# Effect of Amylolytic and Cellulolytic Enzymes on Whole Plant Corn Silage: Characteristics of Silage and Animal Digestion

**JEFFERSON RODRIGUES GANDRA** (jeffersongandra@unifesspa.edu.br)
Universidade Federal do Sul e Sudeste do Para https://orcid.org/0000-0002-4134-5115
**Alanne T. Nunes**
USP FMVZ: Universidade de Sao Paulo Faculdade de Medicina Veterinaria e Zootecnia
**Euclides R. Oliveira**
UFGD: Universidade Federal da Grande Dourados
**Mávio S. J. Silva**
UFGD: Universidade Federal da Grande Dourados
**Cibeli A. Pedrini**
UFGD: Universidade Federal da Grande Dourados
**Fabio S. Machado**
UFGD: Universidade Federal da Grande Dourados
**Geleice K. R. Silva**
UFGD: Universidade Federal da Grande Dourados
**Erika R. S. Gandra**
UFGD: Universidade Federal da Grande Dourados
**Paulo V. C. Mendes**
UNIFESSPA: Universidade Federal do Sul e Sudeste do Para
**Alzira G. S. Pause**
UNIFESSPA: Universidade Federal do Sul e Sudeste do Para

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# Effect of amylolytic and cellulolytic enzymes on whole plant corn silage: characteristics of silage and animal digestion

**Jefferson R. Gandra[a*], Alanne T. Nunes[c], Euclides R. Oliveira[b], Mávio S. J. Silva[b], Cibeli A. Pedrini[b], Fabio S. Machado[b], Geleice K. R. Silva[b], Erika R. S. Gandra[d], Paulo V. C. Mendes[d], Alzira G. S. Pause[a]**

[a] Universidade Federal do Sul e Sudeste do Pará, Instituto de Estudos em Desenvolvimento Agrário e Regional, Faculdade de Agronomia de Marabá, Marabá, PA, Brazil

[b] Faculdade de Ciências Agrarias, Universidade Federal da Grande Dourados, Rodovia Dourados-Itahum, Dourados, Brazil. 79804-970

[c] Department of Animal Nutrition and Animal Production, School of Veterinary Medicine and Animal Science, University of Sao Paulo. Pirassununga, Brazil. 13635-900

[d] Universidade Federal do Sul e Sudeste do Pará, Instituto de Estudos no Trópico Umido, Faculdade de Zootecnia e Medicina Veterinária, Xinguara, PA, Brazil

*Corresponding author: jeffersongandra@unifesspa.edu.br

## ABSTRACT

Enzyme additives have been added to forage at ensiling to improve nutritive value. The aim of this study was to evaluate effects of adding exogenous enzymes to silage on fermentative losses and profile, aerobic stability, chemical composition, in vitro degradation, microbial quality, and nutrients intake and digestibility. Treatments were control (CON); addition of exo-1,4-α-glycosidase glucoamylase (GLU; Kerazyme 3035, Kera Nutrição Animal, Bento Gonçalves, Brazil); addition of β-glucan 4-glucanhydrolase (CEL); and GLU + CEL. Data from the silo experiment were analyzed using PROC MIXED of SAS, with fixed effects of glucoamylase and cellulase, and interaction effect between them. In vivo experiment analyses also included fixed effect of Latin Square and period, and random effect of animal within Latin Square. CEL increased ($P \leq 0.038$) gas losses and effluents production, CEL and GLU decreased ($P = 0.039$) DM recovery compared to control but not differ from GLU+CEL. CEL silage had higher ($P \leq 0.021$) starch and crude protein and in vitro digestibility of DM and NDF ($P \leq 0.032$), while GLU had higher ($P = 0.001$) ADF. CEL showed lower ($P = 0.012$) ethanol content and higher ($P = 0.02$) anaerobic bacteria counts, while GLU showed higher ($P = 0.012$) lactate concentration and lower ($P \leq 0.002$) counts of bacteria and fungi. Lambs fed with CEL presented higher ($P \leq 0.012$) digestibility coefficients for DM, OM, CP and NDF. Decrease on DM recovery indicates no improvements on the nutritive value of silage. On the other hand, cellulolytic enzyme positively affected animal digestion.



**Keywords:** aerobic stability, fermentative losses, microbial quality, nutrient digestibility.

## INTRODUCTION

A variety of enzyme additives have been added to forage at ensiling to improve fermentation and the nutritive value of silage. Inclusion of enzyme additives to forage aims to break down plant cell walls at ensiling, which can improve silage fermentation once provide sugars for homofermentative lactic acid bacteria. Besides that, enzymes may also increase the digestibility of cell walls, enhancing the nutritive value of silage (Muck et al., 2018).

Cellulase is an enzyme that breaks down cellulose into beta-glucose and short-chain polysaccharides. Cellulase is made up of a complex of several different enzymes, including exoglucanases (also called cellobiohydrolases), endoglucanases, and beta-glucosidases. Fibrolytic enzymes added to silages can increase silage digestibility and decrease aerobic stability, as released sugars are rapidly used by spoilage yeasts and molds (Kung and Muck, 2015). Cellulolytic enzymes may act on the more-digestible components of NDF, leaving indigestible components intact what reduces the overall digestibility of consumed NDF (Nadeau et al., 2000; Dehghani et al., 2012; Jin et al., 2015).

Glucoamylases are amylolytic enzymes considered exoamylases, which cleave 1,4-α-glycosidic bonds from the nonreducing end of the glycosidic chains releasing d-glucose. Thus, these enzymes can increase the content of fermentable carbohydrates and reduce the

42 nonfermentable dextrins (Oliveira et al., 2019). Glucoamylase (1,4-α-D-glucan glucohydrolase) is extensively used to hydrolyze starch
43 solubilized, being particularly important in cereal silages, mainly of rehydrated corn grain (Gandra et al., 2019).

44 The addition of amylolytic and cellulolytic enzymes to silage with a high content of starch and NDF, such as corn whole plant, can favor
45 the fermentation process, increasing the digestibility of starch and fiber. We hypothesized that the inclusion of amylolytic and cellulolytic
46 enzymes simultaneously in whole plant corn silage improves the fermentation process and animal digestion. This trial aimed to evaluate
47 the effects of amylolytic, and cellulolytic enzymes added to whole-plant corn silage on fermentative losses, aerobic stability, nutritional
48 value, fermentative profile, microbiological population, and animal intake and digestion.

