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Xylanase - complex efficacy in high-energy diet for bulls finished in feedlot

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ABSTRACT. Enzymes can be an interesting additive in high energy diets for feedlot cattle. However, literature is inconsistent on this subject. Thus, this study was conducted to evaluate animal performance of feedlot bulls receiving high energy diet, composed of a mixture of 85% whole corn grain and 15% protein-mineral-vitamin nucleus, without or with xylanase included in the diets. Diets consisted of: CON - diet without enzymes (Control) and ENZ - diet with enzymes (5 g animal day⁻¹). Thirty-two bulls were used, with an average age of 11 ± 2 months, average initial weight of 365 ± 5 kg, and finished for 119 days in feedlot. The experimental design was completely randomized, consisting of two treatments and eight replications, where each replication was represented by a stall with two animals. ENZ increased the weight gain (1.69 vs. 1.33 kg day⁻¹) and improved the feed conversion (4.60 vs. 6.03 kg⁻¹) in the adaptation period of the animals. Animals receiving ENZ increased 1.65% of carcass yield and were 7.57% more efficient in the conversion of dry matter consumed into carcass gain in relation to CON. Carcass traits of feedlot-finished bulls were not altered by inclusion of enzymes. Xylanase-complex could increase efficiency in feedlot bulls.

Keywords: beef cattle, feed additive, ruminant nutrition.

Eficiência do complexo xilanase em dietas de alta energia para touros terminados em confinamento

RESUMO. As enzimas podem ser aditivos interessantes em dietas de alta energia para gado em confinamento. No entanto, a literatura é inconsistente para este assunto. Assim, este trabalho foi realizado para avaliar o desempenho de touros em confinamento com dietas de alta densidade energética, composto apenas de uma mistura de 85% de grãos de milho inteiros e 15% de núcleo proteico-vitâmico-mineral, com ou sem inclusão de enzimas à base de xilanase. Os tratamentos foram CON - dieta sem enzimas (controle) e ENZ - dieta com enzimas (5 g animal dia⁻¹). Foram utilizados 32 touros, com idade média de 11 ± 2, peso inicial médio de 365 kg ± 5 e terminados por 119 dias. O delineamento experimental foi inteiramente casualizado, consistindo em dois tratamentos e oito repetições, em que cada repetição foi representada por uma baia com dois animais. ENZ aumentou o ganho de peso (1,69 vs. 1,33 kg dia⁻¹) e melhorou a conversão alimentar (4,60 vs. 6,03 kg⁻¹) na fase de adaptação dos animais em confinamento. Os animais que receberam ENZ aumentaram 1,65% do rendimento de carcaça e foram 7,57% mais eficientes na transformação da matéria seca consumida no ganho de carcaça em relação ao CON. As características de carcaça de touros terminados em confinamento não foram alteradas em função da inclusão de enzimas. O complexo de xilanase pode aumentar eficiência de touros de confinamento.

Palavras-chave: bovinos de corte, aditivo alimentar, nutrição de ruminantes.

Introduction

Energy-dense diets for cattle are diets with higher concentrate levels. Notably, these diets are characterized by roughage exclusion, where whole corn grain makes up 80-90% of animal feed. Although in Brazil it is not a common practice (Millen, Pacheco, Arrigoni, Galyean, & Vasconcelos, 2009), these diets are becoming more present in feedlot systems due to several improvements observed in performance, carcass traits, and

convenience of feedlot operations (Neumann, Leão, Horst, Figueira, & Ribas 2015; Monteschio et al., 2017; Rivaroli et al., 2016).

However, Brazilian corn has a predominance of vitreous endosperm, being a corn of low degradability in the ruminal environment and lower total starch digestibility as previously described by Correa, Shaver, Pereira, Lauer and Kohn (2002), which could decrease performance. Therefore, tools that optimize the use of corn starch are necessary.

