Effects of Tween 80 and Fibrolytic Enzymes on Ruminal Fermentation and Digestibility of Feeds in Holstein Cows

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ABSTRACT : The effects of the nonionic surfactant Tween 80 and a mixture of fibrolytic enzymes on total tract digestion, *in situ* disappearance (ISD) and ruminal fermentation characteristics of orchardgrass hay and barley grain were investigated in a 4×4 Latin square experiment with 4 non-lactating Holstein cows and 4 diets in 4 periods. Cows were offered a total mixed ration of 50% rolled barley grain and 50% orchardgrass hay treated with either 1) water (control), 2) 0.2% (vol/wt) Tween 80, 3) 0.2% (vol/wt) hydrolytic enzyme, or 4) 0.2% hydrolytic enzyme plus 0.2% Tween 80. Total tract digestibility coefficients of DM, nitrogen, NDF and ADF were not affected ($p>0.05$) by dietary treatment. Compared to the control, the rate of ISD of DM from orchardgrass hay was faster ($p<0.05$) in cows receiving diets treated with the enzyme alone or with enzyme plus Tween 80 (0.06/h vs. 0.076 and 0.069/h). The rate of digestion was lower ($p<0.05$) as compared to control when barley grain was treated with these additives. Ruminal fluid pH and concentrations of total VFA, acetate, isobutyrate and butyrate were not affected ($p>0.05$) by treatments. Cows that consumed diets treated with enzyme plus Tween 80 had higher ($p<0.05$) ruminal concentrations of propionate and isovalerate, and lower ($p<0.05$) acetate:propionate ratios. Compared to the control, microbial protein synthesis tended ($p = 0.13$) to increase with the addition of enzyme to the diet while non-ammonia nitrogen flow to the duodenum increased ($p<0.05$) with both enzyme and Tween 80 treatments. The study indicated that fibrolytic enzymes alone or in combination with Tween 80 could enhance ISD of orchardgrass hay and ruminal concentrations of propionate, valerate and iso-valerate, but do not affect total tract digestibility. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 816-824)***

Key Words : Tween 80, Fibrolytic Enzyme, Digestibility, Dairy Cow

INTRODUCTION

The nonionic surfactant Tween 80, has been shown in *in vitro* studies to increase cellulase activity and stability (McAllister et al., 2000; Lee and Ha, 2003; Lee et al., 2003), numbers of viable bacteria (Akin, 1980), microbial growth rates (Lee et al., 2003), and enzymatic accessibility and degradation of grain and forage substrates (Goto et al., 2003a, b). In addition, Kamande et al. (2000) reported that Tween 80 promoted the binding of enzymes to their substrates and increased microbial protease and cellulase activities *in vitro*. Wang et al. (2003) observed that Tween 80 increases the rate of gas and volatile fatty acid production during *in vitro* fermentation of corn and orchardgrass silages. Despite these reports of enhanced enzyme and microbial activities, attempts have been made only recently to determine if this surfactant will elicit similar responses *in vivo*, with consequent improvements in ruminant production (Kamande, 1994; McAllister et al., 2000; Lee et al., 2003; Wang et al., 2003; Kim et al., 2004).

Tween 80 has been shown to enhance ruminal fermentation and total tract digestibility (Kim et al., 2004) and to increase DM intake and milk production (Lee et al., 2003) in cattle. Whereas McAllister et al. (2000) observed no any positive response in growth performance of sheep fed either forage or concentrate diets supplemented with Tween 80. Wang et al. (2003) reported that Tween 80 increased average daily gain in backgrounding feedlot cattle. Kamande (1994) reported that adding 0.5% Tween 80 (wt/wt) to orchardgrass hay significantly increased DM intake in sheep from 1.9% to 2.6% of body weight, while digestibility increased from 55.6% to 64.7%. Increased feed intake is usually associated with a rapid flow of digesta from the rumen and a concomitant reduction in cell wall digestibility (Van Soest, 1982). Since intake and digestibility of the orchardgrass diet increased simultaneously (Kamande, 1994), it is possible that Tween 80 improved the efficiency of the digestive enzymes as evidenced by the *in vitro* results reported above.

