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Fermentative Characteristics, Nutritional Aspects, Aerobic Stability, and Microbial Populations of Total Mixed Ration Silages Based on Relocated Sorghum Silage and Cactus Pear for Sheep Diets

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Abstract: Total mixed ration silage has been used as a strategy to optimize the use of dry and wet feed in ruminant feeding. Another promising technique is silage reallocation, which allows producers to divide the ensiled material in large silos into smaller units that can be easily transported and marketed. Thus, this study aimed to improve food preservation through the development of total mixed rations (TMRs) based on relocated sorghum silage (RSS) and cactus pear for sheep diets. A completely randomized design was used with five treatments (0, 15, 25, 30, and 35% RSS inclusion on a dry matter basis) and five replicates. Ninety days after ensiling, the silos were opened. The fermentation characteristics, nutritional aspects, aerobic stability, and microbial populations of TMR silages were evaluated. The inclusion of RSS showed a quadratic effect on pH, density, permeability, lactic acid bacteria and yeast counts, and total carbohydrates ($p < 0.05$). It reduced gas and effluent losses, porosity, ammonia nitrogen, buffer capacity, ash, crude protein, ether extract, and non-fibrous carbohydrates ($p < 0.05$) while increasing dry matter, neutral and acid detergent fiber, hemicellulose, and cellulose contents ($p < 0.05$). There was an interaction effect between the levels of RSS inclusion and exposure times to air on CO_2 and dry matter content ($p < 0.05$). Regarding carbohydrate fractionation, there was a reduction in fraction A + B1 (non-fibrous carbohydrates) and an increase in fractions B2 (fibrous carbohydrates from the cell wall and of slow ruminal availability, susceptible to the effects of the passage rate) and C (indigestible neutral detergent fiber) ($p < 0.05$). For protein fractionation, a quadratic effect was observed for fractions A (non-protein nitrogen) and C (insoluble protein, indigestible in the rumen and intestine), an increase in fraction B1 (soluble protein rapidly degraded in the rumen) + B2 (insoluble protein with intermediate degradation rate in the rumen), and a reduction in fraction B3 (insoluble protein with slow degradation rate in the rumen) ($p < 0.05$) as RSS levels increased. Under the experimental conditions, it is recommended to include up to 30% RSS in the total mixed ration silage to improve microbiological characteristics, reduce gas and effluent losses, and increase dry matter recovery and nutritional aspects of silage when associated with cactus pear.

Keywords: mixed silage; *Opuntia stricta* Haw; silage relocation; *Sorghum bicolor* L.

1. Introduction

Food preservation through the ensiling process is essential for ruminant production, particularly since forage and concentrate feed production is seasonal in most regions. However, for ensiling to be efficient, the feed must possess characteristics that allow for proper fermentation [1]. In this context, a globally adopted alternative is the preparation of silage-based rations, aiming to improve the utilization of feed resources and intensify ruminant production [2].

The use of total mixed ration (TMR) silages involves creating a homogeneous mixture by combining various ingredients in the silo. This minimizes ingredient selection by the animals, enhances herd performance, increases feed acceptability, allows for ensiling both wet and dry feeds, and reduces effluent production during fermentation. In the nutritional context, one of the advantages of using the TMR technique is to improve the feed efficiency of ensiled diets in the form of feed, due to greater use of energy and protein from the diet in the rumen, in addition to stabilizing microbial activities [3]. Other points that deserve attention are the reduction in labor costs and, consequently, animal feed costs, as well as the possibility of marketing silages with high added value, after 60 days of ensiling [4].

Among the forages with high moisture for the preparation of TMR, cactus pear (*Opuntia stricta* Haw) stands out for presenting characteristics peculiar to the good fermentation process, such as the content of water-soluble carbohydrates (WSC = 150 g/kg dry matter) that provides substrates available for lactic modification; thus, a rapid acidification of the ensiled mass occurs, inhibiting the growth of undesirable microorganisms [5]. Furthermore, cactus pear presents mucilage, a complex polymeric heteropolysaccharide with high molecular weight that contains neutral sugars (D-galactose, D-xylose, l-rhamnose and l-arabinose) and D-galacturonic acid, which has hydrophilic properties that create a barrier against the water evaporation from plant tissue and retains fluids resulting from the rupture of plant cells during milling [6], promoting a reduction in losses during ensiling [7].

Sorghum (*Sorghum bicolor* (L.) Moench) is a crop that can be considered an alternative to corn for producing silage for ruminant feeding because it is better adapted to drought and low soil fertility, producing approximately 27% more dry matter under severe climate stress conditions compared to corn [8]. Sorghum cultivars are generally recommended for the production of high-quality silage due to their high water-soluble carbohydrate content (WSC = 140–484 g/kg dry matter [8]), resulting in forage with high energy content, in addition to the dry matter content (DM = 330.6 g/kg fresh matter [9]) [10], which can complement silages made exclusively with cactus pear.

In Brazil, the commercialization of silage has become more frequent due to the reduction in crop productivity resulting from climatic variations during the rainy season, especially in semi-arid and arid regions. The silage purchased by producers is transported and unpacked at its destination, where the re-ensiling of the conserved material will take place [11]. Re-ensiling or relocation offers the possibility of dividing large quantities of silage stored in bunkers, trenches, or piles into smaller units that can be easily transported and sold [12]. This practice has also been used in other parts of the world, such as in Israel [13].

Relocated silage can be advantageous for producers who do not have available land for planting or who lack labor and machinery for silage production [12]. Once exposed to oxygen, the ensiled material can be stored for several months after re-silage. In practice, farmers buy large quantities of silage at low prices and re-silage the material for use throughout the year [11,13,14].

However, the nutritional value of relocated sorghum silages may be compromised due to exposure to air, which promotes the growth of microorganisms that metabolize the soluble sugars and lactic acid produced during fermentation. This increases the temperature and pH and causes an undesirable loss of highly digestible soluble carbohydrates while increasing the proportion of fibrous components [13]. This issue can be circumvented by using forage cactus, as forage cactus provides fermentable substrates for lactic acid bacteria, maintaining the pH within the desirable range, which, according to Muck [15], is between 3.8 and 4.2. Macêdo et al. [7], evaluating rations based on cactus pear and buffel grass in silage form, found that all assessed silages had a pH close to 4.0, considered an ideal range for lactic acid bacteria development. Another benefit of using cactus pear in TMR silage is its ability to increase aerobic stability. This is due to the production of acetic acid during the fermentation of cactus within the silo. This acid inhibits the growth of yeasts, which are precursors of spoilage in ensiled mass when exposed to air [16,17].

