



Chemical and biological additives in high moisture triticale silages: Nutritional value and ingestive behavior in sheep



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Abstract:

Triticale high moisture grain triticale silage is an excellent option for ruminant diets, but loss control during its fermentation process should be further investigated. Thus, the objective of this study was to evaluate the effects of chemical and biological additives on high moisture triticale silages under chemical-bromatological composition, aerobic stability, and *in vivo* digestibility and ingestive behavior in sheep. The treatments were: high moisture triticale silage without additive (HMTC); high moisture triticale silage with

enzyme-bacterial inoculant (HMTEB); high moisture triticale silage with 0.5 % urea in natural matter (HMTU); and high moisture triticale silage with 1.5 % sodium benzoate in natural matter (HMTSB). Four sheep were housed in appropriate metabolic cages according to the ethical principles of animal experimentation. The addition of urea as additive to high moisture triticale silage provided an increase in crude protein and ammoniacal silage (189.7 and 106.2 g kg MS⁻¹, respectively) but did not affect digestibility (699.6 g kg MS⁻¹ for HMTU, with a general average of treatments of 687.5 g kg MS⁻¹) and ingestive behavior of sheep. Fiber consumption by sheep increased with the addition of the enzyme-bacterial additive in the silage (431.87 *versus* 388.06 g d⁻¹ of FDN for HMTEB and HMTC, respectively). All additives helped to preserve crude protein contents after silo opening, but none interfered in aerobic stability time of silage.

Key words: Sodium benzoate, Nutritional quality, Aerobic stability, Enzyme-bacterial inoculant, Urea.

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High moisture triticale silage shows potential for substitution or supplementation in ruminant diets, mainly because it has higher solubility of the starch⁽¹⁾ and higher levels of lysine⁽²⁾ when compared to maize. However, the high epiphytic concentration of yeast present in the triticale grain⁽³⁾ favors nutrient losses and decrease the aerobic stability of silage after silo opening. Linked to this, the use of chemical additives, such as sodium benzoate and urea, have been tested for control of these deteriorating microorganisms and consequent maintenance of food quality.

Sodium benzoate has been tested for quality control of silages in low doses, due to concerns about possible sterilization of the food, which would impair its fermentation process⁽⁴⁾. However, these claims are contradicted by other authors⁽⁵⁾. Under the same view, Jobim *et al*⁽⁶⁾ reported that addition of urea to silage of high moisture corn silages reduced dry matter losses and increased aerobic stability, but information on high moisture triticale silages is still scarce.

In order to alleviate losses and increase the aerobic stability of silages, the use of bacterial inoculants, with or without enzymes, has become the objective of several studies^(7,8), however, according to Bernardes *et al*⁽⁴⁾ results that existing are inconclusive. In this context, the objective of this study was to evaluate the effects of different chemical and one biological additives containing fibrolytic enzymes in the high moisture triticale silages under chemical-bromatological composition, aerobic stability, nutrient digestibility and ingestive behavior in sheep.

The harvest of triticale grains (*X. Triticosecale* Wittmack cv. IPR 111) for silage production occurred when they reached approximately 30 % humidity. Soon after harvest the grains were crushed in 8 mm sieves, adding the additives according to the defined treatments. The treatments were: high moisture triticale silage without additive (HMTC); high moisture triticale silage with enzyme-bacterial inoculant (HMTEB); high moisture triticale silage with 0.5 % urea in natural matter (HMTU); and high moisture triticale silage with 1.5 % sodium benzoate in natural matter (HMTSB).

Twenty-four (24) polyethylene pails with a capacity of 4.5 L were used as experimental silos. The mean compaction density was 1,067 kg of MV m⁻³. The silos were stored for 11 mo until opening. The enzyme-bacterial inoculant used was Silotrato[®], composed of *Lactobacillus curvatus*, *L. acidophilus*, *L. plantarum*, *L. buchneri*, *Pediococcus acidilactici*, *Enterococcus faecium*, *Lactobacillus lactis* at concentrations of 109 CFU g⁻¹ and 4% of complex enzymatically based on cellulase. The application was made with the additive diluted in non-chlorinated water at a concentration of 4.3 g L⁻¹, according to the manufacturer's recommendation. The chemical additives, urea and sodium benzoate, were homogenized to the triticale grains manually after processing. In order to exclude the effect of the addition of water in the silage with enzyme-bacterial inoculant, in the others also it was included pure water in the same volume.

