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Exogenous amylase increases gas production and improves *in vitro* ruminal digestion kinetics of sorghum and corn grains

[*Amilase exógena aumenta a produção de gases e melhora a cinética de digestão ruminal* in vitro *de grãos de sorgo e milho*]

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ABSTRACT

The aim of this study was to evaluate the effect of exogenous amylase on gas production, *in vitro* dry matter digestibility (IVDMD), and *in vitro* digestion kinetics of sorghum (*Sorghum vulgaris*) and two corn hybrids of different grain textures. Ruminal fluid was collected from two rumen-fistulated cows receiving or not exogenous amylase (0.7g kg^{-1} of dry matter (DM basis)), provided to achieve 396 kilo Novo units kg^{-1} for amylase activity (DM basis). Gas production was measured after 1, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42 e 48 hours of incubation. Amylase increased gas production (mL) by 5.4%. Corn hybrids have higher *in vitro* dry matter digestibility than sorghum. Exogenous amylase increased the potential of gas production (A) (P=0.01). There was an effect of hybrid for IVDMD (P<0.01). The addition of exogenous amylase increases the *in vitro* gas production, improves fermentation kinetics, and increases the production of the ammonia nitrogen of corn and sorghum grains, but does not affect in vitro and dry matter digestibility or the short-chain fatty acids production.

Keywords**:** digestibility, ruminal digestion, starch granule

RESUMO

O objetivo deste estudo foi avaliar o efeito da amilase exógena na produção de gases, a digestibilidade in vitro *da matéria seca (DIVMS) e a cinética de digestão* in vitro *de sorgo (Sorghum vulgaris) e de dois híbridos de milho de diferentes texturas de grãos. O líquido ruminal foi coletado de duas vacas fistuladas no rúmen recebendo ou não amilase exógena (0,7g kg^{-1} de matéria seca (MS)), fornecida para atingir 396 kg Novo unidades kg^{-1} para atividade de amilase (base na MS). A produção de gás foi medida após uma, três, seis, nove, 12, 15, 18, 21, 24, 30, 36, 42 e 48 horas de incubação. A amilase aumentou a produção de gás (mL) em 5,4%. Híbridos de milho apresentam maior DIVMS que o sorgo. A amilase exógena aumentou o potencial de produção de gás (A) (P=0,01). Houve efeito de híbrido para DIVMS (P<0,01). Amilase exógena aumenta a produção de gás* in vitro, *melhora a cinética da fermentação e aumenta a produção de nitrogênio amoniacal de grãos de milho e sorgo, mas não afeta a digestibilidade* in vitro *da matéria seca ou a produção de ácidos graxos de cadeia curta.*

Palavras-chave: digestibilidade, digestão ruminal, grânulo de amido

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INTRODUCTION

Cereal grains rich in starch, such as corn and sorghum, are high-cost energy feedstuffs commonly used in ruminant diets. Studies have been conducted to evaluate strategies to maximize starch utilization (Gallo *et al.,* 2016) using additives that improve starch digestibility or by using genotypes of lower vitreousness that favor digestion.

There is a difference in starch utilization related to grain texture and concentration of vitreous endosperm. These differences are associated with the presence of prolamins and grain density (Kljak *et al.,* 2018). However, most of these studies evaluated the digestion of the whole-plant, with possible effects of other dietary components. Lanzas *et al.* (2007) observed lower *in vitro* gas production and digestion rate for sorghum, justified by its harder endosperm compared to corn. Also, a classic study demonstrated a great difference between corn grains produced in temperate conditions for those produced in tropical settings, where the corn hybrids produced in temperate conditions had higher vitreousness and density, interfering on starch availability (Corrêa *et al*., 2002).

