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Ingestive behavior of lambs fed relocated and inoculated whole-plant corn silage

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ABSTRACT. We aimed to determine the effect of relocation of whole-plant corn silage (WPCS) with different fermentation profiles on ingestive behavior of lambs. Twenty-four male Santa Inês lambs, were used in a completely randomized block design based on initial body weight (17.5 ± 1.8 kg) for the following treatments: CS: WPCS (not inoculated and not relocated); R-12h: WPCS relocated for 12h; IR-12h: WPCS inoculated with *Lactobacillus plantarum* and relocated for 12h; and R-24h: WPCS not inoculated and relocated for 24h. Ingestive behavior was observed as continuous (feeding, ruminating, and idling) and specific (urination, defecation, and water and salt access frequency) activities for 48h (3h intervals for a total of eight periods in 24h). The behavioral activities were affected only by periods (p = 0.0001). Feeding peaks was high at 8:00–11:00am (54.2%) and 5:00-8:00pm (54.9%) in compare to ruminating and idling at the same period. Rumination time was intense at night, at 63.7% (2:00–5:00am) and 69.7% (5:00–8:00am). Idling time (49.7%) was long after silage supply. The specific activities were intense during the morning periods. Supply of WPCS relocated up to 24h did not alter the ingestive behavior.

Keywords: continuous activity; feeding; *Lactobacillus plantarum*; lamb; relocation.

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Introduction

Conserved feeds, in particular silage crops such as corn, is commonly used as feedstuff in animal production (Bernardes & Rêgo, 2014). The use of silage is one of the most effective methods to ensure the feeding of animals during the dry season or throughout the year and guarantee the sustainability of production systems and animal performance. However, poor management or lack of forage on farms are still common situations in different animal production systems. Thus, numerous strategies have been used to resolve the deficit of forage supply during part of the year, such as the leasing of new pasture areas and purchase of *in natura* forages, fibrous by-products, hay, and silage. Therefore, the relocation of silages has become a daily practice among producers that experience problems in forage production (Chen & Weinberg, 2014).

Silage commercialization include silo unloading, re-compaction, transport, and biomass storage in plastic bags or transportation until relocation at larger silos. During the relocation process, the silage is exposed to air for hours or days (Chen & Weinberg, 2014). The exposure of silage to air allows for the growth of undesirable microorganisms that cause of silage deterioration owing to their use of lactic acid and soluble carbohydrates for their metabolism (Borreani, Tabacco, Schmidt, Holmes, & Muck, 2018).

Tropical regions can experience increased losses by the silage deterioration process due to the high microbial metabolic activity (Daniel, Bernardes, Jobim, Schmidt, & Nussio, 2019). The changes that occur in the silage with exposure to air can promote a proportional increase in the cell wall components, reducing the nutritional value (Tabacco, Righi, Quarantelli, & Borreani, 2011), and consequently affecting the ingestive behavior of the animals. The effects of aerobic exposure can be more intense in well-fermented silages (e.g., whole plant corn silage [WPCS]) when these silages are inoculated with homofermentative bacteria (e.g., *Lactobacillus plantarum*), which increase the lactic acid content, hence, reducing aerobic stability (Chen & Weinberg, 2014). This situation can be common when a fermentation profile of corn silage with a dry matter (DM) content of up to 325 g kg^{-1} is desired. Therefore, it is

important to understand the consequences of air exposure in the transported material during biomass relocation, and the possible effects of silage ingestion in the animals.

Hence, evaluation of the feed provided to the animals is important, as the different characteristics of these feeds can cause variations in feed quality and ingestive behaviors by the animals. Small ruminants are highly selective animals, and subtle changes in feed characteristics can influence feed intake (Gerlach, Rob, Weib, Büscher, & Südekum, 2014). Thus, one of the options for data recording techniques to express patterns in feed consumption is the evaluation of parameters concerning the ingestive behavior of these animals (Figueiredo et al., 2013). We hypothesized that the relocation and inoculation process, or not, of whole-plant corn silages can influence the ingestive behavior of lambs fed with these silages, throughout the day. The aim of this study was to determine the effect of relocation of WPCS with different fermentation profiles on the ingestive behavior of lambs.

