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Ruminants

Exogenous enzymes improve performance and carcass traits of feedlot cattle fed high-grain diet

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ABSTRACT - The objective of this study was to evaluate the effects of supplementation of two different enzymatic complexes, fibrolytic (NSPases) or amylolytic (EXP3066) enzymes, on growth performance, digestibility, behavior, and carcass characteristics of feedlot cattle. Thirty-six ½ Angus yearling bulls with an average initial weight of 391±5.0 kg were used in this experiment blocked by weight in a completely randomized design divided in three treatments: control, NSPases, and EXP3066. The basal diet was composed of 85% whole corn grain and 15% nucleus. NSPases increased average daily gain on days 63 and 84, and gain:feed ratio on days 42, 63, and 84. Total carcass weight and carcass daily gain were improved by 4.8% and up to 6.0% with EXP3066 and NSPases supplementations, respectively. The group that received NSPases supplementation showed even greater carcass feed efficiency when compared with animals in the control group. There was an increase in apparent dry matter digestibility and a decrease of fecal whole grain residual percentage with enzyme supplementation. Enzyme supplementation affected hot carcass weight and EXP3066 provided greater values for ribeye area and marbling compared with control. Exogenous enzymes improve performance and carcass traits in feedlot cattle fed high-grain diet.

Key Words: amylase, corn, high-energy diet, non-starch polysaccharides, xylanase

Introduction

Energy-dense diets (high grain diets) for cattle, although in Brazil is not a common practice (Millen et al., 2009), is becoming more present in feedlot due to the improvements observed on performance, carcass characteristics, and the convenience for feedlot operations (Neumann et al., 2015).

However, Brazilian corn has a predominance of vitreous endosperm, being a corn of low degradability in the ruminal environment and with lower total starch digestibility as shown by Correa et al. (2002), and this could decrease performance. Therefore, tools that optimize corn starch use are necessary.

In this context, exogenous enzymes may be an important tool to increase the digestibility of corn grain and improve feed efficiency of feedlot cattle. Numerous studies, however, have shown inconsistent results regarding the use of enzymes on animal performance (Beauchemin et al., 1995, 1999; Hristov et al., 2000; DiLorenzo et al., 2011; Oliveira et al., 2015). The lack of benefits when supplementing exogenous enzymes as well as the inconsistency of responses can mainly be attributed to differences in activity and characteristics of supplemented enzymes in each study. Moreover, physical and chemical properties of the substrates may also play a role (Meale et al., 2014; Tadele and Animut, 2015).

Therefore, the objective of this study was to evaluate the effects of the supplementation of two different enzymatic complexes: one composed of fibrolytic enzymes with action on non-starch polysaccharide (NSPases) and an extract with a predominant activity of amylase (EXP3066) on growth performance, digestibility, behavior, and carcass characteristics of feedlot yearling bulls fed an energy-dense diet.

Material and Methods

The experiment was conducted in Guarapuava city, Paraná, Brazil (25º23'02" S, 51º29'43" W, and 1100 m altitude) from February to May, 2015.

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All experimental procedures were submitted and approved (case no. 001/2015) by the Animal Care and Use Committee (CEUA).

Thirty-six ½ Angus bulls sourced from a single herd, with initial body weight (BW) of 391±5 kg and averaging 12 months of age, were used in this experiment. The animals were housed in 18 semi-covered confinement pens (15 m^2), with concrete feeders, and float-regulated waterers.

Bulls were dewormed and allotted according to BW and body condition in a completely randomized design consisting of three treatments with six replicates. Each replicate consisted of a pen with two animals. Experimental treatments were grouped as follows: control – diet without the use of enzymes; EXP3066 – control diet supplemented with 10 g of EXP3066 extract per animal per day; and NSPases – control diet supplemented with 10 g of NSPases complex per animal per day.

The enzymatic extract EXP3066 was obtained by the fermentation of the fungi *Aspergillus oryzae* and *Trichoderma viride* and the bacteria *Bacillus subtilis* and contained a predominant activity of amylase (Table 1). The NSPases complex was obtained by the fermentation of the fungi *Aspergillus niger* and *Trichoderma reesei* with predominant activity of xylanase (JBS-United; Sheridan, IN, EUA). The dose used followed manufacture indication.