The use of exogenous amylase increased the true ruminal digestibility of organic matter (OM), ruminal starch digestibility (Nozière *et al.,* 2014), and neutral detergent fiber (NDF) digestibility (McCarthy *et al.,* 2013) in diets for dairy cows. However, most of these studies evaluating exogenous amylase were made in temperate conditions and additional studies must be conducted in tropical conditions. We hypothesize that the *in vitro* gas production, volatile fatty acids, and digestibility of corn grains differing in vitreousness and sorghum would be improved by the addition of an exogenous amylase. So, the aim was to evaluate the effect of exogenous amylase on *in vitro* ruminal digestion of sorghum or corn grains of different vitreousness.

MATERIAL AND METHODS

The experiment was performed at the Multi-use Complex on Livestock Bioefficiency and Sustainability of Brazilian Agricultural Research Corporation (Embrapa), located in Coronel Pacheco, Minas Gerais, Brazil (21°33'22 "S, 43°06'15 "W). All procedures used were approved by the Ethics Committee of Embrapa Dairy Cattle, under protocol n. 03/2014. To define the different vitreousness, two corn hybrids (AG1051-dent and 1N1932-semiflint) were taken. The grains were evaluated for vitreousness by manual dissection. For each hybrid, 12 grains visually homogeneous in size and shape had the pericarp and germ removed with a scalpel (Dombrink-Kurtzman and Bietz 1993). The vitreous endosperm was expressed as a percentage of the total endosperm. Samples of grains were ground (Wiley mill; A. H. Thomas, USA) at 2-mm and stored for further analysis. Before the *in vitro* study, a statistical analysis was performed to evaluate if the corn grains were different for vitreousness using the ANOVA procedure of SAS (Statistical…, 2012) according to a completely randomized design with 12 replications. The dent and semi-flint type hybrid had 572 and 739g kg^{-1} of vitreousness, respectively (P<0.01).

The following parameters were determined: DM (Official…, 2006; method 934.15); ash (Official…, 2006; method 930.15); crude protein (CP) (Official…, 2006; method 990.03) and ether extract (EE) (Official…, 2006; method 920.39). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were sequentially determined using the ANKOM 220 fiber analyzer (ANKOM 220 fiber analyzer, ANKOM Technology, USA) with the inclusion of thermostable amylase. The lignin contents were quantified by the sulfuric acid solubilization method. The organic matter (OM) was calculated by difference through the equation: OM = DM − ash (Table 1).

Starch concentration in grains samples was quantified by acid hydrolysis and enzymatic method using glucose oxidase–peroxidase (Glucose PAP Liquiform) kits (Labtest, Lagoa Santa, Brazil). Briefly, 400 mg of dried milled (1-mm) sample was added 25 mL of hydrochloric acid (0.6 M) and starch was gelatinized in autoclave for 15 min at 121°C. After cooling at room temperature distilled deionized water (q.s.p. 100 mL) was added. Samples were filtered (Whatman #51, Whatman Inc, USA) and 0.01 mL was transferred to a test tube and added 1 mL of glucose reagent (Labtest-133) and kept under a water bath at 37°C for 10 min. After cooling, absorbance was

read at 505 nm (YSI-2700 Analyzer Inc. Life Sciences, Yellow Springs, OH) and the results were estimated with glucose standards containing 0, 20, 40, 60, 80 and 100 mg glucose dL^{-1} of distilled deionized water. Starch was calculated by multiplying the glucose concentration by 0.9.

For the *in vitro* evaluation, two non-lactating animals were pre-adapted to diets for 14 days before the incubation started and were distributed in a 3 × 2 Latin square design (3 runs x 2 inoculum). The donor animals were fed ad libitum with a forage:concentrate ratio of 53:47 (180g/kg CP and 300 g/kg NDF), being one under control (CTRL) diet and the other supplemented with exogenous amylase (Ronozyme RumiStar™; DSM Nutritional Products Brazil S.A, Brasil) at 0.7g kg^{-1} DM to achieve 396 kilo Novo units kg^{-1} for amylase activity of TMR DM (Table 2). So, the effect of exogenous amylase on ruminal digestion kinetics on two corn hybrids (AG1051-dent and 1N1932-semiflint) and sorghum grain (BRS 332-semiflint; Embrapa Corn and Sorghum, Sete Lagoas, Brazil) were assessed.

