Fibrolytic Enzymes and Parity Effects on Feeding Behavior, Salivation, and Ruminal pH of Lactating Dairy Cows¹

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ABSTRACT

Four multiparous and four primiparous lactating dairy cows fitted with ruminal cannulas were used in a duplicated 4 × 4 Latin square design to study the effects of parity and inclusion of a fibrolytic enzyme product (Agribrands International, St. Louis, MO) on feeding and chewing behavior, salivation, and ruminal pH. Diets consisting of rolled barley, barley silage, and alfalfa haylage (55% forage, DM basis) differed in enzyme application: 1) control, 2) enzyme applied to concentrate (45% of TMR), 3) enzyme applied to supplement (4% of TMR), and enzyme applied to a premix (0.2% of TMR). Enzyme supplementation did not alter daily time spent eating or ruminating, but when enzymes were added to the ration daily, saliva production increased, with no difference among enzyme application treatments. Multiparous cows consumed a greater amount of feed, but spent a similar amount of time eating, compared to primiparous cows. Primiparous cows had shorter ruminating episodes, resulting in lower daily ruminating time compared with multiparous cows. Primiparous cows had lower daily saliva output compared with multiparous cows. These results indicate that application of this fibrolytic enzyme product did not alter the physical structure of the feed, as measured by feeding and chewing variables. The increase in total saliva production observed in cows fed enzyme-supplemented diets may be attributed to a physiological response to compensate for the increase in fermentation products during digestion. The increased intake for multiparous cows is attributed to increased eating rate and not to increased time spent eating. The higher DMI of multiparous cows resulted in increased

rumination time needed to process the additional feed and increased salivation to buffer the greater production of VFA.

(Key words: enzyme, chewing behavior, feeding behavior, ruminal pH)

Abbreviation key: CONC = enzyme product added to concentrate (45% of TMR, DM), CTRL = control treatment, MP = multiparous cows, PP = primiparous cows, PREM = enzyme product added to premix (0.2% of TMR, DM), SUPP = enzyme product added to supplement (4% of TMR, DM).

INTRODUCTION

There is increasing evidence that exogenous fibrolytic enzymes improve fiber digestion within the rumen, thereby increasing feed utilization of ruminants (Lewis, 1999; Rode, 1999). However, exogenous fibrolytic enzyme products are usually applied to the diet before ingestion, potentially causing pre-ingestive effects. Applying exogenous enzymes directly to feed causes a release of reducing sugars (Hristov et al., 1996), and, in some cases, partial solubilization of NDF and ADF (Krause et al., 1998). Hydrolysis of the fiber prefeeding may indicate a modification of the plant cell wall structure, which could decrease the physical effectiveness of the fiber in the diet. Thus, applying fibrolytic enzymes to feed before feeding may decrease both chewing time and saliva output and increase the risk of acidosis.

Saliva is continually produced by cattle and is integral in providing buffering and fluidity within the rumen. Lactating dairy cows produce larger amounts of saliva (Cassida and Stokes, 1986) than nonlactating beef cattle (Bailey, 1961). As well, differences in saliva production between parity groups have been reported (Maekawa et al., 2002), with multiparous cows having a higher salivation rate than primiparous cows. Saliva production increases as eating and ruminating time increases (Beauchemin, 1991). Alterations in mechanical processing (Beauchemin and Rode, 1994) and chemi-

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cal properties (Beauchemin and Buchanan-Smith, 1989) of feed can significantly alter chewing behavior, and consequently saliva production. Therefore, the use of exogenous fibrolytic enzymes in dairy cow diets may alter feeding behavior and saliva production.

Increasing the rate of fermentation within the rumen leads to a decrease in ruminal pH, which decreases fiber digestion (Russell and Wilson, 1996). Supplemental fibrolytic enzymes have been shown to increase fiber digestion (Rode et al., 1999; Yang et al., 2000a), and ruminal pH has been lowered in some cases (Lewis et al., 1996), but not always (Yang et al., 1999).

The objective of this study was to investigate the effects of enzyme supplementation on the chewing and feeding behavior, saliva secretion, and ruminal pH of lactating dairy cows. A second objective was to determine whether the response to enzyme supplementation differed for multiparous and primiparous cows.

MATERIALS AND METHODS

Cows and Diets

Four primiparous and four multiparous (in their second lactation) lactating Holstein cows were surgically fitted with ruminal cannulas, measuring 10 cm in diameter that were constructed of soft plastic (Bar Diamond, Parma, ID). Multiparous and primiparous cows averaged 134 ± 23 , 128 ± 22 DIM and 602 ± 10 , 602 ± 41 kg of BW at the start of the experiment, respectively. Estimated mature body size of the primiparous cows was larger than the multiparous cows because they were purchased as replacement stock.

