Investigation note

Nutritional quality of silage apple bagasse with organic and inorganic nitrogenous sources

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Abstract

The nutritional quality and potential use of apple bagasse (BM) in diets for ruminants when silage (microsilos) with two nitrogenous sources (FN) [urea (U), sow (C)], a commercial inoculant (I) was evaluated and *Chloris gayana* as moisture adherent [80/20 w/w, dry matter (MS)]. The study was conducted in Aguascalientes, Mexico (2014-2016). The treatments: a) BM; b) BM+I; c) BM+U; d) BM+U+I; e) BM+C and f) BM+C+I were analyzed in a completely randomized design (DCA) with factorial arrangement [3 FN (U, C, without FN) × 2 I (with I, without I)]. At 45, the MS of silage was similar between treatments, and lactic and acetic acids increased in greater proportion to propionic and butyric acids. The addition of C (BM+C and BM+C+I) increased the final crude protein (PC) (increments of 8 and 7.44 g 100 g^{-1} MS) although the pH increased (4.33 and 3.91 vs. 3.41 ±0.03) of the ensilage. Considering the content of PC and fermentation standards, the BM silage with C has the stability and sufficient quality to be used in the feeding of ruminants.

Keywords: apple bagasse, bacterial inocula, nitrogen sources, ruminants, silage.

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The potential use of by-products has the purpose of increasing the avoidance of loss of milk and meat production and improving the cost-benefit ratio in extensive production systems, especially at times of the year when there is a low availability of good quality food. The apple bagasse (BM) is a by-product of the juice extraction process (5-19 kg of BM 100 kg^{-1} of apple) composed of husks, seeds, pulp fibrous remains and juice depleted in sugars; the state of maturity and differences in the processing of the apple affect its composition of dry matter (MS, 14-26%), crude fiber (FC, 14-23%) and crude protein (PC, 4-8%) (Mirzaei-Aghsaghali *et al*., 2011). In Mexico, approximately 661 tons of BM are produced per year (SIAP, 2014) and can be considered as an important resource for the feeding of cattle, sheep and goats (Tiwari *et al*., 2008; Mirzaei-Aghsaghali *et al*., 2011; Ajila *et al*., 2015).

The silage process is a viable alternative to prolong the use of BM throughout the year (Skladanka *et al*., 2012; Ajila *et al*., 2015) and the addition of inocula and sources of non-protein nitrogen can improve lactic acid formation , the profile of volatile fatty acids (AGV) (Skládanka *et al*., 2012; Schroeder, 2013), the content of crude protein (PC) (Ajila *et al*., 2015), the aerobic stability at the moment of opening the silo (Barrena and Jiménez, 2013) and the nutritional quality of the BM (Rodríguez-Muela *et al*., 2006; Becerra *et al*., 2008).

The present study evaluated the effect of the addition of nitrogenous sources (organic and inorganic in nature) and of an inoculant during the BM silage process on the variables of nutritional quality PC, MS, pH, lactic acid and AGV (acetic, propionic and butyric).

Location of the experiment

The work was carried out between 2014-2016, at the facilities of the Autonomous University of Aguascalientes, the Llano Aguascalientes Technological Institute and the Agricultural Technological Baccalaureate Center num. 61 [Aguascalientes, Mexico (Central-North Region)].

Experimental material

The BM was used, by-product of the juice extraction of the company "Juice extractor Valle Redondo SA of CV de Aguascalientes". The BM silage lasted 45 d, for which: a base mixture was prepared containing a proportion of 80 g 100 g^{-1} MS of BM (with 18% MS) and 20 g 100 g^{-1} MS of *Chloris gayana* hay (with 90% MS) as moisture adherent material. Nitrogenous sources (FN) were added to the base mixture: 1) urea, 3.75% of the total MS of the mixture, and 2) sowing, with 23.5% of PC (from fattening animals) in proportion equivalent to the nitrogen contributed for urea. In addition, were included 10 g t^{-1} MS of a commercial inoculum (Sil-All 4x4®) containing lactic acid bacteria (*Streptococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Lactobacillus salivarius*) and exogenous enzymes (cellulases, hemicellulases, pentosanas and amylase).

An electromechanical mixer was used. The mixtures were deposited in microsilos of polyvinyl chloride (PVC) pipe 5.08 cm in diameter and 30 cm long, compacting with a metallic tamper to eliminate oxygen and closing with a bell-type plug reinforced on both sides; the microsomes were stored at room temperature of 20 °C.

