The Proportion of the Diet to which Fibrolytic Enzymes are Added Affects Nutrient Digestion by Lactating Dairy Cows¹

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ABSTRACT

Eight lactating Holstein cows, four with ruminal cannulas, were used in a duplicated 4 × 4 Latin square design to investigate a fibrolytic enzyme product characterized by xylanase and cellulase activities (Promote N.E.T. Agribands International, St. Louis, MO). The diet consisted of concentrate containing rolled barley and supplement, barley silage and alfalfa haylage (55% to 45% DM basis, forage to concentrate ratio) and differed in enzyme application: 1) control, 2) enzyme applied to concentrate (45% of TMR), 3) enzyme applied to supplement (4% of TMR), and 4) enzyme applied to premix (0.2% of TMR). All diets that were supplemented with the enzyme product delivered about 1.0 grams per cow per day. Digestibility of OM, NDF and ADF in the total tract was increased in comparison to the control when enzymes were added to the entire concentrate. Enzyme treatments that were applied to a smaller portion of the diet showed only numerical increases in digestibility over the control. However, there was an increase in microbial N synthesis for cows fed enzymes added to the premix. The effects of enzyme supplementation on milk production and composition were not statistically significant, but cows receiving the enzyme product added to the concentrate had a numerically higher FCM compared to the control cows. These results indicate that enzyme supplementation increases total tract digestibility of organic matter and fiber. The proportion of the diet to which the enzyme is applied must be maximized to ensure a beneficial response.

(Key words: dairy cows, enzymes, digestibility, application method)

Abbreviation key: CMC = carboxymethyl cellulose, CONC = enzyme product added to concentrate (45% of TMR, DM basis), CTRL = control treatment, DDMI = digestible dry matter intake, PD = urinary purine derivative, PREM = enzyme product added to premix (0.2% of TMR, DM basis), SUPP = enzyme product added to supplement (4% of TMR, DM basis).

INTRODUCTION

The use of exogenous enzymes to improve the growth and production of nonruminants is common; the mode of action is generally to remove antinutritional factors, thereby increasing the nutritive value of the feed. The rumen contains numerous microbes, which overcome the factors that limit feed utilization by nonruminants. Thus, supplementing the diet of mature ruminants with exogenous enzymes has been met with skepticism. However, studies using various enzyme products fed to ruminants have been recently reported (Beauchemin et al., 1999; Feng et al., 1996; Lewis et al., 1999; Rode et al., 1999; Yang et al., 2000). Various factors such as enzyme type and preparation, application method, amount of enzyme applied and to what fraction of the diet, and animal differences have lead to inconsistencies and variation of results. For enzyme supplementation of ruminant diets to gain acceptance by the dairy cattle industry variability of results must be reduced.

An increase in milk production was reported in some studies when dairy cow diets were supplemented with fibrolytic enzymes (Rode et al., 1999; Yang et al., 2000), but not in others (Beauchemin et al., 2000). Milk production is not the only inconsistent variable. DMI was reported to be both increased (Beauchemin et al., 2000) and unchanged (Beauchemin et al., 1999; Kung et al., 2000) when enzymes were added to the diet. Similarly, effects of supplemental enzymes on digestibility have been inconsistent. Use of enzyme products comprised mainly of xylanases and cellulases have been shown to increase digestibility (Rode et al., 1999; Yang et al., 2000), or have no effect on digestibility (Lewis et al., 1999). It is essential to determine the conditions neces-

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Table 1. Ingredients and chemical composition of the diet.

Item	Diet
	(% of DM)
Ingredient	
Barley silage	36.9
Alfalfa silage	18.2
Barley grain, steam-rolled	33.4
Barley grain, ground ¹	0.4
Soybean meal ¹	1.4
Blood meal ¹	1.4
Corn gluten meal ¹	6.3
Dicalcium phosphate ¹	0.93
Beet molasses ¹	0.24
Vitamins and minerals ^{1,2}	0.82
Chemical	
DM, %	54.1 ± 1.1
OM, % of DM	92.5 ± 0.5
CP, % of DM	17.2 ± 0.7
NDF, % of DM	29.8 ± 1.2
ADF, % of DM	15.7 ± 0.8
Lignin, % of DM	3.10 ± 0.7

¹Ingredients that were in the pelleted supplement.