The design of the experiment was a double 4×4 Latin Square with each period lasting 28 d. One square was comprised of multiparous cows and the other of primiparous cows. Cows received a diet consisting of 45% concentrate and 55% forage (DM basis) (Tables 1 and 2). The diet was formulated using the Cornell-Penn-Miner System (CPMDairy, Version 1.0) and balanced to provide sufficient metabolizable energy, metabolizable protein, vitamins, and minerals to produce 35 kg/d of milk with 3.5% fat and 3.2% CP. Presence or absence of a fibrolytic enzyme product and the percentage of TMR to which the enzyme product was added made up the four treatments. The treatments were: 1) no enzyme (CTRL), 2) enzyme added to the concentrate portion of the TMR consisting of 45% of the dietary DM (CONC), 3) enzyme added to the pelleted portion of the TMR which made up 4% of the dietary DM (SUPP) and 4) enzyme applied to a premix, offered at 50 g/head/d, which made up 0.2% of the dietary DM (PREM).

Cows receiving the enzyme-treated TMR all received approximately the same dose; 1 g of enzyme product per head per day. The enzyme used was Promote N.E.T.

Table 1. Ingredients and chemical composition of the diet.

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

¹Ingredients that were in the pelleted supplement.

²Contained 2000 IU of vitamin A/g, 200 IU of vitamin A/g, 200 IU of vitamin D/g and 20 IU of vitamin E/g. Contained 58.8% NaCl, 2.0% ZnSO₄, 2.4% MnSO₄·4H₂O, 0.8% CuSO₄·H₂O, 90 mg/kg CoSO₄·5H₂O, 88 mg/kg Na₂SeO₃, 16% Dynamate (Pitman Moore, Inc., Mundelein, IL; 22% S, 18% K, and 11% Mg) and 120 mg/kg of ethylenediamine dihydroiodide.

(Agribrands International, St. Louis, MO). This commercial enzyme product contains high xylanase and cellulase activities and has been fully characterized previously (Bowman et al., 2002).

The appropriate amount of enzyme product was diluted into water, and then added at the time of milling. For the CONC treatment, the enzyme solution (93 g/20 L of water) was added slowly into a 1-tonne mixer containing steam-rolled barley and pelleted supplement. For the SUPP treatment, the diluted enzyme (93 g/15 L) was added to 250 kg of ingredients in the mixer before pelleting the supplement. Due to the small volumes that were required for the PREM treatment, diluted enzyme (4 g/16 ml) was added to 200 g of wheat

Table 2. Chemical composition of forages.

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

bran and mixed using a food processor. Enzyme-feed mixtures were prepared at the beginning of each period.

Cows were cared for according to the Canadian Council on Animal Care guidelines (Ottawa, ON). Animals were housed individually in tie stalls bedded with wood shavings. Animals were milked twice daily at 0700 and 1700 h in their stalls. Cows were exercised daily for 1 to 2 h. Cows were fed *ad libitum* intake and feed was offered three times daily. Daily feed allotment was distributed with approximately 25% offered at 0800 h, 30% at 1500 h and 45% at 1800 h.

The first 11 d of each period were for adaptation; d 19 was used to determine saliva produced while eating, d 21 to 23 to electronically measure rumen pH, feeding behavior and ruminating time, and d 28 for saliva produced while resting.

Feed offered and refused was measured and recorded daily. Feed and orts were sampled daily and composited weekly, the composite was dried in a fan forced oven at 55°C for 48 h to determine DM, which was used to calculate DMI. Barley silage and alfalfa silage was sampled weekly, and DM was determined to adjust diet composition when required.

Eating and Ruminating Saliva Production

A sufficient amount of rumen contents was removed from each animal to expose the cardial sphincter. The anterior sac was emptied to approximately the level of the anterior pillar. Removed digesta was held in closed plastic bags that were submerged in warm water to minimize any change in temperature. Once contents were removed, the cows were allowed to eat uninterrupted for 5 min before the start of collection. A plastic bag attached to a rigid hoop, similar to that used by Cassida and Stokes (1986), was placed over the cardial sphincter for 2 min for collection of eating boluses. Care was taken to minimize contact with the rumen wall or area around the cardial opening, as tactile stimulation is known to invoke an artificial increase in saliva production (Kay, 1966). A minimum of three samples was taken while the cow was eating. If fewer samples were obtained all samples for that cow were discarded and the collection was repeated at a later date. Animals were allowed to rest 5 min between each collection. Samples contaminated with ruminal fluid were discarded and repeated. Each sample collected during the 2-min interval was weighed and subsampled. Dry matter was determined by placing the sample in a fan forced oven at 55°C for 48 h.

