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Effect of storage condition and time on the quality of relocated whole-plant corn silages in bags

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ABSTRACT - We aimed to evaluate the effects of storage condition (SC) and storage time (ST) on the fermentative parameters and nutritional value of relocated whole-plant corn silage (WPCS) in plastic bags. A completely randomized design was used in a 2 × 5 factorial arrangement, with two SC (in the field without cover and in a barn as covered space) and five ST (10, 30, 60, 90, and 120 d), with four replications. Corn was harvested (321 ± 10.3 g kg^{-1} dry matter (DM)) and relocated in plastic bags (50 kg) after 210 d of fermentation. The mold count of silage stored in the barn displayed a quadratic adjustment (P = 0.008) and reached its lowest value at 60 d of storage. Yeast count decreased (P = 0.001) linearly as a function of time in the silage stored in the barn. Lactic (P<0.001) and acetic (P<0.001) acid concentrations increased linearly as a function of silage ST in the field. A quadratic relationship was observed between protein concentration (P = 0.039) and *in vitro* dry matter digestibility (IVDMD; P<0.001) and ST for silage in the field, with the lowest value observed at 90 (58.3 g kg^{-1} DM) and 60 (529.3 g kg^{-1} DM) days, respectively. The concentration of ammonia nitrogen increased linearly by 6.80 g kg^{-1} TN (P<0.001) as a function of ST in relocated silage in the field. The IVDMD increased linearly (P<0.001) as a function of time in silage stored in the barn. Silage in the field had 1.00 log CFU g^{-1} more lactic acid bacteria (LAB) than those stored in barns. A quadratic adjustment was observed (P<0.05) as a function of ST on LAB count, aerobic stability, maximum temperature, time to reach maximum temperature, DM, and organic matter. It is recommended that silage be stored in bags in the field for use within 60 d. Silage stored in bags in a barn can be used within 120 d.

Keywords: aerobic deterioration, fermentation parameters, marketing, plastic bag, silage

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1. Introduction

Silage relocation involves crop production, feed-out, transportation, compaction, and sealing in a new silo (Chen and Weinberg, 2014). Silage can be relocated to different types of silos, but plastic bags (30–50 kg) are commonly used for commercialization and transportation.

The plastic films used to seal the silos must meet certain basic criteria, such as maintenance of anaerobiosis, resistance to physical damage caused by weather, and ability to withstand transportation (Bernardes et al., 2018; Borreani et al., 2018). These criteria apply when choosing plastic bags used for storing relocated silage. The bags are manufactured using polyethylene, which is permeable to oxygen (Borreani et al., 2018). Brazil has no suitable materials for manufacturing bags to relocate silage, and recycled materials are often used.

Plastic bags used to store silage in the field are constantly exposed to environmental conditions, such as temperature, radiation, wind, rain, humidity, and birds and rodents, which can cause physical damage. The oxygen permeability of plastic films increases when exposed to radiation, owing to heat absorption and pore dilation (Tabacco and Borreani, 2002), resulting in airflow into the silo. Therefore, paying attention to the storage condition (SC) of silage in plastic bags is essential to prevent silage quality from being compromised.

Furthermore, hot and humid climates can enhance aerobic spoilage due to the rapid growth of yeasts and molds (Bernardes et al., 2018). If the SC of plastic bags affect the preservation of relocated silage, storage time (ST) may intensify this effect. Santos et al. (2023) evaluated the ST after relocating corn silage and observed that the ideal range for silage use was between 30 and 90 d, considering the fermentation parameters and nutritional value. It is worth mentioning that all research involving relocated silage available in the literature has been conducted using plastic drums and anaerobic jars as experimental silos (Santos et al., 2023; Queiroz et al., 2021; Chen and Weinberg, 2014) and do not evaluate the possible effects of ST and ST on the quality of relocated silage packed in plastic bags.

Therefore, it was hypothesized that plastic bag silos produced at the farm level affect the preservation quality of relocated silage when stored under different conditions over the ST, and that SC over the ST affect the nutritional and fermentative characteristics of relocated whole-plant corn silage (WPCS) in plastic bags. The objective of this study was to evaluate the effects of SC and ST on the microbial counts, fermentation parameters, and chemical composition of relocated WPCS in plastic bags.

2. Material and methods

2.1. Silage preparation and experimental design

The silages were prepared on a farm located in Paragominas, Pará, Brazil (03°02'2" S, 47°20'18" W, and 90 m altitude). According to the classification of Köppen and Geiger (1928), the climate of the region is tropical rainy (Awi). The average annual rainfall is 1742.9 mm, with annual average temperature and humidity values of 26.3 °C and 81%, respectively (Alvares et al., 2014).

The experiment was conducted using a completely randomized design in a 2 × 5 factorial arrangement with four replicates. The SC and ST after relocation (10, 30, 60, 90, and 120 d) were evaluated.

