



Full Length Article

Feed Alternatives with Cactus Forage Silage for Animal Nutrition

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Abstract

Wheat bran is commonly used as an absorbent additive in high moisture silages and urea for its possible actions against undesirable microorganisms. Thus, this study was conducted to evaluate the effects of wheat bran and urea on the fermentation profile, silage losses and chemical composition of *Opuntia* silage. Four levels of wheat bran (0, 50, 100 and 200 g kg⁻¹ of dry matter) with urea (10 g kg⁻¹ dry matter) or without urea were added in *Opuntia* silage. Wheat bran negatively influenced gas losses (GL) and the levels of organic acids. The addition of urea ($P < 0.05$) reduced GL, but increases the concentrations of lactic acid and propionic acid. Populations of lactic acid bacteria (LAB), moulds and yeasts were reduced by the addition of urea and wheat bran ($P < 0.05$). Regarding the bromatological constituents, an interaction effect between the factors was only observed ($P < 0.05$) for the variables of lignin (LIG) and total soluble carbohydrates (TSC), with increased contents. In conclusion, a dose of 200 g kg⁻¹ of wheat bran associated with 10 g kg⁻¹ of dry matter in *Opuntia* silage seemed viable option to reduce the silage losses along with sizable improvement in the nutritional value of silage. © 2019 Friends Science Publishers

Keywords: Lactic acid; Lactic acid bacteria; *Opuntia ficus-indica*; Silage additives; Yeast

Introduction

In semiarid regions, opuntia (*Opuntia ficus-indica*) is being widely used and has become one of the main sources of forage for herds in production systems with low and medium technological levels (Tegegne *et al.*, 2007; Costa *et al.*, 2009). However, when its use is intensified, especially in the dry period, the costs related to cutting and supply are increased. Harvesting may also compromise the potential of opuntia to regrow, since its use is unevenly distributed throughout the dry season (Ramos *et al.*, 2015).

Ensiling is the most used technique for forage conservation in semiarid regions, especially to maintain the moisture and nutritional value of the food, besides maintaining the uniformity of plants (Pinho *et al.*, 2013; Silva *et al.*, 2015). According to Gusha *et al.* (2013), forage palm is recognised for its conservation in the form of silage, although it has high moisture contents. This is only possible due to the mucilage (gelatinous substance) that decreases the water activity, controlling the development of clostridia and enterobacteria and reducing effluent losses. However, despite the occurrence of mucilage, the use of an additive in the form of bran could further minimise losses during ensiling. The use of chemical additives and moisture absorbents,

such as urea and wheat bran (*Triticum aestivum*), can be effective in wet material silages, ensuring substrate supply throughout the process and the maintenance of the fermentation process, besides minimising undesirable fermentations (Zanine *et al.*, 2006a; Lopes *et al.*, 2007; Zanine *et al.*, 2007; Zambom *et al.*, 2014). Zanine *et al.* (2006b) have reported that wheat bran could be used in silages as an absorbent additive to minimise silage effluent losses, besides adding nutritional value. In addition to its action in the fermentation process, it could also be an additional source of fibre, since *Opuntia* has a reduced amount of neutral detergent fibre (Vieira *et al.*, 2008).

Another property of *Opuntia* is the high amount of sugars, which could imply a predisposition to alcoholic fermentation (Gusha *et al.*, 2013; Macêdo *et al.*, 2018). In this sense, the use of urea in forage cactus silages would be justified for promoting pH control, favouring the production of lactic acid to the detriment of ethanol. Consequently, alcoholic fermentation is inhibited, and the aerobic stability of the silage is increased in the discharge phase, reducing the production costs (Neumann *et al.*, 2010; Dias *et al.*, 2014; Martins *et al.*, 2014). Therefore, this study was conducted to evaluate the effects of wheat bran and urea on the fermentation profile, silage losses and chemical composition of *Opuntia* silage.