The use of exogenous enzymes such as xylanase and β-glucanase in monogastric feeding systems has led to significant improvements in feed efficiency and increased ability to use a wide range of feed ingredients. Results from the use of exogenous enzymes in ruminant production systems have been less consistent. Lewis et al. (1999) observed higher DM intake and lactational performance in early and mid-lactation cows receiving diets treated with exogenous fibrolytic enzymes. Growth performance of ruminants fed forage or concentrate-based diets also increased when the diets were treated with exogenous

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Table 1. Chemical composition of barley grain and orchardgrass hay fed to cows[1]

Component	Barley grain	Orchardgrass hay
Dry matter (%)	88.8	88.0
Nitrogen (g/kg DM)	19.8	24.0
Acid detergent fiber (g/kg DM)	78.4	373.2
Neutral detergent fiber (g/kg DM)	179.4	623.5

[1] In addition, each cow's ration was top-dressed daily with 100 g of a mineral-vitamin mix containing (g/kg, as fed): Ca (180); P (180); Mg (34); Zn (6); Cu (4); Mn (2.5); Fe (10); F(2); Co (0.1); I (0.15); and vitamins A (500,000 IU/kg); D (50,000 IU/kg) and E (500 IU/kg).

fibrolytic enzymes (Beauchemin et al., 1995; Pritchard et al., 1996). The results from experiments with surfactants and exogenous fibrolytic enzymes suggest that surfactants could potentially be used to enhance the effects of exogenous enzymes in ruminant feeding systems.

The objectives of the present experiment were to determine the effects of combining the nonionic surfactant, Tween 80 and exogenous fibrolytic enzymes on ruminal fermentation, *in situ* disappearance and total tract digestibility of feeds in dairy cows.

MATERIALS AND METHODS

Tween 80, enzyme and dietary treatments

Tween 80 was obtained from Sigma Chemical Company, St. Louis, MO, USA. The enzyme was a 2:1 (vol/vol) mixture of two commercial fibrolytic enzyme preparations derived from fungal extracts and marketed as enzymes A and D by Finnfeeds International Ltd. (Marlborough, UK). The enzyme mixture had CMCase, xylanase and amylase activities of 61.7, 246.6 and 6.8 μmol/ml/min, respectively. Feeds were treated with the enzyme mixture, (E; 2 ml/kg), Tween 80, (T; 2 ml/kg), or the combination of the enzyme mixture plus Tween 80 (TE; 4 ml/kg). Aqueous solutions of E, T and TE were prepared and applied at the rate of 20 ml/kg of feed. The control feed was treated with 20 ml of water per kg of feed. The feed consisted of 50% rolled whole barley grain and 50% orchardgrass hay (chopped through a 5-cm screen). The hay and grain portions of the diets were prepared separately each morning and combined to form a total mixed ration (TMR). Grain was weighed into a cement mixer each morning and an appropriate quantity of aqueous solution was applied with a hand sprayer while the grain was mixed continuously for 5 min. The hay portion of each TMR was spread on polyethylene sheets in an enclosed area and the appropriate aqueous solution applied with a Wagner Heavy Duty Paint Sprayer (Wagner Spray Tech Corporation, Minneapolis, MN, USA) with intermittent hand mixing for approximately 5 min.

Animals and feeding

All animals used in this study were cared for according to the standards set by the Canadian Council on Animal Care (1993). Four non-lactating Holstein cows [795±41.3 kg (mean±SD)], fitted with ruminal and simple T-shaped duodenal cannulas were used in 4×4 Latin square design with four diets and four periods. Each period consisted of 21 d with 14 d of adaptation and 7 d of sampling. The diets were offered three times daily in equal allotments at 8-h intervals. Cows had *ad libitum* access to their diets, water, and a mixture of cobalt-iodized salt (99% NaCl and 1% Co-I which was made up of 150 mg/kg I and 50 mg/kg Co). In addition, the ration of each cow was top-dressed with 100 g of a mineral-vitamin mix (Table 1).

In vivo digestibility determination

Total tract digestibility was determined through total fecal collection from d 16 to 21. After weighing and thorough mixing of the whole feces, a subsample (200 g) was dried at 55°C in a forced air oven to constant weight before further analyses. Samples of each diet were collected daily and composited on a weekly basis for chemical analysis. Total urine output was collected through Foley catheters (Rusch Canada Inc., Scarborough, ON, Canada) during the same period in receptacles containing 700 ml of 5 M H_2SO_4. Duplicate samples of the urine were collected after thorough mixing. Rumen microbial protein was estimated from purine derivatives (allantoin and uric acid) in the urine as previously described by Hristov et al. (1998). Dry matter and nitrogen were analyzed using AOAC (1990) procedures, whereas NDF and ADF were determined by the method of Van Soest et al. (1991), with amylase included in the procedure.

