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Effect of the inclusion of additives on the quality of sugarcane silage

Efecto de la inclusión de aditivos sobre la calidad del ensilado de caña azúcar

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ABSTRACT:

Objective. The objective of the study was to evaluate the effect of the addition of an additive and an inoculum bacterial in the chemical composition and *in vitro* digestibility dry matter (IVDMD) and organic matter (IVOMD) of sugarcane silage. **Materials and methods.** Experimental treatments were: a) sugarcane silage with 1% of bacterial inoculum and 1% of additive (SCS1); b) sugarcane silage with the bacterial inoculum 3% and 1% of additive (SCS3). The bacterial inoculum consists of 10.0% molasses, 1.0% of commercial yogurt containing *Lactobacillus plantarum*, *L. bulgaricus*, *L. casei*, *L. acidophilus* and *L. bifidus*, 5.0% poultry manure, 0.5% urea and 83.0% of water; the additive is formulated with 1.0% urea, 0.1% sulfate of ammonium and 0.25% phosphorus. Each treatment was determined to its chemical composition and *in vitro* digestibility of dry and organic matter and the results obtained were analyzed using T Student test. **Results.** There were differences between treatments ($p < 0.05$) in crude protein, neutral detergent fibre, hemicellulose, ammoniacal nitrogen and pH, as well as on IVDMD, but without changes in the IVOMD, presenting the better values for SCS1. **Conclusions.** The 1% concentration of bacterial inoculum and additive added to sugarcane silage showed better values on *in vitro* digestibility of dry matter, and chemical composition, indicating an adequacy fermentation, but, at concentrations higher than 1% of additive and an inoculum bacterial decreases the quality and digestibility of the silage.

KEYWORDS: Silage, forage, fermentation.

RESUMEN:

Objetivo. El objetivo de este estudio fue evaluar el efecto de la adición de un inóculo bacteriano y un aditivo químico en la composición y digestibilidad *in vitro* de la materia seca (DIVMS) y orgánica (DIVMO) en el ensilado de caña de azúcar. **Materiales y métodos.** Los tratamientos experimentales fueron: a) ensilado de caña de azúcar con 1% de inóculo bacteriano y 1% de aditivo (ECA1); b) ensilado de caña de azúcar con el 3% de inóculo bacteriano y 1% de aditivo (ECA3). El inóculo bacteriano está compuesto por 10.0 % melaza, 1.0 % de yogurt comercial que contiene *Lactobacillus plantarum*, *L. bulgaricus*, *L. casei*, *L. acidophilus* y *L. bifidus*, 5.0 % pollinaza, 0.5% urea y 83.0 % de agua; el aditivo está formulado con 1.0% urea, 0.1% sulfato de amonio y 0.25% fósforo. A cada tratamiento se le determinó su composición química y la digestibilidad *in vitro* de la materia seca y orgánica y los resultados obtenidos fueron analizados mediante una prueba de T student. **Resultados.** Existieron diferencias entre los tratamientos ($p < 0.05$) en proteína cruda, fibra detergente neutro, hemicelulosa, nitrógeno amoniacal y pH, así como en DIVMS, pero sin cambios en la DIVMO, presentando los mejores valores para ECA1. **Conclusiones.** La concentración del 1% de inóculo bacteriano y aditivo adicionado a caña de azúcar ensilada mostró mejores valores en digestibilidad de la materia seca, y composición química, indicando una fermentación adecuada por lo que a concentraciones mayores a 1% de aditivo e inóculo bacteriano se disminuye la calidad y digestibilidad de los ensilados.

PALABRAS CLAVE: Ensilaje, forraje, fermentación.

INTRODUCTION

Sugarcane is a crop produced in more than 100 countries worldwide, and its biomass production exceeds that of any other forage, which can be used as animal feed as a strategy for sustainable agricultural development in many countries (1,2)

Silage is a method of preservation of forage with high moisture content, which is based on fermentation lactic acid of the fodder under anaerobic conditions. Bacteria acid lactic acid, typical of the material to be self-propelled, they ferment soluble carbohydrates of forage, mainly producing lactic acid and, to a lesser extent, acetic acid and to make this fermentation process optimal, is mainly required one sufficient amount of lactic acid bacteria and a proper concentration of soluble carbohydrates in forage producing lactic acid, so that the pH is kept low and the silage is preserved best (3). Sugarcane silage, is usually done without additives, causing losses of up to 30% of the dry matter, in addition to concentrating the components of the cell wall and the reduce the *in vitro* digestibility of dry matter (4).

