

University of São Paulo
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The use of fibrolytic enzymes on the performance, metabolism and feeding behavior of feedlot cattle fed diets which differ in terms of level and source of roughage

Ludmila de Souza Monteiro

Dissertation presented to obtain the degree of Master in Science. Area: Animal Science and Pastures

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2018

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RESUMO

Uso de enzimas fibrolíticas no desempenho, metabolismo e comportamento ingestivo de bovinos em confinamento alimentados com diferentes fontes e níveis de volumoso

Haja vista as barreiras na degradabilidade da fração fibrosa dos alimentos, dois experimentos foram conduzidos com o objetivo de avaliar o uso de enzimas fibrolíticas exógenas (EFE) com atividades de xylanase e celulase em dietas de confinamento para gado de corte, dando origem a um capítulo desta dissertação. No primeiro experimento, o objetivo foi determinar o efeito da enzima fibrolítica na dieta de machos inteiros da raça Nelore terminados em confinamento com dietas contendo duas fontes e dois níveis de volumoso. Duzentos e sessenta e quatro animais foram distribuídos em 48 baias pelo peso vivo inicial ($371 \pm 18,7$) em um delineamento em blocos casualizados com arranjo fatorial $2 \times 2 \times 2$. O período de terminação foi de 95 dias e as dietas continham, na base seca, EFE ou não (0,75 ml/kg de MS; ABVista, Marlborough, UK), 8,5 ou 12,5% de bagaço-de-cana-de-açúcar (SCB) ou de feno de gramínea (GH), 59 ou 55% de milho moído, 15% de farelo proteinoso de milho (corn gluten feed), 15% de casca de soja, 1,5% de minerais e vitaminas com monensina e 1% de ureia. A adição de EFE não afetou significativamente os dados de desempenho animal ($P > 0,10$). O CMS foi maior para as dietas com 12,5% de volumoso ($P < 0,01$) e para as dietas com feno ($P = 0,01$), mas a EA (GPD/CMS) foi maior com 8,5% de volumoso ($P < 0,01$) e tendeu a ser maior com SCB ($P = 0,07$). As energias líquidas para a manutenção (ELm) e para o ganho (ELg) observadas foram maiores para o nível de inclusão de 8,5% ($P < 0,01$) e para o SCB ($P = 0,04$). No segundo experimento, o objetivo foi avaliar parâmetros ruminais, digestibilidade do trato digestivo total e comportamento alimentar de oito novilhos Nelore ($396 \pm 1,4$ kg) recebendo as mesmas dietas que no experimento de desempenho. Os novilhos foram designados a dois Quadrados Latinos (LSQ) 4×4 independentes com arranjo fatorial 2×2 , em que cada LSQ recebeu uma fonte de volumoso diferente. Para os bovinos alimentados com SCB, a digestibilidade dos nutrientes não foi afetada pelo nível de volumoso nem pela presença de EFE ($P > 0,10$). Para os animais alimentados com GH a digestibilidade da PB foi maior com 8,5% do que com 12,5% de volumoso na dieta ($P = 0,01$). A suplementação com EFE tendeu a aumentar as digestibilidades da MS ($P = 0,08$) e da PB ($P = 0,06$) das dietas com GH. Para os animais alimentados com SCB a proporção molar de isovalerato ($P < 0,01$) foi menor com 12,5% de volumoso na dieta e houve tendência de redução na concentração total de AGV ($P = 0,06$) e na proporção molar de valerato ($P = 0,07$) em comparação com 8,5% de SCB. A suplementação com EFE tendeu a aumentar a proporção molar de isovalerato ($P = 0,09$) para dietas com SCB. Nos tratamentos com GH, a relação acetato:propionato foi menor com a inclusão de 8,5% de volumoso ($P = 0,04$). A suplementação com EFE tendeu a diminuir a proporção molar de propionato ($P = 0,06$) e aumentou a relação acetato:propionato ($P = 0,03$) e as proporções molares de isobutirato e isovalerato ($P < 0,01$). Concluindo, a suplementação com EFE não melhora o desempenho de bovinos confinados com dietas contendo SCB ou GH, mas resulta em alguns efeitos positivos na digestibilidade e altera alguns parâmetros ruminais de animais alimentados com GH. A inclusão de 12,5% de SCB ou GH na dieta de bovinos confinados contendo milho flint moído, casca de soja e farelo proteinoso de milho, aumenta o CMS, mas diminui a EA comparada com a inclusão de 8,5% dessas fontes de volumoso. Nos níveis de inclusão de 8,5 ou 12,5% da MS da dieta, o SCB reduz o CMS, sem alteração no GPD e conseqüentemente melhora a EA de bovinos confinados com dietas contendo milho flint moído, casca de soja e farelo proteinoso de milho.

Palavras-chave: Enzima fibrolítica; Fonte de volumoso; Gado de corte; Nível de volume

ABSTRACT

The use of fibrolytic enzymes on the performance, metabolism and feeding behavior of feedlot cattle fed diets which differ in terms of level and source of roughage

Given the barriers in the degradability of the fibrous fraction of feed, two experiments were conducted with the objective of evaluating the use of fibrolytic enzymes (EFE) with xylanase and cellulase activities in feedlot diets for beef cattle, originating this dissertation. In the first experiment, the objective of the study was to determine the effect of the fibrolytic enzyme in the diet of finishing feedlot Nellore bulls with diets containing two sources and two levels of roughage inclusion. Two hundred and sixty-four Nellore bulls (371 ± 18.7 kg) were distributed in 48 pens by initial BW in a randomized complete block design with a $2 \times 2 \times 2$ factorial arrangement of treatments. The finishing period was 95 days and diets were composed, on DM basis, of EFE or not (0.75 ml/kg DM; ABVista, Marlborough, UK), 8.5 or 12.5% of sugarcane bagasse (SCB) or grass hay (GH), 59 or 55% of ground corn, 15% of corn gluten feed, 15% of soybean hulls, 1.5% of minerals and vitamins with monensin, and 1% of urea. Animal performance was not significantly affected by the addition of EFE ($P > 0.10$). Dry matter intake was higher for treatments with 12.5% of roughage ($P < 0.01$) and for treatments with GH ($P = 0.01$), but G:F was higher for 8.5% of roughage ($P < 0.01$) and tended to be higher for SCB ($P = 0.07$). Observed net energy concentrations were higher for 8.5% of roughage inclusion ($P < 0.01$) and for SCB ($P = 0.04$). In the second experiment, the objective was to evaluate ruminal parameters, total tract digestibility, and feeding behavior of 8 Nellore steers (396 ± 1.4 kg) receiving the same diets that the performance trial. The steers were assigned to two independent 4×4 Latin Squares (LSQ) with a 2×2 factorial arrangement of treatments, in which each LSQ received one different source of roughage. For steers fed SCB, digestibility of nutrients were not affected by the level of roughage nor by the presence of EFE ($P > 0.10$). For steers fed GH the digestibility of CP was higher for 8.5% than for 12.5% of dietary roughage ($P = 0.01$). The supplementation of EFE tended to increase the digestibilities of DM ($P = 0.08$) and of CP ($P = 0.06$) of GH diets. For animals fed SCB the molar proportion of isovalerate ($P < 0.01$) was lower with 12.5% of dietary roughage and there was a tendency of reduction on total VFA concentration ($P = 0.06$) and on molar proportion of valerate ($P = 0.07$) compared to 8.5% of SCB. The EFE supplementation tended to increase the molar proportion of isovalerate ($P = 0.09$) for SCB diets. In GH treatments, the acetate:propionate ratio was lower with the inclusion of 8.5% of roughage ($P = 0.04$). The EFE supplementation tended to decrease the molar proportion of propionate ($P = 0.06$), and increased the acetate:propionate ratio ($P = 0.03$) and the molar proportions of isobutyrate and isovalerate ($P < 0.01$). To conclude, the EFE supplementation do not improve the performance of feedlot cattle fed diets containing SCB or GH, but result in some positive effects on digestibility and in some effects on ruminal parameters of animals fed GH. The inclusion of 12.5% of SCB or GH in diets of feedlot cattle containing ground flint corn, soybean hulls, and corn gluten feed increase DMI, but decrease G:F compared to the inclusion of 8.5% of these sources of roughage. On level of inclusion of 8.5 or 12.5% of DM, SCB reduces DMI, with no alteration on ADG, and consequently improves G:F of feedlot cattle containing ground flint corn, soybean hulls, and corn gluten feed.

