

RUMINANT NUTRITION

Fibrolytic enzymes improve the nutritive value of high-moisture corn for finishing bulls

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Abstract

Exogenous fibrolytic enzymes (EFE) improve the energy availability of grains for nonruminant animals by reducing encapsulation of the endosperm nutrients within grain cell walls; however, these benefits are unknown in the treatment of corn-based silage for cattle. The objective of the present study was to evaluate the effects of adding EFE at ensiling on the nutritive value of high-moisture corn (HMC) and snaplage (SNAP) for finishing Nellore bulls. The EFE dose was 100 g/Mg fresh matter in both HMC and SNAP. Diets were 1) a SNAP + HMC control (without enzyme addition); 2) SNAP + HMC EFE (with enzymes); 3) a whole-plant corn silage (WPCS) + HMC control (without enzyme addition); and 4) WPCS + HMC EFE (with enzymes). In addition to the silages, the diets were also composed of soybean hulls, soybean meal, and mineral–vitamin supplement. The statistical design was a randomized complete block with a factorial arrangement of treatments, and the experiment lasted 122 d. For in situ and in vitro analyses, 2 cannulated dry cows were used. There was no interaction between the diets and EFE application (ADG, $P = 0.92$; DMI, $P = 0.77$; G:F, $P = 0.70$), and there was no difference between the SNAP and WPCS diets regarding the DMI ($P = 0.53$), ADG ($P = 0.35$), and feed efficiency (ADG:DMI, $P = 0.83$). Adding EFE to the HMC and SNAP at ensiling did not affect ADG but decreased DMI ($P = 0.01$), resulting in greater feed efficiency by 5.91% ($P = 0.04$) than that observed in animals fed diets without the addition of EFE. Addition of EFE to HMC resulted in reduced NDF content and increased in vitro and in situ DM digestibility compared with untreated HMC. No effects were found for the addition of EFE to SNAP. Fecal starch decreased with EFE application ($P = 0.05$). Therefore, the diet energy content (TDN, NE_m , and NE_g) calculated from animal performance increased ($P = 0.01$) with the addition of EFE to HMC. In conclusion, exchanging the NDF from WPCS with that from SNAP did not affect the performance of finishing cattle, whereas the addition of EFE to HMC at ensiling improved animal performance by increasing the energy availability of the grain.

Key words: feed efficiency, fermentation, high-moisture corn, snaplage, xylanase

Abbreviations:

DM corr	dry matter corrected for silage volatile compounds
EE	ether extract
EFE	exogenous fibrolytic enzymes
HMC	high-moisture corn
ISDMD	in situ digestibility of dry matter
ISNDFD	in situ digestibility of neutral detergent fiber
ISStarchD	in situ starch digestibility
IVTDDM	in vitro true digestibility of dry matter
N	nitrogen
NE _m	net energy for maintenance
NE _g	net energy for gain
NFC	nonfiber carbohydrate
NSPs	nonstarch polysaccharides
pef	physically effective factor
rNDF	roughage neutral detergent fiber
SBW	shrunk body weight
SNAP	snapple
TDN	total digestible nutrients
TMR	total mixed ration
VU	visco-units
WPCS	whole-plant corn silage
WSC	water-soluble carbohydrates

Introduction

The use of corn-based silages has increased in Brazilian feedlots. The number of feedlot consulting nutritionists that have chosen to use high-moisture corn (HMC) as the primary processed grain has increased from zero (Millen et al., 2009) to 3% (Oliveira and Millen, 2014) and then to 6% (Pinto and Millen, 2016). In the United States, approximately 17% of feedlots use HMC (Samuelson et al., 2016). Moreover, the same pattern occurred for whole-plant corn silage (WPCS), increasing from 25.8% in 2009 to 27.3% in 2014 and then to 63.6% usage in 2016; since then, it has been considered the primary roughage source in Brazil. Although snapple (SNAP) has not been mentioned in the surveys, it seems that it is an economical silage alternative (Mahanna, 2008) for improving animal performance (Akens and Shaver, 2014). SNAP is defined as a product containing corn grain, cob, husk, ear shank, and other parts, such as leaves, that are harvested and processed with a self-propelled harvester equipped with a snapper head. Additionally, use of SNAP permits an increase in the stocking rate by approximately 10% when compared with the rate for the same area for corn grain and WPCS production (Daniel et al., 2019).

Studies conducted in Brazil determined that the inclusion of ensiled corn kernels in diets can improve the feed efficiency of growing/finishing cattle by an average of 14% as a result of an average 12% feed intake reduction (Berndt et al., 2002; Costa et al., 2002; Putrino, 2006; Henrique et al., 2007; Silva et al., 2007; Almeida Júnior et al., 2008; Carareto, 2011; Caetano et al., 2015; Silva, 2015; Tres, 2015; Silva, 2016). However, these benefits of grain ensiling are based on three major factors (Gomes et al., 2018): moisture content (Owens et al., 1997), particle size (Rémond et al., 2004), and length of storage time (Hoffman et al., 2011).

HMC and SNAP are harvested when corn kernels are fully mature, which means that the starch granules are embedded in a matrix of storage proteins and accompanied by cell walls and other minor components that are necessary for embryo

germination. The flinty endosperm has shown decreased ruminal degradability; however, the benefits of increased starch digestibility provided by grain ensiling seem to be greater for flinty than for floury endosperm (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002; Pereira et al., 2004). The proteins in the grain endosperm are hydrophobic zeins, which are classified into four subclasses: α , β , γ , and δ (Hoffman et al., 2011). The cell walls in the endosperm consist mainly of arabinoxylan, which encapsulates nutrients, creating a cage effect (Evers and Millar, 2002; Le et al., 2013). According to Kim et al (2005), nonstarch polysaccharides (NSPs) may decrease nutrient digestibility by entrapping the starch and protein in nonruminant diets. Applying carbohydrases to pig diets, which are composed mainly of cereal grains, has been well-documented to increase the utilization of diet energy by breaking down the NSPs present in the ingredient cell walls (Cozannet et al., 2018). Because the pH of the silage is lower and its temperature is relatively higher than those of the rumen, the application of exogenous fibrolytic enzymes (EFE) at ensiling rather than being provided directly to the animal can optimize the enzyme activity and its effects (Adesogan et al., 2014).

