R. Bras. Zootec., 52:e20220059, 2023
https://doi.org/10.37496/rbz5220220059

Forage crops
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

How do relocation time and length of storage after relocation affect fermentation and nutritive value of corn silage?

Rosana Ingrid Ribeiro dos Santos[1], Rita de Cássia Almeida de Mendonça[1], Amanda Caroliny Marques de Queiroz[1], Thiago Fernandes Bernardes[2], Felipe Nogueira Domingues[3], Cristian Faturi[1], Thiago Carvalho da Silva[1], Aníbal Coutinho do Rêgo[4]

[1] Universidade Federal Rural da Amazônia, Instituto da Saúde e Produção Animal, Belém, PA, Brasil.
[2] Universidade Federal de Lavras, Departamento de Zootecnia, Lavras, MG, Brasil.
[3] Universidade Federal dos Vales do Jequitinhonha e Mucuri, Unaí, MG, Brasil.
[4] Universidade Federal do Ceará, Departamento de Zootecnia, Fortaleza, CE, Brasil.

***Corresponding author:**
anibalcr@ufc.br

Received: April 11, 2022
Accepted: November 16, 2022

How to cite: Santos, R. I. R.; Mendonça, R. C. A.; Queiroz, A. C. M.; Bernardes, T. F.; Domingues, F. N.; Faturi, C.; Silva, T. C. and Rêgo, A. C. 2023. How do relocation time and length of storage after relocation affect fermentation and nutritive value of corn silage? Revista Brasileira de Zootecnia 52:e20220059. https://doi.org/10.37496/rbz5220220059

ABSTRACT - This study aimed to determine the effects of relocation time (RT; Experiment 1) and storage time after relocation (ST; Experiment 2) on microbial population, fermentative characteristics, and chemical composition of corn silage. In experiment 1, corn silage was stored for 30 d, subjected to different RT (0–60 h), and stored again for 30 d. Thirty experimental silos were used in a completely randomized design, with three replicates per treatment. In experiment 2, after 150 d of ensiling, silage was removed from a bunker silo, exposed to air for 9 h, relocated to experimental silos, and stored for periods ranging from 0 to 128 d. Twenty-eight experimental silos were used in a completely randomized design, with four replicates per treatment. Relocation time had no effect on fungi counts and concentrations of lactic and propionic acids in corn silage but resulted in a significant increase in dry matter content. In experiment 2, dry matter recovery and concentration of non-fiber carbohydrates decreased in corn silage stored for more than 32 d after relocation. Exposure of corn silage to air during relocation for up to 60 h followed by 30 d of storage did not compromise the fermentation profile or nutritive value of the silage. Increased storage time of relocated corn silage (up to 128 d) consistently decreases its nutritional value. The storage period seems to have an increased impact on nutrient loss in relocated silage than the relocation period.

Keywords: aerobic exposure, fermentation, organic acids, silage transportation

1. Introduction

Silage relocation is a common practice among farms across the world. It involves moving the silage to a new silo for different purposes. In tropical regions, farmers often face low forage availability because of incorrect feed planning or field crop losses caused by climatic occurrences, pests, diseases, and other factors, forcing them to buy silage from other farms (Michel et al., 2017). Selling silage is a common practice in some regions of Brazil because of the climatic variation, low availability of area, and adequate machinery and labor for the ensiling process (dos Anjos et al., 2018).

Silage relocation involves unloading from the original silo, transportation, re-compaction, and sealing in the new silo (Chen and Weinberg, 2014; Queiroz et al., 2021). These steps can take hours or days to complete, which exposes the silage to air during the process (Chen and Weinberg, 2014). This delay can result in a reduction in the nutritional value of the silage due to the oxidation of residual soluble sugars and degradation of lactic acid produced during fermentation, increasing the proportion of cellular content. Corn is the most common crop used for silage production in dairy farms in Brazil (Bernardes and do Rêgo, 2014) and is more prone to aerobic deterioration than other types of silage, especially in tropical conditions (Bernardes et al., 2009; Kung Jr. et al., 2018). However, research has shown that relocation lasting up to 48 h has no effect on fermentation, chemical composition, and dry matter recovery (DMR) of corn silage (Lima et al., 2016).

More studies are required to understand the changes in silage caused by prolonged exposure to air that can occur in different field situations, such as long-distance transportation, excessive rain, broken machinery, and labor unavailability. In addition, changes in the relocated silage over time can strongly affect silage quality, but this topic has not yet been studied.

We hypothesized that relocation and storage times could increase nutrient loss and modify the chemical composition of relocated corn silage. Therefore, we aimed to evaluate the effects of relocation time (RT; Experiment 1) and storage time after relocation (ST; Experiment 2) on the fermentation and nutritive value of the relocated corn silage.

2. Material and Methods

2.1. Experimental design and procedures (experiment 1)

Corn used as silage in this experiment was cultivated in a region located at 3°2'2" S latitude and 47°20'18" W longitude in the city of Paragominas, PA, Brazil. The climate of this region, according to Köppen classification, is tropical dry winter (Aw), characterized by a rainy tropical climate with an extensive dry season. This region has an annual average maximum and minimum temperature of 33 and 22 °C, respectively, and average relative humidity of 81%. The average total annual rainfall is less than 1.743 mm, with the rainy season recorded from January to May (Bastos et al., 2006).

The corn hybrid (PIONEER 30F90H®) was planted in January 2016 with a spacing of 60 cm between rows and harvested at 324 g/kg of dry matter (DM) using a self-propelled harvester (FX40, New Holland Agriculture, Italy).