Table 1. Bromatological composition of corn and sorghum grains

Item[a] (g kg^{-1})	Hybrids[b]		
	AG1051	1N1932	BRS332
DM	884	887	889
OM	977	986	982
CP	105	104	132
EE	76.8	56.3	45.6
ash	22.8	13.7	18.1
NDF	138	103	152
ADF	41.2	31.9	68.3
Lig	14.6	11.7	37.6
Starch	649	624	626
NFC	657	724	652
Vitreousness	572	739	–

[a]Item: DM – dry matter; OM – organic matter; CP - crude protein; EE – ether extract; NDF – neutral detergent fiber; ADF – acid detergent fiber; Lig – lignin; NFC – non-fibrous carbohydrates; vitreousness – g kg^{-1} of total endosperm; –, not determined. [b]Hybrids: AG1051 – dent type corn hybrid; 1N1932 – flint type corn hybrid; BRS332 – sorghum grain.

Table 2. Composition of diets offered to donor cows used to collect ruminal liquor

Item (g kg^{-1} of DM)	Diet	
Maize silage	480	
Tifton hay	52.6	
Corn meal	211	
Soybean meal	210	
Limestone	9.70	
Urea	5.30	
Ammonium sulfate	5.30	
Mineral mix[a]	26.3	
	Control	Amylase[b]
Amylase	-	0.7
Composition		
Organic Matter	915 ± 9.4	924 ± 4.1
Crude Protein	195 ± 3.7	193 ± 7.9
Ether extract	37.0 ± 2.20	37.0 ± 1.70
NDF[c]	308 ± 13.1	292 ± 9.5
ADF[d]	155 ± 6.3	149 ± 4.0
Starch	249 ± 20.1	251 ± 19.6

[a]Mineral mix, 88g kg^{-1} of Ca; 42g kg^{-1} of P; 18g kg^{-1} of S; 45g kg^{-1} of Mg; 20g kg^{-1} of K; 123g kg^{-1} of Na; 14mg kg^{-1} of Co; 500mg kg^{-1} of Cu; 20mg kg^{-1} of Cr; 1,050mg kg^{-1} of Fe; 28mg kg^{-1} of I;: 1,400mg kg^{-1} of Mn; 18mg kg^{-1} of Se; 2,800mg kg^{-1} of Zn; 420 mg kg^{-1} of F; 80 mg kg^{-1} of biotin; 200,000 UI kg^{-1} of vitamin A; 70,000 UI kg^{-1} of vitamin D_3; 1,200 UI kg^{-1} of vitamin E; 600mg kg^{-1} of monensin. [b]Amylase: exogenous at 0.7g kg^{-1} DM to achieve 396 kilo Novo units kg^{-1} for amylase activity of TMR DM. DSM Nutritional Products Brazil SA. [c]NDF - neutral detergent fiber. [d]ADF - acid detergent fiber.

To avoid confounding treatment effects, the cows receiving the additives in diets were swapped between runs. To perform the *in vitro* evaluations, a gas production technique was used, varying in the use of ruminal fluid collected from the rumen-fistulated adult cows fed a CTRL or additive. At 14 days of adaptation, the rumen fluid was collected two hours after morning feeding, filtered through three layers of cheesecloth and transported in pre warmed (39°C) thermos flasks that were previously flushed with CO_2. Then, rumen fluid was immediately transferred to a controlled temperature (39°C) room for buffered rumen fluid preparation and in vitro incubation.

Buffered rumen fluid was prepared by mixing rumen fluid and a mineral buffer with 0.5mL of sodium sulphide solution (Menke *et al.*, 1979) in a ratio of 1:2. The buffered rumen fluid was then transferred (25mL) into samples flasks under a stream of O_2-free N gas. Approximately 800 mg of sample were weighed into the filter bags (F57; Ankom, USA) in triplicate for each hybrid and ruminal fluid (CTRL or additive).

Flasks were sealed and placed on an orbital shaker set at 90 oscillations min^{-1} for two min in every two hours. Gas production was measured after 1, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42 e 48 hours of incubation using a water displacement apparatus (Fedorah and Hrudey 1983).

