Chemical composition of corn silages inoculated with lactic acid bacteria and *Bacillus subtilis*

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Introduction Silage is a common method of preserving forage and is based on the conversion of soluble carbohydrates into organic acids (mainly lactic acid) by the action of lactic acid bacteria (LAB, as *Lactobacillus plantarum*) under anaerobic conditions. As a result, the pH decreases and the forage are preserved against deterioration caused by microorganisms. However, silages resultant of homo-fermentation presents low aerobic stability. Therefore, inoculants containing heterofermentative LAB, such as *L. buchneri* are used to improve the aerobic stability of the silage by producing high levels of acetic acid (inhibit action of yeasts and molds). *Bacillus subtilis* is used to control aerobic spoilage because this bacterium produces substances anti-microorganisms (e.g. bacteriocins) (Katz and Demain, 1977). However, the combination of the homo and heterofermentative microorganisms may improve the silage quality. Thus, our objective was to evaluate the chemical composition of corn silages inoculated with lactic acid bacteria and *B. subtilis*.

Material and Methods The hybrid Impacto Víptera (Syngenta) was harvested with average of 403 g/kg of dry matter (DM) and chopped by a conventional forage harvester to 0.5 cm. The treatments evaluated were: control (uninoculated); forage inoculated with *L. plantarum* MA18/5U and *L. buchneri* CNCM I-4323 at a rate of $1x10^5$ cfu/g fresh forage (LB + LP); forage inoculated with *L. plantarum* MA18/5U and *B. subtilis* AT553098 at a rate of $1x10^5$ cfu/g fresh forage (BS + LP). Inoculants were dissolved in water (0.7 L/t) and then applied with spray mounted on the fresh forage under constant mixing. The similar amount of water was applied in control silage. Three silos were filled with 40 t of corn forage each. Inoculation and packing was completed on the same day. After 88 days of ensiling, the silos were opened and samples were taken for determination of DM, mineral matter (MM), organic matter (OM), total nitrogen (TN) and ether extract (EE) contents following the recommendations of AOAC (1996). The crude protein (CP) content was obtained by the product between the TN and the factor 6.25. Neutral detergent fiber (aNDF) content was estimated using the techniques described by Van Soest et al. (1991). The total carbohydrates (CHO) and non-structural carbohydrates (NSC) contents were calculated according to Sniffen et al. (1992). Data were analyzed using a completely randomized design with 12 replicates using the MIXED procedure of SAS (v. 9.0). The treatment means were compared by Tukey test at 5% significance level, and tendencies between 5 and 10%.

Results and Discussion Corn silages inoculated with LAB and *B. subtilis* presented higher DM content (Table 1). This result is more related to the DM content in the moment of harvest of corn plants (Control = 379.0; LB + LP = 394.2; BS + LP = 435.5 g/kg of DM) than the inoculants applications. We observed difference in relation to ash and OM contents, where the BS + LP silage presented higher OM content and lower ash content than control and LB + LP silages. Silage still presented higher EE, CP and CHO contents in relation the LB + LP silage. Inoculants containing homofermentative LAB such as *L. plantarum* are often used to control the ensiling fermentation by rapid production of lactic acid and the consequent decrease in pH (Filya, 2003). The rapid decreases of pH values can inhibit the action of

spoilage microorganisms in anaerobic condition, and ensure the chemical characteristics of the forage used in the ensiling process, and consequently the silage quality. However, the *B. subtilis* is used to control spoilage because mainly by production of substances anti-microorganisms (Katz and Demain, 1977). Already the *L. buchneri* presents higher effect after opening of the silos because of the acetic acid concentration. This inoculant also can change the fiber content because of the ferulic acid production from some strains (Nsereko et al., 2008), although the *L. plantarum* and *B. subtilis* also can produce this enzyme (Cavin et al., 1997; Donaghy et al., 1998). However, this result was not observed in this research.

Table 1 Chemical composition (g/kg of DM) of corn silages inoculated with lactic acid bacteria and *Bacillus subtilis*.

Item	Control	LB + LP	BS + LP	SEM[1]	P-value
DM	345.6^{c}	362.1^{b}	392.0^{a}	0.324	<0.0001
Ash	51.7^{a}	50.0^{a}	29.5^{b}	0.446	0.0004
OM	948.2^{b}	950.0^{b}	970.4^{a}	0.446	0.0004
EE	34.6^{ab}	33.1^{b}	37.1^{a}	0.108	0.0366
CP	92.7^{b}	91.3^{b}	96.8^{a}	0.127	0.0121
CHO	821.2^{ab}	810.7^{b}	836.0^{a}	0.461	0.0017
aNDF	372.3^{a}	392.4^{a}	382.8^{a}	0.642	0.1024
apNDF[2]	331.1^{b}	339.8^{ab}	352.4^{a}	0.623	0.0663
NSC	492.3^{a}	473.6^{a}	484.9^{a}	0.711	0.1751

Means followed by different letters differ by Tukey test (P<0.05).
[1]SEM = standard error of the mean; [2]apNDF = NDF corrected for residual ash and protein.

Conclusions The microbial inoculation did not changed the chemical composition of corn silages.

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