Material and methods

Silage production and treatments

The experiment was conducted at the Small Ruminants Metabolic Studies Unit at the Federal Rural University of Amazon (UFRA), Belém, Pará State, Brazil (1°27′07″ S, 48°26′13″ W). The procedures and handling methods adopted with animals were approved by UFRA's Animal Ethics Committee (CEUA), protocol No. 022/2016 (CEUA) and 23084. 006712/2016-21 (UFRA). The corn crop used in silage production was harvested in an area located in Paragominas, Pará State, Brazil (03°02′2″ S, 47°20′18″ W). The PIONEER 30F90H® corn hybrid harvested using a self-propelled harvester (FX40, New Holland Agriculture, Italy), and with 325 g kg^{-1} DM (close to 2/3 of the milk line) was used for WPCS production.

The experimental treatments comprised CS: WPCS (not inoculated and not relocated), R-12h: WPCS relocated for 12h, IR-12h: WPCS inoculated with *L. plantarum* and relocated for 12h, and R-24h: WPCS not inoculated and relocated for 24h. The relocation process involved moving the silage from one silo to another. The relocation time (air exposure) was defined as the total time to complete this process. The inoculation was performed at the ensiling process, and not at the relocation process, by diluting the product in distilled water, following the manufacturer's recommendation, which was *L. plantarum* (CH6072 and L286) at 1×10^5 cfu g^{-1} of *L. plantarum* per gram of fresh forage. Accordingly, a total solution volume of 5 mL kg^{-1} of fresh forage was uniformly applied by spraying and subsequent manual homogenization of the forage mass.

As experimental silos, 24 plastic drums of 200 L were used and filled with 125.7 ± 2.3 kg of fresh forage, achieving an average density of 628.4 ± 11.7 kg m^{-3}. The silos were opened after 30 days of ensiling, and the silage was removed and exposed to air at specific times for each treatment and then relocated, except for the CS treatment. The silos were then opened 45 days after the relocation. The silage supply to the animals was initiated for evaluation of the ingestive behavior, where each animal received the silage from their respective treatment. The dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), silage pH value, and fermentation end-product concentrations were analyzed (Table 1).

Table 1. Characterization of WPCS, relocated or not, with or without bacterial inoculant.

Variables[1]	Fresh forage	Silage[2]			
		CS	R-12h	IR-12h	R-24h
DM (g kg^{-1})	325	317	332	317	323
OM (g kg^{-1} DM)	960	962	962	961	960
CP (g kg^{-1} DM)	76	71	70	72	74
NDF (g kg^{-1} DM)	496	561	531	590	576
pH 1	-	-	3.6	3.5	3.6
pH 2	-	3.6	3.6	3.7	3.7
NH_3-N (g kg^{-1} total N)	-	7.1	7.8	6.9	9.0
Lactic acid (g kg^{-1} DM)	-	36	48	51	40
Acetic acid (g kg^{-1} DM)	-	38	38	4.1	35
Propionic acid (g kg^{-1} DM)	-	8.6	6.1	7.0	8.1
Total VFA (g kg^{-1} DM)	-	47	44	48	43

[1]DM - dry matter; OM - organic matter; CP - crude protein; NDF - neutral detergent fiber; pH 1 - pH value after aerobic exposure; pH 2 - pH value at silos opening after relocation; NH_3-N - ammoniacal nitrogen; Total VFA - total volatile fatty acids (acetic acid plus propionic acid); [2]CS: whole-plant corn silage (WPCS, not inoculated and not relocated); R-12h: WPCS relocated for 12h; IR-12h: WPCS inoculated with *Lactobacillus plantarum* (1×10^5 cfu g^{-1} per gram of fresh forage) and relocated for 12h; and R-24h: WPCS not inoculated and relocated for 24h.

Samples preparation and analyses

The fresh forage and the silage were split into two subsamples. One subsample was oven-dried at 55°C for 72h to determine the DM content, weighed, and then milled in a Wiley-type (STAR-FT-80/2, Piracicaba, São Paulo State, Brazil) knife mill with a sieve screen of diameter 1 mm. The DM (official method 934.01) and OM (official method 923.03) were determined, and CP (official method 978.04) were estimated according to Association of Official Analytical Chemists (AOAC, 1990) official methods. The NDF was determined in an autoclave with the use of thermostable alpha-amylase (Barbosa, Detman, Rocha, Franco, & Valadares Filho, 2015).

