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ORIGINAL ARTICLE

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Effects of exogenous amylase on the in vitro digestion kinetics of whole-crop maize silages made from flint or dent grain type at different phenological stages grown in tropical condition

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Abstract

The effect of exogenous amylase on the in vitro rumen digestion kinetics of whole-crop maize silage made from dent (RB9004) or flint grain type (RB9308) was evaluated at different phenological stages: soft dough (SOD), early dent (EAD), ½ milkline (½M) and ¾ milkline (¾M). Forage was harvested from 70 to 110 days after sowing. Two rumen-cannulated cows receiving or not exogenous amylase (0.7 g/kg dry matter—DM, provided to achieve 396 kilo Novo units of amylase activity/kg of TMR DM) were used as donor of ruminal fluid. The in vitro gas production kinetics was evaluated according to a dual-pool logistic model. The chemical composition and gas production kinetics were affected by the hybrid and phenological stages. The flint hybrid had lower range for chemical analysis among physiological stages. Harvesting at ½M and ¾M improved DM content, bromatological composition and silage quality parameters compared to dent or flint types. Amylase (i) increased methane (CH_4) production and in vitro dry matter digestibility (IVDMD) in ½M stage, (ii) improved digestion kinetics by reducing lag time and increasing total gas production and fermentation rates of non-fibrous carbohydrates (NFC) and fibrous carbohydrates (FC), and (iii) increased extent and fermentation rate of NFC and increased fermentation rate of FC fraction in whole-crop maize silages produced from dent or flint types in all phenological stages. Harvesting between ½M and ¾M is the best phenological stage to improve chemical composition and silage quality parameters. Exogenous amylase showed improvements on fibre digestion of silages at ½M and ¾M phenological stages in both grain types of corn.

KEYWORDS
carbohydrates, digestibility, enzyme, harvesting time, methane

1 | INTRODUCTION

In most regions, starch is the main energy component of cereals and the most important fraction of the non-fibrous carbohydrates (NFCs) of maize silage, which is the main forage source used in ruminant feeding. It has been demonstrated that the maximization of rumen starch digestion to the detriment of the post-ruminal digestion is beneficial due to the higher energetic efficiency of use of propionate/synthesis and ruminal microbial protein (Arieli, Abramson, Mabjeesh, Zamwel, & Bruckental, 2001; Reynolds et al., 2001).

In addition, increased intestinal flow of starch leads to risks of intestinal acidosis, which is detrimental to animal health and more difficult to control than ruminal acidosis (Gressley, Hall, & Armentano, 2011). The phenological stage and the cultivar affect starch concentration and may interact among themselves, influencing the digestibility of maize silage (Cone, Van Gelder, Van Schooten, & Groten, 2008; Macome et al., 2017).

The starch present in the hybrids with flint-type endosperm is more resistant to enzymatic attack than in dent-type hybrids (Kaczmarek, Cowieson, Jozefiak, & Rutkowski, 2014), and in tropical conditions where maize hybrids used are generally flint-type, exogenous amylase can have better response than in temperate conditions.

The use of exogenous amylase increases rumen starch degradation (Nozière, Steinberg, Silberberg, & Morgavi, 2014; Vargas-Rodriguez, Engstrom, Azem, & Bradford, 2014). However, responses to exogenous amylase are influenced by the inclusion level and particle size of maize silage (Gallo, Giuberti, Duval, Moschini, & Masoero, 2016) and these studies were made all in temperate conditions using basically dent-type maize hybrids.

The hypothesis is that the exogenous amylase acts differently in vitro digestion kinetics of whole-crop maize silages obtained at different phenological stages of hybrids with different grain vitreousness. Therefore, the aim of this study was to evaluate the effect of exogenous amylase on the in vitro rumen digestion kinetics of maize silage made from plants at different phenological stages from hybrids with different grain textures produced in tropical conditions.

2 | MATERIALS AND METHODS

2.1 | Experimental area

The maize hybrids used in this trial were sowed at the José Henrique Bruschi Experimental Field, Embrapa Dairy Cattle, Minas Gerais, Brazil (21°33'22 "S, 43°06'15 "W). According to Köppen's classification, the climate of the region is Cwa, mesothermic, with hot and rainy summer and cold and dry winter. The average annual rainfall is approximately 1,500 mm. Two commercial maize hybrids were used: RB9004—dent type; and RB9308—flint type (RIBER KWS®, Patos de Minas, Brazil).

2.2 | Harvesting

Phenological stage evaluations of corns plants were performed by two observers, which evaluated the grain hardness from 70 to 110 days after sowing (Wiersma, Carter, Albrecht, & Coors, 1993). The days after sowing for the soft dough (SOD), early dent (EAD), ½ milkline (½M) and ¾ milkline (¾M) stages were: 76, 90, 105 and 110 for the dent hybrid and 84, 91, 99 and 105 days for the flint hybrid respectively.

After the achievement of the harvest stage, five replications of 1 m^2 for each hybrid were randomly sampled within each phenological stage. The materials were processed with a stationary chopper and ensiled in duplicates using polyvinyl chloride (PVC) silos (30 cm of height; 10 cm of diameter) equipped with Bulsen valves. The ensiled mass was standardized at a density of 600 kg/m^3 (fresh basis). The silos were remained closed for 56 days until opening and sampling (silage trial).

2.3 | Laboratory analysis

Dried samples were ground (Wiley mill; A. H. Thomas, Philadelphia, PA) through 1-mm screen sieve. The silage samples were analysed for dry matter (DM), organic matter (OM), total nitrogen (TN) and ether extract (EE) according to methods 930.15, 942.05, 984.13 and 920.39 respectively (AOAC, 2005). To estimate the crude protein (CP), a conversion factor of 6.25 was used to convert N values into CP. Concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined sequentially using Ankom 220 Fiber Analyzer (Ankom Technology, Fairport, USA). For NDF analysis, thermostable α-amylase was used without addition of sodium sulphite. The NFC (g/kg of DM) contents were calculated by difference as:

$$NFC = 100 - (CP + NDF + EE + ash).$$

The lignin contents were analysed by solubilization in sulphuric acid (Gomes et al., 2011).

Starch concentration in silage samples was quantified by acid hydrolysis and enzymatic method (Chow & Landhäusser, 2004) using glucose oxidase–peroxidase (Glucose PAP Liquiform) kits (Labtest, Lagoa Santa, Minas Gerais, Brazil). Briefly, 400 mg of dried milled (1-mm) sample was added to 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, Clifton, NJ), and 0.01 ml was transferred to a test tube and added 1 ml of glucose reagent (Labtest-133) and kept under water bath at 37°C for 10 min. After cooling at room temperature, absorbance was read at 505 nm (YSI-2700 Analyzer Life Sciences, Yellow Springs, OH) and the results were estimated with glucose standards containing 0, 20, 40, 60, 80 and 100 mg glucose/dl of distilled deionized water. Starch was calculated by multiplying the glucose concentration by 0.9.