49

50 **MATERIALS AND METHODS**

51 This experiment was carried out between May and August 2018 at the Department of Animal Science of the Federal University
52 of Grande Dourados, located at 22°14′S, 54°49′W and 450 m of altitude.

53

***Harvesting, Treatments and Ensiling***

Whole plant corn silage (hybrid corn DKB 353 DEKALB$^{TM}$) was produced in a 5ha experimental field divided in 20 locations, until reaching at 105 d. Approximately 100 kg of whole corn plant from each location was manually harvested (ground level) and chopped to a theoretical cut of 10 mm using a stationary cutter. Samples (1,000 g) of chopped corn plant were assessed for contents of DM (method 950.15), ash (method 942.05), OM (DM − ash), CP (N × 6.25; method 984.13), and ether extract (EE; method 920.39) according to AOAC International (2000; Table 1). Neutral detergent fiber, acid detergent fiber, and lignin (sulfuric acid method) were determined according to Van Soest et al. (1991). Net energy content of lactation was calculated NRC (2001).

Four treatments in a factorial arrangement were randomly assigned to 40 experimental silos (plastic buckets, 30 cm height, and 30 cm diameter) equipped with Bunsen valves. Two kilograms of sand was placed in the bottom of the buckets and covered with a nylon mesh screen (500 μm) to drain effluents. Inoculant and chitosan were applied individually to forage assigned for each bucket to generate true replications. Forage was added to the buckets at a compaction rate of 600 kg/m$^3$ and silos were sealed, weighed, and stored at room temperature (24.6 ± 2.7°C; mean ± SD) for 60 d.

Treatments consisted of no enzymes (control; **CON**); 300 ml of fresh forage of exo-1,4-α-glycosidase glucoamylase, obtained from a selected strain of *Aspergillus niger,* enzymatic activity 300 U / mL (**GLU**; Kerazyme 3035, Kera Nutrição Animal, Bento Gonçalves, Brazil); 300 ml of fresh forage of β-glucan 4-glucanhydrolase, obtained from a selected strain of *Trichoderma reesei* (**CEL**; Kerazyme 3035, Kera Nutrição Animal, Bento Gonçalves, Brazil); and GLU + CEL. All treatments were inoculated with microbial

additive (4 g/ton *Lactobacillus plantarum*: $4x10^{10}$ cfu/g + *Pediococcus acidilactici*: $4x10^{10}$ cfu/g; KERAsil, Kera Nutrição Animal, Bento Gonçalves, Brazil). Microbial inoculant was diluted in water (2 g/L) and sprayed on the forage, according to manufacturer's information (https://www.kerabrasil.com.br/laminas/Kerasil.pdf).

***Fermentative Losses***

After 70 days of fermentation, mini silos were weighed to calculated gas losses. Effluent losses were calculated based on the difference between weight of silo assembly (plastic bucket, nylon screen, and sand layer) before the storage and weight of silo assembly (plastic bucket, nylon screen, and sand layer containing silage effluent) after 60 d.

The gas losses, effluent losses and dry matter recovery were calculated according to Jobim et al. (2007), as follows:

$$GL\left(\frac{g}{kg}DM\right) = \frac{SWE(g) - SWO(g)}{DME(kg)}$$

in which: SWE is the silo weight at the ensiling, SWO is silo weight at the opening, and DME is total DM ensiled.

$$EP\left(\frac{g}{kg}DM\right) = \frac{WSAO(g) - WSAE(g)}{DME(kg)}$$

where: WSAO is the weight of silo assembly after the opening (g) and WSAE is the weight of silo before the ensiling (g).

$$DMR\ \left(\frac{g}{kg}\right) = \frac{DMO\ (g)}{DME\ (kg)}$$

in which: DMO is total DM after the opening of silo (kg) and DME is total DM before the ensiling (kg).

***Silage Aerobic Stability***

Aerobic stability was considered as the period (h) in which corn silage temperature remained less than 1°C above the room temperature (Driehuis et al., 2001). During the 5 days period of aerobic stability evaluation, silos were maintained at room temperature (28.55 ± 4.27, mean ± SD), and temperature of silage was measured every 12 h after oxygen exposure using an infrared thermometer (MS6530, Wiltronics Research Pty. Ltd., Victoria, Australia). In addition, samples (100 g) from silos of each treatment were collected every 24 h to determine pH (Kung et al., 1984).

***Chemical Composition and In Vitro Degradation***

Forage samples (500 g) from each experimental silo were collected to assess DM, OM, NFC, CP, EE, NDF, ADF, lignin, ash, NEL and macro minerals as previously described. Dry matter and NDF *in vitro* digestibility were determined using filter bags and artificial rumen incubator (TE-150, Tecnal, Piracicaba, Brazil) according to Tilley and Terry (1963) and adapted by Holden (1999).

97 Briefly, filter bags with samples were incubated for 48 h at 39°C in a buffer-inoculum solution (1,600 mL of buffer solution and 400
98 mL of rumen inoculum). Jars containing the buffer-inoculum solution were purged with $CO_2$ and lids had gas relief valves. After the
99 incubation period, the buffer-inoculum was drained from the jars and the filter bags were gently squeezed against the sides of jar to
100 remove the gas trapped in inflated bags. Afterward, bags were rinsed in jars with 3 changes of warm tap water.

101

102 ***Fermentative Profile***

103 Silage liquid was extracted from forage samples using a hydraulic press and pH was measured using a digital potentiometer
104 (MB-10, Marte, Santa Rita do Sapucai, Brazil). Silage liquid aliquots (2 mL) were mixed with 1 mL of sulfuric acid (1 *N*) for
105 determination of ammonia nitrogen concentration through the colorimetric method described by Foldager (1977).

106 Volatile fatty acids, ethanol, and lactic acid concentrations in silage juice were determined at the Department of Applied
107 Chemistry of Federal University of Sao Carlos (Araras, Brazil) according to the methods described by Rodrigues et al. (2012). Briefly,
108 aliquots (1 mL) of silage juice were mixed with formic acid (0.2 mL) in amber glass bottles and frozen until analysis. Volatile fatty acids
109 and ethanol concentrations were determined in a gas chromatograph (Focus GC, Thermo Fisher Scientific Inc., Waltham, MA) equipped
110 with an automatic sample injector (model AS-3000, Thermo Fisher Scientific Inc.), a glass column (2.0 m × 0.5 cm 80/120 Carbopack
111 B-DA/4% Carbowax 20M phase; Sigma-Aldrich, St. Louis, MO), and a flame ionization detector set at 270°C. The chromatograph oven

112 and injector temperatures were set to 190°C and 220°C, respectively. Hydrogen was used as the carrier gas flowing at 30 mL/min. The
113 lactic acid concentration was measured by HPLC (LC-10ADVP Shimadzu HPLC system, Shimadzu Inc., Kyoto, Japan) according to
114 Ding et al. (1995).