Keywords: Beef cattle; Fibrolytic enzyme; Roughage level; Roughage source

1. INTRODUCTION

Animal nutrition researchers have directed several studies to the improvement of diet digestibility, in order to minimize nutrient loss and to enhance animal performance. One of the strategies that have been adopted is the use of feed additives. Among the additives capable of degrading the cell wall of plants are the inoculants, fungal treatments, and exogenous enzymes (Krause et al., 2003). The first studies with enzymes for ruminants date from the 1960s, showing potential for improvement in beef cattle performance (Burroughs et al., 1960). Exogenous fibrolytic enzymes (EFE) have been an additive relatively commonly studied for ruminant nutrition since the 1990s.

Exogenous fibrolytic enzymes can be different according to each commercial product, and also its proportions and activities, which may impact on the efficacy of cell wall degradation (Beauchemin et al., 2003). Effectiveness vary considerably among treatments. Beauchemin et al. (1995) fed high forage based diets for steers reported improved digestibility of dry matter intake (DM) and acid detergent fiber (ADF), associated with improved animal performance when the forage was alfalfa hay or timothy hay, however when barley silage was fed average daily gain (ADG) and feed efficiency were not altered. Salem et al. (2013) observed greater nutrients digestibility, higher rumen pH, total volatile fatty acids (VFA) concentration and ammonia nitrogen (NH₃-N), corroborating the superior performance of steers fed high grain diets with EFE compared to the control. On the other hand, Miller et al. (2008) showed no effect of EFE on finishing performance and carcass characteristics when EFE was added to diets high in concentrate. However, information is still needed regarding the use of EFE in feedlot diets containing roughage sources typical of tropical environment fed to zebu cattle.

The low availability of forage in the agrostological winter combined with the low cost of net energy for gain (NEg) of concentrated ingredients when compared to forage harvested or grazed (Owens, 2008) makes it interesting including the lowest level possible of fibrous ingredients in diets of finishing cattle in feedlots.

Although this concept is widely spreaded and adopted in US feedlot diets, typical Brazilian feedlot diets contain around 20% roughage (Pinto and Millen, 2018), a relatively high value compared to finishing diets in US feedlots where average levels of inclusion are around 8% in summer and 10% in winter (Samuelson et al., 2016).

According to Fox and Tedeschi (2002) the minimum inclusion of 7 to 10% of physically effective fiber (peNDF) is necessary to avoid or at least reduce the incidence of metabolic disorders, especially acidosis. The content of peNDF is determined by the product between the amount of neutral detergent fiber (NDF) of the ingredient and its effectiveness factor (Mertens, 1997). Even though inclusion levels of roughage ingredients in diets of US and Brazilian feedlots are distinct, the most common forage source is the same, being corn silage (Samuelson et al., 2016; Pinto and Millen, 2018). However, in some Brazilian states, due to the presence of sugarcane ethanol plants, it is possible to use sugarcane bagasse as a bulky ingredient that due to the high concentration of NDF and to the high factor of effectiveness, allows it to be included in smaller amounts (in terms of percentage of roughage) in diets. Among other bulky alternatives grass hay, similar to sugarcane bagasse, is a good alternative for finishing diets because it allows lower inclusion levels in the diet due to its high effectiveness factor.

The two sources of roughages mentioned - sugarcane bagasse and hay - have a high effectiveness factor and high concentrations of NDF making both good alternatives in diets of cattle in feedlot. However, the energy participation of both in total diet NEg is small. Therefore, a study evaluating diets containing different levels and sources of tropical roughages applying EFE to Nelore cattle may contribute to the understanding of the effects of this enzyme on animal performance, carcass characteristics, ruminal parameters, and nutrient digestibility.

1.1. Objectives

The overall objective was to evaluate the inclusion of EFE in finishing diets containing two different levels and two different sources of roughage on animal performance, carcass characteristics, ruminal parameters, apparent total tract digestibility, and feeding behavior of finishing feedlot cattle.

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2. THE USE OF FIBROLYTIC ENZYMES ON THE PERFORMANCE, METABOLISM AND FEEDING BEHAVIOR OF FEEDLOT CATTLE FED DIETS WHICH DIFFER IN TERMS OF LEVEL AND SOURCE OF ROUGHAGE

ABSTRACT

There are inconsistent responses to the use of exogenous fibrolytic enzymes (EFE) in beef cattle diets. Two experiments were conducted with the objective of evaluating finishing performance, carcass characteristics, total tract digestibility, ruminal parameters, and feeding behavior when applying EFE into high concentrate feedlot diets containing two sources and two levels of roughage inclusion. For the performance experiment, 264 Nellore bulls (371 ± 18.7 kg) were distributed in 48 pens by initial body weight in a randomized complete block design with a $2 \times 2 \times 2$ factorial arrangement of treatments. Diets were composed, on dry matter basis, of EFE or not (0.75 ml/kg of dry matter; ABVista, Marlborough, UK), 8.5 or 12.5% of sugarcane bagasse (SCB) or grass hay (GH). The inclusion of EFE had no effect on the feedlot performance and carcass characteristics ($P > 0.10$). Dry matter intake was higher for treatments with 12.5% of roughage ($P < 0.01$) and for treatments with GH ($P = 0.01$), but gain:feed was higher for 8.5% of roughage ($P < 0.01$) and tended to be higher for SCB ($P = 0.07$). Observed net energy concentrations were higher for 8.5% of roughage inclusion ($P < 0.01$) and for SCB ($P = 0.04$). For the metabolism experiment 8 ruminally cannulated Nellore steers (396 ± 1.4 kg) were assigned to 2 independent but simultaneous 4×4 Latin Squares (LSQ). The test diets were the same used in the performance trial, in which each LSQ received one different source of roughage (SCB or GH), and 4 different diets in a 2×2 factorial arrangement of treatments. For steers fed GH the digestibility of crude protein was higher for 8.5% than for 12.5% of dietary roughage ($P = 0.01$). The supplementation of EFE tended to increase the digestibilities of dry matter ($P = 0.08$) and of crude protein ($P = 0.06$) of GH diets. For animals fed SCB the total volatile fatty acids concentration ($P = 0.06$) tended to be lower with 12.5% of dietary roughage compared to 8.5% of SCB. In GH treatments, the acetate:propionate ratio was lower with the inclusion of 8.5% of roughage ($P = 0.04$). The EFE supplementation tended to decrease the molar proportion of propionate ($P = 0.06$), and increased the acetate:propionate ratio ($P = 0.03$) for GH diets. Supplementing EFE to feedlot cattle fed diets with SCB or GH showed no improvement for animal performance, however minor differences on digestibility and ruminal parameters for GH were noticed.

Keywords: Beef cattle; Feedlot; Fibrolytic enzyme; Roughage level; Roughage source

2.1. Introduction

Due to the competitive cost per amount of net energy for gain (NEg) of concentrate ingredients when compared to roughages (Owens, 2008), the inclusion of roughage just enough to avoid metabolic disorders (Van Soest, 1994) and stimulate the intake of dry matter and most important, to stimulate the intake of energy (Galyean and Defoor, 2003) is a recommended nutritional practice (Samuelson et al., 2016).