The use of EFE with xylanase as the main active enzyme improves in vitro digestion (Phakachoe et al., 2013; Romero et al., 2015) of WPCS and bermudagrass haylage. However, to the best of our knowledge, no study has investigated the application of EFE at ensiling to HMC and SNAP that are fed to finishing bulls.

Therefore, our hypothesis assumed that EFE applied at ensiling could increase animal performance by increasing the digestibility of silages combined with a high percentage of grains, such as HMC and SNAP, by reducing the cage effect of the cell walls of starch granules. The objective was to evaluate the performance of Nellore bulls fed finishing diets composed of HMC silage and SNAP supplemented with EFE at ensiling.

Materials and Methods

A performance trial was carried out at the Nutripura Research Center (Pedra Preta, MT, Brazil). All animal procedures were performed in accordance with the guidelines of the Animal Care and Use Committee of the Luiz de Queiroz College of Agriculture.

Ensiling

HMC, SNAP, and WPCS were stored for at least 45 d before the start of a feeding trial. The corn crop was purchased from a commercial farm near the experimental beef feedlot. The SNAP and WPCS silages were ensiled in drive-over silos, whereas HMC was ensiled in Ag-Bags (2.74 m in diameter and 60 m in length; Ipesasilos, São Paulo, Brazil). The ensiling period occurred from June 20 to July 4 2017. All silo experiments were conducted in duplicate, and the first set of silos was opened at the beginning of the performance trial (day 0). The storage periods for HMC, SNAP, and WPCS were 50, 45 and 55 d, respectively. The second set of HMC, SNAP, and WPCS silos was opened after 118, 126 and 108 d of fermentation, respectively. Each silo of HMC, SNAP, and WPCS had, on average, 125, 121, and 345 tons of material, respectively.

At ensiling, HMC and SNAP were treated with an EFE complex (Rovabio Advance P, Adisseo, Antony, France) at a dose of 100 mg/Mg fresh matter. The EFE complex was dissolved in chlorine-free water at a concentration of 0.025 g/L to facilitate the distribution of EFE onto HMC and SNAP. For the HMC, the product was applied by using an onboard device for additive application in a bagger and adjusted to a dose of 100 g/Mg fresh matter. In the SNAP silos, the product was sprayed and mixed

onto the material using a tractor-mounted sprayer during silo filling. To ensure an adequate dose, the product was applied according to the batch weight of each truck.

The EFE complex was composed of 2 major active enzymes, endo-1,4- β -xylanase (E.C. number 3.2.1.8) and endo-1,3(4)- β -glucanase (E.C. number 3.2.1.6), obtained from *Talaromyces versatilis* strains. The applied dose resulted in a concentration of more than 2,500 visco-units (VU) of endo-1,4- β -xylanase and 1,720 VU of endo-1,3(4)- β -glucanase per kg of fresh matter. One VU of xylanase or β -glucanase is defined as the quantity of enzyme required to hydrolyze a standard substrate (wheat arabinoxylan or barley β -glucan, respectively) and reduce the solution viscosity by one arbitrary unit per minute at 30 °C and pH 5.5. The β -glucanase to xylanase ratio was 0.688:1. Additionally, 2 control silos (without EFE) were made for both HMC and SNAP, at the same time that the EFE-treated silos were made. The WPCS received no treatment at all. The HMC was harvested with 68% dry matter (DM) using a combine harvester and transported to the research center. It was processed right before ensiling using a roller mill mounted on a bagger machine. The rolls were adjusted to crack grains into 4 to 5 parts. The SNAP was harvested when it reached 67% DM using a self-propelled forage harvester combined with a snapper head. The theoretical cut length of the SNAP was 14 mm, with grain processing at a 1-mm roll clearance. WPCS was harvested with 42% DM using a self-propelled forage harvester with a theoretical cut length of 16 mm at a 1-mm roll clearance.

The environmental conditions for the ensiling period were an average temperature of 25.5 °C (minimum of 18 °C and maximum of 33 °C) and an average rainfall of 12.5 mm. However, there was no rainfall during the silage-making period.

Animals and housing

The feeding trial started on August 18 2017 and ended on December 21 2017 (122 d). Four hundred sixty-eight Nellore bulls that were 30 mo old were allocated to 16 feedlot pens (10 pens with 29 animals; 5 pens with 30 animals; 1 pen with 28 animals). The animals were weighed individually using a hydraulic squeeze chute equipped with a scale (Beckhauser Manejo Racional e Produtivo, Paranaíba, PR, Brazil) upon arrival to determine the shrunk body weight (SBW) (after 16 h of water and feed deprivation), which was 405 ± 42.9 kg. Furthermore, upon arrival, the bulls were dewormed (Combo and Ticson Ivermectin 1%, Ceva Saúde Animal, Paulínia, SP, Brazil) and vaccinated against botulism and carbuncle (Botulinomax, Ceva Saúde Animal, Paulínia, SP, Brazil) and foot-and-mouth diseases (Aftomune, Ceva Saúde Animal, Paulínia, SP, Brazil). Each feedlot pen was 420 m², with an uncovered soil floor and a feed bunk length of 12 m (approximately 40 cm per animal). During the trial, the animals had free access to a water trough.

