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Fermentative characteristics and chemical composition of cochineal nopal cactus silage containing chemical and microbial additives

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Abstract

The objective of this study was to evaluate the fermentative characteristics and chemical composition of cochineal nopal cactus silage additives with urea or *Lactobacillus buchneri* (LB), as well as the association of both additives in four storage times (7, 15, 60 and 120 days) and during aerobic stability, with evaluations at 0, 48 and 96 h. Four silages were used: no additive, addition of 2% urea, addition of LB and addition of 2% urea and LB. The study was divided into two experiments: the first experiment evaluated the silages at different storage times, and the second experiment evaluated the silages during the aerobic stability test. In both experiments, the experimental design was completely randomized in a factorial scheme (4 × 4 and 4 × 3) with three replicates per treatment. After the ensiling process, lactic acid bacteria predominated in all treatments. The concentration of lactic acid increased significantly from 60 days of ensiling. The concentration of acetic acid varied significantly between the storage times only for the silages treated with urea and LB alone. The silage treated with urea maintained a constant pH value up to 120 days of storage. During the 96 h aerobic stability test, no breaking in the stability of silages was observed. The exclusive or associated use of urea and LB promotes improvement in the fermentative characteristics of cochineal nopal cactus silage, without major alterations in the chemical composition or interfering with the aerobic stability of the silages.

Introduction

The cochineal nopal cactus has specific characteristics, such as low dry matter (DM) content (<200 g/kg) and high water-soluble carbohydrates (WSC) content (>120 g/kg of DM), that are unfavourable to ensiling and favourable to the growth of undesirable microorganisms (Nogueira *et al*., 2019). In addition, the aerobic stability of the ensilage is low after the silo is opened (Brito *et al*., 2020). When the silage is exposed to oxygen, yeasts present in the silage are reactivated and start aerobic deterioration in which the residual WSC and lactic acid are consumed, which increases the temperature and pH of the silage (Pahlow *et al*., 2003). According to Nogueira *et al*. (2016), cochineal nopal cactus silage was stable during a period of 24 h of air exposure, while Brito *et al*. (2020) reported that cochineal nopal cactus silage without additives was stable for 28 h under aerobic conditions.

McDonald *et al*. (1991) classify lactic acid bacteria and urea as additives that inhibit aerobic deterioration. Heterofermentative lactic acid bacteria such as *Lactobacillus buchneri* have been used extensively to help control yeast populations through the acetic acid production (Miranda, 2006). The addition of urea to the silage produces a similar effect. In contact with forage silage, urea is transformed into ammonia, which has an inhibitory effect on the yeast populations (Pedroso *et al*., 2007).

Therefore, it was hypothesized that the use of urea, *L. buchneri*, or a combination of the two additives can promote improvements in the fermentation profile, chemical composition and aerobic stability of the cochineal nopal palm silage in four storage times. Thus, the objective of the current study was to evaluate the fermentation characteristics, chemical composition and aerobic stability of cochineal nopal cactus silages supplemented with urea, *L. buchneri*, or a combination of both additives.

Materials and Methods

The study was conducted in the Forage Production Sector of the Department of Animal Science of the Federal University of Paraíba (UFPB) in Areia – Paraíba, Brazil. Laboratory

analyses were performed in the forage production and animal nutrition laboratories of the same institution.

The cochineal nopal cactus (*Nopalea cochenillifera* Salm Dyck), commonly known in Brazil as *palma Miúda*, was used. The cultivated cactus had a regrowth age of two years and was obtained from a crop that was previously established crop on the experimental farm of the *Empresa Estadual de Pesquisa Agropecuária da Paraíba* (Paraiba's Enterprise for Agricultural Research, EMEPA), located in the Agreste of Paraíba mesoregion, Curimataú microregion, municipality of Soledade, at coordinates 7° 8′ 18″ S and 36° 27′ 2″ W.Gr. at an altitude of 534 m. Based on the Köppen classification, the climatic type of the region is Bsh (hot semiarid) with rainfall from January to April, an average annual temperature of 24°C, average relative humidity of ∼68%, and average annual rainfall of ∼400 mm; drought conditions persist during almost the entire year (Oliveira Jr. *et al.*, 2009).

The cochineal nopal cactus was harvested manually, preserving the primary cladodes. Later, the cladodes were processed in a stationary forage machine (MC1001N Laboremus®), obtaining particles of ∼2 cm. Additives were mixed with the cochineal nopal cactus as follows: Control: no additive; urea: addition of urea to 2% of the DM weight; LB: addition of *L. buchneri* CNCM I – 4323 (1.0×10^{11} cfu/g); this was applied as a commercial inoculant (Lalsil® AS, Lallemand) that was developed to increase the aerobic stability of silage, and it was applied as recommended by the manufacturer; and U + LB: addition of urea and *L. buchneri* in the above-mentioned proportions.

Two experiments were performed in this study. The first experiment evaluated the silages at various storage times, and the second experiment evaluated the properties of the silages during the aerobic stability test.

First experiment: The silages were prepared in 48 experimental PVC (polyvinyl polychloride) silos, 10 cm in diameter and 30 cm in height, to achieve a final packing density of 600 ± 20 kg of fresh matter/m^3. After addition of the silage material, the silos were closed and stored at room temperature in a covered, dry and ventilated place until being opened at 7, 15, 60 and 120 days after ensiling. At each opening time, the microbial populations, the fermentation profile, the fermentation losses and the chemical composition of the silage were determined.