²Contained 2,000 IU of vitamin A/g, 200 IU of vitamin D/g and 20 IU of vitamin E/g. Contained 58.8% NaCl, 2.0% ZnSO₄, 2.4% MnSO₄·4H₂O, 0.8% CuSO₄·H₂O, 90 mg/kg CoSO₄·5H₂O, 88 mg/kg Na₂SeO₃, 16% Dynamate (Pitman Moore, Inc., Mundelein, IL; 22% S, 18% K, and 11% Mg) and 120 mg/kg of ethylenediamine dihydroiodide.

sary for supplemental fibrolytic enzymes to have beneficial effects on animal performance.

The objectives of this study were to compare the effects of applying a fibrolytic enzyme product on different proportions of the TMR on nutrient intake and digestibility, ruminal fermentation, microbial N synthesis, digesta passage kinetics, and milk production and composition.

MATERIALS AND METHODS

Cows and Diets

Eight multiparous lactating Holstein cows were used. Four cows were surgically fitted with ruminal cannulas, measuring 10 cm in diameter that were constructed of soft plastic (Bar Diamond, Parma, ID). Cows averaged 111 ± 32 DIM and 649 ± 52 kg BW.

The design of the experiment was a double 4 × 4 Latin Square with each period lasting 28 d. Four of the cows were fitted with ruminal cannulas. Cows received a diet consisting of 45% concentrate and 55% forage (DM basis) (Table 1 and 2). The concentrate contained steam-rolled barley and a pelleted supplement. The diet was formulated using the Cornell-Penn-Miner System (CPMDairy, Version 1.0) and balanced to provide sufficient metabolizable protein, metabolizable energy, vitamins, and minerals to produce 35 kg/d of milk with 3.5% fat and 3.2% CP. Presence or absence of a fibrolytic enzyme product and the proportion of TMR to which

Table 2. Chemical composition of forages.

Item	
Barley Silage	
DM, %	41.6 ± 1.5
OM, % of DM	93.2 ± 0.2
CP, % of DM	11.3 ± 0.1
NDF, % of DM	36.1 ± 0.5
ADF, % of DM	22.1 ± 0.2
Alfalfa Silage	
DM, %	42.9 ± 5.2
OM, % of DM	87.8 ± 0.3
CP, % of DM	17.1 ± 0.02
NDF, % of DM	38.1 ± 0.2
ADF, % of DM	31.3 ± 0.3

the enzyme product was added made up the four treatments. The treatments were: 1) no enzyme (**CTRL**), 2) enzyme added to the concentrate portion of the TMR consisting of 45% of the dietary DM (**CONC**), 3) enzyme added to the pelleted supplement portion of the TMR which made up 4% of the dietary DM (**SUPP**) and 4) enzyme applied to a premix, offered at 50 g/head/d, which made up 0.2% of the dietary DM (**PREM**).

Cows receiving the enzyme-treated TMR all received approximately the same dose; 1 g of enzyme product per cow per day. Dose rate was based on cows consuming 10.7 kg of concentrate and 2.7 kg of supplement, which contained 1 g of enzyme supplement for CONC and SUPP treatments. The enzyme used was Promote N.E.T. (Agribands International, St. Louis, MO). This commercial enzyme product is characterized with primarily xylanases and cellulases activities.

The appropriate amount of enzyme powder was dissolved in water, and then added at the time of milling. For the CONC treatment, the enzyme solution (93 g/20 L water) was added slowly into a 1-t mixer containing steam-rolled barley and pelleted supplement. For the SUPP treatment, the diluted enzyme (93 g/15 L) was added to 250 kg of ingredients in the mixer prior to pelleting the supplement. Due to the small volumes that were required for the PREM treatment, diluted enzyme (4 g/16 ml) was added to 200 g of wheat bran and mixed using a food processor. All three enzyme-fed mixtures were prepared at the beginning of each period. Treated feed was discarded at the end of each period to ensure that the time between applying exogenous enzymes to feed and feeding was the same for all treatments.

The enzyme product activities (Table 3) were assayed using carboxymethyl cellulose (**CMC**, medium viscosity, Sigma, St. Louis, MO); Avicel PH105 20 µm (FMC Corporation, Philadelphia, PA); oat spelt xylan (Sigma, St. Louis, MO); and wheat arabinoxylan, xyloglucan, and barley β-glucan (Megazyme International Ireland Ltd., Wicklow, Ireland) as substrates. Assays were con-

Table 3. Polysaccharidase and glycosidase activities of enzyme preparation¹.