Diets

Adaptation diets

Initially, in the first 6 d, all bulls were adapted to the feedlot facilities with a common receiving diet and then fed to step-up adaptation diets for 21 d prior to starting the trial. During the adaptation, the diets were altered to decrease the forage:concentrate ratio (Table 1). In the first week of the adaptation period, the animals received a diet with a 55:45 forage:concentrate ratio (diet 1), in the second week, a 45:55 forage:concentrate ratio (diet 2), and in the last week, a 35:65 forage:concentrate ratio (diet 3). During this phase, the

Table 1. Composition of the adaptation diets (DM basis) in the first 21 d with HMC and SNAP with or without the addition of EFE at ensiling

Ingredient, % DM	Diet 1 (7 d)		
	Diet 2 (7 d)	Diet 3 (7 d)	
Whole-plant corn silage	47.00	37.00	27.00
Snaplage (½ control and ½ EFE)	32.00	32.00	32.00
High-moisture corn (½ control and ½ EFE)	–	10.00	20.00
Soybean hulls	9.10	9.10	9.10
Soybean meal	8.30	8.30	8.30
Mineral and vitamin supplement ¹	3.60	3.60	3.60

¹Mineral and vitamin supplement composition (DM basis): 150 g/kg urea, 150 g/kg Ca, 4.5 g/kg P, 37 g/kg S, 10 g/kg Mg, 6 g/kg K, 38 g/kg Na, 350 g/kg Cl, 21 mg/kg Co, 250 mg/kg Cu, 6.7 mg/kg Cr, 19 mg/kg I, 620 mg/kg Mn, 4.5 mg/kg Se, 1,050 mg/kg Zn, 45 mg/kg F, 85,000 IU/kg vitamin A, 350 IU/kg vitamin E, and 800 mg/kg monensin. Manufactured by Nutripura, Rondonópolis, Brazil.

contents of the control silos and the silos inoculated with EFE were mixed in a 50:50 ratio to compose the SNAP and HMC used for the diets.

Experimental diets

After 21 d of adaptation, the bulls were deprived of their water and feed for 16 h and then weighed to determine the SBW. The initial SBW was 420 ± 48.2 kg. After weighing, bulls were assigned randomly to 4 blocks according to their SBWs (heaviest to lightest). Four feedlot pens composed each block. The number of animals per pen ranged from 28 to 30. To create the experimental diets, the silages were combined as follows: 1) SNAP control + HMC control; 2) SNAP with EFE + HMC with EFE; 3) WPCS + HMC control; and 4) WPCS + HMC with EFE. Although SNAP is considered an intermediate between high-cut corn silage and high-moisture shelled corn (Akens and Shaver, 2014), in the current experiment, it was considered a source of roughage instead of a source of starch because of its relatively high fiber content. Other diet ingredients were soybean meal, soybean hulls, urea, and mineral and vitamin supplements that contained monensin (Table 2). The pens within each block were assigned randomly to 1 of the 4 treatments. The diets were formulated to achieve the bull's requirements to gain approximately 1.5 kg/d SBW (NASEM, 2016). Additionally, diets were formulated to reach the same content of roughage neutral detergent fiber (rNDF) (12.6%) and to be iso-nitrogenous (13.0%). Ingredients and their inclusion levels in the experimental diets were chosen to represent diets used in most commercial feedlots in Brazil, as described by the surveys of Oliveira and Millen (2014) and Pinto and Millen (2016).

Feeding and animal performance

During the experiment, diets were fed twice daily, at 08:00 and 15:00. The feed was delivered to the animals using a pull-type wagon (model 3120, Kuhn, Saverne, France) equipped with a horizontal auger and a digital scale (± 2 kg). First, concentrate was premixed in the wagon (1 min), and then, the silages were added and mixed for 5 more minutes. To monitor the daily feed intake, a feed bunk score was used. Every morning, the target was score 1, which represented at least 300 g of DM per animal as orts per day (3% orts). Once a week, samples of silages and other ingredients were collected and dried overnight at 105 °C to correct the DM of the diets offered to the animals. To stabilize feed intake, feed bunks were monitored at night to ensure sufficient feed was present until the following morning. Orts

Table 2. Composition of the experimental diets (DM basis) with HMC and SNAP with or without the addition of EFE at ensiling

Ingredient, %	SNAP + HMC ¹		WPCS ² + HMC	
	CON	EFE	CON	EFE
Whole-plant corn silage	–	–	25.0	25.0
Snaplage	27.7	27.7	–	–
High-moisture corn	51.1	51.1	53.2	53.2
Soybean hulls	12.0	12.0	12.0	12.0
Soybean meal	5.40	5.40	6.00	6.00
Mineral and vitamin supplement ³	3.80	3.80	3.80	3.80
Nutrients, %				
Dry matter, % FM	72.2 ± 3.94	71.8 ± 3.88	67.6 ± 3.55	66.9 ± 3.40
Ash	5.00 ± 0.19	5.04 ± 0.09	5.28 ± 0.09	5.33 ± 0.05
Crude protein	12.9 ± 0.99	12.6 ± 0.13	13.00 ± 0.84	12.8 ± 0.21
Ether extract	3.60 ± 0.15	3.66 ± 0.06	3.39 ± 0.07	3.47 ± 0.09
Neutral detergent fiber	23.1 ± 2.34	22.8 ± 1.40	26.9 ± 1.14	26.0 ± 0.83
Roughage neutral detergent fiber	9.56 ± 2.17	10.19 ± 1.60	13.16 ± 0.42	13.16 ± 0.42
Starch	46.7 ± 3.2	47.1 ± 2.9	43.0 ± 2.6	44.2 ± 2.4
Nonfiber carbohydrates	48.6 ± 6.1	52.6 ± 2.7	45.3 ± 4.1	50.9 ± 1.1
pef > 8 mm ⁵ , %	20.2 ± 5.5	19.0 ± 5.7	33.8 ± 6.0	31.1 ± 6.0
pef > 4 mm ⁵ , %	53.9 ± 6.5	50.7 ± 4.6	62.9 ± 5.7	58.1 ± 5.1

¹Dose of EFE/animal = 1.05 g/d.²Dose of EFE/animal = 0.72 g/d; WPCS = whole-plant corn silage without EFE.³Mineral and vitamin supplement composition (DM basis): 250 g/kg urea, 150 g/kg Ca, 4.5 g/kg P, 37 g/kg S, 10 g/kg Mg, 6 g/kg K, 38 g/kg Na, 350 g/kg Cl, 21 mg/kg Co, 250 mg/kg Cu, 6.7 mg/kg Cr, 19 mg/kg I, 620 mg/kg Mn, 4.5 mg/kg Se, 1,050 mg/kg Zn, 45 mg/kg F, 85,000 IU/kg vitamin A, 350 IU/kg vitamin E, and 800 mg/kg monensin. Manufactured by Nutripura, Rondonópolis, Brazil.

were removed from the feed bunk whenever needed (old feed, when it was warm and visually deteriorated, or that soaked by rain). After removal, the orts were weighed and sampled for DM and then discounted from the quantity offered to that feedlot pen the day before. After the last day of the experiment, the orts were weighed and sampled for DM to calculate the feed intake of the period.