For the chemical-bromatological evaluation of the silages, a completely randomized design with four replicates was used, and the buffer capacity (BM), ammoniacal nitrogen (N-NH₃), hydrogenation potential (pH), dry matter organic matter (OM), crude protein (CP), ethereal extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (FDA). The determination of ammoniacal nitrogen and buffer capacity was performed using the technique described by Playne and McDonald⁽⁹⁾. The pH values were determined according to Cherney and Cherney⁽¹⁰⁾. The contents of dry matter, organic matter and ethereal extract according to AOAC⁽¹¹⁾. Neutral and acid detergent fiber according to Van Soest⁽¹²⁾ methodology.

Aerobic stability was evaluated 340 d after silo sealing. Samples of 1.0 kg of each replicate were placed in polypropylene containers coated with plastic bag and conditioned in an environment with uncontrolled temperature. Silage temperatures were measured twice a day at 0900 and 1500 h for 7 d, with a thermometer inserted at 10 cm in the center of the mass. Loss of aerobic stability was defined as the time required for the silage to rise 2 °C relative to room temperature⁽¹³⁾. From another container set, representing each replicate, daily samples were taken for pH, DM and CP determination. The design was a completely randomized design with three replications, in a scheme of subdivided plots, where the factors attributed to the plots were the silages and to the subplot the exposure time to the air.

The silages used to feed the sheep followed the same manufacturing procedures described above, and were stored in 16 concrete silos with a capacity of 250 L. In the evaluation of the ingestive behavior and apparent digestibility of nutrients, the method of total fecal

collection was used, for which four castrated male sheep with an average weight of 25 kg were housed in appropriate metabolic cages equipped with a drinking fountain and individual feeders and mineral mixers. Procedures were adopted in accordance with the ethical principles of animal experimentation, approved by the Committee of Ethics in Animal Experimentation of the State University of Londrina, according to document number 26014.2012.79.

The evaluations with sheep were conducted in a 4x4 Latin square design, with four treatments and four periods. Each collection period lasted 5 d and preceded 10 d of adaptation. The diets maintained a roughage-concentrate ratio of 50:50, formulated solely with the foods described in Table 1, according to requirements of the NRC⁽¹⁴⁾ for weaned sheep category with daily average gain of 300 g. As a bulky source out used Coast-cross hay (*Cynodon dactylon* (L.) pers) chopped and supplied as mixed feed.

Table 1: Chemical-bromatological composition of Coast-cross hay and diets containing high moisture triticale silages with different additives and hay in the 50:50 ratio

	Diet				Hay
	HMTC	HMTEB	HMTU	HMTSB	
DM, g kg FM ⁻¹	780.3	793.9	787.8	789.3	823.2
CP, g kg DM ⁻¹	158.5	154.8	177.1	154.2	129.7
EE, g kg DM ⁻¹	19.1	20.3	19.3	18.5	11.7
NDF, g kg DM ⁻¹	433.5	441.8	436.1	441.1	677.1
ADF, g kg DM ⁻¹	228.8	233.8	231.1	230.9	337.7
MM, g kg DM ⁻¹	49.5	49.9	49.2	51.7	83.8
OM, g kg DM ⁻¹	950.6	950.1	950.9	948.4	-

HMTC= control; HMTEB= enzyme-bacterial inoculant; HMTU= urea; HMTSB= sodium benzoate; DM= Dry matter; CP= Crude protein; EE= Ethereal extract; NDF= Neutral detergent fiber; FDA= Acid detergent fiber; MM= Mineral matter; MO= Organic matter.

The animals were fed twice a day at 0800 and 1700 h with water and mineral salt *ad libitum*. At the beginning of each period the animals were weighed considering the average weight for the calculation of the metabolic size.