In situ disappearance

On d 18 of each period, 5 g of the barley grain or orchardgrass hay ground to pass through a 2 mm screen was loaded into duplicate monofilament polyester bags (53 μm pore size, 5 cm×20 cm, Ankom, Fairport, NY, USA) and placed sequentially in the rumen of each cow to incubate for 72, 48, 24, 12, 8, 4 and 2 h. Staggered introduction enabled removal of all bags at the same time on d 21. Immediately after removal, bags were placed in a domestic washing machine and washed in cold water using three 10-min delicate wash cycles without the spin cycle. A duplicate set of duplicate bags containing unincubated samples of barley grain and orchardgrass hay was included with the other bags for washing. This was used to estimate 0 h disappearance. Bags were then dried in a forced-air oven at 55°C for 48 h. Percentage disappearances of DM and NDF were calculated from the proportions remaining in the bag after each incubation time.

Rate of passage and rumen fermentation characteristics

The following markers were infused into the rumen

from d 15 (0800) to d 20 (0630) of each period: Co-EDTA (17.33 g Co-Li-EDTA/cow/d) as a liquid marker and Yb (11.2 g $YbCl_3 \cdot 6H_2O$/cow/d) as a solid marker in order to estimate ruminal outflow rates of fluids and solids. All markers were dissolved in distilled water and infused simultaneously into the rumen through a peristaltic pump (Technicon Instruments Corp., Tarrytown, NY, USA). The total amount of infusate was 1 L per cow per day.

During d 18 and d 19 of each period, 6 samples each of rumen and duodenal contents were taken at the following times: d 18 - 09.30, 17.30, 01.30 (next day); d 19 - 13.30, 21.30, 05.30 (next day). Seven samples of ruminal and duodenal contents were taken again on d 20 at the following times: 05.30, 09.30, 13.30, 17.30, 21.30, 03.30 (d 21), 09.30 (d 21). Ruminal contents were filtered through a screen with mesh size of 105 μm. The liquid fraction was centrifuged at 11,220×g for 15 min, and the Co in the supernatant analyzed as previously described (Hristov et al., 1998) by atomic absorption spectrophotometry (Perkin Elmer Corporation). The solid fraction was freeze-dried, ground to pass through a screen with a mesh size of 1 mm, and ashed at 550°C. Subsamples (0.5 g) of the ash were weighed into tubes containing 20 ml of a solution of 1.91 g/L KCl in 2% (vol/vol) nitric acid. The tubes were capped and mounted on a rotary shaker for incubation at 22°C for 2 h. The mixtures were centrifuged at 11,220×g for 15 min, and supernatants analyzed for Yb as described for Co.

Each duodenal sample was divided into two subsamples. One was strained manually through a 105 μm screen into liquid and solid fractions. The fractions were weighed and freeze-dried separately. The second subsample was weighed and freeze-dried without further processing. These duodenal samples were used to estimate the flow of OM, bacterial N and non-ammonia N to the duodenum as described by Hristov et al. (1998).

Ruminal content samples were squeezed through four layers of cheesecloth and the pH was measured immediately. Microbial pellets were isolated from the filtrate by differential centrifugation. Feed particles were removed from subsamples (200 ml) by centrifugation at 500×g for 10 min. The supernatant fluid was centrifuged at 28,099×g for 20 min, and the resulting precipitate (microbial pellet) was freeze-dried. Subsamples of the filtrate were analyzed for total reducing sugars and enzyme activities (CMCase, xylanase and amylase) as described by Hristov et al. (1998). Samples of ruminal fluid obtained at each sampling time were acidified with 0.25 volumes of 25% *m*-H_3PO_4, then centrifuged at 28,099×g for 15 min. The supernatant was analyzed for total VFA, acetate, propionate, isobutyrate, butyrate, isovalerate and valerate (McAllister et al., 1999). Additional samples (10 ml) of ruminal fluid were combined with 0.75 ml of 65% trichloroacetic acid and stored overnight at 4°C. Each mixture was centrifuged at 28,099×g for 15 min, and the supernatant fluids were analyzed for ammonia (NH_3) and total free amino acids (TFAA) using methods outlined by Broderick and Kang (1980). Samples of duodenal and ruminal fluid were strained through a 105-μm screen and viscosities after straining were determined with a cone plate viscometer DV-II+ (Brookfield Engineering Laboratories, Stoughton, MA).

At the end of each sampling period, 40 kg of ruminal content was removed from each cow, and replaced with ruminal content from two ruminally cannulated donor cows that were being maintained throughout the study on a diet of barley grain and orchardgrass hay (1:1). This procedure was adopted to minimize carryover effects between periods in the cows involved in the digestibility trial.

