Strategic Addition of Dietary Fibrolytic Enzymes for Improved Performance of Lactating Dairy Cows

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General Introduction

This paper presents the results of two experiments that investigated how enzyme application affects the performance of dairy cattle and how some management factors affect the response.

Experiment 1: Effect of method of adding a fibrolytic enzyme to diets on dairy cow performance (Dean et al., 2007).

Introduction

Unlike abomasal or ruminal enzyme infusion (Hristov et al., 1998; 2000), enzyme addition to the dietary forage (Lewis et al, 1999; Kung et al., 2000) or concentrate (Rode et al, 1999; Yang et al., 2000) has improved the performance of cows, reflecting the importance of a close association between the feed and enzymes for fiber hydrolysis (Beauchemin et al, 1999). However, few studies have determined whether enzymes are most effective when added to the dietary concentrate or forage. In theory, enzyme application to the dietary forage or the TMR should be more effective than application to the concentrate because of greater opportunity for enzyme-induced fiber hydrolysis. Exogenous enzymes should also be more effective when applied to high-moisture feeds such as silages than to dry feeds because of the importance of water for enzymatic cell wall hydrolysis (Beauchemin et al, 1999). However, one study has demonstrated that enzyme application to the concentrate was more effective than application to the TMR (Yang et al., 2000). Whereas, others indicated that application to one dietary fraction was not more effective than application to another fraction or the TMR (Sutton et al., 2003; Vicini et al., 2003). It is also thought that enzyme efficacy is positively correlated with the proportion of the diet to which enzymes are applied (Bowman et al., 2001), but this is not always true (Yang et al., 1999). These conflicting results emphasize the importance of further examination of how the method of enzyme application affects efficacy.

Though most recent enzyme application studies have examined enzyme application at feeding, there is a sound theoretical basis for applying fibrolytic enzymes to forages at ensiling. This application method allows cell wall hydrolysis into fermentable substrates that improve homolactic fermentation, while improving

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digestibility. Fibrolytic enzyme application at ensiling has improved the fermentation and nutritive value of corn silage (Colombatto et al., 2003) and bermudagrass silage (Dean et al., 2005), and the intake of bermudagrass silage (BS, *Cynodon* spp.) by beef cattle (Bates et al*.,* 1989). However few, if any studies have compared how applying enzymes at ensiling versus at feeding affects the performance of dairy cows. The objective of this study was to determine how method of dietary addition of a cellulose-xylanse enzyme affects the performance of dairy cows.

Materials and methods

Cows and management

Thirty lactating Holstein cows in mid-lactation (129 ± 6 DIM) were balanced for parity and milk production and allocated randomly to 5 dietary treatments for 2 consecutive 28-d periods. Each period consisted of 14 d for adaptation to a new diet and 14 d for sample collection. Cows were individually fed twice daily (0700 and 1430 h) and feed refusals were measured daily at 0600 h. Cows were milked 3x daily at 0200, 1000, and 1800 h and milk production (MP) was measured during the last 14 d of each period. Milk samples were collected twice daily (1000 and 1800 h) on two consecutive days during each week in the last two weeks of each period.

Forage production, diets and treatments

Tifton 85 bermudagrass was mowed after 35 d of regrowth, wilted for 2 h, and chopped (5-cm particle size) using a forage harvester. Sixty-two tons of bermudagrass were packed into each of two, 3.6 m-wide Ag Bags (AG Bag International, Warrenton, OR) and stored for 35 d. A cellulase-xylanase fibrolytic enzyme complex (Promote®, Cargill, Minnetonka, MN) was diluted in water (1:5 ratio v/v) and sprayed on 46 tons of bermudagrass forage at a rate of 1.3 g/kg DM as the forage was being packed into an additional Ag-bag.