The feces of each animal were collected twice a day, by means of collection bags, and weighed. A subsample of 20 % of the total was stored in a freezer at -20 °C, as well as subsamples of food supplied and leftovers. For the laboratory analyzes, the subsamples were pooled to form samples composed by animal, treatment and period. The digestibility coefficients of the different diets were obtained using the system of equations cited by Silva and Leão⁽¹⁵⁾. Chemical and bromatological analyzes of high moisture triticale silages, total diet, leftovers and feces included dry matter, organic matter, crude protein, ethereal extract, neutral detergent fiber and acid.

On the last day of each period the animals were observed for 24 h to investigate the ingestive behavior⁽¹⁶⁾, where each observation consisted of five-minute intervals⁽¹⁷⁾. The behaviors evaluated were ingestion of solids (IS), water intake (WI), mineral salt intake (MSI), standing rumination (SR), lying rumination (LR), standing leisure (SL), lying leisure (LL) and atypical behavior (AB). In addition to these parameters, the number of chewing chews (CHEWS), the duration of ruminal cycle (DUR) and the number of chewing chews per second (CHEWS / S) were also evaluated.

Data relating to chemical and bromatological composition of silages and experimental rations, animal performance and ingestive behavior had their means compared by the Tukey test at 5% significance by were submitted to the Tukey test at 5% significance by the GLM procedure of the SAS program (2001 The results of the aerobic stability were submitted to the regression analysis by program R (2013).

The addition of urea caused a significant increase in the ammoniacal nitrogen contents of high moisture triticale silage (Table 2). This increase is due to the solubilization of urea in the presence of urease, an enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia, and this increase provided a higher buffer capacity of the ensiled mass, but did not differ from the control silage (364.2 and 323.3 meq NaOH 100g MS⁻¹, respectively). Crude protein content was also higher ($P<0.05$) in urea added silage (189.7 g kg MS⁻¹), which is a source of non-protein nitrogen.

Table 2: Chemical and bromatological composition, pH and buffer capacity of high moisture triticale silages with different additives (g kg DM⁻¹)

	Silage				Average	CV	P
	HMTC	HMTEB	HMTU	HMTSB			
DM, g kg FM ⁻¹	686.9	714.1	702.0	704.9	701.9	2.93	0.2208
CP	172.6 ^b	165.2 ^b	189.7 ^a	164.0 ^b	172.9	3.21	<0.0001
EE	16.0	18.5	16.4	14.9	16.5	13.72	0.2286
NDF	109.3	126.0	114.5	124.5	118.6	10.70	0.3081
ADF	39.1	49.0	43.6	43.3	43.7	15.85	0.4390
MM	19.6 ^b	20.5 ^{ab}	19.0 ^b	24.0 ^a	20.8	8.20	0.0092
OM	980.4 ^a	979.5 ^{ab}	981.0 ^a	976.0 ^b	919.2	0.17	0.0092
pH	4.50 ^c	4.35 ^c	4.84 ^b	5.67 ^a	4.84	1.30	<0.0001
N-NH ₃ , % N total	58.7 ^b	46.4 ^b	106.2 ^a	42.1 ^b	63.4	17.07	<0.0001
BC, meq NaOH 100g DM ⁻¹	323.3 ^{ab}	294.1 ^b	364.2 ^a	216.7 ^c	299.6	6.25	<0.0001