Activities of xylanase, cellulase, β-glucanase, mannanase, α-galactosidase, and amylase from the products were determined by the 3,5-dinitrosalicylic acid method and expressed in international units per grams (IU/g), in which 1 IU of activity corresponds to the amount of enzyme hydrolyzing 1 mmoL of the glycosidic linkages of the substrate per minute.

The experiment consisted of a 10-day adaptation period to the diets and housing, followed by an 84-day experimental period. On the first three days of the adaptation period, animals received 1.2% of BW of concentrated mix (85% of whole corn grain plus 15% of the protein-vitamin-mineral premix) and corn silage *ad libitum*. From day 4 to 7, animals received 1.6% of BW of the concentrated mix and corn silage *ad libitum*. On day 8, the corn silage supply was interrupted, and the animals received only the concentrated mix *ad libitum* with daily adjustments.

The control diet was composed of 85% whole corn grain and 15% of protein-vitamin-mineral premix, formulated to sustain a 1.5 kg/day average daily gain (ADG), according to NRC (2000). The products containing the exogenous enzymes (EXP3066 and NSPases) were used in a topdressed form in the diet at feeding time to ensure accuracy and intake of the prescribed dosages.

The protein-vitamin-mineral premix was prepared in the commercial feed mill Cooperativa Agraria (Guarapuava, Paraná, Brazil), formulated with a soybean meal base along with wheat meal, malt byproduct, limestone, calcium phosphate, urea, salt, mineral and vitamin premix, monensin, and virginiamycin and was presented in pelleted form.

Bulls were fed twice daily (06:00 and 17:00 h), and intakes were recorded daily by subtracting the difference between the amount offered and orts from the previous day. Feed was adjusted daily and offered *ad libitum* by ensuring a 10% refusal every day on a dry matter (DM) basis.

Diet samples from each treatment were collected during the experimental period (Table 2). Composite feed samples were dried in a forced-air oven at 55 °C for 72 h and sequentially ground in a Wiley mill using a 1-mm diameter sieve. Samples were analyzed for DM, inorganic matter, crude protein (CP), and fat following AOAC (1995)

Table 2 - Ingredient and chemical composition of the control diet

Ingredient	%
Whole corn grain	85
Protein-vitamin-mineral premix[1, 2]	15
Analyzed composition	% DM
DM (%)	90.06
CP	13.04
Ashes	3.090
Fat	3.300
ADF	6.880
NDF	18.25
Starch	57.74
TDN	81.59

DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; TDN - total digestible nutrients.
[1]Premix composition: DM, 90.22%; CP, 42.23%; NDF, 24.61%; ADF, 12.28%; inorganic matter, 16.31%; fat, 2.95; TDN, 69.70%; phosphorus, 1.11%; calcium, 2.77%; monensin, 75 mg/kg; and virginiamycin, 75 mg/kg.
[2]Guaranteed levels per kg of premix: vitamin A, 42000 IU; vitamin D3, 5400 IU; vitamin E, 225 IU; biotin, 9 mg; sulfur, 2.1 g; magnesium, 0.36 g; sodium, 4.68 g; cobalt, 3 mg; copper, 54 mg; chrome, 0.9 mg; fluorine, 23.4 mg; iodine, 3.3 mg; manganese, 87 mg; selenium, 1.05 mg; and zinc, 216 mg.

Table 1 - Enzymatic activity of EXP3066 extract and NSPases complex

Complex	Enzymatic activity (IU/g)[1]					
	Xylanase	Cellulase	β-glucanase	β-mannanase	α-galactosidase	Amylase
EXP3066	9	-	-	-	-	107
NSPases	3117	2870	2210	372	11	21

[1]Xylanase: tested in pH 4.5 at 40 ºC; Cellulase: tested in pH 4.8 at 50 ºC; β-glucanase, β-mannanase, and Amylase: tested in pH 5.0 at 40 ºC; α-galactosidase: tested in pH 5.5 at 37 ºC.

procedures. The neutral detergent (NDF) and acid detergent (ADF) fiber fractions were analyzed using thermo-stable α-amylase according to Van Soest et al. (1991) and Goering and Van Soest (1970), respectively. The estimated total digestible nutrients (TDN) were obtained according to Weiss et al. (1992).