The diets contained Tifton 85 bermudagrass silage, corn silage, and concentrate mixed at 35, 10 and 55% of dietary DM, respectively (Table.1). The Promote enzyme was applied to different fractions of the diet to give the following treatments: 1) no enzyme added (Control), 2) enzyme applied to bermudagrass at ensiling (ES 1.3 g/kg DM), and enzyme applied at feeding at the rate of 4 g/head/d to (3) the concentrate (EC), 4) the TMR (ETMR) or 5) the forage (EF). Diets were mixed for 5 min prior to feeding in three 250-kg Calan data rangers (American Calan Inc., Northwood, NH). The enzyme-treated concentrate was prepared weekly by dissolving the enzyme in water (1:5 ratio v/v) and spraying the solution on 140 kg of ground corn while the corn was being mixed in a Marion Mixer (Rapids Machinery Co., Marion, IA). The rest of the concentrate ingredients were subsequently mixed with the enzyme-treated corn in a 900-kg New Holland 355 mixer (New Holland North America, New Holland, PA). For the EF and ETMR treatments, the enzyme was dissolved in water (1:10 ratio v/v) and sprayed on the forage and the TMR, respectively within a data ranger that was operated for 5 min to ensure thorough mixing.

Five additional ruminally-cannulated Holstein cows were used to evaluate the effect of the dietary treatments on ruminal fermentation and *in situ* ruminal TMR

degradation during three consecutive 15-d periods. Each period consisted of 12 d of adaptation to a new diet, 1 d of ruminal fluid collection and 2 days of *in situ* rumen DM degradability measurements, but the latter was only measured in periods 2 and 3.

Statistical analysis

These experiments involved partially balanced, incomplete block cross over designs and the model used for analyzing the production trial data included effects of treatment, period and cow. In addition, the model used for rumen fermentation and degradability analysis included the effect of time and the treatment x time interaction. Contrast statements were used to compare each of the enzyme treatments to the Control.

Results And Discussion

Crude protein, NFC, NEL, and organic acid concentrations were greater and fiber and NH_3-N concentrations were lower in enzyme-treated than in untreated BS (Table 2). This suggests that the enzyme improved the fermentation and nutritive value of the BS and supports the findings of Dean et al. (2005). The low organic acid concentrations, high pH, and ammonia-N concentration of the untreated silage may reflect reduced substrates for fermentation or utilization of fermentation products by aerobic organisms such as lactate-utilizing yeasts.

Enzyme addition did not affect intake or apparent digestibility of DM, NDF or CP (Table 3). Consequently, milk production was not improved by enzyme addition (Table 4). Milk fat and true protein yields were unaffected ($P > 0.05$) by enzyme supplementation. However, cows fed ETMR tended ($P < 0.09$) to have greater milk fat and protein concentration than cows fed the Control diet. Cows fed EC also tended ($P = 0.076$) to have greater concentration of milk protein than those fed the Control diet.

Plasma glucose concentrations (Table 5) were unaffected by treatment. However, cows fed EF and ES had lower ($P < 0.05$) BUN concentrations than those fed the Control diet, and cows fed ETMR had a similar tendency ($P = 0.12$). This suggests that these treatments increased the efficiency of N utilization by ruminal microbes since N intake was similar among treatments. Furthermore, cows fed ETMR treatment had lower ($P < 0.01$) BHBA concentration than control cows, indicating decreased fat mobilization. Therefore, despite lack of responses in feed intake, digestibility and milk production, enzyme addition to the TMR improved the status of key blood metabolites that indicate that the N and energy status of the cows was improved.

Mean ruminal fluid pH tended to be lower in cows fed EC ($P = 0.11$) than in those fed the Control diet, presumably due to easier enzymatic hydrolysis of the concentrate than the forage into readily fermentable substrates that depress pH when fermented. Ruminal pH fell below 6 within 6 h of feeding in cows fed EC and remained at 5.5 between 8 and 10 h after feeding (Figure 1). A pH of 5 - 5.8 indicates sub-clinical ruminal acidosis in dairy cows (Oetzel et al., 1999), therefore, cows fed EC were most likely to have experienced sub-clinical ruminal acidosis. However, feed intake and digestion were not affected by the EC diet, and the mean rumen pH diet was 6.11,

suggesting that the periods of subclinical acidosis in cows fed the EC diet were not pronounced.

