating the hypothalamic neurons in the satiety center (Silva et al., 2020).

The increase in the concentration of propionate in the rumen reduced the production of methane enteric, reducing the concentration of acetate being the main precursor for the production of methane, however the formation of propionate does not produce carbon dioxide, conserving more energy and decreasing the production of methane (Chandrakar et al., 2021).

All treatments had ruminal pH close to 5.8, being influenced by the production of lactic and propane, which is considered a low value, which may be indicative of ruminal acidosis, caused by the high amount

of starch present in the diets and its high digestibility (< 90%), increasing rumen fermentation and consequently lowering the pH, in addition to influencing DM Intake (Silva et al., 2020).

The increase in propionate is related to the high passage rate found in treatments containing enzyme with an average of 50% readily available starch, which influenced the increase in starch intake, being of rapid fermentation, influencing the production of lactic acid being one of the main drivers for the production of propionate (Giubert et al., 2013).

The lower production of ammonia in the rumen of animals fed with RCGS (23.59%) is associated with lower hydrolysis of sorghum grain protein, contributing to lower fermentation and consequently lower production of ammonia in the rumen (Chandrakar et al., 2021).

Conclusion

The use of amylolytic enzymes improved the fermentation profile of the silages, reducing the pH values and the concentration of N-NH3 (%NT). The enzyme-containing silages showed a significant increase in effective degradability (DF).

Rehydrated sorghum grain silage can replace rehydrated corn grain silage in sheep feed since no significant difference was found in nutrient intake and digestibility.

Declarations

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Competing Interests

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Data Availability

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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