In addition, the silage, have high levels of lactic acid and residual carbohydrates, which can be potentially usable by silage spoilage microorganisms, such as yeasts, after the opening of the silo (5,6). In recent years, interest has grown by the use of sugarcane silage additives capable of inhibiting the growth of yeasts that promote the alcoholic fermentation (4), there are various additives that have been studied for silage forage in general, including urea (7), strains of homofermentative of the species *L. plantarum* and *L. paracasei* and heterofermentative of the species *L. brevis* that have shown greater growth during fermentation of sugarcane. However, *L. plantarum* is the most widely used in tropical forages silages (8). Recently, the addition of heterolactic bacteria, mainly of the species *L. buchneri*, has shown good results, especially in greater aerobic stability and inhibition of yeasts and proliferation of fungi (9,10).

Therefore, the main focus of the studies with sugarcane silage is the search for additives which, associated with silage, inhibit the alcoholic fermentation of fodder in order to reduce losses, for this reason in the present work we evaluated the effect of the addition of bacterial inoculum and an additive to the sugarcane during the process of silage, on the chemical composition and digestibility of dry matter of the silage.

MATERIALS AND METHODS

Study site. This study was carried out in the Agricultural Farm “Dos Pivotes” located to the southwest of the Municipality of Zapotlan El Grande, in the State of Jalisco, Mexico; with geographic coordinates 19°27'13” north latitude and meridians 103°27'57” west longitude, with an altitude of 1.520 m. The climate is warm, with average annual rainfall of 732 mm distributed in the months of June to September, and occasional winter or summer rainfall. Its average temperature is 20.2°C (11). Chemical determinations were carried out in the laboratory of nutrition of the University Center of the South of the University of Guadalajara located in the same municipality.

Preparation of samples and treatments. In order to observe the effect of the addition of inoculum and an additive to silage sugarcane *in vitro* digestibility of dry matter set out the following treatments: a) sugarcane silage with 1% of bacterial inoculum and additive (SCS1); b) sugarcane silage with 3% of bacterial inoculum and 1% of additive (SCS3). The inoculum consisted of 10.0% molasses, 1.0% of commercial yogurt (containing: *L. plantarum*, *L. bulgaricus*, *L. casei*, *L. acidophilus*, and *L. bifidus*), 5.0% chicken manure, 0.5% urea, and 83.0% water, and the additive was formulated with 1.0% urea, 0.1% ammonium sulfate, and 0.25% phosphorus. The poultry manure in Mexico is commonly used as a source of non-protein nitrogen to feed ruminants, including cattle, but there are countries that restrict its use in animal feed by the presence of

pathogenic organisms, viruses, hormones, among others that may be contained in this waste, however, there are studies that the silage process decreases the microbial load (12) and also the non-transmission of prion of transmissible spongiform encephalopathy or negative effects in the carcasses in lambs in growth (13,14). The poultry manure used in this study was according to the Norma Oficial Mexicana promulgated in the Official Journal of the Federation (NOM-044-ZOO-1995).

The yogurt was homemade preparation; mixed 250 ml of commercial natural yogurt that contains containing *L. plantarum*, *L. bulgaricus*, *L. casei*, *L. acidophilus* and *L. bifidus*, with 5 L of cow's milk. Silage for each treatment process was carried out in two silos, in layers of fodder of 50 cm, sprinkling the inoculum and applying to volley the additive, and compacted with tractor to make the volume above mentioned to finally cover and seal with a canvas plastic. After 30 days, 20 samples at random from each of the silos were collected and deposited in a black plastic bag with an airtight seal for further analysis. Samples of ingredients were dried in a circulating air oven at 60 °C for 48 hours and then milled in a hammer mill equipped with a 2 mm sieve for further analysis

Chemical analysis. Each sample was determined dry matter (DM), crude protein (CP) was determined by the method described by Kjeldahl. Ash (A) and organic matter (OM) were calculated by difference using the technique described by the AOAC (15). The determination of the fiber fractions (NDF and ADF) was performed using alpha amylase without ash correction as specified by Van Soest et al (16), and ammonia nitrogen (NH₃-N) was determined by spectrophotometry according to McCullough (17).

Extraction of rumen fluid for inoculation. Were used as donors of inoculum, two 4-year-old Holstein cows (525±43 kg) equipped with permanent rumen cannula with a core diameter of 10 cm (Bar Diamond Lane, Parma, ID, USA), fed silage sugarcane *ad libitum* plus 1.0 kg of commercial dairy concentrate split into two sessions (8:00 y 16:00 h) to ensure increased activity of microorganism in the rumen. Fresh clean water was available *ad libitum*. The water and feed supply was suspended 16 h before the extraction of the ruminal fluid.

In vitro incubation. The samples were incubated for 48 h, using the technique of Tilley and Terry (18). He was twice the incubation, with three replicates for each treatment and each replica with 5 subsamples. With the data obtained was determined *in vitro* digestibility of dry matter and organic matter.

Statistical analysis. The chemical composition and digestibility data were analyzed using the T Student test (19).

RESULTS

Table 1, shows the chemical analysis of the experimental materials. The concentration of MS and MO were similar for both treatments ($p>0.05$), however, SCS3, showed a higher content of CP, but also of NH₃-N, which causes a greater buffering effect and making requiring greater acidity to lower the pH of the silage, which is consistent with the findings in this study where treatment with 1% inoculum and additive showed lower pH, as well as PC and ammoniacal nitrogen value.