Mean total VFA concentration was lower ($P < 0.05$) in cows fed ETMR and ES rather than the Control diet. The low total VFA concentration in cows fed ETMR partly explains why their milk yields were no greater than those of cows fed the Control diet. The VFA responses in this study did not reflect the digestibility responses, probably because VFA concentration reflects ruminal fermentation while digestibility values reflect total tract digestion. Mean ruminal NH_3-N concentration was lower in cows fed ETMR ($P < 0.01$) than in cows fed the Control diet, supporting the tendency for lower BUN concentration in cows fed ETMR. This suggests that there was enhanced uptake of NH_3-N by the ruminal microbes probably due to greater fermentable metabolizable energy availability from this diet.

Mean ruminal concentration of acetic acid was lower in cows fed ETMR ($P < 0.05$) and EF ($P < 0.01$) than in those fed the Control diet. Butyric acid concentration was unaffected ($P > 0.05$) by enzyme treatment but propionic acid concentration tended to be greater ($P = 0.07$) in cows fed ETMR versus the Control diet. Consequently, acetate:propionate ratio of ruminal fluid was lower in cows fed ETMR diets ($P < 0.05$) rather than the Control diet. This indicates that the ETMR diet promoted a more efficient fermentation in the rumen, probably due to greater fibrolysis and greater consequent release of non-structural carbohydrates by the ETMR treatment. Fermentation of such carbohydrates typically yields relatively higher ruminal propionate proportion as compared to acetate, and thereby lowers the acetate:propionate ratio. This lower ratio partly explains the lower BHBA concentrations and the tendency for higher milk fat and protein concentrations from cows fed ETMR rather than the Control diet.

Efficacy of methods of enzyme addition

Compared to the control treatment, the main benefits of the dietary treatments were as follows: The EC diet tended ($P < 0.15$) to increase milk protein concentration; the ES diet reduced ($P < 0.05$) BUN concentration; the EF diet reduced ($P < 0.05$) BUN and acetate concentrations; whereas the ETMR diet tended ($P < 0.15$) to reduce BUN concentration and increase concentrations of propionate, milk fat and milk protein, and it reduced ($P < 0.05$) acetate to propionate ratio and concentrations of ruminal NH_3N, acetate and BHBA. Therefore though none of the enzyme application methods sufficiently improved animal performance, enzyme application to the TMR produced more desirable effects than the other modes of enzyme application. This may have been because the enzyme was applied to the greatest proportion of the diet in this treatment, thus allowing greater enzyme-diet interaction. However such benefits were not directly attributable to increased feed intake or fiber hydrolysis since enzyme treatment did not affect any measure of feed intake, digestibility or in situ degradability. Rather enzyme application to the TMR improved several indices of rumen function, probably through improved bacterial attachment (Yang et al., 1999), stimulation of rumen microbes (Nsereko et al., 2000; Wang et al., 2001) and synergy between exogenous and endogenous enzymes (Morgavi et al., 2000). Nevertheless, the

beneficial effects of enzyme application to the TMR were minimal, and other methods of application produced even fewer benefits in diet utilization or milk production.

Conclusions

Enzyme supplementation did not improve in vivo digestibility or voluntary intake of the diets, therefore, milk production and constituent yield, and blood glucose concentration were not improved by enzyme addition. However, unlike other modes of application, enzyme addition to the TMR treatment reduced fat mobilization and improved the efficiency of ruminal energy use, decreased rumen ammonia-N concentration, tended to improve milk fat and protein concentrations and nonsignificantly improved FCM yield. Therefore the enzyme was most effective when it was added to the TMR, but even this method of application produced only minimal improvements in rumen function that did not improve milk production by the cows.

Experiment 2: Effect of esterase-xylanase fibrolytic enzyme application to diets with high or low levels of concentrates on the performance of dairy cattle (Kim et al., 2007).