Second experiment: For the aerobic stability test, the silages were produced using the same compaction density mentioned above in 12 PVC silos (15 cm diameter and 30 cm height) fitted with *Bunsen* valves for release of the gases resulting from fermentation. At the bottom of each silo, 1 kg of dry sand was added and covered with a TNT screen (nonwoven fabric) to collect the effluents. At the end of this process, the silos were closed, weighed and stored at room temperature in a covered, dry and ventilated place until they were opened 120 days after ensiling.

The aerobic stability test lasted 96 h; the microbial populations, fermentation profile, chemical composition and recovery of DM from the silage were determined after 0, 48 and 96 h of air exposure. At each evaluation time in both experiments, the pH of the silage was determined according to the methodology described by Bolsen *et al.* (1992), and samples (∼400 g) of the silage were collected for subsequent evaluations.

The microbial populations were quantified before ensiling and in the silages using selective culture media for each microbial group. MRS (de Man, Rogosa and Sharpe) agar containing 0.4% nystatin was used for lactic acid bacteria, Violet Red Bile agar was used for enterobacteria and potato dextrose agar containing 1% of a 10% tartaric acid solution was used for moulds and yeasts.

The quantification of the microbial groups was performed using 10 g of a sample obtained from the replicates of each treatment. To each sample, 90 ml of sterile distilled water was added to obtain a 10^{-1} dilution, and the sample was homogenized for 1 min. Serial dilutions ranging from 10^{-1} to 10^{-9} were then prepared, and the culture was performed in sterile disposable Petri dishes. The Petri dishes were incubated at specific incubation temperatures for each microbial group (Ávila *et al.*, 2014; Santos *et al.*, 2014). For lactic acid bacteria, the dishes were incubated at 37°C for 48 h; for enterobacteria, they were incubated at 30°C for 24 h, and for moulds and yeasts, they were incubated at 28°C for 72 h. Petri dishes containing 30–300 colony-forming units (cfu) were considered appropriate for counting.

Organic acids (lactic, acetic, propionic and butyric acids) and ethanol were determined using the methodology described by Siegfried *et al.* (1984) in a Shimadzu high-performance liquid chromatography system that included a model SPD-10^{a} VP detector coupled to an ultraviolet detector, at a wavelength of 210 nm. For the evaluation of ammoniacal nitrogen and the buffering capacity (BC) of the silages, the methods described by Bolsen *et al.* (1992) and Playne and McDonald (1966), respectively, were used.

Approximately 300 g of silage samples were pre-dried in a forced air oven (60°C), processed in a Willey mill with a 1 mm sieve and analysed according to the AOAC protocols (1990) for the determination of DM (ID 930.15) and crude protein (CP) (ID 954.01) content. The WSC content of the samples was determined using the concentrated sulphuric acid method described by Dubois *et al.* (1956) as modified by Corsato *et al.* (2008). The methodologies described by Van Soest *et al.* (1991) were used to determine the level of neutral detergent fibre (NDF), not analysed with heat-stable amylase and sodium sulphite (NDFom) and not expressed exclusive of residual ash.

The chemical composition of cochineal nopal cactus before ensiling was 159.9 g/kg of DM, 21.6 g/kg DM of CP, 226.4 g/kg DM of NDF not analysed with heat-stable amylase and sodium sulphite and 173.5 g/kg DM of WSC. The DM losses in the silages in the form of gas and effluent were quantified by weight difference using the equations described by Zanine *et al.* (2010):

$$G = (\mathrm{WFc} - \mathrm{WFo})/(\mathrm{FMc} \times \mathrm{DMc}) \times 10000$$

where G is the gas losses (% of DM), WFc is the weight of the filled silo at closing (kg), WFo is the weight of filled silo at opening (kg), FMc is the forage mass at silo closing (kg) and DMc is the forage DM concentration at silo closing (%).

$$E = [(\mathrm{WEf} - \mathrm{Tb}) - (\mathrm{WEi} - \mathrm{Tb})]/\mathrm{FMi} \times 100$$

where E is the effluent losses (kg/t of fresh matter), WEi is the weight of the empty silo + sand at closing (kg), WEf is the weight of the empty silo + sand at opening (kg), Tb is the weight of the empty silo (kg) and FMi is the forage mass at silo closing (kg).

The DM recovery was estimated based on the difference in feed weight and DM concentration before and after ensiling using the equation described by Zanine *et al.* (2010):

$$\mathrm{DMR} = (\mathrm{FMo} \times \mathrm{DMo})/(\mathrm{FMc} \times \mathrm{DMc}) \times 100$$

where DMR is the DM recovery rate (%), FMo is the forage mass at silo opening (kg), DMo is the forage DM concentration at silo