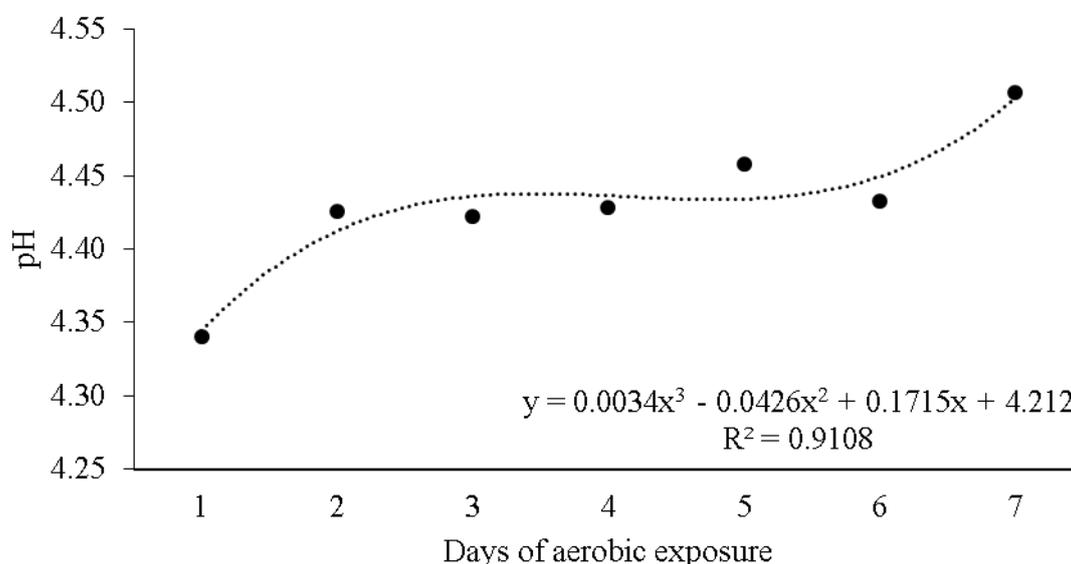
HMTC= control; HMTEB= enzyme-bacterial inoculant; HMTU= urea; HMTSB= sodium benzoate; DM= Dry matter; CP= Crude protein; EE= Ethereal extract; NDF= Neutral detergent fiber; FDA= Acid detergent fiber; MM= Mineral matter; MO= Organic matter; N-NH₃: Ammoniac nitrogen; BC= Buffer capacity.

^{ab} Means followed by different letters, in the line, differ ($P<0.05$).

The above mentioned fermentation alterations provided higher pH of the urea treated silage in relation to the control silage (4.84 and 4.50, respectively), but lower than that found in silage supplemented with sodium benzoate. The justification for the silage with sodium benzoate had high pH value and low buffer capacity, would be the pKa of lactic acid. Bernardes *et al*⁽⁴⁾ affirm that when pH values are higher than pKa, the efficiency of the acid in the solution is reduced because the acid form decreases and the ionized form predominates.

When compared than control silage, it was observed that the addition of enzyme-bacterial inoculant did not alter the chemical-bromatological composition (Table 2), and as described by Bernardes *et al*⁽⁴⁾, the addition of inoculants with homo and heterofermentative bacteria does not seem to influence the aerobic stability of the same (Figure 1).

Figure 1: Value of pH during the aerobic stability test



When evaluating high moisture silages of different winter grains, some researchers found higher content of acetic and propionic acid in the high moisture triticale⁽¹⁸⁾. In agreement, Ni *et al*⁽³⁾ cited by Leão *et al*⁽¹⁹⁾ affirm that there is a predominance of heterofermentative bacteria in the epiphytic community of this species, which could, according to the same authors, justify the prolonged aerobic stability of silage.

Figure 1 shows the average pH of the high moisture triticale silage during the aerobic stability evaluation period. Although there is no difference between the treatments, it is possible to observe a constant increase of pH due to the consumption of organic acids.

None of treatments showed a temperature increase above 2 °C compared to the ambient temperature during the entire aerobic exposure period (Table 3), which is a good indicator of deterioration control⁽¹³⁾. On the seventh day all silages reached their maximum

temperature, being this a consequence of the microbiological development priority. This temperature oscillation observed in silages follows the behavior of ambient temperature, counteracting a possible gradual increase constant and independent of external environment.

Table 3: Ambient temperature and temperature averages (°C) of high moisture triticale silages with different additives during aerobic exposure