115

116 ***Microbiological Quality and Enzymatic Activity***

117 Samples (200 g) from the middle layer within each mini silo were collected at the opening for microbiological population counts.
118 Ten grams from samples were diluted in sterilized sodium chloride solution (0.9%, 90 mL) and a serial dilution was performed.
119 Microorganism counts were carried out in triplicate through decimal dilution series in plates with De Man, Rogosa, Sharpe agar for
120 LAB (Briceño and Martinez, 1995), nutrient agar for aerobic and anaerobic bacteria (48 h of incubation at 30°C), and potato dextrose
121 agar (120 h of incubation at 26°C) for mold and yeast as described by Rabie et al. (1997). The absolute values were obtained as colony-
122 forming units and then log-transformed.

123 For enzymatic activity evaluation, samples (5 g) were constantly shaked at 100 rpm for 1 h with distilled water (40 mL). Then,
124 it was filtered through nylon cloth and centrifuged (3000×g for 5 min at 5 °C). The enzymatic activity was determined by adding 0.1
125 mL of enzymatic suspension (supernatant) to 0.9 mL of sodium acetate buffer (0.1M and pH 5.0). The measurement for glucoamylase
126 activity is in accordance with Gandra et al. (2019) and cellulase activity according to Nidetzky and Claeyssens (1994).

127

128 ***In Vivo Nutrients Intake and Digestibility***

129 Twelve castrated lambs (32.4 ± 2.86 kg body weight and 6.1 ± 0.4 mo) were assigned to three contemporary 4 × 4 Latin square
130 design trial, consisting of 19-d periods, with the last 5 d for data record and sampling. Diet was formulated for 200 g average daily gain,
131 using Small Ruminants Nutritional System (SRNS) (Table 2). Lambs within each square were randomly assigned to diets **CON**, **GLU**,
132 **CEL,** and **GLU+CEL**, previously demonstrated in section 2.1. Silage was produced in 200 L tubs (3 tubs per treatment). Silages were
133 produced as previously described, microbial inoculant was individually weighted, diluted in water, and manually mixed with whole-
134 plant corn silage. Animals were housed in metabolic cages and fed twice daily, at 0700 and 1300 h, targeting refusals between 10 to
135 15%. Samples of feeds and refusals were collected daily during the sampling period and pooled in a composite sample for chemical
136 analyses.

137 On days 15–17 of each experimental period, total fecal collections were performed through a metabolic cage. The feces were
138 weighed every 24 h of collection and a 10% aliquot of each day collection was destined to further analysis of digestibility of dry matter,
139 crude and neutral detergent fiber. Samples of silages, dietary ingredients, orts, and feces were analyzed for DM (method 950.15) and
140 crude protein (CP, N × 6.25; Kjeldahl method 984.13) according to AOAC (2000), and for neutral detergent fiber (without sodium
141 sulfite) according to Van Soest et al. (1991). Nutrient digestibility (NuD) was estimated as:

$$NuD\ \left(\frac{g}{kg}\right) = \frac{Nu_{intake}(g) - Nu_{Fecal}(g)}{Nu_{intake}(kg)}$$

where $Nu_{intake}$ is the nutrient intake and $Nu_{Fecal}$ is the nutrient fecal excretion.

***Statistical Analysis***

Statistical analysis of silage evaluations were performed using PROC MIXED of SAS (SAS Institute Inc, 2011). Data from the silo experiment were analyzed using the following model:

$$Y_{ijl} = \mu + G_i + C_j + G_i{*}C_j + e_{ijl}$$

with $e_{ij} \approx N(0, \sigma_e^2)$, where: $Y_{ij}$ is the observed value; $\mu$ is the overall mean; $G_i$ is the fixed effect of glucoamylase ($i$ = 1 and 2); $C_j$ is the fixed effect of cellulase ($j$ = 1 and 2), $G_i{*}C_j$ is the interaction effect of glucoamylase by cellulase and $e_{ijl}$ is the random residual error ($l$ = 1 to 10); $N$ stands for Gaussian deviation; and $\sigma_e^2$ is the variance of error. The treatment effect was evaluated by analysis of variance with 5% significance.

Data of nutrients intake and digestibility were analyzed according to the following model:

$$Y_{ijklm} = \mu + S_i + a_{j:i} + G_k + C_l + G_k{*}C_{l+}\ P_m + e_{ijklm},$$

with $a_{j:i} \approx N\ (0,\ \sigma_a^2)$; $e_{ijklm} \approx N\ (0,\ \sigma_e^2)$, where: $Y_{ijkl}$ is the value of the dependent variable; $\mu$ is the overall mean; $S_i$ is the fixed effect of Latin Square ($i$ = 1, 2 and 3); $a_{j:i}$ is the random effect of $j^{th}$ animal within the $i^{th}$ Latin Square ($j$ = 1 to 12); $G_k$ is the fixed effect of glucoamylase ($k$ = 1 and 2); $C_l$ is the fixed effect of cellulase ($l$ = 1 and 2); $G_k$*$C_l$ is the interaction effect of glucoamylase by cellulase; $P_m$ is the fixed effect of experimental period and $e_{ijklm}$ is the random experimental error; $N$ stands for Gaussian deviation; $\sigma_a^2$ is the variance of animals; and $\sigma_e^2$ is the variance of error. The significance level of 5% was considered for all statistical analyses.

## RESULTS

### Experiment 1

Cellulases increased ($P \leq 0.038$) gas losses and effluents production (Table 3). Interaction effect ($P \leq 0.039$) was observed on losses by gases (DM) and total (DM), which was greater for silages treated with cellulases and glucoamylases compared with CON but not differ from GLU+CEL. At the same way, recovery DM was smaller for CEL and GLU compared with CON but not differ from GLU+CEL ($P = 0.039$).

