

Effect of a Direct-Fed Fibrolytic Enzyme Formulation on Nutrient Intake, Partitioning, and Excretion in Early and Late Lactation Holstein Cows

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ABSTRACT

The effect of a fibrolytic enzyme formulation on N and P intake, partitioning, and excretion was evaluated in dairy cows in early and late lactation. Twelve lactating Holstein cows (6 early lactation, 6 late lactation) were fed diets with or without the enzyme formulation in a switchback design with three, 4-wk periods. Diets for the early lactation group contained 45% forage, and late lactation diets contained 61% forage. Cows fed diets containing the enzyme formulation gained more weight than those on the control diet; this weight gain with enzyme addition was greater in early lactation cows than in late lactation cows. The main effect of enzyme treatment did not significantly affect apparent digestibility or excretion of N and P, or retention of these nutrients in body tissue. Interactions observed between the effects of group (stage of lactation) and treatment indicated differences in the nature of the milk yield and manure excretion responses to enzyme treatment between early and late lactation cows. These interactions were due to numerical increases in milk yield, feces excretion, and N excretion in early lactation cows fed diets containing the enzyme formulation compared to control, and slight decreases in these measures in late lactation cows with enzyme addition. Cows fed diets containing a direct-fed fibrolytic enzyme formulation had increased body weight gain, but the effect of addition of the enzyme formulation on milk yield and manure nutrient excretion differed for early and late lactation cows.

(Key words: fibrolytic enzyme, lactating cows, nitrogen excretion, phosphorus excretion)

INTRODUCTION

Environmental concerns with N and P are associated with pollution of water resources. Excess N or P in

surface water causes algae populations to grow rapidly, or to “bloom”. The decomposition of this algae consumes dissolved oxygen in the water, impairing the survival and productivity of fish, clams, crabs, oysters, and other aquatic species. In addition, nitrate-N contamination of drinking water supplies can cause methemoglobinemia, impairing oxygen transport to peripheral tissues.

Nutrient losses from livestock farms account for as much as 47% of P loading and 21% of N loading to bodies of surface water, depending on watershed (Smith and Alexander, 2000). Reducing the N and P content of livestock manure, and/or the quantity of manure excreted is a powerful, cost-effective approach to reducing potential nutrient losses from farms (Kohn et al., 1997). Phytase treatment of cereal grains for monogastrics, and application of amino acid nutrition of monogastrics and ruminants are two such nutritional techniques to reduce N and P excretion and potential nutrient losses from livestock farms.

Another nutritional approach to reducing nutrient excretion is to supplement ruminal enzyme activity to enhance digestion of feedstuffs. Cellulose and hemicellulose are digested by cellulase and xylanase produced by ruminal bacteria and protozoa. These fibrolytic enzymes have been isolated from fungal cultures and have improved fermentation during the ensiling process when added to chopped forages (Stokes, 1992; Chen et al., 1994; Sheperd and Kung, 1996a; Sheperd and Kung, 1996b). When fibrolytic enzymes were applied to forage, grain, or TMR immediately prior to feeding lactating cows, increased digestibility (Beauchemin et al., 1999; Rode et al., 1999; Yang et al., 1999; Yang et al., 2000), feed intake (Nussio et al., 1997; Lewis et al., 1999), and milk yield (Lewis et al., 1999; Rode et al., 1999; Yang et al., 1999; Kung et al., 2000; Yang et al., 2000) have been observed.

The effects of direct-fed fibrolytic enzymes in lactating cow diets are somewhat inconsistent, however, with some studies reporting no effect on feed intake (Luchini et al., 1997; Zheng and Stokes, 1997; Rode et al., 1999; Schingoethe et al., 1999; Yang et al., 1999; Kung et al.,

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2000; Yang et al., 2000) or milk yield (Luchini et al., 1997; Nussio et al., 1997; Zheng and Stokes, 1997; Schingoethe et al., 1999). Inconsistency of results may be due to differences in energy status of the cows, diet composition, type and activity of enzyme used, and method of application (Yang et al., 2000).

The increased *in vivo* digestibility frequently observed with direct-fed fibrolytic enzymes implies decreased fecal output, but few studies have reported manure production data. There is no published data on the effect of direct-fed fibrolytic enzymes on excretion of N and P by ruminants. The objectives of the current study were to evaluate the effect of a direct-fed fibrolytic enzyme formulation on N and P retention, efficiency of nutrient utilization, and excretion of feces, urine, N and P by dairy cows in early and late lactation.