The amount of saliva added to the masticated TMR was calculated as the difference in moisture of the masticated bolus and the feed offered:

$$\text{saliva (g)} = \text{weight of bolus (g)} - \text{weight of feed as fed (g)}.$$

In this case, the DM content of the feed was determined by sampling the TMR from the feeder just before masticates were collected. Ensalivation of feed was calculated as the amount of saliva added per gram of DM ingested. Salivation rate (ml/min) was calculated as the ratio of saliva obtained and duration (min) of the sample collection. The total amount of saliva secreted each day during eating was calculated as the eating salivation rate (ml/min) multiplied by the time (min) spent eating each day. A similar calculation was made for the total amount of saliva secreted each day during rumination. It was assumed that the salivation rate was similar for eating and ruminating (Bailey and Balch, 1961a; Seth et al., 1974).

Resting Saliva Production

Rumen contents were completely removed for measurement of resting saliva. Contents were handled in the same manner as for collections during eating. Animals were not handled for 5 min between rumen evacuation and the start of saliva collection. Feed and water were removed from each cow to prevent ingestion during the collection period. Plastic bags attached to rigid hoops were used to collect the saliva at the cardia. Each collection lasted 2 min with a 5-min rest period between each sample. Six samples were collected each period for each cow. The volume of saliva was measured immediately once the sample was taken. Care was taken to minimize contact with the rumen wall and cardial opening during the collection. The daily total saliva secreted during resting was calculated as the resting salivation rate (ml/min) multiplied by the time (min) spent resting each day.

Ruminal pH Measurements

Ruminal pH was monitored for 48 h using an industrial probe (model PHCN-37, OMEGA Engineering Inc., Stamford, CT) that had been modified for use in the rumen environment. The wire from the electrode to the pH meter was threaded through the rumen cannula plug and anchored at approximately 60 cm from the electrode, minimizing the opportunity for the electrode to relocate itself in the reticulum. Probes were weighted down to ensure submersion within the rumen contents; however, extent of their movement within the rumen was not determined. Thus, it should be recognized that these measurements of rumen pH might not correspond to manual measurements of pH taken from precise locations within the rumen. The probes were covered with

a mesh guard to prevent them from coming into direct contact with the rumen wall. Continuous measurements from the indwelling probe were sent to a datalogger (Campbell Scientific, Inc., Logan, UT) every 5 s and averaged every 15 min. The pH probes were calibrated using standard pH solutions 4 and 7 before insertion into the rumen; pH probes were removed and calibrated every 24 h thereafter. Ruminant pH data were summarized daily for each cow as mean pH, minimum pH, maximum pH, area under the curve, area between the curve and pH 5.8 or 5.5, time (h) under pH 5.8 or 5.5, and percentage of the day under pH 5.8 or 5.5. The area was calculated by adding the absolute value of negative deviations in pH from 5.5 or 5.8 for each 15-min interval. Rumen pH was monitored at the same time as chewing activity and feeding behavior. In the event of equipment malfunction, all corrupted data were deleted and measurements were repeated at a later time during the period.

Chewing Behavior

Animals were fitted with leather halters for 48 h that were equipped to measure jaw movements. Each halter contained a piezo disk (Edmund Scientific Company, Barrington, NJ), which was inserted within the halter and positioned under the jaw. Chewing action places stress on the disk generating an electrical signal, which is then processed and counted as a single jaw movement. A datalogger (Campbell Scientific, Inc.) was used to receive the output signal from each cow. The number of jaw movements was summed each minute and stored until retrieval. Cows were fitted with nylon halters 48 h before using the leather halters for data collection; this allowed animals time to adjust to the apparatus. In the event of electrical or mechanical damage, corrupted data were deleted and measurements were made at a later time. Total time spent chewing was calculated as the sum of time spent eating and time spent ruminating. Total time spent resting was calculated as 24 h minus total time spent chewing.