At the end of the experiment (days 119–122), shrunk bodyweights were collected following 16 h removal from water and feed to determine average daily gain (ADG) for the feeding period. The bulls from blocks 1, 2, 3, and 4 were weighed on December 18, 19, 20, and 21; respectively. Diet total digestible nutrients (TDN), net energy for maintenance (NE_m), and net energy for gain (NE_g) were calculated using mean values of the observed SBW, dry matter intake (DMI), and ADG of the bulls in each pen (Zinn and Shen, 1998). Those same energy variables were estimated for the HMC and SNAP silages with or without the EFE. This estimation proceeded by subtracting the observed energy of the diets (as mentioned above) from the energy of the diets without the energy contributions of the HMC and SNAP. For this, the TDN value of each ingredient was estimated using the equations of Weiss et al., (1992).

From December 18 to 21, during weighing and before slaughtering, the carcass quality of the animals was evaluated by using ultrasound scanning technology approved by the Ultrasound Guidelines Council (Pleasantville, IA). The measurements in the animals were made using an Aloka 500 ultrasound scanner (Aloka Co, Tokyo, Japan). Postanalysis of the data was performed using BIA software (Design Genes Technologies Brasil, Presidente Prudente, SP, Brazil). The Nellore bulls had their ribeye area, fat thickness, and marbling measured using this technology. For ribeye area and backfat thickness measurements, an ultrasound probe was placed between the 12th and 13th ribs, above the *Longissimus* muscle. Regarding the marbling, the probe was placed between the 11th and 13th ribs.

Before each measurement, the animal was properly contained, and soy oil was applied to the skin surface to better conduct the ultrasound waves. Subsequently, the bulls were shipped to a commercial slaughterhouse (JBS S.A., Pedra Preta, MT, Brazil), and the hot carcass weight (HCW) was determined by summing the weight of the 2 carcass halves.

Fecal collection

From days 57 to 59 of the first half of the experimental period and from days 101 to 103 of the second half, the feces of the bulls were collected to estimate the concentration of starch in feces. Ten fresh fecal spots of different animals were sampled directly from the ground, avoiding dirt, to compose 1 sample per pen per day (Sartec, 2007). The collection times in the three consecutive days followed the following sequence: day 1—07:00, day 2—12:00, and day 3—18:00. Samples from the 3 d were combined, resulting in 1 sample per pen per period (first half or second half of the experimental period). Right after the collection, the samples were dried to a constant weight at 60 °C in an air-forced oven and then ground to pass a 1-mm screen of a Wiley mill (Arthur H. Thomas, Philadelphia, PA).

Chemical and physical analysis

During the experimental period, samples of the silages and ingredients were collected once per week and subsequently frozen. The collected samples were dried in a forced-air oven for 72 h at 55 °C and ground to pass through a 1-mm mesh screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Subsamples were analyzed for DM, ether extract (EE), and ash according to the Association of Official Analytical Chemists (AOAC) (1990; methods 934.01, 920.39 and 924.05, respectively). The NDF was analyzed according to Mertens (2002) using amylase and sodium sulfite for the silages. Nitrogen was analyzed by the Dumas method 990.03 (AOAC, 2006) using a nitrogen analyzer (FP-2000A, Leco Corp., St. Joseph, MI). Crude protein was obtained

by using the multiplication factor $6.25 \times \text{N\%}$. Soluble protein was estimated from the difference between the total nitrogen of the samples and the insoluble nitrogen after the application of a borate-phosphate buffer solution (Krishnamoorthy et al., 1982). The nonfiber carbohydrate (NFC) was calculated according to Hall (2000) as $\text{NFC} = 100 - (\text{CP} + \text{EE} + \text{ash} + \text{NDF})$. For the silages, soluble carbohydrates were extracted using an 80% ethanol solution (Hall, 2000), and the starch content was analyzed using an enzymatic hydrolysis method according to Hall (2009).

Twenty-five grams of HMC, SNAP, and WPCS subsamples were weighed, and 225 g of deionized water was added and mixed for 4 min at 152 rpm using an automatic agitator. The resulting extract was filtered with 3 layers of cheesecloth, and its pH was measured (DM 20 pH meter, Digimed Analítica, SP, Brazil). Then, it was centrifuged at $10,000 \times g$ for 15 min at 4 °C, and the concentrations of lactic acid (Price, 1969) and volatile fatty acids (VFA) were quantified. Concentrations of VFA, alcohols, and esters were analyzed using a gas chromatograph coupled to a mass spectrometer (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) and were separated using a capillary column (Stabilwax, Restek, Bellefonte, PA; 60 m, 0.25 mm, i.d., 0.25 μm). The DM content was corrected for the volatile compounds according to the equation proposed by Weissbach (2009).

To separate the grain fraction from the stover in SNAP and WPCS, a hydrodynamic separation method was applied (Savoie et al., 2004). The grains were weighed (250 g), dried at 60 °C in an air-forced oven for 72 h, and then analyzed for particle size distribution using a Ro-Tap shaker (Bertel Ltda., Caieiras, SP, Brazil) equipped with 9 sieves with nominal square apertures of 9.50, 6.70, 4.75, 3.35, 2.36, 1.70, 1.18, and 0.59 mm and the bottom pan.