MATERIALS AND METHODS

Cows and Diets

Twelve lactating Holstein cows (6 early lactation, 6 late lactation) were assigned randomly to experimental diets with or without the addition of a fibrolytic enzyme formulation. To evaluate the enzyme formulation, early lactation cows were fed diets containing 44.9% forage, while late lactation diets were 60.7% forage on a dry basis. The enzyme formulation was a commercial preparation from fungal extracts, with 15,000 units of cellulase activity per g (Loveland Industries, Greeley, CO). One unit is defined as the cellulase activity that produced a relative fluidity change of 1.0 in 100 minutes in a 0.2% (w/v) sodium carboxymethyl cellulose (CMC type 7HP, Hercules, Inc., Wilmington, DE) solution under assay conditions (pH 4.5 and 40°C) as measured with a Size 100 Calibrated Cannon-Fiske type viscosimeter.

The granular enzyme formulation was mixed with a corn grain carrier, and the enzyme-corn grain mixture or control (an equal quantity of corn grain containing no enzyme formulation) was added to the grain portion of the diet prior to mixing of the TMR (204 g enzyme formulation/tonne DM fed). Ingredient and nutrient composition of diets are presented in Table 1. The protein content of the alfalfa silage fed during the experiment was lower than indicated by pre-trial analysis, causing a lower than intended dietary CP content in both the early and late lactation cow diets. This alfalfa silage made up a greater proportion of the late lactation diets than of the early lactation diets, causing greater underfeeding of CP to this group of cows. Cows were fed once a day at 0700 h to achieve 15% feed refusals. This experiment was conducted with approval from the Virginia Tech Animal Care Committee.

Experimental Design and Sampling

Six cows in early lactation (average DIM = 30 ± 10.6 ; average parity = 3.3 ± 0.8) and six cows in late lactation (average DIM = 194 ± 9.7 ; average parity = 2.5 ± 0.8) were randomly assigned to treatment. A switchback design with three, 4-wk periods was used to separate cow and treatment effects, and to maximize statistical power. The first 24 d of each period were for adaptation to treatment diets, and the last 4 d were used for data collection. During the first collection period, two early lactation cows fed diets containing the enzyme formulation developed mastitis. Data from this period for these two cows were not included in statistical analyses. Both cows recovered fully and were included in the remaining two collection periods. No other health problems were observed.

Cows were housed in freestalls between collection periods. On d 25 of each period, cows were moved to a tie-stall area for total collection. During each collection period, cows were housed in individual stalls for 4 d. On d 25, a sterile Foley catheter (22 french, 75 cc; C. R. Bard, Inc., Covington, GA) was placed into the urethra for total collection of urine. Total collection of feces, urine, and milk was conducted on d 26, 27, and 28 of each period. Urine was weighed at 4-h intervals, acidified (22 ml of 6 N HCl/kg of urine), pooled, subsampled after 24 h, and stored frozen for later analysis. All excreted feces were collected at 4-h intervals and stored in a sealed container, then weighed, thoroughly mixed, and subsampled daily. Feed ingredients (forages and concentrates) were sampled once each week, and feed refusals were weighed and sampled daily. Feed, feed refusals, and feces samples were dried and ground for later analysis. Milk weights were recorded and milk samples obtained at six consecutive milkings on d 26, 27, and 28 of each collection period. Cows were weighed at the start of the first period, and on d 25 and 28 of each collection period. The two body weight observations on d 25 and 28 of each period were averaged, and the change in body weight between periods was calculated.

To detect changes due to addition of the enzyme formulation, dietary NE_L content was calculated from daily milk yield, milk fat concentration, and body weight change (National Research Council, 1989). This back-calculation of NE_L content from measured animal performance assumes that the quantity of NE_L available from body weight loss is 4.92 Mcal/kg BW loss, the NE_L required for body weight gain is 5.12 Mcal/kg BW gain, the NE_L required for maintenance is 80 kcal/kg $BW^{0.75}$, and the NE_L required for milk yield is 0.74 Mcal/kg 4% FCM.

Table 1. Ingredient and nutrient composition of diets.