Starch analysis was performed according to the methodology described by Hendrix (1993). This method is based on the hydrolysis of starch contained in the sample, after extraction of soluble carbohydrates with successive 80% alcohol washes and colorimetric analysis of reducing sugars (glucose), with subsequent conversion of the results for starch.

Animals were weighed at trial initiation, days 21, 43, and 64, and at the end of the experiment, after a 12-h fasting period, to determine ADG. Amount of feed offered and orts from the previous day were weighed daily to determine dry matter intake (DMI) and gain to feed ratio (G:F).

Total carcass weight gain (CWG), carcass daily gain (CDG), and carcass feed efficiency (CG:F) were calculated using ADG, DMI, and hot carcass weight (HCW). Carcass total weight gain was calculated as the difference between HCW and initial carcass weight (iCW), which was estimated considering an initial carcass yield of 50% (iCW = initial BW*0.50). Carcass daily gain was calculated for the 89 days of confinement (CDG = CWG ÷ 89). Carcass feed efficiency was represented by the ratio between CDG and DMI (CG:F = DMI ÷ CDG).

A behavioral analysis was performed for 48 continuous hours on day 42 of the experiment. Observations were performed by nine individuals per shift in a 6-h rotation cycle. Readings were collected every 3 min. Behavioral data included time spent eating (hours per day and number of times per day), drinking water (hours per day and number of times per day), ruminating (hours per day), and resting (hours per day), following adapted methodology from Johnson and Combs (1991). The events of xylophagy (behavior that the animal tries to consume wood to stimulate salivation in response to a low ruminal pH), and solid and liquid excretions per day were also evaluated.

Between 42 and 44 days of the experimental period, over two consecutive days, total fecal production was collected from the ground of the experimental units to calculate apparent total tract DM digestibility (DMD). Total fecal matter was weighed and sampled each six hours and stored in a freezer at −18 ºC until further analysis. A sample from the composite feed and orts from the previous day were also collected.

The DM, CP, and starch concentration in the composite feed, orts, and feces from each experimental unit were determined using the same procedures from the feed analysis. Apparent DMD was calculated using the following equation: DMD (%) = [(DM consumed – DM excreted) ÷ DM consumed] × 100.

The same formula was used to calculate the CP and starch digestibility, multiplying the consumed feed, orts, and fecal matter by the specific proportions of each variable as obtained from the laboratory.

A 300-g feces sub-sample of each experimental unit was collected at the end of each 6-h shift, washed through a 5-mm sieve to capture whole corn kernels from the stool in the sieve. Following washing, whole corn kernels were counted to determine the number of excreted whole grains (NEWG). Afterwards, the material was dried in a forced-air oven at 55 °C for 72 h and subsequently weighed to obtain the weight of fecal whole grain. Additionally, whole corn kernels in ten 100-g sub-samples of composite feed were counted to determine the number of consumed whole grain (NCWG). The fecal whole grain residual percentage (RWG) was calculated from the NEWG and NCWG data using the following equation: RWG (%) = 100 − 100*[(NCWG − NEWG) ÷ NCWG].

At the end of the experimental period, ribeye area (REA) and fat thickness were evaluated using an ultrasound machine (Aloka SSD-500 Vet) with a 17 cm, 3.5 MHz probe. Measurements were made between the 12th and 13th rib transversally to the *longissimus dorsi* according to Herring et al. (1994). For REA measurements, a ratio was calculated as the relationship between the height and width of the ribeye. Ultrasound images were interpreted by a laboratory responsible for data quality (Designer Genes Technology) through the "BIA/DGT Brazil" software. Marbling was evaluated according to the existence of fat deposits in the *longissimus dorsi* and scored in a 1 (non-existent) to 5 (excessive) scale, adapted from Müller (1987).

