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Short communicaton. Effects of adding different protein and carbohydrates sources on chemical composition and *in vitro* gas production of corn stover silage

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Abstract

The use of protein-rich by-products based in swine manure (SM), poultry waste (PW) or chemicals compounds as urea (U), as well as energy products like molasses (M) and bakery by-product (BB), is a viable method to produce good quality silage. In addition, the use of a bacterial additive can improve the fermentation characteristics of silage. The objective of this study was to determine chemical composition, *in vitro* gas production (GP) and dry matter disappearance (DMd), using different sources of protein and energy in silage. The silages were made using SM, PW or U as protein sources and M or BB as energy source, with corn stover and with or without a bacterial additive. The organic matter (OM) content was higher ($p < 0.001$) in silages with UBB, UM and SMBB compared with the rest of the treatments; meanwhile crude protein content was higher ($p < 0.001$) in silages with U. The addition of a bacterial additive increased ($p < 0.05$) OM content and decreased ($p < 0.05$) fiber content. Total GP was higher ($p < 0.05$) in silages containing BB, but DMd was higher ($p < 0.05$) in silages with U and SMBB. The inclusion of a bacterial additive decreased ($p < 0.05$) GP and DMd. The use of alternative sources of protein such as poultry and swine manure or urea, and of by-products of sugar industry and bakery is an alternative for silages based on corn stover. The results show that when properly formulated, the silages can provide more than 16% of crude protein and have DMd values above 60%.

Additional key words: bakery by-product; molasses; poultry waste; swine manure; urea.

Because nitrogen (N) is a limiting nutrient in low-quality forages, supplementation with N sources has been used to supply ammonia to meet the requirements of rumen microbial population. The use of manure from livestock is an alternative method of supplying N in the feed of ruminants (Ortiz *et al*., 2007); however, they usually have low energy content for optimal rumen bacteria growth. An increase in cellulolytic activity of microorganisms in the rumen can increase the digestible energy due to better utilization of fibrous feeds, and otherwise improving the supply of microbial protein. Bakery by-products and sugar industry are an attractive source of energy for ruminants.

The method of silage preservation is based on converting the soluble carbohydrates in organic acids, mainly lactic acid under anaerobic conditions by lactic acid bacteria. The technique of *in vitro* gas production (Menke & Steingass, 1988), or the modifications by Theodorou *et al*. (1994) simulating the digestive processes generated from microbial production, allows us to know the fermentation and degradation of food according to the nutritional quality and availability of nutrients for ruminal bacterial. The aim of this study was to evaluate the effect of swine manure (SM), poultry waste (PW) and urea (U) as nitrogen sources and of molasses (M) or bakery by-product (BB) as a carbohydrate sources, with and without the addition of a bacterial additive, on chemical composition and *in vitro* gas production of silages based on corn stover (CS).

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Abbreviations used: ADF (acid detergent fiber); BB (bakery by-product); CS (corn silage); DM (dry matter); DMd (dry matter disappeared); FM (fresh matter); M (molasses); ME (metabolizable energy); NDF (neutral detergent fiber); PVC (polyvinyl chloride); PW (poultry waste); RGP (relative gas production); SM (swine manure); U (urea).

Table 1. Proportion of the ingredients used (g kg^{-1} dry matter) for the preparation of the micro-silages

Treatments[1]	Ingredients[2]					
	PW	SM	U	BB	M	CS
PWBB	384			231		385
PWM	414				172	414
SMBB		250		281		469
SMM		274			214	512
UBB			64	338		598
UM			72		262	666

[1] All treatments were performed with and without a bacterial additive (Sill-All$^{4\times4®}$, 10 mg kg^{-1} DM). [2] PW: poultry waste; SM: swine manure; U; urea; BB: bakery by-product; M: molasses; CS: corn stover.

Six micro silages were performed using nitrogen (SW, PW and U) and energy sources (M and BB), and mixed with corn stover in different proportions as a fiber source. The proportion of ingredients in each silage is given in Table 1. Each combination was ensiled either without or with a bacterial additive, Sill-All$^{4\times4®}$ (Alltech®), which contains *Streptococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Lactobacillus salivarius* and enzymes cellulase, hemicellulase, pentosanase and amylase.