Ingredient	Early lactation	Late lactation
	% of diet DM	
Alfalfa silage	22.43	30.34
Corn silage	22.43	30.34
Corn, ground	38.23	28.21
Soybean meal, 48% CP	11.70	6.67
SoyPass	2.69	2.84
Sodium bicarbonate	0.51	0.53
Vitamin-mineral mix	2.02 ¹	1.07 ²
	% of diet DM	
Nutrient		
CP	16.8	14.8
RUP ³	6.67	5.56
ADF	14.3	16.5
NDF	27.3	31.1
Ash	6.68	6.18
Ca	0.80	0.70
P	0.46	0.42

¹Contained 191 g of Ca/kg, 69 g of P/kg, 38.3 g of Na/kg, 60 g of Cl/kg, 2 g of K/kg, 15 g of Mg/kg, 1.78 g of S/kg, 0.6 g of Cu/kg, 2 g of Zn/kg, 2 g of Mn/kg, 5 mg of Co/kg, 30 mg of I/kg, 4.6 g of Fe/kg, 15 mg of Se/kg, 330,000 IU of vitamin A/kg, 110,000 IU of vitamin D/kg, and 1320 IU of vitamin E/kg.

²Contained 161 g of Ca/kg, 100 g of P/kg, 57.5 g of Na/kg, 90 g of Cl/kg, 1.78 g of K/kg, 3.9 g of Mg/kg, 2.87 g of S/kg, 1.1 g of Cu/kg, 4 g of Zn/kg, 4 g of Mn/kg, 10 mg of Co/kg, 65 mg of I/kg, 6.67 g of Fe/kg, 28 mg of Se/kg, 661,000 IU of vitamin A/kg, 220,000 IU of vitamin D/kg, 2640 IU of vitamin E/kg.

³Estimated (National Research Council, 2001).

Laboratory Analyses

Feed, feed refusals, and fecal samples were dried at 60°C to constant weight, and then ground through a 1-mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). Feed, feed refusals, and fecal samples were analyzed in duplicate for N, ash, Ca, and P (Association of Official Analytical Chemists, 1984), and for ADF and NDF (with α -amylase and without sodium sulfite; Van Soest et al., 1991). Urine samples were analyzed in duplicate for P and N using standard procedures (Association of Official Analytical Chemists, 1984). Milk samples were analyzed for fat, protein, lactose, SNF content (Dairy Herd Improvement Association, Blacksburg, VA), and for P content (Association of Official Analytical Chemists, 1984).

Statistical Analyses

All data were statistically analyzed using PROC MIXED of SAS (SAS Institute, 1999), with the model

$$Y_{ijklm} = \mu + T_i + P_j + G_k + (T \times G)_{ik} + O_l + (G \times O)_{kl} + C(G \times O)_m + E_{ijklm}$$

where

μ = overall mean,

T_i = effect of dietary treatment (enzyme vs.

control, $i = 1$ to 2),

P_j = effect of period ($j = 1$ to 3),

G_k = effect of group (early vs. late lactation, $k = 1$ to 2),

$(T \times G)_{ik}$ = effect of interaction of T_i and G_k ,

O_l = effect of order of treatment (i.e., "control, enzyme, control" vs. "enzyme, control, enzyme"; $l = 1$ to 2),

$(G \times O)_{kl}$ = effect of interaction of G_k and O_l ,

$C(G \times O)_m$ = random effect of cow within the interaction of G_k and O_l ; and

E_{ijklm} = residual error, assumed to be normally distributed.

Residual error was used to test main effects and interactions. Differences were considered significant at $P < 0.05$ and trends at $P < 0.15$ unless otherwise indicated. All results are reported as least squares means.

RESULTS

Feed Intake and Manure Production

DMI tended to be greater in early lactation cows than in late lactation cows ($P < 0.07$; Table 2). Within these early lactation cows, DMI was numerically, but not significantly, greater in cows fed the diet containing the enzyme formulation compared to those fed control diets (+1.2 kg/d). The main effect of enzyme addition had no

Table 2. Effect of stage of lactation and direct-fed fibrolytic enzyme formulation on dry matter intake, excretion, and digestibility, and urine output of lactating Holstein cows.