Material and Methods

Study Area, Silage Material and Treatments

The experiment was performed at the Research Center for the Semi-arid Tropics (CPATSA) of the Brazilian Agricultural Research Agency (EMBRAPA) and the Forage Laboratory of the Federal University of Paraíba (UFPB). The *Opuntia ficus-indica* cv. 'Gigante' used in the present research was harvested after approximately 2 years of regrowth. Soon after the harvest, the material was ensiled in polyvinyl chloride silos (30 cm high and 15 cm in diameter) equipped with a Bunsen valve for the exhaust of gases. At the bottom of the silos, sand was added for draining the effluent, as well as a cotton cloth to avoid contact of the forage with the sand. The material was compacted with wooden sockets; we used approximately 2 kg of fresh forage per silo. The silos were closed, weighed and stored in a covered area at room temperature until being opened. The experiment was performed in a 4×2 factorial design (four levels of wheat bran: 0 (control), 50, 100 and 200 g kg⁻¹ dry matter; with or without urea in the silage (10 g kg⁻¹ dry matter) through a completely randomised design with four replicates. After 45 days of ensiling, each silo was opened and the content was thoroughly homogenised, discarding the upper and lower portions (approximately 5 cm each) of every silo. Samples were taken from the homogenised central portion and taken to the laboratory.

Microbial Populations and Fermentation Profile

Enumeration of the microbial groups was performed on the aqueous extract, obtained by homogenising 10 g of silage sample and 90 mL of phosphate buffer solution in an industrial blender for 1 min, obtaining the dilution of 10⁻¹. Afterwards, the samples were successively diluted to obtain the concentration range from 10⁻¹ to 10⁻⁹, counting the plaques with values between 30 and 300 colony-forming units (CFU). Plating was performed in duplicate on sterile Petri dishes immediately after preparing the aqueous extract. Microbial populations were quantified using selective culture media for each microbial group, where: MRS (Difco) for enumeration of lactic acid bacteria (LAB) after incubation for 48 h in a BOD oven at 39°C; Violet Red Bile Agar (Difco) for enumeration of enterobacteria (ENT) after incubation for 24 h in a BOD oven at 30°C; and potato dextrose agar plus 1 to 10% tartaric acid after sterilisation for mould and yeast counts (M and Y) after incubation for 3-7 days at room temperature.

To determine the organic acids, an aliquot of approximately 10 mL aqueous extract was acidified with three drops of 50% H₂SO₄ and stored in a freezer (-20°C). In this extract, the organic acids (lactic, acetic, propionic and butyric) were determined according to Siegfried *et al.* (1984) and water-soluble carbohydrates according to Deriaz (1961). The buffer capacity (BC) of the ensiled mass was determined

according to a methodology proposed by Playne and McDonald (1966). To determine the total water-soluble carbohydrates (WSC), the concentrated sulfuric acid method was used, adapted from Corsato *et al.* (2008).

Dry matter (DM) loss in the form of gas was determined according to the equations described in Zanine *et al.* (2010).

Chemical Analysis

To evaluate the chemical composition, samples of the fresh material (*Opuntia* and wheat bran) were collected, and the silos were opened. These samples were pre-dried for 72 h in a convection oven at 65°C, ground in a Thomas Wiley mill with a 1-mm sieve and placed in plastic flasks.

The concentrations of dry matter (DM), mineral matter (MM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), ether extract and lignin were determined according to the methodologies described in Detmann *et al.* (2012). The NDF corrections for ashes and protein (NDFap) were performed according to Licitra *et al.* (1996) and Mertens (2002), described in Detmann *et al.* (2012). The total carbohydrate (TC) concentration was estimated by the equation $TC = 100 - (\text{dag/kg CP} + \text{dag/kg EE} + \text{dag/kg MM})$ and that of non-fibrous carbohydrates (NFC) by the equation $NFC = (\text{dag/kg TC} - \text{dag/kg NDF})$, according to Mertens (1997). The chemical compositions of *Opuntia* and wheat bran before ensiling are shown in Table 1.

