The Effect of Treating Forages with Fibrolytic Enzymes on its Nutritive Value and Lactation Performance of Dairy Cows[1]

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ABSTRACT

Forages (corn silage and alfalfa hay) were sprayed with liquid enzymes prior to combining with a concentrate to form a total mixed ration (50% forage:50% concentrate, dry matter basis) and fed to lactating cows. In the first year, treatments were 1) no enzymes, 2) an enzyme complex containing 3500 carboxymethyl cellulase (CMCase) and 16,000 xylanase units per kilogram of forage dry matter, or 3) an enzyme complex containing 8800 CMCase units and 40,000 xylanase units. In the second year, the treatments were 1) no enzymes, 2) an enzyme complex as in yr 1 containing 3700 CMCase and 14,000 xylanase units, or 3) an enzyme complex using an alternative cellulase and containing 3600 CMCase and 11,000 xylanase units. In the first year, cows fed diet 2 tended to produce more milk (39.5 kg/d) than those fed diet 1 (37.0 kg/d) or those fed diet 3 (36.2 kg/d). The high level of enzyme treatment in diet 3 decreased the output of milk protein and fat compared to the low level of enzyme treatment. In the second year, cows fed diet 3 produced more milk (35.4 kg/d) than did those fed diet 1 (32.9 kg/d) and numerically more than those fed diet 2 (33.6 kg/d). Milk fat and protein were similar among treatments but numerically lower for cows fed enzyme-treated forages. Dry matter intake (kg/d) was similar among treatments in both years. Spraying certain doses and combinations of enzymes directly onto forages prior to feeding can improve milk yields but enzyme sources and dose levels are of critical importance.
(**Key words:** enzymes, cellulase, xylanase, forage)

Abbreviation key: CMCase = carboxymethyl cellulase, **EA2** = low level of a cellulase and xylanase enzyme mix, **EA5** = high level of a cellulase and xylanase enzyme mix, **EB1.2** = low level of an alternative cellulase and xylanase enzyme mix.

INTRODUCTION

Traditionally, many nutritionists have questioned the feeding of enzyme complexes to ruminants because of the potential for breakdown by ruminal microorganisms. Kopecny et al. (12) reported that a cellulase enzyme complex was rapidly degraded by rumen bacterial proteases, and addition to ruminal fluid had no effect on in vitro fiber digestion. However, a growing body of evidence shows that spraying enzymes directly onto feeds, just prior to feeding, can improve animal performance. Binding the enzymes to substrates before their introduction into the rumen may protect them from degradation by ruminal proteases. For example, Stokes and Zheng (23) sprayed fibrolytic enzymes on the forage portion of a TMR. When fed to cows, treated forage increased DMI by 2.0 kg/d and milk production by 4.2 kg/d. Yang et al. (27) reported marked improvements in nutrient digestion when a barley-based concentrate was sprayed with a cellulase and xylanase enzyme complex and fed to lactating cows. Milk production was 3.6 kg/d more in cows fed treated feed compared with untreated feed in that study.

The objective of this study was to evaluate 1) the effect of spraying various enzyme combinations and amounts on the forage portion of a TMR and its subsequent effect on the production and composition of milk by dairy cows, and 2) possible differences in efficacy between similar, but subtly differing (source), enzymes (in this case cellulases).

MATERIALS AND METHODS

Enzyme Assays

Carboxymethyl cellulase (**CMCase**) activities of the enzyme concentrates were analyzed by Finnfeeds International (Marlborough, UK), incubating enzyme and carboxymethyl cellulose at 50°C for 10 min at a pH of

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4.8 and determining the reducing sugars released per minute as glucose equivalents by the dinitrosalicylic acid procedure (18). Xylanase activity was determined by incubating the enzyme complexes with oat spelts xylan at 50°C for 30 min at a pH of 5.3 and determining the reducing sugars released per minute as xylose equivalents by dinitrosalicylic acid procedure (18).