The content of fermentation end-products was analyzed. An aqueous extract was prepared with 25 g of silage and 300 mL of deionized water and homogenized in a Stomacher® (MARCONI-MA 440/CF). Then, it was centrifuged at 10,000 × *g* for 15 min. at 4°C, and the lactic acid content (Prince, 1969) and volatile fatty acids were quantified. Volatile fatty acids content was determined using a gas chromatograph coupled to a mass spectrometer (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) and were detected using a capillary column (Stabilwax; Restek, Bellefonte, PA, USA; 60 m, 0.25 mm, i.d., 0.25 m). Butyric acid was not detected in the silages. Butyric acid content was not detected in the silages.

To determine ammoniacal nitrogen (NH_3-N) content, 25 g of silage was weighed in an Erlenmeyer flask to which 200 mL of sulfuric acid solution (0.2 N, v.v.) was added, and stored for 48h a refrigerator at 8°C (Bolsen et al., 1992). Afterward, the sample was filtered, and 4 mL of the aqueous extract and 20 mL of distilled water was added to Falcon® centrifuge tubes. Distillation was performed with 10 mL of potassium hydroxide (2 N, p.v.) and 10 mL of boric acid solution 2%, p.v. (receptor solution). Then titration was performed with hydrochloric acid 0.005 N, v.v., according to official method No. 920.03 (AOAC, 1990).

To measure silage pH values, an aqueous extract was prepared by weighing 9 g of silage into a beaker to which 60 mL of distilled water was added (Silva & Queiroz, 2002). The sample was then homogenized and allowed to stand for 30 min. for pH reading using a benchtop pH meter (Tekna T-1000, São Bernardo do Campo, São Paulo State, Brazil).

Experimental design and data collection

In this study, 24 non-castrated, male, Santa Inês lambs with an initial body weight of 17.5 ± 1.8 kg were used. The animals were maintained in individual wooden metabolism cages (0.79 m^2), equipped with a slatted floor, a water fountain, and feeder for diet and salt. The experimental design was in randomized blocks, according to the body weight (BW) of the animals (group 1 = animals above 17.5 kg BW; group 2 = animals below 17.5 kg BW), with four treatments and six replications, totaling 24 experimental units. The total experimental period was 23 days: 14 days for animal adaptation; 7 days for feed and orts sample collection for determination of diet ingestion; and 2 days for ingestive behavior evaluation.

The animals were fed exclusively the silage. The silage was provided *ad libitum* during two periods: at 8:00am and 5:00pm. The quantity of feed provided was calculated based on the previous day ensuring approximately 100 g kg^{-1} of orts. During the collection period, orts from each animal were weighed and stored in freezer (-20°C) for subsequent DM and NDF content analyses. Water and white salt were provided *ad libitum* and changed every day.

The evaluation of ingestive behavior was conducted uninterrupted for 48h, on the last 2 days of the experimental period through visual observations. These observations were made by four previously trained observers, with each observation lasting 5 min., during which the feeding, ruminating, and idling behavior of the animals were recorded (Segabinazzi et al., 2014).

During data tabulation, the total hours (48h) were divided into intervals of 3h each, starting at 8:00am, to evaluate the behavioral variation throughout the day. Thus, eight evaluation periods were obtained: 8:00-11:00am, 11:00am-2:00pm, 2:00-5:00pm, 5:00-8:00pm, 8:00-11:00pm, 11:00pm-2:00am, 2:00-5:00am, and 5:00-8:00am. The following equations were used to calculated some variables.

$$\text{TCT}\left(\text{h day}^{-1}\right)=\text{TFT}\left(\text{h day}^{-1}\right)+\text{TRT}\left(\text{h day}^{-1}\right)$$

$$\text{NRB}\left(\text{nº day}^{-1}\right)=\frac{\text{TRT (h day}^{-1})}{\text{RTB (sec bolus}^{-1})}$$

$$\text{CN}\left(\text{nº day}^{-1}\right)=\text{CN}\left(\text{nº bolus}^{-1}\right)\times\text{RTB}\left(\text{sec bolus}^{-1}\right).$$

Where the total feeding time (TFT), total rumination time (TRT), and total idling time (TIT) of each animal during 24h were calculated by summing the observations, in minutes, of each activity performed during the data collection period. The chews numbers (CN) in n° bolus^{-1} and rumination time per bolus (RTB) in s bolus^{-1} of each animal were determined using a digital chronometer. Observations were recorded at four different periods of the day (8:00am-2:00pm, 2:00-8:00pm, 8:00pm-2:00am, and 2:00-8:00am), with three observations per period for each animal. The total chewing time (TCT) in h day^{-1}, number of ruminal bolus (NRB) in n° day^{-1}, and CN in n° day^{-1} were determined according to Burguer et al. (2000).