Meal Duration and Eating Behavior

Eating behavior was monitored for 48 h, during which chewing and ruminal pH was also monitored. Feed mangers were attached to load cells (OMEGA Engineering Inc.), which were connected to a computer. The load cells monitored feed weight continually and an average weight was obtained every 11 s and stored with Collect software (Labtronics, Inc., Guelph, ONT, Canada). A meal episode was defined as eating activity greater than 30 s and more than 300 g of feed being removed from the feeder. Meals within close proximity

had to be greater than 10 min apart to be considered separate and distinct meals. Rate of DMI was calculated as the ratio of DM ingested and duration of the episode. Eating behavior was further characterized by creating three feeding blocks (A, 1500 to 1800 h; B, 1800 to 0800 h; and C, 0800 to 1500 h) each one starting with one of the three daily feedings. The first and second meal of each feeding block was further characterized by DM ingested (kg), rate of DM ingested per meal (kg DM/min), and total time spent eating (min).

Statistical Analysis

Means were calculated for all variables by cow within period. Data were analyzed using the MIXED procedure of SAS (1999). Period and cow were considered random effects; parity and diet effects were considered fixed. Estimation method was restricted maximum likelihood and the degrees of freedom method was Kenward-Roger (SAS, 1999). Differences were declared significant at $P < 0.05$; trends were discussed at $P < 0.15$, unless stated otherwise. Contrasts were used to test for differences between control and combined enzyme effects. Because no interactions occurred between parity and diet, only the main effects are reported.

RESULTS

Chewing Activities and Eating Behavior

Chewing behavior variables were not significantly affected by enzyme treatment (Table 3), except for the number of ruminating episodes, which was significantly reduced for cows receiving SUPP and PREM treatments compared with cows receiving the CTRL and CONC treatments. Enzyme supplementation did not alter DMI, the amount of DM consumed within a feeding episode, rate of feed intake or duration of a feeding episode (Table 4). However, a trend ($P < 0.06$) for lower total feed consumption within block C (0800 to 1500 h) was observed for SUPP and CONC compared with CTRL and PREM treatments.

Despite greater DMI of multiparous cows compared with primiparous cows the number of episodes during which feed was consumed was similar for both parity groups; ($x \pm SD$) 13.5 ± 1.5 (Table 3). Furthermore, multiparous cows spent the same amount of time eating as primiparous cows.

In contrast, multiparous cows spent a greater portion of the day ruminating ($P = 0.09$) and the duration of a single ruminating episode was longer than with primiparous cows (Table 3). A significant difference was observed between parities for the rate at which feed was ingested (Table 4). Primiparous cows consumed feed more slowly than multiparous cows. Multiparous cows

Table 3. Chewing behavior profiles of multiparous and primiparous lactating cows receiving an enzyme supplement.

Item	Enzyme ¹					Parity ²		
	CTRL	CONC	SUPP	PREM	SE	MP	PP	SE
Eating								
DMI, kg/d	21.8	21.9	21.0	22.2	0.5	23.0 ^a	20.5 ^b	0.5
Number of episodes	13.6	13.9	12.8	13.6	0.6	13.5	13.4	0.4
Time, min/d	339	326	336	339	13	323	347	14
Ruminating								
Chewing rate, chews/min	68.1	67.4	68.0	66.6	1.6	69.1	65.9	1.9
Number of episodes	14.6 ^a	14.9 ^a	13.8 ^b	13.8 ^b	0.5	14.1	14.4	0.6
Time, min/d**	529	530	514	534	18	556	497	21
Time, min/kg DM	24.4	24.2	24.6	24.1	1.0	24.3	24.3	1.2
Time, min/episode	37.5	36.7	38.2	39.1	1.4	40.5 ^b	35.2 ^a	1.6
Chewing								
Total, min/d	868	855	850	873	21	879	844	21
Resting								
Time, min/d	572	585	590	567	21	561	596	21

^{a,b}Enzyme or parity means in the same row with different superscripts differ ($P < 0.05$).

**Parity means in the same row differ ($P = 0.09$).

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

²MP = Multiparous, PP = primiparous.

also tended to ingest larger amounts of feed each episode ($P = 0.10$) than did primiparous cows, and the largest differences occurred within block B (1800 to 0800 h; $P = 0.07$). Differences for the first two meals within feeding block B account for over 76% of the total difference in DMI between parities (Figure 1). Multiparous cows had a greater ability to consume feed within a given meal than did primiparous cows. Significant differences in intake rate between parities were evident in the first meal in block B, 83 and 67 g of DM/min, and block C 83 and 57 g of DM/min, for multiparous and primiparous cows, respectively. Both parity groups exhibited variation in eating rate throughout the day, with the first meal generally having the highest rate

of intake. The duration of a single meal did not differ between parities, with the first meal being the longest meal within a block and subsequent meals being shorter.