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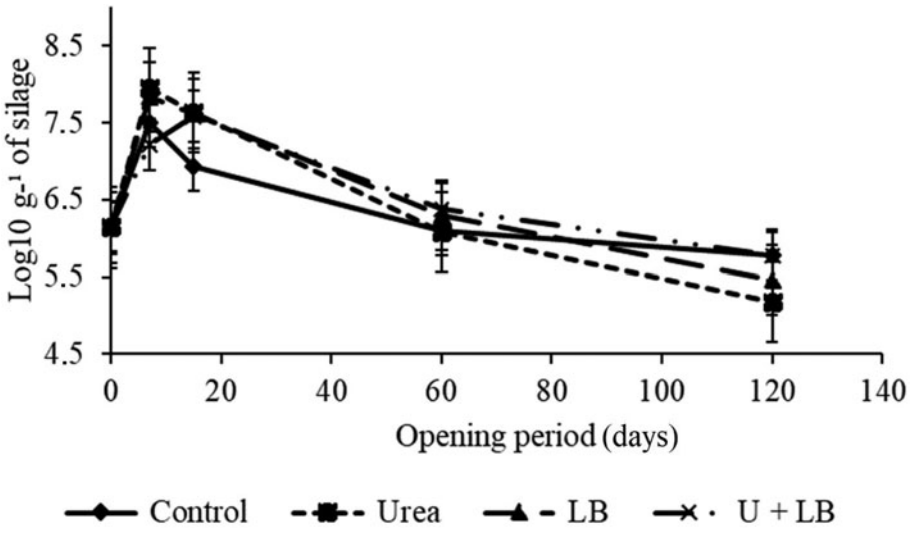

Fig. 1. Number of lactic acid bacteria present in cochineal nopal cactus silages containing chemical and microbial additives as a function of opening time (days). Control, without additive; Urea, addition of urea; LB, addition of *Lactobacillus buchneri*; U + LB, addition of urea and *Lactobacillus buchneri*.

opening (%), FMc is the forage mass at silo closing (kg) and DMc is the forage DM concentration at silo closing (%).

After 120 days of ensiling, the aerobic stability of the silages (expressed in hours) was evaluated by monitoring the surface and internal temperatures of the silages air exposure. The silage samples were placed without compaction in unlidded PVC experimental silos and maintained in a controlled environment (25°C). Temperatures were recorded hourly using digital laser and digital immersion thermometers positioned at the centre of the silage mass. The beginning of deterioration was defined as the point at which the internal temperature of the silage reached 2°C above room temperature (Kung Jr. *et al.*, 2000).

For both experiments, the experimental design was completely randomized in a factorial design. First experiment adopted a 4 × 4 (four additives and four opening times) design with three replicates per treatment. For the variables associated with aerobic stability (second experiment), a 4 × 3 factorial design (four additives and three air exposure times) with three replicates per treatment was used. The data were statistically analysed by analysis of variance, and when significant, the means were compared using Tukey's test at a significance level of 5% using SISVAR software (Ferreira, 2008).

The statistical model used was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$

where μ is the population mean, α_i is the treatment effect, β_j is the time effect, γ_{ij} is the treatment × time interaction effect and ϵ_{ijk} is the residual error.

Data regarding the temperatures in the aerobic stability test and the quantification of the microbial groups (in logarithmic units, $\log_{10}$) were analysed in a descriptive manner.

Results

First experiment

Lactic acid bacteria numbers increased following ensiling to reach a peak of between 7 and 8 $\log_{10}$ cfu/g by 7–14 days post ensiling. Peak counts in silage treated with urea plus LAB was delayed compared to other treatments. Thereafter LAB counts declined in all silages to between 5 and 6 cfu/g $\log_{10}$ by 120 days (Fig. 1).

Moulds and yeasts numbers increased following ensiling to reach a peak of between 6 and 7 $\log_{10}$ cfu/g by 7–14 days post ensiling. Peak counts in silage treated with urea and control plus MY was delayed compared to other treatments. However, at 60 days MY counts increasing in silages treated only with urea and *L. buchneri* to between ND and 5 cfu/g $\log_{10}$ (Fig. 2).

On day 7 of ensiling, the pH was reduced to about 4 and remained around this level throughout the opening periods (Fig. 3(*a*)). There were changes in the concentrations of acetic and lactic acids during the opening periods. In all silages, except for control silage, the highest levels of acetic acid were observed at 120 days of ensiling (Fig. 3*b*)). A similar result was observed for lactic acid, except for silage treated with urea, which presented the highest concentration of lactic acid at 60 days of ensiling (Fig. 3(*c*)).

At 60 days of ensiling, the pH ($P < 0.003$) and BC ($P < 0.010$) were significantly lower in the control silages and treated with urea and *L. buchneri*. The ethanol content ($P < 0.001$) was lower in the silage treated with *L. buchneri*. The highest acetic acid ($P < 0.006$), NH_3-N ($P < 0.001$) and CP ($P < 0.001$) contents were observed in urea-treated silage. However, there was no effect of treatments on lactic ($P = 0.438$), propionic ($P = 0.685$) and butyric ($P = 0.512$) acids contents. The control silage had the highest DM content ($P < 0.002$), while in the silage treated with urea had the lowest WSC content ($P < 0.046$). However, the silage treated with *L. buchneri* had the lowest NDF content ($P < 0.044$; Table 1).

At 120 days of ensiling, the control silage showed the lowest BC ($P < 0.004$), ethanol ($P < 0.001$), acetic acid ($P < 0.025$), propionic acid ($P < 0.046$) and NH_3-N ($P < 0.001$) contents. However, there was no effect of treatments on the pH ($P = 0.890$), lactic acid ($P = 0.551$) and butyric acid ($P = 0.086$; Table 2) contents. The highest CP ($P < 0.001$) and lowest WSC ($P < 0.019$) and NDF ($P < 0.042$) contents were observed in the silage treated with urea. The control silage was the one with the highest DM content ($P < 0.001$) when compared to the other silages (Table 1).