Treatments and response variables

The treatments were: apple bagasse (BM), apple bagasse with inoculum (BM+I), apple bagasse with urea (BM+U), apple bagasse with urea and inoculum (BM+U+I), bagasse apple with sowing (BM+C), and apple bagasse with sowing and inoculum (BM+C+I). Samples collected at 0 and 45 d were dried at 60 °C in a forced air oven until constant weight was obtained and processed in a Thomas Wiley mill with a 1 mm sieve. The MS was obtained by difference between the initial and final weight [AOAC (1999) protocol 930.15-1930], the PC was determined through protocol 990.03-2002 of the AOAC (2002), the pH was measured in a homogeneous aqueous solution composed of 10 g of silage and 100 ml of distilled water (one hour after its preparation) using a digital pH-meter (Cherney and Cherney, 2003), lactic acid was determined by the colorimetric method (Madrid *et al*., 1999) and AGV by gas chromatography (Perkin Elmer® Co., Clarus 560 D Gas Chromatograph) (Erwin *et al*., 1961).

Statistical analysis

We used a completely randomized design (DCA) with a factorial arrangement (3×2) and four repetitions per treatment according to the model (1). The treatment means were compared with the Tukey test ($p< 0.05$). In addition, a test of orthogonal contrasts was applied to compare: 1) BM *vs*. BM with FN o/e I; 2) BM *vs*. BM with FN; 3) BM *vs*. BM with I;4) FN vs. treatments without FN; 5) I *vs*. treatments without I. The general linear procedure (Proc GLM) of the SAS package (Statistics Analysis System V. 9.2) was used.

$$Y_{ij} = \mu + FN_i + I_j + (FN*I)_{ij} + E_{ij} \quad 1)$$

Where: Y_{ij}= response variable; FN_i= effect of the ith nitrogen source; I_j= effect of the jth inoculant; $(FN*I)_{ij}$ = interaction between the ith nitrogen source and the jth inoculant; E_{ij}= random error.

The MS content was similar in all the treatments ($p> 0.05$, Table 1) and adequate for the release of lactic acid and pH decrease during silage fermentation (Schroeder, 2013).

There was FN×I interaction for PC content ($p< 0.001$). The treatments with C had better PC increases (BM+C without I and BM+C + I: 8.00 and 7.44 g 100 g^{-1} MS) than the treatments with urea (BM+U without I and BM+U + I: 3.2 and 1.6 g 100 g^{-1} MS) or with BM without FN (BM and BM+I: 0.92 and 2.02 g 100 g^{-1} MS), the final PC contents of some treatments were comparable to those of corn silage (8-10 g 100 g^{-1} of MS, Guedes *et al*., 2012). Considering the PC, the silage BM could be used to replace some types of grain and reduce feed costs. Mirzaei-Aghsaghali *et al*. (2011) analyzed *in vitro* gas production (contents of FDN, FDA and non-fibrous carbohydrates of 61.2, 46.7 and 23.8%, respectively) of BM with PC similar to that found in the present study and considered it viable to be used in the feeding of ruminants. Tiwari *et al*. (2008) found no negative effects on the production of milk and its fat and protein contents by supplying up to 33% of corn grain with BM (per 300 d).

Silages with I or some FN had higher initial and final pH values than the rest of the treatments ($p< 0.002$), although at the end of the fermentation all the treatments had pH values within the desirable ranges for the variations of microorganisms and production of fatty acids (Weinberg and Ashbell,

2003; Skladanka *et al*., 2012). The treatments with C recorded the highest pH (3.91-4.33 *vs* 3.38-3.44), but similar to those desirable in corn silage whit pH= 3.8-4.5 (Kolver *et al*., 2001; Skladanka *et al*., 2012; Schroeder, 2013).

Table 1. Contents of dry matter, crude protein and hydrogen potential of silage apple bagasse with nitrogenous sources or an inoculum at the beginning and end of fermentation.

Treatment	Dry material (g 100 g^{-1})		Crude protein (g 100 g^{-1} de MS		Potential hydrogen (pH)	
	Initial±	Final	Initial	Final	Initial	Final
BM*	22.03 a	22.67 a	4.82 f	5.75 f	3.11 c	3.4 b
BM+I	22.23 bc	22.43 a	5.08 e	7.1 d	3.43 b	3.44 b
BM+U	21.58 ab	22.47 a	7.42 d	9.04 e	3.34 b	3.38 b
BM+U+I	20.89 c	21.22 a	7.65 c	10.85 c	3.41 b	3.42 b
BM+C	21.54 abc	21.96 a	12.88 a	20.89 a	4.55 a	3.91 a
BM+C+I	22.18 a	22.57 a	11.87 b	19.31 b	4.96 a	4.33 a
CV (%)	1.14	6.92	1.028	0.987	33.25	12.92
Contrast by nitrogen source (P of F)						
N *vs* U y C	0.006	>0.932	< 0.001	<0.001	<0.001	<0.001
U *vs* C	>0.229	>0.751	< 0.001	<0.001	<0.001	<0.001
Contrast by inoculum (P of F)						
Con I *vs* sin I	>0.055	>0.695	<0.001	<0.001	0.002	0.002

*= control (apple bagasse without nitrogenous source or inoculum); BM= apple bagasse; I= inoculum; U= urea; C= sowing; abcde= treatments with different letters represent statistically different means (Tukey $p< 0.05$); CV= coefficient of variation; P of F = probability value; ±= Start and end, 0 and 45 d after fermentation.