Statistical Analysis

The obtained data were subjected to analysis of variance and regression. Significant interactions among the factors were detailed. The effect of wheat bran addition was verified via regression analysis. The regression equations were chosen based on the coefficient of determination and the significance of the regression coefficients through the t-test, using $\alpha = 0.05$. The urea effect was compared by the F test at 5% probability level. All analyses were performed with the System for Statistical and Genetic Analyses (SAEG, 2007).

Results

Chemical Composition before Ensiling

The concentrations of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), ether extract (EE), non-fibre carbohydrates (NFC) and water-soluble carbohydrates (WSC) (g kg⁻¹ DM) of forage cactus and wheat bran before ensiling are shown in Table 1. The WSC quantity of *Opuntia* (120 g kg⁻¹) was higher than 100 g kg⁻¹ DM.

The DM content ranged from 108.2 to 265.1 g kg⁻¹ (Table 1). The TC, in turn, ranged from 6.03 to 22.65 mEq/100 g DM; based on this, *Opuntia* can be classified as a low-TC material.

Table 1: Chemical composition (g kg⁻¹ DM) of *Opuntia* and wheat bran

		<i>Opuntia</i> (g kg ⁻¹ DM)				
DM	Ash	CP	NDF	EE	NFC	WSC
144.9	128.3	44.8	394.3	9.2	423.4	120
		Wheat bran (g kg ⁻¹ DM)				
DM	Ash	CP	NDF	EE	NFC	WSC
873.7	38.4	175.9	311.8	28.3	313.1	60
		<i>Opuntia</i> silage untreated or treated with urea and wheat bran				
		DM (g kg ⁻¹)				
Urea		Wheat bran				
		0	50	100	200	
Without		114.8	187.1	218.9	265.1	
With		108.2	180.1	211.9	255.0	
		BC mEq/100g DM				
Urea		Wheat bran				
		0	50	100	200	
Without		22.65	13.89	13.84	6.03	
With		14.42	12.21	16.32	7.69	

DM – Dry matter on a fresh matter basis; CP – Crude protein; NDF – neutral detergent fibre; EE – Ether extract; NFC – non-fibre carbohydrates

Fermentation Quality and Dry Matter Loss of Silages

The addition of wheat bran to the ensilage significantly influenced ($P < 0.05$) gas losses (GL) and the contents of lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA). The addition of wheat bran had a linear decreasing effect ($P < 0.05$) on GL. The addition of urea ($P < 0.05$) influenced the GL variables and the concentrations of LA and PA; the presence of urea in *Opuntia* silage decreased the GL. There was an interaction effect among the factors ($P < 0.05$) for LA, AA, PA and BA (Table 2).

An interaction effect ($P < 0.05$) was observed on all organic acid variables of the *Opuntia* silage (Table 3). The use of wheat bran in the *Opuntia* silage had a quadratic effect ($P < 0.05$) on most variables, except on BA, which showed a linear decrease. An effect of urea ($P < 0.05$) was observed for LA, AA, PA and BA at the levels of 50 and 100 g kg, 0 and 50 g kg, 50, 100 and 200 g kg and 0 g kg of wheat bran, respectively (Table 3).

However, the addition of urea did not alter the populations of LAB, moulds and yeasts in the silage (Table 4). Populations of LAB close to 7 log CFU/g silage were observed after the fermentation process. With respect to ML proliferation, all treatments averaged 5 log CFU/g silage (Table 4). Wheat bran, in turn, decreased the LAB population, probably due to the reduced amount of TSC of the ensiled mass (Table 4).

Chemical Composition of Silages

Regarding the chemical constituents, the use of wheat bran significantly influenced ($P < 0.05$) most of the analysed variables, with the exception of NFC. On the other hand, the addition of urea influenced the variables DM, CP, NFC and TSC. An interaction among the factors was observed ($P < 0.05$) only for the variables LIG and TSC (Table 5).