After aerobic exposure, no differences were observed between silages to measure temperature of all treatments (Table 3). There was no difference in pH between silages in the first 24 h of aerobic exposure. Control and GLU silages had higher pH value, since 48 h

169 until the end of evaluation period and silages treated with CEL and GLU+CEL showed lower values until the end of oxygen exposure
170 (Figure 1).

171 Control silages showed lower activities of glucoamylase and cellulases enzymes, as GLU silage for cellulase activity. Silages
172 treated with GLU + CEL showed intermediate activity of both enzyme complexes (Figure 2).

173 Silages treated with cellulases showed higher ($P \leq 0.021$) starch and crude protein and lower ($P = 0.001$) ADF content, besides
174 higher ($P \leq 0.032$) in vitro digestibility of DM and NDF (Table 4). However, GLU silages presented higher ($P = 0.001$) ADF and
175 intermediate ($P = 0.003$) starch content. An interaction effect was observed ($P \leq 0.007$) for DM, NDF, NFC and NEL content. CEL and
176 GLU silage showed lower DM and NFC content than CON, but not differ from GLU + CEL silages. Unlike CON silages presented
177 lower NDF content compared with GLU and CEL silages, not differing from GLU + CEL. Additionally, silages treated with cellulases
178 demonstrated higher levels of NEL compared to CON, but not differ to GLU and GLU + CEL.

179 Corn silages treated with cellulases presented lower ($P = 0.012$) ethanol content and GLU silages showed higher ($P = 0.012$)
180 lactate concentration (Table 5) and lower ($P \leq 0.002$) counts of anaerobic, aerobic, total bacteria, and fungi (Table 6). However, CEL
181 silage presented higher anaerobic bacteria counts ($P = 0.02$). An interaction effect ($P = 0.003$) was observed for lactic acid bacteria.
182 GLU+CEL silage showed greater counts than GLU silage, but not differ from CON and CEL.

183 **Experiment 2**

In the intake and digestion trial, an interaction effect ($P \leq 0.043$) was observed for feed intake. Lambs fed CEL silage showed greater intake of DM, OM, CP and NDF than those in the GLU + CEL group, but not differ from animals fed CON and GLU silages. For nutrient digestibility, lambs fed CEL silages presented higher ($P \leq 0.012$) digestibility coefficients for DM, OM, CP and NDF.

## DISCUSSION

This study hypothesized that inclusion of amylolytic and cellulolytic enzymes simultaneously in whole plant corn silage improves the fermentation process and animal digestion. Enzymes additives showed a significative response on fermentation, mainly because glucoamylase increased lactic acid concentration. But the decrease on DM recovery indicates no improvements on the nutritive value of corn silage. Besides that, cellulolytic enzyme positively affected animal digestion trough an improvement on digestibility of DM, OM, CP and NDF, while amylolytic had no effects.

Enzymes incorporation increased gas and total losses (DM) resulting in 6,31% drop in DM recovery. CEL increased gas and effluents losses, probably due to enhances on anaerobic bacteria count, as a greater microbial activity in silages treated with enzymes is likely related to increases on the fermentative losses observed in this study. In contrast, despite of greater total losses in GLU treatment, corn silage with GLU showed lower counts of anaerobic, aerobic, total bacteria and fungi.

198 Enzymes can decrease aerobic stability because of excessive release of WCS, increasing available sugars that can be quickly
199 used by undesirable microorganisms, such as spoilage yeasts and molds (Kung and Muck, 2015). According to Higginbotham et al.
200 (1998) yeasts usually initiate aerobic deterioration, and molds continue the deterioration process, because yeasts grow faster but tolerate
201 less heat than molds. In this study, fungi counts were reduced in GLU and no altered in CEL treatment, consequently no effects on
202 aerobic stability were observed.

203 Cellulolytic enzyme added to corn silage increased starch and crude protein content and reduced ADF. The last can be related to
204 the increase in the degradation of fiber fractions, which is also confirmed for improvements on in vitro degradation of DM and NDF by
205 CEL. Amylolytic enzyme increased ADF and starch content, with no effects on in vitro degradation. Dry matter content was greater in
206 CON, compared to GLU and CEL, but not differ from GLU + CEL. CEL probably showed a lower dry matter content because of greater
207 effluents losses, but the same was not observed in GLU. This is also observed by Lynch et al. (2015) when adding cellulase and xylanase
208 to corn forage before ensiling alone, causing a decrease on DM recovery in the enzyme-treated silage.

209 Exogenous enzymes hydrolyze complex carbohydrates into different products (malto-, cello-, and xylo-oligosaccharides),
210 supporting growth of fibrolytic microorganisms, which was called cross-feeding mechanism and could cause a synergistic effect between
211 fibrolytic and amylolytic enzymes (Zilio et al., 2019). However, in the present study the combination treatments resulted in no further
212 beneficial effects, which agrees with the low cellulase activity observed on GLU treatment.

GLU caused an improvement on lactic acid concentration, despite of reduced bacteria count had been observed. According to Ning et al. (2017), amylolytic enzymes can contributes to starch hydrolysis during the ensiling processes, which can explain the increase on lactic acid concentration. In addition, no effects on silage pH were observed when adding enzymes to silage, differently from the observed by Lynch et al. (2015) who added cellulase and xylanase to corn forage and showed lower pH and higher WSC after 70 d of ensiling.

The overwhelming majority of studies with enzymes have applied cellulases and hemicellulases for improve the release of plant cell wall carbohydrates, increasing its availability for LAB to ferment to lactic acid (Muck et al., 2018). However, different than expected, in the present study CEL did not affect LAB count, but increased anaerobic bacteria count and reduced ethanol concentration. Eun et al. (2017) demonstrated that fibrolytic enzymes products could greatly improve forage utilization, but the optimum doses and the activities supplied are critical for achieving this response.

Exogenous fibrolytic enzyme products can greatly improve forage utilization (Muck et al., 2018). In fact, lambs fed silages containing CEL had greater total tract digestibility. CEL positive effects on DM and NDF digestibility were somewhat expected, as demonstrated by the in vitro assay. Despite of increases on NDF degradability, which could allow greater voluntary intake by reducing physical fill in the rumen (Dado and Allen, 1995), feed intake was not influenced by adding none of the enzymes. On the other hand, increased NDF degradability could also enhance the energy density of diets and stimulates microbial N production (Oba and Allen,

228 2000) being economically viable. Thus, the increases in NDF degradation observed in our study have the potential to substantially
229 improve the performance of animals fed diets containing corn silage.