Hybrid corn DEKALB 177 was cultivated with a spacing of 50 cm between rows and three plants per linear meter (60,000.00 plants ha^{-1}). The corn was harvested when the grains were in the semi-hard stage (dry matter [DM] = 321.6 ± 10.3 g kg^{-1}; organic matter (OM) = 968.8 g kg^{-1}; crude protein (CP) = 53.0 g kg^{-1}; ether extract (EE) = 17.8 g kg^{-1}; neutral detergent insoluble fiber (NDF) = 411.3 g kg^{-1}; starch = 204.3 g kg^{-1}; and *in vitro* dry matter digestibility (IVDMD) = 509.7 g kg^{-1}), with a self-propelled harvester (FX40; New Holland Agriculture, Italy) adjusted to harvest 45 cm above the soil, with a theoretical particle size between 1 and 2 cm.

Corn was ensiled in a bunker silo (50 m long; width: smaller base 10.44 m and larger base 13.09 m; height: 3.07 m) and mechanically compacted with a tractor. Five bags with 4 kg of forage were placed on the surface, center, and base of the panel at a distance of 10 m from the beginning of the silo to determine the silage dry matter recovery (DMR). After filling, the silo was sealed with a double-faced PVC plastic film. The silo was opened after 210 d, and the bags were removed and weighed after a further 6 d, and the silage contained in them was sampled to determine the silage DMR (Table 1).

Relocation was carried out 15 d after opening the silo, following a process commonly used in practice. The silage feed-out rate was 1.50 m day^{-1} before relocation. Relocation was carried out in the morning and lasted for approximately 2 h. Before relocation, the silo face was exposed to oxygen for 12 h, predominantly at night, as it was used to feed the animals. The relocation was repeated, and four piles were prepared from different portions of the silo panel (surface, center, and base). The silage unloaded from the silo was mixed (total amount of silage = 1700 kg) and the piles were prepared.

Silage (43.31 ± 0.80 kg) was added to each bag. The silages were placed in light green plastic bags (80% polyethylene and 20% recycled material; 200 µm thick). Recompaction was performed manually, and the samples were sealed using plastic tape. At the time of relocation, 400 g of silage was collected from each bag to characterize its chemical composition and organic acids (Table 1). Furthermore, 200 g of silage was collected from each pile to evaluate microbiological counts.

The silages relocated in plastic bags were transported and stored in the forage sector of an institution located in Belém, Pará, Brazil (1°27'07" S, 48°26'13" W, and 11 m altitude). According to the classification of Köppen and Geiger (1928), the climate of the region is humid tropical (Af). The average annual rainfall is 2774.33 mm, with annual average temperature and humidity values of 26.9 °C and 82%, respectively (Alvares et al., 2014).

The bags with the relocated silage were stored inside or outside the barn for opening at different ST. The barn was constructed using masonry, covered with fiber cement tiles, and had no side walls. The bags stored in the field were placed in direct contact with the ground in a vertical position in rows without any protection or cover, simulating the conditions observed on farms selling relocated silage. Upon opening, the visibly deteriorated portion was removed, and the silage was homogenized for sample collection. The ambient temperature was 30.3, 28.3, 26.4, 28.2, and 26.0 °C for each opening time of 10, 30, 60, 90, and 120 d, respectively.

Table 1 - Characterization of whole-plant corn silages before relocation

Chemical composition, nutritional value, and losses (g kg^{-1} dry matter [DM])	Mean
DM (g kg^{-1} fresh matter)	309.1
Organic matter	961.4
Crude protein	59.2
Ether extract	23.5
Neutral detergent fiber	446.4
Starch	236.6
In vitro dry matter digestibility	531.4
Dry matter recovery	952.0
Microbiology (log_{10} CFU g^{-1}) and fermentation parameter (g kg^{-1} DM)	
Yeast	4.0
Mold	5.0
pH	4.0
Lactic acid	106.8
Acetic acid	14.5
Propionic acid	4.6
Butyric acid	42.9
Lactic acid:acetic acid	74.0

2.2. Microbial counts and fermentation parameters

The numbers of lactic acid bacteria (LAB), yeasts, and molds were analyzed in all collected samples. An aqueous extract (1:10) of the sample (25 g) was prepared by adding 0.1% (w/v) sterile peptone water and then homogenized for 3 min in a sterile bag. Microbial counts were determined by pour-

plating 10-fold serial dilutions (five per sample) of the extract onto MRS agar and potato dextrose agar (Sigma-Aldrich). After incubation at 35 °C for 3 d (LAB) and at 26 °C for 3 d (yeasts) and 5 d (molds), the colonies of yeasts and molds were counted separately based on their morphological characteristics. The pH of each sample (a 1:4 water extract [25 g of silage to 100 mL of water] manually homogenized and incubated for 15 min) was determined using a digital potentiometer (T-1000; Tekna, São Bernardo do Campo, Brazil) (Bolsen et al., 1992, adapted from Bernardes et al., 2019).