Thereby, using exogenous enzymes may be an important tool to improve corn grain digestibility and feed efficiency by the animals. Nevertheless, results obtained in the literature are inconsistent regarding the enzymes use on performance of the animals in these dietary conditions (Beauchemin, Rode, & Sewalt, 1995; DiLorenzo et al., 2011; Hristov, McAllister, & Cheng, 2000; Oliveira et al., 2015). This inconsistency of results can be attributed in part to differences in activity and characteristics of the enzymes used in each study, as well as physical and chemical properties of the diet, since enzymes performance are related with substrate specificity (Meale, Beauchemin, Hristov, Chaves, & McAllister, 2014).

Identifying exogenous enzymes acting on substrates present in corn grain may be a strategy to increase feed efficiency, improving productive potential of animals. In this context, the objective of this study was to evaluate productive performance, animal behavior, and apparent digestibility of the diet and carcass traits of finished bulls fed xylanase complex in high energy density diet.

Material and methods

Experiment was conducted in Guarapuava, State of Paraná, Southern Brazil (25°23'02" S, 51°29'43" W, 1098 meters altitude) from December 2015 to March 2016. The climate of the Guarapuava is humid mesothermal subtropical (Cfb), without dry season, with fresh summers and moderate winter. Guarapuava presented an average annual of 12.7 and 23.5°C for minimal and maximal temperature, respectively, and average relative humidity 77.9% (Köppen & Geiger, 1928).

All experimental procedures were previously submitted and approved by the Ethics Committee on Animal Use (CEUA) (Protocol 001/2015).

Thirty-two Angus bulls were used with average initial body weight (BW) of 365 ± 7 kg and mean age of 11 ± 2 months. Animals belonged to the same herd and were housed in 16 feedlot stalls, semi-covered, with an area of 15 m², with a concrete feeder and drinking fountain controlled by a float.

Animals were previously dewormed and distributed in a completely randomized experimental design, composed of two treatments: CON - control diet without use of enzymes; and ENZ - diet with enzyme extracts (5g animal day⁻¹). Enzymatic complex used was an extract obtained from the fermentation of the fungi *Aspergillus niger* and *Trichoderma reesei* (Potenzia Grano®, JBS-United; Sheridan, IN, EUA), with predominant activity of xylanase (2,700 µg⁻¹), thiamine (B1: 2000

mg kg⁻¹) and pyridoxine (B6: 2000 mg kg⁻¹). Dose used followed manufacture indication.

Xylanase activity of the product was determined by 3,5-dinitrosalicylic acid (DNS) assay, and expressed in international units (U g⁻¹) wherein one U of activity corresponds to the amount of enzyme hydrolyzing 1 µ mole of glycosidic bonds of the substrate per minute.

Experiment lasted 105 days (d), preceded by 14-d adaptation of the bulls to the diet. During adaptation, experimental diet was fed gradually in proportion of BW (1.2% BW to 1-4d; 1.6% BW to 5-9d; and 2.0% BW to 10-14d). After adaptation, experimental diet was fed *ad libitum* with daily supply adjustments.

Experimental diet consisted of 85% whole grain corn and 15% protein-vitamin-mineral nucleus (Table 1), formulated in view of the requirements of 1.5 kg daily weight gain, according to National Research Council [NRC] (2000). Enzyme was added on the diet during feeding in a top-dressed form.

Protein-vitamin-mineral nucleus was prepared at Cooperativa Agrária (Guarapuava, Paraná State, Brazil), formulated based on soybean meal, wheat bran, malt radicle, calcitic limestone, dicalcium phosphate, salt, mineral vitamin premix, monensin and virginiamycin, presented in pelleted form.

Feed management was performed twice a day (6:00 a.m. and 4:00 p.m.) and dry matter intake (DMI) was recorded daily, by difference in weight between the amount offered and leftovers from the previous day. Supply adjustment was performed daily, aiming at *ad libitum* supply, considering leftovers of 10%, based on the dry matter (DM) of the diet.

Table 1. Proximate and chemical composition of the basal diet.