230 Despite of amylolytic enzymes have potential to increase nutrients digestibility by acting on starch-protein matrix, which could
231 enhance microbial attachment and enzymatic digestion of starch granules (Giuberti et al., 2014), no beneficial responses were observed
232 on nutrient intake and digestibility. The same was observed by Lara et al. (2018), evaluating lambs fed corn silage with inoculant alone
233 or in combination with amylolytic enzymes.

234 Enzyme aditives are mostly applied in combination with bacterial inoculants (Muck et al., 2018), as observed in this study, where
235 we added to all treatments microbial aditive composed by *Lactobacillus plantarum* and *Pediococcus acidilactici*. These bacteria are
236 common facultative heterofermentative strains, which are commonly associated with reduction on pH and acetic and butyric acid
237 contents and increases on latic acid contents and DM recovery (Muck and Kung, 1997). The association realized in this study turned it
238 difficult to differentiate between bacterial versus enzyme-mediated ensiling responses.

239

240 **DECLARATIONS**

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**Conflicts of interest/Competing interests (include appropriate disclosures)**

The authors declare no competing interests.

**Ethics approval (include appropriate approvals or waivers)**

All the procedures in the present study involving animals were in accordance with the Animal Ethics Committee of the Federal University of Grande Dourados, Brazil, number 0285/2017.

**Consent to participate (include appropriate statements)**

Not applicable

**Consent for publication (include appropriate statements)**

All the authors give consent for publication

**Availability of data and material (data transparency)**

All data generated and analyzed during this study are included in this published article

**Code availability (software application or custom code)**

Not applicable

**Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)**

All authors made substantial contributions to the accomplishment of the work, drafted, edited, or revised the work critically, and approved the final manuscript.

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328

329 **Table 1.** Chemical composition of the whole corn plant before the ensiling process (g/kg DM, unless stated)

| Item | Diet |
|---|---|
| Dry matter, g/kg as-fed | 254 |
| Organic matter | 939 |
| Neutral detergent fiber | 556 |
| Acid detergente fiber | 327 |
| Starch | 274 |
| Crude Protein | 101 |
| Lignin | 56.2 |
| Ether extract | 25.4 |
| Net energy[1], Mcal/kg DM | 1.72 |
| Buffering capacity, mEq/kg of DM | 213 |

330 [1]Calculated according with NRC (2001).

331

332 **Table 2.** Ingredients and chemical composition of diets (g/kg DM, unless stated)

| Item | Diet |
|---|---|
| Ingredients | |
| Corn silage | 750 |
| Corn meal | 120 |
| Whole raw soybean | 100 |
| Mineral mix[1] | 30.0 |
| Chemical | |
| Dry matter, g/kg as-fed | 427 |
| Organic matter | 918 |
| Neutral detergent fiber | 467 |
| Acid detergent fiber | 257 |
| Crude Protein | 112 |
| Lignin | 65.6 |
| Ether extract | 43.0 |
| Net energy[2], Mcal/kg | 1.67 |

333 [1]Contained per kilogram: 134 g Ca, 60 g P, 10 g Mg, 110 g Na 12 g S, 30 mg Se, 60 mg I, 150 mg Co, 6,000 mg Zn, 2,500 mg Fe, and
334 4,500 mg Mn.
335 [2]Calculated according to NRC (2001).
336

337**Table 3.** Amylolytic and cellulolytic enzymes effects on corn silage fermentation losses and aerobic stability

| Item | Treatments[1] | | | | SEM[2] | P-value[1] | | |
|---|---|---|---|---|---|---|---|---|
| | CON | GLU | CEL | GLU+CEL | | GLU | CEL | INT |
| | | | | Losses (g/kg) | | | | |
| Gases (fresh) | 12.0 | 17.4 | 39.4 | 23.6 | 0.29 | 0.296 | 0.019 | 0.101 |
| Effluents (kg/ton) | 11.6 | 14.3 | 23.9 | 21.0 | 1.16 | 0.969 | 0.035 | 0.192 |
| Gases (DM) | 97.5$^{a}$ | 170$^{b}$ | 165$^{b}$ | 148$^{ab}$ | 0.61 | 0.085 | 0.117 | 0.035 |
| Effluents (DM) | 10.6 | 12.2 | 21.8 | 18.7 | 0.11 | 0.638 | 0.038 | 0.223 |
| Total (DM) | 108$^{a}$ | 183$^{b}$ | 187$^{b}$ | 167$^{ab}$ | 0.65 | 0.087 | 0.068 | 0.031 |
| Recovery (DM) | 913$^{a}$ | 869$^{b}$ | 845$^{b}$ | 873$^{ab}$ | 0.52 | 0.047 | 0.377 | 0.039 |
| | | | | Aerobic Stability | | | | |
| | | | | Temperature (°C) | | | | |
| Sum (5d) | 674 | 667 | 666 | 684 | 2.30 | 0.101 | 0.410 | 0.224 |
| Maximum | 32.0 | 32.6 | 30.8 | 31.9 | 0.25 | 0.432 | 0.326 | 0.157 |
| Stability | 28.9 | 31.1 | 28.6 | 29.1 | 3.51 | 0.321 | 0.741 | 0.321 |
| | | | | Hours | | | | |
| Stability | 95.0 | 105 | 112 | 108 | 0.35 | 0.654 | 0.765 | 0.321 |

338[1]CON (control); GLU exo-1,4-α-glycosidase glucoamylase (Kerazyme 3035, enzymatic activity 300 U / mL); CEL β-glucan 4-
339glucanhydrolase (Kerazyme 5052 enzymatic activity 700 U / mL).