Anjos et al. [11] emphasizes that there is limited information about the re-silage process, and studies typically do not take into account the characteristics of the tropical climate, While Lima et al. [12] state that, to the best of their knowledge, there are no technical recommendations for the technique of silage relocation. Therefore, research evaluating the re-silage technique and showing the results obtained with the use of relocated silage, whether for exclusive use or as a complement in the preparation of TMR for ruminant feeding, is relevant.

Effluent losses are a significant challenge when ensiling forages with high moisture content. However, studies have demonstrated reduced losses in silages based on cactus pear. This reduction is associated not only with increased dry matter content but also with the effect of the mucilage in cactus pear, which envelops the fluids from cladode grinding [18], preventing effluent loss. Given the aforementioned facts, we hypothesize that the combination of cactus pear and relocated sorghum silage in TMR silage can be complementary, improving nutritional characteristics and reducing fermentative losses.

Therefore, this study aimed to improve food preservation through the development of total mixed rations based on relocated sorghum silage and cactus pear for sheep diets.

2. Materials and Methods

2.1. Experimental Location

The experiment was conducted at the Universidade Federal do Vale do São Francisco (UNIVASF), Petrolina—PE, Brazil (9°19′28″ South latitude, 40°33′34″ West longitude, 393 m altitude). Chemical analyses were performed at the animal nutrition laboratory of the Brazilian Agricultural Research Corporation (EMBRAPA—Semi-Arid), Petrolina—PE, Brazil (09°8′8.9″ South latitude, 40°18′33.6″ West longitude, 379 m altitude), and at the Forage Laboratory of the Universidade Federal da Paraíba (UFPB), Areia—PB, Brazil (06°57′46″ South latitude, 35°41′31″ West longitude, 684 m altitude).

The experimental period, with the preparation of silages and laboratory analyses, occurred from April 2022 to August 2023.

2.2. Preparation of Total Mixed Ration (TMR) Silages

The experimental design was completely randomized with five treatments and five replicates. To prepare the silages, five rations were formulated using cactus pear cv. Mexican

Elephant Ear and relocated sorghum silage (BRS Ponta Negra) as forage and concentrate. The concentrate consisted of corn meal, soybean meal, ammonium sulfate, and urea, comprising of treatments with 0, 15, 25, 30, and 35% inclusion of relocated sorghum silage. The TMR silages were designed to meet the requirements for a 200 g/day weight gain in sheep [19] (Tables 1 and 2).

Table 1. Nutritional composition of the ingredients used in the ensilage of a total ration based on relocated sorghum silage and cactus pear.

Variables (g/kg Dry Matter)	Cactus Pear	Sorghum Silage	Soybean Meal	Corn Meal
Dry matter *	100.51	274.21	929.12	910.03
Ash	142.50	87.57	64.00	14.06
Organic matter	857.50	912.43	936.00	985.94
Crude protein	74.31	78.96	519.56	86.41
Ether extract	15.20	24.04	39.77	53.92
Neutral detergent fiber	242.77	550.76	165.88	99.62
Acid detergent fiber	137.40	337.35	77.40	33.55
Hemicellulose	11.10	21.65	15.57	6.61
Total carbohydrates	767.98	809.43	376.67	845.61
Non-fibrous carbohydrates	525.22	258.67	210.79	745.99

* In g/kg natural matter.

Table 2. Percentage of ingredients and chemical composition of the experimental diets.

Ingredients	Relocated Sorghum Silage (% Dry Matter)				
	0	15	25	30	35
Cactus pear	52.75	37.83	27.83	22.77	17.77
Relocated sorghum silage	0.00	14.99	24.99	29.99	34.99
Soybean meal	15.41	31.23	31.24	31.24	31.24
Corn meal	31.23	15.44	15.48	15.59	15.62
Urea	0.56	0.46	0.42	0.37	0.35
Ammonium sulfate	0.06	0.05	0.05	0.04	0.04
Chemical composition (g/kg dry matter)					
Dry matter *	196.40	241.15	280.01	299.47	299.93
Ash	88.17	88.22	78.36	65.00	67.58
Organic matter	911.83	911.78	921.64	935.00	932.42
Crude protein	178.10	179.78	180.21	178.27	178.72
Ether extract	23.79	24.28	27.19	28.64	29.83
Neutral detergent fiber	187.61	208.63	313.30	284.45	312.91
Acid detergent fiber	94.48	137.85	162.28	169.90	183.80
Hemicellulose	9.47	5.93	14.30	10.81	11.53
Total carbohydrates	735.68	706.88	738.51	764.96	757.54
Non-fibrous carbohydrates	548.07	498.26	425.21	480.52	444.63
Total digestible nutrients	812.26	781.90	764.80	759.47	749.74
Metabolizable Energy (Mcal/kg DM)	2.94	2.83	2.77	2.75	2.71

* In g/kg natural matter; DM = dry matter.

For silage preparation, cactus pear was harvested from a pre-established experimental area, collected 18 months after regrowth. The relocated sorghum silage was sourced from a surface silo, relocated 24 months after ensiling, aiming to simulate the scenario observed on Brazilian rural properties, where farmers purchase large quantities of silage at low prices. The silos were opened, and the material was removed and then re-ensiled after 8 h of exposure to air, stored in 200-micron bags (51 × 110 cm). The cactus pear and relocated sorghum silage were individually processed in a stationary forage chopper (PP-35, Pinheiro

Máquinas, Itapira, São Paulo, Brazil) into particles approximately 2.0 cm long. Samples of the chopped forage were collected for subsequent laboratory analysis.