A completely randomized design with ten treatments and three replicates, totaling 30 experimental units, was used. The treatments were: non-relocated (NR), and relocation times (RT) of 0 (silage was removed from the silo, homogenized, and immediately placed back in the silo), 6, 12, 18, 24, 30, 36, 48, and 60 h. After relocation, silos were stored in a covered barn with an ambient temperature of 28.1±0.167 °C for another 30 d. The total storage time (ST; ST after ensiling + ST after relocation) was 60 d.

Thirty-five experimental silos (plastic buckets with a capacity of 15 L each) were filled with 9.11±0.0681 kg of fresh forage to reach a density of 600 kg/m^3 on a fresh weight basis. After filling, the silos were weighed and stored in a covered barn at ambient temperature. After 30 d of storage, the silage destined for relocation was removed from the experimental silos, mixed, and exposed as piles. After exposure, each pile was mixed again and returned to the original silo. Five extra buckets of silage were exposed to air to top up the buckets after the relocation process in case of excessive shrinkage or losses due to dehydration. During aerobic exposure of silages, the temperature of the piles was measured at two equidistant points with a thermometer inserted into the silage mass during relocation. The initial temperature (IT) was recorded at the beginning of the relocation process, and final temperature (FT) was recorded before silage was returned to silos.

2.2. Experimental design and procedures (experiment 2)

The corn used in this experiment was cultivated in the city of Igarapé Açu, PA, Brazil, located at 1°7'44"S latitude and 47°37'12" W longitude. The climate of this region, according to Köppen classification, is also tropical dry winter (Aw), characterized by a rainy tropical climate with an extensive dry season. The annual average temperature and total rainfall are 27.3 °C and 2.270 mm, respectively. The corn variety (AL Bandeirante) was planted at a spacing of 60 cm between rows, with a total plant density of 55 plants/ha. Corn was harvested at 350 g/kg DM using a tractor (3500, New Holland, Curitiba, Brazil) and an automatic harvester (JF C120, JF Máquinas, São Paulo, Brazil).

A completely randomized design with seven treatments and four replicates, totaling 28 experimental units, was used. Treatments were: NR silage, collected from the silo panel before relocation, and different ST after the relocation of 4, 8, 16, 32, 64, and 128 d. Silos were stored in a covered barn at ambient temperature, and total ST (ST after ensiling + ST after relocation) was 154, 158, 166, 182, 214, and 278 d for each respective ST.

Corn silage was prepared in a bunker silo that was opened after 150 d. At the time of first opening, any visibly spoiled silage at the top of the bunker was discarded, and a 20 cm layer was removed from the silo panel. Subsequently, the silo panel and top were exposed for 9 h to simulate a typical relocation process. Subsequently, the exposed silage was relocated to 24 experimental silos, and plastic buckets with a capacity of 15 L each were filled with 8.87±0.0542 kg of fresh forage to reach a density of 600 kg/m^3 on a fresh weight basis.

2.3. Experimental analysis

In both experiments, fresh silage (1.5 kg) was collected from each silo in the second opening of the experimental silos and transferred to a 9 L cleaned plastic bucket. In experiment 1, silages were exposed to air for 12 d in a room with a controlled temperature of 22.1±0.0281 °C; silage temperature was recorded every 30 min with a thermometer (TESTO, Campinas, Brazil) inserted into the geometric center of the silage mass. In experiment 2, silages were exposed to air for 7 d in a room with a controlled temperature of 25.8±0.0780 °C; silage temperature was recorded every 30 min using a *data logger* thermometer (Klimalogg, Incoterm, São Paulo, Brazil). In both experiments, the ambient temperature was recorded. Aerobic stability (AS) was defined as the time (hours) the silage temperature remained stable before increasing by more than 2 °C above the ambient temperature (O'Kiely, 1993). The following variables were determined: time to reach maximum temperature (HMaxT) in h, maximum (MaxT) and minimum (MinT) temperature in °C, and amplitude in °C, which was the difference between maximum and minimum temperatures.

2.4. Microbial populations and fermentation profiles

After the final opening of silos and relocation, samples from both experiments were collected to analyze microbial counts (molds and yeasts) and fermentation profile. The aqueous extract (1:10) of the sample was prepared by adding peptone water (1 g per L of water) and a Stomacher homogenizer (MA 440/CF; Marconi, Piracicaba, São Paulo, Brazil) for 4 min in a sterile bag. The counts of molds and yeasts were determined by pour-plating 10-fold serial dilutions of the extract on potato dextrose agar (Sigma-Aldrich Brasil LTDA). After incubation at 26 °C for 5 d, the colonies that grew were counted separately for yeasts and molds, based on their morphological characteristics.

The pH was measured after each RT and 30 d after relocation when the silos were opened (Experiment 1), and after each ST (Experiment 2). The pH was also measured after 12 d (pH-12d, Experiment 1) or after 7 d (pH-7d, Experiment 2) of aerobic exposure. Approximately 25 g of fresh silage was mixed with 100 mL of distilled water (Bolsen et al., 1992), and the pH was measured with an electrode after 30 min (Tekna model T-1000). Dry matter recovery was estimated according to the equation described by Zanine et al. (2010).

To determine the concentration of organic acids (lactic, acetic, propionic, and butyric acids), we prepared an aqueous extract with 25 g of silage and 225 mL of deionized water and homogenized it in a Stomacher for 4 min (MA 440/CF; Marconi, Piracicaba, São Paulo, Brazil). The extract was then filtered, and only the liquid fraction was obtained, from which 2 mL of the sample was removed. Lactic acid was quantified by the colorimetric method according to Pryce (1969), and acetic, propionic, and butyric acids were determined using a gas chromatograph coupled to a mass spectrometer (GCMS QP 2010 plus; Shimadzu, Kyoto, Japan) and separated using a capillary column (Stabilwax; Restek, Bellefonte, PA, USA; 60 m, 0.25 mm, internal diameter 0.25 m).