Table 2: Statistical analysis of the fermentative profile of *Opuntia* silage untreated or treated with urea and wheat bran

Item	GL	LA	AA	PA	BA
	P-value				
Wheat bran	0.001	0.000	0.000	0.000	0.000
Urea	0.000	0.000	0.371	0.000	0.198
Wheat bran × Urea	0.202	0.000	0.000	0.000	0.005

GL – Gas losses; LA – lactic acid; AA – acetic acid; PA – propionic acid; BA – butyric acid

Table 3: Concentrations of lactic acid, acetic acid, propionic acid and butyric acid in *Opuntia* silage untreated or treated with urea and wheat bran

		Lactic acid (g kg ⁻¹ DM)				
		Wheat bran levels				
Urea		0	50	100	200	LSD
Without		80.2a	44.3d	47.0d	73.7b	5.9
With		80.8a	57.6c	49.0d	75.0ab	
		Acetic acid (g kg ⁻¹ DM)				
		Wheat bran levels				
Urea		0	50	100	200	LSD
Without		22.5b	12.8cd	10.7d	16.2c	4.7
With		48.6a	15.2cd	11.2d	16.0c	
		Propionic acid (g kg ⁻¹ DM)				
		Wheat bran levels				
Urea		0	50	100	200	LSD
Without		8.1a	5.4b	1.9d	2.1d	1.9
With		8.5a	3.9c	3.0c	5.8b	
		Butyric acid (g kg ⁻¹ DM)				
		Wheat bran levels				
Urea		0	50	100	200	LSD
Without		0.5b	0.4c	0.2e	0.2e	0.8
With		0.7a	0.3d	0.2e	0.2e	

Means followed by different letters differ significantly by the LSD test ($P < 0.05$)

Table 4: Microbial populations (log cfu/g silage) in *Opuntia* silage untreated or treated with urea and wheat bran

		Lactic acid bacteria			
		Wheat bran			
Urea		0	50	100	200
Without		7.80	7.02	7.91	7.11
With		7.80	6.97	8.21	6.34
		Yeasts and moulds			
		Wheat bran			
Urea		0	50	100	200
Without		5.30	4.54	5.92	4.95
With		5.25	4.48	6.23	4.87

There was an increasing linear effect ($P < 0.05$) for the variables DM, CP and EE with increasing wheat bran contents in the silages, with variations ranging from 129.26 to 272.31 g kg⁻¹, 58.26 to 137.80 g kg⁻¹ and 15.91 to 25.22 g kg⁻¹ in the DM of the silage, respectively. A decreasing linear effect ($P < 0.05$) was observed for the contents of ash and NDF, ranging from 118.65 to 68.62 g kg⁻¹ and 315.70 to 255.01 g kg⁻¹, respectively. In relation to the NFC contents in the silage mass, a quadratic effect was observed, with averages ranging from 491 to 519 g kg⁻¹ (Table 6).

The addition of urea significantly influenced ($P < 0.05$) the nutritive value of the silages, in which the contents of DM, CP and TSC increased with the additive.

Table 5: Statistical analysis of the chemical composition of *Opuntia* silage untreated or treated with urea and wheat bran

¹ FV	DM	Ash	CP	NDF	EE	LIG	NCF	TSC
Wheat bran	0.0000	0.0000	0.0000	0.0090	0.0001	0.0004	0.0676	0.0000
Urea	0.0075	0.8933	0.0000	0.0966	0.9131	0.9488	0.0130	0.0050
Wheat bran × Urea	1.0000	0.2812	1.0000	0.5140	0.5341	0.0296	0.4416	0.0058
Coefficient of variation (%)	5.73	7.04	5.50	14.02	17.27	8.55	9.36	58.31

DM – Dry matter on a fresh matter basis; CP – Crude protein; NDF – neutral detergent fibre; EE – Ether extract; LIG – lignin; NFC – non-fibre carbohydrates; TSC – Total soluble carbohydrates

