Effects of dietary exogenous fibrolytic enzymes on ruminal fermentation characteristics of beef steers fed high- and low-quality growing diets¹

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ABSTRACT: The effects of dietary pretreatment with fibrolytic enzyme-based cocktail were evaluated in 2 studies: (1) in vitro true digestibility; and (2) intake, digestibility, feeding behavior, and ruminal fermentation of beef steers fed growing diets. For the in vitro assessment, the ruminal in-oculum was collected from 2 steers (BW = 543 ± 45 kg; 4-h after feeding; growing diets) and enzymes included or not (*Trichoderma reesei* fermentation extract; 0.75 µL/g of substrate DM). Within in vitro batches ($n = 4$), 12 substrates were incubated and in vitro true nutrient digestibility was evaluated. For study 2, 5 ruminally cannulated beef steers (BW = 520 ± 30 kg) were used in a 5 × 4 unbalanced Latin square using a 2 × 2 factorial arrangement of treatments: (a) diet quality (high = HQ; and low = LQ) and (b) enzyme inclusion (0 or 0.75 mL/kg of diet DM). Steers were fed ad libitum during four 21-d periods consisting of 14-d of adaptation and 7-d of collections. An enzyme × substrate was observed ($P < 0.01$), in which DM, OM, and NDF disappearance of sorghum grain increased with enzymes addition. Addition of enzymes increased ($P < 0.01$) ADF digestibility for all substrates. No diet quality × enzyme ($P \geq 0.18$) was observed for intake variables in

study 2. Enzyme-fed steers increased ($P \leq 0.05$) intake of DM, digestible DM, NDF, and ADF compared with steers not fed fibrolytic enzymes. Addition of enzyme did not affect ($P \geq 0.28$) apparent total tract digestibility of beef steers. Steers fed HQ diets consumed more ($P \leq 0.04$) DM, digestible DM and OM, and less ($P \leq 0.03$) total and digestible fiber than steers fed LQ diets. Ruminal pH average decreased ($P = 0.01$) for steers fed HQ or enzyme-fed diets compared with other treatments. A tendency ($P = 0.06$) toward improved total VFA was observed on enzyme-fed steers with HQ diets, but not for LQ diets. The molar proportion of ruminal propionate increased ($P = 0.01$) when steers were fed enzyme. Steers fed HQ diets had greater ($P < 0.01$) propionate and valerate molar proportions, lower ($P < 0.01$) acetate and acetate:propionate ratio than steers fed LQ diets. In vitro methane and total gas production were not affected ($P \geq 0.50$) by dietary treatments. Fibrolytic enzymes positively affected digestion of multiple roughage sources commonly fed to cattle and might have additional benefit when used on unprocessed sorghum grain. Fibrolytic enzymes in beef cattle growing diets stimulated intake and generated positive impacts on ruminal fermentation.

Key words: cellulase, digestibility, enzyme, feeding behavior, growing diet

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INTRODUCTION

A large portion of beef cattle growing diets is composed of forages. The low digestibility of forages in ruminant diets may limit the supply of energy and nutrients. Several strategies may be

used to improve fiber digestion in cattle diets, such as selection of forage hybrids (Bean et al., 2013), chemical treatments (Shreck et al., 2015), and use of direct-fed microbials (Ovinge et al., 2018). In addition, some exogenous fibrolytic enzymes have also been developed to improve fiber digestibility by ruminants (Krueger et al., 2008). However, the efficacy of enzymes depends on factors, such as diet composition, enzyme dosage, and application strategy (Chung et al., 2012). The conferred potential mechanism may be improving the solubility of the fiber contents and increasing the surface area available for attachment by ruminal microbes. Subsequently, if cattle can readily degrade more fiber and increase the amount of digestible nutrients consumed, growth performance may also be positively affected (He et al., 2014, 2015).

The effect of exogenous fibrolytic enzymes on the feed efficiency of both beef and dairy cattle has been studied (Balci et al., 2007; He et al., 2014), and improvements in fiber and/or diet digestibility have been reported (Krause et al., 1998; Rode et al., 1999). However, the distinct feeding strategies employed when fibrolytic enzymes were tested have generated considerable variability as far as effects of enzymes on animal dietary intake. For instance, both a decrease (Beauchemin et al., 1999; Holtshausen et al., 2011) and an increase in dry matter intake (DMI) (Lewis et al., 1999) have been reported.

Many of the studies cited previously focused on high concentrate diets, and in only a few situations were ruminal characteristics assessed, in which none factored quality of diets. In addition, such digestion assessment involving a more comprehensive list of forage types exposed to fibrolytic enzymes treatment has not been performed yet. Therefore, the objectives of the current study were to: (1) assess in vitro digestion potential of 12 forage substrates commonly fed to beef cattle and (2) investigate the effects of exogenous fibrolytic enzymes on ruminal fermentation characteristics of beef steers fed high-quality (HQ) and low-quality (LQ) growing diets.

MATERIALS AND METHODS

All experimental procedures were conducted in accordance with an approved Texas Tech University Institutional Animal Care and Use Committee (IACUC protocol # 16025-04).

Study 1: In vitro True Digestibility

In vitro true digestibility (IVTD) of DM, OM, NDF, and ADF with and without addition of

fibrolytic enzymes were evaluated for 12 substrates in 4 subsequent batches. Substrates consisted of corncobs, intact sorghum grain, wheat straw, cotton burrs, corn stalks, cottonseed hulls, grape pomace, soybean hulls, HQ alfalfa, LQ alfalfa, sorghum stalks, and sorghum dry distillers' grains. Approximately 0.5 g (predehydrated in a forced-air oven for 24 h at 55 °C, and ground through a 1-mm screen) of each substrate sample was placed into a preweighed, acetone-washed filter bag (F57, ANKOM Technology Corp., Macedon, NY) and heat sealed. Each incubation jar (DAISYII Incubator, ANKOM Technology Corp.) contained 24 bags (12 substrates in duplicate) plus 1 blank bag. Two of the 4 incubation jars also contained 1.6 L of prewarmed (39 °C) McDougall's artificial saliva solution (McDougall, 1948) and 18 µL of fibrolytic enzymes [product of *Trichoderma reesei* fermentation extract; exogenous xylanase (EC 3.2.1.8) with declared minimum activity of 21,000 µmol/min of reduced xylose equivalents) and cellulose (EC 3.2.1.4) with declared minimum activity of 600 µmol/min of reduced glucose (from hydroxyethyl cellulose); both at pH 4.8 and 50 °C [AB Vista, Marlborough, UK], while the other 2 remaining jars (control jars) of the same batch did not contain enzymes. All samples (sealed bags) within each batch were submerged in buffer solution inside an incubator (39 °C) for approximately 2 h before the addition of ruminal inoculum to the system. The ruminal fluid inoculum was collected from donors (ruminally cannulated beef steers; $n = 2$; BW = 543 ± 45 kg) 4-h after feeding, and inoculum transported to the Ruminant Nutrition Laboratory inside prewarmed (39 °C) 1 L thermos within ~30 min after collection. Four batches were performed, in which donors were assigned and maintained on respective forage-based diets both LQ and HQ; without exogenous fibrolytic enzymes (diets are depicted in Table 1) for 16 d before ruminal fluid collections. One liter of ruminal fluid from a steer fed HQ growing diet and 1 L from a steer fed LQ growing diet, both without addition of enzyme, were collected, filtered through 4 layers of cheesecloth, mixed, and 400 mL of inoculum was added in each jar, purged each digestion jar with CO₂ for 30 s, and then placed into DAISYII Incubator. Incubations followed a randomized complete block design (block = in vitro batch "experimental unit"). Following 48-h of incubation, bags were placed on ice for 1 h to stop microbial activity and frozen until further procedures. When all batches were processed, bags were thawed and rinsed with neutral detergent solution (ANKOM 200 Fiber Analyzer,