Fiber carbohydrates and lignin constitute the plant cell wall, and the covalent bounding between these carbohydrates with lignin decreases their ruminal degradation rates and extension (Cornu et al., 1994). Exogenous fibrolytic enzymes (EFE) could be used to reduce these barriers. However, studies have shown variable results when EFE were offered to cattle due to different type and activity of enzymes, diet composition, different applied doses, different time of application in relation to feeding, and methods of applying (Beauchemin et al., 2004; Meale et al., 2014). Actually, little is known about the effects of EFE on the performance of finishing beef cattle, especially for animals raised under tropical conditions, whose cattle and dietary components are different from those used in temperate regions. Two experiments were conducted with the objective of evaluating the inclusion of EFE in finishing diets containing ground flint corn, fibrous by-products and different levels and sources of roughage on feedlot cattle performance, carcass characteristics, ruminal parameters, apparent total tract digestibility, and feeding behavior. The

hypothesis was that EFE would increase digestibility of nutrients, mainly fiber portions, and hence, improve animal performance.

2.2. Materials and methods

These studies were conducted at the Experimental Feedlot Cattle facilities of the Animal Science Department of the “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo (USP), in Piracicaba, State of São Paulo, Brazil. All procedures using animals followed the guidelines recommended by the Animal Care and Use Committee of the ESALQ/USP, protocol number 2017.5.124.11.2.

2.2.1. Performance experiment

A finishing experiment with 264 Nelore bulls [initial body weight (BW) = 371 ± 18.7 kg] was conducted to evaluate the effect of EFE in feedlot diets containing ground flint corn, fibrous by-products, and two different sources and two different levels of roughages. A randomized complete block design with a $2 \times 2 \times 2$ factorial arrangement of treatments was used, with 6 replicate pens per treatment. The animals were fed 8 different test diets that were composed of two sources of roughage [either sugarcane bagasse (SCB) or grass hay (GH)], two levels of roughage inclusion [either 8.5 or 12.5% dry matter (DM) basis], and the inclusion or not of 0.75 mL/kg DM of a commercial EFE (Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom). The commercial EFE tested was composed of cellulase and xylanase. The composition and chemical analyses of the feed ingredients and the diets are presented in Tables 1 and 2 respectively.

Animals were dewormed with ivermectin (Ranger LA 3.5; Vallée S/A Produtos Veterinários, Montes Claros, Brazil) and vaccinated against clostridiosis (Poli-Star; Vallée S/A Produtos Veterinários, Montes Claros, Brazil) before the trial began. A 17 d step-up adaptation period preceded the feeding trial. During adaptation, the roughage used was the SCB and its inclusion in the diet was decreased from 25 (5 d), to 20 (4 d), to 17 (4 d), to 12.5% (4 d).

Bulls were weighed after a 16 h fasting from both food and water and blocked by initial BW and randomly assigned to 32 partially roofed concrete-floor pens (32 m²), 8 pens with soil surfaces and partially roofed (84 m²), and 8 pens with soil surfaces and no roof (84 m²).

Ingredients were mixed in a feed wagon, EFE was diluted with water in the proportion of 1:10 and added to the total mixed ration (TMR) with a watering can, diets were weighed individually per pen in a precision scale, then delivered to each pen once a day in the morning. Bunks were managed for a maximum of 3% ortos. Diets were adjusted for 70% DM content. Sugar cane bagasse was stockpiled in a non-roof area and it was not packed, as in commercial Brazilians feedlots. All the other feed ingredients were stored in a feed barn. Samples of SCB were collected twice a week and dried at 105°C for 24 h for diet adjustment. Orts were recovered twice a week, weighed, and then samples were stored (-18°C) for DM analyses. Samples of other feed ingredients were collected weekly throughout the experiment and stored (-18°C). Samples were thawed and composed for the whole experimental period for subsequent analyses. Samples of SCB and GH were composed by the entire trial (Table 3) for distribution and mean particle size, which was determined according to the Penn State Particle Size Separator method (Lammers et al., 1996) using sieves of 19, 8, and 4 mm.

On d 95 of the feeding trial, bulls were weighed after a 16 h fasting from both feed and water and slaughtered at a commercial abattoir. Hot carcasses were weighed, dressing percent was calculated as the ratio between hot carcass weight (HCW) and final shrunk BW. After a 12 h chill at 2°C the back fat thickness (BFT) and the Longissimus muscle area (LMA) were measured. To determine LMA and BFT an image across the Longissimus thoracis muscle was taken by a digital camera coupled to a 15 × 20 cm steel rectangle with a rod of 10 cm height. Digital camera images were recorded and then interpreted using Lince software (M&S Consultoria Agropecuária, Pirassununga, São Paulo, Brazil) by an experienced technician.

2.2.2. Metabolism experiments

Two metabolism trials were conducted to examine ruminal fermentation, total tract digestibility, and feeding behavior of cattle. Eight ruminally cannulated Nellore steers (396 ± 1.4 kg) were assigned to 2 independent but simultaneous 4 × 4 Latin Squares (LSQ) with a 2 × 2 factorial arrangements of treatments. In LSQ 1, SCB was fed at 8.5 or 12.5% of diet DM with or without EFE. In LSQ 2, GH was fed at 8.5 or 12.5% of diet DM with or without EFE. The test diets were the same used in the performance trial (Tables 1 and 2). Animals were housed in individual pens with a solid roof and concrete floors and given free choice access to water during the experiment.

Diets were prepared daily and offered ad libitum once a day at 0800 h. Ingredients of the concentrates used in the metabolism experiments were mixed in a commercial feed mill, composed of ground flint corn, soybean hulls, corn gluten feed, and minerals and vitamin with monensin. Feed ingredients of the TMR were weighed individually in a precision scale and mixed manually. The EFE inclusion was 0.75 mL/kg DM diluted with water in the proportion of 1:10 and added on top of the mixed diet before feeding. Each period of the LSQ was consisted of 14 d of adaptation and 10 d of collection (24 d/period). During the 10 d of the collection period, daily feed intake was restricted to 85% of the observed on the last 5 d of the adaptation period for steers fed the respective diets.

Feed ingredients were collected each collection period. The composition and chemical analyses of the feed ingredients and the diets are presented in Tables 1 and 2 respectively. For particle size distribution analyses SCB and GH samples were composed for the entire experimental period (Table 3). Orts were collected for chemical analyses.

The feeding behavior was recorded at 5 min intervals over 24 h on d 1 of the collection period. During the d 2 to 5 of the collection period, samples of ruminal fluid and of feces from rectum were obtained from each steer at 0, 1.5, 3, 6, 9, 12, and 18 h post feeding. Approximately 200 mL of ruminal fluid were collected manually from each steer via ruminal cannula (from the ventral, caudal, cranial and dorsal portion), squeezed and filtrate through nylon cloth, and immediately measured for pH using a portable pH meter (Digimed DM20; Digicrom Analítica Ltda., São Paulo, Brazil). Two microtubes of 2 mL and a 50 mL aliquot of ruminal fluid were reserved and stored at -18°C, for subsequent analyses of volatile fatty acids (VFA) and ammonia nitrogen (NH₃-N). Fecal grab samples (200 g) from the rectum were collected to determine apparent digestibility of nutrients. Animals were weighted on the d 3 of collection period. During the d 6 to 10 of the collection period the total amount of feces produced per steer was collected from the previously scraped concrete floor, every 6 h to determine total daily fecal production.