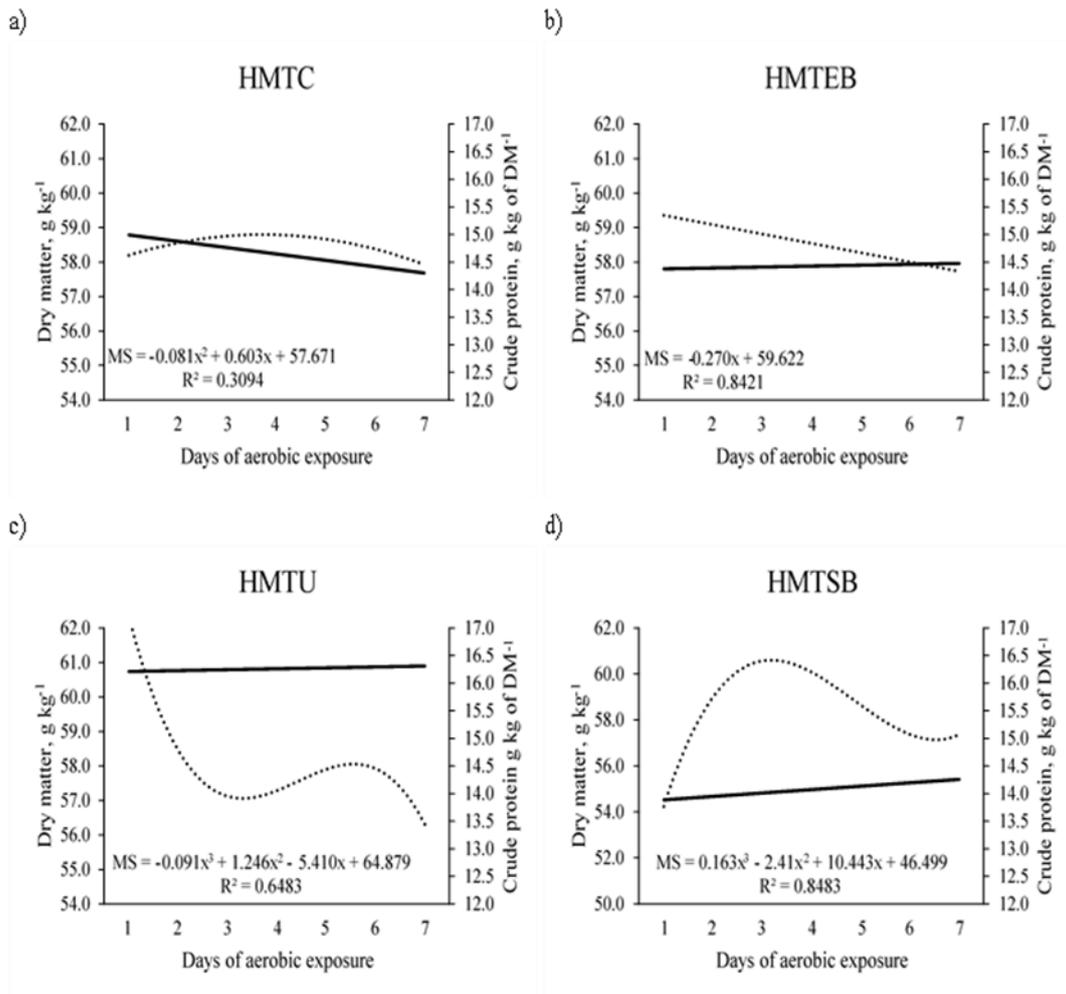
Silage	Days of aerobic exposure						
	1	2	3	4	5	6	7
HMTC	28.83	28.35	29.02	29.13	27.45	28.83	30.28
HMTEB	28.92	27.92	28.45	28.57	26.93	28.30	29.73
HMTU	28.92	28.17	28.72	28.93	27.27	28.30	30.02
HMTSB	28.58	27.97	28.57	28.70	27.08	28.23	30.03
Ambient temperature	27.10	28.10	28.15	27.30	27.20	30.25	29.80

HMTC= control; HMTEB= enzyme-bacterial inoculant; HMTU= urea; HMTSB= High sodium benzoate.

The high density of compaction reached in the silos may have been a determining factor for good apparent control of deterioration; this favors that the yeast population is reduced during the anaerobiosis phase of the silo, providing a more stable food when exposed to oxygen⁽²⁰⁾.

Figure 2 shows the behavior of dry matter and crude protein during days of aerobic exposure. In relation to dry matter there was interaction between silages and days of aerobic exposure. Note the quadratic behavior for control silage (a), linear for silage with enzyme-bacterial additive (b), and cubic for silages treated with urea (c) and sodium benzoate (d).