Table 1. Ingredients and nutritional composition of growing diets fed to beef steers

Item	Low quality (LQ)	High quality (HQ)
Ingredients, % DM basis		
Corn silage	—	36
Alfalfa hay, early vegetative	—	15
Cotton burrs	20	—
Sorghum stalks, hay	25	—
Urea	0.7	—
Molasses, cane	5	—
Steam-flaked corn	26.75	26.6
Wet corn gluten feed (sweet bran ¹)	15	15
Cottonseed meal	4	4
Yellow Grease	1	0.4
Limestone	0.55	1
Supplement ²	2	2
Analyzed ³ nutritional composition		
NE maintenance, Mcal/kg ⁴	1.59	1.81
NE gain, Mcal/kg ⁴	1.01	1.19
CP, % DM	13.68	14.62
NDF, % DM	38.47	27.60
ADF, % DM	21.84	13.51
EE ⁵ , % DM	3.02	3.06
Ca, % DM	0.75	0.75
P, % DM	0.37	0.45
K, % DM	1.41	1.13
Mg, % DM	0.19	0.29
S, % DM	0.18	0.2

¹Cargill Corn Milling, Blair, NE.

²Supplement contained (DM basis) 67.7538% carrier (cottonseed meal), 0.5% antioxidant (Endox; Kemin Industries, Inc., Des Moines, IA), 3.76% urea, 10% potassium chloride, 15% sodium chloride, 0.0022% cobalt carbonate, 0.1965% copper sulfate, 0.0833% iron sulfate, 0.0031% ethylenediamine dihydroiodide, 0.167% manganous oxide, 0.1 25% selenium premix (0.2% Se), 0.9859% zinc sulfate, 0.0099% vitamin A (1,000,000 IU/g), and 0.157% vitamin E (500 IU/g) and provided (dietary) 30 mg/kg of monensin (0.75% Rumensin-90 in supplement; Elanco Animal Health, Indianapolis, IN) and 9 mg/kg of tylosin (0.5063% Tylan-40 in supplement; Elanco Animal Health).

³Analyzed composition from a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX) with the exception of CP, which was analyzed using LECO.

⁴Calculated composition using tabular values (NASEM, 2016).

⁵EE = ether extract.

ANKOM Technology Corp.). Original subsamples and incubation residues were then analyzed in sequence for NDF and ADF (Van Soest et al., 1991), corrected for DM (forced-air oven, 24 h at 100 °C, according to AOAC (1995)), and ashed (ash-oven, 550 °C overnight, AOAC (2005)).

Study 2: Treatments, Design, and Feeding

Ruminally cannulated crossbred beef steers ($n = 5$; BW = 520 ± 30 kg) were assigned to 1 of

4 treatments. A 5 × 4 unbalanced Latin square (5 steers and 4 diets) was used. Steers were housed individually at the Ruminant Nutrition Center located at New Deal Research and Education Center, Texas Tech University (Idalou-TX). The indoor facility contained individual stalls (3.5 × 3.5 m) equipped with automatic water troughs, a concrete floor with a drainage system, and a heater/cooling system. The facility was washed twice daily, once before feeding and again in the afternoon. Steers were fed ad libitum (expected 5% refusals, DM basis), once daily (1,000 h) through 4 to 21 d periods (14 d for adaptation and 7 d for collection). At the end of each period, steers had access to outside individual paddocks (15 × 25 m) for 48 h, in which diets were offered ad libitum following the study treatment arrangement. Treatments were arranged as a 2 × 2 factorial, with factors including growing diet quality (high = HQ and low = LQ) and addition of pretreatment enzyme cocktail to the diets, as shown in Table 1 (0 and 0.75 mL/kg of DM; enzymatic activity previously described). Diets were mixed twice weekly and stored under refrigeration at 5 °C. Diets without enzymes were prepared first using a tractor-pulled mixer wagon (Roto-Mix 84-8, Roto-Mix, Dodge City, KS; scale readability of 0.45 kg). Enzyme extract products were applied to diets after dilution (1:10 with distilled water) and mixed for 5 min. Intake was calculated based on DM offered after subtracting DM refused. Dietary samples were collected daily during the 7-d of the collection periods, assessed DM content, and fresh subsamples composited across the 7 d to be used for further analysis.

Ruminal pH and Volatile Fatty Acids

On day 14 of each period, ruminal pH probes (DASCOR, Escondido, CA) were calibrated and adjusted to continuously assess the ruminal pH (every 6 min) for entire collection period (7 d). On day 18 and 19, ruminal fluid (45 mL, Falcon centrifuge conical tubes) was collected from each steer and filtered through 4 layers of cheesecloth at 2, 4, 8, 16 and 24 h after-feeding. Samples (triplicate) were frozen (−20 °C), then transported to Ruminant Nutrition Laboratory for VFA analyses. Samples were thawed and centrifuged to analyze VFA in ruminal fluid (10,000 × g; 10 min; 4 °C). The supernatant from each sample (4 mL) was treated (deproteinized) with 0.8 mL of 25% metaphosphoric acid containing 2-ethylbutyrate (0.2005 g in 100 mL) as internal standard (Erwin et al., 1961). Individual VFA were analyzed in

duplicate utilizing gas chromatography (Shimadzu GC-8A, Shimadzu Scientific Instruments Inc., Columbia, MD; Supelco SP-1200, 2 m × 5 mm × 2.6 mm glass column, Supelco/Sigma-Aldrich Inc., Bellefonte, PA) according to procedures of [Goetsch and Galyean \(1983\)](#).

In Vitro Gas Production and Methane

On day 17 of each period, rumen fluid was collected to quantify in vitro total gas and methane production ([Quinn et al., 2010](#)). One liter of rumen fluid from each steer in each experimental period ($n = 4$) was collected 4 h after-feeding, filtered through 4 layers of cheesecloth, and placed in a pre-warmed thermos (39 °C). Samples were immediately transported to Ruminant Nutrition Laboratory to be used for in vitro procedure. A 50 mL aliquot of rumen fluid from each thermos (1 per steer) was incubated in a 160 mL serum vial (Wheaton Science Products, Millville, NJ); a total of 4 serum vials (quadruplicate) was used for each steer per incubation period. Results obtained from quadruplicate were averaged for data analysis. The serum bottles were then flushed with CO₂ for 15 s (high-purity, analytical grade), sealed with a rubber stopper and aluminum ring, and incubated at 39 °C for 24 h with constant agitation at 125 rpm (Lab-Line Environ-Shaker, LabLine Instruments Inc., Melrose Park, IL). Following 24 h incubation, total gas production was measured by water displacement by piercing the rubber stopper in the serum vial with a needle attached to 250 mL inverted burette. The sample of headspace gas remaining in each bottle after total gas was retained for measuring methane concentration as outlined by [Ponce et al. \(2012\)](#) using gas chromatography.