The ration ingredients were homogenized manually according to the treatments. The material was then ensiled in 25 experimental silos made of polyvinyl chloride (PVC), with dimensions of 10 cm in diameter and 50 cm in height, and a volume of 3.925 cm^3. A Bunsen valve was attached to the top of each silo to eliminate gases during fermentation. At the base of each silo, 1 kg of sand covered with cotton fabric was placed to drain and quantify effluents, preventing contact between the ensiled mass and the sand. After ensiling, the silos were sealed and stored for 90 days.

2.3. Chemical Composition

Samples of fresh and ensiled material were pre-dried (50–55 °C for 72 h) and processed to 1 cm in a knife mill. Dry matter (DM), ash, crude protein (CP) [20], ether extract (EE) [21], neutral detergent fiber (NDF), and acid detergent fiber (ADF) (Van Soest et al. [22], modified by Senger et al. [23]), lignin [22], total carbohydrates (TC) [24], non-fibrous carbohydrates (NFC) [25], hemicellulose (HEM = NDF − ADF), cellulose (CEL = ADF − lignin), total digestible nutrients (TDN) [26], and metabolizable energy (ME) [27] were evaluated. All analyses were performed in triplicate.

2.4. Determination of Density, Fermentative Losses, and Fermentative Profile of Silages

The silos were weighed before and after forage deposition and reweighed 90 days after ensiling during silo opening. Parameters evaluated included density, effluent losses, gas losses, dry matter recovery [28], porosity [29], and permeability [30]. For the fermentative profile evaluation, pH and temperature (T, in °C) were measured immediately after silo opening and sample collection [31]. Ammoniacal nitrogen was determined according to Bolsen et al. [32], and buffering capacity (BC) was determined following Playne and McDonald [33]. All analyses were performed in triplicate.

2.5. Aerobic Stability

Aerobic stability (AS, expressed in h) was evaluated using a methodology adapted from Taylor and Kung [34], utilizing plastic containers (4 L) as experimental units. Approximately 1 kg of silage was placed in each container, which were then kept in a closed room under controlled temperature at 24 ± 1 °C for 96 h. The internal temperature was monitored at 2 h intervals, and pH measurements were performed at 6 h intervals. All analyses were performed in triplicate.

2.6. Carbon Dioxide Quantification

To evaluate CO_2 production, 100 systems were prepared using plastic bottles with a capacity of 2 L, following the methodology described by Ashbell et al. [35]. Each system contained 300 g of silage and was sealed with adhesive tape after attaching plastic cups containing 10 mL of 20% potassium hydroxide (KOH) solution. The exposure times to air were 24, 48, 72, 96, and 120 h. The amount of CO_2 produced at each exposure time was determined by titrating the KOH solution with 1 N hydrochloric acid (HCl), maintaining the pH of the titrated solution between 3.0 and 3.3, and recording the volume of HCl used. At each exposure time, silage samples were collected to determine dry matter content. All analyses were performed in triplicate.

2.7. Microbial Populations

For microbial evaluation, 80 silos were prepared using 1 kg plastic bags, with 20 silos per opening time (15, 30, 60, and 90 days). The silos were opened after 90 days of fermentation, using silage from the central part of the silo. Microbial groups were evaluated following the methodology proposed by González [36]. All analyses were performed in triplicate.

Microbial populations were quantified using 10 g of composite silage samples from each treatment, to which 90 mL of sterile distilled water was added and homogenized for 1 min, achieving a 10^{-1} dilution. Serial dilutions were performed by transferring 1 mL from the higher to the lower concentration, achieving dilutions from 10^{-1} to 10^{-6}.

The microorganisms were cultured in sterile disposable Petri dishes by pipetting 1 mL onto odd-numbered plates and 0.1 mL onto even-numbered plates. Approximately 10–15 mL of culture medium was added to each plate according to the microorganism type (LAB—MRS agar; fungi and yeasts—potato dextrose agar; enterobacteria—Violet Red Bile agar). The plates were incubated (LAB, 37 °C for 48 h; fungi and yeasts, 28 °C for 48 h; enterobacteria, 30 °C for 24 h). After incubation, colony-forming units (CFUs) were counted, considering values between 30 and 300 CFU.

2.8. Carbohydrate and Protein Fractions

Total carbohydrates (TC) were fractionated following Sniffen et al. [24]: fraction A + B1 (non-fibrous carbohydrates, NFC); fraction B2 (fiber carbohydrates with slow ruminal availability, affected by passage rate); and fraction C (indigestible NDF, iNDF). iNDF was obtained after 288 h of in vivo incubation in rumen-fistulated cattle [37]. After incubation, the material was washed and residual NDF was determined. Fraction B2 represents available fiber, calculated as the difference between ash- and protein-corrected NDF (NDFap) and fraction C. All analyses were performed in triplicate.

Nitrogenous compounds were fractionated [24]: fraction A (non-protein nitrogen, NPN), fraction B1 (soluble protein rapidly degraded in the rumen) + B2 (insoluble protein with intermediate ruminal degradation), fraction B3 (insoluble protein with slow ruminal degradation), and fraction C (insoluble, indigestible protein in the rumen and intestine). Fraction A was determined by the difference between total nitrogen and trichloroacetic acid (TCA)-insoluble nitrogen. Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined following Licitra et al. [38]. Fraction B1 + B2 was calculated using the equation: B1 + B2 = 100 − (A + B3 + C). Fraction B3 was obtained as the difference between NDIN and ADIN, and fraction C was considered as ADIN. All analyses were performed in triplicate.

2.9. Statistical Analysis

The data were subjected to analysis of variance and regression at the level of $p < 0.05$ for Type I error using PROC GLM and PROC REG of the Statistical Analysis System University Software (version 14.1) [39]. As the RSS inclusion levels in the composition of the evaluated TMRs were not equidistant, it was also necessary to use the PROC IML procedure to formulate the vector. Comparisons between the different levels of relocated sorghum silage inclusion were conducted by decomposing the treatment sum of squares into linear and quadratic effects, followed by regression equation fitting. The following statistical model was used:

$$Y = \mu + Tj + eij \tag{1}$$

where μ = overall mean; Tj = effect of relocated sorghum silage; and eij = residual error.