After a 12-h fasting period, all animals were weighed to obtain the final BW and transported to a commercial meat plant located 5 km away. Slaughter was performed according to plant protocols and state laws. All carcasses were evaluated individually according to Müller (1987) to determine HCW, carcass yield, carcass length, arm length and circumference, top round diameter, and fat thickness (*longissimus dorsi*, round, rib cage, and chuck).

Performance, digestibility, behavior, and carcass characteristics data were subjected to analysis of variance (ANOVA) using the PROC GLM of SAS (Statistical Analysis System, version 6.09), and means were compared with Tukey adjustment for $P<0.05$.

The following statistical model was used:

$$Y_i = \mu + T_i + E_i,$$

in which Y_i = response variable; μ = overall average of all observations; T_i = treatment effect (i = 1 to 3); and E_i = residual random error.

For performance and digestibility variables, each pen (containing two animals) represented one experimental unit. For the remaining variables, one experimental unit was represented by each animal individually.

Results

The supplementation with exogenous enzymes did not affect (P≥0.98) DMI (Table 3). However, the addition of the complex NSPases to the diet improved the ADG on days 63 (P = 0.01) and 84 (P = 0.04), and the G:F on days 42, 63, and 84 (P≤0.07). Considering the total period of the experiment, there was an improvement of 11.4% (1.60 vs. 1.44 kg day^{-1}) for ADG and 11.7% (0.200 vs. 0.179 g kg^{-1}) for G:F with the supplementation of the complex NSPases, when compared with the control diet. The variables CWG and CDG were improved (P = 0.05) by 4.8% and up to 6.0% with the supplementation of EXP3066 and NSPases, respectively. The group NSPases supplementation showed even greater (P = 0.07) CG:F values (0.144 g kg^{-1}) when compared with control animals (0.136 g kg^{-1}).

Table 3 - Performance of feedlot finishing yearling bulls fed an energy-dense diet supplemented with exogenous enzymes

Item	Diet[1]			SEM	P-value
	Control	EXP3066	NSPases		
ADG (kg)					
Days 0-21	1.70	1.69	1.73	0.02	0.84
Days 0-42	1.51	1.54	1.67	0.02	0.15
Days 0-63	1.51b	1.60ab	1.73a	0.02	0.01
Days 0-84	1.44b	1.51b	1.60a	0.02	0.04
DMI (kg/day)					
Days 0-21	8.08	8.00	7.99	0.87	0.98
Days 0-42	7.96	7.91	7.88	0.73	0.99
Days 0-63	7.92	7.97	7.94	0.67	0.99
Days 0-84	8.03	8.09	8.01	0.73	0.98
G:F (g/kg)					
Days 0-21	0.211	0.211	0.217	0.05	0.27
Days 0-42	0.189b	0.195b	0.212a	0.01	0.06
Days 0-63	0.191b	0.200ab	0.218a	0.01	0.05
Days 0-84	0.180b	0.187ab	0.201a	0.02	0.07
CWG (kg)	94.90b	99.50a	100.6a	0.92	0.05
CDG (kg/day)	1.091b	1.143a	1.156a	0.02	0.05
CG:F (g/kg)	0.136b	0.141ab	0.144a	0.01	0.07

ADG - average daily gain; DMI - dry matter intake; G:F - gain to feed ratio (feed efficiency); CWG - carcass total weight gain; CDG - carcass daily gain; CG:F - carcass feed efficiency; SEM - standard error of the mean.
[1] Diets = control: diet without the use of enzymes; EXP3066: enzymatic extract (*Aspergillus oryzae* × *Trichoderma viride* × *Bacillus subtilis*) with a predominant activity of amylase; NSPases: enzymatic complex (Enspira®; JBS-United; Sheridan, IN, EUA) composed of enzymes with action on non-starch polysaccharides.
a,b - Means followed by different letters in the same row are significantly different by Tukey test (P≤0.07).