2.5. Chemical composition

In both experiments, the chemical composition of silages was analyzed before and after relocation. To determine the chemical composition, we collected 200 g of silage from openings 1 and 2 of the silos and froze them at −20 °C for further analysis. Samples were dried in a forced-air oven at 55 °C for 72 h and subsequently processed in a mill with a 1 mm sieve (Thomas Wiley Mill Model 4; Thomas Scientific, Swedesboro, NJ, USA).

Concentrations of DM (at 105 °C, method 934.01), organic matter (OM; at 600 °C/4 h, method 923.03), crude protein (CP; method 978.04), and neutral detergent fiber (NDF; method 2002.04) were determined according to AOAC (1990). Ether extract (EE) content was determined using a fat extractor (model XT10, Ankom®). Values of NDF content were corrected for ash (Mertens, 2002) and protein content (Licitra et al., 1996). The NFC concentration was calculated according to the method described by Detmann and Valadares Filho (2010). The *in vitro* dry matter digestibility (IVDMD) was determined using the two-stage fermentation technique described by Tilley and Terry (1963). Rumen fluid was obtained from two ruminally fistulated dry Nellore cows fed whole-crop corn silage. The ammonia nitrogen (NH_3-N) content was analyzed according to the AOAC (1990), method 920.03. The concentration of water-soluble carbohydrates (WSC) was determined as described by DuBois et al. (1956).

2.6. Statistical analyses

The pH and temperature data during relocation were subjected to descriptive analysis. Microbial count data were $\log_{10}$-transformed for analysis. Data from the two experiments were analyzed separately as completely randomized designs using the PROC GLM procedure in SAS (Statistical Analysis System, version 9.2). Data were subjected to analysis of variance (ANOVA) according to the following statistical model:

$$y_{ij} = \mu + T_i + e_{ij}, \quad (1)$$

in which y_{ij} is the observed value j of a dependent variable after an RT of i, μ is the overall mean of the dependent variable, T_i is the fixed effect of treatment (RT for Experiment 1 and ST for Experiment 2), and e_{ij} is the random error associated with each observation.

Differences between control and treatments were evaluated using Dunnett's test ($P<0.05$). Orthogonal contrast analysis was used to determine whether RT and ST had linear or quadratic effects on the measured parameters. Differences were considered statistically significant at $P<0.05$.

3. Results

3.1. Experiment 1

The DM concentration of fresh corn before ensiling was 325 g/kg. Corn contained 75.5 g/kg of CP, 18.6 g/kg of EE, 523 g/kg of NDF, and 343 g/kg of NFC (Table 1). The DM concentrations ranged between 295 and 320 g/kg. The pH after RT treatments ranged from 3.44 to 3.75. Silages with higher RT showed higher FT and MaxT values. Values of MinT showed no significant variation among silage piles.

An overall significant effect of RT (P>0.05) was not observed on the counts of molds or yeasts, the concentration of lactic and propionic acids, and the aerobic stability, amplitude, HmaxT, MaxT, and pH-12d values of the silage (Table 2). Concentration of acetic acid in silage with an RT of 12 h was lower than that in control. Silage pH after 60 h of RT was also higher than that of the control. The pH increased linearly as a function of RT. Aerobic stability had a quadratic relationship with RT. In contrast, increased RT resulted in a linear reduction in HMaxT. The DMR at RT of 12, 48, and 60 h was higher than that in the NR silage (P<0.01).

Table 1 - Chemical composition (g/kg of dry matter) of corn silage before relocation and temperature (°C) and pH of the silage piles after relocation time (RT; Experiment 1)

Item	Chemical composition (g/kg of DM)										
	RT (h)										
	FC	0	6	12	18	24	30	36	48	60	SEM
DM	325	310	305	315	320	315	314	295	307	310	2.22
OM	960	964	960	965	961	961	962	961	961	959	0.846
EE	18.6	21.5	29.4	25.3	29.4	25.9	31.3	28.8	32.3	34.1	1.58
CP	75.5	68.8	71.4	71.1	68.2	73.0	70.1	71.1	70.6	69.7	0.506
NDF	523	485	495	488	473	438	417	499	473	499	9.91
NFC	343	494	463	480	391	424	445	362	385	356	10.7
WSC	ND	30.8	30.0	26.5	18.0	20.7	15.7	18.4	11.6	15.1	1.40
Item		Temperature and pH after air exposure after RT									
		0	6	12	18	24	30	36	48	60	SEM
pH	ND	3.75	3.69	3.74	3.74	3.44	3.48	3.55	3.62	3.69	0.0392
IT	ND	26.1	27.1	27.0	27.0	27.2	27.0	27.0	27.2	27.5	0.126
FT	ND	26.1	27.3	28.0	28.0	29.5	28.7	32.4	36.4	35.0	1.46
MaxT	ND	26.1	27.3	28.1	28.5	31.1	31.4	34.0	41.5	39.6	2.06
MinT	ND	26.1	27.0	26.7	26.7	27.0	27.0	26.9	27.0	27.1	0.102

FC - fresh-chopped corn plant; ND - not determined; DM - dry matter; OM - organic matter; EE - ether extract; CP - crude protein; NDF - neutral detergent insoluble fiber; WSC - water-soluble carbohydrates; IT - initial temperature (moment when silages were exposed to air); FT - final temperature (moment when silages were recompacted - after their respective times of exposure to air); MaxT - maximum temperature; MinT - minimum temperature; SEM - standard error of the mean.