An aqueous extract was prepared at a ratio of 1:9 (30 g silage to 270 mL distilled water), homogenized for 4 min, and filtered three times to analyze the organic acids (lactic [LA], acetic [AA], propionic [PA], and butyric [BA]). Acids were analyzed using high-performance liquid chromatography (Shimadzu LC-10Ai; Shimadzu Corp., Tokyo, Japan) equipped with a dual detection system consisting of an ultraviolet radiation detector (UV-Vis SPD – 10Ai) and a refractive index detector (RID 10A). A Shimadzu ion exclusion column (Shim-pack SCR-101H; 7.9 mm × 300 mm) operating at 50 °C was used for the chromatographic separation. The analysis of ammonia nitrogen (NH_3-N) was carried out according to method 941.04 (AOAC, 1990) using an aqueous extract prepared at a ratio of 1:10 (25 g of silage to 250 mL of 0.2N sulfuric acid).

2.3. Aerobic stability

Silage (1.5 g kg) was placed in 9-L plastic buckets to determine aerobic stability. The buckets were stored in an acclimatized room at a temperature of 26.5±0. 2 °C for 168 h. The temperature was measured every 4 h using a digital thermometer at two points in the geometric center of the mass, and the average was calculated. The aerobic stability (AS, h), i.e., the time taken for the silage to reach 2 °C above ambient temperature; maximum silage temperature (maxT, °C); time for silage to reach maximum temperature (HmaxT, h); and amplitude (AMP, °C), i.e., the difference between the highest and lowest silage temperatures during the period of aerobic exposure, were determined.

2.4. Chemical composition

The samples were weighed and pre-dried in a forced-air circulation oven (55°C/72 h) and then ground using a knife-type mill (STAR-FT-80/2; Fortinox, Piracicaba, São Paulo, Brazil) with a 1-mm diameter sieve to determine the chemical composition of the silages. The DM (934.01), OM (923.03), and CP (978.04) per storage were conditioned following official methods (AOAC, 1990). The NDF was determined using an autoclave, and thermostable alpha-amylase was used without sodium sulfite (INCT-CA F-002/1). The EE content was determined using a fat extraction device (XT10 extractor; Ankom, Macedon, NY, USA) and Ankom XT4 filter bags. These were washed with a degreaser and neutral detergent before use. The non-fibrous carbohydrates (NFC) in the silages were calculated using the following equation:

$$NFC = 100 - (CP + EE + NDF + ash) \quad (1)$$

Starch concentration was measured according to the method described by Hall and Mertens (2008). The IVDMD was determined using a DAISY apparatus (Ankom Technology Corp., Fairport, NY), after the material was incubated for 30 h at 39 °C (Holden, 1999). Rumen fluid was obtained from two Nellore cows, fistulated in the rumen, and fed corn silage and a protein-energy concentrate.

2.5. Statistical analyzes

Statistical analyses were performed using the statistical program R (R Core Team, 2019) after testing for residual normality and variance homogeneity. The microbial count data were transformed to $\log_{10}$ to obtain a normal distribution of the data. Data were analyzed according to the following model:

$$Yijk = \mu + SCi + STj + (SC \times ST)ij + eijk \quad (2)$$

in which Yijk = dependent variable, μ = mean, SCi = fixed effect of SC, STj = fixed effect of ST after relocation, SC × ST= fixed effect of interaction between SC and ST, and eijk = residue. Tukey's test was

used to evaluate the effect of SC, while regression equations were used to evaluate the effect of ST. The equations were chosen based on the significance of the parameter and the determination coefficient. For all statistical analyses, statistical significance was set at P≤0.05.

3. Results

There was no interaction (P = 0.342) between SC and ST on LAB counts (Table 2). There was an effect of the storage method (P<0.001), in which the LAB population was 1.00 log CFU g^{-1} higher in relocated silage in the field (Table 3) than in those stored in the barn. With quadratic adjustment (P = 0.001), the LAB population had the lowest value at 30 d of storage.

There was an interaction between SC and ST (P = 0.002) on yeast counts (Table 2). Yeast counts were 1.18, 0.80, and 1.57 log CFU g^{-1} at 60, 90, and 120 d of storage, respectively, which were significantly higher (P = 0.047) in relocated silage in the field than in those stored in the barn (Table 3). Yeast count decreased linearly (P<0.001) depending on the ST of the silages in the barn. The yeast population in relocated silage in the field did not change (P = 0.610) with ST (Table 2).