There was no effect (P≥0.33) of the use of enzymes on apparent starch and CP digestibility (Table 4). However, the supplementation with EXP3066 and NSPases promoted greater (P = 0.05) values (87.52 and 87.31%, respectively) of apparent DM digestibility, compared with the control diet (84.15%). There was also an effect (P≤0.02) of the supplementation on the presence of fecal whole corn (number kg^{-1} and g kg^{-1} of fecal matter), in which the lowest values were found in animals receiving EXP3066. The supplementation with enzymes reduced (P<0.01) the fecal whole grain residual percentage, with lower values observed for EXP3066 supplementation (2.25%) when compared with NSPases (2.72%), both lower than the percentage observed in control group (4.11%).

There was no effect (P≥0.20) of the inclusion of exogenous enzymes in the diet on time spent drinking water, resting, or number of solid and liquid excretions. However, animals fed diet supplemented with NSPases complex spent less (P = 0.02) time eating (1.83 vs. 2.60 h day^{-1}) compared with non-supplemented animals, and more (P = 0.04) time ruminating (1.92 vs. 0.94 h day^{-1}), compared with animals fed diet supplemented with EXP3066 (Table 5). Animals from EXP3066 group showed lower (P = 0.04) xylophagy events (5.7 times day^{-1}) compared with animals from control and NSPases groups.

Regarding carcass characteristics, there was an effect (P = 0.04) of both enzymatic treatments on HCW, in which the greatest values (294.7 and 296.0 kg for EXP3066 and NSPases, respectively) were observed in animals fed diet supplemented with exogenous enzymes (Table 6). The supplementation with EXP3066 also increased REA

Table 4 - Apparent dry matter, crude protein, and starch digestibilities and analysis of fecal whole grain of feedlot finishing yearling bulls fed an energy-dense diet supplemented with enzymes

Item	Diet[1]			SEM	P-value
	Control	EXP3066	NSPases		
Digestibility (%)					
Dry matter	84.15b	87.52a	87.31a	0.41	0.05
Crude protein	78.10	81.44	81.05	0.67	0.33
Starch	92.38	93.35	92.54	0.34	0.69
Fecal whole grain					
n/kg of fecal matter	136.7a	78.9b	98.9ab	5.38	0.02
g/kg of fecal matter	54.51a	28.78b	36.47ab	1.99	0.01
Fecal whole grain residual percentage (%)	4.11a	2.25c	2.72b	0.21	<0.01

SEM - standard error of the mean.
[1] Diets = control: diet without the use of enzymes; EXP3066: enzymatic extract (*Aspergillus oryzae* × *Trichoderma viride* × *Bacillus subtilis*) with a predominant activity of amylase; NSPases: enzymatic complex (Enspira®; JBS-United; Sheridan, IN, EUA) composed of enzymes with action on non-starch polysaccharides.
a,b - Means followed by different letters in the same row are significantly different by Tukey test (P≤0.05).

Table 5 - Behavioral evaluation of feedlot finishing yearling bulls fed an energy-dense diet and supplemented with enzymes

Item	Diet[1]			SEM	P-value
	Control	EXP3066	NSPases		
	h/day				
Eating	2.60a	2.12ab	1.83b	0.10	0.02
Drinking water	0.30	0.43	0.42	0.04	0.44
Ruminating	1.14ab	0.94b	1.92a	0.14	0.04
Resting	19.97	20.54	19.81	0.17	0.24
	Number of times/day				
Eating	20.50	21.20	16.50	1.06	0.20
Drinking water	10.70	8.50	8.60	0.77	0.43
Xylophagy	8.20a	5.70b	7.50a	0.52	0.04
Liquid excretion	4.70	4.60	3.50	0.54	0.63
Solid excretion	4.60	5.20	4.10	0.24	0.20

SEM - standard error of the mean.
[1] Diets = control: diet without the use of enzymes; EXP3066: enzymatic extract (*Aspergillus oryzae* × *Trichoderma viride* × *Bacillus subtilis*) with a predominant activity of amylase; NSPases: enzymatic complex (Enspira®; JBS-United; Sheridan, IN, EUA) composed of enzymes with action on non-starch polysaccharides.
a,b - Means followed by different letters in the same row are significantly different by Tukey test (P≤0.04).