Table 2 - Microbial populations (log_{10} cfu/g of fresh weight), fermentation products (g/kg of dry matter), aerobic stability (h), and dry matter recovery (DMR; %) of corn silage ensiled for 30 days, subjected to relocation times (RT), and stored again for 30 days (Experiment 1)

Item	RT (h)										SEM	P-value[2]	Contrast	
	NR[1]	0	6	12	18	24	30	36	48	60			L	Q
Molds	0.00	0.00	0.167	0.500	0.933	1.00	1.50	3.00	3.00	3.00	4.76	0.81	0.15	0.38
Yeasts	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	-	-	-	-
Lactic acid	56.3	47.7	37.5	53.7	44.3	41.5	62.7	59.2	31.9	52.4	3.13	0.08	0.72	0.43
Acetic acid	16.7	17.5	23.3	7.19‡	11.6	9.85	33.6	14.6	22.4	13.5	2.51	0.03	0.84	0.83
Propionic acid	7.84	9.21	13.0	2.39	5.72	7.94	16.0	8.63	14.1	8.95	1.28	0.47	0.47	0.94
pH	3.68	3.69	3.67	3.65	3.69	3.72	3.80	3.85	3.89	3.95‡	0.0342	0.01	<0.01	0.77
Aerobic stability	82.3	113	115	132	133	233	224	257	112	111	23.9	0.13	0.69	0.01
Amplitude	8.50	9.50	8.00	9.00	7.33	3.83	4.00	1.50	7.50	7.67	0.668	0.20	0.11	0.06
HmaxT	135	163	150	152	173	216	237	241	136	143	15.6	0.17	0.02	0.42
MaxT	30.2	30.3	28.7	29.7	27.5	23.5	23.5	20.7	27.2	27.0	1.03	0.20	0.11	0.06
pH-12d	6.29	4.83	5.39	5.56	5.43	5.57	4.60	4.45	6.14	6.53	0.181	0.10	0.08	0.12
DMR	93.9	102	100	106‡	103	104	103	100	107‡	112‡	3.94	<0.01	0.01	0.38

HmaxT - time to reach the maximum temperature; MaxT - maximum temperature; pH-12d - pH measured after 12 days of aerobic exposure; DMR - dry matter recovery; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.
[1] Non-relocated silage stored for 60 days after ensiling.
[2] Compared with a significance level of 5%.
‡ Differs from the control (NR) by Dunnett's test.

We did not observe a significant effect of RT (P>0.05) on concentrations of OM, NFC, IVDMD, and WSC in silage (Table 3). The RT of 60 h resulted in a significant increase in DM (P = 0.05) and CP (P = 0.04) concentrations compared with that in the control silage. The concentration of EE in silage with an RT of 36 h also differed significantly from that of the control (P = 0.01). Silage exposed for 60 h during relocation showed a higher CP value than that of the control. The concentration of NDF in the silages with RT of 30 and 48 h was higher than that in the control (P<0.01). The RT of 6–60 h resulted in higher levels of NH_3-N in the silage than that in the control (P<0.01). The EE concentration decreased linearly as a function of increasing RT.

Table 3 - Chemical composition (g/kg of dry matter) and *in vitro* dry matter digestibility (IVDMD) of corn silage stored for 30 days, subjected to relocation times (RT), and stored again for 30 days (Experiment 1)

Item	RT (h)										SEM	P-value[2]	Contrast	
	NR[1]	0	6	12	18	24	30	36	48	60			L	Q
DM	312	323	316	333	338	332	341	324	331	353‡	3.85	0.05	0.01	0.86
OM	960	961	962	960	961	914	965	964	962	957	4.78	0.11	0.98	0.49
EE	37.4	35.2	36.7	34.1	43.3	41.9	31.1	29.0‡	26.2	28.2	1.81	0.01	0.01	0.27
CP	86.3	85.0	81.6	83.9	85.9	84.7	80.0	86.2	82.1	87.7‡	0.768	0.04	0.24	0.17
NDF	413	442	426	438	426	413	487‡	442	449‡	436	6.74	<0.01	0.14	0.24
NFC	424	399	418	404	407	375	367	406	405	404	5.53	0.61	0.86	0.29
IVDMD	714	728	725	724	704	695	726	716	707	696	3.94	0.75	0.18	0.56
NH_3-N/TN	52.6	60.3	65.5‡	63.6‡	61.2	67.3‡	72.9‡	71.1‡	71.4‡	74.2‡	2.14	<0.01	<0.01	0.45
WSC	13.1	13.4	16.1	24.5	32.3	6.66	19.7	13.0	14.3	19.2	1.99	0.21	0.96	0.81

DM - dry matter; OM - organic matter; EE - ether extract; CP - crude protein; NDF - neutral detergent insoluble fiber; NFC - Non-fibrous carbohydrates; NH_3-N - ammonia nitrogen; TN - total nitrogen; WSC - water-soluble carbohydrates; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.
[1] Non-relocated silage stored for 60 days after ensiling.
[2] Compared with a significance level of 5%.
‡ Differs from the control (NR) by Dunnett's test.

3.2. Experiment 2

Our results showed no significant effect of ST on yeast count or lactic acid (P>0.05) (Table 4). Counts of molds in silages with ST of 4, 8, 16, 32, and 64 d were lower than those in the control (P<0.01). Concentrations of propionic and butyric acids in silages with ST 0–128 d were less than 1 g/kg of DM (not shown in Table 4). The acetic acid concentration in silages with ST of 8, 32, 64, and 128 d was greater than that in the control (P<0.01). The pH measured at the opening of silos was higher in the control silage than that in silages with ST of 4, 8, and 128 d. Only the silage with 32 d of ST showed a higher pH value than that of the control silage. Aerobic stability in silages with ST of 8, 16, 32, 64, and 128 d was significantly greater than that in the control (P<0.01). In contrast, the amplitude in silages with ST of 64 and 128 d was lower than that in the control (P<0.01). The HmaxT of the silage with ST for 4 d was higher than that of the control (P<0.01), whereas the MaxT was lower than that of the control only in the silage with 128 d of ST (P<0.01). pH-7d differed from that of the control in silages with 4 and 128 d of ST (P<0.01). An increase in ST resulted in a linear reduction in amplitude, MaxT, pH-7d, and DMR of the silage, whereas mold counts, concentration of acetic acid, and values of aerobic stability and HmaxT increased linearly with ST.