	Early lactation		Late lactation		SEm ¹	<i>P</i> <		
	Control	Enzyme	Control	Enzyme		Group	Trt	Group × trt
n	9	7	9	9				
DMI, kg/d	25.5	26.7	24.3	24.3	0.8	0.07	0.42	0.44
Feces DM, kg/d	9.2	9.6	10.1	9.6	0.3	0.25	0.97	0.16
Urine, kg/d	20.8	21.5	21.1	20.3	0.9	0.69	0.98	0.33
Apparent DM digestibility, %	64.0	63.9	58.2	61.1	1.4	0.01	0.30	0.27

¹Unequal n, largest SEm (n = 7) reported.

effect on excretion of feces, but a trend was observed for an interaction of the effects of enzyme treatment and stage of lactation on this measure ($P < 0.16$). This interaction occurred because the direction of the response to enzyme treatment was different in the two groups of cows. A numerical increase in feces excretion was observed in early lactation cows fed diets containing enzyme compared to those fed control diets, but feces excretion was reduced somewhat in late lactation cows with enzyme addition. Excretion of urine was not affected by the addition of fibrolytic enzymes in either group of cows.

Apparent DM digestibility was higher in early lactation cows than in later lactation cows. This reduced DM digestibility in late lactation cows was likely a consequence of the higher fiber diet they were fed. Digestibility of DM was similar for control and enzyme-supplemented diets in early lactation cows, but in late lactation cows, DM digestibility was numerically greater with the enzyme addition compared to control.

Body Weight, Milk Yield, and Milk Composition

Body weight tended to be lower in early lactation cows than in late lactation cows ($P < 0.12$; Table 3).

There was no overall effect of enzyme addition on body weight, but an interaction was observed between the effects of stage of lactation and enzyme treatment ($P < 0.07$). This interaction was due to a numerical increase in body weight in early lactation cows fed diets containing the enzyme formulation compared to those fed the control diet, while body weights were similar in late lactation cows fed diets containing the enzyme and control formulations.

Cows fed diets containing the enzyme formulation gained more weight than those on the control diet (+16.9 vs. -0.8 kg/28 day period). The magnitude of the change in weight gain was greater in early lactation cows than in late lactation cows (significant group by treatment interaction).

As expected, milk yield was lower in cows in late lactation compared to those in early lactation. The main effect of enzyme addition did not affect milk yield, but an interaction between group and enzyme treatment was observed ($P < 0.08$). This interaction occurred because the direction of the response to enzyme treatment was different in the two groups of cows. A numerical increase in milk yield was observed in early lactation cows fed diets containing the enzyme formulation com-

Table 3. Effect of stage of lactation and direct-fed fibrolytic enzyme formulation on body weight, milk yield and milk composition of lactating Holstein cows.

	Early lactation		Late lactation		SEm ¹	<i>P</i> <		
	Control	Enzyme	Control	Enzyme		Group	Trt	Group × trt
Body weight, kg	577	592	634	632	20	0.12	0.17	0.07
Change in BW, kg/28 d	-9.9	22.7	8.3	11.1	6.6	0.60	0.02	0.04
Milk yield, kg/d	41.1	42.9	32.6	31.4	2.3	0.02	0.72	0.08
4% FCM, kg/d	35.7	37.5	31.4	30.7	2.6	0.14	0.60	0.23
Calculated dietary NEL, mcal/kg ²	1.38	1.61	1.45	1.41	0.08	0.52	0.12	0.06
Milk fat, %	3.11	3.14	3.74	3.83	0.24	0.07	0.64	0.79
Milk protein, %	3.00	3.07	3.29	3.28	0.10	0.09	0.47	0.38
Milk lactose, %	4.85	4.88	4.58	4.60	0.10	0.04	0.76	0.96
Milk SNF, %	8.58	8.69	8.58	8.62	0.15	0.86	0.40	0.70
Milk fat, kg/d	1.29	1.35	1.22	1.21	0.12	0.54	0.60	0.45
Milk protein, kg/d	1.22	1.30	1.27	1.14	0.09	0.60	0.67	0.12
Milk lactose, kg/d	1.99	2.09	1.50	1.44	0.12	0.01	0.69	0.10
Milk SNF, kg/d	3.52	3.73	2.80	2.70	0.19	0.01	0.50	0.06

¹Due to unequal n, largest SEm (n = 7) reported.