Samples were then collected at 24 hours of fermentation for analysis of pH, short chain fatty acids (SCFA), ammonia nitrogen (NH_3-N) and *in vitro* dry matter digestibility (IVDMD). For the SCFA analysis, the remaining rumen fluid was centrifugated for 10 min at 12,000 × g at environmental temperature (4°C). SCFA analysis was performed using HPLC (Ultimate 3000, Dionex Corporation, USA) equipped with a Shodex RI-101 refractive index (RI) kept at 40°C, using Phenomenex Rezex ROA column, 300 × 7.8mm kept at 45°C. The NH_3-N concentration was quantified after Kjeldahl distillation with magnesium oxide and calcium chloride according to method 2.065 (Official…, 1980). After 48 hours of incubation, the flasks were opened and pH was measured (MPA 210, MS Tecnopon®, Brasil).

The cumulative gas production (48 hours) was adjusted by hybrid and inoculum to the model proposed by France *et al*. (1993) using the Gauss-Newton algorithm to describe the potential gas production.

$$Y=A \times 1\text{-}e^{[-b\times(t-L)-c \times (\sqrt{t}-\sqrt{L})]}$$

Where: A= maximum cumulative gas production (mL); L= lag time (h); b (h^{-1}) and c (0.5 h^{-1}) = constant fractional rates; t= time (h). The average gas production fractional rate (μ) was calculated as:

$$\mu=b+c/2\sqrt{t}$$

Effective digestibility of dry matter was estimated considering the rate of passage of 5%/h, using the IVDMD at 48 hours as:

$$ED5 = S_0\, e^{-kt}(1 - kI)/\,(S_0 + U_0)$$

Where: K= rate of passage; S_0 and U_0= initially fermentable fractions and non-fermentable fractions, respectively, where:

$$I= \int L^{\infty} \exp\text{-}[(b+k)(t-T)+c(\sqrt{t}-\sqrt{T})]dt$$

The IVDMD at 24 and 48 hours were determined gravimetrically using the equation:

$$IVDMD = 1000\, x \frac{DMi - DMf}{DMi}$$

Where DMi and DMf are the DM incubated and the final (mg).

Data were tested for normality after model fitting using the UNIVARIATE procedure of SAS version 9.3 (Statistical…, 2012). All data were analyzed using the MIXED procedure of SAS. For comparisons between the addition or not of exogenous amylase and between hybrids, the data were analyzed according to the model:

$$Y_{ijk} = \mu + H_i + A_j + H \times A_{ij} + R_k + \varepsilon_{ijk}$$

Where:

Y_{ijk}= dependent variable; μ= overall mean, H_i= fixed effect of hybrid, A_j= fixed effect of additive; $H\times A_{ij}$= is the fixed effect of interaction between hybrid x additive; R_k= random effect of run; ε_{ijk}= random error. The model included the fixed effects of hybrids and additives and its interaction. The run was included in the model as a random effect. The statistical significance was considered when $P<0.05$. Pearson's correlation analysis among NH_3-N and gas production and digestion kinetics was performed.

RESULTS AND DISCUSSION

Averages of pH were not influenced by the hybrids tested (*P*=0.05) or by amylase addition (*P*=0.11). This is expected *in vitro* studies due to the high buffer capacity of the medium (Mould *et al*., 2005). There was no effect of hybrid on gas production potential (A). But the use of amylase improved the fermentation parameters of the hybrids tested by the increase (+11.4%; *P*=0.01) of A (Table 3). Gallo *et al.* (2016) also observed improvements in fermentation kinetics with amylase addition, with emphasis on the reduction of lag time. In contrast, amylase did not decrease lag time in the present study. Possibly related to sample particle size, since in the study of Gallo *et al.* (2016) the lag time increases according to particle size increased and in present study particle size was the same for three grains tested. Amylase addition modifies the availability of the metabolite profile of starch digestion, contributing to microbial multiplication and, consequently, to improvements in the fermentation process (Tricarico *et al.*, 2008).