Table 6 - Carcass characteristics of feedlot finishing yearling bulls fed an energy-dense diet and supplemented with enzymes

Item	Diet			SEM	P-value
	Control	EXP3066	NSPases		
Hot carcass weight (kg)	290.4b	294.7a	296.0a	2.83	0.04
Carcass yield (%)	56.84	56.77	56.72	0.35	0.99
Carcass length (cm)	132.9	133.8	134.0	0.50	0.81
Top round diameter (cm)	21.58	20.83	21.04	0.15	0.38
Arm length (cm)	39.42	41.67	39.92	0.32	0.16
Arm circumference (cm)	41.08	43.08	43.42	0.44	0.30
Fat thickness (mm)					
Longissimus dorsi	4.500	4.330	4.250	0.08	0.65
Round	5.170	4.830	4.920	0.11	0.67
Rib cage	4.420	4.330	4.750	0.15	0.69
Chuck	3.670	3.500	3.330	0.11	0.68
Top Sirloin	7.890	7.709	8.262	0.33	0.78
Ribeye area (cm^2)	84.76b	92.43a	86.43ab	1.21	0.03
Ratio	0.485	0.477	0.492	0.01	0.28
Marbling	2.555b	3.016a	2.740ab	0.08	0.07

SEM - standard error of the mean.
[1] Diets = control: diet without the use of enzymes; EXP3066: enzymatic extract (*Aspergillus oryzae* × *Trichoderma viride* × *Bacillus subtilis*) with a predominant activity of amylase; NSPases: enzymatic complex (Enspira®; JBS-United; Sheridan, IN, EUA) composed of enzymes with action on non-starch polysaccharides.
a,b - Means followed by different letters in the same row are significantly different by Tukey test (P≤0.07).

(P = 0.03) and marbling (P = 0.07) when compared with the control diet. However, no differences were found between EXP3066 and NSPases supplementations.

Discussion

The use of exogenous enzymes in ruminant nutrition has been identified as a possible strategy to increase digestibility and improve feed utilization efficiency (Krueger et al., 2008; Tang et al., 2008; Meale et al., 2014). However, the effect of the supplementation with enzymes on animal performance has been inconsistent.

Beauchemin et al. (1999) reported a 7% improvement in G:F and a 9% improvement in ADG in feedlot steers fed diets supplemented with enzymes. McAllister et al. (1999), in a study with high-energy diets, also reported an improvement in performance (increased ADG by 9%) in animals that received diets supplemented with enzymes (cellulases and hemicellulases). Several studies, however, showed no effect of enzyme supplementation on animal performance (ZoBell et al, 2000; Eun et al, 2009; DiLorenzo et al., 2011; Oliveira et al., 2015).

The inconsistency of results can be attributed, in part, to the differences in the activity and characteristics of the supplemental enzymes employed in each study, as well as the physical and chemical properties of the substrates, since each of the enzymes has its own individual substrate specificity (Beauchemin et al., 2004; Salem et al., 2012; Tadele and Animut, 2015).

Pursuant to this fact, Beauchemin et al. (1995) reported that mixtures of fibrolytic enzymes (xylanase and cellulase) added to alfalfa hay-based diets increased the ADG of steers, but no effect was found when the mixtures were added to barley silage. When evaluating the same enzymatic formulation of Beauchemin et al. (1995) in high-energy diets, Beauchemin et al. (1997) observed an improvement of 11% in the G:F of animals when the enzymes (high- and low-xylanase activity included in manufacturing at a rate of 4.0 L t^{-1} of concentrate DM) were added to a barley grain-based diet; however, the performance of the animals was not affected when the mixture of enzymes was added to corn-based diet.

In the present study, there was a positive effect of enzyme supplementation on animal performance. In general, the performance improvement (average of 8.8%) of the animals fed diet supplemented with NSPases complex was more pronounced compared with those fed diet supplemented with EXP3066 extract (average of 4.8%), and only the NSPases complex was efficacious for improving the ADG, G:F, and CG:F of these animals. These results are possibly related to the greater quantity of substrates in the diet available for NSPases activity, as well as the higher enzymatic activity (IU/g) of the product itself. As Meale et al. (2014) reported, the amount of enzymes and substrates as well as the interaction between the two are important in improving feed utilization.