$$\text{DMFE}\left(\text{g DM h}^{-1}\right)=\frac{\text{DMI}\left(\text{g day}^{-1}\right)}{\text{TFT}\left(\text{h day}^{-1}\right)}$$

$$\text{NDFFE (g NDF h}^{-1}) = \frac{\text{NDFI (g day}^{-1})}{\text{TFT (h day}^{-1})}$$

$$\text{DMRE (g DM h}^{-1}) = \frac{\text{DMI (g day}^{-1})}{\text{TRT (h day}^{-1})}$$

$$\text{NDFRE (g NDF h}^{-1}) = \frac{\text{NDFI (g day}^{-1})}{\text{TRT (h day}^{-1})}$$

$$\text{DMI} = \text{feed provide (g DM)} - \text{orts (g DM)}$$

$$\text{NDFI} = \text{feed provide (g NDF)} - \text{orts (g NDF)}$$

Where the dry matter feed efficiency (DMFE) in g DM h^{-1}, neutral detergent fiber feed efficiency (NDFFE) in g NDF h^{-1}, dry matter rumination efficiency (DMRE) in g DM h^{-1}, neutral detergent fiber rumination efficiency (NDFRE) in g NDF h^{-1}, dry matter intake (DMI) in g day^{-1}, and neutral detergent fiber intake (NDFI) in g day^{-1}.

Statistical analyses

The data were tested for assumptions of normality errors and homogeneity of variance using the Cramer–von Mises and Brown Forsythe test, respectively. All variables were analyzed using PROC MIXED in Statistical Analysis System (SAS, 2017), with treatments and blocks as fixed effects.

The variables feeding time, rumination time, idling time, urination, defection, and water and salt access frequency were analyzed used a 4 × 8 factorial arrangement (four silages × eight periods). The interaction effect was analyzed when the effect was significant. The means were compared by Tukey's test at a 5% significance level. The statistical model adopted was as follows:

$$\hat{Y}_{ijk} = \mu + B_i + T_j + P_k + (T * P)_{jk} + e_{ijk}$$

where: $\hat{Y}_{ijk}$ = estimated value, μ = mean; B_i = block effect, T_j = treatment effect, P_k = period effect, $(T \times P)_{jk}$ = interaction between treatment × period, and e_{ijk} = residue of the observation.

The means of variables TFT, TRT, TIT, TCT, NRB, RTB, CN, DMI, NDFI, DMFE, NDFFE, DMRE, and NDFRE were compared by Tukey's test at a 5% significance. The statistical model adopted was as follows:

$$\hat{Y}_{ij} = \mu + B_i + T_j + e_{ij}$$

where: $\hat{Y}_{ij}$ = estimated value, μ = mean, B_i = block effect, T_j = treatment effect, and e_{ijk} = residue of the observation.

Results and discussion

There was no interaction ($p > 0.05$) for treatments × period on feeding, rumination, and idling times. The continuous activity of lambs was not affected ($p > 0.05$) by the different silages provided, probably owing to the similarity in chemical composition of silages, which was proven by the absence of butyric acid in these silages. However, there was a period effect ($p < 0.05$) on feeding, rumination, and idling times (Table 2).

Silage intake was higher during the first 12h of the day after the first feeding (8:00am-8:00pm) than during the subsequent 12h (8:00pm-8:00am), corresponding to 79.0% of the total feeding time during the 24-h period. The intake peaks occurred mainly between 8:00-11:00am and 5:00-8:00pm.

Table 2. Continuous activity at different periods of the day in lambs fed with WPCS, relocated or not, with or without bacterial inoculant.