Sorting behavior was assessed once per week according to the method of Leonardi and Armentano (2003). The roughage silages (SNAP and WPCS), the diets, and the orts underwent particle size distribution measurements using 3 sieves (19, 8, 4 mm, and the bottom pan) of the Penn State Particle Size Separator according to the procedure described by Maulfair et al. (2011). Physically effective factors (pef's) were obtained through the sum of the as-fed fractions retained on the sieves with apertures of 19 mm and 8 mm ($\text{pef} > 8 \text{ mm}$) and of 19, 8, and 4 mm ($\text{pef} > 4 \text{ mm}$).

Silage digestibility

Subsamples of HMC, SNAP, and WPCS silages were subjected to in situ and in vitro digestibility assays. For the in situ incubation, 15 g of the dry ground samples were placed into $10 \times 20 \text{ cm}$ woven bags (R1020 Forage Bag, ANKOM Technology, Macedon, NY). According to the producer, the porosity of the bag was $50 \pm 10 \text{ micron}$. The ratio of the sample size to the free bag surface area was 37.5 mg/cm^2 . Each bag was tied 1 cm below the top with rubber bands, and clips were used to attach it to a chain. To permit adequate degradation, the chain with the bags was incubated in the ventral sac of the rumen for 6 and 12 h for HMC and 24 and 48 h for both SNAP and WPCS; bags were removed simultaneously. Two cannulated dry cows fed 55% (DM) corn silage and 45% (DM) concentrate were used to incubate the silages. Each cow received a replicate of the samples, and blank bags were added to correct the weight for the bag tare weight. After removal, all bags were washed 5 times using a washing machine to remove adherent feed particles and bacteria. Bags were dried for 48 h at 60 °C and then weighed to calculate DM digestion. Residues of the bags were analyzed for NDF and starch content, as described previously, to estimate the respective digestibilities.

The in vitro true digestibility (IVTD) of the silages was determined using F57 filter bags and a DAISY II incubator (ANKOM Technology, Macedon, NY). Rumen inoculum was collected from a fistulated Nellore bull fed a diet composed of 10% (DM) sugarcane bagasse and 90% (DM) concentrate. The bags were incubated for 48 h. To remove microbial debris and any remaining soluble fractions, the bags were placed in a TE-149 fiber analyzer (Tecnal Equipamentos, Piracicaba, Brazil) with a neutral detergent solution.

The chemical and physical characteristics, digestibility, and fermentative profile of WPCS are presented in Table 3.

Statistical analysis

Means and SD are shown for the chemical, physical, digestibility, and fermentative profile analyses of HMC and SNAP treated or not treated with EFE.

The statistical design for the analysis of animal performance was a randomized complete block with a factorial arrangement of two diets (SNAP + HMC or WPCS + HMC) and 2 enzyme treatments (control or EFE). Data were analyzed using the PROC MIXED procedure of SAS software using the following model: $Y_{ijk} = \mu + B_i + D_j + T_k + DT_{jk} + e_{ijk}$, where μ = the overall mean, B_i = random effects of blocks ($i = 1, 2, 3, \text{ or } 4$), D_j = fixed effects of diets ($j = \text{SNAP or WPCS}$), T_k = fixed effects of EFE ($k = \text{control or EFE}$), DT_{jk} = interaction between the diet and EFE, and e_{ijk} = residual error. The experimental unit for all performance variables was the pen. Means were considered statistically

Table 3. Data for the whole-plant corn silage

Nutrient	Mean \pm SD
Starch, % DM	24.7 \pm 4.3
Neutral detergent fiber, % DM	46.6 \pm 1.7
Crude protein, % DM	6.75 \pm 0.56
Ether extract, % DM	2.51 \pm 0.28
Ash, % DM	3.20 \pm 0.33
Soluble protein CP, % of CP	48.8 \pm 11.5
Digestibility in vitro and in situ	
IVTDDM, % DM	70.7 \pm 2.8
ISDMD 24 h, % DM	70.2 \pm 1.2
ISDMD 48 h, % DM	81.4 \pm 1.3
ISNDFD 24 h, % NDF	43.2 \pm 1.4
ISNDFD 48 h, % NDF	64.8 \pm 2.2
ISStarchD 24 h, % starch	77.8 \pm 2.1
ISStarchD 48 h, % starch	86.5 \pm 1.2
Fermentative profile (DM basis)	
DM corr ¹ , % as fed	48.1 \pm 6.0
pH	4.28 \pm 0.12
WSC, %	1.86 \pm 0.19
Lactic acid, %	0.77 \pm 0.29
Acetic acid, %	0.26 \pm 0.08
Ethanol, %	0.06 \pm 0.02
1-Propanol, mg/kg	136 \pm 146
2,3-Butanediol, mg/kg	23.4 \pm 13.2
Propionic acid, mg/kg	47.6 \pm 30.9
Ethyl lactate, mg/kg	13.4 \pm 6.8
Butyric acid, mg/kg	4.85 \pm 5.18
Ethyl acetate, mg/kg	3.00 \pm 2.17
Grain and fiber processing	
Grains < 4.75 mm, %	54.9 \pm 7.6
pef > 8 mm, %	65.2 \pm 5.0
pef > 4 mm, %	87.0 \pm 2.4

¹DM corr = dry matter corrected for silage volatile compounds using Weissbach's (2009) equation.

significant when $P \leq 0.05$, and tendencies were declared when $P > 0.05 \leq 0.10$.

Results

Table 4 presents the chemical composition, digestibility, and physical characteristics of HMC and SNAP. In the HMC, the NDF content of the control and EFE treatments was 7.36% and 5.70%, respectively (difference of 22.55%). When the percentages of pefs of SNAP were compared with those of WPCS, pef > 8 mm was 31.8% vs. 65.2%, and pef > 4 mm was 74.1% vs. 87.0%.