2.2.3. Laboratory analyses and calculations: performance and metabolism studies

Feed and fecal samples were thawed, composited for each trial period, dried in a forced-air oven at 55°C for 72 h, and ground through a 1 mm screen using a Wiley-type mill (MA-680; Marconi Ltda, Piracicaba, SP, Brazil). All samples were analyzed for DM (method 930.15; AOAC, 1986), ash (method 942.05; AOAC, 1986), ash-corrected neutral digestible fiber [(a)NDF]; Van Soest et al., 1991] using sodium sulfite and heat-stable α -amylase, acid detergent fiber [(ADF); Goering and Van Soest, 1970], and nitrogen [(N); Leco FP-528; Leco Corp., St Joseph, MI]. The crude protein (CP) content was calculated by multiplying nitrogen content by 6.25. Feed ingredients were analyzed for ether extract [(EE); method 920.85; AOAC, 1986]

Ruminal fluid samples were thawed and centrifuged at $15,000 \times g$ for 30 min at 4°C. The supernatant fluid was analyzed for VFA by gas-liquid chromatography (Palmquist and Conrad, 1971), and for NH₃-N (Chaney and Marbach, 1962).

For the performance experiment, net energy observed for maintenance (NEm) and for gain (NEg) for each treatment were calculated according to (Zinn and Shen, 1998) using average values for shrunk BW, dry matter intake (DMI), and average daily gain (ADG) of the bulls in each pen. The expected NEm and NEg were estimated with the equations proposed by NASCEM (2016) with addition of ionophore from the sum of TDN values from each ingredient calculated using NRC (2001), according to the equations described by Weiss et al. (1992).

2.2.4. Statistical analyses

Data from performance and carcass characteristics experiment were analyzed using the PROC MIXED procedure of SAS software (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The statistical model included the fixed effect of treatment and the random effect of weight block. Data from the metabolism experiments were also analyzed using the PROC MIXED procedure of SAS including the fixed effect of treatment and the random effects of animal and period. Ruminal fermentation parameters (pH, VFA, and NH₃-N) were analyzed as repeated measures over time. Results are reported as least-square means and significance was set at $P \leq 0.05$ and tendencies were reported when $0.05 < P \leq 0.10$. Means were compared using Tukey test.

2.3. Results

2.3.1. Performance and carcass characteristics

Cattle performance and carcass characteristics are presented in Table 4. Dry matter intake was affected by level ($P < 0.01$) and source ($P = 0.01$) of roughage inclusion, being higher for animals fed diets containing 12.5% roughage compared with 8.5%, and for animals fed GH compared with SCB diets. Efficiency of feed utilization (G:F) was affected by roughage level ($P < 0.01$) and tended to be affected by roughage source ($P = 0.07$). Cattle fed diets with 8.5% roughage presented greater gain:feed (G:F) compared with cattle fed diets with 12.5% roughage, while cattle fed SCB diets tended to be more efficient than cattle fed GH diets. There were changes for level ($P < 0.01$) and source ($P = 0.04$) on observed dietary NE concentrations from animal performance (Table 5), for which roughage level 8.5% was higher than 12.5% and SCB diets higher than GH diets. Whereas the observed:expected NE ratios pointed only source of roughage inclusion being affected ($P < 0.01$), with SCB being higher than GH. A tendency was detected for the interaction of level and source on ADG and also on final BW ($P = 0.07$), despite no difference between treatments

when Tukey test was applied, final BW and ADG were better when diets contained 12.5% of SCB (541 kg and 1.794 kg/d) or 8.5% of GH (539 kg and 1.774 kg/d) compared to 12.5% of GH (536 kg and 1.735 kg/d) and 8.5% of SCB (534 kg and 1.713).

Neither of the performance results (DMI, ADG, G:F, NEm and NEg) were affected by feeding EFE ($P > 0.10$). In addition, no differences were observed on any carcass characteristic ($P > 0.10$), as expected from growth performance results. Also, interactions were not detected between the three factors (level, source, and enzyme; $P > 0.10$), except on dressing percentage ($P = 0.10$), which 12.5% of GH with EFE (57.85%) and 12.5% of SCB without EFE (57.81%) had the highest values for dressing, and 12.5% of GH without EFE (57.21%) and 12.5% of SCB with EFE (56.85%) had the lowest values, however, no difference was detected when applied the Tukey test. None of the animals presented liver abscess.

2.3.2. Metabolism

In the two metabolism experiments, there were no interactions between level of roughage and the inclusion of EFE ($P > 0.10$). Dry matter intake, fecal excretion, and digestibility of nutrients are presented in Table 6 and Table 7, for cattle fed SCB and GH respectively. Intake of dry matter was not affected by roughage level or EFE for both roughage sources ($P > 0.10$) despite the decrease in fecal excretion when EFE was fed for cattle on SCB diets ($P = 0.05$; Table 6) and on GH diets ($P < 0.01$; Table 7). Digestibility of nutrients were not affected by roughage level or by EFE supplementation ($P > 0.10$) when cattle were fed SCB (Table 6). On the other hand, when cattle were fed GH (Table 7), including EFE in the diet tended to increase both DM ($P = 0.08$) and CP ($P = 0.06$) digestibility while feeding 8.5% GH resulted in higher CP digestibility compared with 12.5%. Neither roughage level nor EFE supplementation affected apparent digestibility of NDF and ADF ($P > 0.10$).

For rumen parameters, when cattle were fed SCB (Table 8; Figure 1) neither roughage level nor EFE affected pH, molar proportions of propionate, acetate, butyrate, isobutyrate, acetate:propionate ratio, and $\text{NH}_3\text{-N}$ ($P > 0.10$). Feeding 8.5% SCB tended to increase total VFA concentration ($P = 0.06$), increased molar proportion of isovalerate ($P < 0.01$) and tended to increase molar proportion of valerate ($P = 0.07$). Supplementing EFE to SCB diets had no effects on all the rumen parameters ($P > 0.10$), excepted on isovalerate which tended to increase ($P = 0.09$). When GH was fed (Table 9; Figure 2), the level of roughage fed had no effect on any rumen parameter ($P > 0.10$), except on acetate:propionate ratio ($P = 0.04$) which was lower for the 8.5% compared with the 12.5% diet. Feeding EFE increased isobutyrate ($P < 0.01$), isovalerate ($P < 0.01$) and the acetate:propionate ratio ($P = 0.03$) while it tended to decrease molar proportion of propionate ($P = 0.06$).

Ingestive behavior data from both metabolism trials are presented in Table 10. For both roughage sources tested, feeding either level 8.5 or 12.5% with or without EFE, had no effect on all the ingestive behavior parameters evaluated ($P > 0.10$), despite a tendency for the interaction of level and enzyme on rumination time for SCB LSQ ($P = 0.10$), which level 12.5% with EFE had the highest ruminating time (370.00 min/d) and level 8.5% supplemented with EFE had the lowest ruminating time (268.75 min/d), although no difference was detected by Tukey test.

2.4. Discussion

2.4.1. Performance and carcass characteristics

For the performance experiment, the objective was to evaluate the benefits of applying EFE into a feedlot diet with either sugarcane bagasse or low quality grass hay as the roughage sources fed at either 8.5% or 12.5% of diet DM.

The greater DMI, as roughage inclusion is increased (Stock et al., 1990; Guthrie et al., 1996; Defoor et al., 2002; Parsons et al., 2007; Ponce et al., 2016), as well as differences on DMI for different sources of roughage (Bartle et al., 1994; Guthrie et al., 1996; Defoor et al., 2002) have been reported. In this study, the greater DMI of cattle fed diets containing 12.5% roughage is in accordance with published literature. Grass hay had greater DMI than SCB, it may have been related to differences in concentration of roughage NDF, and in physical characteristics of the dietary fiber, which might have impacted passage rate, or ruminal metabolism, not sufficiently to impact ADG, but G:F tended to be greater for SCB. Gain:feed was also greater for level 8.5% than for 12.5%.

Comparing 3 sources of forage (alfalfa hay, corn silage, or corn stalks), and 2 levels of roughage NDF (inclusion based on 4% and 8% of DM of alfalfa hay), Benton et al. (2015) noticed that DMI and ADG were greater for the highest roughage inclusion than for the lowest, and observed no differences on G:F for roughage level and roughage source. Benton et al. (2015) reported no differences for roughage source on the performance variables, and on carcass characteristics, but back fat thickness was lower for corn silage compared with alfalfa hay and corn stalks. Swanson et al., (2017) also evaluated the effect of different sources of roughage with the same level of roughage NDF inclusion (alfalfa hay, corn silage, wheat straw, and corn stover). They observed no differences on animal performance among treatments.