The mixing process was performed by adding water (480 mL kg^{-1} fresh matter) to SM, PW and U, mixing with M or BB, and adding or not the bacterial additive (10 mg kg^{-1} DM). Once diluted, they were mixed with corn stover in different proportions by triplicate in tubes of polyvinyl chloride (PVC; 20 × 10 cm), with a capacity of 1.5 kg. The mixture was compacted and tubes were sealed with plastic bags and tape to prevent the ingress of air. After 60 days the micro-silages were opened, a 200-g sample was taken from each silage and the pH was determined. Samples were dried in a forced air oven (60°C, 48 h) and ground in a Willey mill (2 mm diameter). Silage samples were analyzed for dry matter (DM), ash and N according to AOAC (1997), references 934.01, 942.05 and 954.01, respectively. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (AOAC, 1997; reference 973.18) were analyzed using an ANKOM200 fiber analyzer unit (ANKOM Tech. Co., Macedon, NY, USA) according to Van Soest *et al*. (1991). NDF was assayed with α-amylase and sodium sulfite. Both NDF and ADF are expressed without residual ash. Moisture content of the silages was determined through distillation with toluene (Haigh & Hopkins, 1977).

In vitro gas production (GP, mL gas g^{-1} DM) and *in vitro* dry matter disappeared (DMd) were determined following the technique described by Theodorou *et al.* (1994). A DM sample of 800 mg was placed in a 125 mL flask with 90 mL of incubation solution (Menke & Steingass, 1988). Each treatment was run in triplicate. Rumen fluid was drawn from three fistulated dairy cattle (500 ± 20 kg LW) fed alfalfa hay, corn stover, concentrate (16% CP, 11.7 MJ ME kg^{-1} DM) and a mineral supplement with *ad libitum* access to water drink. The rumen fluid was filtered thought a triple layer of gauze, homogenized under CO_2 flushing for 5 min, and finally 10 mL or rumen fluid were added to each bottle. Bottles were closed, incubated in a water bath at 39°C, and gas production was recorded at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h using a pressure transducer (HD 8804, DELTA OMS, Casselle di Selvazzano, Italy). Additionally, three blanks were included in each of the three series conducted. At the end of the incubation, the residue was filtered, washed with distilled water and dried in an oven (65°C, 48 h) to determine the DMd. Relative gas production (RGP) was calculated as milliliter of gas per gram of DMd after 96 h incubation. Results of gas production were fitted to the equation proposed by France *et al.* (1993):

$$Y = A\ [1 - exp\ (-B^{(t-T)} - C^{(\sqrt{t}-\sqrt{T})})],$$

where Y represents the cumulative gas production (mL), A is the asymptote of the curve (total gas production, mL), B (h^{-1}) and C ($h^{-1/2}$) are the initial and later gas production rate constants, t is the incubation time (h), and T represents the lag time (h), which is the time when the food begins to be degraded by microorganisms in the rumen.

Chemical composition and *in vitro* rumen gas production data were analyzed as a completely randomized design with factorial arrangement of treatments (6 × 2) and their interaction for each variable using the GLM procedure (SAS, 1999). The statistical model was: $Y_{ijkl} = \mu + T_i + Ad_j + TAd_{ij} + \varepsilon_{ijk,}$ where Y_{ijkl} = response variable, μ = overall mean, T_i = effect of the silage, Ad_j = effect of the additive inclusion; TAd_{ij} = effect of the interaction T × Ad and ε_{ijkl} = experimental error. Comparison of means was conducted by Tukey test.

Chemical composition (g kg^{-1} DM) of ingredients before ensiling was PW: 926 DM, 318 CP and 360 NDF; SM: 275 DM, 181 CP and 455 NDF; BB: 929 DM, 124 CP and 75 NDF; M: 645 DM and 43 CP and CS: 929 DM, 37 CP and 795 NDF. Chemical composition of silages is presented in Table 2. The pH was affected by treatment ($p = 0.026$), being higher ($p < 0.05$)

Table 2. Chemical composition (g kg^{-1} dry matter) and parameters of *in vitro* gas production (IVGP) of silages with and without the inclusion of bacterial additive (Ad)