Total Tract Apparent Nutrient Digestibility

Samples of diets and refusals were collected daily, and spot fecal samples were collected twice daily (0800 and 1700 h) during the last 5 d of each collection period. Diets, refusals, and feces were composited across days, within the same steer within each experimental period. Samples were stored at (−20 °C) until analyzed. Frozen samples were thawed and dried at 55 °C, in a forced-air oven. After 72 h of drying, samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1 mm screen. Acid-insoluble ash (AIA) was used as an internal marker and analyzed in diets, refusals, and fecal samples to estimate total fecal output, which was used for the apparent total tract digestibility calculation. The concentration of

AIA in offered diets was adjusted for the AIA remaining in daily refusals. Such adjustment yielded the amount of AIA consumed by animals, which was used for further calculations. For AIA procedure, a 5 g aliquot of each ground sample was dried at 135 °C for 2 h, weighed, and ashed at 450 °C overnight, then samples were transferred to a Berzelius beaker, and 2 N HCl (100 mL) was added to each unit. After boiling for 5 min on a condensation digestion apparatus, the sample was filtered using a Whatman ash-less filter (No. 41) and washed with boiling distilled water ([Van Keulen and Young, 1977](#)). The washed filter with its residue was placed into the muffle furnace (450 °C) overnight. Improved laboratory logistics and method precision can be achieved if the same crucible used for the initial sample incineration is used to hold the washed filter with its residue. After incineration, residues were weighed to determine the AIA concentration. Total fecal output was calculated by the following equation: (AIA intake/concentration of AIA in feces) × 100. Samples, DM, ash, CP, NDF, ADF, and hemicellulose (HEM) were also analyzed for each sample. The AIA concentration consumed and observed in fecal samples were used to calculate total tract apparent digestibility of individual nutrients, as described by [Ovinge et al. \(2018\)](#).

Laboratory Analyses

Daily diet subsamples were dried at 100 °C in a forced-air oven for 24 h (only used to adjust DMI), and all other samples were predehydrated at 55 °C in a forced air oven for 48 h before analyses. Later, all analyses were corrected using the DM (100 °C; forced-air oven) to yield values on a DM basis. Samples were ground through a 1-mm screen (Wiley Mill; Thomas Scientific, Swedesboro, NJ) before analysis. Fiber contents of NDF and ADF were analyzed in sequence (duplicate), according to [Van Soest et al. \(1991\)](#) with the modifications proposed in the Ankom device manual (ANKOM Technology Method, Macedon, NY), of using sodium sulfite, alpha-amylase, and correcting for ash in the residue. Crude Protein was analyzed in LECO TruMac N (LECO, St. Joseph, MI; Method 4.2.10; [AOAC, 1997](#)). Ash content was determined by burning each sample in a muffle furnace at 600 °C for 4 h and used to determine OM (method 942.05; [AOAC, 1990](#)).

Feeding Behavior

During d 15 to 16 of each period, a 24 h behavioral evaluation was performed. Visual observations

were taken every 5 min, for the following behaviors: eating, drinking, ruminating, resting, and active, as described by Campanili et al. (2017). Chewing activity was accounted for by adding time spent eating with total time spent ruminating. All animals were evaluated for all periods during 24 h; thus, missing values were not present in data. Time spent in each activity was expressed as minutes per day. Rumination and chewing times per kilogram of DM, OM, NDF, ADF, HEM, and digestible nutrients intake were also calculated and corrected for nutrients consumed.

Statistical Analyses

Data were analyzed using the GLIMMIX procedures of SAS (SAS Inst. Inc., Cary, NC). For study 1 (IVTD), substrate type and interactions (enzyme $\times$ substrate) were used as fixed effects. In vitro batch was considered the experimental unit. For study 2, the fixed effects of diet quality, enzyme addition, and the interactions (diet quality $\times$ enzyme addition) were used. In the analyses of intake and ruminal pH, day was treated as a repeated measure, and for the ruminal VFA concentration analysis, collection time was treated as a repeated measure. Covariance structures for repeated measures were chosen based on the smallest Akaike information criterion (AIC). Steer within a sequence of dietary treatments was used as a random effect. For both studies, when interactions were not significant ($\alpha > 0.05$), they were removed from the model. The general degrees of freedom procedure Kenward–Rogers was used to adjust for any bias on standard errors caused by multiple terms in the random statement. Significant differences were considered if $P \leq 0.05$ and tendencies declared if $0.05 > P \leq 0.10$.

RESULTS

IVTD: Study 1

An enzyme $\times$ substrate interaction resulted in a 13.2% increase ($P < 0.01$) in IVTDMD of intact sorghum grain. In addition, a tendency ($P = 0.10$) for greater digestion in corn stalks was also observed, while the other 10 substrates DM digestibility was not affected ($P > 0.10$) by the exogenous enzymes (Figure 1A). Similar pattern was observed for the IVTOMD (data not shown), in which intact sorghum grain was enhanced ($P < 0.01$) with addition of enzymes, and a tendency for increased digestion of corn stalks ($P = 0.06$) were also

observed with the inclusion of enzymes, while no effect was observed for the others substrates ($P > 0.10$). Intact sorghum grain also showed improvement in IVTNDFD when treated with enzymes ($P < 0.01$) of approximately 16.4% compared with the same substrate not treated with enzymes. Other substrates NDF digestibility was not affected ($P > 0.10$; Figure 1B) by enzyme treatment. Regardless of substrate type ($P = 0.45$), the inclusion of enzymes increased ($P < 0.01$) IVTADFD by approximately 13.6% for all substrates (Figure 1C).

Intake and Feeding Behavior: Study 2

No diet $\times$ enzyme interaction ($P \geq 0.18$) was observed for intake variables (Table 2). Except for HEM intake ($P = 0.05$), in which steers fed the LQ diet with enzymes increased HEM consumption, but not when the HQ diet was fed. Regardless of diet quality ($P \geq 0.51$), steers fed growing diets with enzymes had greater DMI ($P = 0.04$), digestible DMI ($P = 0.05$), and digestible NDF or ADF intakes ($P \leq 0.02$) by approximately 6, 14, 21, and 24%, respectively, compared with steers fed growing diets without enzymes. A tendency ($P < 0.09$) for a greater intake of OM and digestible OM when enzymes were included was also observed. Intake of NDF, ADF, and HEM was not affected ($P > 0.24$) by dietary pretreatment with exogenous fibrolytic enzymes. Regardless of dietary pretreatment with enzymes, steers fed HQ diets consumed more DM, OM, and digestible DM ($P \leq 0.04$), and exhibited lower ($P \leq 0.03$) intake of total fiber and digestible fiber variables than steers fed LQ diets.