There was a reduction in DM and WSC contents in all silages during the opening periods, except for urea and *L. buchneri* treated silage. However, all silages had the lowest DM and WSC contents at 120 days with the control silage showing the highest value when compared to other treatments (Figs. 4(*a*) and (*b*)). At 60 days of ensiling all silages presented the lowest NDF contents with the silage treated with urea presenting the lowest value (Fig. 4(*c*)).

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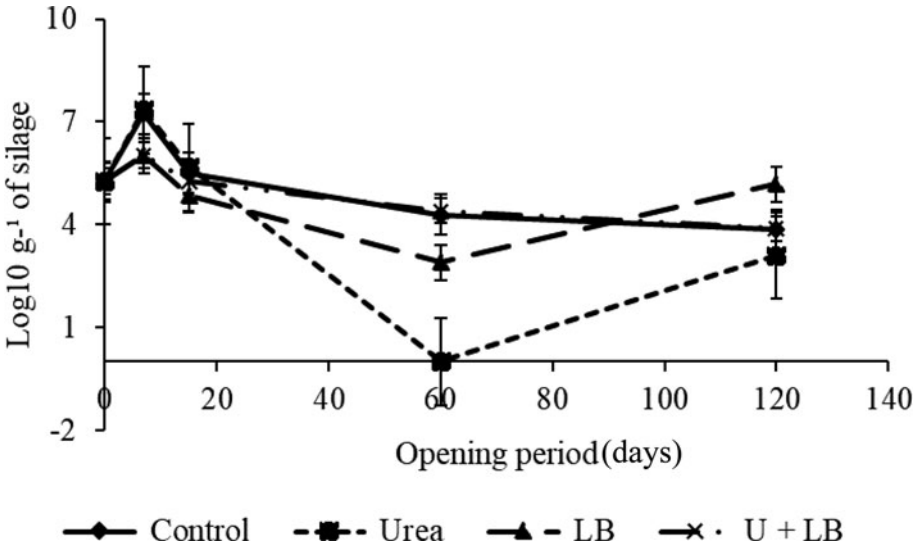

Fig. 2. Quantification of moulds and yeasts present in cochineal nopal cactus silages containing chemical and microbial additives as a function of opening time (days). Control, without additive; Urea, addition of urea; LB, addition of *Lactobacillus buchneri*; U + LB, addition of urea and *Lactobacillus buchneri*.

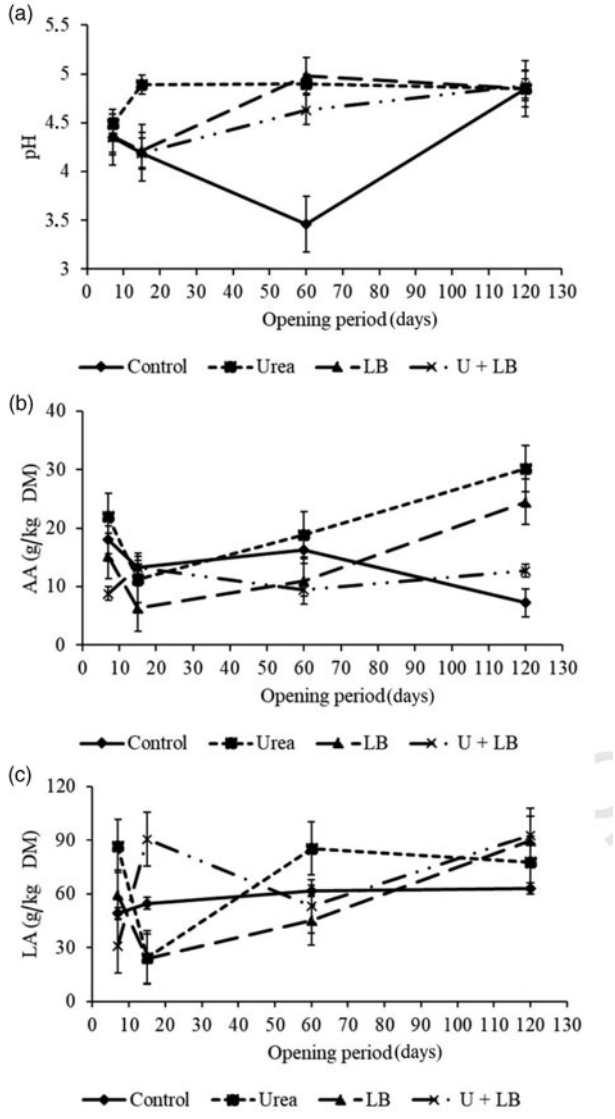

Fig. 3. Mean values of treatments according to the opening periods for pH (a); acetic acid (b) and lactic acid (c). Control, without additive; Urea, addition of urea; LB, addition of *Lactobacillus buchneri*; U + LB, addition of urea and *Lactobacillus buchneri*.

There was a treatment effect for CP content of cochineal nopal cactus silage containing chemical and microbial additives ($P < 0.001$). However, there was no effect of treatments on DM recovery ($P = 0.980$), gas losses ($P = 0.593$) and effluent losses ($P = 0.999$), presenting values of 961.8 g/kg, 27.1 g/kg and 11.9 kg/t of DM, respectively (Table 2).

Second experiment

Figures 5(*a*) and (*b*) show the results of aerobic stability tests of the surface and internal layers of the silages obtained in all treatments. None of the silages obtained in the current study showed deterioration during the 96 h evaluation of aerobic stability.

