Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle

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ABSTRACT

The objective of this study was to examine the effect of applying a fibrolytic enzyme preparation to diets with high (48% of diet dry matter, DM) or low (33% of diet DM) proportions of concentrate on production performance of lactating dairy cows. Sixty lactating Holstein cows ($589 \text{ kg} \pm 20$; $22 \pm 3 \text{ d}$ in milk) were stratified according to milk production and parity and randomly assigned to 4 treatments with a 2×2 factorial arrangement. Dietary treatments included the following: 1) low-concentrate diet (LC); 2) LC plus enzyme (LCE); 3) high-concentrate diet (HC); and 4) HC plus enzyme (HCE). The enzyme was sprayed at a rate of 3.4 mg of enzyme/g of DM on the total mixed ration daily and the trial lasted for 63 d. A second experiment with a 4×4 Latin square design used 4 ruminally fistulated cows to measure treatment effects on ruminal fermentation and in situ ruminal dry matter degradation during four 18-d periods. Enzyme application did not affect dry matter intake (DMI; 23.9 vs. 22.3 kg/d) or milk production (32.8 vs. 34.2 kg/d) but decreased estimated CH_4 production, increased total volatile fatty acid concentration (114.5 vs. 125.7 mM), apparent total tract digestibility of DM (69.8 vs. 72.6%), crude protein (CP; 69.2 vs. 73.3%), acid detergent fiber (50.4 vs. 54.8%), neutral detergent fiber (53.7 vs. 55.4%), and the efficiency of milk production (1.44 vs. 1.60 kg of milk/kg of DMI). Feeding more concentrates increased DMI (21.5 vs. 24.8 kg/d), milk yield (32.2 vs. 34.7 kg/d), milk protein yield (0.89 vs. 0.99 kg/d), and DM (69.9 vs. 72.6%), but decreased ruminal pH (6.31 vs. 6.06). Compared with cows fed HC, those fed LCE had lower DMI (20.8 vs. 25.7 kg/d) and CP intake (3.9 vs. 4.8 kg/d), greater ruminal pH (6.36 vs. 6.10), and similar milk yield ($33.2 \pm 1.1 \text{ kg/d}$). Consequently, the efficiency of milk production was greater in cows fed LCE than those fed HC (1.69 vs. 1.42 kg of milk/kg of

DMI). This fibrolytic enzyme increased the digestibility of DM, CP, neutral detergent fiber, and acid detergent fiber and the efficiency of milk production by dairy cows. Enzyme application to the low-concentrate diet resulted in as much milk production as that from cows fed the untreated high-concentrate diet.

Key words: fibrolytic enzyme, cellulase, esterase, xylanase

INTRODUCTION

Fibrolytic enzymes have been added to forage and ruminant diets to improve forage digestibility and animal performance, respectively. Enzyme application to forage immediately before in vitro incubation improved digestion of DM and NDF (Feng et al., 1996) suggesting that applying fibrolytic enzymes at or just before feeding may enhance digestion of forage by cattle. However, results of application of fibrolytic enzymes to ruminant diets have been equivocal. Some studies showed that enzyme addition increased milk production by dairy cattle (Rode et al., 1999; Kung et al., 2000; Yang et al., 2000) but others did not (Beauchemin et al., 2000; Kung et al., 2002; Sutton et al., 2003). Adding fibrolytic enzymes increased digestibility in some studies (Rode et al., 1999; Yang et al., 2000) but not in others (Lewis et al., 1999). These discrepancies may be due to differences in enzyme activity, application rate and composition, stage of lactation of dairy cows, mode and time of enzyme delivery, ruminal activity and stability of enzymes, enzyme-feed specificity, and the portion of the diet to which enzymes are applied (Beauchemin et al., 2004; Adesogan, 2005).

Most previous studies on effects of fibrolytic enzymes on animal performance focused on and only analyzed their xylanase and cellulase activities. These activities cannot hydrolyze ester linkages between hydroxycinnamic acids and sugars in plant cell walls. Ferulic acid esterases can hydrolyze such linkages (Williamson et al., 1998; Krueger et al., 2008a), thus releasing ferulic acid and opening the remainder of the polysaccharides to further hydrolytic attack by other enzymes (Yu et al., 2005). Various studies have demonstrated that esterase enzymes play an important role in ruminal cell

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wall degradation (Nsereko et al., 2000; Dhiman et al., 2002; Krueger and Adesogan, 2008), particularly following pretreatment or co-incubation with xylanases (Bartolomé et al., 1995, 1997a, b; Yu et al., 2002). Most of such studies have been done in vitro. Consequently, little is known about effects of enzymes containing cellulase, xylanase, and esterase activities on the performance of dairy cows.