Yr 1

The objective of this experiment was to study the effect of a cellulase and xylanase complex at two concentrations on milk production in lactating cows. Whole-plant corn was harvested at the two-thirds milk line stage of maturity from a single field and stored in a bag silo (Ag-Bag International, Warrenton, OR) for 4 mo. Second-cutting alfalfa, in the one-tenth bloom stage of maturity, was harvested as hay and stored as rectangular bales prior to feeding. Twenty-seven multiparous and three primiparous lactating Holstein cows were housed in a barn with Calan gates (American Calan, Northwood, NH) and comfort stalls. Cows were allowed to exercise in a dirt lot twice daily for a total of 3 h. A 1-wk period was used for adaptation to the Calan gates and was followed by a 2-wk pretreatment period. During these periods cows were fed a TMR twice daily (0600 and 1600 h) composed of 45% (DM basis) corn silage, 5% alfalfa hay and 50% pelleted-concentrate (Table 1). Cows were allowed ad libitum access to fresh water and the TMR that was fed to achieve a 5% refusal on an as-fed basis. The diets were balanced to meet NRC (17) requirements (pretreatment milk production of 36 kg of 3.5% FCM). At the end of the pretreatment period, cows were randomly assigned to one of three treatments based on parity, pretreatment milk production, and DIM (average ± SD, 100 ± 45). Cows were then fed the same TMR for 12 wk, during which time the forages were treated with various combinations of enzymes. Enzymes were sprayed onto forages prior to mixing with the concentrate to form a TMR. Forages were treated with either 1) 10 L of water per 1000 kg of forage DM (control), 2) 10 L of water and enzyme mix containing 2 L of an enzyme complex of cellulase and hemicellulase enzyme complexes per 1000 kg of forage DM (**EA2**), or 3) 10 L of water and enzyme mix containing 5 L of an enzyme complex of cellulase and hemicellulase enzyme complexes (**EA5**). The applied enzyme activity for EA2 was 3500 CMCase units/kg of forage DM and 16,000 xylanase units/kg of forage DM. The applied enzyme activity for EA5 was 8800 CMCase units/kg of forage DM and 40,000 xylanase units/kg of forage DM. Enzymes (Finnfeeds Intl., Marlborough, UK) were from *Trichoderma longibrachiatum* and stored at 5°C until diluted for use. The solutions were applied with a garden sprayer set to disperse fine droplets onto corn silage and hay while mixing in a TMR wagon for about 5 min. Care was taken to ensure even distribution of the solutions onto the forage masses. A pelleted concentrate (Table 1) was added and mixed with the treated forage to form a TMR prior to feeding. The total mixing time was no more than 10 min for each treatment. Cows were offered their respective diets twice daily (0700 and 1600 h) within 10 to 20 min of preparing the TMR to achieve approximately 5% refusal. Fresh water was available at all times and the care of animals was via accepted protocols (10). High and low ambient temperatures were recorded daily.

Table 1. Ingredient composition of the pelleted-concentrate fed in both years.

Ingredient	(% of dietary DM)
Cornmeal	30.3
Wheat midds	21.2
Soybean meal, 48%	16.5
Roasted soybeans	10.6
Distiller's dried grains	9.7
Limestone, 37% Ca	2.8
Animal protein blend[1]	2.6
SQ-810™[2]	1.8
Corn gluten meal, 60% CP	1.1
Fat blend[3]	1
Urea	0.9
Salt	0.8
Sodium bicarbonate	0.3
Magnesium oxide	0.2
Selenium, 0.06%	0.1
Trace minerals[4]	0.04
Calcium phosphate[5]	0.03
Vitamin mix[6]	0.03

[1]Contained a combination of blood meal, feather meal, and fishmeal.

[2]Sodium sesquicarbonate (43.4% sodium carbonate and 34.4% sodium bicarbonate).

[3]Contained 90% fatty acids.

[4]Contained (DM basis) 14.3% S, 7.5% Ca, 1.1 g of cobalt/kg, 27.2 g of Cu/kg, 4.1 g of I/kg, 9.5 g of Fe/kg, 108.8 g of Mn/kg, and 108.8 g of Zn/kg.

[5]Contained (DM basis) 27.5% Ca and 20.4% P.

[6]Contained (DM basis) 28,696 KIU of vitamin A/kg, 7173 KIU of vitamin D/kg, and 179,346 IU of vitamin E/kg.

Milk production was recorded by a computer twice daily at 0500 and 1500 h. Once weekly, milk was sampled proportionately to milk yield from consecutive p.m. and a.m. milkings and analyzed by the Maryland DHIC Laboratory for fat and protein, (Milk-O-Scan, Foss Technology, Hillerød, Denmark). Cows were weighed and scored for body condition on 2 consecutive days at the start and end of treatment period.