Characteristics related to silage fermentation are presented in **Table 5** for both the HMC and SNAP silages. According to the results, the DM values of the HMC and SNAP silages with EFE or not and corrected for the volatile compounds (DM corr) presented very similar means. Ethyl acetate increased by 57.14% in EFE-treated HMC silages compared with the untreated HMC (2.75 vs. 1.75 mg/kg). Acetic acid, one of the precursors of ethyl acetate, was found in the enzyme-treated HMC, but had contents of 0.12% and 0.07% in the untreated HMC. Less acetic acid was found in EFE-treated SNAP than in the corresponding control (0.11% vs. 0.16%).

The sorting index of the bulls is shown in **Table 6**. Regarding large particles (larger than 19 mm), medium particles (between 19 and 4 mm), and small particles (smaller than 4 mm), there was no intake preference by the bulls fed the experimental diets ($P > 0.11$).

Outcomes related to animal performance and carcass characteristics of the total experimental period (27–122 d) are shown in **Table 7**. The initial SBW (27 d) and final SBW (122 d) did not differ among treatments ($P > 0.31$). Diet and EFE treatments did not affect carcass characteristics such as HCW, carcass yield, ribeye area, ribeye area ratio, marbling, or fat thickness ($P > 0.22$).

The DMI of the bulls was affected by the EFE only (**Table 7**). There was no difference ($P = 0.35$) between the DMI of the SNAP- and WPCS-fed bulls. The EFE application reduced ($P = 0.01$) DMI by 3.98% but did not affect the ADG ($P = 0.53$). As a result, the

feed efficiency was higher ($P = 0.04$) by 5.91% for diets with EFE than for diets without EFE addition. The SNAP and WPCS diets did not alter the ADG ($P = 0.53$) and, consequently, the feed efficiency ($P = 0.83$) of the bulls.

Data regarding the energy fractioning of the silages and diets are presented in **Table 8**. Bulls that were fed the diet with SNAP silage consumed more starch (265 g) than those consuming diets composed of WPCS ($P = 0.01$). The feces of the animals fed SNAP diets had 13.82% more starch content than that of the animals fed diets with WPCS ($P = 0.11$). The addition of EFE in the silages tended to reduce ($P = 0.06$) the starch intake by 95 g and reduced ($P = 0.05$) the starch content of the feces by 15.17% when compared with those of silages without EFE addition. The variables related to energy content (TDN, NE_m , and NE_g) were lower ($P < 0.01$) for SNAP than for HMC. Regarding the TDN of the experimental diets, the diet factor (SNAP or WPCS) had no effect, but EFE diets increased it ($P = 0.01$) by 3.54% when compared with that of the diets without EFE addition. Likewise, EFE addition in the silages increased ($P = 0.01$) the NE_m (by 4.36%) and NE_g (by 5.77%) of the diets ($P = 0.01$).

Discussion

The presented results partially refute the null hypothesis; however, the EFE can affect animal performance by reducing the fecal starch content and thus reducing the DM intake, which improves starch availability and therefore feed efficiency.

When applied to the HMC silages, EFE treatment resulted in reduced NDF content and higher values for in vitro and in situ DM digestibility after 6 h of incubation. [Eun et al. \(2007\)](#) stated that the ratio between endoglucanase and xylanase is essential for improving the digestibility in WPCS, and that it should be more than 0.4:1. The ratio for the EFE in the current study was 0.69:1. According to [Romero et al. \(2016\)](#), this treatment, which provided high xylanolytic capacity, increased the DMI in early-lactation dairy cows when applied directly onto TMR, which was composed of 35% corn silage and 10% bermudagrass silage

Table 4. Chemical composition (mean \pm SD) of the HMC and SNAP silages with or without the addition of EFE at ensiling

Nutrient, % DM	HMC		SNAP	
	CON	EFE	CON	EFE
Starch, %	69.4 \pm 5.6	71.4 \pm 3.2	41.6 \pm 6.7	39.7 \pm 5.8
Neutral detergent fiber, %	7.36 \pm 1.25	5.70 \pm 0.71	34.6 \pm 7.9	36.9 \pm 5.8
Crude protein, %	7.88 \pm 1.53	7.38 \pm 0.59	7.27 \pm 1.10	7.26 \pm 0.87
Soluble protein CP, % CP	78.6 \pm 9.5	80.4 \pm 3.2	48.3 \pm 10.5	44.7 \pm 12.5
Ether extract, %	4.83 \pm 0.16	4.94 \pm 0.18	3.43 \pm 0.27	3.40 \pm 0.27
Ash, %	1.28 \pm 0.17	1.17 \pm 0.10	2.12 \pm 0.41	2.42 \pm 0.53
Digestibility in vitro and in situ				
IVTDDM, % DM	95.6 \pm 1.9	96.8 \pm 0.7	79.8 \pm 2.9	77.4 \pm 3.2
ISDMD 6 h/24 h, % DM	91.7 \pm 1.8	93.8 \pm 0.6	75.3 \pm 5.7	68.4 \pm 6.9
ISDMD 12 h/48 h, % DM	94.2 \pm 1.9	94.7 \pm 0.6	86.6 \pm 2.2	83.8 \pm 2.5
ISNDFD 6 h/24 h, % NDF	13.4 \pm 4.2	8.89 \pm 5.9	37.1 \pm 5.0	28.5 \pm 3.7
ISNDFD 12 h/48 h, % NDF	22.9 \pm 5.8	18.7 \pm 6.9	62.0 \pm 3.0	60.9 \pm 3.0
ISStarchD 6 h/24 h, % starch	95.1 \pm 2.4	96.4 \pm 1.6	82.8 \pm 5.3	77.1 \pm 8.7
ISStarchD 12 h/24 h, % starch	97.2 \pm 1.1	97.3 \pm 1.2	92.0 \pm 1.8	88.8 \pm 2.5
Grain and fiber processing				
Grains < 4.75 mm, %	77.2 \pm 6.0	84.1 \pm 3.7	77.1 \pm 4.1	76.3 \pm 3.4
pef > 8 mm, %	–	–	32.5 \pm 2.3	31.1 \pm 2.9
pef > 4 mm, %	–	–	74.6 \pm 3.1	73.5 \pm 5.7

Table 5. Fermentative profile (mean \pm SD) of HMC and SNAP silages with or without the addition of EFE at ensiling