Introduction

Schingoethe et al. (1999) noted that a 9 to 15% enzyme-induced increase in milk production occurred during the first 100 d postpartum but not in mid-lactation dairy cows. Beauchemin et al. (1999) also reported that when cows were in positive energy balance, enzyme-induced increases in digestible energy intake did not affect milk production. Therefore the advanced stage of lactation (129 DIM) of cows in Experiment 1 may have limited the milk response to enzyme addition. Furthermore, the enzyme preparation used in Experiment 1 contained only cellulose and xylanase activities, which can not hydrolyze etherified or esterified ferulic and coumaric acid linkages that bind digestible arabinoxylans in plant cell walls to lignin. Such linkages have been compared to the molecular equivalent of spot-welding on a steel mesh frame (Iiyama et al., 1994). Though etherified cross linkages are not known to be degraded by anaerobic microorganisms, esterified cross linkages can be degraded by ferulic acid esterase enzymes. Our recent findings (Krueger et al., 2004; Adesogan et al., 2005; Krueger and Adesogan, 2006) support those of others (Bartolome et al., 1997; Rodrigues et al., 2001; Eun and Beauchemin, 2006), which suggest that esterase enzymes can complement cellulose and xylanase enzyme effects on plant cell walls, thereby increasing digestibility. To our knowledge, esterase enzymes have not been included in the fibrolytic enzyme preparations that have been tested on dairy cows. The objective of this experiment was to determine the effect of a fibrolytic enzyme preparation containing xylanase and esterase activities on the performance of dairy cows fed low or high concentrate diets. The enzyme tested had improved the in vitro digestibility of DM and NDF of alfalfa hay and corn silage by 7 – 9% and 28 - 31% in earlier studies (Eun and Beauchemin, 2006). The enzyme was applied to diets with high or low levels of concentrates that were typical of diets fed to dairy cows in the US and Western Europe, respectively. The hypotheses were 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 and management

Sixty lactating Holstein cows (15 per treatment) in early lactation (22 ± 1 DIM) were grouped by milk production and randomly assigned to four treatment groups for a continuous 77-d trial. The first 14 d were used for adaptation to a new diet and the last 63 d for sample collection. Cows were individually fed twice daily (0700 and 1200 h) and feed refusals were measured daily at 0600 h. Cows were milked 2x daily at 1100 and 2300 h and milk samples from both milkings were collected on the fourth day of each week.

Diets and enzyme application

Three weeks after calving, cows were fed either a high or low concentrate total mixed ration (TMR) that was or was not treated with an esterase-xylanase enzyme (Dyadic International Inc., Jupiter, FL) such that the following treatments were investigated:

1. Low concentrate, untreated diet (LC; 67:33 roughage to concentrate ratio)
2. Low concentrate, enzyme-treated diet (LCE; 67:33 roughage to concentrate ratio)
3. High concentrate, untreated diet (HC; 52:48 roughage to concentrate ratio)
4. High concentrate, enzyme-treated diet (HCE; 52:48 roughage to concentrate ratio)

Prior to the daily am and pm feedings, the enzyme solution was diluted in water (1:3 ratio v/v) and sprayed at a rate of 5 mg/g of DM on the TMR while it was mixed for 5 min in a 250-kg Calan data ranger (American Calan Inc., Northwood, NH). Separate data rangers were used for the enzyme-treated and untreated diets. The roughage portion of the diets contained approximately 20% alfalfa hay, 70% corn silage, and 10% cottonseed hulls. The experimental diets were formulated to be isonitrogenous and to meet NRC (2001) guidelines. The experimental design was completely randomized with a 2 x 2 factorial arrangement of treatments and 15 replicates per treatment.

Four additional ruminally-cannulated, nonlactating Holstein cows were used to determine dietary treatment effects on indices of rumen fermentation and degradation. This aspect of the experiment had a 4 x 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 day 15, cows were sampled for ruminal pH and concentrations of volatile fatty acids and ammonia-N at 0, 2, 4, 6, 8 and 10 h after they were fed in the morning. On days, 16 -18, in situ ruminal degradation of DM and NDF were measured.

Statistical analysis

Statistical models containing treatment, week, and treatment x week interaction were used to analyze the intake and milk production data, whereas the model for analyzing rumen fermentation and degradation data included treatment, cow, time and period. Contrast statements were used to determine the effects of enzyme application

and dietary concentrate level, and to compare the LCE and HC diets. Effects of enzyme application at each level of concentrate supplementation was examined with the PDIFF statement of SAS (2002). The experiment was conducted from September to December, 2006, therefore samples are still being analyzed and only a few key results will be presented.