The objective of this study was to determine the effect of dietary addition of a fibrolytic enzyme preparation containing cellulose, xylanase, and esterase activities on the performance of dairy cows fed low- or high-concentrate diets. We hypothesized that 1) enzyme application to the high-concentrate diet would improve milk production, whereas application to the low-concentrate diet would improve the efficiency of milk production; 2) enzyme application to the low-concentrate diet would result in as much milk production as that from the high-concentrate diet that was not treated with the enzyme.

MATERIALS AND METHODS

Cows, Treatments, and Design

Care of animals used in this study followed protocols approved by the University of Florida Institutional Animal Care and Use Committee. Sixty lactating Holstein cows in early lactation (22 ± 3 DIM) were grouped by milk production and parity (multiparous and primiparous) and randomly assigned to 1 of 4 treatments arranged in a 2×2 factorial design. Cows were fed either a high- or low-concentrate TMR that was treated with or without a prototype fibrolytic enzyme from Dyadic International Inc. (Jupiter, FL). The following treatments were investigated: 1) low-concentrate untreated diet (**LC**; 67:33 roughage-to-concentrate ratio); 2) LC plus enzyme (**LCE**); 3) high-concentrate untreated diet (**HC**; 52:48 roughage-to-concentrate ratio); and 4) HC plus enzyme (**HCE**). The dietary proportions of concentrate were designed to approximate those typical of diets fed to dairy cows in the United States (HC) and western Europe (LC). Prior to the daily a.m. and p.m. feedings, the enzyme solution was diluted in water (1:3 ratio vol/vol) and sprayed at a manufacturer-recommended rate of 3.4 mg of enzyme/g of DM on the TMR during mixing for 5 min in a 250-kg capacity Calan data ranger (American Calan Inc., Northwood, NH). Separate data rangers were used to mix the enzyme-treated and untreated TMR. The enzyme tested was a preparation from a strain of *Trichoderma longibrachiatum*. It was chosen because it was one of the most effective enzymes at increasing the 24 h NDF digestibility of corn silage and alfalfa hay in a previous study

(enzyme FF + FT in Eun and Beauchemin, 2007). The roughage portion of the diets contained approximately 20% of alfalfa hay, 72% of corn silage, and 8% of cottonseed hulls (DM basis). The experimental diets were formulated to be isonitrogenous (Table 1) and to meet NRC (2001) guidelines for a dairy cow in early lactation producing 40 kg of milk with milk fat and protein concentrations of 3.5 and 2.85%, respectively. The experiment was conducted from September 9 to December 29, 2006 at the University of Florida Dairy Unit and it lasted for 63 d per cow. Cows were adapted to diets for the first 14 d and the last 49 d of the trial were used for sample collection. During the trial, ranges in ambient temperature and humidity were 9 to 26°C and 48 to 96%, respectively. Cows were housed in a freestall open-sided barn fitted with fans and misters for cooling when the ambient temperature exceeded 18 and 24°C, respectively, to prevent heat stress. Calan gates (American Calan Inc.) for individual feed intake were used. Freestalls were bedded with sand and maintained daily. Sufficient freestalls were available to provide at least 1 freestall per cow. Water and diets were available in ad libitum amounts.

Enzyme Activity

Xylanase activity (EC 3.2.1.8) measured using the assay of Bailey et al. (1992) was 3,630 U/mL with oat spelt xylan as substrate (Sigma Chemical Company, St. Louis, MO). Endoglucanase (EC 3.2.1.4) and exoglucanase (EC 3.2.1.91) activities measured with the Wood and Bhat (1988) assays were 733 U/mL and 2 U/mL using 1% (wt/vol) carboxymethylcellulose or microcrystalline cellulose (Avicel) as substrates, respectively (Sigma Chemical Company). One unit of activity of the respective enzymes was defined as micromoles of xylose or glucose released per minute per milligram. Assay conditions were 39°C and pH 6.0 to reflect ruminal conditions. Aryl and carboxyl esterase activities measured using the methods described by Gonzalez et al. (2006) and *p*-nitrophenyl acetate and α -naphthyl acetate as substrates were 0.38 $\mu\text{mol}/\text{min}$ per 1 mg and 0.28 $\mu\text{mol}/\text{min}$ per 1 mg, respectively. Release of ferulic acid from ethyl ferulate measured using the HPLC assay described by Mastihuba et al. (2002) was 0.2 nmol/min per 1 mg.