Item	HMC		SNAP	
	CON	EFE	CON	EFE
DM corr ¹ , % AF	68.6 \pm 2.9	67.7 \pm 1.3	67.0 \pm 5.0	68.6 \pm 3.1
pH	4.37 \pm 0.12	4.28 \pm 0.06	4.40 \pm 0.12	4.40 \pm 0.13
WSC, %	1.06 \pm 0.24	1.37 \pm 0.99	1.52 \pm 0.12	1.47 \pm 0.20
Lactic acid, %	0.53 \pm 0.13	0.56 \pm 0.13	0.40 \pm 0.24	0.32 \pm 0.11
Acetic acid, %	0.07 \pm 0.02	0.12 \pm 0.05	0.16 \pm 0.07	0.11 \pm 0.02
Ethanol, %	0.07 \pm 0.04	0.07 \pm 0.02	0.03 \pm 0.01	0.02 \pm 0.01
1,2-Propanediol, mg/kg	115 \pm 74	631 \pm 593	299 \pm 210	152 \pm 60
1-Propanol, mg/kg	39.7 \pm 30.1	35.6 \pm 20.8	113.7 \pm 73.9	62.2 \pm 33.7
2,3-Butanediol, mg/kg	7.67 \pm 6.30	6.50 \pm 4.22	17.2 \pm 7.18	17.5 \pm 9.02
Ethyl lactate, mg/kg	18.2 \pm 6.07	19.7 \pm 5.59	7.08 \pm 3.25	5.75 \pm 2.37
Propionic acid, mg/kg	18.2 \pm 12.5	15.1 \pm 13.4	42.2 \pm 17.9	33.1 \pm 16.6
Butyric acid, mg/kg	3.58 \pm 3.41	2.33 \pm 2.98	3.33 \pm 1.38	2.33 \pm 1.55
Ethyl acetate, mg/kg	1.75 \pm 1.23	2.75 \pm 1.02	1.50 \pm 0.79	1.33 \pm 0.84

¹DM corr = dry matter corrected for silage volatile compounds using [Weissbach's \(2009\)](#) equation.

Table 6. Sorting index of the bulls fed experimental diets with HMC and SNAP with or without the addition of EFE at ensiling

Item	SNAP + HMC		WPCS + HMC		SEM	P-value		
	CON	EFE	CON	EFE		Diet	Enzyme	D \times E ¹
> 19 mm	101	101	101	101	0.20	0.67	0.49	0.32
19–4 mm	101	101	101	100	0.32	0.11	0.22	0.88
<4 mm	98.3	98.2	98.5	99.4	0.44	0.12	0.33	0.27

¹D \times E = diet \times enzyme interaction.

(DM basis). However, EFE application did not improve nutrient digestibility in the mentioned study. The increase in digestibility was associated with the increased availability of soluble carbohydrates generated by the hydrolysis of the arabinoxylan chains. In situ starch and NDF digestibility differences were only numerical in the HMC treated with fibrolytic enzymes. The increase in grain starch digestion was expected because according to [Watson \(1987\)](#), the breakage of corneous endosperm (flint grain hybrids) using a dry-milling process occurs along the cell walls as a result of the strength of the protein matrix. Thus, the endosperm cell wall represents a second barrier to starch digestion. The NDF fraction of the corn grain is encountered mostly in the pericarp and in the endosperm. The pericarp comprises approximately 51% grain fiber, including the starchy endosperm, aleurone endosperm, and germ, which are responsible for 12%, 15%, and 16%, respectively, of the fiber grain content. Hemicellulose accounts for 70% of the NDF in the grains ([Watson, 1987](#)), and xylose and arabinose account for 90%–95% of hemicellulose ([Oomiya and Imazoto, 1982](#)). Therefore, the EFE used in the current experiment, primarily exhibiting xylanase activity, could hydrolyze the hemicellulose, liberating xylan monomers for fermentation in the silo. However, the resistant NDF would decrease the digestible fraction for the rumen bacteria.

Another product that confirms fiber fermentation in HMC was the derivative ester of acetic acid, ethyl acetate, which increased numerically after EFE treatment. Ethyl acetate is formed during the fermentation of silages by combination of ethanol and acetic acid, but its generation is more dependent on the alcohol concentration than on the carboxylic acid concentration ([Weiss](#)

[et al., 2016](#)). Since the ethanol concentration remained relatively constant between the EFE-treated and control samples, the acetic acid concentration was a major factor in increasing the ethyl acetate content in the present study. According to studies by [Fred et al. \(1919\)](#) and [Dehghani et al. \(2012\)](#), the increase in acetic acid could be associated with the fermentation of xylans in the silo. Using doses of xylanase in sugarcane silage, [Del Valle et al. \(2018\)](#) also found increased acetic acid content with intermediate levels of the fibrolytic enzyme (185 mg/kg DM).

In contrast, these benefits were not observed when EFE were applied to the SNAP silages. This likely occurred because of the starch content, which was approximately 44% less than that in the HMC silages, since the same dose (100 g/ton FM) was used for both the HMC and SNAP silages and the major effect was expected to result from the grain fraction. In addition to grains, cobs, and husks, some of the upper plant material is also present in SNAP ([Nigon et al., 2016](#)). The corn cob composition is 41.2% cellulose, 36.0% hemicellulose, only 30% xylan, and 6.1% lignin ([Bagby and Widstrom, 1987](#)). EFE affected SNAP silages in only the digestibility of NDF after 24 h of incubation, and they tended to reduce this digestibility. Similarly, [Lynch et al. \(2015\)](#) applied EFE to corn silages at ensiling and found a reduction in NDF digestibility after 24 h of ruminal incubation. The authors claimed that EFE act on compounds that are particularly susceptible to hydrolysis during fermentation, leaving only the resistant fraction of the fiber. Conversely, the starch content presented in the SNAP was much less than expected. Comparing the performances of dairy cows fed diets with HMC and those fed diets with SNAP, [Akins and Shaver \(2014\)](#) reported that the value of starch content for SNAP was approximately 61%. In the present study, the starch