²Dietary NEL concentration calculated from fat-corrected milk yield and change in body weight (National Research Council, 1989).

pared to control, but milk yield decreased slightly in late lactation cows with enzyme feeding. Yield of protein, lactose, and SNF followed the same pattern, tending to increase in early lactation cows fed diets containing the enzyme formulation, and to decrease somewhat with enzyme addition in late lactation cows. Concentrations of fat, protein, lactose, and SNF, and yield of milk fat were unaffected by enzyme addition.

To detect changes in energy content of the diet due to addition of the enzyme formulation, dietary NE_L content was calculated from daily milk yield, milk fat concentration, and body weight change (National Research Council, 1989). The response in this measure to enzyme addition varied between early and late lactation cows (interaction between group and treatment, $P < 0.06$). Calculated NE_L content tended to increase with enzyme addition to the early lactation (low forage) diets, but was similar in the late lactation diets containing the enzyme formulation or control. The calculated NE_L contents of the diets fed to late lactation cows and the control diet for early lactation cows were lower than would be estimated by the current Dairy NRC model from feed composition data (National Research Council, 2001).

Nutrient Intake, Digestion, and Excretion

Early lactation cows consumed less NDF and tended to excrete less NDF in feces than did late lactation cows (Table 4). These changes were likely due to the lower forage, lower fiber diets fed to the early lactation cows compared to the diets fed to late lactation cows (Table 1).

While dietary NDF content was unchanged by enzyme addition at the time of feeding, intake of NDF was greater in cows fed diets containing the enzyme formulation than in those on the control diet (Table 4). This increase in NDF intake was due to the numerical increase in DMI in early lactation cows fed diets containing the enzyme formulation (Table 2) combined with small changes in the NDF content of feed refusals. Fecal excretion of NDF and apparent NDF digestibility were not affected by enzyme treatment. There were no interactions of stage of lactation and enzyme treatment on fiber digestion.

As expected, P intake was higher in early lactation cows than in later lactation cows (Table 5) due to greater dietary P content of early lactation diets (Table 1) and numerically higher DMI in these cows (Table 2). Fecal P excretion was unaffected by stage of lactation. Urinary P, although low (< 1 g/d), was greater in early lactation cows than in late lactation cows. Apparent P digestibility, net absorbed P, milk P, and P retained in body tissue were all higher in early lactation cows than in late lactation cows. The negative P balance observed in late lactation cows occurred despite the expected adequacy of dietary P (National Research Council, 2001).

Phosphorus intake and excretion in feces and urine was not affected by addition of the enzyme formulation (Table 5). Apparent P digestibility, milk P, and P retained in body tissue were not significantly affected by enzyme treatment or by the interaction of stage of lactation and treatment.

Nitrogen intake was higher in early lactation cows than in later lactation cows (Table 6) because early lactation cows were fed diets higher in CP (Table 1). Urinary N excretion, apparent N digestibility, absorbed N, milk N as a proportion of N intake, and N retention were all higher in early lactation cows than in later lactation cows as well. The negative N balance observed in late lactation cows reflects the lower than intended CP content of the diet, and is similar to the negative metabolizable protein balance predicted for these cows fed this diet by the current NRC model (National Research Council, 2001).

Intake of N and N excretion in feces and urine was not affected by addition of the enzyme formulation (Table 6). A trend for an interaction was observed between the effects of group and enzyme treatment for total N excretion ($P < 0.15$), indicating that early and late lactation cows responded somewhat differently to the enzyme treatment. This interaction was due to a numerical increase in total N excretion in early lactation cows fed diets containing the enzyme formulation compared with the control, and a slight decrease in N excretion with enzyme addition in late lactation cows (-30 g/d). Similarly, secretion of N in milk was numerically increased in early lactation cows fed the enzyme mixture, but declined slightly with enzyme treatment in

Table 4. Effect of stage of lactation and direct-fed fibrolytic enzyme formulation on fiber digestion of lactating Holstein cows.

	Early lactation		Late lactation		SEm ¹	<i>P</i> <		
	Control	Enzyme	Control	Enzyme		Group	Trt	Group × trt
NDF intake, kg/d	6.11	6.54	7.13	7.28	0.21	0.01	0.05	0.33
Fecal NDF excretion, kg/d	3.74	4.01	4.61	4.67	0.34	0.06	0.59	0.73
Apparent NDF digestibility, %	44.9	44.8	37.8	40.5	3.2	0.09	0.67	0.64

¹Due to unequal n, largest SEm (n = 7) reported.