Calculations and statistical analyses

The DM and NDF disappearance data were fitted to a modified version of the exponential model of Ørskov and McDonald (1979) with a lag phase:

$$p = a+b\ [1-e^{-c(t-lag)}] \text{ for } t > lag$$

where p is the disappearance (%) of a component of the substrate say, DM or NDF after t hours; a is the rapidly disappearing fraction (%); b is the slowly disappearing fraction (%); and c is the fractional rate of disappearance (/h) of fraction b. Estimates of a, b, c and lag were calculated by an iterative non-linear procedure (Marquardt method) with the SAS (1990) software package. Effective disappearance (EFFD) of each component was determined using the following equation:

$$EFFD = a+b\ [c/(c+k)]$$

where k is the particle flow rate constant assumed to be 6%/h.

The estimated fractions and all other data were subjected to analysis of variance for 4×4 Latin square design using GLM procedures of SAS (1990). Data from the digestibility and disappearance experiments were analyzed using the following model:

$$Y_{ijk} = \mu+\alpha_i+\beta_j+\gamma_k+\varepsilon_{ijk}$$

where, Y_{ijk} is the observation; μ is the overall mean; α_i is the effect of treatment (control, E, T or TE); β_j is the effect of period (1, 2, 3 and 4); γ_k is the effect of cow (A, B, C and D) and ε_{ijk} is the residual error. The model used in the analysis of the ruminal fluid pH and fermentation products included the effects of day and hour of sampling in addition to the factors in the model above. Treatment and period

Table 2. Effect of Tween 80 and exogenous fibrolytic enzymes on total tract digestibility coefficients in dairy cows fed a mixture of barley grain and orchardgrass hay (%)

Item	Treatment[1]				SEM[2]
	Control	T	E	TE	
Dry matter	69.8	68.2	69.5	67.7	0.65
Nitrogen	66.5	64.0	65.4	63.7	0.85
Neutral detergent fiber	60.6	58.1	58.3	57.5	0.93
Acid detergent fiber	49.9	47.9	49.3	46.1	1.17

[1] Control (treated with water); T = 0.2% (vol/wt) Tween 80; E = 0.2% (vol/wt) enzyme preparation. TE = 0.2% (vol/wt) Tween 80 and 0.2% (vol/wt) enzyme preparation.
[2] Standard error of the mean.

Table 3. Effect of Tween 80 and exogenous fibrolytic enzymes on kinetics of dry matter disappearance in dairy cows fed a mixture of rolled barley grain and orchardgrass hay

Item[1]	Treatment[2]				SEM[3]
	Control	T	E	TE	
Orchardgrass hay					
a (%)	29.5^{b}	30.0^{a}	29.4^{b}	29.3^{b}	0.09
b (%)	53.9^{b}	54.8^{b}	55.1^{ab}	56.4^{a}	0.5
c (/h)	0.060^{c}	0.067^{bc}	0.076^{a}	0.069^{b}	0.003
lag (h)	5.7^{d}	8.3^{a}	6.6^{c}	7.2^{b}	0.18
EFFD (%)	48.5^{a}	46.2^{c}	47.7^{ab}	46.9^{bc}	0.50
Barley grain					
a (%)	46.4^{b}	50.0^{a}	49.3^{a}	46.9^{b}	0.55
b (%)	43.7^{a}	40.4^{c}	41.4^{b}	43.9^{a}	0.52
c (/h)	0.368^{a}	0.294^{bc}	0.318^{b}	0.272^{c}	0.011
lag (h)	0.0	0.0	0.8	0.0	0.11
EFFD (%)	83.6	82.5	83.1	82.6	0.37

[a-d] Within a row, means lacking a common superscript differ ($p<0.05$).
[1] Parameters calculated from the fitted equation: $p = a - b[1-e^{-c(t-lag)}]$ for $t > lag$; where p is the proportion (%) of dry matter disappearing from nylon bags after t hours of incubation; a is the rapidly soluble fraction (%); b is the slowly disappearing fraction (%); c is the fractional rate of disappearance (/h) of fraction b; and EFFD is the effective disappearance based on an assumed outflow rate of 6%/h.
[2] Control (treated with water); T = 0.2% (vol/wt) Tween 80; E = 0.2% (vol/wt) fibrolytic enzyme preparation; TE = 0.2% (vol/wt) Tween 80 and 0.2% (vol/wt) fibrolytic enzyme preparation.
[3] Standard error of the mean.

were considered fixed effects while cow, day and hour of sampling were considered random effects. In all cases significance was declared at $p<0.05$. Least square means were separated using the PDIFF option of the GLM procedure of SAS (1990) when fixed effects were significant.