The data from the CO_2 dynamics were subjected to analysis of variance and Tukey's test using the following statistical model:

$$Yijk = \mu + ai + bj + abij + eijk \tag{2}$$

where μ = overall mean; *ai* = effect of relocated sorghum silage; *bj* = effect of exposure time; *abij* = interaction effect between *a* and *b*; and *eijk* = residual error.

The normality of the data (Shapiro–Wilk test at a 5% probability) was verified using the UNIVARIATE procedure (PROC UNIVARIATE) in SAS. Differences between treatments were considered significant ($p < 0.05$) based on the models and determination coefficient values. The means for each variable were estimated and compared using Tukey's test at a 5% probability level. When isolated effects for the variables opening time and inclusion levels of sorghum silage were detected, interaction unfolding was performed.

3. Results

3.1. Fermentation Profile, Fermentative Losses, and Physical Characteristics

The inclusion of relocated sorghum silage resulted in a quadratic effect for pH ($p = 0.003$), density ($p = 0.036$), and permeability ($p = 0.020$), with the highest pH value observed in the TMRs without RSS (0% = 3.98) and the highest density and permeability values obtained with the inclusion of 25% RSS in the composition of the TMRs, presenting 756.71 kg/m^3 of density and 1220.18 µm^2 of porosity (Table 3).

Table 3. Average values and standard deviation of fermentation profile, fermentative losses, and physical characteristics of total mixed rations based on cactus pear and relocated sorghum silage opened after 90 days of ensiling.

Variables	Relocated Sorghum Silage (% Dry Matter)					SEM	*p*-Value	
	0	15	25	30	35		L	Q
pH	3.98 ±0.03	3.83 ±0.01	3.81 ±0.02	3.89 ±0.07	3.78 ±0.08	0.03	0.001	0.003
T	28.70 ±0.03	28.70 ±0.05	28.50 ±0.30	28.50 ±0.17	20.50 ±0.71	0.13	0.140	0.825
D	414.97 ±24.33	528.95 ±20.64	756.71 ±13.24	743.77 ±12.49	704.05 ±16.71	12.60	0.003	0.036
GL	31.26 ±1.08	20.65 ±0.30	13.23 ±0.42	13.20 ±0.17	12.42 ±1.09	0.77	<0.001	<0.001
EL	76.44 ±1.84	52.69 ±1.73	20.49 ±1.09	17.27 ±1.31	11.48 ±0.79	7.76	0.001	0.266
DMR	77.73 ±2.26	78.18 ±1.42	87.56 ±1.34	86.29 ±1.40	93.70 ±1.04	1.42	<0.001	0.005
K	961.78 ±7.53	1043.71 ±32.33	1220.18 ±58.55	1194.08 ±50.45	1131.99 ±50.62	12.83	0.003	0.020
POR	82.03 ±6.06	78.83 ±1.81	73.71 ±2.13	72.39 ±1.86	70.15 ±1.05	0.36	0.008	0.388
NH_3-N	22.63 ±0.79	16.62 ±0.02	19.35 ±3.07	12.70 ±0.40	14.64 ±0.81	0.53	<0.001	0.242
BC	1043.78 ±56.57	996.08 ±18.70	954.10 ±28.11	919.09 ±17.46	677.96 ±7.85	16.31	<0.001	<0.001
AS	>120.0	>120.0	>120.0	>120.0	>120.0	0.00	0.998	0.998

pH = hydrogen potential; T = temperature (°C); D = density (kg/m^3); NH_3-N = ammonia nitrogen (g/kg of total nitrogen); GL = gas losses (% dry matter); EL = effluent losses (kg/t fresh matter); DMR = dry matter recovery (% dry matter); K = permeability (µm^2); POR = porosity (µm); BC = buffering capacity (E.mgNaOH/100 g dry matter); AS = aerobic stability (h); SEM = standard error of the mean; L = linear effect; Q = quadratic effect; significant at the 5% probability level.

Reductions were observed in gas losses ($p < 0.001$), effluent losses ($p = 0.001$), porosity ($p = 0.008$), ammonia nitrogen ($p < 0.001$), and buffer capacity ($p < 0.001$) as RSS levels in the TMR composition increased. No effect of different levels of relocated sorghum silage inclusion was observed on the temperature and aerobic stability ($p > 0.05$) of the total mixed ration silages (Table 3).

3.2. Dynamics of DM and CO_2 Production

There was an interaction effect between relocated sorghum silage inclusion levels and exposure times to air on dry matter content ($p < 0.05$). Air exposure times influenced ($p < 0.05$) the dry matter contents of total mixed ration silages containing 15% and 30% relocated sorghum silage inclusion (Table 4).

Table 4. Average values and standard deviation of dynamics of dry matter and carbon dioxide production of total mixed rations based on cactus pear and relocated sorghum silage during exposure to the aerobic environment for 24, 48, 72, and 96 h.

Times (Hours)	Relocated Sorghum Silage (% Dry Matter)					SEM	*p*-Value		
	0	15	25	30	35		L	Q	RSS × T
			Dry matter (g/kg fresh matter)						
24	169.17 b ±2.89	223.12 ±6.19	223.72 a ±3.82	244.37 ±2.89	217.83 b ±6.29	3.08	<0.001	<0.001	<0.001
48	184.20 a ±3.99	225.13 ±10.44	226.96 a ±7.10	254.28 ±3.05	235.60 ab ±10.26	5.34	<0.001	0.020	
72	176.21 b ±4.21	214.42 ±7.17	214.94 ab ±7.60	262.43 ±1.42	255.46 a ±14.93	5.41	<0.001	0.454	
96	170.09 a ±2.46	219.68 ±5.80	215.09 ab ±6.37	256.69 ±7.52	233.58 ab ±8.76	4.29	<0.001	0.006	
120	177.64 b ±0.99	214.95 ±2.11	204.81 b ±3.13	260.44 ±4.35	244.43 ab ±5.59	4.93	<0.001	0.625	
SEM	2.21	4.73	3.94	5.04	6.46	-	-	-	
p-value	0.001	0.428	0.010	0.153	0.012	-	-	-	
			Carbon dioxide (g/kg dry matter)						
24	32.84 a ±2.88	29.50 a ±3.60	12.81 b ±1.22	11.09 d ±2.05	31.43 a ±2.62	2.34	0.001	0.001	<0.001
48	31.12 a ±3.754	23.29 b ±1.760	22.29 a ±1.41	23.15 a ±2.24	15.68 b ±2.11	0.93	<0.001	0.830	
72	27.61 ab ±0.80	22.42 b ±2.03	21.18 a ±2.67	17.78 b ±1.72	18.11 b ±1.26	1.26	<0.001	0.673	
96	31.13 a ±1.24	22.01 b ±1.01	21.86 a ±1.50	16.29 bc ±1.64	14.19 b ±1.49	2.44	<0.001	0.996	
120	19.02 b ±2.25	24.33 b ±1.21	21.45 a ±1.63	13.19 c ±1.47	19.99 b ±1.72	0.73	0.009	<0.001	
SEM	2.28	0.92	0.90	1.04	2.55	-	-	-	
p-value	0.004	0.001	<0.001	<0.001	0.002	-	-	-	