Substrate or activity	Enzyme (lot 1)		Enzyme (lot 2)	
	pH 5	pH 6	pH 5	pH 6
CMC	510	414	463	381
Avicel	241	181	300	177
Arabinoxylan	241	181	299	177
β -Glucan	932	689	754	623
Xylan	1307	1230	1313	1069
Xyloglucan	280	258	266	258
Acetyl esterase	55	84	53	82

¹Polysacchridase activities are expressed as nmol glucose equivalents liberated from the substrate/min/mg protein and glucosidase activities as nmol of nitrophenol released from the p-nitrophenyl substrate/min/mg protein.

ducted by adding 50 μ l of enzyme solution to a tube containing 100 μ l of 0.1 M sodium citrate and phosphate buffer (pH 5.0 and 6.0) and 50 μ l of 2% substrate. The contents were then incubated at 39°C for 10 min; the reaction was stopped with the addition of Somogyi reagent and boiling. Blanks were also used for corrections. Reducing sugars liberated from the hydrolysis of the various substrates were detected using the Nelson-Somogyi method (Somogyi, 1952). Acetyl-esterase (EC 3.1.1.6) was assayed with p-nitrophenyl substrates obtained from Sigma (St. Louis, MO). The assays were carried out in microtiter plates and consisted of 20 μ l of diluted enzyme sample and 80 μ l of 1 mM substrate in 0.1 M citrate and PO₄ buffer (pH 5.0 and 6.0). The mixture was incubated at 39°C for up to 30 min; the reaction was then stopped with 100 μ l of 0.5 M glycine and NaOH buffer, pH 10.6. Release of p-nitrophenol was measured at 420 nm using a MRX-HD plate reader (Dynatech Laboratories, Inc., Chantilly, VA).

Cows were cared for according to the Canadian Council on Animal Care guidelines (Ottawa, ON). Animals were housed individually in tie stalls bedded with wood shavings. Animals were milked twice daily at 0700 and 1700 h in their stalls. Cows were exercised daily for 1 to 2 h, except during urine collection and digestibility measurements. Cows were offered feed three times daily at 0800, 1500 and 1800 h and fed for ad libitum intake with 10% of daily feed refused.

The first 11 days of each period were for adaptation, d 12 to 17 were used to determine rate of passage, d 12 to 14 for digestibility measurements, d 14 to 16 for urine collection, d 21 to 28 for milk yield and composition determination and d 26 for rumen fermentation measurements.

Feed offered and refused was measured and recorded daily to determine DMI. Barley silage and alfalfa silage was sampled weekly and DM was determined to adjust diet composition when required. The TMR and ort samples were collected daily for 1 wk coinciding with milk sampling. The TMR and ort samples were dried at 55°C

and then ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA) and composited for each cow by period and subsequently analyzed for NDF, ADF, and OM. Milk production was recorded daily and sampled morning and evening for 1 wk. Milk samples were preserved with potassium dichromate and stored at 4°C until sent to Central Alberta Milk Testing Laboratory (Edmonton, AB, Canada). Milk was analyzed for milk fat, CP, and lactose (AOAC, 1990) using an infrared analyzer (Milk-O-Scan 605; Foss Electric, Hillerød, Denmark).

Cows were weighed at the beginning and end of each period at approximately 0900 h; these weights were then used to calculate mean BW for each period.

Ruminal Fermentation and Rate of Passage

Ruminal fluid was collected from cannulated cows at 0730 and 1300 h. Samples were taken from four locations within the rumen, composited and then squeezed through four layers of cheesecloth with a mesh size of approximately 250 μ m. Five milliliters of filtered rumen fluid was added to 1 ml of 25% HPO₃ for VFA determination and 5 ml of filtrate was added to 1 ml of 1% sulfuric acid for NH₃ determination. Samples were stored at -20°C until analysis.

Cr-mordanted NDF was used to measure the rate of passage of particulate matter and Co-EDTA was used as a liquid phase marker. Barley silage was boiled in detergent and rinsed until the NDF content of the residual fiber exceeded 85%. The resulting fiber was dried at 55°C. Chromium was mordanted to the barley silage fiber and Co-EDTA was prepared according to Udén et al. (1980).

Each cannulated cow received 225 g of Cr-mordanted barley silage and a 300 ml solution containing 15 g of Co-EDTA via the rumen cannula on d 11. Rectal samples of feces were collected at 0, 3, 6, 9, 12, 16, 20, 24, 28, 32, 36, 40, 48, 60, 72, 96 and 120 h after dosing. Samples were oven dried at 55°C and ground to pass

a 2-mm screen (standard model 4) and stored until analysis.