RESULTS

Total tract digestibility and *in situ* disappearance

The TMR contained 86.6% DM, and its OM, NDF, ADF and nitrogen contents (g/kg DM) were 923.8, 395.7, 215.2 and 21.9, respectively. Average DM intake of the total mixed ration (TMR) per cow was 12.5 kg/d. Total tract apparent digestibility coefficients of DM, nitrogen, NDF and ADF were unaffected ($p>0.05$) by dietary treatments (Table 2). Apparent DM digestibility coefficients in cows on the control, T, E and TE treatments were 69.8, 68.2, 69.5 and 67.7%, respectively.

Orchardgrass hay incubated in the rumen of cows on the T treatment had the highest ($p<0.05$) rapidly disappearing fraction (a) of DM compared with hay incubated in the rumen of cows receiving the other treatments (Table 3). Fraction a in barley grain was also higher ($p<0.05$) in cows that received either T (50%) or E (49.3%), compared to the fractions in cows on the control (46.4%) or TE (46.9%) treatments. The slowly disappearing fraction (b) of DM of the hay was higher ($p<0.05$) when incubated in the rumen of cows on the enzyme treated diets. Fraction b was 55.1% and 56.4% in cows receiving E and TE, respectively, compared with 53.9% for those on control and 54.8% for those on T. Barley grain incubated in the rumen of cows on treatment T had lower ($p<0.05$) b DM fractions. Compared to the other treatments, the fractional rate of disappearance of DM from orchardgrass hay was the highest ($p<0.05$) when incubated in cows on treatment E (0.076/h, vs. 0.069, 0.067 and 0.060/h for TE, T and control, respectively). However, in the case of barley grain, fractional rate of DM disappearance was the fastest when the grain was incubated in the rumen of cows on the control treatment and the slowest in those on the T treatment. The fractional rate of DM disappearance of the grain in control cows was 0.368/h, whereas the rates in cows on treatments T and TE were 0.294/h and 0.272/h, respectively.

Table 4. Effect of Tween 80 and exogenous fibrolytic enzymes on neutral detergent fiber disappearance in the rumen of dairy cows fed a mixture of barley grain and orchardgrass hay

Item[1]	Treatment[2]				SEM[3]
	Control	T	E	TE	
Orchardgrass hay					
a (%)	9.6[a]	7.2[c]	7.5[bc]	8.0[b]	0.19
b (%)	68.7[c]	76.1[a]	73.0[b]	75.0[a]	0.73
c (/h)	0.063	0.063	0.071	0.062	0.003
lag (h)	6.6[a]	6.8[a]	6.2[b]	6.7[a]	0.13
EFFD (%)	32.7	30.2	31.5	30.6	1.36
Barley grain					
a (%)	7.7[b]	9.7[a]	9.9[a]	4.0[c]	0.19
b (%)	63.7[a]	61.9[a]	56.8[b]	62.4[a]	0.88
c (/h)	0.056[c]	0.043[d]	0.058[b]	0.064[a]	0.001
lag (h)	0.0[b]	0.0[b]	0.0[b]	0.1[a]	0.01
EFFD (%)	35.8	34.6	37.8	34.6	1.62

[a-d] Within a row, means lacking a common superscript differ ($p<0.05$).

[1] Parameters calculated from the fitted equation: $p = a+b[1-e^{-c(t-lag)}]$ for $t>lag$; where p is the proportion (%) of neutral detergent fiber from nylon bags after t hours of incubation; a is the rapidly soluble fraction (%); b is the slowly disappearing fraction (%); c is the fractional rate of disappearance (/h) of fraction b; and EFFD is the effective disappearance based on an assumed outflow rate of 6%/h.

[2] Control (treated with water); T = 0.2% (vol/wt) Tween 80; E = 0.2% (vol/wt) fibrolytic enzyme preparation; TE = 0.2% (vol/wt) Tween 80 and 0.2% (vol/wt) fibrolytic enzyme preparation.

[3] Standard error of the mean.

Hay incubated in the rumen of cows that received Tween 80 had lower ($p<0.05$) EFFD DM values. The lag time in DM disappearance of hay incubated in rumen of cows on the control treatment was the shortest ($p<0.05$), 5.7 h; compared to the lag in cows receiving treatment E (6.6 h), T (8.3 h) or TE (7.2 h). However, the lag time and EFFD in DM disappearance from grain were unaffected ($p>0.05$) by dietary treatments.