Table 6: Chemical composition of *Opuntia* silage with added wheat bran

Item (g kg ⁻¹)	Wheat bran levels (g kg ⁻¹)				r ²
	0	50	100	200	
DM	129.26	161.13	203.14	272.31	99.84
Ash	118.65	111.05	85.68	68.62	94.90
CP	58.26	85.28	113.98	137.80	95.19
NDF	315.70	332.46	285.25	255.01	80.02
EE	15.91	18.46	23.27	25.22	90.59
NCF	491.43	452.70	491.80	519.61	67.70

DM – Dry matter on a fresh matter basis; CP – Crude protein; NDF – neutral detergent fibre; EE – Ether extract; NFC – non-fibre carbohydrates

However, the NFC values were significantly higher ($P < 0.05$) for the silage without urea (Table 7). A quadratic effect ($P < 0.05$) was observed on the LIG contents in the silages with wheat bran without urea; however, when urea was added, the LIG contents decreased linearly ($P < 0.05$). Silage without wheat bran showed a significantly lower lignin content ($P < 0.05$) without the addition of urea. Regarding the TSC contents, a linear decreasing effect ($P < 0.05$) was observed when wheat bran was added to the silage in combination with urea (Table 8).

Discussion

Wheat bran is commonly used as an absorbent additive in high-moisture silages, increasing silage DM and the availability of CP. *Opuntia* showed a low TC content, allowing a lower resistance to pH decrease by the action of organic acids, which may be associated with the high WSC content. The WSC quantity of *Opuntia* (120 g kg⁻¹) was higher than the 100 g kg⁻¹ DM recommended by McDonald et al. (1991) to obtain well-fermented silages.

In addition to the WSC content being within the range recommended in the literature, *Opuntia* contains a hydrocolloid compound known as mucilage, which, among other characteristics, has a high water retention capacity (Saag et al., 1975). Such capacity is due to the formation of a viscous gel in the presence of Ca²⁺ ions as well as in the presence of water and ions in the medium (Trachtenberg and Mayer, 1982). Mucilage tends to prevent the flow of fluid components present in the cladodes of the plant after cutting. In this study, this gelatinous substance, associated with wheat bran and urea, possibly inhibited the losses (Driehuis and Wikselaar, 2000), which explains the reduction in gas losses (EL) during *Opuntia* ensiling (Table 2).

The concentrations of lactic acid found in the studied silages (Table 3) were higher than the values observed by

Table 7: Chemical composition of *Opuntia* silage with added urea

Item (g kg ⁻¹)	Urea	
	Without	With
DM	182.1 ^b	193.8 ^a
CP	85.8 ^b	106.7 ^a
Ash	97.0 ^a	97.4 ^a
EE	20.4 ^a	20.5 ^a
NDF	284.7 ^a	311.3 ^a
NCF	511.8 ^a	466.6 ^b
TSC	105.3 ^b	213.8 ^a

Means within a row with different superscripts differ significantly ($P < 0.05$)

DM – Dry matter on a fresh matter basis; CP – Crude protein; EE – Ether extract; NDF – Neutral detergent fibre; NFC – non-fibre carbohydrates; TSC – Total soluble carbohydrates

Table 8: Concentrations of lignin and total soluble carbohydrates in *Opuntia* silage untreated or treated with urea and wheat bran

Urea	Lignin (g kg ⁻¹ DM)				r ²
	Wheat bran levels (g kg ⁻¹ DM)				
	0	50	100	200	
Without	59.12B	65.80	65.10	50.90	97.71
With	69.45A	66.52	59.62	55.33	96.78
Urea	Total soluble carbohydrates (g kg ⁻¹ DM)				r ²
	Wheat bran levels (g kg ⁻¹ DM)				
	0	50	100	200	
Without	20.04B	8.88	7.41	6.66	-
With	53.24A	19.91	10.01	4.35	73.89