Several enzymes (xylanase, cellulase, β-glucanase, β-mannanase, and α-galactosidase) with activity in NSP were found in the enzymatic complex NSPases composition,

while in EXP3066 extract composition, only xylanase (9 IU/g) and amylase (107 IU/g) were found. According to Krause et al. (2003), Beauchemin et al. (2003), and Elghandour et al. (2015), the diversity of enzymes is advantageous as it facilitates the segmentation of several feed substrates. Collins et al. (2006) and Barletta (2010) reaffirmed that the enzymes cellulase, xylanase, and β-glucanase (present in NSPases complex composition) break the β-1,4 bond of cellulose, arabinoxylan, and β-glucan, respectively, which are the main NSP in corn grain.

According to Akin and Rigsby (2008) and Barletta (2010), NSP not only are less digestible, but they can also prevent the digestion of other carbohydrates (such as starch), proteins, and other nutrients, as these nutrients are encapsulated by NSP, thus preventing physical access of digestive enzymes. Therefore, the increased degradation of NSP present in the corn grain cellular wall can improve the utilization of other nutrients.

The starch and CP digestibilities were not affected by the use of enzymes in the present study; however, there was an improvement in apparent DM digestibility. Lower fecal whole grain residual percentage was also observed in animals fed the EXP3066 and NSPases supplementation. These results explain, in part, the observed improvement in the performance of the animals receiving supplementation with enzymes in the present study. Mendoza et al. (2013) also observed an improvement in DM digestibility in ruminants receiving a diet supplemented with enzymes. In their case, the addition of glucoamylases to a diet composed of a 45% corn and sorghum mix resulted in an increase of 4.13% in DM digestibility in lambs compared with animals fed the non-supplemented diet. Salem et al. (2012, 2013) also reported improvements in diet DM digestibility in ruminants (sheep and beef steers, respectively) receiving supplementation of a commercial mixture of exogenous enzymes (endoglucanase, xylanase, α- amylase, and protease activity). The same effect was observed in *in situ* (Krueger and Adesogan, 2008) and *in vitro* (Moharrery et al., 2009) studies. Nonetheless, the mechanism by which exogenous enzymes improve diet digestibility is still not fully understood. Several hypotheses, among them an increase in ruminal microbial colonization and adhesion to the surface of feedstuffs (Colombatto et al., 2003; Jalilvand et al., 2008), stimulation of ruminal microbial population and synergy with ruminal microbial enzymes (Morgavi et al., 2000; Tadele e Animut, 2015), and direct hydrolysis of substrates by the enzymes (Beauchemin et al., 2003; Moharrery et al., 2009), have been suggested.

Hristov et al. (2000) and McAllister et al. (2001) proposed a possible action of these enzymes in post-ruminal diet digestion. According to these authors, the exogenous enzymes can remain active in the lower gastrointestinal tract and, thus, could potentially contribute to post-ruminal digestion.

The NSPases complex contains enzymes originated from the fungi *Aspergillus niger* and *Trichoderma reesei*, which (especially those formed by the latter) possess an optimum pH range below 6.0 (Paloheimo et al., 2010) and, therefore, ensure a degree of activity in post-ruminal digestion. Furthermore, in energy-dense diets (as in the diet from the current study), it is expected that even in the rumen, the pH values are below typical levels that generally result in a decrease in the population of fibrolytic microorganisms (Brown et al., 2006; Fernando et al., 2010). Therefore, as the enzymes from the NSPases complex exhibit activity even at low pH, those populations were probably unaffected, which may partially justify the improved performance observed in animals fed diet supplemented with this enzymatic complex.

According to McAllister et al. (2001), exogenous enzymes can also decrease the viscosity of the duodenal contents, which contribute to a greater digestion and absorption of nutrients. Hristov et al. (2000) reported decreased digesta viscosity in addition to the 30% increase in xylanase activity in the intestine when exogenous enzymes were added to the diet.