During air exposure, lactic acid bacteria numbers increased in all silages to reach a peak between 5 and 6 $\log_{10}$ cfu/g by 0–48 h post opening the silo. However, a reduction in the population of LAB was observed only in silage treated with urea at 96 h of air exposure (Fig. 6(*a*)).

There was a reduction in the mould and yeast numbers in silage treated with *L. buchneri* from 5 to 2 $\log_{10}$ cfu/ g by 0–96 h post opening the silo. However, other silages showed an increase in MY numbers by 0–48 h keeping stable until 96 h of air exposure (Fig. 6(*b*)).

There were no significant variations in the ethanol and NH_3-N contents during air exposure, although the control silage showed the lowest ethanol and NH_3-N contents (Figs. 7(*a*) and (*b*)). However, changes in acetic acid content were observed throughout the aerobic stability test, with the silage treated with urea and *L. buchneri* showing the lowest content of acetic acid at 96 h of air exposure (Fig. 7(*c*)).

Discussion

First experiment

The results obtained in Fig. 1 suggest that the treatments evaluated did not affect the growth of lactic acid bacteria. However, the population of moulds and yeasts in silage treated with urea disappeared after 60 days of storage, reappearing at 120 days (Fig. 2). This was expected since both urea and *L. buchneri* have an inhibitory effect on the growth of moulds and yeasts

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Table 1. Fermentation profile and selected nutritive value parameters for silage after 60 and 120 days ensiling

	Treatments[a]					
Variables	Control	Urea	LB	U + LB	SEM	*P* value
After 60 days ensiling						
pH	3.46	4.90	4.98	4.63	0.071	0.003
BC (E.mg/100 g DM)	100.00	140.00	110.00	100.00	0.013	0.010
Ethanol (g/kg DM)	13.8	36.9	8.5	24.4	0.28	0.001
Lactic acid (g/kg DM)	6.2	8.5	4.5	5.3	0.80	0.438
Acetic acid (g/kg DM)	16.3	18.9	10.9	9.5	0.12	0.006
Propionic acid (g/kg DM)	0.32	0.38	0.36	0.24	0.010	0.685
Butyric acid (g/kg DM)	0.34	0.32	0.24	0.40	0.021	0.512
NH_3-N (% TN)	0.09	0.31	0.12	0.11	0.013	0.001
CP (g/kg DM)	23.4	39.4	23.8	26.8	0.13	0.001
DM (g/kg FM)	148.8	128.6	135.5	129.6	0.16	0.002
WSC (g/kg DM)	100.5	38.8	72.5	51.4	0.40	0.046
NDF (g/kg DM)	190.9	187.3	186.0	198.6	0.51	0.044
After 120 days ensiling						
pH	4.85	4.85	4.85	4.88	0.012	0.890
BC (E.mg/100 g DM)	100.00	240.00	180.00	120.00	0.015	0.004
Ethanol (g/kg DM)	27.7	68.1	44.1	35.7	0.43	0.001
Lactic acid (g/kg DM)	6.3	7.8	9.0	9.3	0.44	0.551
Acetic acid (g/kg DM)	7.2	30.2	24.5	12.7	0.32	0.025
Propionic acid (g/kg DM)	0.13	0.49	0.17	0.21	0.030	0.046
Butyric acid (g/kg DM)	0.44	0.44	0.46	0.33	0.010	0.086
NH_3-N (% TN)	0.10	0.43	0.13	0.18	0.022	0.001
CP (g/kg DM)	21.2	42.4	23.6	24.5	0.13	0.001
DM (g/kg FM)	142.8	122.5	127.9	132.2	0.17	0.001
WSC (g/kg DM)	70.3	50.1	53.4	70.8	0.31	0.019
NDF (g/kg DM)	192.9	187.6	200.8	218.4	0.42	0.042

pH, hydrogen potential; BC, buffering capacity; NH_3-N, ammonia nitrogen; CP, crude protein; DM, dry matter; WSC, water-soluble carbohydrates; NDF, neutral detergent fibre; EM, standard error of the mean.
[a]Control = without additive; Urea = addition of urea; LB = addition of *Lactobacillus buchneri*; U + LB = addition of urea and *Lactobacillus buchneri*.

Table 2. Mean crude protein (CP), dry matter recovery (DMR), gas losses (GL) and effluent losses (EL) in cochineal nopal cactus silage containing chemical and microbial additives after 120 days of storage

	Treatments[a]					
Variable	Control	Urea	LB	U + LB	SEM	*P*-value
CP (g/kg DM)	22.1[b]	42.4[a]	23.6[b]	24.5[b]	0.13	0.001
DMR (g/kg DM)	964.4	958.4	961.0	962.9	0.46	0.980
GL (g/kg DM)	25.4	29.3	28.6	24.9	0.14	0.593
EL (kg/t DM)	11	12	12	12	3.5	0.999

DM, dry matter; SEM, standard error of the mean.
[a]Means followed by the same letters in the row do not differ according to Tukey's test at the 5% probability level. Control = without additive; Urea = addition of urea; LB = addition of *Lactobacillus buchneri*; U + LB = addition of urea and *Lactobacillus buchneri*.

(Neumann *et al.*, 2010; Santos *et al.*, 2013). Their reappearance is probably related to the ambient conditions and the substrate becoming favourable for spore activation.