Alfalfa hay, corn silage, concentrate, the corn silage and hay mix (after addition of the enzyme solutions),

and TMR samples were collected three times per week and composited on a biweekly basis. Forages were analyzed for DM (60°C for 48 h). Dietary ingredients and enzyme applications were adjusted on a weekly basis based on the DM content of the feeds. After drying, samples were ground in a Wiley Mill to pass through a 1-mm screen and analyzed for ADF (9) and NDF (26) with an Ankom^{200} Fiber Analyzer incubator (ANKOM Technology, Fairport, NY). Crude protein content was calculated (N × 6.25) after determination of N by total combustion (FP-2000 Analyzer, Leco Corporation, St. Joseph, MI). The concentrate was analyzed for DM (100°C for 24 h) and CP (as described). Neutral detergent fiber digestion was determined on samples of the untreated and treated corn silage and hay mix that had been composited on a biweekly basis. Samples (0.25 g) were digested in triplicate in F-57 incubation bags that had been prerinsed in acetone with a Daisy^{II} incubator (ANKOM Technology) for 12 and 48 h. Rumen fluid was collected from a steer fed a diet consisting of 45% corn silage, 5% hay, and 50% pelleted concentrate and combined with an in vitro buffer (26). A composite sample of the TMR was analyzed for minerals by inductively coupled plasma emission spectroscopy.

Data from the 2-wk pretreatment period were used as covariates for all variables. Lactation data were analyzed as a completely randomized design by the procedure of SAS (22). The main effect of diet was declared significant at $P < 0.10$ and trends were discussed at $P < 0.15$. For analytical data, the effect of treatment was declared significant at $P < 0.05$. Least square means were used to compare treatment differences.

Yr 2

The objective of this study was to compare the effect on milk production of two different cellulase enzyme complexes combined with a single xylanase enzyme complex. In this study, the xylanase enzyme complex was the same as used in yr 1 but the two cellulase complexes were derived from differing fermentations of the same organism. Whole-plant corn was harvested in the one-half milk line stage of maturity from the same field and stored in a bag silo (as in yr 1). Silage was stored for 5 mo prior to use. Second-cutting alfalfa, in the one-tenth bloom stage of maturity, was harvested as hay and stored as rectangular bales prior to feeding. Twenty-four multiparous and six primiparous lactating Holstein cows were used in a continuous lactation trial. Cows (average ± SD, 112 ± 58 DIM) were managed and housed as described in yr 1. The TMR differed slightly from that fed in yr 1 in that it was composed of 45% (DM basis) corn silage, 5% alfalfa hay, and 50% pelleted-concentrate (same formulation as in yr 1). Diets were balanced to meet the pretreatment requirements for cows producing 34 kg of 3.5% FCM. During a subsequent 12-wk treatment, cows were randomly assigned to one of three treatments: 1) water (control), 10 L of fresh water/1000 kg of the fresh forage, 2) (EA2) 2 L of an enzyme complex EA containing cellulase and hemicellulase enzyme complexes (3700 CMCase units and 14,000 xylanase units/kg of forage), or 3) (**EB1.2**) 1.2 L of an enzyme complex EB containing an alternative cellulase and hemicellulase complexes (3600 carboxymethyl cellulase units and 12,000 xylanase units/kg of forage DM). Treatments 2 and 3 were diluted in water and applied at a final rate of 10 L/1000 kg of fresh forage.

During the last week of treatment ruminal fluid was collected from all cows by stomach tube approximately 4 h after the a.m. feeding. Ruminal fluid was sampled in a manner that minimized contamination from saliva. The fluid was kept on ice until processed in the laboratory by addition of 1 ml of 25% meta-phosphoric acid to 5 ml of ruminal fluid. The acidified fluid was analyzed for VFA by gas chromatography (flame ionization detector) using a 530-μm macro bore Carbowax M column (Supelco, Bellefonte, PA). The chromatograph oven was programmed as follows: 70°C for 1 min, 5°C increase/min to 100°C, 45°C increase/min to 170°C, and a final holding time of 5 min. The molar proportions of VFA were calculated by dividing the individual acid concentrations by the total VFA concentration.

One cow from each treatment was removed from the data set due to various health reasons not associated with the treatment. Data from the pretreatment period were used as covariates for all variables. Lactation data were analyzed as a completely randomized design subject to ANOVA according to the procedures of SAS (22). The main effect of diet was declared significant at $P < 0.10$ and trends were discussed at $P < 0.15$. For analytical data, the effect of treatment was declared significant at $P < 0.05$. Least square means were used to compare treatment differences.