Particulate and liquid kinetic parameters were estimated for each cannulated cow for each period from the concentration of Cr and Co, respectively in the feces. A double compartmental model (Grover and Williams, 1973) was fitted using the non linear regression procedure of SAS (SAS, 1999).

Apparent Digestion

Apparent total tract digestion of nutrients was determined for all cows using YbCl₃ (Rhône-Poulenc, Inc., Shelton, CT). Ytterbium solution was incorporated directly into the concentrate at the time of milling. Cows received approximately 1 g of Yb/cow daily throughout the entire study. Fecal samples were taken beginning on d 12 at 0900 h then 3, 6, 9, 12, 16, 20, 24, 28, 32, 36, 40, 48 and 60 h later. Samples were individually dried and ground to pass a 2-mm screen (standard model 4), composited across sampling times for each cow and stored for chemical analysis.

The concentration of Yb was analyzed in feed, orts and feces and then digestibility was calculated using the indicator method (Schneider and Flatt, 1975).

Microbial Nitrogen Synthesis

One kilogram of fresh rumen contents obtained from each cannulated cow during rumen evacuation the last day of each period was blended (Waring Products Division, New Hartford, CT) with 1 liter of 0.9% NaCl for 2 min and then squeezed through four layers of cheesecloth. The filtrate was centrifuged (800 × g for 15 min at 4°C) to remove feed particles and then the supernatant was further centrifuged (20,000 × g for 45 min at 4°C) to obtain a bacterial pellet. Bacterial pellets were freeze-dried and ground using a ball mill (Wig-L-Bug; Crescent Dental Mfg. Co., Lyons, IL). The samples were then stored for analysis of purines-N and total-N.

Total output of urine was collected from cannulated animals on d 14 to 16 using a balloon catheter (Rüsch Canada, North York, ON, Canada). Urine was collected in vessels containing sufficient amounts of 4 N H₂SO₄ to maintain pH below 3. Urine volume was measured daily and samples were kept at -20°C until analysis.

Total purine derivatives (PD) excreted (mmol/d) were estimated as the sum of uric acid and allantoin. Excretion of the endogenous PD was assumed to be constant at 0.385 mmol/kg BW^{0.75} for individual cows (Chen and Gomes, 1992). Purine absorption of microbial origin (mmol/d) was calculated as:

$$(\text{total PD excreted} - \text{endogenous factor})/0.85$$

(Chen and Gomes, 1992).

Synthesis of microbial N within the rumen was calculated as:

$$(\text{purine absorption} \times 70)/(\text{purine-N:total-N} \times 0.83 \times 1000)$$

(Chen and Gomes, 1992).

The purine-N and total-N were determined from bacterial pellets obtained from individual cows and the average purine N: total N measured in this study was 0.136.

Chemical Analysis

Analytical DM was determined by drying samples at 135°C for 3 h. Oven DM was determined by drying samples at 55°C for 48 h. Organic matter was determined by ashing (AOAC, 1990). The NDF and ADF contents were determined using an ANKOM^{200/220} Fiber Analyzer (ANKOM Technology, Fairport, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulphite and heat-stable amylase were used in the analysis of NDF. The N content was determined by flash combustion (Carlo Erba Instruments, Milan, Italy). Contents of Co, Cr and Yb were determined using atomic absorption according to the AOAC procedure (AOAC, 1990). Ruminant VFA were separated and quantified using gas chromatography (5890; Hewlett Packard, Mississauga, ON, Canada) using a 30-m (0.32-mm i.d.) column (Nukol column; Supelco, Oakville, ON, Canada). Rumen ammonia was determined as described by Rhine et al. (1998).

Allantoin in urine was determined by autoanalyzer using the procedure of Pentz (1969) with modifications by Lindberg and Jansson (1989). Uric acid was determined using a commercial kit (Sigma No. 292; Sigma Chem. Co., St. Louis, MO). Purine content in the microbial pellet was determined using the procedure of Zinn and Owens (1986).

Statistical Analysis

Means were calculated for all variables by cow within period. Data were analyzed using the MIXED procedure of SAS (SAS, 1999). Period and cow were considered random effects; diet and cannulation effects were considered fixed. Estimation method was restricted maximum likelihood and the degrees of freedom method was Kenward-Roger (SAS, 1999). Differences were tested using the PDIFF option in SAS (SAS, 1999) using a protected ($P < 0.10$) LSD test. Differences were declared significant at a $P < 0.05$; and trends were discussed at a $P < 0.15$, unless stated otherwise.