Eating and Resting Salivation

There was a numerical increase in eating salivation rate ($P = 0.07$) with enzyme supplementation compared with the CTRL (Table 5). Total daily saliva production was 5.7 to 16.5% higher for cows receiving enzyme treatments compared with CTRL ($P = 0.03$; Table 6).

Salivation rates (ml/min) while eating ($P = 0.15$) and resting ($P = 0.10$) tended to be higher for multiparous

Table 4. Eating behavior profiles of multiparous and primiparous lactating cows receiving an enzyme supplement.

Item	Enzyme ¹					Parity ²		
	CTRL	CONC	SUPP	PREM	SE	MP	PP	SE
DMI, kg								
A block, (1500 to 1800 h)	6.39	6.45	6.46	6.40	0.42	6.62	6.23	0.42
B block, (1800 to 0800 h)**	9.49	9.75	9.13	9.73	9.51	10.46	8.59	0.64
C block, (0800 to 1500 h)	5.89	5.75	5.38	6.12	0.24	5.90	5.67	0.26
DMI, kg/episode**	1.65	1.62	1.69	1.69	0.08	1.76	1.57	0.07
Intake rate, g DM/min	65.1	68.1	63.0	66.3	2.7	71.9 ^a	59.4 ^b	2.9
Time, min/episode	25.87	24.07	27.12	25.83	1.70	24.80	26.65	1.62

^{a,b}Parity means in the same row with different subscripts differ ($P < 0.05$).

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

²MP = Multiparous, PP = primiparous.

**Parity means in the same row differ ($P < 0.10$).

Figure 1. Dry matter intake, intake rate, and meal duration for multiparous (MP) and primiparous (PP) cows for individual daily meals (A = 1500 to 1800 h, B = 1800 to 0800 h, C = 0800 to 1500 h, 1 = first meal, 2 = second meal).

cows than for primiparous cows (Table 5). Consequently, total daily saliva output was higher ($P = 0.05$) for multiparous cows compared with primiparous cows (Table 6). Much of the increase in total saliva production for multiparous cows was due to higher ($P = 0.09$) saliva production during ruminating. Masticate DM content and ensalivation of feed were not affected by parity.

Ruminal pH Measurements

Ruminal pH measurements were lower than expected across all diets (Table 7). Mean pH averaged 5.62 and minimum pH averaged 5.19, with no effect of enzyme treatment. However, a significant enzyme effect was observed for area under pH 5.5. Cows receiving the CONC treatment had the largest area under pH 5.5 ($\text{pH} \times \text{h/d}$), which was significantly greater than for SUPP and PREM. However, area under pH 5.5 for the enzyme treatments did not differ from the CTRL treatment. The rumen pH of cows on this study was under 5.8 for a substantial part of the day, approximately 17 h.

None of the pH measurements was affected by parity (Table 7). However, all pH variables indicated a higher risk of acidosis for multiparous cows than for primiparous cows. Multiparous cows spent almost 50% of the day with the ruminal pH below 5.5 compared with 38% for primiparous cows (Figure 2).

DISCUSSION

Maximizing feed intake of dairy cows is necessary to maintain high levels of milk production. In this study, DMI was maintained at a high level throughout the experiment across all enzyme treatments, indicating that this enzyme product did not alter palatability of the feed. Unfamiliar factors such as smell and taste may lead to reductions in DMI (Mertens, 1996), which is undesirable. Inspection of individual meal blocks and DMI during individual feeding events further indicates that enzyme supplementation did not alter feeding behavior any time of the day.

In a study using a different enzyme product, time spent eating per unit of NDF and ADF were decreased (Beauchemin et al., 2000). This change was attributed to pre-ingestive effects of the enzyme product upon the feed, indicating that enzyme application to feed may have altered the fragility of the feed and, subsequently, its ability to promote chewing. A similar trend was not seen in this study, indicating that any possible alterations to the feed before ingestion were not manifested in the physical effectiveness of the TMR to stimulate chewing. The total daily chewing time was over 850 min for all treatments and given an upper physiological limit of 1000 min/d (Mertens, 1997), sufficient physical

Table 5. Eating and resting salivation parameters for primiparous and multiparous lactating cows receiving an enzyme supplement.

Item	Enzyme ¹					Parity ²		
	CTRL	CONC	SUPP	PREM	SE	MP	PP	SE
Eating								
Masticate DM, %	19.8	19.4	18.3	18.9	0.9	18.2	20.0	1.2
Salivation rate, ml/min ^{a,**}	207	248	250	217	16	247	214	15
Ensalivation of feed, ml/g DM	3.30	3.48	3.79	3.62	0.29	3.76	3.33	0.34
Resting								
Salivation rate, ml/min*	138	145	156	154	9	160	136	9

^aContrast comparison CTRL versus CONC, SUPP, PREM ($P = 0.07$).