No diet $\times$ enzyme interactions ($P \geq 0.17$) were observed for feeding behavior variables, except for a tendency ($P = 0.09$) for eating time, in which the inclusion of fibrolytic enzymes on LQ diets increased steers eating time (min/d), but not for HQ diets (Table 3). Regardless of enzyme inclusion, steers fed HQ diets spent less time eating ($P < 0.01$), chewing ($P < 0.01$), chewing/kg of DMI and OMI (both; $P < 0.01$), and chewing/ kg of digestible DMI and OMI (both; $P < 0.01$); and greater time resting ($P < 0.01$) than those fed LQ diets.

Total Tract Apparent Digestibility

No diet quality $\times$ enzyme addition interaction ($P \geq 0.21$) was observed for digestibility of nutrients (Table 2). The addition of fibrolytic enzymes to the diet of beef steers did not affect the digestibility of nutrients ($P \geq 0.28$). However, a tendency ($P = 0.06$) for interaction between diet quality $\times$

Figure 1. IVTD of 12 byproducts commonly fed to beef cattle, incubated with or without fibrolytic enzyme (ALFH = HQ alfalfa hay; ALFL = LQ alfalfa hay; CTBR = cotton burrs; CSTK = corn stalks; CSH = cottonseed hulls; GRPM = red grape pomace; SBH = soybean hulls; SDDGS = sorghum dry distillers grains plus solubles; ISG = intact sorghum grain; SRST = sorghum stalks; and WHST = wheat straw). (A) Dry matter–DM [enzyme $\times$ substrate ($P < 0.01$) enzyme increased ($P < 0.01$) DM digestibility for ISG and tended ($P = 0.10$) to increase for CSTK]. (B) NDF [enzyme $\times$ substrate ($P < 0.01$) enzyme increased ($P < 0.01$) NDF digestibility for ISG]; and (C) ADF [enzyme $\times$ substrate ($P = 0.45$); greater ($P < 0.01$) ADF digestibility was observed for substrates added with enzymes compared with those without enzyme].

Table 2. Intake, total fecal output and total tract apparent nutrient digestibility of beef steers fed LQ or HQ growing diets with or without pre-dietary treatment with exogenous fibrolytic enzymes (Enz)

	High Quality		Low Quality			P -values		
Variables	Control	Enz	Control	Enz	SEM ₁	Diet	Enz	Diet × Enz
Intake, kg/d								
DM	14.95	15.59	13.51	14.79	0.912	0.02	0.04	0.51
OM	13.74	14.47	12.16	13.09	0.808	<0.01	0.07	0.82
NDF	4.24	4.19	5.27	5.74	0.304	<0.01	0.24	0.19
ADF	2.06	2.07	3.05	3.28	0.167	<0.01	0.25	0.28
HEM	2.19	2.11	2.25	2.49	0.140	0.01	0.28	0.05
CP	2.46	2.59	1.98	1.97	0.139	<0.01	0.36	0.37
Digestible intake, ² kg/d								
DM	10.78	12.72	9.37	10.77	0.846	0.04	0.05	0.74
OM	10.34	12.12	8.98	10.08	0.802	0.09	0.09	0.65
NDF	2.28	2.87	3.25	4.10	0.283	<0.01	0.02	0.65
ADF	1.03	1.45	1.75	2.22	0.155	<0.01	<0.01	0.87
HEM	1.29	1.40	1.54	1.84	0.143	0.03	0.16	0.52
CP	1.61	1.99	1.27	1.38	0.145	<0.01	0.11	0.36
Fecal output, kg/d of DM	3.7	2.9	4.1	3.1	0.54	0.50	0.87	0.06
Digestibility, %								
DM	76.8	79.1	70.6	70.4	2.00	<0.01	0.39	0.27
OM	78.7	80.8	74.5	73.8	2.06	<0.01	0.56	0.21
NDF	64.1	66.4	67.3	66.1	3.27	0.45	0.78	0.34
ADF	62.7	66.8	64.6	63.7	4.09	0.78	0.44	0.22
HEM	65.2	65.8	70.4	68.6	4.71	0.41	0.91	0.80
CP	73.5	71.5	67.7	66.1	2.21	<0.01	0.28	0.90

¹Standard error of the mean ($n = 5$ steers/treatment).

²Digestible intake calculated using current data for nutrient digestibility averages from each dietary treatment.

³Main effects presented inside the table. For hemicellulose (HEM), the interaction diet type × enzyme inclusion ($P = 0.05$) is depicted in results.

enzyme addition was observed for fecal output, in which addition of enzymes on HQ diets decreased fecal output compared with steers fed other treatments, while the greatest fecal output was observed for steers fed LQ diet with addition of enzymes. Regardless of enzyme inclusion, as expected, steers fed HQ diets showed greater total tract apparent digestibility of DM, OM, and CP ($P < 0.01$) than steers fed LQ diets.

Ruminal pH

No interactions ($P \geq 0.17$) for diet quality × enzyme inclusion were observed for ruminal pH, except for a tendency ($P = 0.07$) to decrease pH magnitude of change (maximum–minimum) with inclusion of enzymes in steers fed HQ diets only (Table 4). The inclusion of enzymes decreased ($P = 0.03$) the peak value of ruminal pH and increased ($P = 0.03$) the time in which the ruminal pH was below the pH threshold of 6.0. However, enzymes showed no effect on ruminal pH minimum value ($P = 0.54$), time below ruminal pH 5.6 ($P = 0.30$), and area below ruminal pH 6.0 and 5.6 ($P > 0.24$). Regardless of dietary pretreatment

with enzymes, steers fed HQ diets exhibited a lower ($P < 0.01$) ruminal pH peak, pH minimum ($P \leq 0.01$), and a greater ($P < 0.02$) time and area in which the ruminal pH was below thresholds of 6.0 and 5.6 compared with LQ diets. A diet quality × enzyme interaction ($P = 0.01$) was observed for ruminal pH average, in which steers fed HQ diets with enzymes had the least (5.77; SEM = 0.057) average (Figure 2), followed by those fed HQ diets without enzymes (5.97; SEM = 0.057), LQ diets with enzymes (6.29; SEM = 0.057), and the greatest values for those fed LQ diets without enzymes (6.40; SEM = 0.057), respectively. Regardless of enzyme addition, the ruminal pH average reached the lowest levels between 5 to 11 h after-feeding for LQ diets (5.91; SEM = 0.086), while steers fed the HQ diets presented least ruminal pH average (5.55; SEM = 0.086) between 8 to 14 h after-feeding (time × diet, $P < 0.01$; Figure 2).

