

Short Communication

Wasted cabbage (*Brassica oleracea*) silages treated with different levels of ground corn and silage inoculant

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ABSTRACT - Our objective was to evaluate the chemical composition, fermentation profile, and aerobic stability of cabbage silages treated with ground corn and inoculant. The evaluated treatments were: addition of 200, 300, 400, 500, and 600 g of ground corn per kilogram of cabbage (fresh matter basis), with or without a bacterial inoculant composed of *Lactobacillus plantarum* and *Pediococcus pentosaceus*. As expected, ground corn additions increased the dry matter (DM) content of cabbage silage, and high values were observed for the highest level of addition (540 g kg⁻¹). Conversely, the crude protein, neutral detergent fiber, acid detergent fiber, and lignin contents decreased with ground corn additions. The *in vitro* dry matter digestibility coefficients increased slightly with ground corn additions, but all cabbage silages had digestibility higher than 740 g kg⁻¹ of DM. In the fermentation process, the pH values of cabbage silages increased linearly because of the high levels of ground corn addition. Cabbage ensiled with 200 and 300 g kg⁻¹ of ground corn had lower maximum pH values during aerobic exposure, but all silages had constant temperature during aerobic exposure. The ensiling of wasted cabbage is possible and we recommend the application of 400 g kg⁻¹ ground corn to improve the silage quality, whereas the use of the inoculant is unnecessary.

Key Words: additives, by-product, lactic acid bacteria, nutritive value

Introduction

In Brazil, cabbage production was 377,108 t in 2006 and 1.3% was lost in the field (IBGE, 2006), whereas approximately 16% of cabbage is lost during commercial processing (Carvalho et al., 2013). Moreover, approximately 45% of the 60 million tons of cabbage produced in the world is wasted or lost during cultivation in the field (FAO, 2011). Thus, the ensiling process may be an alternative to preserve wasted foods in agriculture to use as feed for animal nutrition (Makkar and Ankers, 2014) or to be a source of substrates for energy production (e.g., biogas) Kafle et al., 2014).

However, the dry matter (DM) content of cabbage is below 120 g kg⁻¹, and cabbage has a low content of water-

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soluble carbohydrates (WSC) (Freyman et al., 1991). The ensiling of cabbage without additives leads to low production of lactic acid and pH reduction is not satisfactory to avoid the action of undesirable microorganisms for silage (Cao et al., 2011), which results in an inefficient fermentative process and low silage quality (Woolford, 1984). Thus, the use of absorbent material (e.g., ground corn) could increase the DM content of the silage and improve the fermentation, as suggested for mango, lemon, and banana (Aguilera et al., 1997; Khattab et al., 2000). A study reported increases of 163% in the WSC content of grass at ensiling with the addition of 7 kg ground corn per ton of grass (Van Onselen and Lopes, 1988). In addition, a high-moisture grass exhibited higher DM content, lactic and acetic acid production, and digestibility when ground corn was added at ensiling (Andrade and Melotti, 2004), and similar effects are expected for other forages. Although the evaluation of ground corn as an additive for silage has shown positive results (Van Onselen and Lopes, 1988; Andrade and Melotti, 2004), high levels (>100 g kg⁻¹) have not been assessed. In theory, the use of cabbage silages treated with high amounts

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of ground corn could be addressed for monogastrics and ruminants fed a high-concentrate diet.

Moreover, the addition of bacterial inoculants (homofermentative species) could improve the fermentation, since these bacteria produce large amounts of lactic acid and decrease the silage pH (Kung and Muck, 1997), resulting in a reduction of the DM and energy losses even in high-DM silages (Rabelo et al., 2014).Therefore, our objective was to evaluate the chemical composition, fermentation profile, and aerobic stability of wasted cabbage silages treated with ground corn and inoculant.

Material and Methods

Wasted cabbage was chopped and homogenized, and then ensiled with different amounts of ground corn: 200, $300, 400, 500, and 600 g kg^{-1}$ of cabbage (fresh matter basis). On a farm, the treatments proposed above could simulate common diets used for the nutrition of monogastrics (i.e., concentrate feedstuffs and a fiber source), in addition to being a rich starch source for ruminants kept in feedlot and those supplied in grazing systems. For each level of ground corn, a bacterial inoculant (commercial product Silobac®) containing Lactobacillus plantarum and Pediococcus *pentosaceus* was applied or not at 5×10^5 cfug⁻¹ prior to ensiling. The inoculant was dissolved in water (0.7 Lt⁻¹) and was applied to the cabbage by spraying under constant mixing. The same amount of water was applied to the control. Samples were collected for chemical characterization of the cabbage and ground corn, as well as for levels of ground corn addition prior to ensiling (Table 1).