Feeds	% of the diet
Whole corn grain	85
Nucleus ^{1,2}	15
Chemical composition	% of DM
Dry matter	90.06*
Crude protein	13.04
Ash	3.09
Fat	3.30
Acid detergent fiber	6.88
Neutral detergent fiber	18.25
Starch	57.74
Total digestible nutrients	81.59

¹ Nucleus composition: DM: 90.22%; CP: 42.23%; NDF: 24.61%; ADF: 12.28%; MM: 16.31%; EE: 2.95; TDN: 69.70; P: 1.11%; Ca: 2.77%; monensin: 75 mg kg⁻¹; and virginiamycin: 75 mg kg⁻¹. ² Premix guarantee level per kg of nucleus: vit. A: 42000 IU; vit. D3: 5400 IU; vit. E: 225 IU; biotin: 9 mg; S: 2.1 g; Mg: 0.36 g; Na: 4.68 g; Co: 3 mg; Cu: 54 mg; Cr: 0.9 mg; F: 23.4 mg; I: 3.3 mg; Mn: 87 mg; Se: 1.05 mg; and Zn: 216 mg. * Percentage of natural matter.

During feedlot period, samples were taken from the diet to determine chemical composition. Samples were dried at 55°C for 72 hours, and

sequentially ground in a Wiley mill with a 1 mm sieve. Analysis of DM, crude protein (CP), ash and fat were determined according to Association Official Analytical Chemist [AOAC] (2005). Neutral detergent fiber (NDF) content was obtained according to Van Soest, Robertson and Lewis (1991) with thermostet γ -amylase and acid detergent fiber (ADF), according to Goering and Van Soest (1970). Total digestible nutrient (TDN) coefficient was calculated according to Weiss (1993). After the extraction of soluble carbohydrates with successive washes with 80% alcohol and colorimetric analysis of reducing sugars (glucose), starch analysis was performed according to a methodology described by Hendrix (1993) based on the starch hydrolysis contained in the sample.

Bulls were weighed at the adaptation phase, on days 21, 42, 63, 84 and at experiment end to determine average daily gain (ADG). DMI was expressed in kg of animal day⁻¹ or expressed as percentage of BW. Feed conversion was determined by the ratio between DMI and ADG (DMI ADG⁻¹). Total carcass gains (TCG), average carcass gains (ACG), carcass per dry matter intake (CDMI) and carcass transformation efficiency gain (CTE) were calculated from ADG, DMI, and hot carcass weight (HCW) data. TCG was calculated by the difference between the HCW and initial carcass weight (ICW), which was estimated considering initial carcass yield of 50% (ICW = initial BW x 0.50). CG was calculated based on feedlot period (CG = TCG ÷ feedlot period). CDMI was calculated by the ratio between DMI and HCW (CDMI = DMI ÷ HCW). CTE was represented by the relationship between ACG and ADG (CTE = ACG ÷ ADG).

Behavioral analysis was performed in a continuous time of 48 hours, during 65 to 68 d of feedlot period. Observations were performed by six observers per shift, in a rod system every 6 hours. Observations were taken at regular intervals of 3 minutes. Ingestive behavior evaluated were represented by activities of leisure, rumination, water and feed intake, expressed in day⁻¹ hours. Frequency of occurrence, expressed as number of times day⁻¹, of eating, drinking, solid excretion, liquid excretion and xylophagy activities were also observed, following the same methodology. At night, the environment was maintained with artificial lighting.

Total fecal collection of each experimental unit was performed during two consecutive days, to determine apparent dry matter digestibility (ADMD) of the diet. Feces samples were weighed and stored in a freezer at -18°C until analysis. Feed and leftovers were also collected. Feces were

determined using the same procedures adopted in feed analysis. Apparent dry matter digestibility (ADMD) was calculated by the following formula: ADMD (%) = [(DM ingested - DM excreted) ÷ DM ingested] x 100.