SEM = standard error of the mean; L = linear effect; Q = quadratic effect; RSS × T = interaction effect between the relocated sorghum silage and times; significant at the 5% probability level. Different letters in the same row indicate statistical differences between them according to the Tukey test at a 5% probability level for Type I error.

Different inclusion levels of relocated sorghum silage had a quadratic effect on dry matter content at 24, 48, and 96 h of exposure to the aerobic environment, with a maximum point at 30% of RSS. Conversely, at 72 and 120 h, dry matter content increased ($p < 0.05$) as the levels of RSS inclusion in the TMR composition increased, in relation to the control treatment (0%) (Table 4). An interaction effect was observed between relocated sorghum silage inclusion levels and exposure times to air on CO_2 content ($p < 0.05$). Relocated sorghum silage inclusion had a quadratic effect on CO_2 content at 24 and 120 h of exposure to the aerobic environment, presenting the lowest CO_2 production with the inclusion of 30% of RSS in the TMR composition. There were reductions in CO_2 content at 48, 72, and 96 h ($p < 0.05$) as RSS levels increased in the TMR composition (Table 4).

3.3. Microbial Populations

For lactic acid bacteria populations at different silo opening times, a quadratic effect was observed for 0% ($p = 0.007$), 15% ($p = 0.002$), and 35% ($p < 0.001$) inclusion of relocated sorghum silage. A reduction in lactic acid bacteria population was observed at 25% inclusion ($p = 0.017$). No effect was observed at 30% inclusion for lactic acid bacteria populations at different silo opening times ($p > 0.05$). Enterobacteria did not predominate at any silage opening time (Figure 1).

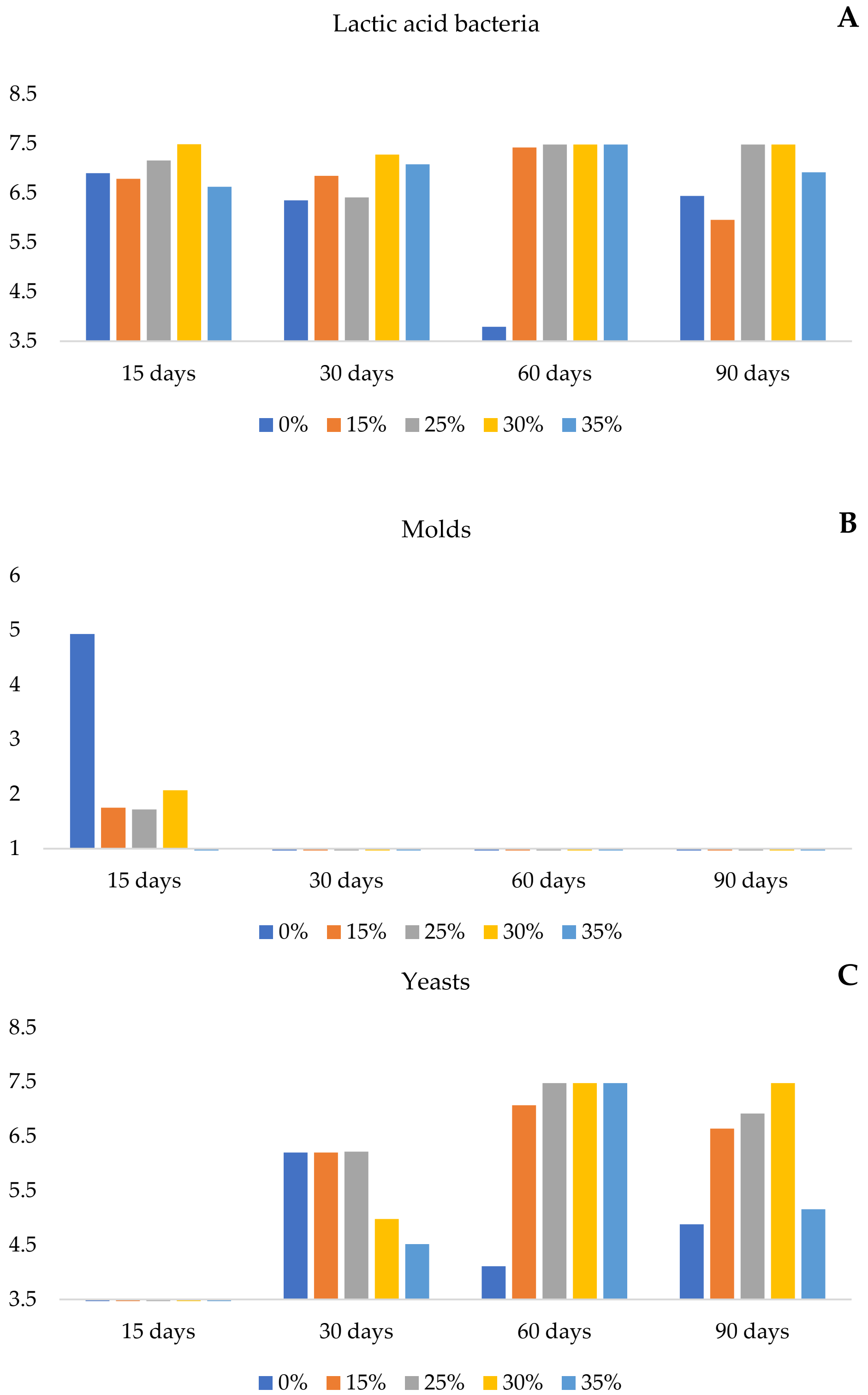

Figure 1. Microbial populations (log CFU/g fresh silage) ((**A**) Lactic acid bacteria; (**B**) Molds; (**C**) Yeasts) of total mixed rations based on cactus pear and relocated sorghum silage (0, 15, 25, 30, and 35%) on different opening days (15, 30, 60, and 90 days).

