Fermentation, microbial occurrence and aerobic stability of corn silages inoculated with *Bacillus subtilis*

M. Morelli[1], F. C. Basso[1], E.C. Lara[1], C. H. S. Rabelo[1], B. L. Cabral[1], R. A. Reis[1]
[1]*Animal Science Department, Faculty of Agricultural Sciences and Veterinary, São Paulo State University-UNESP, São Paulo, Brazil*
Email: fcarvalhobasso@yahoo.com.br

Introduction Corn silage has high concentration of nutrients, which crucially affects its stability after opening the silos under the action of undesirable microorganisms. In this context, the use of microbial inoculants have been proposed in order to increase the aerobic stability of these silages (Kung Jr. et al., 2003). In this sense, many species and strains of the genus *Bacillus* produce a large number of antimicrobial substances including antibiotics. According to Todovora & Kozhuharova (2009), the species *Bacillus subtilis* is one of the most important producers of antifungal and antibacterial compounds from genus *Bacillus*. Strains of *B. subtilis* have been studied in corn silage (Phillip & Fellner, 1992), demonstrating a positive effect on aerobic stability of these bulky. Therefore, the aim of this research was to investigate the effect of *Bacillus subtilis* inoculation on the characteristics and fermentation losses, microbial occurrence and aerobic stability of corn silages.

Materials and Methods The corn was harvested when the grain were in the dough stage. The treatments evaluated were: control (silage without inoculation of *B. subtilis*); inoculation with $5x10^4$ colony forming units - CFU of *B. subtilis*/g of forage, with $1x10^5$ CFU *B. subtilis*/g of forage and with $5x10^5$ CFU *B. subtilis*/g of forage. Silos plastic buckets with a capacity of 7 L were used as experimental. After 60 days of ensiling, the silos were opened, the spoiled forage was discarded, and the remainder was homogenised and sampled to determine the DM content, pH values, ammonia nitrogen (NH_3-TN), lactic acid, acetic acid, yeasts and molds counts. Silage temperature was measured every half hour by a data logger inserted in the center of mass during the aerobic exposure. Room temperature was measured by data logger distributed near of the experimental silos.The data were analysed as a completely randomised design and subjected to an analysis variance with the Statistical Analysis System (1988). The means were separated by the Tukey´s test, and the significance level was $P<0.05$.

Results and Discussion Inoculation of forage with *B. subtilis* levels did not cause changes in the concentrations of dry matter (DM), NH_3/TN, acetic and lactic acid and number of molds from silage ($P>0.05$). However, the pH values, yeasts counts and aerobic stability were affected by inoculation with *B. subtilis* ($P<0.05$). In control silage was observed lower pH value than that silages in inoculated with *B. subtilis* at doses of $5x10^4$ and $1x10^5$ CFU/g of forage. However, the pH values are considered suitable to a desirable fermentation (3.5 to 3.7), as described by Borreani et al. (2002). The yeasts counts was lower in silage inoculated with *B. subtilis* at a dose of $5x10^5$ CFU/g forage compared to the control treatment, which can be explained by production of antifungal compounds by this microorganism (Todovora & Kozhuharova, 2009). However, the *B. subtilis* levels lowest inoculated at the time of ensiling ($5x10^4$ and $1x10^5$ CFU/g forage) were not enough to reduce the proliferation of yeasts in relation to the control treatment. The aerobic stability was improved in silages inoculated with $1x10^5$ and $5x10^5$ CFU of *B. subtilis*/g forage. As it is known, the increase of temperature is caused mainly by the action of yeast and bacteria.

Conclusions The inoculation of $5x10^5$ CFU of *Bacillus subtilis* /g of forage with controls the growth of yeast and improve the aerobic stability in corn silage.

References

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Table 1. Characteristics and losses fermentative, microbiological occurrence and aerobic stability of corn silage inoculated with *Bacillus subtilis*[1]

Vaiable	Control	$5x10^4$	$1x10^5$	$5x10^5$	VC (%)
DM (%)	37.49^A	36.71^A	35.49^A	36.56^A	3.19
pH	3.71^B	3.75^A	3.76^A	3.73^{AB}	0.39
NH_3/NT (%)	5.27^A	6.26^A	6.42^A	5.56^A	19.59
Acetic acid (% of DM)	0.94^A	1.18^A	1.06^A	0.99^A	15.13
Lactic acid (% of DM)	4.07^A	3.32^A	3.52^A	3.15^A	19.47
Yeasts (Log_{10} cfu/g of silage)	4.73^A	4.12^{AB}	3.82^{AB}	3.09^B	16.68
Molds (Log_{10} cfu/g of silage)	4.90^A	5.14^A	4.54^A	4.47^A	21.77
Aerobic stability (hours)	43.6^C	93.75^B	157.82^A	157.87^A	9.66

[1]Means followed by same letter in the line do not differ by Tukey test at 5% significance.