Table 7. Performance and carcass characteristics of Nellore bulls fed diets with HMC and SNAP with or without the addition of EFE at ensiling

Item	SNAP + HMC		WPCS + HMC		SEM	P-value		
	CON	EFE ²	CON	EFE		Diet	Enzyme	D × E ¹
Initial shrunk body weight (SBW), kg	423	420	420	419	22.9	0.45	0.31	0.66
Final SBW, kg	571	569	568	570	19.0	0.87	0.97	0.63
average daily gain (ADG), kg	1.48	1.52	1.52	1.54	0.045	0.53	0.53	0.92
dry matter intake (DMI), kg/day	9.51	9.10	9.58	9.23	0.305	0.35	0.01	0.77
Feed efficiency	0.1568	0.1678	0.1593	0.1670	0.0088	0.83	0.04	0.70
Hot carcass weight, kg	319	320	320	318	13.1	0.91	0.91	0.62
Carcass yield, %	55.8	56.1	56.2	55.8	0.61	0.89	0.82	0.34
Ribeye area, cm	82.8	84.3	84.4	84.1	2.60	0.55	0.61	0.48
Ribeye area ratio ²	0.488	0.489	0.491	0.490	0.0031	0.38	0.93	0.85
Marbling, score	3.48	3.48	3.40	3.46	0.072	0.51	0.65	0.70
Back fat thickness, mm	4.70	4.71	4.86	4.79	0.213	0.22	0.76	0.66

¹D × E = diet × enzyme interaction.²Ribeye area ratio = ribeye area height × width.**Table 8.** Fecal starch and observed energy of the silages and experimental diets

Item	SNAP + HMC		WPCS + HMC		SEM	P-value		
	COM	EFE	CON	EFE		Diet	Enzyme	D × E ¹
Starch intake, kg	4.44	4.29	4.12	4.08	0.14	0.01	0.06	0.24
Fecal starch, %	5.47	4.91	5.08	4.04	0.37	0.11	0.05	0.53
TDN silage, %	68.8	70.7	94.7	99.7	2.37	<0.01	0.25	0.61
NE _m silage, Mcal/kg	1.55	1.62	2.35	2.50	0.09	<0.01	0.22	0.68
NE _g silage, Mcal/kg	0.91	0.99	1.62	1.75	0.08	<0.01	0.19	0.76
TDN, % ²	80.5	83.5	80.6	83.3	1.08	0.94	0.01	0.83
NE _m , Mcal/kg ²	1.95	2.04	1.95	2.03	0.03	1.00	0.01	0.87
NE _g , Mcal/kg ²	1.30	1.38	1.30	1.37	0.03	0.96	0.01	0.81

¹D × E = diet × enzyme interaction.²Estimated from the diets using the equations of Zinn and Shen (1998).

values were 41.6% and 39.7% for the control and EFE-treated SNAP, respectively. According to Mahanna (2008), one of the concerns of SNAP usage is the variation that can occur from one operation to another. The risk of diluting the energy content of SNAP is increased since fibrous fractions, such as leaves above the ear, are susceptible to inclusion in the material. Therefore, the addition of increased doses of fibrolytic enzymes may be necessary to induce the same benefits when other sources of fibers (cob and husks) are included in grain silages. Furthermore, the lack of animal performance studies using SNAP makes it worthwhile to investigate.

In contrast to the planned diets, the SNAP and WPCS diets contained different contents of rNDF. However, feeding diets with SNAP or WPCS did not affect animal performance. The possible reason for this was the different NDF digestibility content between SNAP and WPCS. Both WPCS diets had 13.16% (DM basis) rNDF, in contrast to 9.56% in the SNAP control and 10.19% in the SNAP EFE diets. In the current study, the absence of animal performance results may be attributed to the lower digestibility of the NDF (less than 23.85% in 24-h NDF digestibility) for the SNAP silages than for the WPCS. This reduction in the SNAP silages is related to an increased amount of less-digestible components, such as cob and husks (Klopfenstein et al., 1987; Petzel et al., 2019). Although the pef > 8 mm and pef > 4 mm fractions of the WPCS were higher than those of the SNAP, the decreased digestibility of the SNAP silages might have resulted in similar chewing activity (Corrêa et al., 2003; Sá Neto et al.,

2014), although chewing activity was not measured in our study. Regarding the combination of SNAP with HMC or WPCS with HMC, there was no evidence of an associative effect between the less-processed and immature grains of WPCS and the HMC grains in the diets (Stock and Erickson, 2006).

Regarding the EFE application to ensiling, this treatment indeed altered the performance of the animals. The bulls fed diets treated with EFE had decreased feed intake, the same ADG, and thus greater feed efficiency than those fed the control diets. This could be caused by a reduction in the cage effect of the cell walls in the endosperm (Evers and Millar, 2002; Le et al., 2013), after which the nutrients, such as starch, could be more available for fermentation in the silo or in the rumen. It seems that once the prolamin of the endosperm are hydrolyzed by the plant and bacterial enzymes (Junges et al., 2017), which increases soluble protein content, the proceeding barrier to starch digestion is the cell wall of the endosperm. However, Zahiroddini et al. (2004) found that EFE in combination with a lactic acid bacterial inoculant applied in barley silage at ensiling increased the ADG and the feed efficiency of steers by hydrolyzing the fiber content of not just the grain but the whole silage. However, the results of experiments with sows that used EFE similar to those used in the present study showed an increase in the energy of corn-based and wheat-based diets (Cozannet et al., 2018). In agreement with that, the fecal starch content of the bulls fed EFE was decreased, and the TDN, NE_m, and NE_g of the treated diets were greater than those of the control groups.

The addition of EFE had an effect on only HMC, where it improved the nutrient utilization by finishing Nelore bulls, thus enhancing the feed efficiency by reducing their intake. Moreover, replacing WPCS NDF with SNAP NDF in the diets did not affect animal performance or carcass traits.

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Conflict of interest statement

None declared.

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