Through a compilation of data involving 11 trials with different sources and levels of roughages, Galyean and Defoor (2003) observed that a relationship between DMI and NDF supplied by roughage shows an important indicative for strategies when formulating diets for feedlot cattle. According to the results, they concluded that the changes in DMI caused by different levels and sources of roughage, are more related to the amount of NDF supplied by roughage. In the same study, Galyean and Defoor (2003) assumed that small changes (less than 5% DM basis) in the percentage of roughage supplied for cattle would not change considerably DMI by the attempt of the cattle to maintain energy intake, but might affect ruminal metabolism and digesta kinetics.

Observed NE concentrations were greater for 8.5% of dietary roughage than for 12.5%, and were greater for SCB than for GH, which may have been the reason to the decrease on DMI for level 8.5% and for SCB, based on the chemostatic factor of intake regulation (Mertens, 1987). Similarly to our study, a linear decrease on NE concentrations were noticed by May et al. (2011) when increasing the amount of dietary alfalfa hay (7.5, 10, and 12.5% of DM basis), as well as a tendency for linear decrease on G:F and a tendency for linear increase on DMI. In the same study, no difference were observed in carcass characteristics for level of roughage inclusion. On the other hand, Marques et al. (2016) showed that observed NE concentrations were not altered for cattle fed whole corn with levels of sugarcane bagasse varying from 0, 3 and 6% of inclusion, but noticed a quadratic effect on DMI, ADG, and final BW, and a tendency on HCW and BFT, yet no difference on G:F. Differently from our study, Caetano et al. (2015) observed no alterations on dietary NE concentrations from cattle performance with increasing levels of sugarcane bagasse from 3 to 18% of roughage NDF in the diet, they observed a quadratic effect on ADG, final BW, and HCW, a linear effect on dressing percentage, and no difference on G:F, with level of 13%, approximately, having the best performance.

Unlike our study, Balci et al. (2007) noticed that ADG and feed conversion rate were improved when EFE was applied into the diet of steers fed high grain based diets. According to McAllister et al. (1999), for growing steers fed diet with EFE the overall results for ADG, DMI, and G:F were not affected, but final BW tended to increase linearly as the inclusion of EFE increased in the diet, and for finishing steers EFE had higher ADG than control, but no differences were observed for DMI, feed efficiency, final BW, and carcass characteristics.

High grain based diets were offered to finishing heifers with or without EFE, and even though ADG was higher for EFE treatment than for control, no differences on DMI, feed conversion, and final BW were noticed (Beauchemin et al., 1999). However, Beauchemin et al. (1997) observed that EFE improved performance of finishing cattle fed barley diet but not for cattle fed corn based diet. When enzyme containing high xylanase activity and lower cellulase was added to barley based diets, feed conversion rate was improved compared to the control diet, but no differences were detected for EFE on ADG, DMI, final BW, and on any carcass characteristics.

Gómez-Vázquez et al. (2011) reported that when EFE was increased in high forage based diets, DMI, ADG, feed conversion, and DM and NDF digestibility linearly increased. Gómez-Vázquez et al. (2003) also observed linear improvement on ADG, feed conversion, final BW, and NDF digestibility, however no differences on DMI or DM digestibility.

Krueger et al. (2008) evaluating three different ways of applying EFE (after cutting, at baling, or at feeding) found no significant differences for cattle performance on ADG, G:F, and final BW, but DMI was higher when EFE was applied right after cutting to warm-season grass comparing to control. On the other hand, He et al. (2015) observed a tendency to linear decrease on DMI when EFE was added to the diet, however no difference for ADG and G:F.

As in our results, some studies showed that growing performance was not affected by EFE (ZoBell et al., 2000; Wang et al., 2003; Miller et al., 2008; Eun et al., 2009; Vargas et al., 2013; He et al., 2014) nor carcass characteristics were affected (Wang et al., 2003; Miller et al., 2008; He et al., 2014). Eun et al. (2009), reported that cattle receiving EFE had lower BFT than control diet, even though there were no differences for growing performance of growing and finishing beef steers.

Data from this experiment indicate that the inclusion of EFE had no effect on feedlot performance and carcass characteristics of Nellore bulls fed diets containing either SCB or tropical GH, and either for levels of 12.5% or 8.5% of inclusion of these roughages. Regarding level and source of roughage inclusion, DMI was greater for level 12.5% and for GH, which led to a greater G:F for level 8.5% and a tendency of SCB to be greater, as ADG was not altered. Also, observed NE concentrations were greater for 8.5% of dietary roughage, and for SCB, which may have been the reason to the decrease on DMI for this level and source of roughage.

2.4.2. Metabolism

The objective of these studies were to evaluate if EFE would improve ruminal parameters and apparent total tract digestibility of Nellore bulls fed diets with either 8.5% or 12.5% level of roughage. Two sources of forage were tested in two separate LSQ.

Differences were observed on fecal excretion for treatments receiving EFE for SCB and GH diets, which could lead to a better use of nutrients when applied EFE to the diet. However, despite no significant difference was observed on DMI, numerically EFE treatments consumed less DM than control treatments, in both LSQ. It may explain the lower fecal excretion for EFE treatments, mainly for SCB LSQ, as apparent digestibility of nutrients was not altered. Furthermore, apparent digestibility of CP tended to be higher for EFE treatments than for control

treatments in the GH LSQ, which reflected the response on the DM digestibility. The lack of a consistent effect of EFE on digestibility of nutrients in the metabolism study corroborates the no improvement on performance of feedlot cattle in the present study.

Krueger et al. (2008) observed that applying EFE to high forage diets increased total tract DM apparent digestibility, and also that applying EFE after cutting or at baling to warm-season grasses increased NDF and CP digestibility; when EFE was applied at feeding, NDF digestibility tended to be higher than in control treatment, and no alteration was detected for CP digestibility. He et al. (2015), feeding steers high forage diets with increasing levels of EFE linearly increased CP digestibility, similar to our study. Martins et al. (2006) observed significant improvement on total tract digestibility of NDF, ADF, and cellulose when EFE was added to two sources of roughage.

Applying EFE to the diets did not alter ruminal pH as reported by others (Balci et al., 2007; Romero et al., 2013; He et al., 2015). Miller et al. (2008) also reported no difference on pH and total VFA concentration, but butyrate concentration was higher for barley diet supplemented with EFE and it was lower for sorghum diet supplemented with EFE. Álvarez et al. (2009) evaluating 2 different commercial EFE found that ruminal pH increased with addition of both EFE, and, similarly with our study, found that with one of the commercial EFE molar proportion of propionate was lower than control, leading to a higher acetate:propionate ratio for this EFE, whereas Krause et al. (1998) reported that acetate:propionate ratio tended to decrease when EFE was added to the diet.

Also, no ruminal NH₃-N alteration for EFE treated diets was observed by some authors (Álvarez et al., 2009; Romero et al., 2013). He et al. (2014) noticed that when dietary EFE increased ruminal NH₃-N concentration decreased, and there were no differences for total VFA or molar proportions of individual VFA. Miller et al. (2008) reported that ruminal NH₃-N concentration of steers fed barley grain based diet with EFE was higher than for steers fed the control diet without EFE, yet no difference for the sorghum grain based diets.

Feeding EFE increased isovalerate and acetate:propionate while decreasing molar proportion of rumen propionate, indicating no improvements in rumen energetics. As in the present study, Krause et al. (1998) detected no differences on animal behavior for ruminating and eating time, when supplementing diets with EFE.