RESULTS

Yr 1

Ambient temperatures ranged from –5°C to 20°C during the study (data not shown). The ADF and NDF contents of the forage mixture (corn silage and alfalfa hay) were similar among treatments but forage treated with EA5 had greater hemicellulose content than the other treatments (Table 2). Digestion of the NDF fraction was numerically greater after 12 h of incubation for forages treated with EA2 and EA5, respectively, compared to untreated forage, but these differences were not statistically different. After 48 h, NDF diges-

Table 2. Fiber composition of the forage portion[1] of the TMR (80% corn silage:20% alfalfa hay, DM basis) treated with cellulase/xylanase enzymes in Year 1.

	Enzyme treatment[2]			
Item	Control	EA2	EA5	SE
ADF, %	25.3	24.4	23.2	0.9
NDF, %	40.3	39.4	43.2	1.3
Hemicellulose, %	15.2[b]	15.0[b]	20.0[a]	1.1
NDF digestion, %				
12 h	14.1	17.5	19.2	1.5
48 h	56.4[a]	55.1[ab]	54.2[b]	0.6

[a,b]Means in rows with unlike superscript differ ($P < 0.05$).

[1]Silage and hay were mixed together in a TMR wagon and sprayed with an enzyme solution.

[2]Control = No enzyme application; EA2 = forage treated with a cellulase enzyme (3500 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (16,000 xylanase units/kg of forage DM); EA5 = forage treated with a cellulase enzyme (8800 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (40,000 xylanase units/kg of forage DM).

tion of forage treated with EA5 was lower than control but not different from forage treated with EA2. The nutrient compositions of TMR in yr 1 are shown in Table 3. The diets had similar chemical compositions and averaged 52.4% DM, 17.8% CP, 19.0% ADF, and 33.5% NDF.

Production data from cows in the first year are shown in Table 4. Dry matter intake (kg/d and % of BW) was not affected by treatment and averaged 22.1 kg/d. However, milk production from cows fed the diet containing forage treated with EA2 (39.5 kg/d) was greater than milk production from cows fed the control diet (37.0 kg/d). In addition, cows fed the diet with forage treated with EA2 tended to have greater ($P < 0.15$) 3.5% FCM

Table 3. Nutrient composition of the TMR containing forages treated with enzymes (yr 1).

	Enzyme treatment[1]			
Item	Control	EA2	EA5	SE[2]
DM, %	51.6	53.1	52.4	1.2
CP, %	17.4	18.5	17.6	1.4
ADF, %	19.8	18.6	18.4	1.3
NDF, %	33.7	32.7	34.1	2.6
NE_L,[3] Mcal/kg	1.72	1.72	1.72	. . .
Ca, %	0.77	0.81	0.80	. . .
P, %	0.45	0.47	0.48	. . .
Mg, %	0.24	0.25	0.24	. . .

[1]Control = No enzyme applicaton; EA2 = forage treated with a cellulase enzyme (3500 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (16,000 xylanase units/kg of forage DM); EA5 = forage treated with a cellulase enzyme (8800 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (40,000 xylanase units/kg of forage DM).

[2]n = 14 for DM. n = 7 for all other analyses.

[3]Estimated from NRC (17).

Table 4. Performance of lactating cows fed forages treated with enzymes in Year 1.

	Enzyme treatment[1]			
Item	Control	EA2	EA5	SE
DMI, kg/d	22.0	22.5	21.8	0.7
DMI, % BW	3.48	3.50	3.36	0.10
Milk, kg/d	37.0[b]	39.5[a]	36.2[b]	1.04
3.5% FCM, kg/d	32.5[d]	35.4[c]	30.5[d]	1.3
FCM/DMI	1.47[ab]	1.54[a]	1.44[b]	0.04
Milk component				
Fat				
%	2.80[ab]	2.91[a]	2.52[b]	0.13
kg/d	1.07[ab]	1.13[a]	0.92[b]	0.06
Protein				
%	3.14[ab]	3.19[b]	2.96[b]	0.08
kg/d	1.16[ab]	1.25[b]	1.08[a]	0.05
ADG,[2] kg/d	0.55	0.67	0.70	0.17
BW, kg	647	657	631	11

[a,b]Means with unlike superscript differ ($P < 0.10$).