There was an interaction (P = 0.040) between SC and ST on the mold count (Table 2). The mold count was 1.07 log CFU g^{-1} higher in relocated silage in the barn (P = 0.010) than in the field at 10 d of storage; there was no difference (Table 3; P = 0.900) on the other days. The mold count of relocated silage in the barn showed a quadratic relationship (P = 0.008) and was the lowest at 60 d of storage. The mold count of relocated silage in the field did not change (P = 0.692) with ST (Table 2).

There was an interaction (P<0.001) between SC and ST and pH (Table 2). The pH decreased linearly (P<0.001) depending on the storage time of the silage in the barn. There was a quadratic relationship (P<0.002) in the pH of relocated silage in the field as a function of ST, with the lowest value at 90 d of storage.

Interactions were observed between SC and ST and the concentrations of LA (P = 0.017), AA (P<0.001), PA (P<0.001), and BA (P = 0.029) (Table 2). The concentrations of LA, AA, and PA at 90 days of storage were 31.3, 34.4, and 4.0 g kg^{-1} DM, respectively. At 120 days, they were 18.1, 22.0, and 10.9 g kg^{-1} DM. These concentrations were higher (P<0.043) in the silage relocated to the field than in the barn. The BA concentration was 32.0 g kg^{-1} DM higher in relocated silage in the field at 90 d of storage than in that stored in the barn. The LA and AA concentrations increased linearly (P<0.001) as a function of ST in relocated silage in the field. The PA concentration (P<0.001) was quadratically adjusted, with a lower concentration at day 30 in the field. The BA concentration (P = 0.034) was quadratically adjusted, with the highest concentration at 90 d in the field. The LA, PA, and BA concentrations in relocated silage in the barn did not change (P = 0.890) with ST (Table 2). The AA concentration of the silage stored in the barn differed (P = 0.028) depending on ST, but none of the tested models (linear, P = 0.343; Quadratic, P = 0.060) fitted the data.

There was an interaction (P<0.001) between SC and ST and the concentration of NH_3-N (Table 2). At 90 and 120 d of storage, the NH_3-N concentration was 8.80 and 3.80 g kg^{-1} total nitrogen (TN), respectively, and was higher (P = 0.032) in relocated silage in the field than in the barn. (Table 3). The concentration of NH_3-N increased linearly by 6.80 g kg^{-1} TN (P<0.001) as a function of ST in relocated silage in the field. The NH_3-N concentration of the silage stored in the barn did not change (P = 0.062) with ST (Table 2).

There was no interaction (P = 0.646) between SC and ST on AS, maxT, and HmaxT (Table 2). There was an effect of SC (P<0.001) on these variables, in which relocated silage in the barn was 11.6 h more stable and took 12.6 h longer to reach maxT compared with that in the field (Table 4). Relocated silage in the field had a maxT 1.6 °C higher than relocated silage in the barn. With quadratic adjustment of the data (P = 0.003), relocated silage at 60 d showed greater stability (18.5 h) and delayed time (35.0 h) to reach maxT. The maxT of the silages had a quadratic adjustment (P<0.001) depending on ST, with the lowest maxT (42.85 °C) at 90 d. There was an interaction (P = 0.002) between SC and ST on amplitude (Table 2). There was a quadratic relationship (P<0.001) in the range of relocated silage in barns as a function of ST, with a smaller difference between the maximum and ambient temperatures over 90 d. The amplitude of relocated silage in the field decreased linearly (P<0.001) as a function of ST.

Table 2 - P-values related to the measured variables analyzed for the effects of storage condition (SC) and time (ST) and interaction of these factors