Several studies in the literature show the efficiency of urea as an inhibitor of the yeast population (Alli *et al.*, 1983; Pedroso *et al.*, 2006; Pedroso *et al.*, 2007; Pedroso *et al.*, 2008; Lopes and

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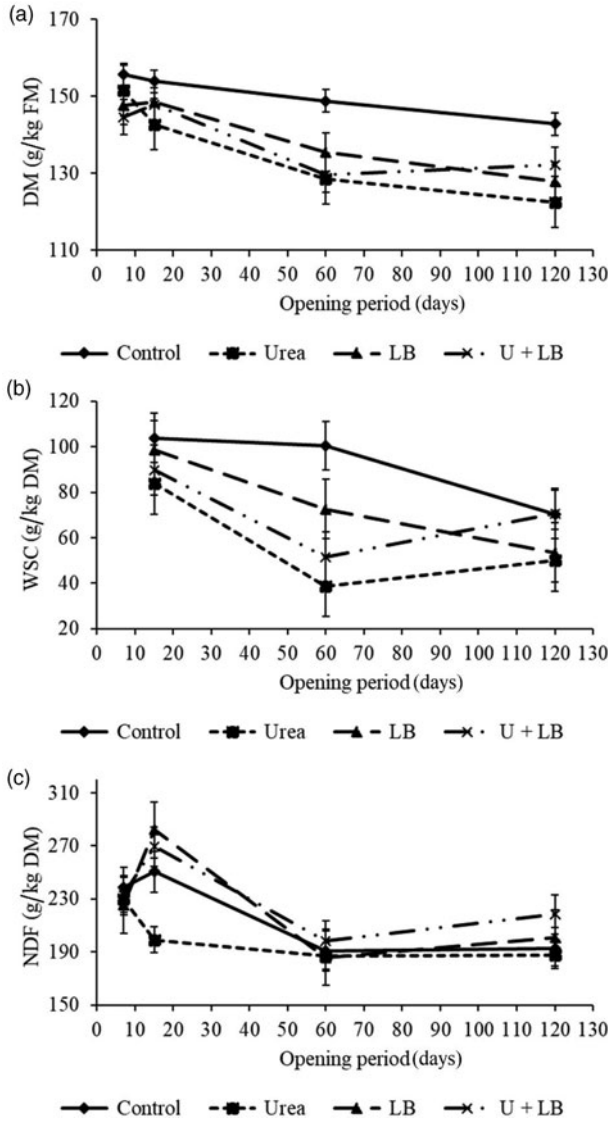

Fig. 4. Mean values of treatments according to the opening periods for dry matter (a); water-soluble carbohydrates (b) and neutral detergent fibre (c). Control, without additive; Urea, addition of urea; LB, addition of *Lactobacillus buchneri*; U + LB, addition of urea and *Lactobacillus buchneri*.

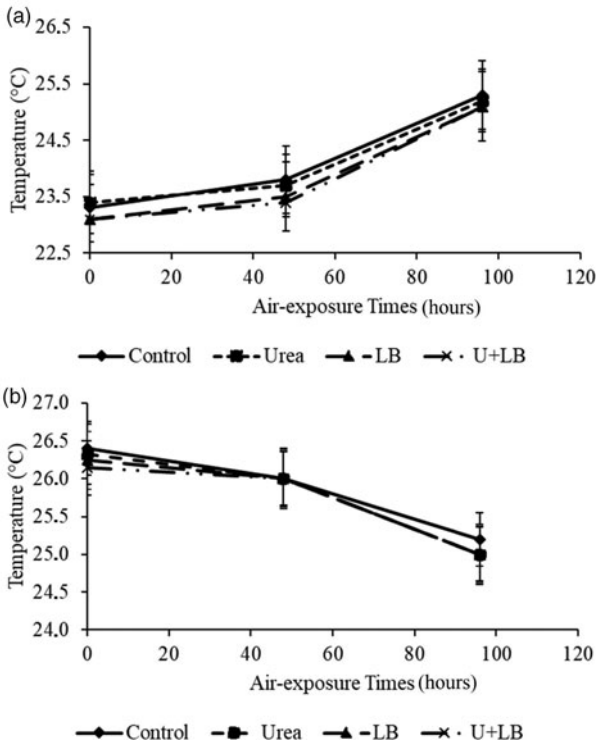

Fig. 5. Surface temperatures (a) and internal temperatures (b) of cochineal nopal cactus silages containing chemical and microbial additives at 0, 48 and 96 h of air exposure. Control, without additive; Urea, addition of urea; LB, addition of *Lactobacillus buchneri*; U + LB, addition of urea and *Lactobacillus buchneri*.

Evangelista, 2010; Pedroso *et al.*, 2011), and its efficiency in combination with microbial inoculants has been demonstrated (Siqueira *et al.*, 2007). In the short term, treatment of silage with *L. buchneri* also proved to be efficient in controlling the yeast population (Ávila *et al.*, 2008).

The pH of the silage treated with urea remained constant until 120 days of storage and was on average 4.78. This average was due to the higher concentration of ammonia nitrogen in this silage due to hydrolyses of urea (Table 1); the increased level of ammonia nitrogen caused an increase in the BC of the silage, thus preventing a rapid decrease in pH. According to Pereira *et al.* (2007), the addition of urea contributes to an increase in mean pH value, ammonia nitrogen level (% NT) and CP content of silage.