Table 4. Milk production and composition for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments ¹				SE
	CTRL	CONC	SUPP	PREM	
Milk production, kg/d					
Actual	29.4 ^{a,b}	30.0 ^a	28.8 ^{a,b}	27.9 ^b	2.2
4% FCM	28.9	29.8	28.9	28.4	1.9
Milk composition, %					
Fat	3.91 ^a	4.01 ^{a,b}	4.06 ^{a,b}	4.18 ^b	0.17
Protein	3.59 ^a	3.61 ^{a,b}	3.62 ^{a,b}	3.68 ^b	0.11
Lactose	4.51 ^a	4.49 ^{a,b}	4.48 ^{a,b}	4.45 ^b	0.04
Milk composition, kg/d					
Fat	1.14	1.19	1.16	1.15	0.07
Protein	1.05	1.07	1.03	1.02	0.05
Lactose	1.33 ^a	1.34 ^a	1.29 ^{a,b}	1.24 ^b	0.09
Milk/kg DMI	1.25	1.26	1.20	1.18	0.08
BW change, kg/d	0.49	0.83	0.36	0.68	0.32

¹CTRL = control, CONC = enzyme applied to concentrate (45% of TMR), SUPP = enzyme applied to supplement (4% of TMR), PREM = enzyme applied to premix (0.2% of TMR).

^{a,b}Means in the same row with different superscripts differ ($P < 0.10$).

RESULTS

Milk Production, Composition and Dry Matter Intake

Milk production of cows receiving enzyme treatments was similar to that of cows fed CTRL (Table 4). However, cows receiving the CONC treatment produced more milk than cows receiving the PREM treatment. Although not statistically significant, cows receiving the CONC treatment had numerically higher FCM; 3% more than the CTRL and SUPP treatments, and 5% more than the PREM treatments. Use of an enzyme supplement tended to increase both fat and protein percentage and decrease lactose percentage in milk, however only the PREM treatment was significantly different from CTRL.

Dry matter and other nutrient intakes were not significantly altered by inclusion of the enzyme product (Table 5). There was a significant interaction between the main effects of treatment and cannulation for DMI. Cannulated cows receiving the CONC treatment decreased DMI in comparison to cannulated cows receiving CTRL, while their intact counterparts increased DMI while receiving the CONC treatment compared to CTRL (Figure 1). Cannulated cows receiving the SUPP and PREM treatments increased DMI compared to the CTRL. Dry matter intake for intact cows increased for SUPP and decreased for PREM in comparison to intact cows receiving the CTRL treatment.

Total Tract Digestibility

Cow receiving CONC had a significantly higher DM and OM total tract digestion compared to cows receiving

CTRL or PREM (Table 5). Total tract digestion of NDF and ADF digestion was also increased in CONC fed cows compared to CTRL. Cows receiving SUPP and PREM treatments showed a nonsignificant, intermediate increase in nutrient digestibility compared with CTRL. Treatment differences in rumen pool size of the various constituents were not significant (Table 6).

Ruminal Fermentation

Ruminal fermentation measurements taken prefeeding showed no treatment differences (Table 7). The SUPP and PREM treatments had differing molar proportions of propionate postfeeding, but neither treatment differed from the control. The differences in propionate concentration led to differences in the acetate to propionate ratio between these two treatments. Ammonia nitrogen concentration in the rumen both pre- and postfeeding did not differ between treatments and was highly variable as shown by the large standard error.

Rate of Passage

Supplementation with this enzyme product had no effect on the rumen fluid outflow or digesta passage from the rumen (Table 8).

Microbial Nitrogen Synthesis

Purine derivatives excreted in urine were similar for all treatments (Table 9). Cows fed PREM produced significantly more microbial nitrogen than did cows receiving the SUPP treatment, with intermediate levels of microbial nitrogen from cows fed CONC. However, microbial nitrogen from cows fed the enzyme treatments

Table 5. Nutrient intake and total tract digestibility for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments ¹				SE
	CTRL	CONC	SUPP	PREM	
Intake, kg/d					
DM	23.6	23.7	24.0	23.7	0.5
DDM	15.4 ^a	17.2 ^b	16.8 ^{a,b}	15.6 ^{a,b}	0.6
OM	21.8	21.9	22.0	21.7	0.4
NDF	7.1	7.0	7.0	7.3	0.2
Digestibility, %					
DM	65.1 ^a	72.6 ^b	70.1 ^{a,b}	65.9 ^a	2.4
OM	66.6 ^a	73.8 ^b	71.4 ^{a,b}	67.2 ^a	2.3
NDF	44.3 ^a	55.6 ^b	50.6 ^{a,b}	46.9 ^{a,b}	4.0
ADF	43.6 ^a	55.6 ^b	50.2 ^{a,b}	47.4 ^{a,b}	4.1

¹CTRL = control, CONC = enzyme applied to concentrate (45% of TMR), SUPP = enzyme applied to supplement (4% of TMR), PREM = enzyme applied to premix (0.2% of TMR).