Figure 2: Dry matter (dotted line) and crude protein (solid line) contents of high moisture triticale silages with different additives during aerobic exposure



HMTC= control (a); HMTEB= enzyme-bacterial inoculant (b); HMTU: urea (c); HMTSB= sodium benzoate (d).

Both control (a) and sodium benzoate (d) silages showed a more abrupt fall from the fourth day of aerobic exposure, whereas silage with enzyme-bacterial inoculant (b) had a linear decrease ($R^2=0.8421$) in the dry matter contents from the opening of the silo. This decrease in dry matter contents in silages with bacterial additives has been shown to be common in the aerobic stability studies, suggesting that losses of the same. These losses are due to the reduction in the concentration of acetate⁽²¹⁾, which is a potent antifungal, and the increase in lactate concentration, which is a growth substrate for yeasts. Wilkinson and Davies⁽²²⁾ still point out that for each molecule of acetic acid formed, an equivalent molecule of carbon dioxide is produced, increasing dry matter losses.

The initial rapid decrease in dry matter contents of urea silage (c) may be due to the evaporation of ammonia formed in larger amounts (Table 2) during the fermentation process of this treatment.

The increase in the dry matter content observed in the first days of silage treated with sodium benzoate (d) indicates a loss of humidity for the environment. Furthermore, this type of additive is characterized by hindering the development of yeasts, delaying the onset of deterioration⁽⁵⁾. Similar behavior was described in sugarcane silage with this same additive⁽²³⁾. In relation to crude protein content, trend lines indicate increasing linear behavior for all silages that were added, which is a dilution reflex, where consumption of carbohydrates is mainly observed, increasing the concentration of other constituents in dry matter. However, control silage showed an opposite behavior, with decrease in crude protein content during the days of aerobic exposure, suggesting that there was degradation by molds, which act at higher pH and consume more complex components⁽²⁴⁾.

Studies about dry matter losses of silages after silo opening are widely diffused in the scientific field⁽⁷⁾, but it is not possible to say in relation to each specific constituent, and a larger number of researches on the subject to come to a more precise conclusion.

The data in Table 4 show that nutrient intake present significant difference only for neutral detergent fiber and acid detergent fiber when expressed in grams per day, where the highest intakes were recorded for the animals fed with silage with enzyme-bacterial additive. The explanation for this result may not be directly related to the treatment, however, it is possible that bacteria present in inoculant altered ruminal environment, allowing greater consumption. The improvement in fiber intake with the use of an enzyme-bacterial inoculant is contradictory among the authors. In review, Oliveira *et al*⁽⁷⁾ concluded that the divergences in results may be related, among other factors, to the different variations of the inoculant used, and also of the diet. Just like McCuistion *et al*⁽²⁵⁾, with the addition of cellulase containing inoculant, it was expected that the fiber would be better utilized, which was not observed since it is a food with low contents of this component.

Table 4: Nutrient intake of diets containing high moisture triticale silages with different additives (g day⁻¹)

	Diet				Average	CV, %	P
	HMTC	HMTEB	HMTU	HMTSB			
DMI	975.78	1056.15	954.12	957.75	985.95	7.33	0.2569
CPI	156.51	164.47	163.66	147.41	158.01	7.73	0.2688
EEI	19.49	22.23	19.73	18.36	19.95	14.95	0.3876
NDFI	388.06 ^b	431.87 ^a	383.64 ^b	381.76 ^b	396.33	5.33	0.0435
ADFI	211.46 ^{ab}	232.54 ^a	208.28 ^b	203.55 ^b	213.95	4.42	0.0197
OMI	927.66	1003.84	908.26	908.97	937.18	7.36	0.2599

HMTC= control; HMTEB= enzyme-bacterial inoculant; HMTU= urea; HMTSB= sodium benzoate; DMI= Dry matter intake; CPI= Crude protein intake; EEI= Ethereal extract; NDFI= Neutral detergent fiber intake; ADFI= Acid detergent fiber intake; OMI= Organic matter intake.

^{ab} Means followed by different letters, in the line, differ ($P < 0.05$).