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

²MP = Multiparous, PP = primiparous.

*Parity means in the same row differ ($P = 0.10$).

**Parity means in the same row differ ($P = 0.15$).

effectiveness for chewing was likely provided by the TMR.

A significant difference in DMI between parity groups was observed, as was previously reported by Dado and Allen (1994). It has been well established in herbivores that there is a strong relationship between BW and gastrointestinal capacity (Van Soest, 1994). Body weight was reported to be the most important factor explaining variation in DMI of first lactation cows (Kertz et al., 1991). Parity differences in DMI attributed to differences in BW are thought to be due to increased rumen fill of primiparous cows (Dado and Allen, 1994). However, in the present study BW was similar between the two parity groups, as was rumen volume (data not shown). Differences in DMI between multiparous and primiparous cows may be due to the differences in energy demands for lactation. The review by Forbes (1996) indicates that DMI is controlled by numerous inter-

acting factors including physical factors in the feed that limit intake (Allen, 1996), as well as the absorption of nutrients that result from digestive processes (Illius and Jessop, 1996). The likelihood of differences between DMI of parities being manifested simply in differences in rumen volume or milk production is remote; rather these two factors likely act together.

Multiparous cows were more efficient while chewing than primiparous cows, because they spent a similar amount of time chewing even though they consumed 2.5 kg/d of DM more. Both groups were of similar BW and had similar rumen volumes (data not shown). Using a variety of ruminant species varying in size, Bae et al. (1983) reported that chewing efficiency increased as body size increased. Using animals similar to this study, Dado and Allen (1994) found multiparous cows to be more efficient during chewing than primiparous cows. Multiparous cows increased the duration of a sin-

Table 6. Total daily saliva output for primiparous and multiparous lactating cows receiving and enzyme supplement.

Saliva	Enzyme ¹					Parity ²		
	CTRL	CONC	SUPP	PREM	SE	MP	PP	SE
Eating, L/d	70	81	84	72	6	80	74	6
Ruminating, L/d**	111	132	129	117	10	137	107	10
Resting, L/d	79	85	92	87	6	90	81	6
Total ^{a,*}	261	298	304	276	14	307	263	13
Eating, % of total	26.9	27.1	27.5	26.2	1.2	25.7	28.1	1.2
Ruminating, % of total	42.1	44.0	41.8	41.6	2.0	44.4	40.4	2.4
Resting, % of total	31.1	28.9	30.6	32.2	2.7	29.9	31.5	2.9

^aContrast comparison CTRL versus CONC, SUPP, PREM ($P = 0.03$).

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

²MP = Multiparous, PP = primiparous.

*Parity means in the same row differ ($P = 0.05$).

**Parity means in the same row differ ($P = 0.09$).

Table 7. Ruminal pH profiles for primiparous and multiparous lactating cows receiving an enzyme supplement.

pH variables	Enzyme ¹					Parity ²		
	CTRL	CONC	SUPP	PREM	SE	MP	PP	SE
Mean pH	5.60	5.55	5.67	5.68	0.06	5.58	5.67	0.07
Min pH	5.18	5.14	5.22	5.24	0.03	5.17	5.22	0.03
Max pH	6.33	6.18	6.33	6.33	0.08	6.29	6.29	0.06
Area under curve, pH $\times$ h/d	133	132	135	135	2	135	132	2
Area under pH 5.5, pH $\times$ h/d	2.1 ^{ab}	2.7 ^a	1.4 ^b	1.3 ^b	0.5	2.3	1.5	0.4
Percent of day under 5.5, %	46.4	53.5	38.1	36.8	7.6	49.5	37.9	8.2
Area under pH 5.8, pH $\times$ h/d	6.5	7.5	5.4	5.0	0.9	6.8	5.4	1.0
Percent of day under 5.8, %	72.1	75.5	69.5	66.1	7.2	73.5	68.1	8.7

^{a,b}Enzyme treatment means in the same row with different superscripts differ ($P < 0.05$).

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

²MP = Multiparous, PP = primiparous.

gle rumination episode rather than increase the number of ruminating episodes in order to process the additional feed ingested, which is in agreement with others (Dado and Allen, 1994).