There was an effect of hybrid for IVDMD (*P*<0.01), in which corn hybrids had higher degradation rates than sorghum, regardless of amylase, whose addition did not influence IVDMD (*P*=0.31) (Table 3). Therefore, the lack of effect of amylase on the IVDMD may be related to incubation of corn or sorghum alone, since the substrate for fermentation was almost completely starch and the addition of amylase may have conferred competitive advantage to non-amylolytic bacterial multiplication (Tricarico *et al.*, 2008), having no substrate available for fermentation. In addition, the absence of differences in IVDMD in the present study may have occurred because the gravimetric technique considers that the soluble fraction is instantaneously degradable, which is not the case for gases, since microorganisms can use this fraction in different ways. Increased NDF digestibility has been reported in *in vivo* studies with amylase addition (McCarthy *et al.*, 2013), possibly these findings occurred because amylase can catalyze the cross feeding in the ruminal environment. In diets with low starch and high NDF content for dairy cows, amylase increased NDF digestibility in relation to a diet with higher starch content (Gencoglu *et al.*, 2010) and increased starch digestibility regardless of its dietary level (200 vs. 300g kg^{-1}; DM basis). However, similarly to the present study, amylase did not increase the total DM digestibility, as also reported by Nozière *et al.* (2014).

Table 3. pH, accumulated gas production, in vitro dry matter digestibility (IVDMD) and ammonia nitrogen (NH_3-N) at 48 hours of incubation and parameters of ruminal kinetics of corn and sorghum grains differing for vitreousness with or without exogenous amylase

Item	CTRL			Amylase			SEM	*P* Value		
	BRS332	AG1051	1M1932	BRS332	AG1051	1M1932		Hybrid	Amylase	Hyb x Amy
pH	5.90	5.80	5.80	6.0	5.9	5.9	0.07	0.05	0.11	>0.50
IVDMD (g kg^{-1})	547[b]	620[a]	606[a]	559[b]	612[a]	642[a]	2.2	<0.01	0.31	0.36
NH_3-N (mg g^{-1})	2.0	2.0	2.1	2.4	2.4	2.4	0.13	>0.50	<0.01	>0.50
Lag (hh:mm:ss)	00:30:19	00:42:40	00:43:08	00:56:39	00:40:38	00:36:39	00:09:45	>0.50	>0.50	0.19
T/2 (hh:mm:ss)	13:45:00	11:17:24	13:07:48	13:52:12	14:51:00	14:55:12	01:01:33	>0.50	0.01	0.14
A (mL)	247	226	232	247	281	257	13.0	>0.50	0.01	0.08
µ (mL h^{-1})	0.063	0.064	0.059	0.054	0.053	0.054	0.01	0.17	0.01	0.09
ED5 (g kg^{-1})	508[b]	572[a]	559[a]	508[b]	563[a]	594[a]	2.1	<0.01	>0.50	0.32

Hybrids: BRS332 - sorghum grain; AG1051 - dent type corn hybrid; 1N1932 - flint type corn hybrid. Lag, lag time. T/2 - time necessary to gas production reaches half of its potential; A - maximum gas production potential; µ - gas production rate; DE5 - effective digestibility to passage rate of 5% h^{-1}; SEM - standard error mean; *P*-value - Hyb x Amy - hybrid x amylase interaction. means within a row not sharing a common superscript differ by Fisher test (*P*<0.05).

The proportion of vitreous endosperm has been reported in the literature as a key factor in the decrease of DM digestibility (Holding, 2014). In the present study, the proportion of vitreous endosperm does not have a great variation among genotypes. Likewise, van Zwieten *et al.* (2008) did not observe an increase in the IVDMD due to small differences in vitreousness

(610 vs. 810g kg^{-1} of total endosperm) among the hybrids evaluated. In the present study the difference in vitreousness among corn hybrids was 572 vs. 739g kg^{-1} of total endosperm to AG1051 and 1N1932, respectively with a statistical difference.