In general, nutrient and dry matter digestibility of high moisture triticale silage were not affected ($P>0.05$; Table 5) by the addition of no additives, however, all diets showed dry matter digestibility compatible with high moisture corn and sorghum silage, which are the reference foods for this variable^(26,27). However, both digestibility values of organic matter and crude protein were lower than those described for the same feed (891 and 907 g kg DM⁻¹, respectively)⁽¹⁸⁾.

Table 5: Apparent digestibility of nutrients from diets containing high moisture triticale silages with different additives (g kg DM⁻¹)

	Diet				Average	CV	P
	HMTC	HMTE B	HMTU	HMTSB			
DMD	679.6	687.4	699.6	683.4	687.5	2.01	0.2898
CPD	707.5	715.2	703.6	689.7	711.5	2.67	0.0823
EED	770.9	782.5	768.6	769.0	772.7	3.97	0.9035
NDFD	548.3	568.4	549.3	551.9	554.5	6.78	0.8583
ADFD	578.4	597.5	607.4	602.2	596.4	7.32	0.8012
OMD	706.8	710.4	721.0	710.6	712.2	1.95	0.5515

HMTC= control; HMTEB= enzyme-bacterial inoculant; HMTU= urea; HMTSB= sodium benzoate; DMD= Dry matter digestibility; CPD= Crude protein digestibility; EED= Ethereal extract digestibility; NDFD= Fiber in neutral detergent digestibility; ADFD= Fiber in acid detergent digestibility; OMD= Organic matter digestibility.

Ingestive behavior is a good parameter for the evaluation of any food⁽²⁷⁾, since often the acquired chemical or fermentative benefits are not reflected at the time of feeding.

The data in Table 6 show that none of the variables related to ingestive behavior of sheep presented significance between the treatments. Longer water intake time was expected for animals fed silage with sodium benzoate, in view presence of sodium of the additive itself, which was not observed. It should be noted that in this study water consumption was not measured. Kozloski *et al*⁽²⁸⁾ observed that ingestion of sheep may be affected by dietary protein, but that there was no difference when protein source was urea, as in the present study.

Table 6: Ingestive behavior of sheep fed with a diet containing high moisture triticale silage with different additives

	Diet				Average	P
	HMTC	HMTEB	HMTU	HMTSB		
CHEWS	78.73	84.69	79.35	79.71	80.62	0.3716
DUR, sec cycle ⁻¹	56.29	59.94	57.52	57.06	57.70	0.4289
CHEWS S ⁻¹	1.40	1.41	1.38	1.40	1.40	0.6047
WI, min d ⁻¹	2.19	4.06	2.19	3.75	3.05	0.7490
IS, min d ⁻¹	57.50	55.00	53.75	52.50	54.69	0.8895
MSI, min d ⁻¹	5.31	3.75	3.13	6.65	4.71	0.1597
SR, min d ⁻¹	7.81	6.25	6.88	5.31	6.56	0.7381
LR, min d ⁻¹	141.56	148.75	147.50	132.50	142.58	0.4305
SL, min d ⁻¹	43.75	42.19	40.94	45.93	43.20	0.8783
LL, min d ⁻¹	99.69	97.81	102.81	109.00	102.34	0.6623
AB, min d ⁻¹	2.19	2.19	2.81	4.36	2.89	0.5053

HMTC= Control; HMTEB= Enzyme-bacterial inoculant; HMTU= Urea; HMTSB= Sodium benzoate; CHEWS= Number of chewing chews; DUR= Duration of the rumination cycle; CHEWS S⁻¹= Number of chewing chews per second; WI= Water intake; IS= Ingestion of solids; MSI= mineral salt intake; SR= Standing rumination; LR= lying rumination; SL= Standing leisure; LL= Lying leisure; AB: atypical behavior.

The fibrolytic action of some enzymes present in certain additives leads to a reduction in the silage fiber content, which may increase the feed intake of the animals⁽²⁷⁾. However, this report did not show this difference in the constitution of fibers (Table 1) and consequently ingestion.

Very expressive effects were not observed with the addition of the studied additives on the chemical-chemical composition, aerobic stability, digestibility of nutrients and ingestive behavior in sheep. Can be highlight the addition of urea as a high moisture triticale additive for promoting increased silage crude protein content.

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