Mini-silos of PVC tubes with a capacity of 4 L were used to ensile the cabbage, which remained sealed and stored in a room for 30 d. Each mini-silo contained 0.5 kg of sand in the bottom separated by a fine screen to avoid the contact between silage and sand, besides a *Bunsen* valve for gas escape. The silages containing ground corn at 200, 300, 400, 500, and 600 g kg⁻¹ of cabbage had a density (DM basis) of 148.3±5.43, 210.3±27.91, 274.4±10.77, 353.2±10.83, and 414.2±24.89 kg m⁻³, respectively. After silos were opened, effluent and gas losses were calculated according to Jobim et al. (2007). The aerobic stability was determined by placing 4 kg of cabbage silage in plastic buckets, which were placed in a room maintained at ambient temperature. The temperature of the silage was measured every 6 h during the 3 d of aerobic exposure with a thermometer placed in the center of the mass. The aerobic stability was defined as the time required for the temperature of the cabbage silage to increase by 2 °C above the baseline (ambient temperature) after exposure to air. During aerobic exposure, the pH values in the silages were also determined using the same buckets while measuring the temperature (approximately 20 g of silage was sampled in each time).

A water extract was prepared from the wet silage samples (Kung et al., 1984), and the pH was determined with a pH meter (MA522 model, Marconi Laboratory Equipment, Piracicaba, Sao Paulo, Brazil). The ammonia N was measured by distillation (AOAC, 1996, method no. 941.04). The silage samples were oven-dried (55 °C for 72 h), processed in a knife mill before passing through a 1-mm screen, and analyzed for DM (105 °C for 12 h). The DM content of cabbage silages was corrected for the volatile losses that occur during oven drying (Porter and Murray, 2001), as follows: true DM content (g/kg) = $1.011 \times \text{oven DM} + 1.24$.

The total nitrogen (TN) content was determined according to the AOAC (1996), method 954.01. The crude protein (CP) content was calculated as TN × 6.25. Neutral detergent fiber (aNDF) and acid detergent fiber (ADF) contents were determined sequentially using the method of Van Soest et al. (1991) in an Ankom 2000 Fiber Analyzer (Ankom Technologies, Macedon, NY, USA) without sodium sulfite and expressed inclusive of residual ash. Heat-stable α -amylase was used in the aNDF assay. The lignin concentration was measured after hydrolysis of the cellulose in ADF residues in a 72% H₂SO₄ solution (Van Soest and Robertson, 1985).

The *in vitro* dry matter digestibility (IVDMD) was determined according to Tilley and Terry (1963), and this assay was conducted according to the procedures of the

Table 1 - Chemical composition (g kg⁻¹ of DM) of wasted cabbage and ground corn and different levels of ground corn addition before ensiling (n = 4)

				Addition of g	round corn, g k	g ⁻¹ of cabbage		
Item	Cabbage	Ground corn	200	300	400	500	600	SEM
Dry matter, g kg ⁻¹ as fed	140.3	870.2	303.7	354.3	400.7	486.2	549.3	1.75
Crude protein	156.3	78.8	103.2	94.9	83.6	82.0	78.7	0.36
Neutral detergent fiber	428.0	256.8	447.7	421.7	377.8	343.4	273.8	1.02
Acid detergent fiber	210.4	29.6	118.3	84.5	67.0	55.8	45.3	0.24
Lignin	22.9	8.5	32.3	24.6	19.3	16.3	13.0	0.88

SEM - standard errors of least squares means.