Treatm.[2]	Period (hours)								Mean	SEM	*p*-value[3]		
	8:00-11:00am	11:00am-2:00pm	2:00-5:00pm	5:00-8:00pm	8:00-11:00pm	11:00pm-2:00am	2:00-5:00am	5:00-8:00am			T	P	T×P
	Feeding time (% of the time in the period)[1]												
CS	56.7	23.8	25.2	57.2	16.7	14.6	6.9	3.0	25.5	1.31	0.09	<0.01	0.53
R-12h	48.2	19.9	15.1	49.3	13.9	11.3	8.3	5.6	21.5				
IR-12h	52.6	19.7	25.7	56.9	19.0	10.0	7.4	8.6	25.0				
R-24h	59.5	24.8	19.0	56.0	6.3	9.0	2.3	4.4	22.7				
Mean	54.2^{a}	22.1^{b}	21.2^{bc}	54.9^{a}	13.9^{cd}	11.2^{de}	6.2^{de}	5.4^{e}	23.6				
	Rumination time (% of the time in the period)[1]												
CS	21.5	31.3	43.1	6.9	39.6	54.4	64.8	69.7	41.4	1.59	0.97	<0.01	0.84
R-12h	20.6	32.2	50.2	9.0	33.3	51.9	57.4	72.0	40.8				
IR-12h	19.0	44.0	43.8	8.8	34.5	53.5	62.0	68.5	41.8				
R-24h	13.4	35.0	43.5	5.3	38.0	52.1	70.4	68.5	40.8				
Mean	18.6^{d}	35.6^{c}	45.1^{bc}	7.5^{e}	36.3^{c}	53.0^{b}	63.7^{a}	69.7^{a}	41.2				
	Idle time (% of the time in the period)[1]												
CS	21.8	44.9	31.7	35.9	43.8	31.0	28.2	27.3	33.1	1.89	0.21	<0.01	0.99
R-12h	31.3	47.9	34.7	41.7	52.8	36.8	34.3	22.5	37.8				
IR-12h	28.5	36.3	30.6	34.3	46.5	36.6	30.6	22.9	33.3				
R-24h	27.1	40.3	37.5	38.7	55.8	38.9	27.3	27.1	36.6				
Mean	27.1^{cd}	42.4^{ab}	33.6^{bcd}	37.6^{abc}	49.7^{a}	35.8^{bcd}	30.1^{cd}	24.9^{d}	35.2				

[1]The sum of the activities is equal to 100%; [2]CS: whole-plant corn silage (WPCS, not inoculated and not relocated); R–12h: WPCS relocated for 12h; IR–12h: WPCS inoculated with *Lactobacillus plantarum* (1×10^5 cfu g^{-1} per gram of fresh forage) and relocated for 12h; and R–24h: WPCS not inoculated and relocated for 24h. Means on the same row followed by different lowercase letters differed from each other ($p < 0.05$) by Tukey's test.

The time spent for feed intake by the animals, especially in feedlot systems, can vary considerably depending on the feed management system adopted (Segabinazzi et al., 2014). The fact that the animals in the present study spent more time feeding in the periods 8:00-11:00am and 5:00-8:00pm, in relation to rumination and idling activities, was due to the silage supply at these intervals, stimulating silage intake. In feedlot systems, the feeding time is an important variable that has be managed in the production systems, as it is highly related to diet intake, and consequently, animal performance (Santos et al., 2018).

The rumination time increased during the night after silage supply (5:00-8:00pm), being high between 2:00-5:00am and 5:00-8:00am in the first 12h (8:00pm-8:00am) after the second feeding, with 68.6% of the total rumination time recorded during the first 24h. A lower rumination time was observed between 8:00-11:00am and 5:00-8:00pm. The rumination time spent by animals presented a crescent-shaped tendency after the periods when the diets were provided, being more intense at night. The rumination time is rhythmic according to the feed supply, with the rumination periods interspersed with feed intake (Pazdiora et al., 2011).

There is a preference by the animals to perform rumination activity, during periods other than the hottest hours of the day, when the animal is calmer, which resulted in the highest rumination frequencies between 10:00pm and 5:00am. Furthermore, the diet composition, forage cell wall content, and high inclusion of forage in the diets, can increase the rumination time spent by animals (Beauchemin, 2018). Lower rumination times were observed during the first 12h of the day, accounting for 31.4% of the total rumination time. This behavior occurred as a result of the silage supply during the same interval, which increased the roughage intake, interrupting the rumination process.

The distribution of idling activity was similar between the first 12h after the first feeding and the subsequent 12h, accounting for 49.8 and 50.2% of the total activity time for the respective intervals. A higher idling time was observed during 8:00-11:00pm, after the silage supply and at the time when the rumination activity was initiating. The idling time was shorter during the periods 5:00-8:00am and 8:00-11:00am.

Idling time in animals is defined as the time when the animals do not perform physical activities (e.g. feeding, rumination, and water intake), and this time, as well as its distribution pattern, is strongly influenced by the diet fractionation and ingestion activities of the animals (Pazdiora et al., 2011). The idling behavior in feedlot systems provides an absence of competition among the animals (e.g. physical space), which is different compared with that of animals that live in groups. In a group, the idling time is spent in exploration activities and competitions for territory or group leadership, which in turn reduce the animals' idling activities during the day. The influence of feeding and rumination activities on idling may explain the fact that the animals had a higher time allocated to this activity between 8:00 and 11:00pm, when the time spent feeding and rumination was lower.