Ruminal Volatile Fatty Acids

A tendency ($P = 0.06$) for interaction between diet quality × enzyme was observed, in which the addition of enzymes increased total

Table 3. Feeding behavior of beef steers fed LQ or HQ growing diets with or without pretreatment with exogenous fibrolytic enzymes (Enz)

Item	High quality		Low quality		SEM ¹	P-value		
	Control	Enz	Control	Enz		Diet	Enz	Diet × Enz
Time, min/d								
Ruminating	430	399	427	441	17.1	0.51	0.77	0.45
Eating	149	149	272	314	5.5	<0.01	0.09	0.09
Drinking	26	28	20	12	3.3	0.15	0.69	0.50
Resting	811	829	691	653	18.6	<0.01	0.77	0.42
Activing	24	35	30	20	3.6	0.58	0.95	0.21
Chewing ²	579	548	699	755	17.7	<0.01	0.68	0.17
Rumination rate, min/kg of intake								
DM	29	27	31	30	3.1	0.34	0.78	0.53
OM	32	29	34	35	3.5	0.23	0.73	0.51
NDF	97	94	85	89	10.0	0.38	0.95	0.70
ADF	190	190	157	164	20.8	0.15	0.88	0.86
HEM	202	187	188	198	21.3	0.94	0.89	0.47
Rumination rate, min/kg of digestible intake								
DM	39	34	47	44	6.1	0.16	0.50	0.90
OM	41	36	49	46	6.4	0.17	0.56	0.86
NDF	153	141	149	142	25.7	0.96	0.71	0.92
ADF	318	288	302	267	58.7	0.74	0.56	0.97
HEM	311	279	300	306	50.3	0.86	0.78	0.68
Chewing rate, min/kg of intake ²								
DM	40	36	50	54	3.6	<0.01	0.98	0.35
OM	43	39	55	60	4.2	<0.01	0.94	0.30
NDF	130	129	139	153	12.0	0.16	0.61	0.51
ADF	257	262	256	279	26.9	0.77	0.61	0.74
HEM	271	256	309	340	24.9	0.01	0.72	0.29
Chewing rate, min/kg of digestible intake ²								
DM	53	47	75	75	7.6	<0.01	0.68	0.75
OM	55	50	79	79	7.9	<0.01	0.74	0.68
NDF	206	192	238	241	33.6	0.23	0.87	0.80
ADF	433	394	480	452	80.9	0.50	0.66	0.95
HEM	418	379	485	520	63.3	0.09	0.98	0.53

¹Standard error of the mean ($n = 5$ steers/treatment).²Chewing activity calculated by adding time spent eating and total time spent ruminating.

VFA production for steers fed HQ growing diets, but not when those were fed LQ diet (Table 5). No 3-way interactions (diet quality × enzyme addition × time; $P = 0.24$), diet quality × time ($P = 0.33$), or enzyme addition × time ($P = 0.90$) interactions were observed. However, regardless of diet quality and enzymes addition, total VFA molar proportion peaked ($P < 0.01$) at 8 to 16 h after-feeding, with intermediate values being observed at 2 and 4 h after-feeding (data not shown). Regardless of diet quality, the addition of enzymes induced an increase ($P = 0.01$) in propionate molar proportion, which led to a tendency ($P = 0.09$) to decrease the acetate:propionate ratio in steers fed HQ or LQ diets. Regardless of enzyme addition, steers fed HQ diets had greater ($P < 0.01$) molar proportion of propionate and valerate ($P < 0.01$), and lower

molar proportion of acetate ($P < 0.01$), resulting in a lower acetate:propionate ratio ($P < 0.01$) compared with steers fed LQ diets.

In Vitro Gas Production and Methane

No diet × enzyme interaction ($P \geq 0.47$) was observed for methane production expressed as a percentage of total gas or methane volume expressed as milliliters per liter of rumen fluid, or total gas and methane expressed as milliliters per gram of rumen fluid DM (Table 6). The inclusion of fibrolytic enzymes on growing diets had no effect ($P \geq 0.50$) on in vitro gas and methane productions. Regardless of addition of fibrolytic enzymes, diet quality did not affect in vitro gas production ($P = 0.82$) or methane (percentage of total gas; $P = 0.64$), while a tendency

Table 4. Ruminal pH variables of beef steers fed LQ or HQ growing diets with or without pre-dietary treatment with exogenous fibrolytic enzymes (Enz)

Item	High-quality diet		Low-quality diet		SEM ¹	P-value		
	Control	Enz	Control	Enz		Diet	Enz	Diet x Enz
pH								
Average	5.97	5.77	6.40	6.29	0.057	<0.01	<0.01	0.01
Maximum	6.73	6.20	6.94	6.80	0.143	0.01	0.03	0.17
Minimum	5.14	5.34	5.62	5.54	0.106	<0.01	0.54	0.16
Magnitude of change ²	1.49	0.85	1.34	1.27	0.161	0.38	0.04	0.07
Time, min/d								
Bellow pH 6.0	713	1034	254	357	99.4	<0.01	0.03	0.25
Bellow pH 5.6	334	580	57	87	127.5	<0.01	0.30	0.41
Area, pH × min								
Bellow pH 6.0	296	456	67	97	78.5	<0.01	0.24	0.43
Bellow pH 5.6	90	131	9	13	39.2	0.02	0.57	0.65

¹Standard error of the mean ($n = 5$ steers/treatment).

²For pH magnitude of change (maximum–minimum) the interaction diet type × enzyme inclusion tendency ($P = 0.07$) is depicted in results.

Figure 2. Average ruminal pH (10 measurements per hour) by hours after-feeding of beef steers fed LQ or HQ growing diets with or without pretreatment with exogenous fibrolytic enzymes. No 3-way interaction was observed (diet quality × enzyme addition × time, $P = 0.98$). An interaction for diet quality × enzyme addition ($P = 0.01$) was observed, in which both HQ and LQ diets had lower ruminal pH average when the enzyme (Enz) was added compared with non-added enzymes diet (Cont). Regardless of enzyme addition, the ruminal pH average reached the lowest levels between 5 to 11 h after-feeding for LQ diets, while steers fed HQ diets presented least ruminal pH average between 8 and 14 h after-feeding (time × diet, $P < 0.01$).

($P = 0.09$) for an increase in methane (milliliters per liter of rumen fluid) was observed for the rumen fluid of steers fed LQ diets. Additionally, steers fed LQ diets showed an increase ($P = 0.01$) in methane production (milliliters per grams of rumen fluid DM) compared with that fed HQ. Despite dietary treatments, total gas production was approximately 1357 mL/L of rumen fluid; of which, methane represented ~25% of the in vitro total gas produced.