Results and discussion

Table 7 shows the ingredient and chemical composition of the experimental diets. There were no interactions between concentrate level and enzyme application for any of the results except for milk fat yield. Enzyme application did not increase DM intake (DMI) but it tended to increase milk yield ($P = 0.063$; Table 8) and therefore increased the efficiency of milk production ($P = 0.008$). The enzyme-induced increase in milk yield was significant ($P = 0.044$) in cows fed the high concentrate diet, but it only numerically increased ($P = 0.538$) milk yield in those fed the low concentrate diet. Whereas the increase in feed efficiency was significant ($P = 0.027$) in cows fed the low concentrate diet but it was a tendency ($P = 0.114$) in cows fed the high concentrate diet. Therefore, these results confirm our first hypothesis that 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.

Increasing the dietary concentrate level increased ($P < 0.05$) DMI, milk yield, and milk protein yield, but tended to reduce ($P = 0.108$) the efficiency of milk production. These responses are attributable to the increase in concentration of dietary non-fiber carbohydrates particularly starch, as the dietary concentrate level increased.
Cows fed the LCE diet consumed less ($P = 0.009$) DM than those fed the HC diet, nevertheless milk production from both of these diets was similar ($P = 0.693$). Consequently the efficiency of milk production was greater ($P = 0.003$) in cows fed the LCE diet than those fed the HC diet. These results confirm our second hypothesis by indicating that enzyme application to the low concentrate diet made it as effective as the untreated high concentrate diet at stimulating milk production.

One concern about fibrolytic enzyme application to diets containing high levels of concentrates is that hydrolysis of such diets may depress ruminal pH and predispose cows to ruminal acidosis. Yet enzyme application did not affect ($P = 0.923$) the ruminal pH of cows fed the high or low concentrate diets in this study. Increasing the level of concentrate supplementation in untreated diets did produce the expected pH decrease ($P < 0.001$), as did feeding the HC diet instead of the LCE diet ($P = 0.006$). However, none of the resulting pH values were low enough to indicate sub-acute ruminal acidosis in the cows.

Conclusions

This study shows that application of the xylanase-esterase enzyme preparation increased milk production in dairy cows fed a high (48%) concentrate diet, and produced a nonsignificant increase effect in cows fed a low (33%) concentrate diet. Enzyme application increased the efficiency of milk production in cows fed the low concentrate diet and had a similar tendency in those fed the high concentrate diet.

Furthermore, enzyme application to the low concentrate diet resulted in as much milk production as from cows fed the untreated high concentrate diet.

General summary and recommendations

The first experiment indicated that adding a cellulose-xylanase enzyme to the TMR was more effective than adding it to dietary components. Enzyme addition to the TMR improved the ruminal utilization of energy and protein and reduced back fat mobilization but did not affect milk production. The second experiment indicates that application of a xylanase-esterase enzyme to the TMR increased milk production and improved the efficiency of milk production. The mode of action of this enzyme is still being investigated. These experiments differed in the enzyme used, diet composition and stage of lactation of cows. Therefore, the better performance of the enzyme used in Experiment 2 may be attributable to better enzyme:substrate specificity and use of cows in early lactation. It is concluded that enzyme efficacy is more likely to result if the following conditions are met:

1. Only enzymes that exhibit high activity under ruminal pH and temperature conditions are used.
2. Proper enzyme-substrate specificity is ensured i.e. activities of the specific enzymes in the enzyme preparation are appropriate for hydrolysis of the nutritional fractions in the feed or diet being investigated.
3. Enzymes are uniformly applied to the TMR rather than to individual components of the diet at feeding, or to the forage component at ensiling.
4. Enzymes are applied to cows in early stages of lactation when they are more sensitive to diet-induced improvements in rumen function.

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Table 1. Ingredient and chemical composition of the basal diet without enzyme supplementation.

Ingredient composition	% dietary DM
Bermudagrass silage	35.0
Corn silage	10.0
Ground corn	27.0
Citrus pulp	5.1
Whole cottonseed	2.8
SoyPlus[1]	6.6
Soybean meal	8.6
Mineral mix[2]	4.4
Biophos (Calcium phosphate)[3]	0.4
Chemical composition	
DM, %	46.4
CP, % of DM	16.1
ADF, % of DM	27.2
NDF, % of DM	46.5
TDN, % of DM	66.0
NEL, Mcal/kg of DM	1.57

[1] West Central Soy, Ralston, IA.