Regarding fungi populations, only the 0% relocated sorghum silage inclusion treatment showed a reduction in fungal counts at 15 days of silo opening ($p < 0.05$). For other inclusion levels, no fungal growth occurred at different evaluated opening times ($p > 0.05$) (Figure 1). For yeast quantification, 0% ($p = 0.022$), 15% ($p < 0.001$), 25% ($p < 0.001$), and 35% ($p < 0.001$) inclusion levels showed a quadratic effect. The inclusion of 30% relocated sorghum silage increased yeast populations ($p < 0.001$) (Figure 1).

3.4. Chemical Composition

As the inclusion levels of relocated sorghum silage in the total mixed ration silage increased, dry matter ($p < 0.001$), organic matter ($p < 0.001$), neutral detergent fiber (NDF, $p < 0.001$), acid detergent fiber (ADF, $p < 0.001$), hemicellulose ($p < 0.001$), and cellulose ($p < 0.001$) contents increased, while ash ($p < 0.001$), crude protein ($p < 0.001$), ether extract ($p < 0.001$), and non-fibrous carbohydrate ($p < 0.001$) contents decreased. A quadratic effect was observed for total carbohydrates ($p < 0.001$) presenting the highest CHOT content (711.68 g/kg DM) with the inclusion of 25% of RSS in the TMR composition. However, lignin concentration was not altered ($p > 0.05$) (Table 5).

Table 5. Average values and standard deviation of chemical composition of total mixed rations based on cactus pear and relocated sorghum silage opened after 90 days of ensiling.

Variables (g/kg)	Relocated Sorghum Silage (% Dry Matter)					SEM	*p*-Value	
	0	15	25	30	35		L	Q
DM	179.63 ±4.07	211.65 ±4.49	265.36 ±9.16	276.07 ±2.80	298.51 ±10.82	4.05	<0.001	0.014
Ash	98.06 ±1.02	87.11 ±3.56	88.68 ±3.35	75.21 ±6.57	74.83 ±3.22	2.36	<0.001	0.370
OM	901.94 ±1.02	912.89 ±3.56	911.32 ±3.35	924.79 ±6.57	925.17 ±3.22	2.36	<0.001	0.370
CP	197.49 ±5.31	195.98 ±8.87	172.14 ±5.44	174.55 ±5.61	180.44 ±4.22	3.67	<0.001	0.635
EE	38.73 ±2.39	38.73 ±2.57	32.64 ±1.08	33.13 ±2.93	35.17 ±3.65	1.19	<0.001	0.678
NDF	245.24 ±11.50	316.17 ±8.41	348.50 ±9.35	359.84 ±6.50	390.17 ±3.90	4.86	<0.001	0.315
ADF	124.22 ±3.01	176.24 ±6.28	191.42 ±6.85	204.73 ±3.84	219.83 ±4.44	2.92	<0.001	0.050
Lignin	1.40 ±0.59	1.99 ±0.62	1.56 ±0.25	1.83 ±0.54	1.80 ±0.56	0.27	0.410	0.467
HEM	122.44 ±8.88	137.39 ±9.11	158.59 ±13.61	160.40 ±8.10	173.83 ±3.75	5.31	<0.001	0.407
CEL	122.82 ±2.97	174.44 ±4.75	188.37 ±6.49	197.61 ±6.26	214.58 ±6.11	3.27	<0.001	0.039
CHOT	673.14 ±48.80	688.39 ±136.88	711.68 ±48.72	633.89 ±98.46	622.30 ±92.26	5.18	<0.001	<0.001
NFC	465.36 ±94.34	414.12 ±163.80	404.36 ±46.09	310.56 ±139.78	274.22 ±102.06	6.45	<0.001	<0.001

DM = dry matter; OM = organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein; HEM = hemicellulose; CEL = cellulose; CHOT = total carbohydrates; NFC = non-fibrous carbohydrates; SEM = standard error of the mean; L = linear effect; Q = quadratic effect; significant at the 5% probability level.

3.5. Carbohydrate and Protein Fractionation

For carbohydrate fractionation, a reduction in fraction A + B1 ($p < 0.001$) and increases in fractions B2 ($p < 0.001$) and C ($p = 0.015$) were observed as relocated sorghum silage levels increased in the total mixed ration silage. Regarding protein fractionation, a quadratic effect was observed for fractions A ($p < 0.001$) and C ($p = 0.029$). It was observed that the inclusion of 15% RSS in the TMR provided a higher content of fraction A (29.77 g/kg CP) and a lower content of fraction C (10.05 g/kg CP), in relation to the other RSS levels tested. There was an increase in fraction B1 + B2 ($p < 0.001$), and a reduction in fraction B3 ($p < 0.001$) as relocated sorghum silage levels increased in the total mixed ration silage (Table 6).

Table 6. Average values and standard deviation of carbohydrate and protein fractionation of total mixed rations based on cactus pear and relocated sorghum silage opened after 90 days of ensiling.