Ground corn, g kg ⁻¹	7	200	ň	300	4	400	Ϋ́.	500	õ	600			P-value		
Inoculant	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	SEM	Corn meal	Inoculant	Interaction	Contrast
Chemical composition, g kg ⁻¹ of DM	of DM														
Dry matter, g kg ⁻¹ as fed	252.3i	261.7i	345.9g	320.9h	388.3f	407.1e	454.7d	456.5c	544.5a	539.7a	7.38	<0.0001	0.9872	0.0646	Γ^{**}
Crude protein	94.9	95.8	89.3	89.6	84.7	88.1	85.3	86.0	82.4	80.8	2.21	<0.0001	0.6195	0.8806	L^{**}
Neutral detergent fiber	449.7a	456.5a	430.3b	427.3b	390.3c	390.5c	341.8e	374.0d	291.3f	273.7g	2.64	<0.0001	0.0363	< 0.0001	C**
Acid detergent fiber	135.1	132.7	94.3	93.5	82.0	81.5	78.5	77.7	72.3	56.8	4.71	<0.0001	0.1903	0.4587	C**
Lignin	38.9	39.1	26.7	25.4	23.2	26.3	22.4	20.8	19.4	14.8	1.88	<0.0001	0.5193	0.4048	C**
IVDMD	754.7	744.7	763.3	749.6	740.6	750.4	751.3	761.7	779.9	782.1	13.16	0.0934	0.9743	0.8358	L*
Fermentative profile															
Hd	3.82	3.85	3.88	3.89	3.93	3.88	3.90	3.90	3.92	3.91	0.02	0.0013	0.7637	0.2150	L**
Ammonia N ²	157.5b	165.4a	150.5c	156.3b	24.3d	20.8d	24.4d	24.3d	20.5d	24.5d	2.09	<0.0001	0.0447	0.0872	C**
Effluent, kg t ⁻¹	45.0	50.3	51.9	50.3	14.5	14.2	17.4	14.5	19.1	15.4	3.39	<0.0001	0.7631	0.6961	C**
Gas, g kg ⁻¹ DM	257.3b	291.7a	72.5d	81.9c	40.5e	38.4e	34.8e	39.7e	19.7f	19.6f	3.37	<0.0001	0.0003	0.0005	C**
Aerobic exposure															
Maximum pH	4.30bc	4.30bc 4.30bc	4.20bc	4.50a	4.35b	4.29bc	4.32b	4.22bc	4.14c	4.15c	0.06	0.0256	0.4397	0.0344	*ð

a-i - means in the same row followed by different letters are significantly different (P<0.05). DM - dry matter; IVDMD - *in vitro* DM digestibility. ^{1*} P<0.05; ** P<0.01; ns - not significant; L - linear effect; Q - quadratic effect; C - cubic effect ² Ammonia N is expressed in relation to total nitrogen. Animal Care Committee of Universidade José do Rosário Vellano. The rumen fluid was obtained from a ruminallycannulated bull fed (*ad libitum*), for 15-d, chopped grass (*Panicum maximum*) daily and a mix of cabbage silages treated with ground corn. After this period, the rumen fluid was collected in the morning before feeding, filtered through four layers of cheesecloth into prewarmed thermos flasks, homogenized, and mixed with the solution medium.

The trial was organized in a 5 \times 2 factorial arrangement, in a completely randomized design with four replicates. The data were analyzed with a mixed model using the MIXED procedure of SAS (Statistical Analysis System, version 9.2), with the level of ground corn and the inoculant as fixed effects and the residual error as a random effect. Differences between means were determined using the DIFF procedure, which differentiates means based on Fisher's F-protected least significant difference test. Contrasts were constructed, and the single degree-of-freedom orthogonal comparisons included the linear, quadratic, and cubic effects of corn meal addition. The covariance structure that best fit the data according to the Bayesian information criterion (BIC) was selected. Differences were declared significant at P≤0.05 and trends at P≥0.05<0.10.

Results

The expected increase in the cabbage silage DM content with the addition of ground corn was confirmed (P<0.01), and higher values of DM were observed for the highest level of addition. Conversely, the CP, aNDF, ADF, and lignin contents of the silage decreased with the addition of ground corn (P<0.01), whereas the inoculant had no effect on the CP, ADF, and lignin contents (P>0.05). Because of the considerable reduction in the aNDF content due to ground corn addition, the IVDMD coefficients increased slightly (P<0.10). However, all values of IVDMD were high and ranged from 740.6 to 782.1 g kg⁻¹ of DM. Additionally, the effect of inoculant on the chemical composition was inconsistent (Table 2).

The pH values of cabbage silage showed a linear increase due to ground corn addition (P<0.01). For ammonia N, the highest values (P<0.01) were observed in the silage containing high moisture contents (200 and 300 g kg⁻¹ of ground corn). At these levels, the inoculant also increased the ammonia N concentrations (P<0.10). Moreover, decreases in the effluent and gas production were related to the ground corn additions (P<0.01), whereas the silage treated with 200 or 300 g kg⁻¹ of ground corn had higher gas losses due to inoculation (P<0.01; Table 2).

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Overall, the cabbage silage treated with 600 g kg⁻¹ of ground corn had a lower maximum pH (P<0.05), whereas the silage treated with 300 g kg⁻¹ of ground corn and inoculant had the highest value (P<0.05; Table 2). All silages had a constant temperature during 72 h of aerobic exposure (Figure 1), and the pH values also remained stable (below 4.5) during the 3 d of aerobic exposure (Figure 2).