Fraction a of the NDF in the hay (Table 4) was the highest ($p<0.05$) when it was incubated in the rumen of cows on the control treatment (9.6%), followed by treatments TE (8.0%), E (7.5%) and T (7.2%). The lowest fraction b in NDF was observed in cows on the control treatment (68.7%), and the highest was observed in those on treatments T and TE; 76.1% and 75.0 %, respectively, while those on treatment E (73.0%) had an intermediate value ($p<0.05$). The lag time for the NDF disappearance was the shortest ($p<0.05$) when incubated in cows on treatment E (6.2 h). The lag was not different ($p>0.05$) between the control (6.6 h), T (6.8 h) and TE (6.7 h) treatments. Fraction a of the NDF in barley grain was higher ($p<0.05$) in cows on treatments E and T. The fastest ($p<0.05$) rate of disappearance of the NDF fraction in barley grain was observed in cows on treatment TE (0.064/h), followed by E (0.058/h), control (0.056/h) and T (0.043/h). As was observed in DM disappearance, the EFFD of NDF was not affected ($p>0.05$) by dietary treatments.

Ruminal fermentation

Concentrations of total VFA, acetate, butyrate and isobutyrate in ruminal fluid of cows were not altered ($p>0.05$) by dietary treatments (Table 5). The concentration of total free amino acids in ruminal fluid from cows on the T treatment (1.00 mM) was not different ($p>0.05$) from that from cows on the control treatment (1.08 mM) but was lower when compared to cows on treatments E and TE (1.26 mM). Ruminal fluid pH was not different ($p>0.05$) between cows on the various treatments and ranged from 5.93 in cows on treatment E to 6.02 in cows on the control treatment. However, Tween 80 and/or enzyme increased ($p>0.05$) propionate, isovalerate and valerate concentrations, and reduced ($p>0.05$) A:P ratio, compared to the control treatment. Ruminal A:P ratio was the lowest in cows on treatment TE (4.7) and the highest in cows on the control treatment (5.0), with cows on treatments T and E having intermediate values ($p>0.05$).

The viscosities of ruminal and duodenal contents were lowest ($p>0.05$) in cows on the control treatment and highest ($p>0.05$) in cows on the T treatment. Ruminal content viscosity in control cows was 5.04 cP compared to 6.40, 5.58 and 6.02 cP in cows on treatments T, E and TE, respectively. The values for duodenal viscosity were 2.28, 2.70, 2.58 and 2.50 cP in cows on treatments control, T, E and TE, respectively.

Microbial nitrogen production was numerically higher ($p = 0.13$) in cows on treatment TE (0.15 kg/d) compared to 0.13 kg/d in cows on the other treatments. Non-ammonia nitrogen flow to the duodenum was in turn higher ($p>0.05$) in cows on treatments T and E, 0.27 kg/d and 0.26 kg/d, respectively, compared to the flow in those on the control (0.23 kg/d) and TE (0.22 kg/d) treatments. Ruminal fluid and solid passage rates, and OM and bacterial nitrogen flow to the duodenum were, however, unaffected ($p>0.05$) by dietary treatments.

Table 5. Effects of Tween 80 and exogenous fibrolytic enzymes on ruminal and duodenal digesta characteristics, and microbial protein synthesis in non-lactating Holstein cows