TABLE 1 Diet composition offered to ruminal liquor donor cows

Item (g/kg of dry matter)	Diet	
Whole-crop maize silage	480	
Tifton hay	52.6	
Cornmeal	210.6	
Soya bean meal	210.2	
Limestone	9.7	
Urea	5.3	
Ammonium sulphate	5.3	
Mineral mix	26.3	
	Control	**Amylase**
Amylase[a]	–	0.7
Composition (g/kg of dry matter)		
Organic matter	915 ± 9.4	924 ± 4.1
Crude protein	195 ± 3.7	193 ± 7.9
Ether extract	37 ± 2.2	37 ± 1.7
NDF	308 ± 13.1	292 ± 9.5
ADF	155 ± 6.3	149 ± 4.0
Starch	249 ± 20.1	251 ± 19.6

Note: Mineral mix: 88 g/kg of calcium; 42 g/kg of phosphorus; 18 g/kg of sulphur; 45 g/kg of magnesium; 20 g/kg of potassium; 123 g/kg of sodium; 14 mg/kg of cobalt; 500 mg/kg of copper; 20 mg/kg of chromium; 1,050 mg/kg of iron; 28 mg/kg of iodine;: 1,400 mg/kg of manganese; 18 mg/kg of selenium; 2,800 mg/kg of zinc; 420 mg/kg of fluorine; 80 mg/kg of biotin; 200,000 UI/kg of vitamin A; 70,000 UI/kg of vitamin D3; 1,200 UI/kg of vitamin E; and 600 mg/kg of monensin.
Abbreviations: ADF, acid detergent fibre; NDF, neutral detergent fibre.
[a]Amylase: provided to achieve 396 kilo Novo units of amylase activity/kg of TMR DM. Ronozyme RumiStar™; DSM Nutritional Products Brazil SA.

The N-NH_3/TN concentration in the silage samples was quantified after the Kjeldahl distillation with magnesium oxide and calcium chloride according to the method 2.065 (AOAC, 1980).

For the SCFA analyses, frozen samples of silage juice (liquid collected after pressed sample) were pre-treated as described by Siegfried, Rückemann, and Stumpf (1984). SCFA analyses were performed using HPLC (Ultimate 3000, Dionex Corporation, Sunnyvale, USA) equipped with a Shodex RI-101 refractive index (RI) kept at 40°C, using Phenomenex Rezex ROA column, 300 × 7.8 mm kept at 45°C.

2.4 | In vitro trial

The effects of exogenous amylase on the in vitro gas production kinetics of maize silage made from dent and flint hybrids were performed using ruminal inoculum from animals supplemented or not with exogenous amylase. All the procedures with animals were approved by the Animal Care and Use Committee of Embrapa Dairy Cattle (Protocol CEUA—EGL No. 03/2014). The in vitro assays were conducted at the Multi-Use Livestock Complex of Bioefficiency and Sustainability at Embrapa, Minas Gerais, Brazil. Five silage samples from trial I were randomly used for each hybrid and phenological stage.

Two non-lactating rumen-fistulated cows with 560 ± 20.9 kg live weight were distributed in a 2 × 2 Latin square design with 14 days of adaptation and used as source of inoculum. The donor animals were fed ad libitum with a forage:concentrate ratio of 53:47 (180 g/kg CP and 300 g/kg NDF), being one under control diet and the other supplemented with exogenous amylase (Ronozyme RumiStar™; DSM Nutritional Products Brazil S.A) at 0.7 g/kg DM to achieve 396 kilo Novo units of amylase activity/kg of TMR DM. (Table 1). Maize silage (RB9004) and Tifton 85 hay (*Cynodon* sp) were used as a basal forage source in the diets (Table 1).

For the in vitro incubations, samples of each maize hybrid and phenological stage were weighted (500 mg, DM basis) into filter bags (one bag per flask) (F57; Ankom®, Macedon, USA) with two replicates per maize hybrid (flint or dent) per phenological stage (SOD, EAD, ½M or ¾M) and inoculum (control or amylase treated). For each run, three blank bags (without sample) were sealed, placed into 50-ml bottles.

At day 15 of each period, rumen fluid used for incubations was taken 2 hr after morning feeding from four distinct sites in the rumen, 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

incubations. Buffered rumen fluid was prepared by mixing rumen fluid (25 ml) and a mineral buffer with 0.5 ml of sodium sulphide solution (Menke et al., 1979) in a ratio of 1:2. The buffered rumen fluid was then transferred (25 ml) into samples flasks under a stream of O_2-free N gas. Flasks were sealed and placed on an orbital shaker set at 90 oscillations/min for 2 min in every 2 hr. The cumulative gas production was measured at 14 times among 2 and 96 hr after incubation, and CH_4 concentrations and production were measured only at 24 hr using a water displacement apparatus (Fedorah & Hrudey, 1983). After 96 hr of incubation, flasks were opened and pH was measured (MS Tecnopon®, MPA 210, Piracicaba—SP-Brasil) and two aliquots (5 ml) of fluid were frozen at −20°C for SCFA and NH_3/TN analysis. The filter bags with sample residues were rinsed thoroughly with distilled water, and residual DM was analysed.

For CH_4 analysis, 10 ml of headspace gas was sampled using a 20-ml syringe. Gas was immediately transferred into a 6.8-ml evacuated exetainer (Labco, High Wycombe, Buckinghamshire, UK). Collected gas after 24 hr of incubation was analysed for CH_4 concentration by gas chromatography (03 CG-FID Agilent Technologies 7820A, Santa Clara, EUA) according to Holtshausen et al. (2009).

The gas production kinetic parameters were estimated using Minitab software version 16.2.4.4, according to the dual-pool logistic model (Schofield, Pitt, & Pell, 1994):

$$Vf(t)=\frac{V_1}{\left(1+e^{(2-4\times C_1\times(t))}\right)}+\frac{V_2}{\left(1+e^{(2-4\times C_2\times(t-L))}\right)}+\varepsilon$$

where V_1 and C_1 are, respectively, the maximum volume of gases produced (ml) and fermentation rate (/h) of the CNF fraction; V_2, C_2 and L are, respectively, the maximum volume of gas production (ml), the degradation rate (/h) and latency (h) of the fibrous carbohydrates (FC); Vf, t and "ε" are, respectively, the total volume of gas (ml), incubation time (h) and random error.

The in vitro DM digestibility (IVDMD; %) was calculated for 96 and 24 hr of incubation according to the equation:

$$IVDMD=100\times\frac{DMi-DMf}{DMi}$$

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

2.5 | Statistical analysis

Data of chemical composition and fermentative profile of silages were analysed using the PROC GLIMMIX (SAS, 9.4; SAS Inst., Cary, NC, USA), according to the factorial model:

$$Y_{ijk}=\mu+H_i+E_j+H\times E_{ij}+\varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, H_i is the fixed effect of hybrid, E_j is the fixed effect of phenological stage, $H \times E_{ij}$ is the fixed effect of interaction between hybrid and phenological stage, and ε_{ijk} is the random error. When the effects of interaction between hybrid and phenological stage were significant, the means were compared within each hybrid by the SLICEDIFF option.

Data of the in vitro trial were analysed using the PROC GLIMMIX (SAS, 9.4), separately for each phenological stage, according to the model:

$$Y_{ijk}=\mu+H_i+I_j+H\times I_{ij}+R_l+\varepsilon_{ijkl}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, H_i is the fixed effect of hybrid, I_j is the fixed effect of inoculum, $H \times I_{ij}$ is the fixed effect of interaction between hybrid and inoculum, R_l is the random effect of run (period), and ε_{ijk} is the random error. Originally, the model included the effects of phenological stage as a third factor, but due to the significance ($p < .05$) of the triple interaction between the phenological stage, hybrid and inoculum, the described model was analysed separately for each phenological stage. When effects of interaction between hybrid and inoculum were significant, the means were compared within each hybrid by the SLICEDIFF option. Pearson's correlation analysis among CH_4 production and digestion kinetics was performed. The comparisons between means were performed by Tukey's test. In all evaluations, the significance was declared at $p < .05$.