The arrangement of proteins in grains with more vitreous endosperm tends to obstruct the attack of microorganisms on starch granules. In addition, increased vitreousness is related to a higher proportion of amylose that is less soluble than amylopectin. Pôssas *et al.* (2015) suggested that the sorghum grain is composed of a compact protein matrix that is denser than the corn grain; therefore, this dense protein matrix justifies the lower IVDMD of sorghum in relation to the corn grains in the present study. Although, vitreousness as an isolated effect should be analyzed with caution, as the degree of vitreousness can be altered by grain processing. Nevertheless, Ngonyamo-Majee *et al.* (2008) in a correlation study, observed that ruminal digestibility had a negative correlation coefficient with vitreousness ($r = -0.65$; $P<0.001$) and it can partially be used to estimate the DM digestibility.

Amylase increased NH_3-N ($P<0.01$), but there was no effect of hybrid. Lag was not influenced by the hybrid ($P>0.50$) or amylase ($P>0.50$). In the present study, in both evaluation times (24 and 48 hours of incubation), amylase increased the NH_3-N content. Higher concentration of NH_3-N in the medium suggests greater proteolysis of nitrogenous compounds and greater microbial multiplication. In the present study, in both evaluation times (24 and 48 hours of incubation), amylase increased the NH_3-N content. Higher concentration of NH_3-N in the medium suggests greater proteolysis of nitrogenous compounds and greater microbial multiplication. Additionally, the exogenous amylase addition may favor the degradation of the protein matrix that embedded the starch granules (Eun and Beauchemin, 2005). In addition, in the present study, the NH_3-N was positively correlated with the gas production ($r=0.59$; $P=0.001$). Amylase increases the microbial population through greater availability of monosaccharides (Tricarico *et al.*, 2008).

This increase in NH_3-N triggered by amylase in the present study may be due to the increased activity of proteolytic enzymes produced by the microorganisms in the medium. This effect seems to have favored the degradation of prolamins, which are hydrophobic proteins that cover the starch granules, inhibiting to some degree the microbial enzymatic attack (Hoffman *et al.*, 2011). From the point of view of the reduced effect of vitreousness, this action of amylase is positive, because the access to the starch granules can be facilitated with the degradation of this protein matrix.

T/2 was higher when amylase was used ($P=0.01$), but it was not influenced by hybrids ($P>0.50$). In contrast with the present study, Giuberti *et al.* (2013) reported lower T/2 as the vitreousness increased in corn grains. The maximum gas production potential (A) increased with amylase addition ($P<0.01$), but it was not affected by the hybrid ($P>0.50$). The μ (mL h^{-1}) decreased with the inclusion of amylase ($P=0.01$), whereas it was not affected by the hybrids ($P=0.17$). On the other hand, ED5 was not influenced by amylase ($P>0.50$), but it was higher for corn hybrids compared to sorghum ($P<0.01$).

Amylase increased the gas production potential, as reported by Gallo *et al.* (2016) and decreased the gas production rate, possibly favoring the fibrolytic microorganisms, which degrade the available substrate more slowly. For this reason, the amylase also increased the time required to reach half of the gas production potential. In contrast to the present study, Gallo *et al.* (2016) reported an increase in gas production rate of corn grains with amylase addition. One of the reasons for the depression of rumen fermentation is the accumulation of gas in the medium, and then amylase may have favored this environment due to the lower gas production rate.

The IVDMD at 24 h was higher for corn hybrids compared to sorghum ($P<0.01$), but it was not influenced by amylase addition ($P>0.50$). NH_3-N was influenced by amylase ($P<0.01$) but was not influenced by hybrid ($P>0.50$). The pH was not influenced by hybrids ($P=0.36$) or amylase ($P>0.50$). There was an effect of hybrid ($P=0.03$) for acetic acid, whereas there was no effect of amylase ($P>0.50$) or the interaction hybrid x amylase ($P>0.05$). An interaction between hybrid x amylase was reported ($P=0.001$) (Table 4) for propionic acid since the amylase addition

increased its concentration for the sorghum hybrid and dent corn. However, the opposite effect occurred for the flint hybrid. When highly fermentable carbohydrates are available for fermentation, the production of propionic acid is expected to be higher. So, feedstuffs with a high content of starch probably produce more propionic acid. In the present study, the interaction hybrid x amylase occurred for propionic acid production was not expected, because the structure of starch in sorghum, as demonstrated by Amanzougarene *et al.* (2017).