Table 5. Effect of stage of lactation and direct-fed fibrolytic enzyme formulation on P intake and partitioning of lactating Holstein cows.

	Early lactation		Late lactation		SEM ¹	P <		
	Control	Enzyme	Control	Enzyme		Group	Trt	Group × trt
P intake, g/d	117.5	119.3	99.6	98.9	4.7	0.01	0.91	0.77
Fecal P excretion, g/d	82.4	83.8	89.1	82.3	3.8	0.49	0.45	0.25
Urinary P excretion, g/d	0.41	0.34	0.14	0.15	0.06	0.01	0.54	0.44
Total P excretion, g/d	82.8	84.3	92.0	82.7	4.3	0.37	0.34	0.19
Apparent P digestibility, %	28.7	29.8	10.2	17.0	3.9	0.01	0.28	0.44
Absorbed P, g/d	35.1	35.7	10.5	16.5	4.7	0.01	0.47	0.55
P in milk, g/d	31.7	36.1	27.7	26.0	3.1	0.07	0.60	0.24
Milk P, % of P intake	27.6	30.6	27.8	26.4	3.2	0.53	0.80	0.45
P retention, g/d	0.13	-1.08	-20.10	-9.83	6.8	0.05	0.49	0.38

¹Due to unequal n, largest SEM (n = 7) reported.

late lactation cows (interaction, $P < 0.13$). Apparent N digestibility and N retained in body tissue were not significantly affected by enzyme treatment.

DISCUSSION

The effect of the addition of a fibrolytic enzyme formulation to the diet of lactating cows varied with stage of lactation. While the main effect of enzyme treatment for many of these parameters was not significant across or within the two groups of cows, interactions were observed because the responses of the two groups of cows were opposite in direction. In early lactation cows, feed intake was numerically increased, and diet digestibility and manure excretion were unchanged. Enzyme addition improved body weight gain and numerically increased milk yield in these early lactation cows, increasing the calculated NE_L content of the diet by 17%. In later lactation cows fed a higher forage diet, enzyme treatment tended to decrease excretion of feces and N compared to control, with no effect on DMI and little effect on body weight gain.

Differences in the response of early and late lactation cows to enzyme treatment like those observed in the

current study have been reported in other published research. In mid-lactation cows, no change in feed intake was observed with the addition of a liquid mixture of cellulases and xylanases to forages 8 to 24 h prior to feeding (Lewis et al., 1999). In a 16-wk companion study with varying amounts of this enzyme mixture applied just before feeding (Lewis et al., 1999), feed intake was increased in early lactation cows fed the enzyme mixture compared to those fed control diets. Interactions of week × treatment were detected, with increased feed intake observed with enzyme addition compared with the control in wk 3 to 7 of lactation, but not in wk 8 to 16 (Lewis et al., 1999). Beauchemin et al. (1999), also observed no change in feed intake in mid-lactation cows with application of liquid fibrolytic enzyme formulation to the ration, but observed increased total tract starch, fiber and OM digestibility. Similarly, addition of a liquid enzyme formulation to alfalfa cubes increased total tract digestion of OM and NDF without affecting feed intake in cows past peak lactation (Yang et al., 1999).

The difference in response to enzyme addition of early and later lactation cows observed in the current study and in other published research may be due to the effect of ruminal fiber digestibility on feed intake. Treatments

Table 6. Effect of stage of lactation and direct-fed fibrolytic enzyme formulation on N intake and partitioning of lactating Holstein cows.