Surprisingly feeding 12.5% SCB or GH did not decrease diet DM digestibility compared to 8.5%, despite lower numerical values for higher forage levels. These results are not in agreement with the lower G:F and observed NE values of 12.5% roughage diets compared to 8.5% diets in the performance study. Benton et al. (2015) evaluated steers fed 2 sources of roughage (alfalfa hay and corn stalks) and 3 levels of roughage inclusion (zero, 4% alfalfa and 3% corn stalks, and 8% alfalfa and 6% corn stalks), as the roughage level increased they observed a linear decrease on apparent total tract digestibility of DM, OM, and CP, and a tendency for NDF; to increase the percentage of roughage in the diet, they decreased the amount of corn, and considered the difference of the total tract digestibility of nutrients a result of the least digestibility of roughage. It may also explain the difference on CP apparent digestibility in our study for grass hay, with level 8.5% of inclusion being more digestible than 12.5%. Ponce et al. (2016) observed that steers fed 6% of alfalfa hay had less fecal excretion, and higher DM and OM digestibility than steers fed 12% of this roughage, and no difference on CP, ADF and NDF digestibility. On the other hand, Gorocica-Buenfil and Loerch (2005) observed no differences on apparent digestibility of nutrients (DM, OM, starch, CP, NDF, and ADF) when corn silage was added to the diet at either 5.2% or 18.2% of DM basis.

In the present study, feeding 12.5% roughage diets did not increase mean ruminal pH compared with feeding 8.5% diets. For both roughage sources and levels rumen pH reached minimum values bellow 5.5 (Figures 1 and 2), considered a threshold for acidosis (REF????). Despite no significance, cattle fed 8.5% roughage remained more time with pH bellow 5.5. On the other hand, cattle fed 8.5% roughage presented greater G:F and observed NE

values than cattle fed 12.5% roughage. Unlike our result for ruminal pH, Benton et al. (2015) detected a linear increase for ruminal pH as dietary roughage increased.

In this study, no differences were observed regarding ingestive behavior. But according to Armentano and Pereira (1997), increasing forage NDF increases chewing time. Gentry et al. (2016) reported that steers fed diets with 10% of corn stalks had more rumination time than steers fed 5%.

Higher rumination time for higher levels of roughage inclusion could have explained the lower total VFA concentration of diets containing 12.5% of SCB due to a dilution caused by the increase of saliva flow to the rumen (Owens et al., 1998), but no difference for rumination time was detected between treatments, which is also in agreement with pH, as it was not altered by treatments. For level of roughage inclusion, it is expected for the acetate:propionate ratio to be higher as roughage is increased in the diet, as fiber fermentation produces more acetate. The acetate:propionate ratio being higher for EFE in GH diets is more related to the decrease of molar proportions of propionate for EFE treatments compared to the control.

Differently from our data for level of roughage inclusion, Weiss et al. (2017) reported that cattle fed diets with 5% of inclusion of corn stalks tended to have more DM and NDF digestibility, lower rumination time, lower average ruminal pH, lower ruminal NH₃-N, lower molar proportion of acetate, and greater proportion of propionate than cattle fed 10% of corn stalks; but, as well as part of our study, greater total VFA concentration, and lower acetate:propionate for 5% compared to 10% of roughage inclusion.

In the performance study, the roughage NDF contents were 6.07, 8.93, 5.58, and 8.22% for the diets containing 8.5% SCB, 12.5% SCB, 8.5% GH and 12.5% GH respectively. Considering these roughage NDF values, the mean particle size of 4.5 mm for SCB and 16.9 mm for GH and the performance and rumen pH values, SCB must have high content of effective fiber compensating its smaller particle size compared to GH.

In general, the inclusion of EFE in diets either containing SCB or GH for both 8.5% or 12.5% inclusion of roughage for Nellore steers decreased the amount of fecal excretion, and, only for GH diets, a tendency to increase apparent digestibility of DM and CP was observed for EFE treatments, as well as an increase on acetate:propionate ratio. For SCB diets, level of roughage inclusion of 8.5% tended to produce more total VFA in the rumen than level 12.5%. For GH diets, level 8.5% had greater apparent digestibility of CP, and level 12.5% had greater acetate:propionate ratio.

2.5. Conclusion

Finishing Nellore cattle fed 8.5% SCB or GH perform better than cattle fed 12.5% roughage, when ground corn, soybean hulls and corn gluten feed are fed in the diet. Sugarcane bagasse improves finishing performance of feedlot cattle when fed at 8.5 or 12.5% of diet compared with low quality grass hay.

Despite the heterogeneity of results on EFE for beef cattle observed in several studies, in our study EFE showed no improvement for animal performance, carcass characteristics, or animal behavior. However, some effects were noticed on digestibility of nutrients and on proportions of ruminal VFA, especially for GH diets. More research is needed to obtain concrete answers regarding the use of these enzymes.

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Table 1. Ingredients and chemical composition of the performance and metabolism experiment diets

Item				
Ingredient, % DM				
Sugarcane bagasse	8.5	12.5	-	-
Grass hay	-	-	8.5	12.5
Ground corn	59.0	55.0	59.0	55.0
Soybean hulls	15.0	15.0	15.0	15.0
Corn gluten feed	15.0	15.0	15.0	15.0
Urea	1.0	1.0	1.0	1.0
Minerals and vitamin supplement ¹	1.5	1.5	1.5	1.5
Analyzed composition				
Performance experiment				
Crude protein, % DM	13.0	12.8	13.3	13.2
Ash, % DM	5.0	5.5	4.6	4.8
Ether extract, % DM	3.1	3.0	3.2	3.0
Neutral detergent fiber, % DM	30.5	32.8	30.0	32.1
Acid detergent fiber, % DM	13.9	15.9	12.1	13.2
Net energy for maintenance, Mcal/kg ²	1.85	1.79	1.90	1.85
Net energy for gain, Mcal/kg ²	1.22	1.16	1.26	1.22
Metabolism experiment				
Crude protein, % DM	11.4	10.6	11.7	11.1
Ash, % DM	5.1	5.2	5.5	5.7
Ether extract, % DM	3.6	3.5	3.6	3.5
Neutral detergent fiber, % DM	34.8	35.1	33.2	32.7
Acid detergent fiber, % DM	15.0	15.5	13.2	12.8

¹ Minerals and vitamin supplement was composed (DM basis) of 270 g/kg Ca, 79 g/kg Na, 6.7 g/kg Mg, 38 g/kg S, 2,240 mg/kg Zn, 750 mg/kg Cu, 1,495 mg/kg Mn, 37.5 mg/kg I, 11 mg/kg Co, 7.5 mg/kg Se, 98,560 IU/kg vitamin A, 2,000 mg/kg monensin. Manufactured by Agrocerec Multimix Nutrição Animal, Rio Claro, São Paulo, Brazil.

²The net energy for maintenance and for gain were estimated with the equations proposed by NASCEM (2016) with addition of ionophore from the sum of TDN values from each ingredient calculated using NRC (2001) according to the equations described by Weiss et al. (1992).

Table 2. Chemical composition of the feed ingredients of the performance and metabolism experiments

Item	CP	Ash	EE	NDF	ADF	TDN ²
Performance experiment						
Sugarcane bagasse	2.92	13.73	0.30	71.44	52.34	38.13
Grass hay	6.63	8.24	0.60	65.72	31.32	53.17
Ground corn	8.61	1.69	4.01	15.07	3.08	86.67
Soybean hulls	10.72	4.62	1.66	63.51	43.32	65.29
Corn gluten feed	21.66	4.61	3.22	40.14	7.60	74.56
Metabolism experiment						
Sugarcane bagasse	2.60	12.48	0.73	78.76	47.34	36.49
Grass hay	6.43	16.22	0.59	59.74	26.04	47.16
Concentrate 1 ³	12.18	4.47	3.89	30.75	11.97	
Concentrate 2 ³	11.74	4.16	3.93	28.83	10.96	

¹ CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; TDN = total digestible nutrients.