Means within a column with different superscripts differ significantly ($P < 0.05$)

Çürek and Özen (2004), who obtained *Opuntia* silages with an average lactic acid content of 25.90 g kg⁻¹. A higher lactic acid and acetic acid production (Table 3) occurred in silages treated with urea, indicating a positive effect of urea on silage fermentation, since it provided a favourable environment for the development of homofermentative and heterofermentative lactic bacteria (Table 4). Evaluating *Opuntia* silages, Çürek and Özen (2004) observed an average acetic acid concentration of 1.53 g kg⁻¹. In this study, similar and higher values were found in silages without additives, with values of 2.25 g kg⁻¹ and 4.86 g kg⁻¹ in the treatments without and with urea, respectively. The moderate presence of acetic acid in the silage (Table 3) is favourable, since it can decrease aerobic deterioration at the time of feeding. There was a significant effect of urea on the concentrations of butyric acid and propionic acid; despite this, the values were relatively low, making it possible to characterise a suitable fermentation process of *Opuntia* silage in relation to the inhibition of unwanted fermentations (Table 3).

Opuntia presented a high WSC concentration of 120 g kg⁻¹ (Table 1), which, in turn, allows a rapid reduction of pH to the silage preservation range (Gusha et al., 2013), creating

a favourable environment for the development of lactic acid bacteria. The LAB values shown in Table 4 were derived from the fermentation of WSC (Table 1), which are classified as homofermentative and heterofermentative according to the products of their fermentation (Gollop *et al.*, 2005). However, the addition of wheat bran negatively influenced the availability of WSC in the treatments (Table 1), which may have negatively influenced the LAB proliferation in the silage (Table 4). Due to the low DM content of silages without additives, high concentrations of moulds and yeasts were observed in the material (Table 4). Although it does not represent high losses, fungi under stress conditions can produce mycotoxins, which are harmful to animal and human health (Ivanek *et al.*, 2006).

Yeasts are the main silage-degrading microorganisms, especially after aerobic exposure of the material, promoting marked losses of DM throughout the ensiling process. These microorganisms can grow at a pH below 3.5, since they can use lactic acid as substrate when in anaerobiosis, thus increasing the pH in the silage, which may favour the emergence of undesirable fermentations (Muck, 2010). However, it is noteworthy that LAB populations were higher than those of moulds and yeasts in all silages evaluated (Table 4).

The increase in the DM contents of the silages (Table 6) is associated with the addition of wheat bran, which in turn is an additive that allows forming silage from forages with low DM contents (Souza *et al.*, 2003). Moreover, its addition increases the nutritional value of the silage (Table 6) and results in lower losses during the ensiling process, inhibiting possible undesirable fermentations (Ribeiro *et al.*, 2008).

Mucilage formation may have influenced the reduction in losses during ensiling and the fermentation profile of the silages evaluated, possibly favouring the maintenance of the PC contents of the silages (Table 6) and preserving the nutritive value of the silage. The NFC values presented in Table 6 were lower than those verified by Duru and Turker (2005), who observed changes in the chemical composition of *Opuntia* silages over time. These authors reported NFC values of around 580 g kg⁻¹ DM for *O. ficus-indica*, corroborating the values observed in the present study (Table 6).

The positive influence of urea on the chemical composition of *Opuntia* silage was expected due to the protein equivalence of urea. Most likely, urea was dissociated in the ensiled mass and remained in the form of ammonium salts. Regarding the alkaline hydrolysis potential of urea, no action of greater availability of soluble material or reduction of the fibrous portion of the ensiled material was observed (Table 7).

Conclusions

The addition of wheat bran and urea reduced gas losses, moulds and yeasts, increase populations of lactic acid bacteria of the silages. We recommend a dose of 200 g kg of

wheat bran associated with 10 g kg of dry matter in *Opuntia* silage seemed viable option to reduce the silage losses along with sizable improvement in the nutritional value of silage.

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