3 | RESULTS

3.1 | Chemical composition and fermentation profile of maize silages

The interaction effect between hybrid and phenological stage was significant ($p < .001$) for all variables of bromatological composition, except EE ($p = .133$). The NDF content of silages was higher at SOD stage and did not differ among other phenological stages for the dent hybrid ($p < .001$), whereas for the flint hybrid, the values were similar among the stages. The NFC content in silage reached maximum values in EAD, ½M and ¾M stages for the dent hybrid ($p < .001$) and did not differ among phenological stages for the flint hybrid ($p > .05$). The starch content in silage was similar in EAD and ½M stages and higher in ¾M stage for the dent hybrid ($p < .001$) and higher in EAD, ½M and ¾M stages for the flint hybrid ($p < .001$) (Table 2).

There was an interaction effect ($p < .001$) for pH, N-NH_3/TN and SCFA of silages. The pH was higher for the dent hybrid at SOD phenological stage, while for the flint hybrid, it was similar at SOD and ¾M stages ($p < .001$). Increased values of ammonia nitrogen were observed in SOD, ½M and ¾M phenological stages for the dent hybrid ($p = .02$). The lactic acid concentration was lower in SOD stage for the dent hybrid, whereas for the flint hybrid, in ½M stage the value was lower ($p < .001$). Higher acetic acid concentration was observed in SOD stage for the dent hybrid, whereas a higher concentration was observed in ½M stage for the flint hybrid ($p < .001$). The concentrations of propionic and butyric acid presented similar profile in silages made from flint hybrid, showing highest concentrations in ¾M stage, while for the dent hybrid, the highest propionic acid values were observed in ¾M stage ($p < .001$) (Table 2).

TABLE 2 Bromatological composition and fermentative profile of dent and flint whole-crop maize silages at different phenological stages

	Hybrid[†]											
	Dent				Flint							
	Phenological stage[‡]				Phenological stage					*p*-value[§]		
Item	SOD	EAD	½M	¾M	SOD	EAD	½M	¾M	*SEM*	H × S	H	S
Bromatological composition (g/kg of DM)												
DM	201^{c}	227^{c}	320^{b}	375^{a}	227^{c}	277^{b}	320^{a}	348^{a}	7.8	<.001	.035	<.001
OM	916^{c}	933^{b}	952^{a}	958^{a}	930^{c}	951^{a}	941^{b}	949^{ab}	2.4	<.001	.091	<.001
CP	99.2^{a}	85.2^{b}	80.8^{b}	65.2^{c}	79.5	70.7	74.7	72.6	2.77	<.001	<.001	<.001
NDF	623^{a}	434^{b}	439^{b}	475^{b}	539	526	497	503	12.0	<.001	<.001	<.001
ADF	374^{a}	252^{b}	244^{b}	253^{b}	328^{a}	302^{ab}	277^{b}	276^{b}	8.3	<.001	.017	<.001
LIG	39.8^{a}	25.5^{b}	24.1^{b}	24.2^{b}	32.0^{b}	29.7^{ab}	26.2^{b}	27.1^{ab}	1.50	<.001	.711	<.001
EE	33.7^{a}	45.5^{b}	40.0^{b}	35.1^{b}	34.8^{a}	37.8^{b}	38.8^{b}	36.5^{b}	2.12	.133	.295	.005
NFC	160^{b}	368^{a}	392^{a}	383^{a}	291	317	331	337	12.0	<.001	.237	<.001
Starch	55^{c}	213^{b}	246^{ab}	258^{a}	111^{b}	195^{a}	207^{a}	220^{a}	8.9	<.001	.129	<.001
Fermentative profile												
pH	4.12^{a}	3.86^{b}	3.91^{b}	3.90^{b}	3.79^{b}	3.98^{a}	4.01^{a}	3.89^{ab}	.050	<.001	.416	.529
N-NH_3/TN (mg/g)	2.21^{a}	1.43^{b}	1.83^{ab}	2.12^{a}	1.27	1.59	1.22	1.65	.175	.020	<.001	.113
Lactic acid (µmol/ml)	105^{b}	182^{a}	179^{a}	184^{a}	199^{a}	168^{ab}	148^{b}	169^{ab}	10.2	<.001	.256	.077
Acetic acid (µmol/ml)	95.2^{a}	57.5^{b}	53.7^{b}	46.6^{b}	52.8^{b}	50.8^{b}	108.5^{a}	70.8^{b}	6.22	<.001	.096	<.001
Propionic acid (µmol/ml)	12.4^{bc}	10.4^{c}	18.2^{ab}	21.1^{a}	11.6^{c}	14.1^{bc}	19.7^{b}	39.6^{a}	1.73	<.001	<.001	<.001
Butyric acid (µmol/ml)	3.92	3.17	4.27	3.50	4.31^{b}	2.90^{b}	4.16^{b}	8.66^{a}	.960	<.001	.032	.003

Note: Different letters in the same line indicate significant statistical differences ($p < .05$).
Abbreviations: ADF, acid detergent fibre; CP, crude protein; DM, dry matter; EE, ether extract; LIG, lignin; OM, organic matter; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; *SEM*, standard error of mean.
[†]Hybrid: dent, RB9004; flint, RB9308.
[‡]Phenological stage. SOD: soft dough; EAD: early dent; ½M: ½ milkline; ¾M: ¾ milkline.s
[§]*p*-value: H = effect of hybrid; E = effect of phenological stage; H × S = Hybrid × Stage interaction ($p < .05$ by the Tukey test).

3.2 | Fermentation kinetics and in vitro digestion

3.2.1 | Soft dough

The value of *L* ($p < .01$) was higher for the dent hybrid ($p < .001$). The interaction between hybrid and amylase was significant only for V_1 ($p < .001$). Values of V_2 ($p < .001$) were higher for the flint hybrid. Exogenous amylase increased the values of C_1, C_2 and *Vf* ($p < .001$) in both hybrids (Table 3).

3.2.2 | Early dent

The interaction between hybrid and amylase was significant for *L* ($p = .001$), V_1 ($p < .001$), V_2 ($p < .005$), C_1 ($p = .015$) and *Vf* ($p < .001$). The use of exogenous amylase reduced *L* ($p < .001$) and increased V_1 ($p < .001$) and V_2 ($p = .02$) for the flint hybrid, without significant alteration in any of these parameters ($p > .05$) for the dent hybrid. The use of exogenous amylase increased the values of V_1 ($p < .001$) and C_1 ($p < .001$) for both hybrids, but in a greater extent for the flint hybrid ($p = .015$). The IVDMD was higher for the dent hybrid compared to the flint ($p = .006$) (Table 3).