It is important to note that McDonald *et al.* (2010) reported that well-preserved silages generally have pH values between 3.7 and 4.2. Many of the pH values recorded in the current study were above the maximum value reported by those authors. However, the pH value alone does not determine the quality of the silage, and it is necessary to consider other variables along with to determine its quality. Furthermore, significant reductions in WSC content and consequent increases in lactic acid concentration at 120 days most likely contributed to the increased BC of these silages (Evangelista *et al.*, 2009).

An increase in acetic acid concentration was expected in silages inoculated with *L. buchneri* because it is a heterofermentative lactic acid bacterium that produces acetic acid along with lactic acid (Reich and Kung Jr. *et al.*, 2010). However, the results show that the urea and *L. buchneri* treated silages had similar acetic acid contents. The acetic acid contents in the current study, even that of the control silage, were consistent with the content up to 20 g/kg DM recommended in the literature for well-fermented silages (McDonald *et al.*, 2010; Pinho *et al.*, 2016).

The highest concentration of ethanol at 60 days of ensiling was observed in the silage treated with urea (36.9 g/kg of ethanol). However, in the opening period at 120 days, all silages with the exception of the control seal reached concentrations greater than 40 g/kg of silage. Kung Jr. *et al.* (2018) reported that ethanol concentrations above 40 g/kg in silage indicate excessive yeast metabolism. At 120 days, the mould and yeast population of the silage increased, which may have contributed to the increase in ethanol concentration.

It is also possible that heterofermentative lactic acid bacteria exhibit high activity in the long term, converting the readily available substrates to ethanol. This interpretation is supported by the acetic acid concentrations in the current study, which were higher in the silages treated with urea and *L. buchneri* after 120 days of storage.

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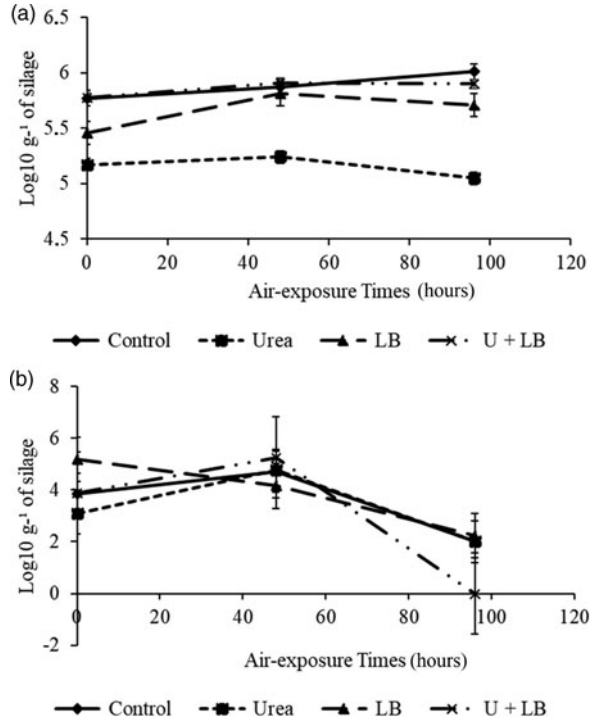

Fig. 6. Quantification of lactic acid bacteria (a) and moulds and yeasts (b) present in cochineal nopal cactus silages containing chemical and microbial additives at 0, 48 and 96 h of air exposure. Control, without additive; Urea, addition of urea; LB, addition of *Lactobacillus buchneri*; U + LB, addition of urea and *Lactobacillus buchneri*.

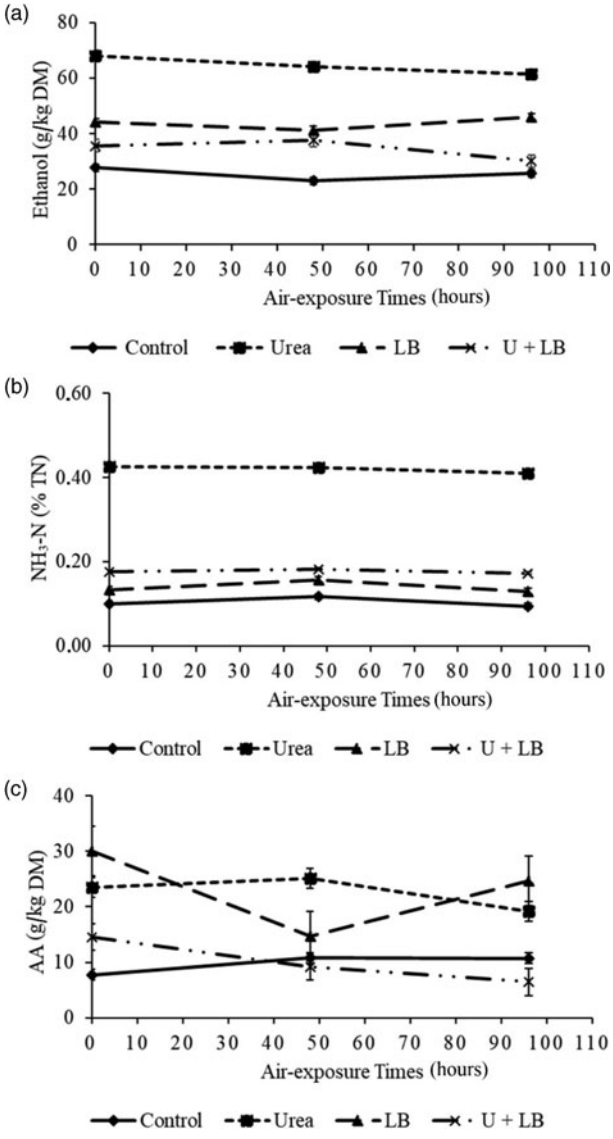

Fig. 7. Mean values of ethanol (a), ammonia nitrogen (b) and acetic acid (c) in cochineal nopal cactus silages containing chemical and microbial additives at 0, 48 and 96 h of air exposure. Control, without additive; Urea, addition of urea; LB, addition of *Lactobacillus buchneri*; U + LB, addition of urea and *Lactobacillus buchneri*.