	Early lactation		Late lactation		SEM ¹	P <		
	Control	Enzyme	Control	Enzyme		Group	Trt	Group × trt
N intake, g/d	701.1	729.5	572.7	572.8	24.0	0.01	0.46	0.46
Fecal N excretion, g/d	302.8	311.9	327.6	307.9	13.0	0.43	0.67	0.25
Urinary N excretion, g/d	200.6	207.8	181.4	171.7	9.3	0.03	0.87	0.28
Total N excretion, g/d	505.6	520.7	510.0	480.0	15.8	0.24	0.63	0.15
Apparent N digestibility, %	56.8	57.5	42.3	46.8	2.4	0.01	0.27	0.42
Absorbed N, g/d	399.0	422.1	245.1	264.8	21.0	0.01	0.29	0.94
N in milk, g/d	191.8	203.0	198.6	178.7	15.0	0.63	0.66	0.13
Milk N, % of N intake	27.4	27.9	35.0	31.8	2.8	0.07	0.60	0.48
N retention, g/d	4.22	6.79	-134.8	-86.3	32.0	0.01	0.41	0.45

¹Due to unequal n, largest SEM (n = 7) reported.

that increase the potentially degradable fraction of DM and fiber may increase DMI when physical fill limits intake, as may occur in early lactation cows (Dado and Allen, 1995). Increased consumption of a diet usually depresses its total tract DM digestibility, however, due to increased rate of passage of feed through the digestive tract (Tyrrell and Moe, 1975). In early lactation cows fed diets varying in potentially degradable fiber, the offsetting effects of increased feed intake and decreased total tract digestibility would minimize differences in fecal output. This may explain why increased feed intake with addition of fibrolytic enzymes is more commonly observed in early lactation cows than in mid-lactation cows (Nussio et al., 1997; Lewis et al., 1999).

If rumen fill is not limiting intake, however, intake is less affected by increasing ruminal digestibility of fiber and DM (Oba and Allen, 1999). In this situation, typical of later lactation cows, total tract digestibility increases with increased ruminal digestibility, and fecal output is likely to be reduced. This might explain the observation that in mid and late lactation cows fed diets containing fibrolytic enzymes, increased total tract nutrient digestibility is often observed without changes in feed intake (Beauchemin et al., 1999; Lewis et al., 1999; Yang et al., 1999).

There is little data in the literature on the effect of the addition of fibrolytic enzymes to the diet on excretion of feces and manure nutrients in lactating cows. Beauchemin et al. (1999), observed reduced fecal OM flow with fibrolytic enzyme treatment in mid-lactation cows. The constant DMI and improved total tract DM and OM digestibility observed by Yang et al. (1999), also implies reduced fecal output. In the current study, the nature of the feces and N excretion response to enzyme treatment tended to differ between early and late lactation cows. Feces and N excretion increased slightly in early lactation cows fed diets containing the enzyme formulation compared with the control, but were numerically lower in later lactation cows fed enzyme-supplemented diets.

Differences in milk yield response to enzyme treatment between the early and late lactation cows were observed in the current study and have been reported by others. In early lactation cows, addition of fibrolytic enzymes has resulted in increased milk yield, even without changes in DMI (Beauchemin et al., 1999; Rode et al., 1999; Yang et al., 1999; Yang et al., 2000). Increases in milk yield with addition of fibrolytic enzymes to the diet are observed less often in mid and late lactation cows. An aqueous mixture of cellulase and xylanase added to alfalfa hay prior to feeding early and mid lactation cows tended to increase feed intake, but only early lactation cows had increased milk yield (+9%) with enzyme treatment (Nussio et al., 1997). In another study, addition of a liquid enzyme mixture (cellulase

and xylanase) to the forage portion of the diet immediately prior to feeding tended to increase milk yield (+10.8%), while feed intake was unaffected (Schingoethe et al., 1999). The production response was only observed in cows that began treatment during the first 100 d of lactation; milk yield was not affected in cows in mid-lactation at the start of treatment. Similarly, addition of a nonstarch polysaccharidase to grains increased feed intake and total tract digestibility in mid-lactation cows (Beauchemin et al., 2000), but cows were in positive energy balance, and no effect on milk yield was observed.

CONCLUSIONS

The main effect of addition of a fibrolytic enzyme formulation to the diet did not consistently influence intake, apparent digestibility, excretion, or retention of N and P, but the response of early and late lactation cows to enzyme treatment differed, as indicated by group \times treatment interactions. These interactions were due to higher milk yield, body weight gain, and 17% greater calculated NE_L content of the fibrolytic enzyme-treated diet in early but not late lactation cows compared with the control. Feces and N excretion were slightly lower in late but not early lactation cows fed diets containing the enzyme formulation. Additional work is needed to clarify the differences in response to enzyme addition in early and late lactation cows.

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