^{a,b}Means in the same row with different superscripts differ ($P < 0.10$).

was not different ($P > 0.05$) from cows fed the control treatment. Although not statistically significant, the CONC and PREM treatments increased microbial nitrogen production by 6% and 9%, respectively, compared to the CTRL ($P = 0.09$).

DISCUSSION

The results from this study indicate that the proportion of the concentrate that this fibrolytic enzyme prod-

uct is applied to plays a key role in determining whether digestion increases. Increases in OM digestibility over the control in this study ranged from 1% to 10%, depending upon the proportion of the TMR that this enzyme was applied to. Rode et al. (1999) reported a 10% increase in DMD for cows in early lactation, while Yang et al. (2000) reported a 5% increase in OM digestibility, when enzymes were applied to the concentrate portion of a TMR. Although Lewis et al. (1999) applied fibrolytic enzymes directly to the forage portion of the diet and reported no increase in digestibility, milk production and DM intake increased.

The application of the enzyme product prior to pelleting, as was the case for the SUPP treatment, appears to have hindered the efficacy of the product based on the lack of improvement in total tract digestibility. The pelleting process involved temperatures as high as 94°C, which may reduce the activity of this enzyme, thereby delivering insufficient activity levels when fed to the animal. Unfortunately we were not able to quantify the possible decline in enzyme activity due to pelleting because reliable methods for detecting exogenous enzymes added to feeds are lacking (McCleary, 2001). Methods that rely on extracting the enzymes from feeds are subject to the errors associated with inadequate enzyme recovery. Furthermore, the background reducing sugar content of supernatant due to the soluble feed substrate greatly increases the potential for errors (Wallace and Hartnell, 2001). Applying the enzyme product to the supplement after the pelleting process may be a viable solution for future studies. Alternatively, decreased efficacy of enzymes for the SUPP treatment may indicate that enzymes are more effective when applied to a larger portion of the diet, as was the case when enzymes were added to concentrate.

Providing the enzyme product to cows in a concentrated premix was also not effective based on the lack

Figure 1. Interaction between cannulation and enzyme treatment effects for DMI. (Top) Dry matter intake for cannulated (□) and intact (■) cows (SE=0.63) and (Bottom) digestible dry matter intake (DDMI) for cannulated (□) and intact (■) cows (SE=0.86). CTRL = control, CONC = enzyme applied to 45% of TMR, SUPP = enzyme applied to 4% of TMR, PREM = enzyme applied to 0.2% of TMR.

Table 6. Ruminal pool size for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments ¹				SE
	CTRL	CONC	SUPP	PREM	
Whole rumen contents					
Wet, kg	90.2	89.2	92.9	93.5	3.8
DM, kg	13.8	13.3	14.6	14.6	0.7
NDF, kg	7.7	7.6	8.1	8.1	0.4

¹CTRL = control, CONC = enzyme applied to concentrate (45% of TMR), SUPP = enzyme applied to supplement (4% of TMR), PREM = enzyme applied to premix (0.2% of TMR).

of improvement in total tract digestibility. The portion of the ration treated with enzyme for CONC was larger, in comparison to SUPP and PREM, which may have maximized the dispersion of the enzyme within the rumen and increased the likelihood of the exogenous enzyme being beneficial. Beauchemin et al. (1999) postulated that applying enzymes to feed creates a "slow release" mechanism releasing the enzyme into the rumen fluid as feed is digested. The decreased size of the premix may have allowed rapid passage out of the rumen, lessening the enzyme effect for cows fed PREM. This hypothesis is further supported by an in vitro study using the same treatments showing 18% and 20% increases in DM digestibility for CONC and PREM, respectively (unpublished). Thus, having the enzyme delivered in a highly concentrated form did not hinder the product in vitro, but residence time is not a factor in batch culture. In contrast, residence time of enzyme in the rumen may have been reduced in the present study for cows receiving PREM, thereby negating potential benefits.