3.2.3 | ½ Milkline

The interaction between hybrid and amylase was significant only for C_2 ($p = .049$), and amylase increased this rate only for the flint hybrid ($p < .001$). The use of exogenous amylase reduced *L* values ($p = .013$) and increased values of V_1 ($p < .001$), C_1 ($p < .001$), *Vf* ($p = .024$) and IVDMD ($p = .033$) for both hybrids. The V_1 values were higher for the flint compared to the dent hybrid ($p = .017$) (Table 3).

3.2.4 | ¾ Milkline

The interaction between hybrid and amylase was significant only for the parameters V_2 ($p = .016$) and *Vf* ($p = .017$), and amylase reduced the value of V_2 only for the dent hybrid ($p < .016$), while it increased *Vf* only for the flint hybrid and decreased for the dent hybrid ($p < .017$). The use of exogenous amylase increased V_1 ($p = .002$), C_1

TABLE 3 In vitro gas production kinetics with or without exogenous amylase of whole-crop maize silages ensiling at different phenological stages

		Hybrid[†]							
		Dent		Flint			*p*-value[§]		
Stage[‡]	Item	Control	Amylase[4]	Control	Amylase	*SEM*	H × A	H	A
SOD	*L* (h:min)	3:44	3:52	2:13	2:12	.362	.826	<.001	.872
	V_1 (ml)	149[b]	179[a]	146[b]	160[a]	2.4	.001	<.001	<.001
	V_2 (ml)	85.8	85.9	111.6	111.6	16.84	.999	<.001	.997
	C_1 (/h)	0.0214	0.0231	0.0217	0.0227	.00173	.203	.872	<.001
	C_2 (/h)	0.100	0.118	0.104	0.108	.0016	.085	.347	.006
	Vf (ml)	235	265	258	271	17.8	.109	.005	<.001
	IVDMD (%)	56.9	57.4	58.2	59.7	2.06	.615	.062	.307
EAD	*L* (h:min)	2:51	2:38	4:41[a]	2:52[b]	.394	.001	<.001	<.001
	V_1 (ml)	148[b]	158[a]	128[b]	157[a]	3.5	<.001	<.001	<.001
	V_2 (ml)	116.9	114.6	84.2[b]	106.2[a]	20.67	.005	<.001	.020
	C_1 (/h)	0.0223[b]	0.0239[a]	0.0200[b]	0.0229[a]	.00218	.015	<.001	<.001
	C_2 (/h)	0.098	0.104	0.099	0.106	.0159	.706	.590	.027
	Vf (ml)	265	272	212[b]	263[a]	23.6	<.001	<.001	<.001
	IVDMD (%)	62.1	63.6	60.5	59.9	1.37	.253	.006	.654
½M	*L* (h:min)	4:31	3:24	3:47	3:16	.324	.336	.170	.013
	V_1 (ml)	134	147	140	157	9.5	.477	.017	<.001
	V_2 (ml)	93.7	98.4	96.8	92.7	17.89	.292	.755	.948
	C_1 (/h)	0.0205	0.0230	0.0204	0.0227	.00309	.780	.661	<.001
	C_2 (/h)	0.094	0.101	0.087[b]	0.104[a]	.0181	.049	.452	<.001
	Vf (ml)	228	246	237	250	10.1	.747	.306	.024
	IVDMD (%)	62.1	64.1	60.7	62.7	2.13	.993	.134	.033
¾M	*L* (h:min)	2:40	3:31	4:18	3:10	.658	.085	.257	.787
	V_1 (ml)	150	156	141	165	5.7	.065	.998	.002
	V_2 (ml)	126.7[a]	95.5[b]	95.4	99.3	12.63	.016	.056	.057
	C_1 (/h)	0.0220	0.0242	0.0213	0.0243	.00337	.522	.642	<.001
	C_2 (/h)	0.086	0.105	0.092	0.101	.0162	.057	.668	<.001
	Vf (ml)	275[a]	252[b]	236[b]	265[a]	11.6	.017	.212	.813
	IVDMD (%)	58.0	61.4	58.4	61.8	1.66	.975	.704	.002

Note.: L: lag time (hours:minutes); V_1: gas volume from rapidly degradable fraction (ml/g DM); V_2: gas volume from slowly degradable fraction (ml/g DM); C_1: degradation rate of rapidly degradable fraction (/h); C_2: degradation rate of slowly degradable fraction (/h); *Vf*: total gas volume (ml/g DM); IVDMD: in vitro dry matter digestibility (g/kg of DM). Amylase: exogenous at 0.7 g/kg DM to achieve 396 kilo Novo units of amylase activity/kg of TMR DM. Ronozyme RumiStar™; DSM Nutritional Products Brazil SA. Different letters in the same line indicate significant statistical differences ($p < .05$).

Abbreviation: *SEM*: standard error of mean.

[†]Hybrid: dent, RB9004; flint, RB9308.

[‡]Stage: SOD: soft dough; EAD: early dent; ½M, ½ milkline; ¾M: ¾ milkline.

[§]*p*-value: H: effect of hybrid; A: effect of Amylase inclusion; H × A: Hybrid × Amylase interaction ($p < .05$ by the Tukey test).

($p < .001$) and C_2 ($p < .001$) and IVDMD ($p < .002$) in both hybrids (Table 3).

3.3 | pH, methane and in vitro dry matter digestibility

The pH values were reduced by adding exogenous amylase at the SOD, EAD ($p = .005$) and ¾M stages ($p = .004$). The use of exogenous amylase increased CH_4 production per unit of DM incubated (DMi) and degraded (DMd) for the flint hybrid at SOD ($p < .001$) and ¾*M* ($p < .001$) stages, without significant effect ($p > .05$) for the dent hybrid at the same phenological stages. The use of exogenous amylase increased CH_4 production per unit of DMd ($p < .009$) in both hybrids at the ½M stage. The IVDMD increased ($p = .035$) with the use of exogenous amylase at the ½M stage in both hybrids.

At SOD stage, there was an interaction effect of Hybrid × Additive for N-NH_3/TN and CH_4 ($p < .001$). Effect of hybrid was observed for pH, total SCFA, acetic and butyric acids, CH_4 and IVDMD ($p < .01$). No treatment effect was observed for the concentrations of propionic acid ($p > .05$) and acetic:propionic ratio (A:P) ($p > .05$).

At EAD stage, the N-NH_3/TN production was higher in the treatment without amylase ($p = .005$). There was an interaction effect for propionic acid production ($p = .028$) and the A:P ratio ($p = .027$). The dent hybrid produced higher amounts of SCFA ($p < .001$) and butyric acid ($p = .009$).

At ½M stage, the interaction occurred only for butyric acid ($p = .036$) and CH_4 (DMi) ($p = .016$). Effect of hybrid was observed for acetic ($p < .001$), propionic ($p < .001$) and butyric ($p = .003$) acids, A:P ratio ($p < .001$), CH_4 (DMd) ($p = .001$) and IVDMD ($p < .001$). There was a decrease in N-NH_3/TN concentration, acetic acid and A:P ratio with amylase inclusion ($p < .001$) and an increase in propionic acid and CH_4 (DMi and DMd) ($p < .001$).

There was an interaction effect for acetic acid ($p = .041$) and propionic acid ($p = .023$) concentrations, A:P ($p = .018$) and CH_4 (DMi) ($p = .006$) and CH_4 (DMd) ($p = .042$). Amylase decreased the pH ($p = .004$) and propionic acid ($p < .009$), and increased the SCFA production ($p < .033$), A:P ratio ($p = .015$), CH_4 (DMi) ($p < .006$) and CH_4 (DMd) ($p = .042$) at ¾M stage (Table 4).