Variable	P-value and coefficient of determination (R^2)																
	Variance analysis			ST related to SC										Isolated effect of ST			
	SC	ST	SC × ST	Barn × time	L	R^2	Q	R^2	Field × time	L	R^2	Q	R^2	L	R^2	Q	R^2
LAB	<0.001	0.004	0.342	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.029	0.277	0.001	0.968
Yeast	0.001	0.003	0.002	<0.001	<0.001	0.725	0.015	0.897	0.610	NS	NS	NS	NS	NS	NS	NS	NS
Mold	0.960	0.340	0.040	0.017	0.031	0.359	0.008	0.924	0.692	NS	NS	NS	NS	NS	NS	NS	NS
pH	0.094	<0.001	<0.001	<0.001	<0.001	0.883	0.076	0.925	<0.001	<0.001	0.536	0.002	0.632	NS	NS	NS	NS
LA	0.032	<0.001	0.017	0.321	NS	NS	NS	NS	<0.001	<0.001	0.663	0.232	0.702	NS	NS	NS	NS
AA	<0.001	<0.001	<0.001	0.028	0.343	0.073	0.060	0.375	<0.001	<0.001	0.713	0.152	0.718	NS	NS	NS	NS
PA	<0.001	<0.001	<0.001	0.890	NS	NS	NS	NS	<0.001	<0.001	0.702	<0.001	1.000	NS	NS	NS	NS
BA	<0.002	0.010	0.029	0.722	NS	NS	NS	NS	<0.001	0.627	0.009	0.034	0.195	NS	NS	NS	NS
NH_3-N	0.009	0.046	<0.001	0.062	NS	NS	NS	NS	<0.001	<0.001	0.764	0.538	0.775	NS	NS	NS	NS
AS	<0.001	0.002	0.646	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.792	0.003	<0.001	0.665
MaxT	<0.001	<0.001	0.167	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.001	0.605	<0.001	0.928
HmaxT	<0.001	0.015	0.158	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.312	0.072	0.003	0.760
AMP	0.989	<0.001	0.002	<0.001	0.001	0.370	<0.001	0.956	<0.001	<0.001	0.569	0.224	0.603	NS	NS	NS	NS
DM	<0.001	0.026	0.692	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.005	0.734	0.967	0.734
OM	0.209	<0.001	0.456	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.001	0.361	<0.001	0.797
CP	0.002	0.689	<0.001	0.017	0.112	0.189	0.120	0.369	<0.001	0.001	0.425	0.039	0.581	NS	NS	NS	NS
NDF	0.089	0.741	0.111	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
EE	0.058	0.123	0.369	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NFC	0.269	0.478	0.086	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Starch	<0.001	0.002	0.150	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.001	0.713	0.017	0.845
IVDMD	<0.001	0.013	<0.001	<0.001	<0.001	0.660	0.449	0.676	<0.001	0.005	0.324	0.001	0.791	NS	NS	NS	NS

LAB - lactic acid bacteria (log_{10} CFU g^{-1}); LA - lactic acid (g kg^{-1} DM); AA - acetic acid (g kg^{-1} DM); PA - propionic acid (g kg^{-1} DM); BA - butyric acid (g kg^{-1} DM); NH_3-N - ammonia nitrogen (g kg^{-1} total nitrogen); AS - aerobic stability (h); MaxT - maximum temperature (°C); HmaxT - time to reach the maximum temperature (h); AMP - amplitude (°C); DM - dry matter (g kg^{-1} FM); OM - organic matter (g kg^{-1} DM); CP - crude protein (g kg^{-1} DM); NDF - neutral detergent insoluble fiber (g kg^{-1} DM); EE - ether extract (g kg^{-1} DM); NFC - non-fibrous carbohydrates (g kg^{-1} DM); IVDMD - *in vitro* dry matter digestibility (g kg^{-1} DM); L - linear effect; Q - quadratic effect; NS - non-significant effects.

Table 3 - Microbiological count and fermentative parameters of relocated whole-plant corn silages in plastic bags stored in different conditions (SC: field and barn) and storage times

SC	Storage time (d)					Mean	CV%	Equation
	10	30	60	90	120			
			LAB (log CFU g^{-1})				8.07	
Barn	6.60	5.75	5.58	6.25	7.00	6.24b		
Field	7.20	6.98	7.20	7.08	7.75	7.24a		
Mean	6.90	6.36	6.39	6.66	7.38			$Y = 0.0003x^2 - 0.03x + 7.09$
			Yeast (log CFU g^{-1})				11.04	
Barn	6.08	4.65	4.30b	4.18b	3.93b	4.63		$Y = -0.02x + 5.64$
Field	5.30	5.13	5.48a	4.98a	5.50a	5.28		NS
Mean	5.69	4.89	4.89	4.58	4.71			
			Mold (log CFU g^{-1})				9.68	
Barn	6.55a	5.98	5.15	5.50	5.73	5.78		$Y = 0.0003x^2 - 0.04x + 6.97$
Field	5.48b	5.93	5.95	5.63	5.88	5.77		NS
Mean	6.01	5.95	5.55	5.56	5.80			
			pH				1.20	
Barn	3.90	3.90	3.80	3.80a	3.65b	3.81		$Y = -0.002x + 3.94$
Field	3.88	3.93	3.76	3.60b	3.75a	3.79		$Y = 0.00003x^2 - 0.006 + 3.99$
Mean	3.89	3.91	3.79	3.70	3.70			
			Lactic acid (g kg^{-1} DM)				11.11	
Barn	95.5	102.8	113.8	108.1b	105.8b	105.2		NS
Field	100.0	92.5	113.4	139.4a	123.9a	113.8		$Y = 0.03x + 9.25$
Mean	97.7	97.7	113.6	123.7	114.9			
			Acetic acid (g kg^{-1} DM)				7.21	
Barn	37.4	30.9	34.5	31.8b	34.3b	33.8		No adjusted model
Field	33.5	34.6	38.6	66.2a	56.3a	45.8		$Y = 0.03x + 2.86$
Mean	35.5	32.8	36.5	49.0	45.3			
			Propionic acid (g kg^{-1} DM)				42.02	
Barn	2.5	2.5	2.0	2.8b	3.3b	2.6		NS
Field	4.1	2.6	3.2	6.8a	14.2a	6.2		$Y = 0.0002x^2 - 0.02x + 0.53$
Mean	3.3	2.5	2.6	4.8	8.8			
			Butyric acid (g kg^{-1} DM)				49.12	
Barn	15.4	14.8	12.6	12.5b	7.1	12.5		NS
Field	23.1	19.5	20.5	44.5a	14.1	24.3		$Y = -0.0004x^2 - 0.05x + 1.32$
Mean	19.3	17.2	16.5	28.5	10.6			
			NH_3-N (g kg^{-1} TN)				9.45	
Barn	26.3	24.5	22.8	21.5b	25.0b	24.0		NS
Field	23.5	22.0	26.0	30.3a	28.8a	26.1		$Y = 0.007x + 2.19$
Mean	24.9	23.3	24.4	25.9	26.9			