Paloheimo et al. (2010) highlighted that the enzymes provided a probiotic effect in addition to the beneficial characteristics mentioned above. Its mechanism would be based on breaking down the cellular wall and the transfer of nutrients, favoring the development of amylolytic bacteria, such as the *Lactobacillus sp*., which, as a result, would generate an increase in starch digestibility. However, in the present study, this probably did not occur, since the starch digestibility was not improved.

Nozière et al. (2014), when evaluating the effect of the use of exogenous amylolytic enzymes on the metabolism of dairy cattle fed diets containing high and low starch content, observed that the ruminal starch degradation was greater in the cows fed supplemented diet (81 vs. 75%) compared with those of control. However, no effect in overall starch digestibility was observed, which may have also occurred in the present study.

According to Beauchemin et al. (2003), the increased protein digestibility with the use of enzymes in the diet may be attributed in part to the greater synthesis of microbial protein in the rumen. This however, was not observed in

the current study as the CP digestibility was not different between treatments.

Therefore, the improved DM digestibility observed with the use of the enzymes may be attributed to enzyme activity affecting other components of the diet, such as NDF. This hypothesis is consistent with several other studies (Beauchemin et al., 1995; Feng et al., 1996; Martins et al., 2006; Klingerman et al., 2009 and Gencoglu et al., 2010) in which the authors associated the increase in diet digestibility to the increase in NDF digestibility. It is possible that the same happened in the present study, since no alteration in starch and CP digestibility was observed.

Regarding the behavioral variables, animals fed diet supplemented with EXP3066 spent less time in rumination and presented a reduced number of xylophagy events. The amount of time (h/day) spent eating was also affected by the enzyme supplementation, whereas the animals fed diet supplemented with NSPases complex spent less time eating. Despite this difference, there was no alteration in DMI of these animals. Bowman et al. (2003) did not observe differences in eating behavior of dairy cows fed diet supplemented with enzymes in different ways of administration.

The use of exogenous enzymes in animal nutrition is known to increase the concentrations of volatile fatty acids (Beauchemin et al., 2003), which can influence fat synthesis and carcass characteristics of animals fed supplemented diets (Vargas et al., 2013). However, there are few studies evaluating the effect of the use of enzymes in carcass variables.

Diet supplementation with exogenous enzymes increased the HCW values of animals compared with the non-supplemented diet. This result is possibly related to the increase in diet digestibility, in which the greater use of nutrients was reflected in greater carcass weight. Parsons et al. (2007) also observed higher carcass weight in animals fed diet supplemented with enzymes.

The supplementation with EXP3066 resulted in increased REA and marbling compared with the control diet. Tricarico et al. (2007) observed a quadratic increase in REA when evaluating increasing values of α-amylase (0, 580, and 1160 DU/kg of DM) supplementation to feedlot cattle, which was attributed to the improvement in carcass weight gain of these animals.

There was no effect of the diet supplementation with exogenous enzymes on the other carcass variables evaluated. However, Vargas et al. (2013) found a quadratic increase in carcass yield when evaluating different concentrations (0, 2, 4, and 6 ppm) of an enzymatic complex (xylanases and cellulases) in the supplementation of confined cattle, with greater values from animals that received intermediate concentrations of enzymes.

According to Wallace et al. (2001) and Beauchemin et al. (2003), dosage is one of the main factors responsible for the ineffectiveness of enzymatic products. Too-low dosage levels are insufficient to produce an improvement in nutrient digestibility, whereas too-high dosage levels may compete with microorganism adhesion sites on the same substrate, thereby lowering its activity. According to these reports, together with the quadratic responses of different enzyme concentrations observed in the studies of Vargas et al. (2013) and Tricarico et al. (2007), future research should be considered to evaluate the effect of different concentrations of NSPases and EXP3066 supplementation.

Conclusions

Exogenous enzymes improve performance and carcass traits of feedlot cattle fed high-grain diet. However, these improvements are more relevant for animals fed NSPases complex.

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