We observed no significant effect of ST on EE, NH_3-N, or WSC concentrations in silage (P>0.05; Table 5). The DM concentration in silages with ST of 4, 16, 32, 64, and 128 d was lower than that in the control (P<0.01). The OM concentration in the silages with ST of 32, 64, and 128 d was lower than that in the control (P<0.01), whereas for the same ST, the CP concentration was higher than that in the control (P<0.01). The NDF concentration in silages with ST of 64 and 128 d was higher than that in the control (P<0.01), whereas for the same ST, the NFC was lower than that in the control. The IVDMD was reduced when the relocated silage was stored for 64 d compared with that of the control. Increased ST resulted in linear reductions in the DM, OM, and NFC concentrations of the silage, whereas the CP and NDF values increased linearly as a function of ST.

Table 4 - Microbial composition (log_{10} ufc/g), fermentation products (g/kg of dry matter), aerobic stability, and dry matter recovery (DMR; %) of corn silage stored for 150 days and subjected to storage times (ST) after relocation (Experiment 2)

Item	ST (days)							SEM	P-value[2]	Contrast	
	NR[1]	4	8	16	32	64	128			L	Q
Molds	2.18	0.540‡	0.566‡	0.929‡	1.43‡	1.73‡	0.00	0.168	<0.01	<0.01	0.01
Yeasts	2.01	1.49	0.575	0.425	1.55	1.55	0.00	0.140	0.09	0.09	0.82
Lactic acid	26.0	31.6	58.2	32.0	22.4	36.3	26.4	4.06	0.06	0.06	0.32
Acetic acid	30.0	13.2	23.9‡	32.1	74.4‡	75.7‡	77.9‡	5.43	<0.01	<0.01	0.10
pH	4.02	3.80‡	3.64‡	3.86	4.36‡	4.02	3.67‡	0.0474	<0.01	0.20	<0.01
Aerobic stability	1.90	9.00	31.3‡	50.4‡	148‡	214‡	252‡	19.8	<0.01	<0.01	<0.01
Amplitude	14.7	17.3	15.4	11.8	10.3	5.20‡	2.20‡	1.09	<0.01	<0.01	0.02
HmaxT	24.5	45.0‡	59.3‡	92.3‡	229‡	258‡	281‡	20.0	<0.01	<0.01	<0.01
MaxT	40.7	43.3	41.4	37.8	36.3	31.2	28.2‡	1.09	<0.01	<0.01	<0.01
pH-7d	7.18	7.88‡	7.15	7.59	7.35	7.54	5.13‡	0.173	<0.01	<0.01	<0.01
DMR	-	90.4	97.1	91.0	85.2‡	84.9‡	82.8‡	2.16	<0.01	<0.01	<0.01

HmaxT - time to reach the maximum temperature; MaxT - maximum temperature; pH-7d - pH measured after seven days of aerobic exposure; DMR - dry matter recovery; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.
[1] Non-relocated silage.
[2] Compared with a significance level of 5%.
‡ Differs from the control (NR) by the Dunnett's test.

Table 5 - Chemical composition (g/kg of dry matter) and *in vitro* dry matter digestibility (IVDMD) of corn silage stored for 150 days and subjected to different storage times (ST) after relocation (Experiment 2)

Item	ST (days)							SEM	P-value[2]	Contrast	
	NR[1]	4	8	16	32	64	128			L	Q
DM	337	304‡	327	306‡	286‡	285‡	278‡	4.10	<0.01	<0.01	<0.01
OM	977	976	977	972	971‡	973‡	970‡	0.655	<0.01	<0.01	0.11
EE	22.7	22.4	24.5	21.9	27.0	28.4	23.9	0.839	0.22	0.16	0.11
CP	54.5	56.1	53.9	56.1	58.8‡	61.7‡	62.4‡	0.875	<0.01	<0.01	0.01
NDF	381	397	371	409	400	426‡	423‡	5.20	<0.01	0.01	0.22
NFC	519	500	528	485	485	457‡	458‡	6.20	<0.01	0.01	0.06
IVDMD	666	651	615	604	589	531‡	604	6.60	0.02	0.80	0.55
NH_3-N/TN	74.0	64.2	64.5	62.6	70.6	68.0	67.2	1.40	0.26	0.45	0.33
WSC	20.0	14.3	25.6	20.5	19.6	19.3	18.8	1.25	0.77	0.80	0.91

DM - dry matter; OM - organic matter; EE - ether extract; CP - crude protein; NDF - neutral detergent insoluble fiber; NFC - Non-fibrous carbohydrates; NH_3-N - ammonia nitrogen; TN - total nitrogen; WSC - water-soluble carbohydrates; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.
[1] Non-relocated silage.
[2] Compared with a significance level of 5%.
‡ Differs from the control (NR) by Dunnett's test.

4. Discussion

4.1. Experiment 1

The action of the hydrolytic enzymes produced by plants is effective when the material is ensiled. Structural and non-structural carbohydrates serve as energy sources for microorganisms during fermentation (Rooke and Hatfield, 2003). This hydrolysis causes a reduction in the NDF values, which was reflected in the results of the silages in the first opening of the silos, 30 d after the first ensiling (Table 1) in the current study.

Aerobic deterioration of silage occurs when it is exposed to atmospheric oxygen, which triggers a series of reactions such as an increase in the population of yeasts, temperature, and pH (Pahlow et al., 2003). Changes in temperature are the first response to aerobic deterioration. An increase in RT may result

in other changes, such as changes in populations of molds and other aerobic bacteria, which intensify the production of heat and increase the temperature of the silages, as observed in the present study. However, this change in microbial population was not observed after relocation, even in silages with longer RT (60 h). Therefore, we discuss whether such changes could influence the characteristics of the silage after silo opening.

