Long storage period and amylases addition on fermentative profile of rehydrated ground corn silage

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Key words: alpha-amylase, enzyme, glucoamylase, starch

Introduction Corn is one of the principal ingredients used to meet the energy demands in the diet of high production ruminants. However, most hybrids grown in Brazil has high vitreousness, which ends up limiting its digestibility. Wet corn grain silage improves aspects of ruminal starch degradation by promoting zein solubilization during the fermentation process (JUNGES et al., 2017). And the addition of enzymatic compounds in the animal diet has been used more frequently in order to improve nutrient performance and profitability (CAMPESTRINI; SILVA; APPELT, 2005). Thus, the objective of the study was to evaluate the action of amylolytic enzymes on the fermentative profile of rehydrated corn silage.

Materials and Methods Corn grain was milled, rehydrated in a ratio of 30:100 (L/kg) and homogenized. One hundred and twenty experimental silos were prepared in plastic buckets (40 cm high, 30 cm in diameter) containing Bunsen valves to avoid gas penetration and allow gas scape. The experiment was composed of the following treatments: Control (CON); AMY, 300 mL t^{-1} of fresh matter (Kerazyme 3035, Kera Nutrição Animal, Bento Gonçalves, RS); GLU, 300 mL t^{-1} of fresh matter (Kerazyme 4560, Kera Nutrição Animal, Bento Gonçalves, RS);. All silos were also inoculated with KeraSIL grão úmido® (Kera Nutrição Animal, Bento Gonçalves, Brazil) added at 4 g t^{-1} of hydrated ground corn. KeraSIL is composed by *L. plantarum* (4.0×10^{10} ufc g^{-1}) and *P. acidipropionici* (2.6×10^{10} ufc g^{-1}). The silos were packed and were opened (5 mini silos per treatment per time point) on days 30, 60, 90, 120, 150, 180, 210, 240 of storage. To measure the pH, 10 g samples were homogenized in 50 ml of distilled water and allowed to stand for 30 minutes. The reading was made with digital potentiometer (MB-10, Mars, Santa Rita do Sapucaí, Brazil). Ammonia nitrogen analysis was performed using a colorimetric method as described by Kulasek (1972) and adapted by Foldager (1977). Lactic acid concentration was assessed by high performance liquid chromatography (HPLC LC-10ADVP Shimadzu system, Shimadzu Inc., Kyoto, Japan) as described by Ding et al. (1995). Organic acid analysis was performed on a gas chromatograph (GC-2010 Plus chromotograph, Shimadzu, Barueri, Brazil), according to the methodology of Erwin et al. (1961). The peaks identified according to the methodology were quantified using GCsolution v. 2.42.00 software (Shimadzu©). Data were submitted to analysis of variance using the PROC MIXED of SAS 9.3 as repeated measures and fixed effects were included: enzyme, time and enzyme by time interaction. The differences between the treatments were studied by orthogonal contrasts, as follows: CON vs. GLU + AMI (C1) and GLU vs. AMI (C2).

Results and Discussion The addition of enzymes reduced ($P \leq 0.013$) pH values and concentrations of N-NH_3, acetate, propionate and butyrate, and increased ($P < 0.001$) ethanol and lactate concentrations of silage (Table 1). With the exception of N-NH3 ($P = 0.438$) there was a time effect ($P \leq 0.008$) for all variables related to the fermentative profile. Regarding α-AMI, GLU tended to increase ($P \leq 0.098$) acetate and propionate concentrations. In addition, there was interaction between enzyme and time effects on pH, ethanol and organic acid concentrations. Silos treated with different enzymes had similar ($P > 0.05$) pH values.

Increasing lactic acid concentration reduced the pH values. Oliveira et al. (2019) observed a reduction in pH in silos treated with amylolytic enzymes, especially when the glucoamylase enzyme was used. With respect to ethanol, the addition of enzymes may cause rapid acidification and favor the development of low pH-sensitive yeasts, leading to the fermentation of residual sugars to ethanol (CHAMBERLAIN, 1987).

Table 1 Fermentation profile according to experimental treatments.

	Treatments[1]			SEM[2]	*P*-value[3]				
	CON	AMY	GLU		Enzyme	Time	Interaction	C1	C2
pH	4.06	3.54	3.52	0.04	<0.001	<0.001	<0.001	<0.001	0.381
N-NH_3 (mg dL^{-1})	11.3	8.21	8.04	0.63	0.046	0.438	0.128	0.013	0.910
Ethanol *(g kg^{-1} MS)*	5.49	23.07	23.44	1.23	<0.001	0.007	0.005	<0.001	0.859
Lactate *(g kg^{-1} MS)*	4.37	6.35	6.94	0.28	<0.001	<0.001	0.006	<0.001	0.205
Acetate *(g kg^{-1} MS)*	31.7	8.31	13.7	1.69	<0.001	<0.001	0.008	<0.001	0.070
Propionate *(g kg^{-1} MS)*	0.96	0.41	0.52	0.05	<0.001	<0.001	<0.001	<0.001	0.098
Butyrate *(g kg^{-1} MS)*	1.91	0.46	0.60	0.14	<0.001	0.008	0.005	<0.001	0.582
SCFA[4] *(g kg^{-1} MS)*	1.40	1.43	1.38	0.03	0.739	<0.001	<0.001	0.851	0.452

[1]CON = Control; AMY = amylase, Kerazyme 3035, enzymatic activity 300 mL t^{-1} of fresh matter); GLU = glucoamylase, Kerazyme 4560, enzymatic activity 300 U mL^{-1}.
[2]SEM = Standard error of the mean.
[3]Orthogonal contrasts C1 = CON vs. α-AMI + GLU; C2 = α-AMI vs. GLU.
[4]SCFA = Total short chain fatty acids.

Conclusions Amylase addition reduced pH and increased lactic acid and ethanol contents in rehydrated corn grain silage.

References E. CAMPESTRINI, V.T.M. SILVA, M.D. APPELT. 2005. Utilização De Enzimas Na Alimentação Animal. **Revista Eletrônica Nutritime**, v. 2, n. 6, p. 259–272.
CHAMBERLAIN, D.G. 1987. The silage fermentation in relation to the utilization of nutrients in the rumen. Process Biochemistry, v.4, n.1, p.60-63.
M.I. DING, H. KOIZUMI, Y. SUZUKI. 1995. Comparison of three chromatographic systems determination of organic acids in wine. **Analytical Sciences**, v. 11, p. 239–243.
E.S. ERWIN, G.J. MARCO, E.M. EMERY. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. **Journal of Dairy Science**, v.44, n.9, p.1768-1771.
J. FOLDAGER. 1977. **Protein requirement and non protein nitrogen for high producing cow in early lactation**. Ph.D. thesis, East Lasing - Michigan State University.
D. JUNGES et al. 2017. Short communication: influence of various proteolytic sources during fermentation of reconstituted corn grain silages. **Journal of Dairy Science**, v. 100, n. 11, p. 9048–9051.
G. KULASEK. 1972. A micromethod for determination of urea in plasma, whole blood and blood cells using urease and phenol reagent. **Polskie Archiwum Weterynaryjne**, v.15, n.4, p.801-810.
E. R. OLIVEIRA et al. 2019. Effects of exogenous amylolytic enzymes on fermentation, nutritive value, and in vivo digestibility of rehydrated corn silage. Animal Feed Science and Technology, v. 251, p. 86–95.