[2] Mineral mix contained 26.4% CP, 10.2 Ca, 0.9% P, 3.1% Mg, 1.5% S, 5.1% K, 8.6% Na, 1698 mg/kg of Zn, 512 mg/kg of Cu, 339 mg/kg of Fe, 2231 mg/kg of Mn, 31 mg/kg of Co, 26 mg/kg of I, 7.9 mg/kg of Se, 147,756 IU of vitamin A/kg, 787 IU of vitamin E/kg (DM basis).

[3] IMC Feed Ingredients, Lake Forest IL; contained 15.9% Ca, 21.2% P.

Table 2. Chemical composition of the enzyme-treated[1] and untreated forages and concentrates (% DM) (n = 4 replicates per mean).

Item[2]	DM, %	CP	NDF	ADF	TDN	NFC[3]	NDF dig[4]	pH	NEL[5]	NH_3-N[6]	Lactic acid	Acetic acid	Propionic acid	Butyric acid
Untreated concentrate	88.3	21.9	15.8	8.55	84.5	-	-	-	1.98	-	-	-		-
Treated concentrate	88.4	21.5	15.6	8.15	84.0	-	-	-	1.97	-	-	-		-
SE	0.3	0.5	2.1	0.87	1.27			-	0.06					
Pre-ensiled BS	23.4	12.7	76.1	45.3	43.0	7.85	-	-	0.58	-	-	-	-	-
SE	3.0	0.9	1.1	2.2	1.5	0.4	-	-	0.05					
Corn silage	28.1	8.8	45.2	27.0	79.0	38.1	54.0	-	1.52	12.0	3.08	6.68	0.89	0.07
SE	4.2	1.2	1.5	3.1	2.1	0.6	4.0	-	0.08	15.4	0.60	0.35	0.034	0.03
Untreated BS	29.9	9.3	81.8	49.9	52.7	5.3	47.8	8.4	0.45	38.0	0.10	0.05	0.00	0.00
Enzyme-treated BS	29.6	11.4	76.2	45.2	55.0	8.6	49.3	4.6	0.77	13.7	1.77	3.08	0.32	0.18
SE	0.6	0.3	0.8	0.6	0.18	0.5	2.0	0.3	0.04	1.3	0.30	0.15	0.06	0.01

[1] Promote®, Cargill, Minnetonka, MN
[2] Bermudagrass silage
[3] Non-fiber carbohydrates
[4] NDF digestibility
[5] Net energy of lactation, Mcal/kg DM
[6] As percentage of total N

Table 3. Effect of method of enzyme[1] addition on voluntary intake and apparent digestibility.

	Intake				Digestibility		
Treatment[2]	DM, kg/d	DM,% BW	NDF, kg/d	CP, kg/d	DM, %	NDF, %	CP, %
Control (C)	20.9	3.35	9.7	3.4	66.4	50.7	65.6
EC	21.6	3.46	9.9	3.5	64.2	51.0	65.7
ETMR	22.4	3.65	10.0	3.5	66.3	50.4	66.9
EF	19.9	3.18	9.0	3.1	64.3	51.6	65.7
ES	21.8	3.41	9.5	3.3	68.3	48.7	67.4
SE	1.0	0.18	0.6	0.2	1.5	2.3	1.5
	P values						
Treatment effect	0.30	0.40	0.37	0.37	0.14	0.92	0.87
Contrasts							
C vs. EC	0.56	0.63	0.62	0.62	0.93	0.92	0.97
C vs. ETMR	0.22	0.21	0.22	0.22	0.78	0.93	0.55
C vs. EF	0.40	0.48	0.44	0.44	0.80	0.76	0.96
C vs. ES	0.45	0.80	0.72	0.72	0.50	0.55	0.40

[1] Promote® , Cargill, Minnetonka, MN

[2] C: Control, EC: enzyme applied to concentrate, ETMR: enzyme applied to the TMR, EF: enzyme applied to forage at feeding, ES: enzyme-treated silage

Table 4. Effect of method of enzyme[1] addition on milk production and composition.