In the present study, modifications that could compromise the fermentation profile and preservation of the silage did not occur, even though they were exposed to air for up to 60 h, as no differences were observed in their microbial populations and fermentation profiles relative to those in the control silage. This demonstrates that aerobic exposure before relocation did not affect the fermentation profile or silage conservation. The highest pH value (3.9) observed was still within the recommended pH range for corn silage (3.8–4.2; McDonald et al., 1991). Other studies have reported few changes in fermentation profile and microbial populations in maize and sorghum silage relocated for up to 48 h (Chen and Weinberg, 2014; Lima et al., 2016; dos Anjos et al., 2018). However, reductions in concentrations of lactic acid and NH_3-N in the silage and increases in effluent losses were observed in sorghum silage relocated for up to 12 h (dos Anjos et al., 2018). The low acetic acid content at RT of 12 h is not biologically explainable.

Although no changes occurred in fermentation products in the silage, its aerobic stability increased up to 36 h of RT and decreased for longer RT, 48 and 60 h. This increase in aerobic stability probably occurred because of oxygen penetration into the silage mass during aerobic exposure at the time of relocation. Oxygen promotes the proliferation of deterioration-initiating microorganisms, such as yeasts, which metabolize soluble carbohydrates, starch (McDonald et al., 1991), and organic acids (Driehuis et al., 1999), producing carbon dioxide, water, and heat (Muck, 2010). With the consumption of fermentative products, the pH of the silage is raised to values above 4.5, favoring the growth of a wide variety of other aerobic microorganisms, which accelerates the deterioration process and causes even greater heating in the silage (Muck, 2010). Thus, the higher stability of the silage with RT longer than 24 h can be explained by the lower availability of substrates for yeasts under these conditions, because these would have been consumed during the relocation and subsequent storage processes, which could be observed in the chemical composition of the silage observed after relocation (Table 3). It is possible that RT longer than 48 h made the silages more prone to aerobic deterioration, considering that their pH was above 5.0 after 5 d of aerobic exposure, whereas silages with RT lower than 48 h had a pH below 4.5 on the same day. It suggests that longer RT increases aerobic deterioration because of the long period that the silage remains exposed to air during the process, which will affect microbial populations and other silage characteristics that accelerate the deterioration process. However, in the current study, the result could not be attributed to any parameter evaluated in the silage.

The increase in the DM content of the silage as a function of RT occurred because of dehydration resulting from the contact with air during the aerobic exposure of silage. Recovery values of DM above 100% demonstrated that prolonged aerobic exposure during relocation probably caused water loss in the silage and concentrated its DM. Chen and Weinberg (2014) also observed the drying of silage during aerobic exposure. Changes in EE, CP, and NDF concentrations in the silage were proportional to the changes in its DM content and were related to decrease in levels of other nutrients, such as soluble carbohydrates, which are included in the NFC.

Small changes in chemical composition did not affect the nutritive value of the silage, demonstrating that its final quality was not compromised by relocation, which corroborates the results of previous studies. Therefore, well-preserved silages, such as corn, are safe to relocate within 60 h without major changes in fermentation characteristics and chemical composition.

4.2. Experiment 2

The acetic acid concentration increased after 32 d of storage compared with that in the control, which resulted in a lower pH than that of the control at silo opening and increased aerobic stability (Table 4). Changes in these components after long periods of storage indicate that some microbial activity persists in silage, even when the pH is low (Kung Jr., 2013).

In a study in which corn silage was stored for up to 360 d, Der Bedrosian et al. (2012) reported an increase in the concentration of acetic acid and 1,2-propanediol in the silage as the storage time increased. The main explanation for this increase is the metabolism of heterofermentative lactic acid species, such as *Lactobacillus buchneri* that can metabolize lactic acid to acetic acid and 1,2-propanediol under anaerobic conditions (Oude Elferink et al., 2001).

Other studies have reported that some strains of *Lactobacillus plantarum, Lactobacillus bifermentans*, and *Streptococcus faecium* under anaerobic conditions and at low WSC concentrations begin to produce formic acid and acetic acid using lactic acid as a substrate (Kandler et al., 1983; Lindgren et al., 1990; London, 1968; Murphy and Condon, 1984).

The antifungal action of acetic acid has been confirmed by the easy penetration of the cell membranes of yeasts when the pH is lower than the dissociation constant (pKa) of these acids. At that point, they take on their dissociated form, causing the release of H^+ ions inside the yeast cells (Walker, 1998). As a consequence of the entrance of H^+ ions, the pH of the yeast cells decreases, compromising their growth due to the energy required for the active transport carried out by the cell in an attempt to eliminate H^+ ions from its interior (McDonald et al., 1991).

The increase in aerobic stability and HMaxT of relocated corn silages can be explained by the increase in acetic acid concentration with storage time. However, the fact that relocated silage is more stable cannot be considered beneficial because the loss of nutrients and reduction in the concentration of NFC were higher in silage with longer ST. This indicates that the relocation of silage increased the degradation of nutrients, even under anaerobic conditions in the silo, as a function of ST. The IVDMD value was also lower for silage with an ST of 64 d, showing that the observed reduction in the NFC reduced the IVDMD value of the silage that was relocated and subsequently stored. Thus, it is important to know the storage period and chemical composition of relocated silage before formulating livestock diets.