² TDN values from each ingredient were calculated using NRC (2001) according to the equations described by Weiss et al. (1992).

³ Concentrate 1 = for diets with 8.5% of roughage inclusion; Concentrate 2 = for diets with 12.5% of roughage inclusion. Concentrates were composed of ground corn, soybean hulls, corn gluten feed, and minerals with monensin.

Table 3. Mean particle size and particle size distribution of roughages

Item	Sugarcane bagasse	Grass hay
Retained/screen, %		
Sieve screen size, mm		
19.0	3.7	54.7
8.0	30.0	29.7
4.0	16.8	10.6
Mean particle size, mm ¹	4.5	16.9

¹ Distribution and mean particle size was determined according to the Penn State Particle Size Separator method (Lammers et al., 1996).

Table 4. Effects of the use of fibrolytic enzymes¹, level, and source of roughage on performance of feedlot Nellore bulls

Item ³	Treatment ²								SEM	<i>P</i> -value						
	B 8.5 Con	B 8.5 Enz	B 12.5 Con	B 12.5 Enz	H 8.5 Con	H 8.5 Enz	H 12.5 Con	F 12.5 Enz		Level	Source	Enzyme	L*S	L*E	S*E	L*S*E
Feedlot performance																
Initial BW, kg	371	371	371	371	372	370	371	371	18.72	0.68	0.50	0.14	0.89	0.89	0.22	0.14
Final BW, kg	535	533	542	541	543	535	537	534	20.19	0.53	0.98	0.25	0.07	0.66	0.56	0.73
DMI, kg	9.91	9.68	10.51	10.66	10.30	10.28	10.80	10.62	0.28	<0.001	0.01	0.57	0.13	0.65	0.79	0.26
ADG, kg	1.726	1.700	1.799	1.789	1.806	1.742	1.748	1.721	0.05	0.52	0.97	0.33	0.07	0.69	0.67	0.87
G:F	0.175	0.176	0.172	0.168	0.176	0.170	0.162	0.163	0.005	0.005	0.07	0.46	0.39	0.87	0.84	0.35
Carcass characteristics																
HCW, kg	308	307	313	307	312	307	307	309	11.56	0.75	1.00	0.28	0.27	0.94	0.62	0.17
Dressing percentage	57.52	57.62	57.81	56.85	57.39	57.43	57.21	57.85	0.38	0.81	0.93	0.85	0.47	0.64	0.13	0.10
LMA, cm ²	63.77	63.84	63.04	63.54	64.20	64.80	61.92	65.16	1.69	0.53	0.69	0.35	0.85	0.52	0.49	0.64
BFT, mm	3.55	3.69	3.96	3.74	4.04	3.74	3.75	4.04	0.24	0.43	0.28	0.88	0.46	0.71	0.89	0.11

¹ Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom.

² B = sugarcane bagasse; H = grass hay; 8.5 = 8.5% of roughage inclusion; 12.5 = 12.5% of roughage inclusion; Con = control (no fibrolytic enzyme addition); Enz = addition of fibrolytic enzyme.

³ BW = body weight; DMI = dry matter intake; ADG = average daily gain; G:F = feed efficiency; HCW = hot carcass weight; LMA = Longissimus muscle area; BFT = back fat thickness.

Table 5. Diet NE values of the performance experiment evaluating the effects of the use of fibrolytic enzymes¹, level, and source of roughage on feedlot Nellore bulls

Item ³	Treatment ²								SEM	P-value						
	B 8.5 Con	B 8.5 Enz	B 12.5 Con	B 12.5 Enz	H 8.5 Con	H 8.5 Enz	H 12.5 Con	H 12.5 Enz		Level	Source	Enzyme	L*S	L*E	S*E	L*S*E
Observed NE ⁴ , Mcal/kg																
Maintenance	2.08	2.09	2.03	2.00	2.07	2.02	1.95	1.95	0.04	0.001	0.04	0.50	0.54	0.93	0.84	0.27
Gain	1.41	1.42	1.37	1.34	1.41	1.36	1.30	1.30	0.04	0.001	0.04	0.50	0.54	0.93	0.84	0.27
Observed:expected NE ⁵ ratio																
Maintenance	1.12	1.13	1.13	1.11	1.09	1.06	1.05	1.05	0.02	0.34	<0.001	0.52	0.36	0.95	0.80	0.26
Gain	1.15	1.17	1.18	1.15	1.12	1.08	1.06	1.07	0.03	0.44	<0.001	0.52	0.21	0.96	0.80	0.26

¹ Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom.

² B = sugarcane bagasse; H = grass hay; 8.5 = 8.5% of roughage inclusion; 12.5 = 12.5% of roughage inclusion; Con = control (no fibrolytic enzyme addition); Enz = addition of fibrolytic enzyme.

³ NE = net energy.

⁴ Calculated according to Zinn and Shen (1998).

⁵ The expected net energy for maintenance and for gain were estimated with the equations proposed by NASCEM (2016) with addition of ionophore from the sum of TDN values from each ingredient calculated using NRC (2001) according to the equations described by Weiss et al. (1992).

Table 6. Initial body weight (BW), dry matter intake (DMI), fecal excretion, and apparent digestibility of feedlot cattle fed diets containing fibrolytic enzymes¹ and different levels of sugarcane bagasse

Item ³	Treatment ²				SEM	P-value		
	B 8.5 Con	B 8.5 Enz	B 12.5 Con	B 12.5 Enz		Level	Enzyme	L*E
Initial BW, kg	399	398	403	398	1.14	-	-	-
DMI, kg	7.80	7.16	7.71	7.28	0.65	0.98	0.32	0.84
Fecal excretion, kg	2.49	2.23	2.55	2.39	0.32	0.32	0.05	0.62
Apparent digestibility								
DM, %	69.8	70.8	68.3	68.7	2.18	0.27	0.65	0.83
NDF, %	67.8	68.4	63.0	66.2	3.11	0.27	0.54	0.69
ADF, %	73.9	72.4	64.9	69.0	4.03	0.15	0.76	0.51
CP, %	61.79	60.87	58.22	58.30	2.66	0.24	0.86	0.84

¹ Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom.

² B = sugarcane bagasse; 8.5 = 8.5% of roughage inclusion; 12.5 = 12.5% of roughage inclusion; Con = control (no fibrolytic enzyme addition); Enz = addition of fibrolytic enzyme.

³ DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein.

Table 7. Initial body weight (BW), dry matter intake (DMI), fecal excretion, and apparent digestibility of feedlot cattle fed diets containing fibrolytic enzymes¹ and different levels of grass hay

Item ³	Treatment ²				SEM	P-value		
	H 8.5 Con	H 8.5 Enz	H 12.5 Con	H 12.5 Enz		Level	Enzyme	L*E
Initial BW, kg	395	392	390	396	1.19			
DMI, kg	8.34	8.09	8.56	8.08	0.76	0.80	0.38	0.78
Fecal excretion, kg	2.79	2.39	3.01	2.52	0.36	0.11	<0.001	0.69
Apparent digestibility								
DM, %	68.6	71.8	66.2	70.7	2.72	0.36	0.08	0.74
NDF, %	64.2	71.3	62.7	64.9	4.93	0.26	0.19	0.47
ADF, %	67.9	76.8	67.2	66.0	6.63	0.26	0.44	0.32
CP, %	60.89	63.26	54.58	58.86	2.69	0.01	0.06	0.53

¹ Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom.

² H = grass hay; 8.5 = 8.5% of roughage inclusion; 12.5 = 12.5% of roughage inclusion; Con = control (no fibrolytic enzyme addition); Enz = addition of fibrolytic enzyme.

³ DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein.