Variables	Relocated Sorghum Silage (% Dry Matter)					SEM	p-Value	
	0	15	25	30	35		L	Q
	Carbohydrate fractionation (g/kg CHOT)							
A + B1	691.28 ±9.68	601.37 ±11.70	568.20 ±6.68	489.58 ±14.79	440.41 ±10.55	6.39	<0.001	<0.001
B2	207.93 ±23.01	270.64 ±10.70	302.19 ±20.44	376.16 ±47.84	400.60 ±24.35	16.38	<0.001	0.097
C	100.79 ±82.17	127.99 ±3.94	129.61 ±18.92	134.26 ±38.43	158.99 ±27.77	14.40	0.015	0.794
	Protein fractionation (g/kg CP)							
A	24.54 ±1.74	29.77 ±1.33	23.56 ±0.52	23.25 ±0.51	23.08 ±0.65	0.57	<0.001	<0.001
B1 + B2	958.02 ±1.12	958.76 ±1.26	962.47 ±1.70	963.39 ±0.73	964.47 ±0.78	0.79	<0.001	<0.001
B3	6.78 ±0.62	5.43 ±1.06	3.37 ±1.82	2.89 ±0.91	1.15 ±0.71	0.63	<0.001	0.184
C*	10.66 ±0.02	10.05 ±0.68	10.61 ±0.49	10.46 ±0.60	11.29 ±0.04	0.30	0.236	0.029

Carbohydrate fractionation: CHOT = total carbohydrates; fraction A + B1 (non-fibrous carbohydrates); fraction B2 (fibrous carbohydrates from the cell wall and of slow ruminal availability, susceptible to the effects of the passage rate); fraction C (indigestible neutral detergent fiber). *Protein fractionation*: CP = crude protein; fraction A (non-protein nitrogen), fraction B1 (soluble protein rapidly degraded in the rumen) + B2 (insoluble protein with intermediate degradation rate in the rumen), fraction B3 (insoluble protein with slow degradation rate in the rumen), and fraction C* (insoluble protein, indigestible in the rumen and intestine); SEM = standard error of the mean; L = linear effect; Q = quadratic effect; significant at the 5% probability level.

4. Discussion

The pH values ranged from 3.78 to 3.98, which is within the acceptable range for a good fermentation process, according to Muck [15], reducing the possibility of undesirable microorganism development. These satisfactory pH results are associated with the use of cactus pear in total mixed ration silage due to its high content of soluble carbohydrates [40]. Lactic acid bacteria utilize these soluble sugars as a substrate for their growth, producing organic acids that lower the pH and acidify the environment. Consequently, deleterious microorganisms are inhibited, ensuring excellent preservation of the ensiled mass [41]. In this context, it is worth emphasizing the importance of using cactus pear in total mixed ration silage, especially when relocated silages are used, as observed by Macêdo et al. [7]. These silages typically have low levels of soluble carbohydrates, which would hinder the fermentation process due to the slow pH drop.

The ammoniacal nitrogen content in silage is an indicator of proteolytic activity, representing the amount of protein degraded during fermentation and serving as an indirect marker of clostridial activity [42]. In this study, all total mixed ration silages had ammoniacal nitrogen levels within the desirable range, varying from 12.70 to 23.63 g/kg of total nitrogen, aligning with Costa et al. [43], who emphasize that well-fermented silages should have $N\text{-}NH_3$ levels below 100 g/kg of total nitrogen.

The reduction in buffering capacity in silages is related to the low content of buffer substances in sorghum plants and the decrease in the diet of forage cactus since forage cactus is rich in minerals, such as K^+, Ca^{2+}, and Mg^{2+}, which strongly influence this variable [44]. This can be observed by the ash content, which was reduced as the participation of forage cactus in the TMR was reduced. However, the reduction in buffering capacity did not negatively affect the reduction in pH and, consequently, the preservation of the ensiled mass.

Despite silages having dry matter content below 30% [45] for proper fermentation, the inclusion of relocated sorghum silage increased the silage density to above 500 kg/m^3. According to Saute et al. [46], silages with a compaction density above 550 kg/m^3 ensure anaerobic conditions, thus not compromising the fermentation process. The inclusion of over 15% relocated sorghum silage enabled the production of total mixed ration silages, meeting established standards, reducing losses, and increasing dry matter recovery.

Gas and effluent losses are directly influenced by forage moisture content [47]. Excess moisture is associated with the development of *Clostridium* bacteria and enterobacteria, increasing silage gas production. Notably, gas and effluent losses were minimized with the inclusion of relocated sorghum silage in the total mixed ration silages, demonstrating its capacity to absorb silage moisture and enhance dry matter recovery. Exclusively ensiling high-moisture feedstuffs can negatively affect fermentation characteristics, increase gas and effluent losses, and reduce dry matter recovery [41], as observed in silages with higher proportions of cactus pear (0% RSS). Therefore, the association of cactus pear with RSS is beneficial since it contributes to reducing nutrient losses through leaching, improving the nutritional value of TMR.

The inclusion of relocated sorghum positively influenced porosity and permeability, as these variables are strongly affected by silage density and dry matter content [48]. Increased dry matter and silage density reduced porosity and improved silage compaction—a satisfactory result, facilitating aeration within the silo, increasing density, and reducing aerobic stability. Aerobic stability loss is generally expressed by temperature rise and high pH values [49]. However, no stability loss was observed in the evaluated silages, as the silage temperature did not exceed 2 °C above ambient temperature during 120 h of air exposure. The high aerobic stability may be attributed to adequate acetic acid concentrations, which inhibit fungal and yeast activity, preventing silage spoilage [50]. This ensures prolonged nutrient preservation for animal consumption. Future studies are necessary to better understand organic acid responses in these silages.

This study demonstrated the successful use of high-moisture forage in ensiled diets without significantly altering pH, temperature, or aerobic stability variables. The aerobic stability results are consistent with those reported by Macêdo et al. [7] in total mixed ration silages based on cactus pear. Similarly, relocated sorghum silage did not compromise the aerobic stability of total mixed ration silage, allowing the use of both high-moisture ingredients like cactus pear and relocated silage. These findings align with Anjos et al. [11], who observed stable material temperatures when using relocated sorghum silage, indicating maintained aerobic stability.

Carbon dioxide production gradually decreased with increased relocated sorghum silage inclusion and silage air exposure. This highlights reduced dry matter and nutrient losses during silage oxygen exposure. The low CO_2 values in this study correlate with silage pH maintenance and likely adequate acetic acid production, preventing spoilage by undesirable microorganisms. According to McDonald et al. [45], nutrient oxidation leads to heat production, pH rise, water, and CO_2 generation. However, high pH values were not observed during oxygen exposure, indicating well-preserved silage with no aerobic stability loss.