4 | DISCUSSION

The results demonstrate a clear evidence that maize phenological stage is a relevant point not only for silage production, but also for a better efficacy of exogenous amylase in ruminants' diets. Overall, the ½M and ¾M stages had more appropriate silage fermentation parameters and improved in vitro digestion kinetics, independently of the grain type.

4.1 | Bromatological composition and fermentative profile of maize silages

The increase in the DM concentration with the advancement in the phenological stage occurs mainly by the inherent accumulation of starch at mature stages with a relative reduction of fibrous carbohydrates (Der Bedrosian, Nestor, & Kung, 2012; Ferraretto, Fonseca, Sniffen, Formigoni, & Shaver, 2015; Maxin, Andueza, Le Morvan, & Baumont, 2017). Subtle variations in DM, NDF and NFC contents for the flint hybrid were found in comparison with the dent type with the advancement in the phenological stage. This characteristic of the flint hybrid is desirable because of the potential advantage of allowing a larger adequate harvesting period for silage production.

One of the factors that can determine the extent and quality of fermentation is the pH of the ensiled mass, which can inhibit heterofermentative bacterial growth (Auerbach, 2003; Muck, 2010). Heterofermentative bacteria are less efficient than homofermentative in the acidification and nutrient preservation during the anaerobic phase, and they are more abundant in silages with pH higher than 4.0 (Holzer, Mayrhuber, Danner, & Braun, 2003). The pH of silages evaluated, even at phenological stages with a lower dry matter content, remained between 3.8 and 4.2, an ideal range for the prevalence of homofermentative micro-organisms (Muck, 2010).

The N-NH_3/TN concentrations may be one indicative of secondary fermentation caused by heterofermentative bacteria of the genus Clostridium with consequent decrease in lactic acid (Silva et al., 2016). In the present study, even though the N-NH_3/TN concentration was higher in SOD and ¾M stages for the dent hybrid, the lactic acid was sufficient to maintain the appropriate fermentation process and low N-NH_3/TN. N-NH_3/TN values below 10 g/kg indicate good preservation of ensiled material for both evaluated hybrids (McDonald, Henderson, & Heron, 1991).

The higher N-NH_3/TN values for the dent than the flint hybrid may indicate degradation of the zein proteins involving the starch granules. Zein proteins can undergo degradation during the fermentation process by solubilization or by the action of proteolytic enzymes (Hoffman et al., 2011). Ferraretto et al. (2015) studying hybrids of different proportion of vitreous endosperm observed an increase in N-NH_3/NT concentrations, the lower the vitreous, similar to the observed in this study.

4.2 | Fermentation kinetics and in vitro dry matter digestibility

The α-amylase is the enzyme responsible for breaking the bonds between starch granules. This hydrolysis occurs through the cleavage of the α-1.4 and α-1.6 bonds of the starch granules (Tricarico, Johnston, & Dawson, 2008). Our results indicate that exogenous amylase increased the rate of fermentation. Likewise, Gallo et al. (2016) observed that amylase decreased lag time and increased rate and amount of gas production. This effect of amylase is due to the greater hydrolysis of starch (Nozière et al., 2014) providing substrates for the multiplication of other microbial species, mainly fibrolytic micro-organisms (Mccarthy, Engstrom, Azem, & Gressley, 2013), a process defined as cross-feeding among rumen micro-organisms (Russell, 1985) that can improve fibre digestibility by increase in fibre digestion rate as shown in our study.

The increase in the proportion of vitreous endosperm is positively related to grain hardness, density and percentage of zein proteins involving starch granules, which negatively affects starch digestibility (Giuberti, Gallo, Moschini, Cerioli, & Masoero, 2013). The higher value of *L* for the dent hybrid, which has less proportion of vitreous endosperm than the flint hybrid, was not expected considering only corn endosperm as an isolated effect. In that situation, the higher concentration of starch was negatively correlated with lag time ($p = .0234$) and positively correlated with gas production of NFC fraction ($p = .0019$). However, when *L* was higher, gas production of FC fraction increased, suggesting greater fibrolytic microbial activity. In contrast to present study, Pôssas et al. (2015) observed longer lag time for hybrids with hard-textured grains compared to hybrids with semi-hard texture grains.

TABLE 4 Ammonia nitrogen, short-chain fatty acids (SCFAs), methane production (CH_4) and in vitro dry matter digestibility (IVDMD) with or without exogenous amylase of whole-crop maize silages ensiling at different phenological stages incubated for 24 hr

		Hybrid[†]							
		Dent		Flint			*p*-value[§]		
Stage[‡]	Item	Control	Amylase	Control	Amylase	*SEM*[§]	H × A	H	A
SOD	pH	6.50	6.48	6.39	6.32	.180	.053	<.001	.005
	N-NH_3/TN (mg/g)	2.40	2.06	1.83[b]	2.39[a]	.364	<.001	.353	.383
	Total SCFA (μmol)	69.9	67.1	76.8	76.0	3.21	.709	.004	.489
	Acetic acid (μmol/ml)	0.60	0.61	0.57	0.59	.017	.908	.014	.255
	Propionic acid (μmol/ml)	0.22	0.21	0.23	0.22	.014	.941	.524	.170
	Butyric acid (μmol/ml)	0.18	0.18	0.19	0.19	.005	.846	<.001	.983
	A:P[6] ratio	2.50	2.52	2.35	2.40	.127	.765	.068	.608
	CH_4 (mg/g DMi)	6.06	6.25	7.93[b]	9.44[a]	.199	.002	<.001	<.001
	CH_4 (mg/g DMd)	17.5	18.3	20.5[b]	25.5[a]	.59	.001	<.001	<.001
	IVDMD (%)	35.2	34.7	39.3	38.1	.85	.651	<.001	.210
EAD	pH	6.36	6.32	6.41	6.37	.215	.885	<.001	.005
	N-NH_3/TN (mg/g)	2.17	2.01	2.16	1.47	.532	.115	.104	.015
	Total SCFA (μmol)	75.8	76.9	63.5	65.7	3.68	.836	<.001	.517
	Acetic acid (μmol/ml)	0.54	0.55	0.55	0.54	.011	.064	.892	.432
	Propionic acid (μmol/ml)	0.25	0.24	0.25[b]	0.27[a]	.012	.028	.110	.219
	Butyric acid (μmol/ml)	0.21	0.21	0.20	0.19	.006	.319	.009	.342
	A:P ratio	2.07	2.11	2.11[a]	1.94[b]	.081	.027	.147	.145
	CH_4 (mg/g DMi)	7.46	6.50	4.91	4.43	.563	.675	.002	.208
	CH_4 (mg/g DMd)	17.5	14.7	12.3	10.7	1.36	.676	.002	.121
	IVDMD (%)	44.6	45.7	41.0	41.9	1.06	.890	<.001	.064
½M	pH	6.40	6.35	6.40	6.39	.196	.337	.291	.116
	N-NH_3/TN (mg/g)	2.05	1.50	2.29	1.60	.570	.472	.084	<.001
	Total SCFA (μmol)	60.8	68.2	62.7	71.2	4.09	.886	.549	.060
	Acetic acid (μmol/ml)	0.51	0.49	0.56	0.52	.011	.644	<.001	<.001
	Propionic acid (μmol/ml)	0.29	0.33	0.27	0.30	.006	.539	<.001	<.001
	Butyric acid (μmol/ml)	0.20[a]	0.18[b]	0.17	0.18	.005	.036	.003	.132
	A:P ratio	1.80	1.50	2.12	1.78	.056	.711	<.001	<.001
	CH_4 (mg/g DMi)	5.10[b]	7.17[a]	6.58	6.95	.334	.016	.068	<.001
	CH_4 (mg/g DMd)	12.3	16.1	16.8	18.2	.94	.214	.001	.009
	IVDMD (%)	43.5	46.0	40.9	41.3	.21	.101	<.001	.035