TABLA 1.
Tabla 1. Chemical composition of experimental samples.

Components	SCS1	SCS3*
	%	
Dry matter	27.00 ^a	29.00 ^a
Organic matter	91.42 ^a	89.96 ^a
Crude protein	14.71 ^b	17.75 ^a
NDF	53.83 ^a	44.91 ^b
ADF	22.30 ^a	18.34 ^a
Hemicellulose	31.53 ^a	26.57 ^b
N-NH ₃	3.50 ^b	16.00 ^a
Ash	8.58 ^a	10.04 ^a
Silage pH	4.30 ^b	4.75 ^a
^{ab} Different letters following the means in the same row indicate differences (p<0.05). *SCS1: sugarcane silage with 1% of bacterial inoculum and additive; SCS3: sugarcane silage with 3% of bacterial inoculum and 1% of additive.		

Acid detergent fiber concentration was similar for both treatments, but treatment with 1% inoculum and additive had a cell wall (NDF) and increased hemicellulose concentration. On the other hand, the silage pH and NH₃-N was lower in this treatment, which indicates accepted good fermentation quality.

Differences were found (p<0.05) in the coefficients of *in vitro* digestibility dry matter, where treatment with 1% was higher compared with the treatment with 3% additive, however, did not find change between treatments for organic matter (Table 2).

TABLA 2.
Tabla 2. Coefficient of *in vitro* dry matter (IVDMD) and organic matter digestibility (IVOMD) of experimental samples (%).

	IVDMD	IVOMD
SCS1*	41.93 ± 0.02 ^a	84.18 ± 0.48 ^a
SCS3	35.06 ± 0.03 ^b	85.28 ± 0.39 ^a
^{ab} Different letters following the means in the same column indicate differences (p<0.05). *SCS1: sugarcane silage with 1% of bacterial inoculum and additive; SCS3: sugarcane silage with 3% of bacterial inoculum and 1% of additive.		

DISCUSSION

The content of crude protein of the silages were lower than those reported by Granda et al (18) evaluated the effect of three inoculant in sugarcane silage, in this study SCS3 presented higher values of CP and N-NH₃ probably favored by the inoculum and additive, thus increasing the microbial activity on the substrate and in the uptake of carbohydrates composed of easily digestible and soluble; similar results reported several authors (6,19,20), using as additive urea 1.5%, benzoate sodium 0.1% and sodium hydroxide 1% and *L. plantarum*, *L. paracasei* and *L. buchneri* respectively.

Santos et al (21) evaluated sugarcane silages treated with additives and observed that values ranging from 32% to 35%, 48% to 52%, for DM and FDN, respectively, which are similar to those observed in this study, however, FDA reported values between 29 and 32% which are higher than those determined in our study. The NDF and hemicellulose was higher in SCS1, but no difference in FDA, which may explain that SCS1 showed higher value of IVDMD in comparison with SCS3, but not change in IVOMD.

The pH values were different ($p < 0.05$) between treatments (Table 1). SCS1 was lower in comparison with SCS3; considering that an acceptable silage should have a pH around 4.0 (22). The inclusion of 1% of inoculum and additive in this study, is among these ranges being acceptable as a fermentation, since a value of low acidity silage, reduces proteolysis and improves the stability of aminoacids (23) that it can be confirmed by the low content of ammoniacal nitrogen found in SCS1, at concentrations less than 11% of the NH₃-N the silage are qualified as acceptable and more than 15% are of poor quality, which showed SCS3 for the concentration of CP and N-NH₃ (16%), and being highly shock-absorbers materials requires high amounts of acids, which probably did not decrease the pH of the SCS3 silage, in ranges referred to above, situation that happens in this study and that was in the low IVDMD.

The increase in pH and N-NH₃ may be related to the greater inclusion of the inoculum and mainly to the additive in this treatment, because there is increase in the concentration of urea, presents a greater hydrolysis of it, transforming it into ammoniacal nitrogen which when mixed with water, it produces ammonium hydroxide, although you can control the growth of yeast, it is an unfavorable reaction during the silage process that the decrease of yeasts, presents naturally, in sugarcane in high quantities, are that begin the fast process of fermentation in silage sugarcane (20, 24).

In conclusion, the 1% concentration of bacterial inoculum additive added to silage sugarcane showed better values of pH and N-NH₃ and chemical composition, as well as greater in vitro digestibility of dry matter, although he showed no changes on in vitro digestibility organic matter. Concentrations above 1% additive and bacterial inoculum increased ammonia nitrogen concentration in silage, modifying the process of fermentation, and concomitantly decreasing the quality and digestibility of the silage.

CONFLICT OF INTERESTS

The author (s) did not declare potential conflicts of interest with respect to the investigation, authorship and/or publication of this article.

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