All silages showed similar LAB production results, averaging 5 to 7 log CFU/g silage, indicating effective ensiled mass fermentation dominated by LAB over other microbial populations. This was facilitated by the high soluble carbohydrate content in sorghum and cactus pear, as reported by Santos et al. [5]. This result provided a reduction in CHOT content, with a greater reduction with the inclusion of 30–35% relocated sorghum, probably due to cell wall catabolism, providing more fermentative potential substrates like glucose for LAB [51].

As expected, fungal counts were highest upon initial opening and absent after the second opening, due to anaerobiosis and intense lactic fermentation within the silo, inhibiting fungal development [11]. The use of relocated sorghum silage likely introduced an active LAB population from prior fermentation. Enterobacteria growth was absent across treatments, attributed to the low silage pH, which inhibits their development.

The association of ingredients used in the preparation of TMRs was beneficial as it improved the nutritional value of the silages, increasing the CP content above the 7% recommended for adequate rumen fermentation, without compromising the efficient use of the fibrous carbohydrates in the silages [7]. Thus, the use of these silages in the feeding of ruminants, especially sheep, is recommended because, in addition to providing a water reserve, it improves the crude protein levels of the diets, since the total mixed ration silages meet the dietary needs of sheep, as the NRC [19] suggests 130 g of CP for 200 g of daily live weight gain in sheep. When the CP level is below 7% in the diet, there is low nitrogen availability, which can reduce the digestion of fibers and reduce consumption due to the slow passage of the food through the rumen [19]. Likewise, to avoid limiting feed intake, ruminant diets must have EE content below 5%, as higher values can reduce dry matter intake [52]. Our results indicate all silages are suitable for ruminant feeding, optimizing intake without limitations due to high energy concentrations [53]. This balance is crucial for ruminal fermentation, fiber digestibility, and passage rate.

Silages without relocated sorghum had the lowest NDF, ADF, hemicellulose, and lignin levels, explained by cactus pear's lower structural carbohydrate concentration and hemicellulose hydrolysis into monosaccharides, providing extra substrate for lactic acid production during fermentation [54]. Increasing relocated sorghum inclusion raised NDF, ADF, hemicellulose, cellulose, and B2 and C fractions, potentially limiting nutrient digestibility by rumen microbes and affecting silage nutritional quality [55]. However, despite the increase in these attributes, NDF and ADF values remained below the 60% [52] and 40% [56] thresholds for ruminant diets, which may benefit dry matter intake and digestibility, providing a higher passage rate.

The determination of carbohydrate and protein fractions is of utmost importance for the animal nutritionist. This information is used in diet formulations for ruminants, maximizing the synchronization between carbohydrates and nitrogenous compounds, minimizing energy and nitrogen losses due to ruminal degradation, and promoting greater microbial synthesis efficiency [57]. Thus, evaluating the nutritional resources available in an animal production system allows for the formulation of diets that meet the animals' requirements so they can express their full potential, according to their physiological stages, following the recommendations of the NRC [19,58].

Due to the nutritional variation in the feed offered to animals, such as the total mixed ration silages evaluated in this research, with the responses obtained through carbohydrate and protein fractionation analysis, it is possible to predict the retention period of the TMR in the fermentation chamber (reticulum–rumen), and the components of this feed that may hinder digestion and fermentation, interfering with the prediction of animal performance. Therefore, for proper characterization, the nutrients used in ruminant feeding must be fractionated [59].

The reduction in the cactus pear content, together with the consumption of soluble carbohydrates for fermentation and consequent reduction in pH, reduced non-fibrous carbohydrates and fractions A + B1. This is significant since NFCs are rapidly digestible in the rumen and provide substrate for non-fibrous carbohydrate fermenting bacteria that produce short-chain fatty acids due to their higher rate of passage from the rumen to the intestine [60]. According to Silva and Silva [61], the increase in the C fraction (indigestible neutral detergent fiber) and the reduction in the A + B1 fractions (non-fibrous carbohydrates) imply a reduction in the availability of energy for microorganisms that ferment fibrous and non-fibrous carbohydrates, which could influence the efficiency of microbial protein synthesis. Since ruminal bacteria require energy for microbial protein synthesis, the low availability of the A + B1 fraction may result in N losses via feces and urine, which is not convenient in a production system [61]. For Van Soest [52], a maximum of 44% NFC is necessary for optimal ruminal function. Therefore, all silages with reallocated sorghum meet this premise, due to the availability of carbohydrates, which are the main source of energy for ruminants in the semi-arid region, especially in periods of prolonged drought.

The determination of nitrogen fractions estimates the non-protein nitrogen (NPN) content, true protein degradation in the rumen, and unavailable nitrogen [24]. The inclusion of reallocated sorghum silage reduced the A fraction and increased B1 + B2, probably due to the fibrous characteristics of sorghum, as mentioned above where the increase in RSS levels increased the proportion of the C fraction of the TMRs. In addition, there was a reduction in the proportion of urea (non-protein nitrogen readily available to be used by ureolytic bacteria for microbial protein synthesis) in the TMRs' composition, as RSS levels in the silage composition increased. Sniffen et al. [24] observed that the B1 + B2 fractions degrade rapidly in the rumen compared to B3, meeting the nitrogen needs of rumen microorganisms. Higher levels of RSS reduced the B3 fractions but did not affect the C fraction. Although digestible, the B3 fraction degrades slowly in the rumen, with a ruminal degradation rate between 0.02 and 1.0% h^{-1}, according to Viana et al. [62]. This protein fraction (B3) is represented by the protein fraction bound to the cell wall, which can pass through the animal's digestive tract and be absorbed in the intestine or excreted by the animal [63]. Therefore, it is possible that, as RSS has a higher proportion of cell wall components compared to prickly pear, the increased levels of RSS in the composition of TMRs provided a reduction in the B3 fraction.

5. Conclusions

It is recommended to relocate sorghum silage in the total mixed ration to improve microbiological characteristics, reduce gas and effluent losses, and increase dry matter recovery and nutritional aspects of silage when associated with cactus pear. Future studies that quantify the organic acids of the studied silages are necessary and pertinent.

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