(Continues)

TABLE 4 (Continued)

		Hybrid[†]							
		Dent		Flint			*p*-value[§]		
Stage[‡]	Item	Control	Amylase	Control	Amylase	*SEM*[§]	H × A	H	A
¾M	pH	6.40	6.36	6.40	6.33	.207	.571	.442	.004
	$N\text{-}NH_3$/TN (mg/g)	1.73	1.93	1.78	1.73	.393	.463	.669	.657
	Total SCFA (µmol)	69.0	70.0	62.3	75.4	3.18	.064	.858	.033
	Acetic acid (µmol/ml)	0.52	0.52	0.51[b]	0.53[a]	.008	.041	.623	.042
	Propionic acid (µmol/ml)	0.26	0.26	0.28[a]	0.25[b]	.012	.023	.600	.009
	Butyric acid (µmol/ml)	0.22	0.22	0.21	0.22	.007	.232	.141	.071
	A:P ratio	1.90	1.90	1.75[b]	2.02[a]	.086	.018	.893	.015
	CH_4 (mg/g DMi)	6.70	5.66	3.05[b]	7.64[a]	.602	<.001	.173	.006
	CH_4 (mg/g DMd)	17.3	14.4	7.7[b]	18.5[a]	1.86	<.001	.145	.042
	IVDMD (%)	41.1	40.7	40.1	39.3	.14	.877	.346	.646

Note: DMi: dry matter incubated; DMd: dry matter degraded; IVDMD: dry matter in vitro digestibility. Amylase: exogenous at 0.7 g/kg DM to achieve 396 kilo Novo units of amylase activity/kg of TMR DM. Ronozyme RumiStar™; DSM Nutritional Products Brazil SA. A:P ratio: acetate: propionate ratio. Different letters in the same line indicate significant statistical differences ($p < .05$).
Abbreviation: *SEM*: standard error of mean.
[†]Hybrid: dent, RB9004; flint, RB9308.
[‡]Phenological stage: SOD: soft dough; EAD: early dent; ½M: ½ milkline; ¾M: ¾ milkline.
[§]*p*-value. H: effect of hybrid; A: effect of amylase inclusion; H × A: Hybrid × Amylase interaction ($p < .05$ by the Tukey test).

The increase in V_1, V_2, C_1 and C_2 with amylase inclusion indicates higher digestibility of both non-fibrous and fibrous fractions of silage, which strengthens the occurrence of cross-feeding interaction among the micro-organisms. Similarly, amylase supplementation increased the total NDF digestibility when evaluated in vivo (Gencoglu et al., 2010; Mccarthy et al., 2013). Contrary to our study, others did not found effect of addition of the exogenous amylase on NDF digestibility for dairy cows (Andreazzi et al., 2018; Zilio et al., 2019). The higher IVDMD of the dent hybrid, at EAD stage, can be justified by the lower proportion of vitreous endosperm in the grain and greater physical availability of the starch at that stage. In the same way, Giuberti et al. (2013) and Corona, Owens, and Zinn (2006) reported a negative effect of vitreous endosperm on grain digestibility. However, when starch proportion was higher in ½M and ¾M stages, amylase equalized the differences in vitreous endosperm of the flint grain compared to the dent type, resulting in increased IVDMD for both hybrids.

Ferraretto, Shaver, Espineira, Gencoglu, and Bertics (2011) did not observe effect of amylase in low-starch diets (230 g/kg DM) in an in vivo study with exogenous amylase supplementation at 324 KNU/kg of DM of activity in the total diet based on maize and alfalfa silage and forage:concentrate of 50:50. On the other hand, Nozière et al. (2014) evaluated two dietary starch concentrations (200 g/kg DM and 300 g/kg DM) and observed an effect of amylase on rumen starch digestion for both diets with 0.5 g/kg of amylase to achieve 300 kilo Novo units (KNU)/kg of DM in the total diet, similar to that used in the present study (396 KNU/kg of DM).

Exogenous amylase is an additive that has shown increase in starch and NDF digestibility (Nozière et al., 2014; Vargas-Rodriguez et al., 2014). The amylase degrades the chains of starch, attacking the α-1,4 glycosidic bonds of the starch polymers. In this way, exogenous amylase supplementation in dairy cows' diets can increase rumen digestion of starch and support the availability of substrates for diverse rumen microbe's species.

In this perspective, exogenous amylase can decrease the amount of starch that pass to the small intestine. This is interesting because a great amount of starch in the intestine can cause hindgut acidosis. So, the increase in starch digestion in the rumen is desirable.

Care must be taken when starch is degraded in the rumen by increase in acute ruminal acidosis. However, an earlier study has reported that amylase does not affect ruminal pH (Nozière et al., 2014) and does not cause episodes of rumen acidosis. Moreover, the buffer capacity of closed systems and the case of our study is high. Although the pH in the present study suffers a small drop, this is not enough to change the ruminal environment and microbiota.

Overall, there are no limiting factors for starch digestion in the rumen (Harmon, Yamka, & Elam, 2004). However, fermentation pathways are variable (Nozière et al., 2014); thus, the exogenous amylase supplementations can promote micro-organisms, which do not utilize starch directly as substrate (Tricarico et al., 2008). These

changes can promote improvements in ruminant production. In this context, Andreazzi et al. (2018) observed an increase of 0.7 kg/day in milk yield of dairy cows with exogenous amylase. These authors explain this effect most likely due to increase in lactose production by greater glucose availability as consequence of exogenous amylase supplementation. The lactose is an important product of uptake mammary glucose (Zhao, 2014), and higher production of propionate can increase the supply of substrate for gluconeogenesis.

The degradation of starch promoted by exogenous amylase in the rumen probably supported the growth of fibrolytic bacteria, because for several species, the simple sugars are the primary preferable substrates. In this perspective, all ruminal microbiota may be benefited. Although the addition of exogenous amylase decreased the Fibrobacter succinogenes (Nozière et al., 2014), the glucose provided by increase in starch ruminal digestibility is mainly fermented by odd-chain pathway, this pathway uses less ATP per mol of fermented carbon (Sauvant & Van Milgen, 1995), contributing for overall microbial growth.

The IVDMD increased with amylase at ½M and ¾M stages, demonstrating that amylase could beneficially affect the digestion of non-fibrous and fibrous carbohydrates, because the higher proportion of starch in whole-crop maize silage at these phenological stages favours the action of amylase.