340 [2]SEM (standard error of the mean).
341[3]Amyloglucosidase effect (GLU); cellulase effect (CEL); amyloglucosidase*cellulase interaction.
342

343**Table 4.** Amylolytic and cellulolytic enzymes effects on corn silage chemical composition and in vitro degradation

| Item | Treatments[1] | | | | SEM[2] | P-value[1] | | |
|---|---|---|---|---|---|---|---|---|
| | CON | GLU | CEL | GLU+CEL | | GLU | CEL | INT |
| Dry matter | 285[a] | 264[b] | 272[b] | 279[ab] | 0.16 | 0.001 | 0.267 | 0.001 |
| Organic matter | 946 | 941 | 941 | 940 | 0.05 | 0.303 | 0.231 | 0.909 |
| Neutral detergent fiber | 576[b] | 589[a] | 600[a] | 582[ab] | 0.29 | 0.681 | 0.111 | 0.007 |
| Acid detergent fiber | 437 | 457 | 379 | 419.5 | 0.53 | 0.001 | 0.001 | 0.531 |
| Non-fiber carbohydrate | 264[a] | 249[b] | 227[b] | 247[ab] | 0.36 | 0.705 | 0.002 | 0.002 |
| Starch | 207 | 222 | 258 | 241 | 0.31 | 0.003 | 0.021 | 0.761 |
| Crude protein | 81.3 | 83.9 | 88.2 | 86.7 | 0.09 | 0.727 | 0.007 | 0.245 |
| Lignin | 64.4 | 67.4 | 68.8 | 62.6 | 0.14 | 0.547 | 0.388 | 0.839 |
| Fat | 24.9 | 23.8 | 25.8 | 24.2 | 0.04 | 0.121 | 0.434 | 0.774 |
| Net energy (Mcal/kg) | 1.48[b] | 1.52[ab] | 1.57[a] | 1.51[ab] | 1.01 | 0.432 | 0.001 | 0.006 |
| In vitro degradation (g/kg) | | | | | | | | |
| Dry matter | 487 | 503 | 565 | 556 | 0.87 | 0.849 | 0.024 | 0.654 |
| Neutral detergent fiber | 468 | 497 | 511 | 507 | 0.76 | 0.543 | 0.032 | 0.653 |

344 [1]CON (control); GLU exo-1,4-α-glycosidase glucoamylase (Kerazyme 3035, enzymatic activity 300 U / mL); CEL β-D-glucan 4-
345 glucanhydrolase (Kerazyme 5052 enzymatic activity 700 U / mL).
346 [2]SEM (standard error of the mean).

347 [3]Amyloglucosidase effect (GLU); cellulase effect (CEL); amyloglucosidase*cellulase interaction.
348

**Table 5.** Amylolytic and cellulolytic enzymes effects on corn silage fermentation profile

| Item | Treatments[1] | | | | SEM[2] | P-value[1] | | |
|---|---|---|---|---|---|---|---|---|
| | CON | GLU | CEL | GLU+CEL | | GLU | CEL | INT |
| pH | 3.26 | 3.14 | 3.11 | 3.09 | 0.02 | 0.543 | 0.661 | 0.871 |
| N-$NH_3$ (% TN) | 3.87 | 3.67 | 3.52 | 3.62 | 0.12 | 0.213 | 0.554 | 0.441 |
| mmol/kgDM | | | | | | | | |
| Ethanol | 2.95 | 2.02 | 1.95 | 2.12 | 0.03 | 0.125 | 0.012 | 0.546 |
| Acetate | 6.56 | 6.04 | 6.01 | 6.13 | 0.15 | 0.554 | 0.554 | 0.441 |
| Propionate | 0.005 | 0.008 | 0.003 | 0.006 | 0.02 | 0.443 | 0.541 | 0.564 |
| Butyrate | 1.02 | 1.08 | 1.00 | 1.02 | 0.01 | 0.441 | 0.442 | 0.551 |
| Lactate | 6.02 | 7.44 | 6.12 | 6.09 | 0.21 | 0.012 | 0.681 | 0.429 |

[1]CON (control); GLU exo-1,4-α-glycosidase glucoamylase (Kerazyme 3035, enzymatic activity 300 U / mL); CEL β-D-glucan 4-glucanhydrolase (Kerazyme 5052 enzymatic activity 700 U / mL).
[2]SEM (standard error of the mean).
[3]Amyloglucosidase effect (GLU); cellulase effect (CEL); amyloglucosidase*cellulase interaction.

355**Table 6**. Amylolytic and cellulolytic enzymes effects on corn silage microbial profile

| Item | Treatments[1] | | | | SEM[2] | P-value[1] | | |
|---|---|---|---|---|---|---|---|---|
| | CON | GLU | CEL | GLU+CEL | | GLU | CEL | INT |
| | *log*$_{10}$ | | | | | | | |
| Lactics | 7.23$^{ab}$ | 6.60$^{b}$ | 7.41$^{ab}$ | 8.26$^{a}$ | 0.02 | 0.001 | 0.432 | 0.003 |
| Anaerobics | 5.45 | 5.15 | 8.00 | 7.28 | 0.02 | 0.002 | 0.002 | 0.422 |
| Aerobics | 7.72$^{a}$ | 4.00$^{c}$ | 6.82$^{ab}$ | 5.00$^{b}$ | 0.01 | 0.001 | 0.434 | 0.021 |
| Total | 7.84 | 6.62 | 7.75 | 8.45 | 0.02 | 0.001 | 0.111 | 0.116 |
| Fungi and molds | 5.26 | 4.80 | 6.08 | 6.08 | 0.03 | 0.001 | 0.881 | 0.431 |

356 [1]CON (control); GLU exo-1,4-α-glycosidase glucoamylase (Kerazyme 3035, enzymatic activity 300 U / mL); CEL β-D-glucan 4-
357 glucanhydrolase (Kerazyme 5052 enzymatic activity 700 U / mL).
358 [2]SEM (standard error of the mean).
359 [3]Amyloglucosidase effect (GLU); cellulase effect (CEL); amyloglucosidase*cellulase interaction.
360

361**Table 7**. Amylolytic and cellulolytic enzymes effects on sheep dry matter and nutrients intake and digestibility