It is possible to observe a certain behavioral pattern in animals in relation to the manner in which the continuous activity is performed by the animals throughout the day. Activity peaks occur at specific times of the day and animals establish a standardized means of performing activities, according to the management system adopted. During the beginning of these activities, there was an intake peak as early as the first observation period (8:00-11:00am), caused by the silage supply. This intake peak was followed by an idling activity peak, which can be interpreted as preparation for the rumination process. Shortly after the second period (11:00am-2:00pm), the idling activity decreased at the same time as the rumination activity increased; therefore, the rumination activity showed a peak after the idling activity peak.

The rumination activity was interrupted by the provision of the diet, presenting a new intake peak, when the second diet fraction was provided at 5:00pm. However, the behavioral pattern of these animals showed the same trend as that of the previous activities performed. An idling peak was observed again after the silage was provided, followed by a new rumination peak that was distributed throughout the night periods as a crescent-shaped trend in comparison to the trends for feeding and idling activities, which decreased simultaneously. The rumination peak was interrupted again the next day when a new feed was provided.

Because of the fermentation characteristics of silage, its use in diets can present characteristics that could influence the forage intake. pH values above 4.4 and DM content below 300 g kg^{-1} can be used as proteolytic fermentative indicators leading to, for example, butyric acid production, which can reduce the feed intake (Borreani et al., 2018). In the present study, the silages showed pH values below 4.4 and DM concentrations above 300 g kg^{-1} (Table 1), probably inhibiting the bacterial proteolytic activity in the silo, because of to the low moisture content in the ensiled forage mass. Therefore, we believe that the silages used in this study did not have organic acids that could influence diet intake, and consequently, the ingestive behavior. The NDF content of the diet is a variable that can influence silage intake. In the present study, the NDF content of silage varied between 531 and 590 g kg^{-1}; however, it did not influence diet intake. In general, the animals spent an average of 8.48, 9.74, and 5.78h day^{-1} in idling, ruminating, and feeding, respectively.

There was no interaction effect ($p > 0.05$) and no treatment effect ($p > 0.05$) on the specific activities. However, there was a period effect ($p < 0.05$) on the same variables (Table 3).

Table 3. Specific activities at different times of the day, of lambs fed with WPCS, relocated or not, with or without bacterial inoculant.

Treatm.[2]	Period (hours)								Mean	SEM	*p*-value[3]		
	8:00-11:00am	11:00am-2:00pm	2:00-5:00pm	5:00-8:00pm	8:00-11:00pm	11:00pm-2:00am	2:00-5:00am	5:00-8:00am			T	P	T×P
Urination (number of times lambs day^{-1})[1]													
CS	1.3	1.1	1.4	0.8	0.7	1.2	0.1	0.5	0.9	0.11	0.09	0.01	0.77
R-12h	0.6	0.8	1.0	0.3	0.6	0.8	0.5	0.3	0.6				
IR-12h	1.7	0.8	1.8	0.3	0.8	0.8	0.4	0.5	0.9				
R-24h	0.9	1.5	1.3	0.9	1.0	0.9	0.8	0.6	1.0				
Mean	1.1^{ab}	1.1^{ab}	1.4^{a}	0.6^{b}	0.8^{ab}	0.9^{ab}	0.5^{b}	0.5^{b}	0.9				
Defecation (number of times lambs day^{-1})[1]													
CS	1.6	1.7	1.0	0.5	0.8	0.9	0.2	0.8	0.9	0.12	0.23	<0.01	0.99
R-12h	1.9	1.8	0.7	0.4	0.7	0.5	0.3	0.2	0.8				
IR-12h	1.4	1.3	0.7	0.2	0.5	0.5	0.4	0.4	0.7				
R-24h	1.9	1.4	1.3	0.4	1.0	0.7	0.6	0.9	1.0				
Mean	1.7^{a}	1.6^{ab}	0.9^{bc}	0.4^{c}	0.8^{c}	0.7^{c}	0.4^{c}	0.6^{c}	0.9				
Water access frequency (number of times lambs day^{-1})[1]													
CS	1.3	0.5	0.3	0.3	0.3	0.0	0.0	0.0	0.3	0.08	0.61	<0.01	0.22
R-12h	1.3	0.3	0.3	0.4	0.1	0.0	0.2	0.1	0.3				
IR-12h	2.8	0.7	0.2	0.1	0.0	0.0	0.0	0.1	0.5				
R-24h	2.2	0.6	0.2	0.2	0.1	0.0	0.0	0.0	0.4				
Mean	1.9^{a}	0.5^{b}	0.3^{b}	0.3^{c}	0.1^{c}	0.0^{c}	0.1^{c}	0.1^{c}	0.4				
Salt access frequency (number of times lambs day^{-1})[1]													
CS	2.4	0.8	0.4	0.8	0.6	0.0	0.0	0.2	0.7	0.11	0.29	<0.01	0.87
R-12h	2.8	0.8	0.3	0.9	0.3	0.1	0.4	0.3	0.7				
IR-12h	2.0	0.2	0.8	0.5	0.1	0.1	0.3	0.0	0.5				
R-24h	1.6	1.0	0.8	0.4	0.1	0.0	0.0	0.0	0.5				
Mean	2.2^{a}	0.7^{b}	0.6^{b}	0.7^{b}	0.3^{b}	0.1^{b}	0.2^{b}	0.1^{b}	0.6				