Treatment[2]	Milk, kg/d	4% FCM, kg/d	Milk fat, %	Milk fat, kg/d	Milk protein %	Milk protein, kg/ d	kg milk/ kg DMI	SCC[3] x 10^3 cells/ml
Control (C)	33.1	31.8	3.67	1.23	2.91	0.96	1.64	339
EC	30.9	29.9	3.78	1.16	3.07	0.95	1.46	488
ETMR	32.3	32.4	3.99	1.29	3.07	1.00	1.42	581
EF	31.2	30.0	3.77	1.64	3.03	0.93	1.64	817
ES	32.3	30.6	3.72	1.19	2.90	0.92	1.59	458
SE	1.0	1.0	0.12	0.05	0.07	0.03	0.04	250
				P values				
Treatment effect	0.43	0.27	0.42	0.20	0.26	0.24	0.26	0.53
Contrasts								
C vs. EC	0.10	0.17	0.53	0.28	0.08	0.82	0.15	0.26
C vs. ETMR	0.52	0.68	0.07	0.33	0.08	0.24	0.08	0.53
C vs. EF	0.13	0.18	0.59	0.27	0.19	0.50	0.96	0.10
C vs. ES	0.51	0.40	0.79	0.50	0.92	0.35	0.70	0.58

[1] Promote® , Cargill, Minnetonka, MN

[2] C: Control, EC: enzyme applied to concentrate, ETMR: enzyme applied to the TMR, EF: enzyme applied to forage at feeding, ES: enzyme-treated silage:

[3] SCC: somatic cell counts.

Table 5. Effect of method of enzyme[1] addition on BW, BW gain, BCS, and blood metabolite concentration.

Treatment[2]	Variable					
	BW, kg	BW gain, kg/d	BCS	BUN, mg/dl	Glucose, mg/dl	BHBA[3], mM/l
Control (C)	633	0.20	2.82	16.9	64.5	0.94
EC	635	0.64	2.61	16.0	62.9	0.84
ETMR	624	0.42	2.77	15.6	64.6	0.68
EF	618	0.21	2.64	15.2	64.5	0.86
ES	623	0.31	2.83	15.2	64.5	0.83
SE	19	0.20	0.12	0.6	1.1	0.07
	P values					
Treatment effect	0.96	0.46	0.48	0.23	0.77	< 0.01
Contrasts						
C vs. EC	0.94	0.14	0.18	0.28	0.29	0.46
C vs. ETMR	0.72	0.45	0.74	0.12	0.97	< 0.01
C vs. EF	0.57	0.98	0.25	0.05	0.99	0.27
C vs. ES	0.72	0.70	0.96	0.05	0.98	0.14

[1] Promote® , Cargill, Minnetonka, MN

[2] C: Control, EC: enzyme applied to concentrate, ETMR: enzyme applied to the TMR, EF: enzyme applied to forage at feeding, ES: enzyme-treated silage:

[3] BHBA: beta hydroxybutyrate.

Table 6. Effect of method of enzyme[1] addition on ruminal pH and concentrations of VFA and NH_3-N (mg/dL).

Treatment[2]	pH	NH_3-N, mg/dL	Acetate, C2, mol %	Propionate C3, mol %	Butyrate mol %	C2:C3 ratio	Iso-butyrate mol %	Valerate mol %	Iso-valerate mol %	Total VFA mmol/L
Control (C)	6.32	15.1	58.9	20.8	11.8	2.8	2.8	3.5	4.0	144
EC	6.11	13.6	57.7	20.5	12.7	2.8	2.4	2.8	3.8	125
ETMR	6.27	10.1	56.0	21.7	12.3	2.6	2.6	3.7	3.8	105
EF	6.26	17.0	55.3	20.8	12.6	2.7	2.6	4.7	4.1	115
ES	6.23	14.9	59.2	20.2	11.7	2.9	2.4	3.1	3.3	106
SE	0.09	1.12	0.91	0.33	0.67	0.05	0.22	0.75	0.45	12.5
					P values					
Treatment effect	0.53	0.01	0.02	0.07	0.78	< 0.01	0.70	0.42	0.72	0.07
Time effect	0.01	< 0.01	0.04	0.10	0.46	< 0.01	0.05	0.55	0.42	0.51
Treatment x time effect	0.45	0.01	0.78	0.92	0.91	0.99	0.45	0.53	1.00	0.92
Contrasts										
C vs. EC	0.11	0.40	0.38	0.58	0.34	0.75	0.23	0.52	0.71	0.25
C vs. ETMR	0.73	< 0.01	0.03	0.07	0.59	0.02	0.52	0.83	0.70	0.02
C vs. EF	0.63	0.29	0.01	0.99	0.38	0.12	0.40	0.23	0.92	0.08
C vs. ES	0.52	0.97	0.78	0.20	0.95	0.05	0.20	0.73	0.26	0.02