Sampling and Analysis

Cows were fed individually (at 0700 and 1200 h) and milked (at 1100 and 2300 h) twice daily. Milk samples from a.m. and p.m. milkings were collected twice every week and analyzed by Southeast Dairy labs (Bellevue, FL) for fat, protein, and SCC using a Bentley 2000 near

Table 1. Ingredient and chemical composition of the untreated experimental diets

Item	Low concentrate	High concentrate
Ingredient composition (% of DM)		
Corn silage	49.20	37.00
Alfalfa hay	13.50	10.00
Cottonseed hulls	4.63	5.00
Corn meal	7.38	17.89
Citrus pulp	2.00	5.01
Whole cottonseed	1.81	4.84
SoyPlus ¹	7.90	5.93
Soybean meal	2.49	6.01
Cottonseed meal	7.80	5.10
Mineral mix ²	3.26	3.25
Roughage:concentrate ratio	67:33	52:48
Chemical composition		
DM (%)	64.9	72.2
Ash (% of DM)	7.1	6.7
CP (% of DM)	18.6	18.5
NDF (% of DM)	34.3	31.0
ADF (% of DM)	22.4	20.9
Hemicellulose ³ (% of DM)	11.9	10.1
NFC ⁴ (% of DM)	36.5	39.7
NE _L (Mcal/kg)	1.53	1.60

¹West Central Soy (Ralston, IA).

²Mineral mix contained 26.4% CP, 10.2% Ca, 8.6% Na, 5.1% K, 3.1% Mg, 1.5% S, 0.9% P, 2,231 mg of Mn/kg, 1,698 mg of Zn/kg, 512 mg of Cu/kg, 339 mg of Fe/kg, 31 mg of Co/kg, 26 mg of I/kg, 7.9 mg of Se/kg, 147,756 IU of vitamin A/kg, and 787 IU of vitamin E/kg (DM basis).

³Calculated as NDF – ADF.

⁴Calculated as NFC = 100 – [CP + ash + fat (NRC, 2001, values) + NDF].

infrared reflectance spectrophotometer (Bentley Instruments Inc., Chaska, MN). Somatic cell scores were generated as described by Norman et al. (2000) for statistical analysis of SCC. Body weight was measured after milking before the trial and weekly thereafter. Body condition was scored by the same person on a 1 to 5 scale (Wildman et al., 1982) before the trial and weekly thereafter. Energy balance was calculated using NRC (2001) equations for NE_L requirements for maintenance according to the BW of cows ($0.08 \times \text{BW}^{0.75}$), NE_L for milk synthesis according to concentrations of fat and protein in milk [$(0.0929 \times \text{fat } \%) + (0.0547 \times \text{protein } \%) + (0.192)$], and NE_L concentration of the TMR. Fat-corrected milk at 3.5% fat [$(0.4324 \times \text{milk yield}) + (16.218 \times \text{milk fat yield})$] was also calculated.

Weights of TMR and orts were recorded daily for each cow. Duplicate samples of corn silage, alfalfa hay, and concentrates were collected weekly and composited monthly. Subsamples from each month were dried at 60°C for 48 h in a forced-air oven, ground to pass the 1-mm screen of a Wiley mill (A. H. Thomas, Philadelphia, PA), and analyzed for DM (105°C for 16 h) and ash (512°C for 8 h) content. Concentrations of NDF and ADF were measured using the method of Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY). Heat-stable α -amylase and sulfite were used in the NDF assay. Nitrogen con-

centration was determined by rapid combustion using a Macro elemental N analyzer (Vario MAX CN, model ID 25.00-5003; Elementar, Hanau, Germany) and CP was calculated as $\text{N} \times 6.25$. Blood samples were collected weekly by coccygeal venipuncture into vacutainer tubes containing sodium heparin anticoagulant (Fisher Scientific, Pittsburgh, PA). The blood was centrifuged at $2,500 \times g$ for 20 min at 4°C and the plasma was frozen (–20°C). A Technicon autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and BUN (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965).

In vivo apparent digestibility was calculated using chromic oxide as a marker (Schneider and Flatt, 1975). Chromic oxide powder (Fisher Scientific, Fairlawn, NJ) was weighed (10 g/dose) into gelatin capsules (Jorgensen Laboratories Inc., Loveland, CO) and dosed twice daily (at 0700 and 1900 h) with a balling gun for 10 consecutive days between d 45 and 60. Fecal samples were collected at 0630 and 1830 h during the last 5 d of dosing. Feces were dried to a constant weight at 55°C in a forced-air oven, ground to pass through a 1-mm screen in a Wiley mill, and a composite sample was made from all 10 fecal samples per cow. Chromium concentration in the feces was determined using a Perkin Elmer 5000

atomic absorption spectrometer (Perkin Elmer, Wellesley, MA), according to the procedure described by Williams et al. (1962). Apparent digestibility of CP, ADF, and NDF were calculated.