	Treatments (T)[1]						**Additive (Ad)**		**SEM[2]**	***p*-value**		
	PWBB	**PWM**	**SMBB**	**SMM**	**UBB**	**UM**	**Yes**	**No**		**T**	**Ad**	**T × Ad**
Chemical composition												
pH	4.64^{b}	4.91^{b}	4.32^{b}	4.63^{b}	5.62^{a}	5.89^{a}	4.84	5.51	0.261	0.026	0.178	0.129
DM[3]	359^{cd}	407^{ab}	357^{cd}	338^{d}	380^{bc}	408^{a}	375	375	5.1	0.001	1.000	0.001
OM	913^{b}	872^{d}	921^{a}	886^{c}	921^{a}	920^{ab}	915^{a}	896^{b}	2.3	0.001	0.001	0.001
CP	170^{bc}	172^{bc}	161^{c}	147^{c}	220^{a}	206^{ab}	174	185	6.0	0.001	0.154	0.026
NDF	529	466	52^{4}	511	525	485	492^{a}	522^{b}	7.7	0.031	0.021	0.111
ADF	263	240	255	241	266	255	248	259	6.8	0.479	0.243	0.967
Lignin	39	38	39	38	40	39	39	39	1.5	0.587	0.365	0.894
In vitro gas production[4]												
A	248^{a}	227^{e}	239^{ab}	200^{c}	243^{ab}	205^{a}	222^{a}	231^{b}	1.7	0.001	0.033	0.001
B	0.045^{ab}	0.041^{b}	0.049^{a}	0.049^{a}	0.041^{b}	0.043^{b}	0.044	0.045	0.0028	0.001	0.233	0.001
C	–0.068	–0.076	–0.061	–0.011	–0.043	–0.081	–0.075	–0.072	0.0180	0.179	0.854	0.241
Lag time	2.78^{b}	3.32^{ab}	1.64^{c}	1.90^{c}	1.47^{c}	3.48^{a}	2.12^{a}	2.74^{b}	0.288	0.001	0.001	0.001
DMd	58.7^{b}	61.7^{b}	68.8^{a}	60.5^{b}	71.7^{a}	68.0^{a}	63.1^{a}	66.7^{b}	0.80	0.001	0.001	0.002
RGP	426^{a}	373^{b}	348^{b}	332^{bc}	338^{bc}	303^{c}	357	350	2.5	0.001	0.492	0.001

[1] PW: poultry waste; SM: swine manure; U; urea; BB: bakery by-product; M; molasses. [2] SEM: standard error of mean. [3] DM expressed as g kg^{-1} of fresh matter. [4] A: total gas production (mL gas g^{-1} incubated DM); B: fermentation rate (h^{-1}); C: fermentation rate (h$^{-1/2}$); Lag time (h); DMd: dry matter disappeared (mg/100 mg DM); RGP: relative gas production (mL gas g^{-1} DMd after 96 h of incubation). [a,b,c,d] Different letters indicate significance ($p < 0.05$). Within a row, means with different letter differ ($p < 0.05$; Tukey's test).

for silages containing U compared with the rest of treatments. Content of OM was higher ($p < 0.05$) for SMBB, UBB and UM than for PWM and SMM. The CP content was higher ($p < 0.05$) for UBB and UM, due to the addition of 6.8% of U. There were no differences ($p > 0.05$) in NDF, ADF and lignin contents among treatments. Evans & Smith (1986) reported that the use of U lead to changes in cell wall components of forages treated, destroying the linkages of phenolic groups between hemicellulose and lignin, which solubilizes the hemicellulose and facilitates cell wall degradation.

The inclusion of additive resulted in lower ($p < 0.05$) OM content and higher NDF content. Similar results were reported by Gutiérrez *et al*. (2003), who evaluated pineapple waste silage (80%) and PW (20%). Borquez *et al.* (2009) evaluated cattle manure silage with BB, and observed a reduction in the amount of DM (396 *vs.* 424 g kg^{-1}). Treatment × additive interactions ($p < 0.05$) were detected for DM, OM and CP contents of silage.

Table 2 also presents the parameters of *in vitro* gas production of silages. Total GP was higher ($p < 0.05$) for PWBB compared to SMBB, SMM and UM. Fermentation rate (B) was higher ($p < 0.05$) for SMBB and SMM than for the rest of the treatments, but parameter C was not affected ($p > 0.05$). Lag time was lower ($p < 0.05$) for SMBB, SMM and UBB, and DMd was higher ($p < 0.05$) for SMBB, UBB and UM than for the rest of the treatments. Values of DMd in PW silages were lower than the 763 g kg^{-1} DM reported by Mendoza & Ricalde (1993), but Borquez *et al.* (2009) reported a value (621 g kg^{-1} DM) similar to that in the present study for a cattle manure silage with 16% BB. Mthinyane *et al.* (2001) ensiled PW (40% inclusion) and obtained 52% *in sacco* DMd. The PWBB silage had the highest ($p < 0.05$) value of RGP. The addition of Sill-All$^{4\times4}$ decreased ($p < 0.001$) total GP, lag time and DMd, but treatment × additives interactions ($p < 0.05$) were detected for most gas production parameters.

The use of alternative sources of protein such as poultry and swine manure and by-products of sugar and bakery are an alternative for ruminant feeding. The results show that properly formulated silages including these by-products can provide more than 16% of crude protein and values of *in vitro* DMd above 60%.

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