It is possible that applying enzyme to concentrate increased digestion by enhancing the digestibility of the barley grain. It is well documented that the close association of enzyme with feed enables a form of preingestive attack of the enzymes upon the plant fiber (Wang et al., 2002). However, this explanation does not account for the increase observed in vitro for the premix treatment (unpublished). Thus, it is unlikely that the digestion response was dependant upon the substrate to which the enzyme was applied.

Previously it was thought that exogenous enzyme sources were susceptible to rumen microorganisms and gastrointestinal degradation. This notion has recently been refuted and it is now known that some exogenous enzyme sources have the ability to withstand both ruminal and intestinal degradation (Morgavi et al., 2001), although resistance to proteolysis is not the same for all enzyme products (Hristov et al., 1998a; Hristov et al., 1998b).

There are few studies in which cannulated and intact animals are studied simultaneously and the interaction

Table 7. Ruminal fermentation for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments ¹				SE
	CTRL	CONC	SUPP	PREM	
Prefeeding (0 h)					
VFA, mM	118.3	122.5	121.1	118.6	7.2
VFA, mol/100 mol					
Acetate (A)	64.9	63.6	63.9	65.3	1.1
Propionate (P)	19.7	19.6	21.4	17.9	1.5
Butyrate	11.1	12.2	10.7	12.6	0.7
A:P	3.35	3.27	3.10	3.68	0.28
NH ₃ N, mg/L	104.5	140.7	104.5	125.3	14.7
Postfeeding (5 h)					
VFA, mM	122.7	126.9	116.3	128.5	8.3
VFA, mol/100 mol					
Acetate (A)	63.8	64.2	63.0	64.9	1.6
Propionate (P)	20.9 ^{a,b}	20.3 ^{a,b}	22.2 ^a	18.7 ^b	1.9
Butyrate	11.1	11.5	10.9	12.5	0.6
A:P	3.17 ^a	3.23 ^{a,b}	2.98 ^a	3.53 ^b	0.36
NH ₃ N, mg/L	56.2	70.4	70.7	77.1	13.0

¹CTRL = control, CONC = enzyme applied to concentrate (45% of TMR), SUPP = enzyme applied to supplement (4% of TMR), PREM = enzyme applied to premix (0.2% of TMR).

^{a,b}Means in the same row with different superscripts differ ($P < 0.10$).

Table 8. Digesta and ruminal fluid passage kinetics for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments ¹				SE
	CTRL	CONC	SUPP	PREM	
Liquid					
LORR, ² %/h	12.1	10.1	10.4	9.9	2.2
RRT, ³ h	9.5	11.5	10.4	10.7	1.8
TRT, ⁴ h	24.2	22.9	20.9	21.7	1.6
Particle					
PORR, ⁵ %/h	4.8	5.1	4.8	5.0	0.6
RRT, h	21.8	19.9	23.8	20.4	3.2
TRT, h	47.8	49.2	50.0	46.1	3.2

¹CTRL = control, CONC = enzyme applied to concentrate (45% of TMR), SUPP = enzyme applied to supplement (4% of TMR), PREM = enzyme applied to premix (0.2% of TMR).

²Liquid outflow rate from the reticulorumen.

³Rumen retention time.

⁴Total tract retention time.

⁵Particle outflow rate from the reticulorumen.

between diet and cannulated effects for DMI were unanticipated (Figure 1). Cannulated cows decreased DMI when receiving CONC, however due to the increase in total tract digestibility, intake of DDM was increased for cows fed CONC compared to cows fed CTRL. It is hypothesized that due to production differences between cannulated and intact cows additional nutrients available due to enzyme supplementation were in excess of requirements and cannulated cows receiving the CONC treatment subsequently lowered DMI. Intake of DDM for cows fed PREM was similar to cows fed CTRL, indicating comparable total tract digestion.

Milk production and composition were reported, but were not the main focus of this study due to the limited number of cows used. Other researchers feeding various enzyme products have shown both an increase (Rode et al., 1999; Yang et al., 2000) and no change (Beauchemin et al., 2000) in milk production. In the present study, the numerical increase in 4% FCM for cows fed CONC was attributed to the increase in diet digestibility because DMI was not affected by diet. Milk production followed the same trend as intake of DDM. This

experiment confirmed previous studies that showed enzyme supplementation did not increase DMI (Beauchemin et al., 1999; Hristov et al., 1998a; Kung et al., 2000). However, the effects of enzyme supplementation on DMI may depend on the specific formulation evaluated, as other studies using different enzyme products fed to beef steers (Feng et al., 1996) or dairy cows (Beauchemin et al., 2000) have shown an increase in DMI.