Item	Treatment[1]				SEM[2]
	Control	T	E	TE	
Ruminal fermentation products (mM)					
Acetate (A)	66.5	66.8	68.0	66.6	0.67
Propionate (P)	13.5^{b}	14.2^{a}	14.1^{a}	14.4^{a}	0.18
Butyrate	15.1	15.0	14.8	14.7	0.16
Isobutyrate	0.75	0.76	0.75	0.74	0.010
Valerate	0.87^{c}	0.94^{a}	0.92^{b}	0.91^{b}	0.010
Isovalerate	0.88^{c}	0.96^{b}	0.97^{b}	1.2^{a}	0.020
Total volatile fatty acids	97.6	98.7	99.5	98.5	0.98
A:P ratio	5.0^{a}	4.8^{b}	4.9^{b}	4.7^{c}	0.04
Ammonia	8.96	8.73	8.50	8.07	0.301
Total free amino acids	1.08^{ab}	1.00^{b}	1.26^{a}	1.26^{a}	0.07
Reducing sugars (RS)	0.57	0.54	0.87	0.59	0.110
pH	6.02	5.93	5.94	5.98	0.030
Enzyme activities, nmol RS/(ml×min)					
CMCase	57.3	55.8	57.2	58.1	0.84
Xylanase	243.6	249.2	249.1	254.8	4.94
Amylase	30.4^{c}	34.0^{b}	32.9^{c}	37.8^{a}	1.13
Fluid passage rate (%/h)	9.0	9.3	9.6	10.5	1.29
Solid passage rate (%/h)	3.9	5.1	3.8	4.9	0.90
Duodenal flow (kg/d)					
Organic matter	7.71	7.77	7.32	6.92	0.680
Bacterial nitrogen	0.13	0.16	0.14	0.14	0.013
Non ammonia nitrogen	0.23^{b}	0.27^{a}	0.26^{a}	0.22^{b}	0.010
Microbial-nitrogen[3] (kg/d)	0.13	0.13	0.13	0.15	0.009
Ruminal content viscosity (cP)	5.04^{d}	6.40^{a}	5.58^{c}	6.02^{b}	0.015
Duodenal content viscosity (cP)	2.28^{d}	2.70^{a}	2.58^{bc}	2.50^{c}	0.039

[a-d] Within a row, means lacking a common superscript differ ($p<0.05$).

[1] Control (treated with water): T = 0.2% (vol/wt) Tween 80: E = 0.2% (vol/wt) fibrolytic enzyme preparation: TE = 0.2% (vol/wt) Tween 80 and 0.2% (vol/wt) fibrolytic enzyme preparation.

[2] Standard error of the mean.

[3] Microbial nitrogen estimated from urinary excretion of purine derivatives (uric acid and allantoin).

DISCUSSION

Although the differences between treatments in total tract apparent digestibility coefficients of DM, nitrogen, NDF and ADF were not significant, cows on treatments T and TE tended ($p = 0.12$) to have lower digestibility coefficients than those in C or E. In contrast, Feng et al. (1996) reported that total tract digestibilities of DM and NDF were higher in cows fed fibrolytic enzyme-treated smooth bromegrass hay. Kim et al. (2004) also reported that although digestibility of crude fibre increased in native Korean cattle (Hanwoo) fed a diet supplemented with 10 g/d f Tween 80, digestibilities of DM, crude protein, NDF, and ADF were unaffected when compared to the control. Furthermore, those authors observed that increasing concentration of Tween 80 tended to increase the digestibility of the above nutrients.

Ruminal passage rate in cows on treatment T tended to be faster than the rate in cows on the control and E treatments by 31% ($p = 0.11$) and 34% ($p = 0.09$), respectively. This tendency towards a decrease in particulate passage rate, or increased residence time of feed in the rumen of cows on the control and E treatments, might have contributed to the marginal increase in total tract apparent digestibility coefficients of nutrients in cows on these treatments.

The *in situ* data indicate that the fractional rate of DM disappearance in orchardgrass hay increased by about 27% when the hay was incubated in the rumen of cows on treatment E compared to those on the control treatment. A higher rate of DM disappearance is usually associated with a higher rate of particle size reduction and a shorter retention time in the rumen (Van Soest, 1982). A shorter retention time results in a decrease in digestibility as the feed does not stay in the rumen long enough for it to be fully digested. However, this was not the situation in the present study.

In addition, the decrease in the fractional rate of ruminal disappearance of DM from barley as a result of the T or E treatment may be advantageous in the case of barley grain. This is because more of the feed (starch) can escape ruminal fermentation and pass directly to the lower gut, thereby preventing the development of digestive conditions such as

rumen acidosis and bloat. The NDF disappearance rate in barley grain was higher when the grain was incubated in the rumen of cows receiving diets treated with enzyme alone or the combination of enzyme and Tween 80. It therefore appears that there was a positive interaction between Tween 80 and the fibrolytic enzymes with respect to the disappearance of the NDF fraction in barley grain.

Relative to the control and treatment TE, the proportion of barley DM that disappeared rapidly (fraction a) was higher when the barley was incubated in the rumen of cows on treatments T and E. This is consistent with the findings of Hristov et al. (1998), and seems to suggest that both T and E may be having a greater effect on the fraction of DM in barley recovered in the soluble fraction (fraction a). It is possible that Tween 80 and the fibrolytic enzymes may be hydrolyzing endosperm cell walls of barley grain thereby enhancing the release of soluble cell constituents, an effect that would not necessarily increase the extent of DM disappearance as evidenced by the similarity in EFFD values reported in this study.