[1]Specific activities; [2]CS: whole-plant corn silage (WPCS, not inoculated and not relocated); R−12h: WPCS relocated for 12h; IR−12h: WPCS inoculated with *Lactobacillus plantarum* (1×10^5 cfu g^{-1} per gram of fresh forage) and relocated for 12h; and R−24h: WPCS not inoculated and relocated for 24h. Means on the same row followed by different lowercase letters differed from each other ($p < 0.05$) by Tukey's test.

The highest urination frequencies were observed between 2:00pm and 5:00pm, whereas the lowest frequencies occurred between 5:00pm and 8:00am, which comprise the first three and the last five periods of the day, respectively. The defecation frequency was high from 8:00am and 11:00am and low between 5:00pm and 8:00am.

The urination frequency is a variable that is closely related to water intake, whether from the feed or direct water intake. Therefore, it is understandable that the higher urination frequencies occurred in the morning periods, consistent with the water access frequency by lambs. The defecation rate, in turn, may be associated with the amount of feed intake, which was higher during the periods 8:00-11:00am and 5:00-8:00pm.

The water access frequency was higher between 8:00 and 11:00am than during the other periods, with less frequency between 5:00pm and 8:00am. The salt access frequency was higher at 8:00-11:00am, probably due to the diet provided during this period, stimulating the mineral supplement intake.

Throughout the day, the salt intake was low, as salt is only considered as a contribution to the supply requirements of micronutrients in the animals. The water access frequency was intense during the early hours of the day (8:00-11:00am), most likely owing to the beginning of the animals' daily activities and the supply of water.

The silages provided did not affect ($p > 0.05$) DMI, chews, and feed and rumination efficiency; however, these silages affected ($p < 0.05$) the NDFI (Table 4). The fact that the animals showed no difference in behavioral activities (feeding and ruminating) explains why there was no difference in TCT in lambs. These animals have a good adaptation to the changes in the feed management and did not present differences in the NRB and CN. These can be strongly influenced by diet NDF and RTB content, which in this study had no effect on the ingestive behavior of lambs. Figueiredo et al. (2013) evaluated the behavior of lambs fed with different fiber sources and observed that when fed sugarcane silage, the CN was 61.31 n° bolus^{-1}, close to the average presented by lambs, 59.29 n° bolus^{-1}, when fed corn silage.

Table 4. Intake, chews, and feeding and rumination efficiency in lambs fed with WPCS, relocated or not, with or without bacterial inoculant.