In Situ Rumen Degradability

Four ruminally cannulated, nonlactating Holstein cows were used to determine dietary treatment effects on ruminal fermentation and *in situ* ruminal DM degradation. This aspect of the experiment had a 4×4 Latin square design with four 18-d periods. The first 14 d of each period were used to adapt cows to a new diet. On d 15, ruminal fluid samples were taken at 0, 2, 4, 6, 8, and 10 h after cows were fed in the morning. Approximately 100 mL of ruminal fluid was immediately filtered through 2 layers of cheesecloth, and pH was measured with an electrode (Accumet pH meter, model HP-71, Fisher Scientific, Pittsburgh, PA). Subsequently, the ruminal fluid was acidified with 2 mL of 50% of H_2SO_4 per 100 mL of ruminal fluid and centrifuged at $11,500 \times g$ for 20 min. The supernatant was frozen (-20°C) and subsequently analyzed for concentrations of lactate and VFA using the method of Muck and Dickerson (1988) and a HPLC system (Hitachi, FL 7485, Tokyo, Japan) coupled to a UV Detector (Spectroflow 757, ABI Analytical Kratos Division, Ramsey, NJ) set at 210 nm. The column was a Bio-Rad Aminex HPX-87H (Bio-Rad Laboratories, Hercules, CA) column with 0.015M sulfuric acid mobile phase and a flow rate of 0.7 mL/min at 45°C . Ammonia-N was determined with a Technicon autoanalyzer (Technicon, Tarrytown, NY) and an adaptation of the Noel and Hambleton (1976) procedure that involved colorimetric N quantification. *In vitro* VFA data were used to estimate the quantity of hexose equivalent fermented (mol/100 mol of VFA produced), as well as CH_4 and CO_2 produced (mol/mol of hexose fermented) using fermentation balance calculations from Wolin (1960).

The ruminal degradation kinetics of the experimental diets was measured *in situ* on d 16 to 18. The TMR samples were dried (60°C for 48 h), ground to pass a 4-mm screen with a Wiley mill and weighed (5 g as is) into preweighed polyester bags (10×20 cm, pore size 50 μm , Bar Diamond Inc., Parma, ID). Samples were incubated for 0, 4, 8, 16, 24, and 48 h in quadruplicate in each of 2 ruminally fistulated cows fed that same diet in each period. All bags were inserted in the rumen simultaneously. After each incubation period, bags were removed and rinsed with cool water and frozen (-20°C). Subsequently, all bags were thawed, washed using a rinse cycle without soap in a Kenmore Series 70 washing machine, dried for 48 h at 60°C and weighed. The exponential model of McDonald (1981) was fitted

to the DM degradation data with the NONLIN procedure of SAS (Version 9.2 SAS Institute Inc., Cary, NC) to generate DM degradation parameters. The model is of the form

$$P = A + B(1 - e^{-c(t-\text{lag})}),$$

where P is the DM degradation (%) at time t , A is the y-axis intercept representing the washout fraction (%), B is the potentially degradable fraction (%), c is the rate of degradation of the B fraction (%/h), and lag is the time that elapsed before degradation commenced.

Statistical Analysis

A completely randomized design with a 2 (control vs. enzyme) $\times$ 2 (LC vs. HC) factorial arrangement of treatments was used to analyze the data. The MIXED procedure of SAS (Version 9.2 SAS Institute Inc.) and a model containing treatment, week (repeated measure), parity, all interactions of these terms, and cow nested in treatment $\times$ parity as the random effect was used to analyze the data from measurements that were repeated weekly. Milk production during the first 21 d of lactation was used as a covariate for analyzing milk production data. A similar model excluding the week effect and its interactions was used to analyze digestibility coefficients. The model for analyzing ruminal fermentation data included treatment, period, time (repeated measure), treatment $\times$ time and cow (random effect). Least squares means and significant interactions are reported and all results are presented on a DM basis. Contrast statements were used to determine the effects of enzyme application (control vs. enzyme), concentrate level (LC vs. HC), the interaction (enzyme treatment vs. concentrate level), and to compare the LCE and HC diets. The slice command of SAS was used to detect differences among means at specific time points and the PDIF statement of SAS also was used to compare enzyme effects within each concentrate level. Treatment significance was declared at $P \leq 0.05$ and tendencies were declared at $0.1 > P > 0.05$.

RESULTS AND DISCUSSION

Multiparous cows had greater ($P < 0.001$) intake of DM (25.4 vs. 20.9 kg/d), CP (4.8 vs. 3.9 kg/d), ADF (5.6 vs. 4.5 kg/d), and NDF (8.4 vs. 6.8 kg/d), and greater milk yield ($P = 0.01$; 34.9 vs. 31.9 kg/d) than did primiparous cows but no parity $\times$ treatment interactions occurred.