[c,d]Means with unlike superscript differ ($P < 0.15$).

[1]Control = No enzyme application; EA2 = forage treated with a cellulase enzyme (3500 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (16,000 xylanase units/kg of forage DM); EA5 = forage treated with a cellulase enzyme (8800 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (40,000 xylanase units/kg of forage DM).

[2]Average daily gain.

production than cows fed the control diet and diet containing forages treated with EA5. Treatment with enzymes did not improve feed efficiency relative to cows fed the control diet. Milk fat composition was low for all treatment groups and was caused by the corn silage being chopped too finely (a subjective assessment). Enzyme treatment did not improve milk composition relative to the control diet. However, cows fed forage treated with EA5 had lower milk fat, protein, protein yield, and fat yield than cows fed EA2. Average daily gain and BW were not different among treatments.

Yr 2

Ambient temperatures ranged from –4°C to 21°C during the study (data not shown). The ADF and NDF contents of the forage mixes are shown in Table 5. The ADF content of the forage mix treated with EB1.2 was greater ($P < 0.05$) than the untreated forage mix. Hemicellulose and NDF content were similar among treatments. Digestion of the NDF fraction of the forages was also similar among treatments. The compositions of the TMR were similar among treatments and are shown in Table 6.

Production data during the treatment period of yr 2 are shown in Table 7. Dry matter intake expressed as kilograms per day was not different among treatments but cows fed EB1.2 consumed more DM on a BW basis than did cows fed the control diet. Cows fed forage

Table 5. Fiber composition of the forage portion[1] of the TMR (90% corn silage:10% alfalfa hay, DM basis) treated with various complexes of cellulase and xylanase enzymes (yr 2).

	Enzyme treatment[2]			
Item	Control	EA2	EB1.2	SE
ADF, %	26.6[a]	27.3[ab]	28.7[b]	0.4
NDF, %	42.7	41.7	44.0	0.8
Hemicellulose, %	15.9	15.7	15.5	0.4
NDF digestion, %				
12 h	19.6	18.4	21.4	
48 h	61.1	59.9	59.0	0.8

[a,b]Means with unlike superscripts differ ($P < 0.05$).

[1]Silage and hay were mixed together in a TMR wagon and sprayed with an enzyme solution.

[2]Control = No enzyme application; EA2 = forage treated with a cellulase enzyme (3700 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (14,000 xylanase units/kg of forage DM); EB1.2 = forage treated with an alternative cellulase enzyme (3600 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (12,000 xylanase units/kg of forage DM).

treated with EB1.2 produced 2.5 kg more milk than did cows fed the control diet. The production of 3.5% FCM was not different among treatments. Milk composition was not affected by treatment.

Ruminal VFA from cows in yr 2 are presented in Table 8. The concentrations and molar proportions of VFA were not different among treatments and thus could not explain differences in milk production among treatments.

DISCUSSION

Feeding unprotected or unbound enzyme preparations to improve ruminal digestion has, in the past, been a questionable practice. Feeding unprotected enzymes may be more useful in immature ruminants when enzyme systems are not fully developed and (or) when liquids bypass the rumen via the esophageal groove. For example, Baran and Kmet (1) reported that a pectinase-cellulase enzyme additive improved ruminal fermentation in newly weaned lambs but not in adult sheep (with established rumen microflora). However, not all enzymes are subject to extensive degradation by microbial proteases in the rumen. Fontes et al. (8) reported that many fungal and bacterial cellulases and xylanases are glycosylated which may partially protect

Table 6. Average composition of the TMR fed to lactating cows during the treatment period (yr 2).

	Enzyme treatment[1]			
Item	Control	EA2	EB1.2	SE[2]
DM, %	52.1	53.2	52.7	0.5
CP, %	17.6	18.4	17.8	0.6
ADF, %	19.2	17.8	20.1	1.4
NDF, %	35.6	34.9	36.4	1.8
NE_L[3], Mcal/kg	1.61	1.63	1.61	. . .
Ca, %	1.05	1.09	0.92	. . .
P, %	0.45	0.48	0.46	. . .
Mg, %	0.34	0.35	0.34	. . .

[1]Control = No enzyme application; EA2 = forage treated with a cellulase enzyme (3700 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (14,000 xylanase units/kg of forage DM); EB1.2 = forage treated with an alternative cellulase enzyme (3600 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (12,000 xylanase units/kg of forage DM).