Any increase in daily feed intake must be the result of changes in the number of daily meals or feed intake per meal (Nielsen, 1999). Dado and Allen (1994) found that the number of eating episodes were similar for parity groups (average 11.0 per day) and that multiparous cows consumed 0.7 kg of DM more each meal than did primiparous cow, although this difference was not significant. The number of daily feeding episodes in the current study was not different between multiparous and primiparous cows and multiparous cows consumed 0.2 kg of DM more than primiparous cows each episode. In the study by Dado and Allen (1994) feeding rate for multiparous cows was over 80 g of DM/min compared with 69.5 g of DM/min for primiparous cows. Feeding rates for the current study also show a difference be-

tween parity groups; multiparous cows ingested 12.5 g DM more per minute than primiparous cows.

Although faster eating rates have been established for multiparous cows (Burt, 1957; Dado and Allen, 1994; Friggens et al., 1998), eating rate is not constant within a meal (Beauchemin, 1991), nor is it constant throughout the day (Gill and Romney, 1994). The first meal after feed is offered is generally the largest meal (Gill and Romney, 1994). Inspection of the first two feeding episodes after fresh feed was offered showed that duration and intake were generally highest for the first meal within a feeding block for both primiparous and multiparous cows. Multiparous cows significantly increased their eating rate during the first meal in blocks B and C, but not block A. Blocks B and C were the first meals after milking, and it may be that the increased eating rate by multiparous cows was related to this activity. Despite the faster eating rate of multiparous cows, the only individual meal that was significantly larger than for primiparous cows was the first meal in block B. The higher intake of multiparous cows in block B may indicate that the physical limitation posed by the feed consumed in block A had less of a filling effect for multiparous cows than for primiparous cows.

The rate of salivation during eating has been estimated at 200 to 300 ml/min (Bailey, 1961), which is in accordance with the mean value of 217 to 250 ml/min observed in the present study. Saliva is continuously secreted during periods of rest at a rate of 50 to 100 ml/min (Bailey and Balch, 1961b). Using a steer with the right parotid gland cannulated, resting salivation rate fluctuated from 5 ml/min after a meal up to 20 ml/min before the start of the next meal (Bailey and Balch, 1961a); however, others have found that the salivation rate while resting is relatively constant (Yarns et al., 1965). Recognizing that salivary secretions are influ-

Figure 2. Ruminal pH measurements for multiparous (x) and primiparous (o) lactating dairy cows obtained using indwelling probes.

enced by feed intake (Beauchemin, 1991; Putnam et al., 1966), Cassida and Stokes (1986) investigated eating and resting salivation in lactating dairy cows and found eating salivation rates were 166 to 188 ml/min and resting salivation rates ranged from 130 to 173 ml/min, depending on stage of lactation. The resting salivation rates of 138 to 156 ml/min for the present study are similar to others using lactating dairy cows (Cassida and Stokes, 1986), but slightly higher than those of Maekawa et al. (2002) who reported resting salivation rates of 114 and 88 ml/min for multiparous and primiparous cows, respectively.

Total daily saliva output has been established at between 98 to 190 L (Bailey, 1961), accounting for 90% of the fluid added to the rumen daily (Bailey, 1961). Early values were obtained from beef or nonlactating dairy cows, creating a void in the literature for high producing dairy cows. Estimates for daily saliva output for multiparous lactating dairy cows range from 252 (Maekawa et al., 2002) to 308 L/d (Cassida and Stokes, 1986), which compare to total daily saliva output for multiparous cows (307 L/d) from the current study.

The tendency for increased eating salivation rate and total daily saliva output when rations were supplemented with a fibrolytic enzyme product was unexpected. Cows receiving CONC and SUPP supplements had the highest eating salivation rate and daily saliva output. These two treatments also had the highest OM digestibility in the total tract (Bowman et al., 2002). The increase in salivation rate may have been a physiological response to offset the increase in digestion products.

Few studies have compared the differences in daily saliva output between multiparous and primiparous cows. Maekawa et al. (2002) reported that multiparous cows produced more saliva daily during eating, ruminating, and resting, resulting in 25 L/d more saliva. In the current study, multiparous cows showed a tendency to have higher salivation rate during both eating and resting, and this resulted in higher total daily saliva output than for primiparous cows. The higher total saliva output observed for multiparous cows may be attributed to their higher level of intake resulting in a higher buffering requirement due to greater ruminal fermentation.