Silages treated with urea had a higher propionic acid concentration than the silage treated with *L. buchneri*; this outcome was probably due to the higher BC of this silage, which caused a delay in pH reduction that may have favoured fermentation by propionic bacteria at the beginning of the ensiling process. The propionic acid concentrations observed after the treatments in the current study were not high enough to indicate poor silage fermentation; according to Kung Jr. *et al.* (2018), only concentrations above 5 g/kg indicate high clostridial fermentation because of the activity of *Clostridium propionicum*.

The ammonia fraction (N-NH_3) of total nitrogen (TN) in all silages in the current study may be considered low. This outcome is true even in the case of the silage treated with urea, which showed a significantly higher ammonia concentration (0.31% of TN) due to the increase in nitrogen caused by the addition of urea. Ammonia nitrogen is naturally found in all silages at relatively low contents (usually less than 10–15% of TN). However, in the current study, the values were very low (below 1%) and may be attributed to the action of lactic acid bacteria, which cannot decarboxylate amino acids to form carbon dioxide (CO_2) and ammonia (McDonald *et al.*, 1991).

The butyric acid concentration decreased by 60 days of storage and was greater than 1.0 g/kg DM, the reference value reported by Pinho *et al.* (2016) as a target to produce stable silage. According to Kung Jr. *et al.* (2018), butyric acid should not be found in silage that has been adequately fermented. However, the butyric acid contents found in the silages in this study may be considered low, not exceeding 0.42 g/kg DM, indicating very low clostridial activity.

The lactic acid content was affected by storage time. It increased significantly after 60 days of ensiling, maintaining without a significant difference at 120 days and ranged from 51.3 to 84.9 g/kg DM. Brito *et al.* (2020), evaluating the fermentation profile of cochineal nopal cactus silage, found 60 g/kg DM lactic acid after 90 days of storage. Nogueira *et al.* (2019) observed 80 g/kg DM of lactic acid after 30 days of cochineal nopal cactus ensiling, corroborating the results of this study, which indicate a predominance of lactic acid fermentation during ensiling of cochineal nopal cactus.

The NDF content decreased with storage time; this outcome can be attributed to hydrolysis by plant enzymes or to acid hydrolysis due to the organic acids produced during fermentation (McDonald *et al.*, 1991). Silages treated with urea showed a significant reduction in NDF content. This reduction could be due to alkaline hydrolysis resulting from the reaction of ammonium hydroxide with the ester bonds within structural carbohydrates (Rosa and Fadel, 2001).

Based on the current results, it can be inferred that losses in this study were small and that they were within the range of losses expected for silages that are predominantly fermented by lactic

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acid bacteria (McDonald *et al.*, 2010). The DM loss in the current study was close to that observed by Brito *et al.* (2020) of 34.0 g/kg in cochineal nopal cactus silage without additives at 90 days of ensiling. A DM loss of ~70.0 g/kg was observed by Nogueira *et al.* (2019) at 30 days of storage. Recently, Carvalho (2017) reported a DM loss of ~90.0 g/kg in cactus silage varieties at 60 days of storage.

Second experiment

The results observed in Figs. 5(*a*) and (*b*) corroborate those found by Carvalho (2017) and Macêdo *et al.* (2018), showing the high stability of these silages. The increase in the number of lactic acid bacteria at 96 h (Fig. 6(*a*)) may have been due to the presence of oxygen, given that these bacteria are facultative anaerobes (Carr *et al.*, 2002). Santos *et al.* (2018) evaluated the lactic acid bacteria population during an aerobic stability test of sorghum silages treated with urea and found an increase in the growth of these microorganisms during 24 and 48 h of air exposure.

In addition to the inhibitory effect of urea and *L. buchneri*, it is possible that maintenance of the size of the lactic acid bacterial population promoted competition between these microorganisms that resulted in the total inhibition of mould and yeast growth (Fig. 6(*b*)).

The decrease in ethanol levels at the end of the stability test at 96 hours (Fig. 7(*a*)) can be attributed to the decrease in the population of molds and yeasts that occurred at the same time due to the inhibitory effect of urea on these microorganisms and that, in combination with L. buchneri, completely inhibited mold and yeast growth in silage treated with a combination of Urea and L. Buchneri. The acetic acid remained higher in silage treated only with urea (Fig. 7(*c*)). This result probably was due to the higher concentration of ammoniacal nitrogen in this silage (Fig. 7(*b*)).

Conclusion

The use of urea, *L. buchneri*, or both in combination improves the fermentation characteristics of cochineal nopal cactus silage without producing major changes in the chemical composition of the silage, and the best results are observed after 60 days of storage. Neither the use of a single additive nor the use of a combination of the two additives affects the aerobic stability of cochineal nopal cactus silage, since non-treated silage presents high aerobic stability

Conflicts of Interest. There is no conflict of interest.

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Ethical Standards. Not applicable.

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