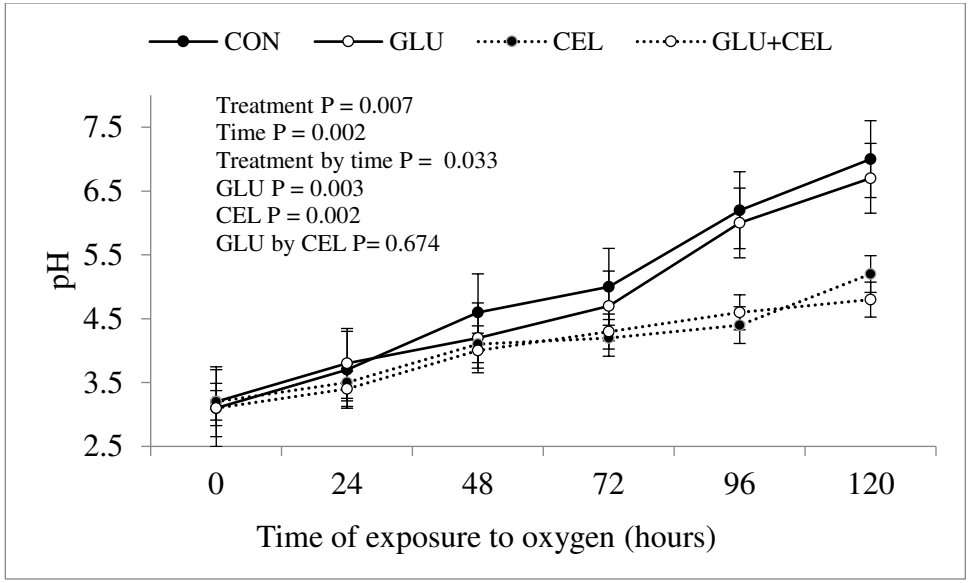
| Item | Treatments[1] | | | | SEM[2] | P-value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | CON | GLU | CEL | GLU+CEL | | GLU | CEL | INT |
| *Intake (kg/day)* | | | | | | | | |
| Dry matter | 1.54$^{a}$ | 1.42$^{ab}$ | 1.65$^{a}$ | 1.38$^{b}$ | 0.69 | 0.765 | 0.632 | 0.034 |
| Organic matter | 1.45$^{a}$ | 1.33$^{ab}$ | 1.54$^{a}$ | 1.22$^{b}$ | 0.96 | 0.732 | 0.564 | 0.033 |
| NDF | 1.23$^{a}$ | 1.06$^{ab}$ | 1.25$^{a}$ | 1.01$^{b}$ | 0.51 | 0.675 | 0.342 | 0.039 |
| Crude protein | 0.123$^{ab}$ | 0.125$^{ab}$ | 0.169$^{a}$ | 0.106$^{b}$ | 0.05 | 0.732 | 0.498 | 0.043 |
| *Digestibility (g/kg)* | | | | | | | | |
| Dry matter | 684 | 757 | 772 | 747 | 0.8 | 0.223 | 0.012 | 0.451 |
| Organic matter | 704 | 775 | 790 | 765 | 0.9 | 0.534 | 0.007 | 0.561 |
| NDF | 687 | 742 | 762 | 742 | 0.9 | 0.431 | 0.009 | 0.453 |
| Crude protein | 564 | 730 | 754 | 747 | 1.0 | 0.341 | 0.011 | 0.548 |

362 [1]CON (control); GLU exo-1,4-α-glycosidase glucoamylase (Kerazyme 3035, enzymatic activity 300 U / mL); CEL β-D-glucan 4-
363 glucanhydrolase (Kerazyme 5052 enzymatic activity 700 U / mL).

364 [2]SEM (standard error of the mean).

365 [3]Amyloglucosidase effect (GLU); cellulase effect (CEL); amyloglucosidase*cellulase interaction.

366

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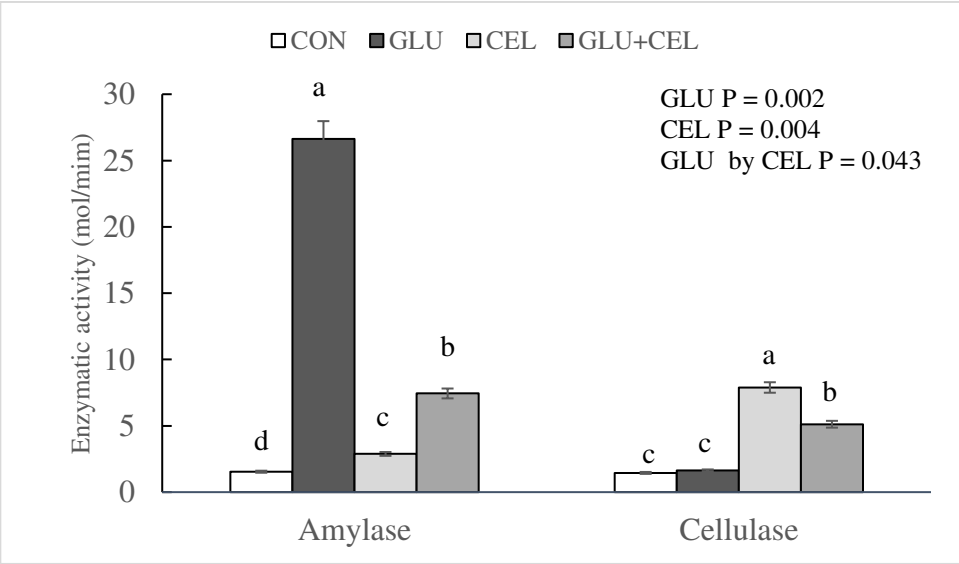
369**Figure 1**. Amylolytic and cellulolytic enzymes effects on corn silage pH after aerobic exposure

370

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373 **Figure 2**. Amylolytic and cellulolytic enzymes effects on corn enzymatic activity