Lewis et al. (1996) reported a decrease in ruminal pH for beef steers consuming a forage-based ration supplemented with enzymes. Animals fed a ration composed of primarily barley grain also exhibited a reduction in ruminal pH when the diet was supplemented with an enzyme product (Hristov et al., 2000). Studies involving lactating dairy cows reported no difference in ruminal pH when cows were fed a diet containing feed enzymes (Yang et al., 1999; Beauchemin et al., 2000),

confirming the findings of this study. All diets in this study produced a mean ruminal pH that was considerably lower than the expected value of 5.9 to 6.1, but there was no effect of enzyme treatment on ruminal pH. Cows receiving CTRL and CONC treatments spent a considerable amount of the day below 5.8, which can be characterized as subclinical acidosis.

The low ruminal pH is not likely to be attributed to methodological errors, nor is it likely a malfunction of the monitoring equipment. Ruminal pH measurements using continuous indwelling electrodes have been reported to be 0.17 units lower than values obtained by manually sampling (Dado and Allen, 1993). This difference has been attributed to elevated pH values from manually sampling due to loss of CO₂ (Smith, 1941). The higher rate of fermentation of barley grain compared with other grain sources may be attributed to the depressed rumen pH. Yang et al. (1997) reported a lower ruminal pH for diets containing barley grain than those utilizing corn grain. However, the pH values observed in the present study were lower than those previously reported for dairy cows fed diets containing barley grain and of similar forage particle size and grain processing index (Yang et al., 2000b). Low-fiber diets and a reduced forage and TMR particle size have been attributed to depressed ruminal pH (Mertens, 1997). In the present study, the percentage of material remaining on both sieves of the Penn State Particle Separator (Lammers et al., 1996) was 56, 70, and 40% for the alfalfa haylage, barley silage and TMR, respectively. This indicated that the alfalfa haylage was processed finer than expected and that the TMR was on the lower limits of recommended particle size (Yang et al., 2001). The nonfiber carbohydrate (NFC) content of the diet was high at 42%; however, the NDF content of 30% with 20% NDF from forage sources exceeds the recommended concentrations of fiber for that level of NFC (NRC, 2001). Thus, it is likely that the reduced particle size of the diet was the main reason for lower than expected rumen pH.

A reduction in ruminal pH can have a negative impact on fiber digestion (Erdman, 1988; Russell and Wilson, 1996). A variety of in vitro studies have shown that the major cellulolytic bacteria species are unable to digest cellulose at a pH below 6.2 (Russell and Wilson, 1996). Cellulose digestion measured in sacco was virtually stopped at a ruminal pH of 6.1 in sheep when rumen contents were artificially lowered using mineral acids (Mould and Orskov, 1983). These studies give clear results on the effects of low pH for pure cultures; however, mixed cultures do not respond like pure cultures and ruminal pH fluctuates throughout the day. An in vitro study using mixed cultures held pH constant at two levels and then allowed drops in pH for short or long

periods of time, and results indicated that mixed cultures are able to withstand periods of low pH without greatly affecting fiber digestion (Calsamiglia et al., 1999). Although not significantly different from the control diet, cows receiving CONC supplements in the current study had the lowest ruminal pH. The cows receiving the CONC supplement also had the highest total tract fiber digestibility across all treatments (Bowman et al., 2002). The low ruminal pH was likely due to increased ruminal fermentation and rates of digestion. It has been suggested that fibrolytic enzyme supplementation may aid in minimizing the effect that pH has on fiber digestion (Lewis et al., 1996).

Although no parity effect for ruminal pH was evident from the variables measured, multiparous cows may be at an increased risk of subclinical acidosis. Their higher DMI leads to increased VFA production, and there appear to be times throughout the day that the acid load in the rumen is larger for multiparous cows compared with primiparous cows (Figure 2). There is large individual cow variation when measuring ruminal pH, thus requiring a substantial number of animals to obtain significant differences. More work is required to determine the effects such differences in rumen pH may cause.

CONCLUSION

Enzyme supplementation did not alter the feeding, chewing, or ruminating behavior of multiparous or primiparous cows. Therefore, the effectiveness of the diet to promote chewing was not reduced due to enzyme supplementation. Furthermore, salivation increased with enzyme supplementation. Despite the effects on digestibility, enzyme supplementation and method of application had no effect on ruminal pH measurements when compared to control.

Time spent eating was similar for multiparous and primiparous cows, but multiparous cows consumed feed faster, thus DMI was higher. Multiparous cows ruminated for a greater portion of the day, leading to an increase in the total daily saliva produced. Despite higher saliva production, ruminal pH was lower for multiparous cows, indicating that the higher saliva production did not fully compensate for the greater need for buffer in the rumen. Under the conditions of this experiment, multiparous cows were at a higher risk for ruminal acidosis than primiparous cows.

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