Discussion

The increases in demand for livestock products impose a huge demand on feed resources, and the efficient use of available feed resources is the key to efficient animal production and food security (Makkar and Ankers, 2014), besides production of alternative sources of energy. Moreover, the commercial processing of vegetables in the world results in many residues, which are a waste of resources that leads to possible environmental problems because of unsuitable disposal (Cao et al., 2011). Thus, considering that approximately 45% of the cabbage

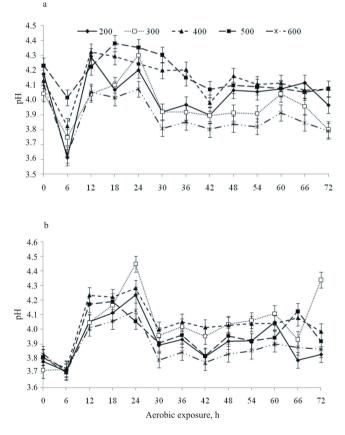
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a - control silages; uninoculated; b - inoculated silages; n = 4.

Figure 1 - Changes in temperature of wasted cabbage silages treated with different levels of ground corn during aerobic exposure.

production is wasted or lost during cultivation in the field before human consumption (FAO, 2011), we investigated the possibility of ensiling cabbage treated with ground corn and a silage inoculant.

As expected, the DM content in the cabbage silage increased considerably because of the addition of ground corn, which has high DM content and a large capacity to absorb moisture from silage (Andrade and Melotti, 2004). Conversely, in this study, as ground corn was added to the cabbage, its moisture-absorption capacity decreased (0.82, 0.71, 0.65, 0.69, and 0.68 g of moisture per gram of ground corn added at 200, 300, 400, 500, and 600 g kg⁻¹ of cabbage, respectively). Normally other products like citrus and beet pulp are used as an additive for grass silages due to their high capacity to absorb moisture and high WSC content, respectively (Moore and Kennedy, 1994; Bernardes et al., 2005). However, the use of a starch-rich source (i.e., ground corn) seems more appropriate for diets used in the ruminant and monogastrics nutrition aiming to reduce costs associated with animal feeding.



a - control silages; uninoculated; b - inoculated silages; n = 4.

Figure 2 - Changes in pH values of wasted cabbage silages treated with different levels of ground corn during aerobic exposure. Moreover, ground corn had low concentrations of fiber and protein compared with cabbage, and this explains the reductions observed for the CP, NDF, ADF, and lignin contents of the cabbage silages. Consequently, the IVDMD coefficients of cabbage silage increased slightly (P<0.10). The negative effect of fiber on digestibility is well known, and lignin is the component most negatively associated with fiber digestion by ruminants (Jung and Allen, 1995); this helps us to understand the obtained results. Our results are in agreement with those reported by Cao et al. (2011), who found increases in DM digestibility of Chinese cabbage silages treated with beet pulp.

Considering the use of cabbage silages supplemented with ground corn for animal nutrition, the increase in digestibility is an important factor to provide superior animal performance. Conversely, the changes in chemical composition are less important when cabbage silages are used for energy production (e.g., biogas), which could stimulate their use (Kafle et al., 2014), especially nowadays, since the search for alternative sources of energy has been increased.

Although the changes in chemical composition of cabbage silage were clear using ground corn, the bacterial inoculant had less obvious effects. After the silos were opened, only DM and NDF were affected by inoculant. Some strains of *L. plantarum* have *p*-coumaric and ferulic acid decarboxylase activities (Cavin et al., 1997), and these enzymes could reduce the fiber content of cabbage silage. However, a reduction in fiber content only occurred in the silage treated with 600 g kg⁻¹ of ground corn. Therefore, it is likely that changes in chemical composition caused by inoculants were more associated with reductions of DM and energy losses (McDonald et al., 1991).

As discussed previously, the ground corn absorbed the free water present in the cabbage silage and the water content decreased, which resulted in the higher pH values in the silages. Although we did not evaluate the presence of spoilage microorganisms, the development of these microorganisms is restricted by low water content (Davey, 1989). Additionally, cabbage silage with high moisture had high ammonia N production (i.e., >150 g kg⁻¹ of total nitrogen), which is typical of high-moisture silages and is predominantly a product of clostridial fermentation of amino acids (Charmley, 2001).