The decreased DM content of the silage with longer storage (Table 5) is related to nutrient losses in general, which can be observed by the reduction in DMR values with increasing ST. When evaluating the storage time of corn silage, Sariçiçek et al. (2016) also observed a decrease in the DM concentration of silage after long periods of storage. The reduction in DMR after long-term storage could be explained by slight modifications in the fermentation profile and an increase in acetic acid concentration. Non-fiber carbohydrate is the main source of fermentable substrates in silage, while WSC, glucose, fructose, sucrose, and fructan are also important sources of energy for microorganisms in the rumen (Bernardes et al., 2018). A reduction in the availability of these compounds in silage decreases their nutritive value.

5. Conclusions

The relocation of well-preserved corn silage for up to 60 h has no effect on the fermentative profile and nutritive value of the silage. However, dry matter content increases as a function of relocation time owing to aerobic exposure during relocation process. Increased storage time of the relocated corn silage, up to 128 days, has no effect on silage preservation. However, it decreases the nutritional value of relocated silage. Storage period after relocation has a greater impact on the nutritional value of relocated corn silage than time of exposure to air during relocation.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: A.C. Rêgo. Data curation: R.I.R. Santos, R.C.A. Mendonça, A.C.M. Queiroz and A.C. Rêgo. Formal analysis: R.I.R. Santos, R.C.A. Mendonça and A.C.M. Queiroz. Funding acquisition:

T.F. Bernardes, F.N. Domingues, C. Faturi, T.C. Silva and A.C. Rêgo. Investigation: R.I.R. Santos, R.C.A. Mendonça, A.C.M. Queiroz, F.N. Domingues, C. Faturi, T.C. Silva and A.C. Rêgo. Methodology: R.C.A. Mendonça, A.C.M. Queiroz and A.C. Rêgo. Project administration: A.C. Rêgo. Resources: T.F. Bernardes and A.C. Rêgo. Software: R.I.R. Santos, R.C.A. Mendonça, A.C.M. Queiroz, C. Faturi and A.C. Rêgo. Supervision: T.F. Bernardes, F.N. Domingues, T.C. Silva and A.C. Rêgo. Validation: R.I.R. Santos, R.C.A. Mendonça, A.C.M. Queiroz, T.F. Bernardes, F.N. Domingues, C. Faturi, T.C. Silva and A.C. Rêgo. Visualization: R.I.R. Santos, R.C.A. Mendonça, A.C.M. Queiroz and A.C. Rêgo. Writing – original draft: R.I.R. Santos, R.C.A. Mendonça, A.C.M. Queiroz and A.C. Rêgo. Writing – review & editing: T.F. Bernardes, F.N. Domingues, C. Faturi, T.C. Silva and A.C. Rêgo.

Acknowledgments

The authors thank the undergraduate and graduate students from the Grupo de Estudo em Ruminantes e Forragicultura da Amazônia (GERFAM) for their technical support. This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; project 423031/2016-3). The authors thank PROCAD (UFRA/UFPA/UFLA) and Universidade Federal Rural da Amazônia (UFRA) for the financial support the publication. We would like to thank Editage for English language editing.

References

AOAC - Association of Official Analytical Chemists. 1990. Official methods of analysis. 12th ed. AOAC, Washington, DC.

Bastos, T. X.; Silva, G. F. G.; Pacheco, N. A. and Figueiredo, R. O. 2006. Informações agroclimáticas do município de Paragominas para o planejamento agrícola. Available at: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/102368/1/Nilza4.pdf>. Accessed on: Apr. 11, 2022.

Bolsen, K. K.; Lin, C.; Brent, B. E.; Feyerherm, A. M.; Urban, J. E. and Aimutis, W. R. 1992. Effect of silage additives on the microbial succession and fermentation process of alfalfa and corn silages. Journal of Dairy Science 75:3066-3083. https://doi.org/10.3168/jds.S0022-0302(92)78070-9

Bernardes, T. F.; Reis, R. A. and Amaral, R. C. 2009. Chemical and microbiological changes and aerobic stability of marandu grass silages after silo opening. Revista Brasileira de Zootecnia 38:1-8. https://doi.org/10.1590/S1516-35982009000100001

Bernardes, T. F. and do Rêgo, A. C. 2014. Study on the practices of silage production and utilization on Brazilian dairy farms. Journal of Dairy Science 97:1852-1861. https://doi.org/10.3168/jds.2013-7181

Bernardes T. F.; Daniel, J. L. P.; Adesogan, A. T.; McAllister, T. A.; Drouin, P.; Nussio, L. G.; Huhtanen, P.; Tremblay, G. F.; Bélanger, G. and Cai, Y. 2018. Silage review: Unique challenges of silages made in hot and cold regions. Journal of Dairy Science 101:4001-4019. https://doi.org/10.3168/jds.2017-13703

Chen, Y. and Weinberg, Z. G. 2014. The effect of relocation of whole–crop wheat and corn silages on their quality. Journal of Dairy Science 97:406-410. https://doi.org/10.3168/jds.2013-7098

Der Bedrosian, M. C.; Nestor Jr., K. E. and Kung Jr., L. 2012. The effects of hybrid, maturity and length of storage on the composition and nutritive value of corn silage. Journal of Dairy Science 95:5115-5126. https://doi.org/10.3168/jds.2011-4833

Detmann, E. and Valadares Filho, S. C. 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 62:980-984. https://doi.org/10.1590/S0102-09352010000400030

DuBois. M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356. https://doi.org/10.1021/ac60111a017

Driehuis, F.; Oude Elferink, S. J. W. H. and Spoelstra, S. F. 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. Journal of Applied Microbiology 87:583-594. https://doi.org/10.1046/j.1365-2672.1999.00856.x

dos Anjos, G. V. S.; Gonçalves, L. C.; Rodrigues, J. A. S.; Keller, K. M.; Coelho, M. M.; Michel, P. H. F.; Ottoni, D. and Jayme, D. G. 2018. Effect of re-ensiling on the quality of sorghum silage. Journal of Dairy Science 101:6047-6054. https://doi.org/10.3168/jds.2017-13687