Table 8. Effects of the use of fibrolytic enzymes¹ and different levels of sugarcane bagasse on ruminal characteristics of feedlot Nellore steers

Item ³	Treatment ²				SEM	P-value		
	B 8.5 Con	B 8.5 Enz	B 12.5 Con	B 12.5 Enz		Level	Enzyme	L*E
Ruminal pH	6.19	6.11	6.35	6.27	0.13	0.21	0.54	0.99
Total VFA, mmol/mL	81.15	90.25	79.55	75.27	7.96	0.06	0.58	0.13
VFA, mol/100mol								
Acetate	59.72	59.09	62.02	60.12	1.74	0.11	0.23	0.54
Propionate	25.00	24.95	24.90	25.12	1.31	0.97	0.92	0.88
Butyrate	10.37	9.71	8.82	9.70	1.41	0.58	0.94	0.59
Isobutyrate	1.07	0.99	1.02	1.06	0.07	0.90	0.79	0.35
Isovalerate	1.19	1.33	1.05	1.12	0.08	0.005	0.09	0.55
Valerate	1.42	1.71	1.20	1.34	0.17	0.07	0.19	0.61
Acetate:propionate ratio	2.47	2.40	2.61	2.45	0.18	0.40	0.34	0.79
Ruminal NH ₃ -N, mg/dL	11.62	12.89	10.87	11.40	1.51	0.41	0.51	0.78

¹ Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom.

² B = sugarcane bagasse; 8.5 = 8.5% of roughage inclusion; 12.5 = 12.5% of roughage inclusion; Con = control (no fibrolytic enzyme addition); Enz = addition of fibrolytic enzyme.

³ VFA = volatile fatty acids; NH₃-N = ammonia nitrogen.

Table 9. Effects of the use of fibrolytic enzymes¹ and different levels of grass hay on ruminal characteristics of feedlot Nellore steers

Item ³	Treatment ²				SEM	P-value		
	H 8.5 Con	H 8.5 Enz	H 12.5 Con	H 12.5 Enz		Level	Enzyme	L*E
Ruminal pH	6.04	6.06	6.00	6.13	0.20	0.91	0.68	0.77
Total VFA, mmol/mL	96.66	91.07	92.22	85.04	7.48	0.30	0.20	0.87
VFA, mol/100mol								
Acetate	54.71	59.31	59.46	60.67	3.32	0.36	0.38	0.61
Propionate	30.55	24.30	25.76	22.56	3.72	0.18	0.06	0.54
Butyrate	10.49	11.66	10.59	11.66	0.83	0.96	0.18	0.96
Isobutyrate	0.76	0.90	0.76	1.00	0.09	0.49	0.008	0.47
Isovalerate	0.90	1.09	0.91	1.27	0.16	0.26	0.002	0.35
Valerate	1.77	1.45	1.58	1.55	0.28	0.75	0.21	0.30
Acetate:propionate ratio	2.13	2.59	2.60	2.81	0.45	0.04	0.03	0.40
Ruminal NH ₃ -N, mg/dL	16.30	18.20	15.91	17.81	2.57	0.85	0.40	0.96

¹ Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom.

² H = grass hay; 8.5 = 8.5% of roughage inclusion; 12.5 = 12.5% of roughage inclusion; Con = control (no fibrolytic enzyme addition); Enz = addition of fibrolytic enzyme.

³ VFA = volatile fatty acids; NH₃-N = ammonia nitrogen.

Table 10. Animal behavior of Nellore steers fed feedlot diets containing fibrolytic enzymes¹ and different levels of sugarcane bagasse or grass hay

Item, min/d	Treatment ²				SEM	P-value		
	8.5 Con	8.5 Enz	12.5 Con	12.5 Enz		Level	Enzyme	L*E
Sugarcane bagasse								
Intake time	183.8	170.0	193.8	186.3	11.42	0.27	0.37	0.79
Ruminating time	361.3	268.8	337.5	370.0	45.26	0.27	0.38	0.10
Resting time	895.0	1,001.3	908.8	883.8	51.87	0.26	0.37	0.17
Grass hay								
Intake time	192.5	208.8	220.0	223.8	19.21	0.11	0.42	0.61
Ruminating time	328.8	311.3	295.0	313.8	28.87	0.25	0.96	0.19
Resting time	918.8	920.0	918.8	902.5	24.86	0.56	0.62	0.56

¹ Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom.

² 8.5 = 8.5% of roughage inclusion; 12.5 = 12.5% of roughage inclusion; Con = control (no fibrolytic enzyme addition); Enz = addition of fibrolytic enzyme.

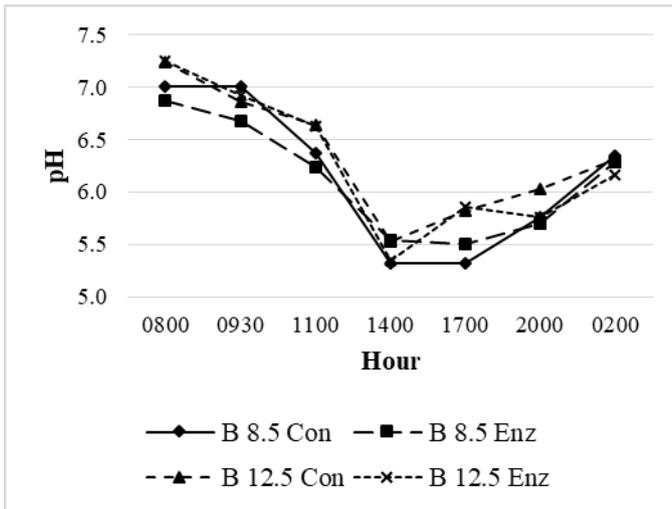


Figure 1. Effects of the use of fibrolytic enzymes (Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom) and different levels of sugarcane bagasse on ruminal characteristics of feedlot Nellore steers. B 8.5 Con = sugarcane bagasse, 8.5% of roughage inclusion, no fibrolytic enzyme addition; B 8.5 Enz = sugarcane bagasse, 8.5% of roughage inclusion, with addition of fibrolytic enzyme; B 12.5 Con = sugarcane bagasse, 12.5% of roughage inclusion, no fibrolytic enzyme addition; B 12.5 Enz = sugarcane bagasse, 8.5% of roughage inclusion, with addition of fibrolytic enzyme

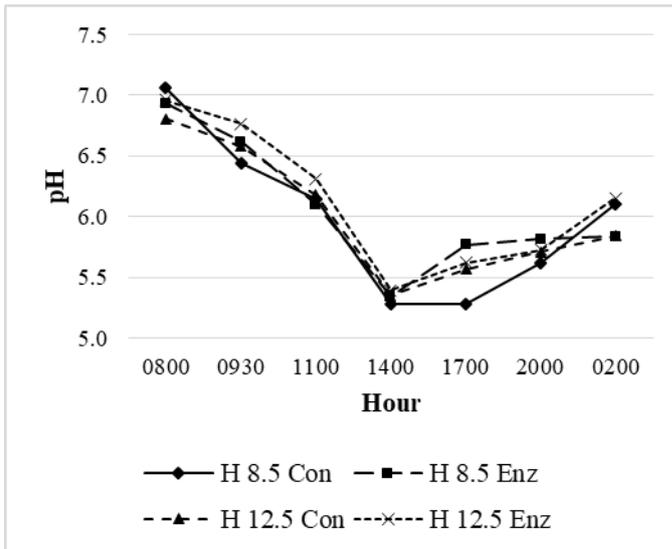


Figure 2. Effects of the use of fibrolytic enzymes (Vista PreT; ABVista, Marlborough, Wiltshire, United Kingdom) and different levels of grass hay on ruminal characteristics of feedlot Nellore steers. H 8.5 Con = grass hay, 8.5% of roughage inclusion, no fibrolytic enzyme addition; H 8.5 Enz = grass hay, 8.5% of roughage inclusion, with addition of fibrolytic enzyme; H 12.5 Con = grass hay, 12.5% of roughage inclusion, no fibrolytic enzyme addition; H 12.5 Enz = grass hay, 8.5% of roughage inclusion, with addition of fibrolytic enzyme