At the end of the feedlot period, animals were sent to a commercial slaughterhouse. After slaughter, some carcass traits were determined: carcass length, which is the distance between the medial cranial edge of the pubic bone and the medial cranial edge of the first rib; leg length, which is the distance between the medial cranial border of the pubic bone and the tibio-tarsal joint; and arm length, which is the distance between the tuberosity of the olecranon and the radio-carpal joint; arm perimeter, obtained in the median region of the arm encircling with a measuring tape; and the thickness of the cushion, measured by means of a compass, perpendicular to the carcass length, taking the longest distance between the cut that separates the two half-carcasses and the lateral thigh muscles, according to the methodologies described by Müller (1987).

The experimental design was completely randomized, composed of two treatments, with eight replications, where each replicate corresponded to a stall with two animals. Data collected for each variable were tested by an analysis of variance with a comparison of means at 5% of significance, through the statistical program SAS. Each variable analysis followed the statistical model: $Y_{ij} = \mu + S_i + E_{ij}$; Where: Y_{ij} = dependent variables; μ = overall mean of all observations; S_i = effect of enzyme of order 'i', being 1 = control diet and 2 = diet with enzyme; and E_{ij} = residual random effect.

Results and discussion

Analysis of variance indicated no interaction ($p > 0.05$) between diets and periods. ENZ group allowed higher ($p < 0.05$) ADG and better FC compared to CON (Table 2). These results suggest a better adaptation to energy-dense diets for ENZ group. However, during the other phases, ENZ did not show a favorable effect ($p > 0.05$) on ADG, DMI in kg day⁻¹, DMI %BW and FC. Performance results were in agreement with studies that have also demonstrated an absence of performance results of animals fed enzymes in feedlot (ZoBell, Wiedmeier, Olson, & Treacher, 2000; Eun, ZoBell, Dschaak, Diaz, & Tricarico, 2009; DiLorenzo et al., 2011; Oliveira et al., 2015), with the exception of adaptation period.

Meale et al. (2014) consider that to obtain results in performance using enzymes, the type and amount

of substrate, amount of enzymes, and enzyme-substrate relationship must be adequate. First of all, in these energy-dense diets, substrate for enzymatic action and enzyme-substrate relationship were not the limiting factors. In this context, the additive used had a relevant theoretical enzymatic activity, since cellulase, xylanase and β -glucanase break β -1,4 bonds of cellulose, arabinoxylan and β -glucans respectively, which are the main non-starch polysaccharides (NSP) of corn (Collins, Gerday, & Feller, 2005; Barletta, 2011; Bedford & Partridge, 2011).

NSPs are less digestible and also can prevent the digestion of other carbohydrates (such as starch), proteins and other feed nutrients, by encapsulating these nutrients, preventing physical access of digestive enzymes (Akin & Rigsby, 2008; Barletta, 2011). Theoretically, NSPs degradation presented in corn cell wall could improve the use of other nutrients, and increase performance, which have not occurred in this study.

Thus, the lack of performance results during the experimental period, possibly, are related to lower enzymatic concentration and may be a reflection of the level used. According to Beauchemin, Colombatto and Morgavi, Yang (2003); Wallace, Wallace, McKain, Nsereko and Hartnell (2001) the level is one of the main factors responsible for the inefficiency of the enzymatic products.

Table 2. Overall performance of bulls finished in feedlot with or without enzymes included in the diet.