The derivation of EFFD values takes into account rumen outflow rate and incorporates all the other disappearance variables. Therefore, EFFD values may be better indicators of the overall effect of the treatments on digestibility than any single parameter, and is probably the reason why the total tract apparent digestibility coefficients determined from the *in vivo* digestibility experiment are more consistent with EFFD values.

The lag time in the disappearance of DM and NDF from barley was less than one hour with all treatments and therefore may have minimal impact on overall digestibility of the grain. However, the longer lag in disappearance of DM in hay incubated in the rumen of cows on treatments containing either Tween 80 and/or enzyme could lead to a reduction in digestibility of the forage and possibly DM intake.

Consistent with the findings of Hristov et al. (1998), ruminal CMCase and xylanase activities were not affected by treatment, although ruminal amylase activities were higher in cows consuming diets treated with either Tween 80 alone or Tween 80 plus enzyme. Ruminal amylase activity in cows on TE was higher than the activity in cows on the control and E treatments by 24 and 15%, respectively. This enhanced amylase activity is consistent with other observations on the effects of Tween 80 on the activities of hydrolytic and proteolytic enzymes (Kamande et al., 2000; McAllister et al., 2000; Goto et al., 2003a; Lee et al., 2003).

Contrary to observations by Hristov et al. (1998), the application of hydrolytic enzymes to the feed did not reduce ruminal fluid viscosity, probably because of the lower content of grain in the rations used in this study. The ration used by Hristov et al. (1998) contained 60% rolled barley grain and 35% corn silage. A diet with a high proportion of grain will obviously have higher levels of components such as β-glucans that are responsible for the higher viscosity of digesta and feces (Classen and Bedford, 1991). Therefore, the extent to which fibrolytic enzymes will reduce the ruminal viscosity in cows on such a diet will be more pronounced than in situations where cows are fed 50:50 (grain:forage) diet.

Microbial protein synthesis tended to increase with the addition of enzyme to the diet. Bacterial-N flow to the duodenum was not affected despite the fact that in cows on treatments T and E, flow was numerically higher by 27 and 12.5%, respectively, compared to the flow in control cows. The inability to detect differences in bacterial-N flow may have been due to the large variation in bacterial-N content of duodenal samples found in this study.

Although some previous studies (Kamande et al., 2000; Wang et al., 2003; Goto et al., 2003a; Lee at al., 2003; Kim et al., 2004) have shown that Tween 80 enhances microbial attachment, ruminal fermentation and feed efficiency, there have been other studies in which Tween 80 did not appear to be of benefit. McAllister et al. (2000) did not observe a positive response in growth performance of sheep fed either forage or concentrate diet supplemented with 0.02% or 0.2% Tween 80. However, Wang et al. (2003) reported that 0.2% Tween 80 increased average daily gain in backgrounding feedlot cattle by 7%. Lee et al. (2003) also showed that lactating Korean native cattle (Hanwoo) receiving 1.2 kg/d of Tween 80 had higher DM intake and milk yield. Goto et al. (2003a) observed that the extent of enzymatic degradation of leaf blade fractions was dependent on the concentration of Tween 80 and the age of the leaf. The above observations are consistent with the hypothesis put forward by McAllister et al. (2000) that the range of concentrations of Tween 80 that promote association or binding of enzymes and/or microbial attachment to feed particles may be narrow, and may depend on characteristics of the feed. Too low a concentration may not promote any significant interaction, whereas too high a concentration may mask the surface of feed particles and impede attachment and subsequent digestion. More fundamental research to elucidate the mode of action of Tween 80 and other surfactants under different feeding regimes is therefore required in order to ensure that the inclusion of surfactants in ruminant diets results in consistent positive response in animal performance.

CONCLUSIONS AND IMPLICATIONS

The specific rate of *in situ* DM disappearance of orchardgrass hay was increased when cows received the

diet treated with exogenous fibrolytic enzymes, however, total tract digestibility of nutrients were unaffected. Ruminal content of cows fed diets treated with Tween 80 and/or fibrolytic enzymes contained higher concentrations of propionate, valerate and *iso*-valerate, implying a shift in ruminal fermentation patterns as a result of treating the diet with Tween 80 and/or fibrolytic enzymes. The *in situ* disappearance data indicate the potential improvement in rate of degradation of hay that could be achieved if hay is treated with exogenous fibrolytic enzymes prior to feeding. In situations where total mixed rations of dairy cows contain higher proportions of forage, the higher rate of degradation of the forage component might lead to an increase in particulate passage rate and allow for greater DM intake.

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