The treatments with C had higher final concentration of lactic acid ($p< 0.0001$, Table 2), which represents better fermentation and stability of the silage and lower probability of contamination and degradation of the proteins (Bautista *et al*., 2007; Nkosi *et al*., 2011; Huntanen *et al*., 2013). Although the proportions of lactic acid of all the treatments of the present study were within the minimum and optimum ranges for the high-quality silages (Kolver *et al*., 2001; Guedes *et al*., 2012).

During fermentation, the increase in acetic acid was greater than that of the butyric and propionic acids ($p< 0.05$), which could help reduce the aerobic deterioration of the silage at the time of opening the silo (Weinberg *et al*., 2002) since acetic and propionic acid concentrations are indicators of silage stability (Huntanen *et al*., 2013). The increase in acetic acid in the treatments of the present study was greater than that published by Guedes *et al*. (2012) and Skladanka *et al*. (2012), especially in the BM+C treatment, followed by the other treatments with C ($p< 0.0001$). In addition, treatments with C and I had propionic acid at the end of fermentation ($p< 0.001$).

On the other hand, the increase of butyric acid in BM+U+I and BM+I during fermentation (of 0.60 and 0.63 mol/100 mol), could represent some contamination by bacteria *Clostridium* spp. in these treatments (Leupp *et al*., 2006; Bautista *et al*., 2007). On the contrary, the proportions of

butyric acid of BM, BM+U, BM+C and BM+C+I did not change through the fermentation time, suggesting that the final contents of butyric acid of BM+C and BM+C+I higher than desirable, Guedes *et al*. (2012), were related to the initial content of the acid in C more than to the growth of *Clostridium* spp.

Table 2. Proportions of lactic, acetic, propionic and butyric acids of silage apple bagasse with nitrogenous sources or an inoculum at the beginning and end of fermentation.

Treatment	Lactic acid (mol 100 mol^{-1})		Acetic acid (mol 100 mol^{-1})		Propionic acid (mol 100 mol^{-1})		Butyric acid (mol 100 mol^{-1})	
	Initial$^{\pm}$	Final	Initial	Final	Initial	Final	Initial	Final
BM*	1.36 ab	4.83 b	0.87 e	1.68 d	0 c	0 d	0 c	0 c
BM+I	1.28 b	3.22 c	0.87 e	1.93 d	0 c	0.44 c	0 c	0.63 b
BM+U	1.6 a	4.58 b	1.37 b	2.74 c	0 c	0 d	0 c	0 c
BM+U+I	1.19 b	5.64 a	1.46 a	2.97 c	0 c	0.97 a	0 c	0.6 b
BM+C	1.47 ab	5.78 a	1.29 c	5.04 a	0.38 a	0.67 b	1.31 a	1.32 a
BM+C+I	1.18 b	5.47 a	1.19 d	4.16 b	0.34 b	0.48 c	1.22 b	1.29 a
CV (%)	8.53	2.36	8.53	2.87	3.91	4.15	5.21	3.63
Contrast by nitrogen source (P of F)								
T *vs* U y C	>0.5639	<0.0001	<0.001	<. 0.001	<0.001	<0.001	<0.001	<0.001
U *vs* C	>0.9413	< 0.0001	<0.001	< 0.001	<0.001	<0.001	<0.001	<0.001
Contrast by inoculum (P of F)								
Con I *vs* sin I	<0.0005	0.0002	>0.811	< 0.021	0.001	<0.001	0.013	<0.001

*= control (apple bagasse without nitrogenous source or inoculum); BM= apple bagasse; I= inoculum; U= urea; C= sowing; a, b, c, d y e= treatments with different letters represent statistically different means (Tukey $p< 0.05$); CV= coefficient of variation; P of F = probability value; ± Start and end, 0 and 45 d after fermentation.

Conclusiones

In the present study the BM silage process increased the PC and in some cases the pH decreased to values comparable to those of other more conventional silage types such as maize, in addition, the increases of lactic acid and acetic acid of silage BM indicate which has the necessary stability to be included as an ingredient in ruminant diets. Mainly, adding C to the ensile BM could be an alternative to improve its quality, in order to be used in diets without adversely affecting the health or productive behavior of dairy cattle and meat.

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