Kandler, O.; Schillinger, U. and Weiss, N. 1983. *Lactobacillus bifermentans* sp. nov. nom. rev., an organism forming CO_2 and H_2 from lactic acid. Systematic and Applied Microbiology 4:408-412. https://doi.org/10.1016/S0723-2020(83)80025-3

Kung Jr., L.; Smith, M. L.; Silva, E. B.; Windle, M. C.; Silva, T. C. and Polukis, S. A. 2018. An evaluation of the effectiveness of a chemical additive based on sodium benzoate, potassium sorbate, and sodium nitrite on the fermentation and aerobic stability of corn silage. Journal of Dairy Science 101:5949-5960. https://doi.org/10.3168/jds.2017-14006

Kung Jr., L. 2013. The effects of length of storage on the nutritive value and aerobic stability of silages. p.7-19. In: Proceedings of the III International Symposium on Forage Quality and Conservation. Campinas.

Licitra, G.; Hernandez, T. M. and Van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57:347-358. https://doi.org/10.1016/0377-8401(95)00837-3

Lima, E. M.; Gonçalves, L. C.; Keller, K. M.; Rodrigues, J. A. S.; Santos, F. P. C.; Michel, P. H. F.; Raposo, V. S. and Jayme, D. G. 2016. Re-ensiling and its effects on chemical composition, in vitro digestibility, and quality of corn silage after different lengths of exposure to air. Canadian Journal of Animal Science 97:250-257. https://doi.org/10.1139/cjas-2016-0005

Lindgren, S. E.; Axelssona, L. T. and McFeeter, R. F. 1990. Anaerobic-lactate degradation by *Lactobacillus plantarum*. FEMS Microbiology Letters 66:209-213. https://doi.org/10.1111/j.1574-6968.1990.tb03998.x

London, J. 1968. Regulation and function of lactate oxidation in *Streptococcus faecium*. Journal of Bacteriology 95:1380-1387. https://doi.org/10.1128/jb.95.4.1380-1387.1968

McDonald, P.; Henderson, A. R. and Heron, S. J. E. 1991. The biochemistry of silage. 2nd ed. Chalcombe Publications, Marlow.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. Journal of AOAC International 85:1217-1240.

Michel, P. H. F.; Gonçalves, L. C.; Rodrigues, J. A. S.; Keller, K. M.; Raposo, V. S.; Lima, E. M.; Santos, F. P. C. and Jayme, D. G. 2017. Re-ensiling and inoculant application with *Lactobacillus plantarum* and *Propionobacterium acidipropionici* on sorghum silages. Grass and Forage Science 72:432-440. https://doi.org/10.1111/gfs.12253

Muck, R. E. 2010. Silage microbiology and its control through additives. Revista Brasileira de Zootecnia 39(supl. especial):183-191. https://doi.org/10.1590/S1516-35982010001300021

Murphy, M. G. and Condon, S. 1984. Comparison of aerobic and anaerobic growth of *Lactobacillus plantarum* in a glucose medium. Archives of Microbiology 138:49-53. https://doi.org/10.1007/BF00425406

O'Kiely, P. 1993. Influence of a partially neutralized blende of aliphatic organic acids on fermentation, effluent production and aerobic stability of autumm grass silage. Irish Journal of Agricultural and Food Research 32:13-26.

Oude Elferink, S. J. W. H.; Krooneman, J.; Gottschal, J. C.; Spoelstra, S. F.; Faber, F. and Driehuis, F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Applied and Environmental Microbiology 67:125-132. https://doi.org/10.1128/AEM.67.1.125-132.2001

Pahlow, G.; Muck, R. E.; Driehuis, F.; Oude Elferink, S. J. W. H. and Spoelstra, S. F. 2003. Microbiology of ensiling. p.31-93. In: Silage science and technology. Buxton, D. R.; Muck, R. E. and Harrison, J. H., eds. American Society of Agronomy, Madison.

Pryce, J. D. 1969. A modification of Barker-Summerson method for determination of lactic acid. Analyst 94:1151-1152. https://doi.org/10.1039/an9699401151

Queiroz, A. C. M.; Mendonça, R. C. A.; Santos, R. I. R.; Cardoso, M. V. S. B.; Domingues, F. N.; Faturi, C.; Silva, T. C. and Rêgo, A. C. 2021. Effects of whole-plant corn silage relocation on quality, chemical composition, and intake, digestibility, and nitrogen balance in sheep. Small Ruminant Research 205:106558. https://doi.org/10.1016/j.smallrumres.2021.106558

Rooke, J. A. and Hatfield, R. D. 2003. Biochemistry of ensiling. p.95-139. In: Silage science and technology. Buxton, D. R.; Muck, R. E. and Harrison, J. H., eds. American Society of Agronomy, Madison.

Sariçiçek, B. Z.; Yildirim, B.; Kocabaş, Z. and Özgümüş Demir, E. 2016. The effects of storage time on nutrient composition and silage quality parameters of corn silage made in plastic mini silo in laboratory conditions. Journal of Institute of Science and Technology 6:177-183. https://doi.org/10.21597/JIST.2016321853

Tilley, J. M. A. and Terry, R. A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Journal of the British Grassland Society 18:104-111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Walker, G. M. 1998. Yeast physiology and biotechnology. Wiley Editorial Offices, London.

Zanine, A. M.; Santos, E. M.; Dórea, J. R. R.; Dantas, P. A. S.; Silva, T. C. and Pereira, O. G. 2010. Evaluation of elephant grass silage with the addition of cassava scrapings. Revista Brasileira de Zootecnia 39:2611-2616. https://doi.org/10.1590/S1516-35982010001200008