Parameter	Experimental diet		Mean	SEM	p-value
	com	ENZ			
ADG, kg day ⁻¹					
Adaptation	1.327	1.688	1.507	34.286	0.0337
0 to 21 d	1.095	1.089	1.092	52.295	0.9725
0 to 42 d	1.194	1.116	1.155	52.188	0.6435
0 to 63 d	1.234	1.183	1.208	52.618	0.7541
0 to 84 d	1.263	1.246	1.254	45.333	0.9026
0 to 105 d	1.254	1.268	1.261	46.277	0.9251
DMI, kg day ⁻¹					
Adaptation	7.47	7.38	7.43	0.093	0.7629
0 to 21 d	6.95	6.89	6.92	0.167	0.9008
0 to 42 d	7.06	6.91	6.99	0.187	0.7875
0 to 63 d	7.14	7.05	7.09	0.186	0.8682
0 to 84 d	7.35	7.26	7.31	0.186	0.8738
0 to 105 d	7.50	7.42	7.46	0.195	0.8888
DM, % BW					
Adaptation	2.01	1.99	2.00	0.015	0.6694
0 to 21 d	1.78	1.74	1.76	0.035	0.7059
0 to 42 d	1.75	1.69	1.72	0.035	0.6226
0 to 63 d	1.71	1.67	1.69	0.032	0.7146
0 to 84 d	1.70	1.66	1.68	0.030	0.6934
0 to 105 d	1.69	1.64	1.67	0.030	0.6479
FC (DMI:ADG)					
Adaptation	6.03	4.60	5.31	0.172	0.0450
0 to 21 d	6.75	6.45	6.60	0.269	0.7197
0 to 42 d	6.22	6.47	6.35	0.275	0.7662
0 to 63 d	5.96	6.46	6.21	0.248	0.5142
0 to 84 d	5.90	6.08	5.99	0.160	0.7108
0 to 105 d	6.04	6.12	6.08	0.159	0.8778

SEM: Standard error of the mean.

On the other hand, ENZ improved ($p < 0.05$) efficiency in carcass gains with higher CTE and lower CDMI (Table 3). In turn, CG, TCG, and feces parameters were similar ($p > 0.05$) between ENZ and CON treatments (Table 3).

A justification could be a possible action of the enzymes in the post-ruminal digestion (Hristov et al., 2000; McAllister, Hristov, Beauchemin, Rode, & Cheng, 2011). According to these authors, exogenous enzymes can remain active in the lower digestive tract, contributing to the post-ruminal digestion of the feed. Enzymes from *Aspergillus niger* and *Trichoderma reesei*, which, especially those formed by *Trichoderma reesei*, have an optimum pH range below 6.0 (Paloheimo, Piironen, & Vehmaanoerra, 2011), which could generate some activity in post-ruminal digestion. Indeed, enzymes of the ENZ treatment can possibly remain active even at low ruminal pH (expected in high energy diets), which may justify this effect on the efficiency of the animals (Brown, Ponce, & Pulikanti, 2006; Fernando et al., 2010).

Table 3. Carcass gains and feces parameters of feedlot bulls with or without enzymes included in the diet.

Parameters	Experimental diet		Mean	SEM	P-value
	CON	ENZ			
Carcass gains					
ACG (kg day ⁻¹)	0.857	0.933	0.985	0.032	0.4083
CTE (%)	68.59	74.23	71.41	0.708	0.0451
TCG (kg)	102.0	111.0	106.5	3.474	0.4106
CDMI (kg of DM kg carcass ⁻¹)	8.84	8.17	8.50	0.201	0.0322
Feces parameters					
Feces production (kg day NM ⁻¹)	4.95	5.17	0.210	5.06	0.7104
Feces dry matter (%)	27.39	26.98	0.175	27.18	0.4515
Feces production (kg day DM ⁻¹)	1.34	1.26	0.004	1.30	0.6644
ADMD (%)	80.90	81.34	0.796	81.12	0.8572

ACG: Average carcass gains. CTE: Carcass transformation efficiency gain. TCG: Total carcass gains. CDMI: Carcass per dry matter intake. ADMD: Apparent dry matter digestibility. SEM: Standard error of the mean.

The mechanisms by which exogenous enzymes improve digestion of diets are not fully understood, suggesting some hypotheses such as increased colonization and microbial rumen fixation on the surface of the feed (Colombatto, Morgavi, Furtado, & Beauchemin, 2003; Jalilvand et al., 2008), ruminal microbial population stimulation and enzymatic synergism (Morgavi, Newbold, Beever, & Wallace, 2000) or the direct hydrolysis of the substrates by enzymes (Beauchemin et al., 2003; Moharrery, Hvelplund, & Weisbjerg, 2009).