CV% - coefficient of variation; LAB - lactic acid bacteria (log_{10} CFU g^{-1}); NS - non-significant effect; NH_3-N - ammonia nitrogen (%TN); TN - total nitrogen.
Means followed by different letters in the column differ (P<0.05) by Tukey's test.

There was no interaction (P = 0.692) between SC and ST for the concentrations of DM, OM, NDF, EE, NFC, or starch in the silages (Table 2). There was an effect of SC (P<0.001) on DM concentration; the relocated silage in the barn had 24.9 g kg^{-1} fresh matter more DM than those stored in the field. There was an effect of ST (P = 0.02) on DM concentration of the silages, which decreased linearly over time (Table 5). There was a quadratic adjustment (P<0.001) in OM concentration of the relocated silages as a function of ST, which decreased until day 30. There was an effect of SC (P<0.001) on starch concentration; silage stored in the field had 44.1 g kg^{-1} DM more starch than relocated silage in a barn. The starch concentration in the silages increased linearly (P<0.001) with ST.

Table 4 - Aerobic stability of relocated whole-plant corn silages in plastic bags stored in different conditions (SC; field and barn) and storage times

SC	Storage time (d)					Mean	CV%	Equation
	10	30	60	90	120			
	Aerobic stability (h)						38.92	
Barn	15.0	16.0	24.0	24.0	16.0	19.0a		
Field	8.0	4.00	13.0	10.0	2.0	7.4b		
Mean	11.5	10.0	18.5	17.0	9.0			$Y = -0.0025x^2 + 0.33x + 6.28$
	Maximum temperature (°C)						2.11	
Barn	45.15	44.33	42.23	41.83	43.50	43.4b		
Field	46.08	45.58	45.13	43.88	44.35	45.0a		
Mean	45.61	44.95	43.68	42.85	43.93			$Y = 0.0004x^2 - 0.0770x + 46.55$
	Time to reach the maximum temperature (h)						21.91	
Barn	27.0	33.0	42.0	41.0	35.0	35.6a		
Field	25.0	19.0	28.0	25.0	18.0	23.0b		
Mean	26.0	26.0	35.0	33.0	26.5			$Y = -0.0026x^2 + 0.36x + 20.84$
	Amplitude (°C)						6.78	
Barn	20.20	17.03	15.95b	15.78	17.12a	17.22		$Y = 0.001x^2 - 0.15x + 21.30$
Field	19.63	16.40	18.48a	17.05	14.55b	17.22		$Y = -0.03x + 19.27$
Mean	19.91	16.71	17.21	16.41	15.84			

CV% - coefficient of variation.
Means followed by different letters in the column differ (P<0.05) by Tukey's test.

There was an interaction (P<0.001) between SC and ST and the concentration of CP and IVDMD in the silages (Table 2). There was a quadratic relationship (P = 0.039) in the CP concentration of relocated silage in the field as a function of ST, which decreased until day 90 (Table 5). The CP concentration of relocated silage in the barn changed (P = 0.017) depending on ST, but none of the tested models (linear, P = 0.112; Quadratic, P = 0.120) fitted the data.

Relocated silage in the field was 52.0 g kg^{-1} DM more digestible than silages in the barn. The IVDMD increased linearly (P<0.001) with ST of relocated silage in the barn. A quadratic adjustment was observed for the IVDMD (P<0.001) of silage stored in the field as a function of ST. Relocated silages for 60 d were less digestible than those stored for other periods in the field (Table 5).