Enzyme application did not affect ($P = 0.14$) DMI but tended to reduce intake of CP ($P = 0.06$; 4.5 vs. 4.2 kg/d), NDF ($P = 0.07$; 7.9 vs. 7.4 kg/d), and ADF (P

Table 2. Effect of adding a fibrolytic enzyme¹ (Enz) to diets containing low (33%) or high (48%) amounts of concentrate (conc) on intake and digestibility in dairy cows

Item	Low-conc		High-conc		SEM	Contrast <i>P</i> -value			
	No Enz	Enz	No Enz	Enz		Enz	Conc	LCE vs. HC ²	Enz × Conc
Intake (kg/d)									
DM	22.1	20.8	25.7	23.8	1.0	0.14	0.003	0.001	0.79
CP	4.2	3.9	4.8	4.4	0.16	0.06	0.001	0.003	0.80
NDF	7.8	7.3	8.0	7.4	0.29	0.07	0.43	0.06	0.87
ADF	5.1	4.7	5.4	5.0	0.19	0.07	0.09	0.01	0.84
Digestibility (%)									
DM	68.5	71.2	71.1	74.0	1.1	0.02	0.02	0.91	0.90
CP	68.4 ^a	71.5 ^b	70.0 ^x	75.1 ^y	1.0	0.002	0.01	0.29	0.31
NDF	52.1	55.2	53.3	57.5	1.7	0.03	0.30	0.43	0.75
ADF	48.6	52.9	52.1	56.6	1.8	0.02	0.06	0.75	0.97

^{a,b,x,y} At the same amount of concentrate supplementation, means with different superscripts differed ($P < 0.05$).

¹Produced by Dyadic International Inc. (Jupiter, FL).

²LCE = low-concentrate diet with enzyme; HC = high-concentrate diet with no enzyme added.

= 0.07; 5.3 vs. 4.9 kg/d). Enzyme application increased ($P \leq 0.02$) digestibility of DM (69.8 vs. 72.6%), CP (69.2 vs. 73.3%), ADF (50.4 vs. 54.8%), and NDF (53.7 vs. 55.4%; Table 2). Krueger et al. (2008b) reported that fibrolytic enzyme application to bermudagrass hay increased DMI and DM and NDF digestibility by beef steers. Yang et al. (1999) also reported that supplementation with a mixed xylanase and cellulase enzyme preparation increased NDF digestibility of an alfalfa-barley silage-based TMR by dairy cows. The enzyme-mediated digestibility increases in this study may have occurred via increased microbial colonization of feed particles or by direct cell wall hydrolysis (Cheng et al., 1995; Yang et al., 1999).

Increasing the concentrate amount increased DMI (21.5 vs. 24.8 kg/d, $P = 0.003$) and CP intake (4.1 vs. 4.6 kg/d, $P = 0.001$) and tended to increase ADF intake ($P = 0.09$; 4.9 vs. 5.2 kg/d). Likewise, increasing the concentrate amount increased digestibility of DM (69.9 vs. 72.6%; $P = 0.02$) and CP (70.0 vs. 72.6%, $P = 0.01$) and tended ($P = 0.06$) to increase ADF digestibility (50.1 vs. 54.4%). Cows fed HC instead of LCE had greater ($P \leq 0.01$) DMI (25.7 vs. 20.8 kg/d), CP intake (4.8 vs. 3.9 kg/d), and ADF intake (5.4 vs. 4.7 kg/d), and tended ($P = 0.06$) to have greater NDF intake (8.0 vs. 7.3). Increasing the amount of concentrate in diets has caused linear increases in DMI in other studies (Llamas-Lamas and Combs, 1991; Weiss and Shockey, 1991). Eun and Beauchemin (2005) also reported that cows receiving high-concentrate diets had greater DMI and greater DM digestibility than those receiving low-concentrate diets. Substitution of concentrates for forage generally increases intake and digestibility because concentrates cause less ruminal fill, have a lower ligni-

fied polysaccharide concentration, a faster passage rate, and require less rumination than does forage.

Enzyme application effects on plasma glucose concentration differed with the amount of concentrates fed (Enzyme × concentrate interaction, $P = 0.006$) but the differences were small. The plasma urea nitrogen concentration was not affected by enzyme application, but feeding more concentrates increased the plasma urea nitrogen concentration (13.5 vs. 15.0 mg/dl, $P = 0.001$; Table 3), reflecting the increased CP digestibility caused by enzyme treatment. Energy intake ($P < 0.001$), milk energy output ($P = 0.07$), and energy balance ($P = 0.001$) increased with the amount of concentrate supplementation.

Enzyme application did not statistically increase milk yield, although numerical increases were evident at both concentrate amounts (Table 4). In some studies, dietary addition of fibrolytic enzymes increased milk production (Lewis et al., 1999; Rode et al., 1999; Yang et al., 2000) but no milk response was reported in others (Beauchemin et al., 2000; Elwakeel et al., 2007). Differences in enzyme activity, form and application rates, and diets across these studies complicate elucidation of the reasons for these discrepancies and highlight the need for caution when comparing studies involving different enzyme preparations.

