Long storage period and organic acids addition on microbial profile of soybean whole-plant silage

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Introduction The increase in interest in silage use of the whole plant of soybean (Glycine max), was because is a leguminous plant of high nutritional value, which can be used in animal diet (Gobetti et al., 2011). However, the conservation of the plant as silage presents factors that hinder proper fermentation, such as the development of undesirable microorganisms that cause quality losses without the use of additives (Arcanjo et al., 2016). Therefore, this study was to evaluate the effects of adding inoculant and organic acids in soybean whole plant silage with different opening times of the microbial population.

Materials and Methods The soybean cultivar used was GMX CANCHEIRO RR. The whole plant soybean was harvested at the R7 vegetative stage under cultivation conditions of Southern Mato Grosso do Sul. The experimental was completely randomized design with repeated measures in time, where the treatments were: 1) CON (no additives added); 2) INO (Bacterial inoculante application: 4g/ton with blend of $4x10^{10}$ cfu/g of *Lactobacillus plantarum* and $2.6x10^{10}$ cfu/g of *Propionibacterium acidipropionici*); 3) AcF (addition of product formic acid based: 2 ml/kg organic acid blend of 35-45% formic acid, 15-45% propionic acid and 15-20% sodium formate); 4) AcP (addition of product propionic acid based: 2 ml/kg blend of 50-60% propionic acid, 15-20% formic acid and 1-5% propionate sodium, 5% 1-propionate of glycerol and 15-25% glycerol). The additives were applied individually on the forage assigned for each bucket to generate true replications. The experimental silos were opened every 30 days (five repetitions per treatment), composing 6 opening times and 180 days of total storage. Ten grams of the samples taken from various parts of the silo, was used to saline to sterile 90 ml serial dilution of 10^{-1} to 10^{-6} in test tubes. Quantification of microorganisms were done in triplicate for each dilution, the culture medium used was the MRS agar (Man, Rogosa and Sharpe) for count of lactic acid bacteria, the Nutrient Agar for total count of aerobic and anaerobic bacteria, and for counting fungi (moulds and yeast) the PDA (Potato Dextrose Agar). The number of microorganisms were counted as colony forming unit (cfu) and expressed as $\log_{10}$. Data were analyzed using SAS software (version 9.1.3, SAS Institute, Cary, NC 2004). For review of experimental treatments outside made orthogonal contrasts where first contrast (C1; CON vs. additives), second contrast (C2; INO vs. blend organic acids), third contrast (C3; AcF vs. AcP), significance level was set at 0.05.

Results and Discussion The microorganism count was influenced by the treatments, storage time, observing the interaction effect (time x treatment) ($P < 0.0001$; Table 1). The silage treated with

organic acids showed lower counts ($P < 0.0001$) of lactic acid bacteria, anaerobic bacteria, and aerobic fungi and molds in relation to treated with microbial inoculant. The highest rate of lactic acid bacteria found in INO, was similar to found by Gandra et al. (2018), that may be related to the composition of the inoculant used, enhancing the development of these microorganisms (Moon, 1983), beyond what the blend of organic acids may have inhibited the growth of other microorganisms. The materials treated with AcP presented higher counts ($P < 0.0001$) of lactic acid bacteria compared to those treated with AcF. However, lower concentrations ($P < 0.0001$) of mold and yeast were observed in AcP treated soybean silages compared to AcF. Chemical additives such as AcF and AcP are used in silage of crops such as corn and sugarcane (Pedroso et al., 2005) to control moulds and yeast, but in whole plant soybean silage this effect was not observed in the AcF treatment.

Table 1 Microbial profile according to experimental treatments.

Item	Treatments[1]					*P*-value[3]					
	CON	INO	AcF	AcP	SEM[2]	Treat	Time	Int	C1	C2	C3
Lactic acid bacteria, log cfu/g	7.245	7.582	6.378	6.730	0.067	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Anaerobic, log cfu/g	6.268	5.901	4.990	6.431	0.120	<.0001	<.0001	<.0001	<.0001	<.0001	< 0001
Aerobic, log cfu/g	4.898	6.759	6.482	6.656	0.172	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Mold and yeast, log cfu/g	5.195	4.916	5.644	3.651	0.201	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

[1]CON = excluding additive; INO = 4g/ton with blend of $4x10^{10}$ cfu/g of *Lactobacillus plantarum* and $2.6x10^{10}$ cfu/g of *Propionibacterium acidipropionici*; AcF = 2ml/kg organic acid blend with 35-45% formic acid, 15-45% propionic acid, 15-20% sodium formate; AcP = including blend of organic acids 2ml/kg as 50-60% acid propionic acid, 15-20% of acid formic, 1-5% propionate sodium, 5% 1-propionate of glycerol, 15-25% glycerol.
[2]SEM = standard error of the mean.
[3]Trat = treatment effect; Int = time * treatment interaction effect; C1 = CON vs. additives; C2 = INO vs. organic acid blend; C3 = AcF vs. AcP.

Conclusions The addition of AcP reduced the fungal count and INO increased the lactic acid bacterial count, presenting the potential to be used in whole plant soybean silage.