4. Discussion

The higher LAB count in relocated silage in the field may be associated with higher temperature that accelerates the growth of these microorganisms, increasing their population (Bernardes et al., 2018). According to Borreani et al. (2018), optimal temperatures for LAB occur between 27 and 38 °C, increasing their growth. Also, this probably explains the higher LA and AA concentrations in these silages compared with relocated silages stored in a barn with 90 and 120 d of storage, due to their higher LAB count. Associated with the higher LA concentration, the lower pH values may be explained by LA ability to reduce the pH more efficiently compared with other acids, acidifying the environment during silage fermentation (Pahlow et al., 2003). Furthermore, the increase in pH of relocated silages after 120 d of storage in the field is possibly related to their lower LA concentration.

Yeasts are the primary initiators of the deterioration process in silages (Kung et al., 2018), which can grow at pH < 4.0, and high temperatures can increase the population (McDonald et al., 1991). The Belém region is characterized by high temperatures throughout the day and year, with a minimum average of 26.3 °C and a maximum average of 31.7 °C (Alvares et al., 2014). This may have contributed to the greater proliferation of yeasts in silages stored in the field throughout the storage time. Koc et al. (2009) observed that the highest yeast counts occur in silages exposed to temperatures between 30 and 37 °C, and Bernardes et al. (2018) reported that hot environments improve yeast growth and accelerate

Table 5 - Chemical composition of relocated whole-plant corn silages in plastic bags stored in different conditions (SC; field and barn) and storage times

SC	Storage time (d)					Mean	CV%	Equation
	10	30	60	90	120			
	Dry matter (g kg^{-1} FM)						4.52	
Barn	319.8	323.8	314.8	318.3	306.8	316.7a		
Field	306.8	298.8	282.3	293.0	278.3	291.8b		
Mean	313.3	311.3	298.5	305.6	292.5			Y = −0.017x + 31.46
	Organic matter (g kg^{-1} DM)						0.27	
Barn	963.8	955.8	961.0	962.5	967.0	962.0		
Field	961.8	957.8	958.5	961.3	965.5	961.0		
Mean	962.8	956.8	959.8	961.9	966.3			Y = 0.0002x^2 – 0.017x + 96.29
	Crude protein (g kg^{-1} DM)						3.15	
Barn	58.8b	56.8b	60.3	61.5a	58.8	59.2		No adjusted model
Field	62.8a	64.8a	59.5	58.3b	60.8	61.2		Y = 0.0001x^2 – 0.01x + 6.55
Mean	60.8	60.8	59.9	59.9	59.8			
	Neutral detergent insoluble fiber (g kg^{-1} DM)						5.14	
Barn	443.3	482.0	445.3	446.8	438.0	451.1		
Field	466.0	454.0	466.8	462.3	471.8	464.2		
Mean	454.6	468.0	456.0	454.5	454.9			
	Ether extract (g kg^{-1} DM)						22.89	
Barn	18.3	22.8	27.0	24.3	20.5	22.6		
Field	21.0	18.3	20.5	22.3	15.8	19.6		
Mean	19.6	20.5	23.8	23.3	18.1			
	Non-fibrous carbohydrates (g kg^{-1} DM)						6.35	
Barn	443.3	388.0	429.3	430.0	444.5	427.0		
Field	412.3	427.5	411.5	418.8	417.3	417.5		
Mean	427.8	407.8	420.4	424.4	430.9			
	Starch (g kg^{-1} DM)						10.30	
Barn	188.7	171.7	197.6	198.1	213.6	193.9b		
Field	204.1	220.1	262.9	259.6	243.3	238.0a		
Mean	196.4	195.9	230.3	228.8	228.4			Y = 0.03x + 19.46
	IVDMD (g kg^{-1} DM)						2.40	
Barn	477.0b	464.0b	503.5b	498.0b	506.5b	489.8		Y = 0.03x + 46.89
Field	568.8a	539.8a	524.3a	529.3a	537.0a	541.8		Y = 0.0008x^2 – 0.13x + 57.59
Mean	522.8	501.9	513.9	518.6	521.8			

CV% - coefficient of variation; IVDMD - *in vitro* dry matter digestibility.
Means followed by different letters in the column differ (P<0.05) by Tukey's test.

silage spoilage. Furthermore, as the material used to manufacture plastic bags is relatively permeable to oxygen, the relocated silages in the field were possibly directly influenced by environmental conditions, where the gas exchange can be more intense in these silages, creating an aerobic environment inside the bags to yeast growth.

The mold count in all relocated silages (stored in the field or barn and opened at any storage time) may indicate silage spoilage. However, the relocated silage did not have visible characteristics of deteriorated silage. In the literature, molds are related to silage quality and there is a variation about mold count in silages: 4 log CFU g^{-1} (Alonso et al., 2013; Ávila et al., 2020), 5 log CFU g^{-1} (Tapia et al., 2005), 6 log CFU g^{-1} (Kung et al., 2018). In the relocation process of silages, contact with aerobic conditions is inevitable, allowing the growth of aerobic microorganisms, possibly explaining the presence of mold in the silages. The higher mold population in relocated silages stored in the barn after 10 d may be related to a possible increase in silage temperature due to the higher accumulation of heat inside the silo, since the barn is an enclosed space, reducing the heat exchange with the external environment. In addition, residual oxygen may have contributed to accelerating mold growth, leading to stabilization of the

mold population, while the available oxygen is reduced. The gas exchange between the environmental condition and the silages in the barn was possibly lower, interrupting the mold and yeast growth in these silages.