Enzyme application effects on milk fat concentration differed with the amount of concentrates (Enzyme × concentrate interaction, $P = 0.04$) but milk fat concentrations did not differ ($P > 0.05$) at the same concentrate feeding amount. Enzyme application increased the efficiency of milk production ($P = 0.04$; 1.44 vs. 1.60) because of increased nutrient supply for milk production. The increased feed efficiency across concentrate

Table 3. Effect of adding a fibrolytic enzyme¹ (Enz) to diets containing low (33%) or high (48%) amounts of concentrate (conc) on BW, ADG, BCS, plasma metabolites, and energy balance in dairy cows

Item	Low-conc		High-conc		SEM	Contrast <i>P</i> -value			
	No Enz	Enz	No Enz	Enz		Enz	Conc	LCE vs. HC ²	Enz × Conc
Growth measure									
BW (kg)	583	580	594	571	14.9	0.38	0.97	0.50	0.49
BW change (kg/d)	0.32	0.42	0.57	0.38	0.14	0.89	0.44	0.63	0.51
BCS (1 to 5)	2.49	2.41	2.43	2.46	0.03	0.49	0.97	0.63	0.11
Plasma metabolite (mg/dL)									
Glucose	69.3 ^a	71.3 ^b	71.9	70.6	0.6	0.53	0.10	0.46	0.006
Urea N	13.4	13.6	14.5	15.4	0.4	0.13	0.001	0.06	0.31
Energy measure (Mcal/d)									
NE _L intake	33.8	33.1	41.1	39.7	1.8	0.50	<0.001	<0.001	0.82
Milk energy output	21.6	22.2	24.2	23.9	1.2	0.86	0.07	0.22	0.70
Maintenance requirement	9.49	9.44	9.62	9.32	0.19	0.35	0.95	0.47	0.46
Energy balance	2.80	1.44	7.35	6.41	1.47	0.41	0.001	<0.003	0.88

^{a,b}At the same amount of concentrate supplementation, means with different superscripts differed ($P < 0.05$).

¹Produced by Dyadic International Inc. (Jupiter, FL).

²LCE = low-concentrate diet with enzyme; HC = high-concentrate diet with no enzyme added.

amounts with enzyme application partly confirms our first hypothesis.

Increasing the dietary concentrate amount increased milk yield ($P = 0.02$; 32.2 vs. 34.7 kg/d) and milk protein yield ($P = 0.02$; 0.89 vs. 0.99 kg/d). These responses are attributable to the increase in concentration of dietary NFC as the dietary concentrate level increased. Starch digestion in the rumen increases propionic acid production, which is a major gluconeogenic precursor in ruminants (Chen et al., 1994). Theurer et al. (1999) reported that feeding dairy cows with steam-flaked versus dry-rolled corn or sorghum improved milk and milk protein yields by enhancing uptake of glucose and amino acids by the mammary gland due to increased ruminal and total starch digestion and increased microbial protein synthesis.

Despite the lower DMI of cows fed the LCE diet relative to that of the HC diet, FCM from both diets did not differ ($P = 0.26$). Consequently, the efficiency of milk production was greater ($P = 0.01$; 1.69 vs. 1.42 kg of milk/kg of DMI) in cows fed the LCE diet than in those fed the HC diet. Yet, cows fed LC had lower DMI ($P = 0.02$) and FCM ($P = 0.06$) and similar efficiency of milk production ($P = 0.75$) to those fed HC. These results confirm our second hypothesis by indicating that enzyme application to the LC diet made it as effective as the HC diet at stimulating milk production. Similarly, Schingoethe et al. (1999) reported that cows fed low-concentrate diets (45% of TMR) treated with a fibrolytic enzyme increased milk production to the same extent as that achieved when cows were fed a high-concentrate (55%) diet without the enzyme. Such

Table 4. Effect of adding a fibrolytic enzyme¹ (Enz) to diets containing low (33%) or high (48%) amounts of concentrate (conc) on milk production, composition, and efficiency of feed utilization by dairy cows

Item	Low-conc		High-conc		SEM	Contrast <i>P</i> -value			
	No Enz	Enz	No Enz	Enz		Enz	Conc	LCE vs. HC ²	Enz × Conc
Milk yield (kg/d)	31.9	32.5	33.6	35.8	1.1	0.21	0.02	0.43	0.47
3.5% FCM (kg/d)	32.5	33.9	35.9	36.2	1.2	0.49	0.03	0.26	0.64
Milk protein (%)	2.79	2.85	2.91	2.89	0.05	0.71	0.15	0.43	0.43
Milk fat (%)	3.60	3.80	3.90	3.56	0.13	0.62	0.80	0.59	0.04
Milk protein (kg/d)	0.88	0.90	0.97	1.00	0.04	0.59	0.02	0.19	0.96
Milk fat (kg/d)	1.14	1.20	1.32	1.25	0.08	0.93	0.13	0.24	0.41
SCC (× 1,000/mL)	412	158	417	465	144	0.88	0.68	0.85	0.47
FCM/DMI	1.46 ^a	1.69 ^b	1.42	1.51	0.08	0.04	0.16	0.01	0.35
Milk NE _L (Mcal/kg)	0.68	0.71	0.70	0.68	0.12	0.61	0.64	0.48	0.03