The cabbage silage treated with 200 and 300 g kg⁻¹ of ground corn had high effluent (>45 kg of fresh matter t⁻¹) and gas losses (>70 g kg⁻¹ of DM). The silages made with wetter plants endure more pressure during compaction, which causes the disruption of cell walls and losses of soluble constituents present in the cells and greatly reduces

the nutritional value of the silage (Khorvash et al., 2006). Moreover, the higher density found in the cabbage silages treated with high amounts of ground corn probably reduced the aerobic respiration from undesirable microorganisms due to the escape of the air trapped in the silo, as often reported for others crops (McDonald et al., 1991), resulting in lower gas losses. In this sense, the addition of large amounts of ground corn successfully resulted in decreased fermentative losses from cabbage silage, which is in agreement with other studies, in which the necessity of including an additive at the ensiling of fruits also was reported (Aguilera et al., 1997; Khattab et al., 2000).

The inoculants used in this study (P. pentosaceus and L. plantarum) are aimed at increasing the production of lactic acid, and as a consequence, reducing the production of ammonia N and DM losses during the fermentation process through more efficient metabolic pathways (McDonald et al., 1991; Muck, 2010). However, the application of the inoculant in cabbage silages treated with 200 and 300 g kg⁻¹ of ground corn increased the production of ammonia N and gas losses. In theory, P. pentosaceus converts hexoses into lactic acid, whereas usually L. plantarum also ferments hexoses into lactic acid. Conversely, under special conditions, L. plantarum can metabolize hexoses into lactic acid, carbon dioxide, and ethanol or acetic acid, whereas pentoses are fermented into lactic and acetic acid via a phosphoketolase pathway (Holzer et al., 2003; Pahlow et al., 2003). It was likely that under conditions of high-moisture, L. plantarum was induced to adopt a heterofermentative pathway, which produced more acetic acid and promoted higher DM losses (McDonald et al., 1991). Indirectly, the pH was not quickly reduced, and higher ammonia N production occurred via enzymes from the ensiled plant itself and/or undesirable microorganisms (McDonald et al., 1991). Positive effects from silage inoculant applied to high-DM silages (>380 g kg⁻¹ of DM) have been reported in the literature for corn (Hu et al., 2009; Rabelo et al., 2014). However, in the present study we did not find positive results when the inoculant was applied in the cabbage silages, perhaps due to low WSC content even including ground corn as an additive, or probably because of the low water activity, which can also reduce the LAB activity during fermentation (Davey, 1989; Pahlow et al., 2003).

Normally, assays of aerobic stability for silage produced in tropical climates are carried out for five or more days (Pedroso et al., 2008; Basso et al., 2012; Rabelo et al., 2015). However, considering the situation of silage management in Brazilian dairy farms, 67.7% of dairy farmers remove the silage from the silo top in three

or fewer days (Bernardes and Rêgo, 2014). Therefore, we performed the aerobic stability assay for three days because it is representative of the majority of dairy farmers in Brazil. In this regard, although the aerobic stability may be defined conceptually as the length of time required for the temperature of the cabbage silage to increase by 2 °C above the baseline (ambient temperature) after exposure to air, all cabbage silages had constant temperature during the 72 h of aerobic exposure despite the occurence of great changes in the ambient temperature during the trial. As is known, aerobic deterioration of silage occurs mainly during the feedout phase due to greater activity of yeasts and molds, which develop on the residual carbohydrates and lactic acid as substrates, increasing the pH and favoring the growth of spoilage microorganisms in addition to yeasts, and decreasing the nutritional value of the silage (Wilkinson and Davies, 2012). Therefore, we expected lower aerobic stability for the silage treated with 600 g kg⁻¹ of ground corn because of higher digestibility. However, when analyzing the data for maximum pH, the cabbage silage treated with 600 g kg⁻¹ of ground corn had low pH values. A possible explanation for this was discussed previously, and the silage with a high DM content had a low moisture content, and the development of spoilage microorganisms was possibly reduced due to low water activity (Davey, 1989).

It is important to stress that the use of vegetable silages (e.g., cabbage) as animal feed has proven economically viable in regions where vegetables residues are the main food by-product resulting not only from a way of disposing of vegetables' residues but also as an alternative for livestock feed (Cao et al., 2011). Conversely, considering the profitability of the use of ground corn as an additive for cabbage silage, their use may be unviable initially, but ground corn is largely used in the nutrition of ruminants and monogastrics, and the use of the cabbage silages supplemented with ground corn could reduce or offset the costs associated with concentrate feedstuffs.

Conclusions

The ensiling of wasted cabbage is possible, and the ensiling time of 30 days seems to be suitable to provide good silage quality. The best approach to ensile wasted cabbage is applying 400 g kg⁻¹ of ground corn at the moment of ensiling because of reductions of fermentative losses. The use of silage inoculant is not recommended because this does not promote improvements in the silage quality.

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