Clostridial fermentation may have occurred in relocated silage in the field, which can be confirmed by BA concentration. According to Kung et al. (2018), bacteria of the genus *Clostridium* grow at pH > 4.5 and DM concentrations < 30%, which can be observed in the silages stored in the field evaluated in the present study. The humid environment of these silages (DM < 30%) probably contributed to the clostridia growth, even in an acidic environment, which increased the BA concentration at 90 d of storage. According to Whittenbury et al. (1967), bacteria of the genus *Clostridium* are osmotic pressure-sensitive and high acid concentration-tolerant in a humid environment, and the inhibition of clostridia growth occurs in an inversely way between pH and humidity. According to that, pH values below 4.5 may not be enough to inhibit the clostridia growth in humid environments. High PA concentration (0.3–0.5%) and NH_3-N are also frequently related to clostridial fermentations, a result of *Clostridium* proliferation (Kung et al., 2018). In the present study, the higher PA and NH_3-N concentrations can indicate clostridial fermentation in relocated silages stored in the field after 90 d (Kung et al., 2018).

When silage is exposed to air, the aerobic stability depends mainly on the aerobic microorganisms population and the organic acids concentration (Chen and Weinberg, 2014). In this study, the instability of relocated silage in the field is due to the fact that they already have a higher population of yeast and lactic acid. Therefore, when exposed to an aerobic environment, the development of yeasts is accentuated, causing the temperature peak to be higher and reach it in less time, as these microorganisms intensify heat production. The increase in AS and HmaxT and reduction in maxT with ST could be explained by the increase in acetic acid and consequent reduction in yeast. Acetic and propionic acids are known for their antifungal effects, preventing yeast growth in silages when exposed to the aerobic environment (Moon, 1983).

The lower DM content of the relocated silage in the field may have occurred due to the infiltration of rainwater and humidity inside silage bags. It is worth mentioning that the region where the experiment was carried out is characterized by high humidity (average of 81%) and well distributed rainfall throughout the year (Alvares et al., 2014), which may have contributed to increase the moisture concentration of the relocated silage in the field. The lower CP concentration of the relocated silage in the field at 90 d may be a result of the higher clostridial fermentation in these silages. At 90 d, the NH_3-N concentration was also higher in relocated silage in the field. According to Kung et al. (2018), some bacteria of the genus *Clostridium* are capable of degrading proteins, producing ammonia, increasing the NH_3-N, and reducing the CP concentrations in silages.

The reduction of CP concentration in relocated silage in the field up to 90 d may have contributed to the proportional increase in starch in these silages. Corn grain starch is covered with a protein-matrix composed mainly of hydrophobic protein zein. According to Junges et al. (2017), bacterial activity (60%) is the main contributor to proteolysis in corn silages, and the main proteolytic bacteria in silages are *Clostridium*, not LAB (McDonald et al., 1991). In the present study, clostridial fermentation, as well as the higher LAB population in relocated silage in the field, may have contributed to the protein-matrix degradation, indicated by the increase in NH_3-N concentration, increasing starch availability and, consequently, the digestibility of these silages. The reduction in DM concentration during ST may be related to the higher effluent losses in these silages. Effluents are composed of proteins and sugars (McDonald et al., 1991), and the leaching of these constituents results in a reduction in the DM concentration of the silages.

The reduction in IVDMD up to 60 d in relocated silage in the field was due to the lower DM content of these silages. Furthermore, the chemical composition of the relocated silage was similar to that before relocation. Therefore, evaluating the acceptability of relocated silages by animals through preference assay and quantifying losses due to deterioration are crucial to reach more accurate conclusions regarding the storage location of the silos. The use of bagged silage in small units of 30 to 50 kg is

common in Brazil, heating up the silage market. However, the plastic film industry still does not offer bags with low permeability, with materials such as polyethylene, which allow air infiltration during storage. This leads to higher silage spoilage when in contact with the plastic film. The situation is worse in tropical regions, where high temperatures increase the permeability of the films, intensifying gas exchange and aerobic deterioration.

5. Conclusions

The characteristics of relocated silages were more affected by storage condition than storage time. Relocated silages in barn provide silages with a high aerobic stability, but a lower IVDMD. It is recommended that relocated silage in the field be used within 60 d. Relocated silage in a barn can be used within 120 d.

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Conflict of interest

The authors declare no conflict of interest.

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