4.3 | pH, methane production and digestibility after 24 hr of incubation

The pH was not below the critical value (<5.6) at any maturity stage for efficient bacterial multiplication, especially those bacteria that digest the cell wall (Ramos, Champion, Poncet, Mizubuti, & Nozière, 2009). Sudden drops in pH cause drastic changes in microbial populations (Gressley et al., 2011).

Higher CH_4 production (mg CH_4/g of DMd) with amylase inclusion may be associated with changes in the fermentation route, related to the increased digestibility of the plant fibrous fraction shown by the positive correlation of CH_4 production with gas production of FC fraction (0.19). In this situation, when FC digestibility increases, more acetic and butyric acid concentration could increase, and this fact implicates in more CH_4 production. This can be indicated by changes in A:P ratio among the maturity stages of the plant. Peyrat, Baumont, Le Morvan, and Nozière (2016) observed an increase in A:P ratio with maturity of the maize. We expected this because increase in non-fibrous carbohydrates favours the propionate production, mainly due to the drop in rumen pH.

The higher CH_4 production for the flint hybrid at SOD stage is possibly associated with higher production of total SCFA, as well as for the dent hybrid at EAD stage. In addition, CH_4 production was higher when more butyric acid was produced. Morgado, Ezequiel, Júnior, and Galzerano (2013) obtained an average of 17.4 mg CH_4/g of DM using maize silage, a similar value to that obtained in the present study (17.2 mg CH_4/g DM). The effect of amylase seems to be associated with the fibrous fraction of the maize plant supported by the cross-feeding mechanism providing substrate to growth of micro-organisms that degraded mainly fibrous substrates. Hence, studies evaluating the use of exogenous amylase with other forages species at different phenological stages and digestible fibre concentrations are necessary.

5 | CONCLUSION

Harvesting between ½M and ¾M is the best phenological stage to improved bromatological composition and silage quality parameters to both dent or flint whole-crop maize silages. Feeding exogenous amylase increases the extent and fermentation rate of NFC and increases fermentation rate of FC of whole-crop maize silages in all phenological stages. Exogenous amylase showed improvements on fibre digestion of silages at ½M and ¾M phenological stages in both grain types of corn.

6 | ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journalss author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and all the procedures with animals were approved by the Animal Care and Use Committee of Embrapa Dairy Cattle (Protocol CEUA—EGL No. 03/2014).

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REFERENCES

Andreazzi, A. S. R., Pereira, M. N., Reis, R. B., Pereira, R. A. N., Júnior, N. N. M., Acedo, T. S., & Cortinhas, C. S. (2018). Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. *Journal of Dairy Science*, *101*(8), 1–9. https://doi.org/10.3168/jds.2017-14331

Arieli, A., Abramson, S., Mabjeesh, S. J., Zamwel, S., & Bruckental, I. (2001). Effect of site and source of energy supplementation on milk yield in dairy cows. *Journal of Dairy Science*, *84*, 462–470. https://doi.org/10.3168/jds.S0022-0302(01)74496-7

Association of Official Analytical Chemists. (1980). *Official methods of analysis* (13th ed.). Washington, DC, USA: Author.

Association of Official Analytical Chemists. (2005). *Official methods of analysis* (18th ed.). Gaithersburg, MD: Author.

Auerbach, H. (2003). Molds and mycotoxins in silages. In T. P. Lyons & K. A. Jacques (Eds.), *Proceedings of the 19th Alltech Annual Symposium on Biotechnology in the Feed Industry* (pp. 247–266). Lexington, KY: Alltech Inc.

Chow, P. S., & Landhäusser, S. M. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology*, *24*, 1129–1136.

Cone, J. W., Van Gelder, A. H., Van Schooten, H. A., & Groten, J. A. M. (2008). Effects of forage maize type and maturity stage on in vitro rumen fermentation characteristics. *NJAS—Wageningen Journal of Life Sciences*, *55*, 139–154. https://doi.org/10.1016/S1573-5214(08)80033-4

Corona, L., Owens, F. N., & Zinn, R. A. (2006). Impact of corn vitreousness and processing on site and extent of digestion by feedlot cattle. *Journal of Animal Science*, *84*, 3020–3031. https://doi.org/10.2527/jas.2005-603

Der Bedrosian, M. C., Nestor, K. E., & Kung, L. (2012). The effects of hybrid, maturity, and length of storage on the composition and nutritive value of corn silage. *Journal of Dairy Science*, *95*, 5115–5126. https://doi.org/10.3168/jds.2011-4833

Fedorah, P. M., & Hrudey, S. E. (1983). A simple apparatus for measuring gas production by methanogenic cultures in serum bottles. *Environmental Technology Letters*, *4*, 425–432. https://doi.org/10.1080/09593338309384228

Ferraretto, L. F., Fonseca, A. C., Sniffen, C. J., Formigoni, A., & Shaver, R. D. (2015). Effect of corn silage hybrids differing in starch and neutral detergent fiber digestibility on lactation performance and total-tract nutrient digestibility by dairy cows. *Journal of Dairy Science*, *98*, 395–405. https://doi.org/10.3168/jds.2014-8232

Ferraretto, L. F., Shaver, R. D., Espineira, M., Gencoglu, H., & Bertics, S. J. (2011). Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. *Journal of Dairy Science*, *94*, 1490–1499. https://doi.org/10.3168/jds.2010-3736

Gallo, A., Giuberti, G., Duval, S., Moschini, M., & Masoero, F. (2016). Short communication: The effect of an exogenous enzyme with amylolytic activity on gas production and in vitro rumen starch degradability of small and large particles of corn or barley meals. *Journal of Dairy Science*, *99*, 3602–3606. https://doi.org/10.3168/jds.2015-9904

Gencoglu, H., Shaver, R. D., Steinberg, W., Ensink, J., Ferraretto, L. F., Bertics, S. J., … Akins, M. S. (2010). Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. *Journal of Dairy Science*, *93*, 723–732. https://doi.org/10.3168/jds.2009-2673

Giuberti, G., Gallo, A., Moschini, M., Cerioli, C., & Masoero, F. (2013). Evaluation of the impact of maize endosperm vitreousness on in vitro starch digestion, dry matter digestibility and fermentation characteristics for pigs. *Animal Feed Science and Technology*, *186*, 71–80. https://doi.org/10.1016/j.anifeedsci.2013.08.011

Gomes, D. I., Detmann, E., Valadares Filho, S. D. C., Fukushima, R. S., de Souza, M. A., Valente, T. N. P., … de Queiroz, A. C. (2011). Evaluation of lignin contents in tropical forages using different analytical methods and their correlations with degradation of insoluble fiber. *Animal Feed Science and Technology*, *168*, 206–222. https://doi.org/10.1016/j.anifeedsci.2011.05.001

Gressley, T. F., Hall, M. B., & Armentano, L. E. (2011). Ruminant nutrition symposium: Productivity, digestion, and health responses to hindgut acidosis in ruminants. *Journal of Animal Science*, *89*, 1120–1130. https://doi.org/10.2527/jas.2010-3460

Harmon, D. L., Yamka, R. M., & Elam, N. A. (2004). Factors affecting intestinal starch digestion in ruminants: A review. *Canadian Journal of Animal Science*, *84*, 309–318. https://doi.org/10.4141/A03-077

Hoffman, P. C., Esser, N. M., Shaver, R. D., Coblentz, W. K., Scott, M. P., Bodnar, A. L., … Charley, R. C. (2011). Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. *Journal of Dairy Science*, *94*, 2465–2474. https://doi.org/10.3168/jds.2010-3562