# Figures

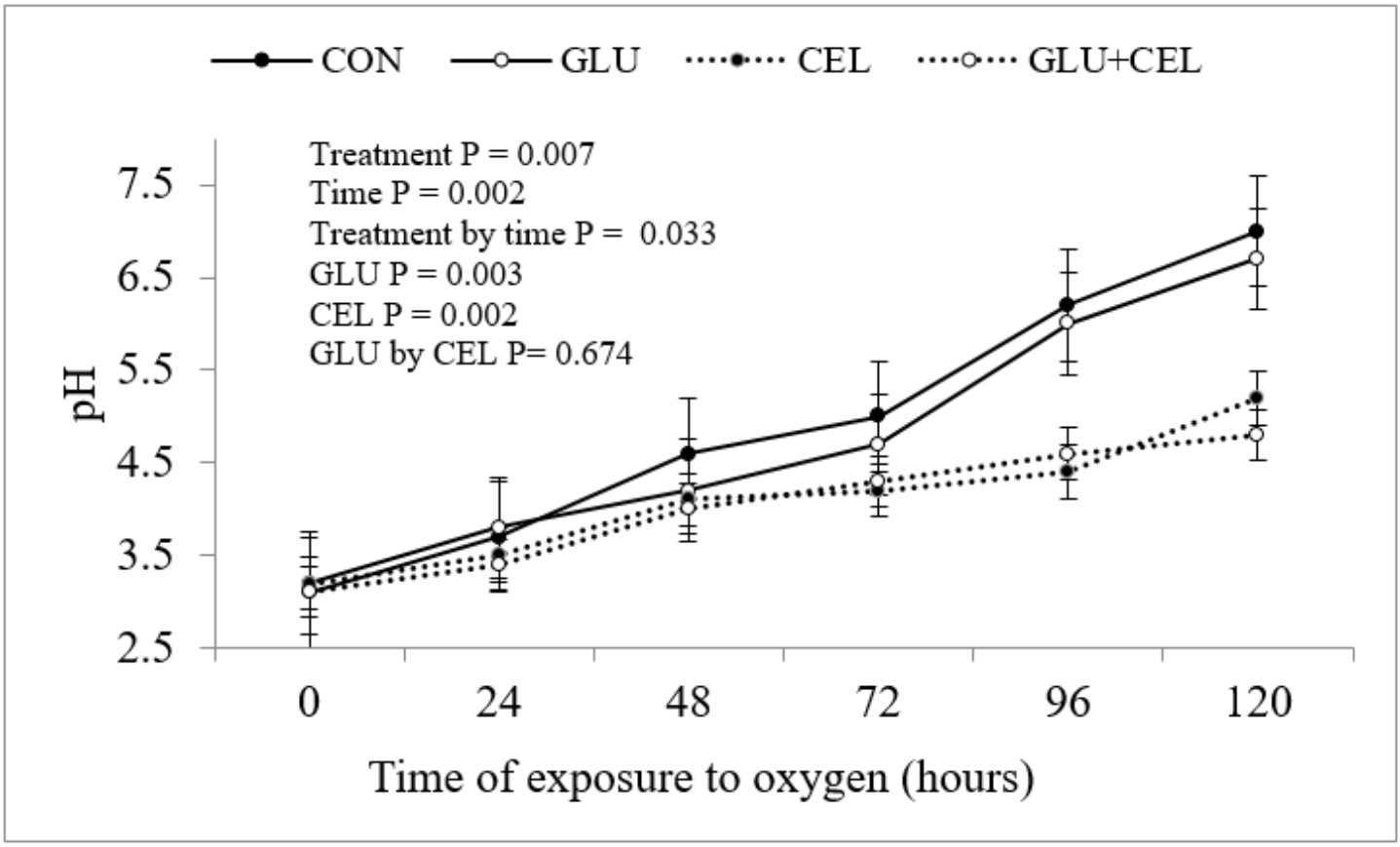


**Figure 1**

Amylolytic and cellulolytic enzymes effects on corn silage pH after aerobic exposure

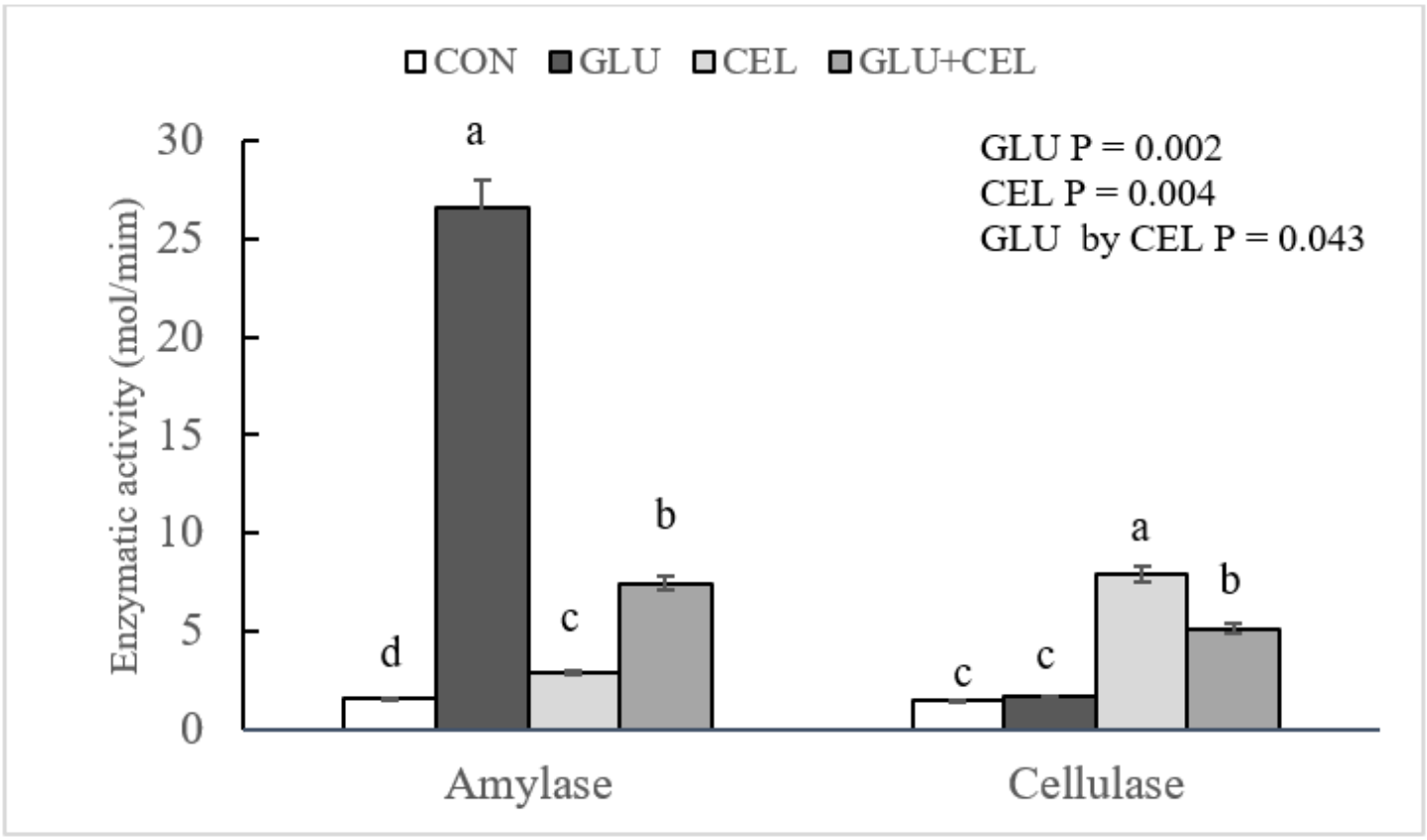


**Figure 2**

Amylolytic and cellulolytic enzymes effects on corn enzymatic activity