^{a,b}At the same amount of concentrate supplementation, means with different superscripts differed ($P < 0.05$).

¹Produced by Dyadic International Inc. (Jupiter, FL).

²LCE = low-concentrate diet with enzyme; HC = high-concentrate diet with no enzyme added.

Table 5. Effect of adding a fibrolytic enzyme¹ (Enz) to diets containing low (33%) or high (48%) amounts of concentrate (conc) on ruminal fermentation characteristics by dairy cows

Item	Low-conc		High-conc		SEM	Contrast <i>P</i> -value			
	No Enz	Enz	No Enz	Enz		Enz	Conc	LCE vs. HC ²	Enz × Conc
Ruminal pH	6.26	6.36	6.10	6.01	0.08	0.92	<0.001	0.006	0.14
Ammonia-N (mg/dL)	12.4	13.0	12.7	13.0	0.78	0.61	0.87	0.81	0.87
Lactate (mM)	6.57	4.41	5.14	8.94	0.55	0.15	0.01	0.37	<0.001
Total VFA (mM)	110.4	118.2	118.6	133.1	4.8	0.03	0.01	0.96	0.49
Individual VFA (mmol/100 mmol)									
Acetate (A)	60.1	59.5	57.7	52.4	1.73	0.10	0.004	0.42	0.18
Propionate (P)	18.6	18.9	19.6	20.2	0.34	0.20	0.005	0.16	0.79
Butyrate	11.7	12.0	12.3	12.7	1.40	0.81	0.67	0.90	0.97
Isobutyrate	2.7	2.6	2.9	2.5	0.22	0.30	0.92	0.39	0.43
Isovalerate	4.1	4.0	3.4	3.5	0.22	0.79	0.007	0.03	0.57
Valerate	2.3	2.1	1.8	2.9	0.19	0.02	0.31	0.41	0.001
A:P ratio	3.23	3.15	2.94	2.59	0.07	0.04	0.003	0.29	0.37
Fermentation balance ³ (mol/100 mol)									
CH ₄	32.1	32.0	31.7	28.1	0.86	0.04	0.006	0.80	0.04
CO ₂	61.8	61.9	59.9	58.3	1.94	0.67	0.05	0.35	0.61
Hexose fermented	57.2	56.9	55.0	53.8	1.99	0.71	0.09	0.42	0.81

¹Produced by Dyadic International Inc. (Jupiter, FL).

²LCE = low-concentrate diet with enzyme; HC = high-concentrate diet with no enzyme added.

³Hexose fermented (mol/100 mol of VFA) and CO₂ and CH₄ (mol/100 mol of hexose fermented) were calculated from VFA data according to Wolin (1960).

results imply that by adding such enzymes, diets with greater forage proportions can be fed without jeopardizing milk production, and this could lower the cost of the diet and reduce the risk of acidosis.

One concern about fibrolytic enzyme application to diets containing considerable amounts of concentrates is that fermentation of starch in such diets may indirectly depress ruminal pH and predispose cows to ruminal acidosis (Eun and Beauchemin, 2005). Hristov et al. (1996) also noted that applying fibrolytic enzymes to feed may decrease both chewing time and saliva output and, thereby, increase the risk of acidosis. Enzyme application did not affect ($P = 0.92$; Table 5) the mean ruminal pH of cows in this study, but caused lower values 4 h after feeding (Figure 1). Therefore, cows fed high-concentrate diets supplemented with enzymes should be monitored closely for signs of subclinical ruminal acidosis.

Enzyme application did not affect ruminal ammonia concentration but increased total VFA concentration ($P = 0.03$; 114.5 vs. 125.7 mM). In contrast, several studies reported that enzyme application had no effect on total VFA concentration (Kung et al., 2002; Sutton et al., 2003; Eun and Beauchemin, 2005). Enzyme application also tended ($P = 0.10$) to reduce acetate proportion (58.9 vs. 56.0 mmol/100 mmol) but did not affect propionate proportion or ammonia-N concentration ($P = 0.20$). Consequently, enzyme application decreased the acetate-to-propionate ratio ($P = 0.04$; 3.09 vs. 2.87), implying improved efficiency of energy

utilization in the rumen. This was confirmed by the fact that enzyme addition decreased the proportion of methane produced ($P = 0.04$), particularly when more concentrates were fed (concentrate × enzyme interaction, $P = 0.04$). Therefore, this study suggests that fibrolytic enzyme treatment of diets may contribute to reduction in greenhouse gas production from dairy cows.