[2]n = 12 for DM. n = 6 for all other analyses. TMR were sampled three times weekly, composited by treatment for each week, and DM was determined. Samples were pooled every 2 wk for nutrient analyses. Minerals were analyzed on a composite of the TMR.

[3]Estimated from NRC (17).

Table 7. Covariately adjusted performance data (lsmeans) of lactating cows during the treatment period (yr 2).

	Enzyme treatment[1]			
Item	Control	EA2	EB1.2	SE
DMI, kg/d	21.0	21.9	21.9	0.4
DMI, % BW	3.56[a]	3.72[ab]	3.77[b]	0.08
Milk, kg/d	32.9[a]	33.6[ab]	35.4[b]	0.9
3.5% FCM, kg/d	32.9	32.8	32.5	1.2
FCM/DMI	1.53	1.51	1.52	0.05
Milk component				
Fat				
%	3.49	3.36	3.11	0.16
kg/d	1.14	1.13	1.06	0.06
Protein				
%	3.26	3.01	3.07	0.09
kg/d	1.11	1.01	1.05	0.04
BW, kg	590	591	586	5
ADG,[2] kg/d	0.32	0.32	0.19	0.11

[a,b]Means with unlike superscript differ $P < 0.10$.

[1]Control = No enzyme application; EA2 = forage treated with a cellulase enzyme (3700 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (14,000 xylanase units/kg of forage DM); EB1.2 = forage treated with an alternative cellulase enzyme (3600 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (12,000 xylanase units/kg of forage DM).

[2]Average daily gain.

Table 8. Ruminal VFA from cows fed forages treated with enzymes sampled during the last week of treatment (yr 2).

	Enzyme treatment[1]			
Item	Control[1]	EA2	EB1.2	SE
Total VFA, mM	103.4	115.5	93.8	11.6
Acetate, %	56.6	56.5	56.3	1.6
Propionate, %	25.7	24.3	25.6	2.1
Iso-butyrate, %	1.1	1.1	1.0	<0.1
Butyrate, %	13.6	14.9	14.1	0.6
Iso-valerate, %	1.4	1.5	1.4	<0.1
Valerate, %	1.6	1.7	1.6	<0.1

[1]Control = No enzyme application; EA2 = forage treated with a cellulase enzyme (3700 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (14,000 xylanase units/kg of forage DM); EB1.2 = forage treated with an alternative cellulase enzyme (3600 carboxymethyl cellulase units/kg of forage DM) and a xylanase enzyme complex (12,000 xylanase units/kg of forage DM).

them from degradation by proteases. In the rumen, Hirstov et al. (11) reported that some cellulase enzymes were relatively stable, but the ability to measure cellulolytic activity suggests that the enzymes were not bound to substrate.

Recently, fibrolytic enzymes have been applied to feeds just prior to feeding. When enzymes are applied to feed in this fashion, binding the enzyme to the substrate may result in a conformational change that makes the enzymes more resistant to proteolysis. This approach offers exciting possibilities for using enzymes to improve nutrient digestion, utilization, and animal productivity and, at the same time, reduce animal fecal material and pollution. Spraying enzymes onto feeds just before feeding may also provide increased management flexibility for feeding and bypasses any negative interactions that the ensiling process may have on enzyme performance. Specifically, treating forages with enzymes just prior to feeding would no longer be seasonal (only associated with the time of ensiling).

In our first lactation study, the low, but not the high, level of treatment with enzymes improved milk production. In fact, milk production and intake were numerically lower for cows fed the later treatment than for the control. Others have reported quadratic production from ruminants when these animals were fed forages treated with enzymes. In lactating cows, Sanchez et al. (20) reported that cows fed forages treated with a medium amount of cellulase and xylanase enzyme complex produced about 6 kg more milk than did cows fed untreated forage, or forage treated with a low or high level of enzymes. In steers, Beauchemin et al. (2) found that low, but not high concentrations of enzyme applied to alfalfa hay increased average daily gain in steers. Enzyme-binding capacity of some cellulases decreases with increasing molecular weight (5). In addition, Tanaka et al. (24) hypothesized that low molecular weight cellulases may move into small pores inaccessible to larger enzymes resulting in a decrease in the synergistic effects among different cellulase enzymes (e.g., endoglucanase and cellobiohydrolase). The end result would be a decrease in hydrolysis. Overtreatment with enzymes could also result in reduced chewing of forages (if they are more readily digested) subsequently resulting in less saliva production and thus, lower rumen pH and fiber digestion that may result in reduced milk production. Treacher et al. (25) also hypothesized that excessive binding of enzymes to substrates might hamper attachment to fiber by rumen microorganisms. Furthermore, they suggested that antinutritional factors (e.g., phenolic compounds) could be released by high concentrations of enzyme treatment, thus reducing microbial digestion.