DISCUSSION

Current IVTD assessment suggests that the addition of current fibrolytic enzymes on the 12

substrates tested (commonly used byproducts on beef cattle diets) has the potential to increase the ADF digestion, and additional digestion benefits on DM, OM, and NDF might be expected depending on substrate type. Moreover, it may have an additional positive effect on intact sorghum grain and corn stalks. Those findings are in accordance to Balci et al. (2007) and Holtshausen et al. (2011), in which an improvement with the addition of fiber degrading enzymes (cellulose- and xylanase-based enzymes) on common forages (wheat straw, alfalfa hay, alfalfa silage, and barley silage) used in cattle diets were observed on in vitro of DM, OM, NDF, and ADF, with additional

Table 5. Molar proportion and profile of ruminal volatile fatty acids (VFA) of beef steers fed LQ or HQ growing diets with or without pre-dietary treatment with exogenous *fibrolytic enzymes* (Enz)

Item	High-Quality Diet		Low-Quality Diet		SEM ₁	P-value		
	Control	Enz	Control	Enz		Diet	Enz	Diet × Enz
Total VFA, mM/L	104.3	112.8	91.8	125.2	3.50	<0.01	0.09	0.06
C2:C3	3.1	2.8	3.5	2.4	0.24	<0.01	0.09	0.79
<i>VFA profile, mM/100 mM tVFA²</i>								
Acetate	63.5	61.6	66.8	58.4	1.18	<0.01	0.17	0.53
Propionate	21.9	22.9	19.3	25.5	1.65	<0.01	0.01	0.86
Butyrate	11.3	12.1	11.2	12.2	0.91	0.41	0.52	0.38
Isobutyrate	0.8	0.8	0.8	0.8	0.08	0.24	0.49	0.87
Valerate	1.2	1.1	0.8	1.7	0.15	<0.01	0.18	0.99
Isovalerate	1.4	1.3	1.3	1.4	0.11	0.33	0.30	0.84

¹Standard error of the mean ($n = 5$ steers/treatment).²Total VFA.³Main effects presented inside the table. For total VFA (mMol/L), the interaction diet type × enzyme inclusion tendency ($P = 0.06$) is depicted in results.**Table 6.** In vitro gas production and methane variables of ruminal fluid collected from beef steers fed LQ or HQ growing diets with or without pre-dietary treatment with exogenous *fibrolytic enzymes* (Enz)

Item	High-quality diet		Low-quality diet		SEM ¹	P - value		
	Control	Enz	Control	Enz		Diet	Enz	Diet × Enz
Methane, % of total gas	29.49	25.04	30.42	29.73	5.963	0.64	0.67	0.76
<i>Volume, mL/L of rumen fluid</i>								
Total gas	1323	1448	1222	1436	248.4	0.82	0.50	0.86
Methane	311	287	363	411	53.2	0.09	0.80	0.47
<i>Volume, mL/g of rumen fluid DM</i>								
Total gas	27.72	28.97	33.86	37.23	5.287	0.18	0.66	0.84
Methane	6.66	5.90	10.08	10.96	1.451	0.01	0.97	0.57

¹Standard error of the mean ($n = 5$ steers/treatment).

positive effects on digestion exhibited for wheat straw and alfalfa hay. In contrast, when fibrolytic enzymes were used for wheat straw and alfalfa hay in study 1, an improvement in ADF digestibility was observed only (~13.6%). These differences might be related to the higher dosage (2 μ L/g of DM) of enzyme with endoglucanase and xylanase activities of 722 and 2,604 μ mol/mL-min of enzyme product, respectively, used by [Holtshausen et al. \(2011\)](#) or by a different source of cellulase and xylanase (*Trichoderma longibrachiatum*) enzymes with an activity of 1,200 μ mol/mL-min used by [Balci et al. \(2007\)](#). Therefore, the addition of fibrolytic enzymes has the potential to improve ruminal fiber degradation; however, dietary selection of ingredients during formulation might be an important factor to consider when aiming for better enzymatic effect on digestion. The fraction that has been degraded may also confer an important aspect. The ADF component of plant cell walls is known for their low digestibility profile (~40% for average reported in the current study), especially

in the stems of crop residues (~25% for average reported in the current study). Therefore, although not limited to the improvement of ADF digestion, the use of such strategy may become very attractive in situations where an ingredient with high ADF content is included in the diet. Assuming the evidence from current in vitro assessment (study 1), in which ADF digestion of substrates was improved, the simulation that follows depicts potential biological importance of such finding. For instance, an improvement of ~10% in ADF digestibility in LQ fibrous byproducts (such as sorghum stalks and cotton burrs, in which NDF and ADF contents within byproduct are similar) would induce an increase in ~2.6 and 1.5 percent-units in total digestible nutrients (TDN), respectively (calculating TDN by equation proposed by [Weiss \(1992\)](#) and using IVTD of ADF to represent improvement in fiber digestion). Assuming 1 kg of TDN = 4.409 Mcal of DE ([NASEM, 2016](#)); $ME = (0.9611 \times DE) - 0.30$ ([Galyean et al., 2016](#)); and the variable ME:DE ratio conversion equations to convert ME to NEm

and NEg (Galyean et al., 2016), such improvements in TDN would induce ~7.8; 13.7; 4.1; and 7.0% improvements in NEm and NEg available for sorghum stalks and cotton burrs, respectively. Therefore, if using the Beef Cattle Nutrient Requirement Model (NASEM, 2016) assuming a nutrient balanced growing diet for beef steer yearlings, with these 2 ingredients representing ~45% of the diet (DM basis), such as in a growing diet, an additional 4.6% ME allowed for gain might be expected (ADG = 0.98 vs. 1.03 kg). Such improvement in ADG (50 g/d) may be biologically important depending on how long animals are on feed. Such calculation assumes that fibrolytic enzymes would be affecting only the 2 ingredients used in the aforementioned simulation example, and DMI of animals is not affected. However, it is known that fibrolytic enzymes will be applied and thus subsequently exposed to the entire diet, in which fiber present on grain matrix could also be positively affected, as suggested by the greater intact sorghum grain *in vitro* digestion observed in the current study. Moreover, the potential additional intake of steers when fibrolytic enzymes are applied can dramatically improve the scenario, and perhaps potentially make such strategy very attractive.

The addition of fibrolytic enzymes on growing diets, regardless of quality, improved the DMI of steers by 6%, while inducing greater intakes of digestible portions of the diets such as digestible DM, OM, NDF, and ADF. Even though there was no significant improvement observed on the DM, OM, and fiber portions digestibility coefficients of growing diets. These observations agree with data observed by Álvarez et al. (2008) and Lewis et al. (1999), in which addition of enzyme on fiber-based diets improved the DMI even when no changes in total tract apparent digestibility were observed. That may be explained by the effect enzymes have on increasing the solubility of fiber portions; therefore, the density of the particles is reduced and the passage rate increased. Consequently, ruminal fill could be affected, and the increase of DMI could induce a similar response on the intake of digestible nutrients as well, which may lead to better utilization of the diets and feed efficiency, as reported by Lewis et al. (1999). If the additional nutrients consumed by steers fed the enzyme-treated diets bypassed the digestive tract, the nutrient total tract apparent digestibility would be negatively affected, which was not perceived in the current study. However, if fiber-degrading enzymes are included in grain-based diets, the opposite effect may be observed (Beauchemin et al. 1997, 1999; Holtshausen

et al., 2011). The addition of enzyme reduced the intake by cattle fed finishing or early lactating-cow diets by an average of 5%. Such effect may be attributed to a greater intake of digestible nutrients when gut fill is no longer the limiting factor, leading to a chemical feedback for satiety due to the quantity of metabolites produced during the additional digestion and absorption of nutrients by the animal, even with a lower intake (Beauchemin et al., 1997, 1999; Holtshausen et al., 2011). Furthermore, HQ diets in the current study also had greater DM and OM intakes, as expected due the greater quality of diet components and lower dietary levels of NDF, ADF, and HEM compared with the LQ diet treatments.