Table 4. in vitro dry matter digestibility (IVDMD), ammonia nitrogen (NH3-N), pH and short-chain fatty acids (SCFA) of corn and sorghum grains differing for vitreousness at 24 hours of incubation with or without exogenous amylase

Item	CTRL			Amylase			SEM	*P* Value		
	BRS 332	AG1051	1M1932	BRS332	AG1051	1M1932		Hybrid	Amylase	Hyb x Amy
IVDMD (g kg^{-1})	229^{b}	270^{a}	265^{ab}	227^{b}	236^{b}	279^{a}	1.5	<0.01	>0.50	0.15
NH_3-N (mg g^{-1})	1.6	1.7	1.7	2.0	1.9	1.9	0.08	>0.50	<0.01	0.29
pH	6.7	6.6	6.6	6.7	6.7	6.6	0.04	0.36	>0.50	>0.50
Acetic acid (µmol mL^{-1})	27.3^{a}	26.2^{a}	25.5^{a}	28.8^{a}	22.6^{b}	28.5^{a}	1.98	0.03	>0.50	>0.05
Propionic acid (µmol mL^{-1})	19.1^{a}	19.7^{a}	18.8^{a}	24.9^{a}	15.7^{b}	22.5^{a}	2.22	0.008	0.10	0.001
Butyric acid (µmol mL^{-1})	9.3^{a}	8.9^{a}	8.8^{a}	11.7^{a}	7.9^{b}	10.5^{a}	1.27	0.06	0.18	0.10
A:P ratio	1.5^{a}	1.4^{a}	1.4^{a}	1.2^{b}	1.6^{a}	1.3^{b}	0.09	0.18	>0.50	0.03
Total SCFA (µmol mL^{-1})	55.6^{a}	54.9^{a}	53.2^{a}	64.0^{a}	46.4^{b}	61.6^{a}	5.17	0.02	0.30	0.02

Hybrids: BRS332 - sorghum grain; AG1051 - dent type corn hybrid; 1N1932 - flint type corn hybrid. SEM - standard error of mean; A:P ratio= acetate:propionate ratio; total SCFA - total short chain fatty acids; *P*-value - Hyb x Amy - hybrid x amylase interaction. means within a row not sharing a common superscript differ by Fisher test ($P<0.05$).

There was no effect of hybrid ($P=0.06$), amylase ($P=0.18$) or interaction ($P=0.10$) for butyric acid. A interaction effect was observed for acetate:propionate (A:P) ratio, the A:P ratio was higher for the dent hybrid compared to sorghum and flint corn ($P=0.03$) when exogenous amylase was used, an opposite effect occurred for total SCFA, since the dent corn had lower values in relation to flint corn and sorghum ($P<0.02$) (Table 4). In the present study, amylase did not increase the production of propionic, acetic and butyric acids, and total SCFA. However, for SCFA, an interactive hybrid x amylase was observed. The endosperm of sorghum grain is more vitreous than corn grain, and it is more difficult to be attacked by the microbial population and enzymes. This may explain the lower SCFA production in sorghum grains. Similar to the present study, 0.20mg mL^{-1} per liquid volume incubated of an amylolytic enzyme did not influence the SCFA (Freiria *et al.*, 2018).

CONCLUSIONS

Corn hybrids have higher *in vitro* dry matter digestibility than sorghum grain. The addition of exogenous amylase increases gas production and the potential of the gas production of corn and sorghum grains. Exogenous amylase improves fermentation kinetics of corn and sorghum grains, but does not affect *in vitro* and dry matter digestibility.

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