The trend of increased fat and/or protein percentage due to fibrolytic enzyme supplementation has been previously reported (Luchini et al., 1997; Nussio et al., 1997; Schingoethe et al., 1999). In contrast, Kung et al. (2000) showed similar fat and protein percentages with enzyme supplementation, while Beauchemin and co-workers reported an increase in protein percentage with no difference in daily production of fat or protein (Beauchemin et al., 2000). To date, most studies that reported an increase in milk production due to enzyme supplementation observed either a decrease or no effect on fat and protein percentage (Rode et al., 1999; Yang et al., 1999; Kung et al., 2000; Yang et al., 2000). In our study, it is hypothesized that the increased intake

Table 9. Urinary purine derivatives and microbial nitrogen synthesis for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments ¹				SE
	CTRL	CONC	SUPP	PREM	
Uric acid, mmol/d	23.5	25.2	24.9	29.6	4.2
Allantoin, mmol/d	309	305	293	322	17
Total PD, 2 mmol/d	332	331	318	352	19
Microbial N, g/d	196 ^{a,b}	209 ^{a,b}	185 ^a	216 ^b	12

¹CTRL = control, CONC = enzyme applied to concentrate (45% of TMR), SUPP = enzyme applied to supplement (4% of TMR), PREM = enzyme applied to premix (0.2% of TMR).

²PD = purine derivatives excreted in urine.

^{a,b}Means in the same row with different subscripts differ ($P < 0.10$).

of digestible energy due to enzyme supplementation (CONC) that was not used for milk production was allocated to milk fat, milk protein, and body reserves. This is also supported by the numerical increase for change in BW for cows fed CONC compared to cows fed CTRL.

Despite the increase in total tract feed digestion, the response in milk production was not observed with enzyme supplementation. The positive energy status of the cows was attributed to the lack of response. However, calculated energy status revealed that cows receiving the CONC treatment had a larger surplus of energy after maintenance energy requirements were met. If this excess energy had been partitioned into milk and milk composition remained unchanged, then daily milk output would have increased 9% in cows receiving the CONC treatment in comparison to cows receiving CTRL. This implies that cows in early lactation and negative energy balance may benefit from receiving supplemental enzymes.

Though no difference was seen for digesta or passage kinetics in this study, others have reported trends of increased digesta passage for dairy cows (Beauchemin et al., 1999) and significantly increased digesta passage for beef steers (Feng et al., 1996) due to enzyme supplementation. However, fibrolytic enzyme supplements have generally been shown not to alter passage of either ruminal fluid or particulate material (Lewis et al., 1996; Yang et al., 1999; Hristov et al., 2000).

The decrease in propionate, subsequently leading to an increase in the acetate to propionate ratio was unexpected for the PREM treatment. Previous studies providing enzyme supplementation have reported no effect (Beauchemin et al., 1999; Kung et al., 2000) to slight differences in molar ratios (Hristov et al., 2000), even when the total VFA concentration was increased (Lewis et al., 1996). Differences in ammonia N concentrations pre- and postfeeding were expected and are explained by decreased synchrony between protein and energy.

Two main factors can affect rumen microbial yield: rate of outflow from the rumen and amount of OM fermented within the rumen. Because rumen flow kinetics were not affected by enzyme supplementation, this is not likely to account for increased microbial yield due to enzyme supplementation. The trend of increased microbial nitrogen synthesis is attributed to an increase in ruminal digestion and overall rumen efficiency.

CONCLUSIONS

The portion of the diet to which a fibrolytic enzyme product is applied must be considered in order to elicit the maximum benefit. Cows in midlactation fed a diet to which an enzyme product was applied to 45% of

the TMR numerically increased milk production. This increase in production is attributed to an increase in digestibility and intake of digestible DM, not to increased DM intake. All treatments containing this enzyme product numerically increased total tract digestibility of OM, NDF, and ADF. However, cows fed the diet in which the enzyme was applied to a reduced portion of the diet did not show the same increased digestibility when compared to cows fed the control. The results are interpreted to indicate that exogenous fibrolytic enzymes should be applied to a substantial portion of the diet to ensure their effectiveness. Future studies are required to understand the mode of action of fibrolytic exogenous enzymes. Studies focusing on method of application, proportion of diet that the enzyme is applied, and mode of action for increased digestion would greatly advance the understanding of exogenous enzymes for ruminants.

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