Increasing the amount of concentrate supplementation produced the expected pH decrease ($P < 0.001$; 6.31 vs. 6.06), as did feeding the HC diet instead of the LCE diet ($P = 0.006$; 6.36 vs. 6.10). None of the average pH values was low enough to indicate acute ruminal acidosis, which is characterized by ruminal pH below 5 (Nagaraja and Town, 1990). However, ruminal pH of cows fed the HC diet reached the subclinical acidosis threshold (pH < 5.8; Ghorbani et al., 2002) 10 h after feeding. Feeding more concentrates decreased ($P = 0.004$) acetate proportion (59.8 vs. 55.1 mmol/100 mmol), and increased ($P < 0.05$) concentration of total VFA (114.3 vs. 125.9 mM) and lactate (5.5 vs. 7.0 mM) and proportion of propionate (18.8 vs. 19.9 mmol/100 mmol). Consequently, acetate-to-propionate ratio was decreased ($P = 0.003$) by feeding more concentrates, implying increased ruminal energetic efficiency. Eun and Beauchemin (2005) also reported that feeding more concentrates had no effect on molar acetate proportion, increased total VFA concentration and molar propionate proportion, and decreased the acetate:propionate ratio.

Table 6. Effect of adding a fibrolytic enzyme¹ (Enz) to diets containing low (33%) or high (48%) amounts of concentrate (conc) on the in situ ruminal degradability of DM by dairy cows

Item ²	Low-conc		High-conc		SEM	Contrast <i>P</i> -value			
	No Enz	Enz	No Enz	Enz		Enz	Conc	LCE vs. HC ³	Enz × Conc
A (%)	31.6	35.5	44.9	41.7	3.38	0.929	0.03	0.11	0.34
B (%)	56.3	51.5	43.3	46.6	3.82	0.85	0.06	0.20	0.33
A + B (%)	87.9	87.0	88.2	88.3	1.17	0.77	0.54	0.55	0.66
C (per h)	0.05	0.06	0.05	0.05	0.01	0.60	0.31	0.30	0.31
L (h)	0.0	0.39	1.16	0.63	0.42	0.88	0.15	0.27	0.33

¹Produced by Dyadic International Inc. (Jupiter, FL).

²A = y-axis intercept representing the washout fraction; B = potentially degradable fraction; A + B = total degradable fraction; C = fractional degradation rate; L = lag phase.

³LCE = low-concentrate diet with enzyme; HC = high-concentrate diet with no enzyme added.

Proportions of CH₄ ($P = 0.006$), CO₂ ($P = 0.05$), and hexose fermented ($P = 0.09$) were decreased as the level of concentrate supplementation increased. The lower CH₄ and CO₂ proportions reflect the reduction in acetate to propionate ratio due to increased concentrate supplementation.

Enzyme application did not affect the ruminal degradation of the diets (Table 6). Similarly, Krueger et al. (2008b) reported no effect of enzyme application on the rate or extent of degradation of bermudagrass hay. However, feeding more concentrates increased the washout fraction ($P = 0.03$; 43.3 vs. 33.6%), and tended to decrease the potentially degradable fraction ($P = 0.06$; 45.0 vs. 53.9%), likely reflecting differences in particle size distribution and NFC and fiber concentration

between low- and high-concentrate diets. That enzyme treatment increased total-tract digestibility but did not affect in situ ruminal degradability suggests that enzyme treatment increased postruminal digestion of the diets. Sutton et al. (2003) also reported that enzyme treatment increased postruminal and total-tract digestion of a TMR but did not increase ruminal digestion.

CONCLUSIONS

This study shows that application of a fibrolytic enzyme preparation did not affect DMI or milk production but increased nutrient digestibility, total VFA concentration, and the efficiency of milk production. Furthermore, enzyme application to the low-concentrate diet

Figure 1. Effect of adding a fibrolytic enzyme to dairy cow diets with low or high levels of concentrates on ruminal pH. LC = low-concentrate; LCE = low-concentrate + enzyme; HC = high-concentrate; HCE = high-concentrate + enzyme; x indicates a difference ($P = 0.07$) between LC and HCE at that time. Treatment × time, $P = 1.00$, SEM = 0.16. Color version available in the online PDF.

resulted in as much milk production and DM digestibility as from cows fed the untreated high-concentrate diet. These beneficial effects of enzyme application were primarily attributable to improved nutrient digestion and improved ruminal energy utilization.

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