The total tract nutrient apparent digestibility of steers fed HQ and LQ growing diets was not affected by the addition of fibrolytic enzymes. Similar findings were also observed by multiple studies (Yang et al., 2000; Álvarez et al., 2008; He et al., 2014, 2015) in which the total tract apparent nutrient digestibility was not affected when xylanases and cellulases were used as pretreatment of diets or the forage portion of the diet. In contrast, Krueger et al. (2008) and Rode et al. (1999) observed improvements in DM and NDF total tract apparent digestibility by ~9.5 and 12.5%, respectively. It is interesting to highlight that Krueger et al. (2008) added the enzyme on hay individually before mixing as an ingredient in the diet (16.5 g/ton, air-dry basis). On the other hand, Rode et al. (1999) applied the enzyme on TMR similar to our current study. It has been previously documented that fibrolytic enzymes increase fiber digestibility, especially in fibrous ingredients as reported within the *in vitro* assessment in the current study. However, kinetics of digestion might be affected by the level of intake (Van Soest, 1994). In the current study, regardless of LQ or HQ, the pretreatment with fibrolytic enzymes induced a meaningfully greater DMI. Improved intake can be related to the improved profile of digestion of less-digestible fractions in the diet, such as fiber. Although animals might also start to consume more due to a potential decrease in the bulkiness of the slow-digested fiber fraction of enzyme pretreated diets. However, it is intuitive to attribute such an increase in consumption to an increased rate of passage of particles as well. The current study provides additional evidence of such increased ruminal metabolic activity when fibrolytic enzymes were used to pretreat growing diets, as further discussed on ruminal pH variables and ruminal VFA profile. In addition, the tendency exhibited by steers fed enzymes in LQ diets to

increase time spent eating, and the greater DMI observed for animals consuming diets pretreated with enzymes, may all be supportive to the hypothesis that overall dietary physical-structure (bulkiness) was somehow affected, especially when enzymes were used in LQ diets. Feeding behavior effects induced by dietary pretreatment with enzymes is unclear in the current study. However, it is known that a longer time spent chewing (which includes time spent eating) or ruminating increases saliva production. The saliva buffering agents neutralizes the acids from the fermentation of carbohydrates in the rumen (Owens et al., 1998), and the balance between the production and absorption of acids in the rumen and the secretion of salivary buffers is the main determinant of ruminal pH (Allen, 1997). For feedlot cattle, it is not known certainly how the relationship between diet NDF content supplied by roughage vs. the total dietary NDF content is associated with time chewing or rumination, saliva production, and finally to the ruminal pH (Vasconelos and Galyean, 2007).

Steers fed diets (regardless if HQ or LQ) pretreated with fibrolytic enzymes spent more time at ruminal pH below 6.0, causing a lower ruminal pH average. Moreover, a VFA molar proportion shifted toward a more energetic path, in which more propionate and a consequent reduction in the acetate:propionate ratio were observed. Such findings indicate an improved energetic efficiency of ruminal fermentation, which agrees with the greater intake of diets observed in the current study. In contrast, ruminal pH and ruminal VFA profile were not affected by other studies (Krause et al., 1998; Balci et al., 2007; Chung et al., 2012; He et al., 2014, 2015). It is interesting to highlight that some of these studies (He et al., 2014, 2015) animals were limit-fed, or ad libitum intake differences were not observed (Krause et al., 1998; Balci et al., 2007; Chung et al., 2012). Current findings suggest that even with no change on overall apparent tract nutrient digestibility, likely correlated to the greater intake observed, the pretreatment with fibrolytic enzymes may change the structure of the fiber exposed to the ruminal environment. McAllister et al. (2010) also reported a similar hypothesis. Such hypothesis could be supported by the rationale that a greater concentration of released sugars and monosaccharides could be utilized by amylolytic bacteria inducing a greater production of propionate, as well as decreasing ruminal pH and stimulating the intake of current growing diets pretreated with fibrolytic enzymes.

Furthermore, ruminal fluid collected from steers fed growing diets with addition of fibrolytic enzymes produced similar in vitro total gas and methane volume per liter of ruminal fluid, compared with those fed growing diets without addition of enzymes. Such findings agree to the results reported by Chung et al. (2012). These observations might be partially explained by the fact that although more propionate molar proportion was observed inside the rumen of steers fed pretreated diets with enzymes, the molar proportion of acetate was not affected. It is known that ruminal methanogenic microbiota potentialize methane synthesis when ruminal acetate is increased, due to its greater H₂ producing fermentation pathway, such as when forage-based diets are fed (Janssen, 2010).

Regardless of enzyme addition, as expected, the ruminal pH and VFA profile variables were in general intensified for steers fed HQ diets than steers fed LQ diets. The greater quality of ingredients selected for such treatment directly reflected current findings. In addition, beef steers fed HQ diets tended to produce less methane per liter of ruminal fluid than those fed LQ diets. This can be attributed to a less fibrous portion provided within the diet and more starch prevalence from corn silage in HQ compared with the LQ diet. Such characteristics would induce a greater propionate derivate from amylolytic bacterial activity, translating into a reduced production of ruminal methane, which is well documented in the literature (Van Soest, 1994; Janssen, 2010; McDonald et al., 2010).

CONCLUSIONS

The use of fibrolytic enzymes (*Trichoderma reesei* extract) in beef cattle growing diets stimulated intake and generated positive aspects for ruminal fermentation, regardless of the quality of growing diets offered. In vitro ADF digestion of multiple roughage substrates commonly available in Texas was positively affected using exogenous fibrolytic enzymes, with additional benefits observed for intact sorghum grain and corn stalks. Beef cattle HQ growing diets positively affected intake and ruminal fermentation characteristics, even though chewing activity was reduced.

Conflict of interest statement. None declared.

LITERATURE CITED

- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447–1462. doi:10.3168/jds.S0022-0302(97)76074-0