Holtshausen, L., Chaves, A. V., Beauchemin, K. A., McGinn, S. M., McAllister, T. A., Odongo, N. E., … Benchaar, C. (2009). Feeding saponin-containing Yucca schidigera and Quillaja saponaria to decrease enteric methane production in dairy cows. *Journal of Dairy Science*, *92*, 2809–2821. https://doi.org/10.3168/jds.2008-1843

Holzer, M., Mayrhuber, E., Danner, H., & Braun, R. (2003). The role of Lactobacillus buchneri in forage preservation. *Trends in Biotechnology*, *21*, 282–287. https://doi.org/10.1016/S0167-7799(03)00106-9

Kaczmarek, S. A., Cowieson, A., Jozefiak, D., & Rutkowski, A. (2014). Effect of maize endosperm hardness, drying temperature and microbial enzyme supplementation on the performance of broiler chickens. *Animal Production Science*, *54*, 956–965. https://doi.org/10.1071/AN13113

Macome, F., Pellikaan, W., Hendriks, W., Dijkstra, J., Hatew, B., Schonewille, J., & Cone, J. (2017). In vitro gas and methane production of silages from whole-plant corn harvested at 4 different stages of maturity and a comparison with in vivo methane production. *Journal of Dairy Science*, *100*, 1–11. https://doi.org/10.3168/jds.2017-12953

Maxin, G., Andueza, D., Le Morvan, A., & Baumont, R. (2017). Effect of intercropping vetch (*Vicia sativa* L.), field pea (*Pisum sativum* L.) and triticale (X Triticosecale) on dry-matter yield, nutritive and ensiling characteristics when harvested at two growth stages. *Grass and Forage Science*, *72*, 1–8. https://doi.org/10.1111/gfs.12277

Mccarthy, M. M., Engstrom, M. A., Azem, E., & Gressley, T. F. (2013). The effect of an exogenous amylase on performance and total-tract digestibility in lactating dairy cows fed a high-byproduct diet. *Journal of Dairy Science*, *96*, 3075–3084. https://doi.org/10.3168/jds.2012-6045

McDonald, P., Henderson, A. R., & Heron, S. J. E. (1991). *The biochemistry of silage* (2nd ed.). Marlow, UK: Chalcombe Publications.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *The Journal of Agricultural Science*, *93*, 217–222. https://doi.org/10.1017/S0021859600086305

Morgado, E. D. S., Ezequiel, J. M. B., Júnior, A. C. H., & Galzerano, L. (2013). Potencial de produção de gás metano e dióxido de carbono in vitro dos ingredientes utilizados em dietas para ovinos. *Ciencia Animal Brasileira*, *14*, 413–417. https://doi.org/10.5216/cab.v14i4.18096

Muck, R. E. (2010). Silage microbiology and its control through additives. *Revista Brasileira de Zootecnia*, *39*, 183–191. https://doi.org/10.1590/S1516-35982010001300021

Nozière, P., Steinberg, W., Silberberg, M., & Morgavi, D. P. (2014). Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. *Journal of Dairy Science*, *97*, 2319–2328. https://doi.org/10.3168/jds.2013-7095

Peyrat, J., Baumont, R., Le Morvan, A., & Nozière, P. (2016). Effect of maturity and hybrid on ruminal and intestinal digestion of corn silage in dry cows. *Journal of Dairy Science*, *99*, 258–268. https://doi.org/10.3168/jds.2015-9466

Pôssas, F. P., Gonçalves, L. C., Pereira, L., Machado, F. S., Jayme, D. G., Rodrigues, J., … Tomich, T. R. (2015). Cinética de fermentação ruminal das silagens de três híbridos de milho comerciais em diferentes estádios de maturação. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, *67*, 159–165. https://doi.org/10.1590/1678-7066

Ramos, B. M. O., Champion, M., Poncet, C., Mizubuti, I. Y., & Nozière, P. (2009). Effects of vitreousness and particle size of maize grain on

ruminal and intestinal in sacco degradation of dry matter, starch and nitrogen. *Animal Feed Science and Technology*, *148*, 253–266. https://doi.org/10.1016/j.anifeedsci.2008.04.005

Reynolds, C. K., Cammell, S. B., Humphries, D. J., Beever, D. E., Sutton, J. D., & Newbold, J. R. (2001). Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. *Journal of Dairy Science*, *84*, 2250–2259. https://doi.org/10.3168/jds.S0022-0302(01)74672-3

Russell, J. B. (1985). Fermentation of cellodextrins by cellulolytic and non-cellulolytic rumen bacteria. *Applied and Environmental Microbiology*, *49*, 572–576.

Sauvant, D., & Van Milgen, J. (1995). Dynamic aspects of carbohydrate and protein breakdown and the associated microbial matter synthesis. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction; Proceedings of the 8th International Symposium on Ruminant Physiology* (pp. 71–91). Stuttgart, Germany.

Schofield, P., Pitt, R. E., & Pell, A. N. (1994). Kinetics of fiber digestion from *in vitro* gas production. *Journal of Animal Science*, *72*, 2980–2991. https://doi.org/10.2527/1994.72112980x

Siegfried, R., Rückemann, H., & Stumpf, G. (1984). Method for determination of organic acids in silage by high performance liquid chromatography. *Landwirtschaftliche Forschung*, *37*, 298–304.

Silva, V. P., Pereira, O. G., Leandro, E. S., Da Silva, T. C., Ribeiro, K. G., Mantovani, H. C., & Santos, S. A. (2016). Effects of lactic acid bacteria with bacteriocinogenic potential on the fermentation profile and chemical composition of alfalfa silage in tropical conditions. *Journal of Dairy Science*, *99*, 1895–1902. https://doi.org/10.3168/jds.2015-9792

Tricarico, J. M., Johnston, J. D., & Dawson, K. A. (2008). Dietary supplementation of ruminant diets with an Aspergillus oryzae α-amylase. *Animal Feed Science and Technology*, *145*, 136–150. https://doi.org/10.1016/j.anifeedsci.2007.04.017

Vargas-Rodriguez, C. F., Engstrom, M., Azem, E., & Bradford, B. J. (2014). Effects of dietary amylase and sucrose on productivity of cows fed low-starch diets. *Journal of Dairy Science*, *97*, 4464–4470. https://doi.org/10.3168/jds.2013-7845

Wiersma, D. W., Carter, P. R., Albrecht, K. A., & Coors, J. G. (1993). Kernel milkline stage and corn forage yield, quality, and dry matter content. *Journal of Production Agriculture*, *6*, 94. https://doi.org/10.2134/jpa1993.0094

Zhao, F. (2014). Biology of glucose transport in the mammary gland. *Journal of Mammary Gland Biology and Neoplasia*, *19*, 3–17. https://doi.org/10.1007/s10911-013-9310-8

Zilio, E. M. C., Del Valle, T. A., Ghizzi, L. G., Takiya, C. S., Dias, M. S. S., Nunes, A. T., … Rennó, F. P. (2019). Effects of exogenous fibrolytic and amylolytic enzymes on ruminal fermentation and performance of mid-lactation dairy cows. *Journal of Dairy Science*, *102*, 1–11. https://doi.org/10.3168/jds.2018-14949

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