Variables[1]	Treatments[2]				Mean	SEM	*p*-value
	CS	R-12h	IR-12h	R-24h			
TFT (h day^{-1})	6.46	5.03	5.89	5.75	5.78	0.54	0.34
TRT (h day^{-1})	9.82	9.50	9.94	9.69	9.74	0.51	0.93
TIT (h day^{-1})	7.72	9.47	8.17	8.56	8.48	0.75	0.42
TCT (h day^{-1})	16.28	14.53	15.83	15.44	15.52	0.75	0.42
NRB (n° day^{-1})	916.35	836.80	945.51	823.37	880.51	49.07	0.25
RTB (sec bolus^{-1})	38.82	41.26	38.07	42.45	40.15	1.80	0.30
CN (n° bolus^{-1})	55.19	61.04	58.72	62.24	59.30	3.05	0.40
CN (n° day^{-1})	50,352.46	51,001.82	55,365.54	51,168.97	51,972.20	3601.12	0.75
DMI (g day^{-1})	512.44	552.73	566.40	581.06	555.40	23.07	0.20
NDFI (g day^{-1})	200.67^{b}	235.66^{ab}	236.58^{a}	225.88^{ab}	224,70	8,91	0.03
DMFE (g DM h^{-1})	83.28	132.39	101.12	102.07	104,72	14,87	0,17
NDFFE (g NDF h^{-1})	32.53	56.45	42.21	39.66	42.71	6.16	0.08
DMRE (g DM h^{-1})	52.63	59.85	57.29	60.23	57,50	3.63	0.43
NDFRE (g NDF h^{-1})	20.60	25.44	23.95	23.42	23.35	1.41	0.13

[1]TFT – total feeding time; TRT – total rumination time; TIT – total idle time; TCT – total chews time; NRB – number of ruminal bolus; RTB – rumination time per bolus; CN – chews number; DMI – dry matter intake; NDFI –neutral detergent fiber intake; DMFE – dry matter feed efficiency; NDFFE – neutral detergent fiber feed efficiency; DMRE – dry matter rumination efficiency; NDFRE – neutral detergent fiber rumination efficiency. [2]CS: whole-plant corn silage (WPCS, not inoculated and not relocated); R–12h: WPCS relocated for 12h; IR–12h: WPCS inoculated with *Lactobacillus plantarum* (1×10^5 cfu g^{-1} per gram of fresh forage) and relocated for 12h; and R–24h: WPCS not inoculated and relocated for 24h. Means on the same row followed by different lowercase letters differed from each other ($p < 0.05$) by Tukey's test.

The DMI and NDFI are important variables that must be monitored in animal nutrition and show a strong relation with the continuous activity of animals and the ability to tolerate variations throughout the day according to the time spent by the animals in these activities. Although there was a higher and/or lower feeding, ruminating, and idling time at specific periods of the day, the DMI was not affected ($p > 0.05$), probably due to the similarity in silage DM content (Table 1), demonstrating the animals' adaptability in relation to feed management, in particular to the silages provided, without compromising their performance.

The unique chemical characteristics of NDF result in this feed fraction having the greatest influence on animal intake, wherein sources with the same NDF content may or may not present positive or negative responses on feed intake. In the present study, the NDF content was higher in the IR-12h silage (Table 1), which explains the higher NDFI by animals.

The behavioral variables related to DMFE, DMRE, NDFFE, and NDFRE can be influenced positively by the chemical composition of diets, mainly because of the DM and NDF content (Oliveira et al., 2015). Nevertheless, the DM content of the silages had similar values and the variation in NDF content did not influence the rumination and feed efficiency of the animals.

Differences could have been expected in the DMFE, DMRE, NDFFE, and NDFRE variables, as these components can increase the average values of these variables (Missio et al., 2010), which was not the case in the present study. Oliveira et al. (2016) observed no effect on DMFE, DMRE, NDFFE, and NDFRE when evaluating the ingestive behavior of lambs fed castor bean meal. These authors also concluded that the DMFE and NDFFE present a direct relation with the nutrient intake concentrations by the animals. Therefore, the absence of a significant effect on DMI may have contributed to the lack of a relationship between dietary efficiency and DM content in the diet, as in the present study, animal intake did not differ among silages and silage DM content had similar values.

When silages are exposed to air, negative changes can occur in the fermentation characteristics and feed chemical composition, reducing the nutritional value. However, no significant changes were observed in the nutritional value of silages that could have influenced the ingestive behavior of the lambs. According to Gerlach, Rob, Weib, Büscher, and Südekum (2013), changes in the feed intake by animals are seen after longer periods of air exposure, with a reduction in the corn silage DM intake by goats from the 4th day of air exposure. However, in corn silages with a more intense fermentation as a result of the use of homofermentative bacterial inoculants, changes in the chemical composition and silage intake can occur with a lower time of exposure to air, making the relocation process a determining factor in the chemical composition and silage fermentation characteristics.

Conclusion

Relocated whole-plant corn silage exposure to air for up to 24h, with or without homofermentative bacterial inoculant, did not alter the ingestive behavior and DM intake by the lambs.

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