In yr 2, we used the same xylanase enzyme complex as in yr 1 and combined it with the same cellulase complex used in yr 1 to make treatment EA or combined it with a different cellulase complex to make EB. Milk production was 0.7 kg greater for cows fed forage treated with EA2 (the same enzyme mix as in yr 1) but 2.5 kg greater for cows fed EB1.2 when compared to cows fed untreated forage. This data suggests that the source and combination of specific enzymes is an important factor in optimizing animal response.

A growing body of evidence suggests improvements in nutrient digestion and animal productivity when a variety of feeds are treated with fibrolytic enzymes just prior to feeding. In forages, fiber digestion has been improved when grass hay was treated with fibrolytic enzymes (7, 13). Beauchemin et al. (2) treated alfalfa cubes and concentrates with fibrolytic enzymes, and milk production was improved. Alfalfa silage treated with fibrolytic enzymes supported greater intake and a tendency for greater gains in beef steers (16). In addition, Pritchard et al. (20) reported linear improvements in intake and gain in steers fed treated grass hay. Improvements in digestion and production have also been observed when grains have been treated with various types of enzymes. For example, Boyles et al. (6) reported that treating steam-flaked sorghum with an enzyme complex with high amylase activity improved gain and feed efficiency in steers by about 10%. Maki et al. (15) reported improvements in digestion when barley was treated with fibrolytic enzymes. In another study (4), an improvement in milk production was observed when fibrolytic enzymes were applied to alfalfa cubes or alfalfa cubes and concentrate.

Yang et al. (28) reported that enzyme treatment resulted in improvements in digestibility and animal performance. In our study, treatment with fibrolytic enzymes did not affect the fiber content of the forage. This was not unexpected because the actual time for hydrolysis between treatment and sampling (or initial feeding) was short (no more than 30 to 45 min). Furthermore, in vitro NDF digestion and rumen VFA could not explain the differences that we observed in milk production between cows fed untreated and those fed treated diets. In a review article, Beauchemin et al. (3) reported that they had performed in vitro and in situ studies to evaluate the effectiveness of enzymes but that their results, like ours, did not always support the in vivo production responses. These findings suggest that other factors may be responsible for some of the responses found when cows are fed forages treated with enzymes. Treating feeds with enzymes just prior to feeding may improve digestibility via a number of different mechanisms including, direct hydrolysis, enhanced microbial attachment, changes in gut viscosity,

complementary actions with ruminal enzymes, and changes in the site of nutrient digestion (3, 24). Improvements in palatability and changes in patterns of feed consumption could also occur.

Not all results with treating forages with enzymes have been positive. For example, Luchini et al. (14) reported no effects of graded levels of a cellulase and xylanase enzyme mix that was sprayed onto forages for lactating cows. They did, however, observe a greater persistency of milk production from cows fed treated forages in a second study. Nuisso et al. (19) sprayed various levels of a cellulase and xylanase enzyme complex onto alfalfa hay and only noted a trend for increased milk production (9%) from cows in early lactation. Forages treated with fibrolytic enzymes also had no effect on milk production in a study reported by Zheng and Stokes (29).

CONCLUSIONS

Growing evidence indicates that improvements in gain and milk production can be achieved when ruminants are fed forages or grains treated with fibrolytic enzyme complexes just prior to feeding. The data from our studies showed that treating a diet in which forage was based on corn silage and alfalfa hay with fibrolytic enzymes improved milk production with no marked effects on milk composition. The increase in milk production occurred without apparent changes in DMI or fibrous fractions of the feeds. Treating forages just before feeding improves management flexibility because treatment can be done at any time of the year and treated forages can be fed only as needed. Our data also suggests that high levels of enzyme treatment may not be beneficial and that various enzyme combinations may be more efficacious than others. Further research is needed to understand how treating forages with enzymes prior to feeding improves productivity of animals and to determine specific, optimal enzyme combinations.

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