Supplementation with exogenous enzymes promotes a greater permanence of microorganisms on food particles (Martins, Vieira, Berchielli, & Prado, 2008). In addition, studies developed by Martins, Vieira, Berchielli, Prado and Garcia (2006), showed an increase in enzymatic activity in liquid phase of ruminal content, and not in the solid

portion where there is a higher concentration of enzymes responsible for feed degradation. According to McAllister et al. (2011), exogenous enzymes may further decrease digest viscosity in duodenum, which contributes to greater digestion and absorption of nutrients. Therefore, it is not clear to state the real reason for enzyme's effect on diet digestion.

In relation to the ingestive behavior, ENZ, in general, did not show an effect ($p > 0.05$) for these variables (Table 4). However, an effect ($p < 0.05$) on solid excretion and xylophagy activity was observed, showing that ENZ group performed more solid excretions and xylophagy compared to CON.

Thereby, these results could be related to an increased availability of soluble carbohydrates and short chain acids production, which leads to a high buffering need. In this way, xylophagy could be a reflex behavior in an attempt to stimulate salivation and buffering. Moreover, these short chain accumulations resulted in high passage rate (Beauchemin, Colombatto, Morgavi, Yang, & Rode, 2004) and, consequently, increased solid excretion. Other results of the present study are in agreement with those found by Bowman, Beauchemin and Shelford (2003), who did not observe differences in the ingestive behavior of animals supplemented or not with enzymes.

Table 4. Ingestive behavior of feedlot-finishing bulls under the effect of enzymes included in the diet.

Parameter	Experimental diet		Mean	SEM	p-value
	CON	ENZ			
	Hours day ⁻¹				
Feed intake	2.06	1.88	1.97	0.062	0.3419
Water intake	0.19	0.18	0.18	0.011	0.9155
Rumination	1.08	1.17	1.13	0.059	0.8124
Leisure	20.71	20.76	20.73	0.155	0.9215
	Number of times day ⁻¹				
Feed intake	13.71	12.75	13.23	0.331	0.3549
Water intake	6.79	6.13	6.46	0.485	0.6508
Liquid excretion	4.00	4.50	4.25	0.159	0.4943
Solid excretion	2.64	3.63	3.13	0.015	0.0239
Xylophagy	2.36	4.50	3.43	0.153	0.0457

SEM: Standard error of the mean.

Regarding carcass traits of finished bulls (Table 5), ENZ had no effect ($p > 0.05$) on the carcass traits. However, ENZ conditioned higher ($p < 0.05$) carcass dressing (56.2 vs. 55.3%) compared to CON.

Table 5. Carcass traits of feedlot-finishing bulls under the effect of enzymes included in the diet.

Parameter	Experimental diet		Mean	SEM	P value
	CON	ENZ			
Hot carcass, kg	281.4	290.8	286.1	4.370	0.4924
Carcass yield, %	55.29	56.22	55.75	0.145	0.0451
Fat thickness, mm					
Longissimus dorsi	6.00	5.38	5.69	0.247	0.4200
Back	6.14	5.00	5.57	0.215	0.2520
Costilhar	8.07	7.00	7.54	0.243	0.3643
Front	3.79	3.75	3.77	0.156	0.9408

SEM: Standard error of the mean.

Carcass yield may have been a reflection from the increase in efficiency as highlighted above. By illustrating this fact, Vargas, Mendoza, Rubio-Lozano and Castrejón (2013) evaluating different levels (0, 2, 4, 6 ppm) of enzymatic complexes (xylanases and cellulases) for feedlot cattle, observed a quadratic increase in carcass dressing of the evaluated animals, and the best values were obtained for animals that received intermediate levels of enzymes in the diet. These data may suggest that lower levels may not generate performance effects, but may be effective in modifying some carcass traits, in accordance with this study.

Conclusion

Xylanase-complex could increase efficiency of feedlot bulls receiving energy-dense diets.

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