- Álvarez, G., J. M. Pinos-Rodríguez, J. G. Herrera, J. C. García, S. S. Gonzalez, and R. Bárcena. 2008. Effects of exogenous fibrolytic enzymes on ruminal digestibility in steers fed high fiber rations. *Livestock Sci.* 121:150–154. doi:10.1016/j.livsci.2008.05.024
- Association of Official Analytical Chemists (AOAC). 1990. Official method of analysis. 15th ed. Arlington (VA): AOAC Int..
- Association of Official Analytical Chemists (AOAC). 1995. Official method of analysis. 16th ed. Washington (DC): AOAC Int..
- Association of Official Analytical Chemists (AOAC). 1997. Official methods of analysis. 16th ed. 3rd rev. Washington (DC): AOAC Int..
- Association of Official Analytical Chemists (AOAC). 2005. Official method of analysis. 18th ed. Gathersburg (MD): AOAC Int..
- Balci, F., S. Dikmen, H. Gencoglu, A. Orman, I. I. Turkmen, and H. Biricik. 2007. The effect of fibrolytic exogenous enzyme on fattening performance of steers. *Bulgarian J. Vet. Med.* 10:113–118.
- Bean, B. W., R. L. Baumhardt, F. T. McCollum III, and K. C. McCuiston. 2013. Comparison of sorghum classes for grain and forage yield and forage nutritive value. *Field Crops Res.* 142:20–26. doi:10.1016/j.fcr.2012.11.014
- Beauchemin, K. A., S. D. M. Jones, L. M. Rode, and V. J. H. Sewalt. 1997. Effects of fibrolytic enzymes in corn or barley diets on performance and carcass characteristics of feedlot cattle. *Can. J. Anim. Sci.* 77:645–653. doi:10.4141/a97-050
- Beauchemin, K. A., L. M. Rode, and D. Karren. 1999. Use of feed enzymes in feedlot finishing diets. *Can. J. Anim. Sci.* 79:243–246. doi:10.4141/a98-124
- Campanili, P. R. B., J. O. Sarturi, M. A. Ballou, S. J. Trojan, J. D. Sugg, L. A. Ovinge, A. U. Alrumaih, L. A. Pellarin, and A. A. Hoffman. 2017. Effects of silage type and inclusion level on ruminal characteristics and feeding behavior of steers fed finishing diets. *J. Anim. Sci.* 95:4623–4637. doi:10.2527/jas.2017.1510
- Chung, Y. H., M. Zhou, L. Holtshausen, T. W. Alexander, T. A. McAllister, L. L. Guan, M. Oba, and K. A. Beauchemin. 2012. A fibrolytic enzyme additive for lactating holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. *J. Dairy Sci.* 95:1419–1427. doi:10.3168/jds.2011-4552
- Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768–1771. doi:10.3168/jds.S0022-0302(61)89956-6
- Galyean, M. L., N. A. Cole, L. O. Tedeschi, and M. E. Branine. 2016. BOARD-INVITED REVIEW: efficiency of converting digestible energy to metabolizable energy and reevaluation of the California net energy system maintenance requirements and equations for predicting dietary net energy values for beef cattle. *J. Anim. Sci.* 94:1329–1341. doi:10.2527/jas.2015-0223
- Goetsch, A. L., and M. L. Galyean. 1983. Influence of feeding frequency on passage of fluid and particulate markers in steers fed a concentrate diet. *Can. J. Anim. Sci.* 63:727–730. doi:10.4141/cjas83-084
- He, Z. X., M. L. He, N. D. Walker, T. A. McAllister, and W. Z. Yang. 2014. Using a fibrolytic enzyme in barley-based diets containing wheat dried distillers grains with solubles: ruminal fermentation, digestibility, and growth performance of feedlot steers. *J. Anim. Sci.* 92:3978–3987. doi:10.2527/jas.2014-7707
- He, Z. X., N. D. Walker, T. A. McAllister, and W. Z. Yang. 2015. Effect of wheat dried distillers grains with solubles and fibrolytic enzymes on ruminal fermentation, digestibility, growth performance, and feeding behavior of beef cattle. *J. Anim. Sci.* 93:1218–1228. doi:10.2527/jas.2014-8412
- Holtshausen, L., Y. H. Chung, H. Gerardo-Cuervo, M. Oba, and K. A. Beauchemin. 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. *J. Dairy Sci.* 94:899–907. doi:10.3168/jds.2010-3573
- Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Anim. Feed Sci. Technol.* 160(1):1–22. doi:10.1016/j.anifeeds.2010.07.002
- Krause, M., K. A. Beauchemin, L. M. Rode, B. I. Farr, and P. Nørgaard. 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. *J. Anim. Sci.* 76:2912–2920. doi:10.2527/1998.76112912x
- Krueger, N. A., A. T. Adesogan, C. R. Staples, W. K. Krueger, S. C. Kim, R. C. Littell, and L. E. Sollenberger. 2008. Effect of method of applying fibrolytic enzymes or ammonia to bermudagrass hay on feed intake, digestion, and growth of beef steers. *J. Anim. Sci.* 86:882–889. doi:10.2527/jas.2006-717
- Lewis, G. E., W. K. Sanchez, C. W. Hunt, M. A. Guy, G. T. Pritchard, B. I. Swanson, and R. J. Treacher. 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. *J. Dairy Sci.* 82:611–617. doi:10.3168/jds.S0022-0302(99)75274-4
- McAllister, T. A., A. N. Hristov, K. A. Beauchemin, L. M. Rode, and K. J. Cheng. 2010. Enzymes in ruminant diets. In: Bedford, M. R. and G. G. Partridge, editor. *Enzymes in farm animal nutrition*. Wallingford (UK): CAB International; pp. 273–298.
- McDougall, E. I. 1948. The composition and output of sheep's saliva. *Biochem. J.* 43:99–109.
- McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, C. A. Morgan, L. A. Sinclair, and R. G. Wilkinson. 2010. *Animal nutrition*. 7th ed. Essex (UK): Pearson Education Limited.
- NASEM. 2016. *Nutrient requirements of beef cattle*. 8th ed. Washington (DC): The National Academies Press.
- Ovinge, L. A., J. O. Sarturi, M. L. Galyean, M. A. Ballou, S. J. Trojan, P. R. B. Campanili, A. A. Alrumaih, and L. A. Pellarin. 2018. Effects of a live yeast in natural-program finishing feedlot diets on growth performance, digestibility, carcass characteristics, and feeding behavior. *J. Anim. Sci.* 96:684–693. doi:10.1093/jas/sky011.
- Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: a review. *J. Anim. Sci.* 76:275–286. doi:10.2527/1998.761275x.
- Ponce, C. H., D. R. Smith, J. S. Schutz, and M. L. Galyean. 2012. Effects of receiving diets based on wet corn gluten feed on performance and morbidity of newly received beef heifers and in vitro fermentation. *Profess. Anim. Sci.* 28:213–220. doi:10.15232/s1080-7446(15)30342-9.

- Quinn, M. J., M. L. May, N. DiLorenzo, D. R. Smith, and M. L. Galyean. 2010. Effects of distillers grains and substrate steam-flaked corn concentration on in vitro dry matter disappearance, gas production kinetics, and hydrogen sulfide production. *Profess. Anim. Sci.* 26:365–374. doi:10.15232/s1080-7446(15)30616-1.
- Rode, L. M., W. Z. Yang, and K. A. Beauchemin. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. *J. Dairy Sci.* 82:2121–2126. doi:10.3168/jds.S0022-0302(99)75455-X.
- Shreck, A. L., B. L. Nuttelman, J. L. Harding, W. A. Griffin, G. E. Erickson, T. J. Klopfenstein, and M. J. Cecava. 2015. Digestibility and performance of steers fed low-quality crop residues treated with calcium oxide to partially replace corn in distillers grains finishing diets. *J. Anim. Sci.* 93:661–671. doi:10.2527/jas.2013–7194.
- Van Keulen, J. and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44:282–287. doi:10.2527/jas1977.442282x.
- Van Soest, P. J. 1994. *Nutritional ecology of the ruminant*. 2nd ed. Ithaca (NY): Cornell University Press.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.
- Vasconelos, J. T. and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. *J. Anim. Sci.* 85:2772–2781. doi:10.2527/jas.2007-0261.
- Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. *Anim. Feed Sci. Technol.* 39:95–110. doi:10.1016/0377-8401(92)90034-4.
- Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. *J. Dairy Sci.* 83:2512–2520. doi:10.3168/jds.S0022-0302(00)75143-5.