[1] Promote® , Cargill, Minnetonka, MN

[2] C: Control, EC: enzyme applied to concentrate, ETMR: enzyme applied to the TMR, EF: enzyme applied to forage at feeding, ES: enzyme-treated silage.

Table 7. Ingredient and predicted[1] chemical composition of the untreated experimental diets.

	Low concentrate	High concentrate
Ingredient composition, % DM		
Corn silage	49.2	37.0
Alfalfa hay	13.5	10.0
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[2]	7.90	5.93
Soybean meal	2.49	6.01
Cottonseed meal	7.80	5.10
Mineral mix[3]	3.26	3.25
Roughage : concentrate ratio	67:33	52:48
Chemical composition		
Net energy of lactation, Mcal/Kg DM	1.56	1.61
Crude protein, % DM	17.3	17.3
Total digestible nutrients, % DM	67	70
Neutral detergent fiber, % DM	37	33
Acid detergent fiber, % DM	23.9	21.6
Non-fiber carbohydrate, % DM	37.1	40.6
Ca, % DM	0.8	0.8
P, % DM	0.4	0.4

[1] Predicted after NRC (2001)
[2] West Central Soy, Ralston, IA.
[3] Mineral mix contained 26.4% CP, 10.2 Ca, 0.9% P, 3.1% Mg, 1.5% S, 5.1% K, 8.6% Na, 1698 mg/kg of Zn, 512 mg/kg of Cu, 339 mg/kg of Fe, 2231 mg/kg of Mn, 31 mg/kg of Co, 26 mg/kg of I, 7.9 mg/kg of Se, 147,756 IU of vitamin A/kg, 787 IU of vitamin E/kg (DM basis).

Table 8. Effect of addition of a xylanase-esterase enzyme[1] (Enz) preparation on the performance of dairy cows fed diets containing low (33%) or high (48%) levels of concentrate (conc).

	Low Conc		High Conc		SEM	Contrast *P values*		
	No Enz	Enz	No Enz	Enz		Enz	Conc	LCE vs. HC
Item	(LC)	(LCE)	(HC)	(HCE)				
DMI, kg	22.9	21.2	25.6	25.3	1.1	0.383	0.005	0.009
Milk yield, kg	32.0	32.9	33.5[x]	36.5[y]	1.0	0.063	0.017	0.693
Feed efficiency (kg milk/kg DMI)	1.40[a]	1.62[b]	1.32	1.48	0.07	0.008	0.108	0.003
Rumen pH	6.26	6.36	6.10	6.01	0.08	0.923	<0.001	0.006
Milk protein, %	2.83	2.83	2.91	2.87	0.05	0.777	0.219	0.285
Milk fat, %	3.62	3.81	3.88	3.65	0.12	0.875	0.683	0.689
Milk protein, kg/d	0.90	0.93	0.97	1.04	0.04	0.238	0.049	0.563
Milk fat, kg/d	1.16	1.25	1.31	1.34	0.08	0.465	0.164	0.636

[a, b; x, y] At the same level of concentrate supplementation, means with different superscripts differed ($P < 0.05$).

[1] Produced by Dyadic International Inc., Jupiter, FL

Figure 1. Effect of method of enzyme addition to diet on ruminal fluid pH after feeding (n = 3).
EC: enzyme applied to concentrate, ETMR: enzyme applied to the TMR, EF: enzyme applied to forage at feeding, ES: enzyme-treated silage.