DIGESTION, RUMINAL PH, SALIVATION, AND FEEDING BEHAVIOR OF LACTATING DAIRY COWS FED A DIET SUPPLEMENTED WITH FIBROLYTIC ENZYMES

by

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Abstract

The objectives were to determine the effects of supplementing different components of the diet with enzymes on dry matter intake, ruminal fermentation, total tract digestion, milk production, and effective fiber. Eight multiparous and four primiparous lactating Holstein cows were used in a replicated 4 x 4 Latin square design to investigate the effects of supplementing a diet with a fibrolytic enzyme product containing mainly xylanase and cellulase activities. A total mixed ration (TMR), consisting of rolled barley, barley silage and alfalfa haylage (forage to concentrate ratio of 55:45, dry matter (DM) basis) differed in enzyme application: 1) no enzyme, 2) enzyme applied to concentrate (45% of TMR), 3) enzyme applied to supplement (4% of TMR), and 4) enzyme applied to premix (0.2% of TMR). Application rate was constant for enzyme treatments at 1g/head/day.

Digestibility of DM measured using multiparous cows increased by 12% compared to the control when enzyme was added to the concentrate. Enzyme treatments that were applied to a smaller component of the TMR did not increase digestibility compared to the control. There were no significant effects of enzyme supplementation on milk production and composition. However, cows fed concentrate supplemented with enzyme produced significantly more milk than cows receiving the enzyme applied to the premix.

Enzyme supplementation did not alter daily time spent eating or ruminating, indicating that this fibrolytic enzyme product does not alter the physical structure of the feed. However, when enzymes were added to the ration daily saliva production increased, which may have been a physiological response to the increase in fermentation products due to increased digestion. Enzyme supplementation did not change the mean ruminal pH or the

amount of time pH was below 5.5, which was attributed to increased saliva production. The higher DM intake of multiparous cows was manifested in a faster eating rate, and resulted in more rumination time and greater saliva secretion.

These results indicate that enzyme supplementation increases digestibility, and consequently improves the energy status of the cow. The component of the diet to which the enzyme is applied must be maximized to ensure a beneficial response.

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List of Abbreviations

ADF	acid detergent fiber
BW	body weight
CMC	carboxymethyl cellulose
Co	cobalt
CONC	enzyme added to the concentrate, 45% of the dietary dry matter
CP	crude protein
Cr	chromium
CTRL	control, no enzyme applied
DDM	digestible dry matter
DDMI	digestible dry matter intake
DIM	days in milk
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
DNDF	digestible neutral detergent fiber
eNDF	effective neutral detergent fiber
FCM	fat corrected milk
INDF	indigestible neutral detergent fiber
MP	multiparous cows
N	nitrogen
NDF	neutral detergent fiber

NFC	nonfiber carbohydrates
NRC	National Research Council
OM	organic matter
PD	purine derivatives
pef	physical effectiveness factor
peNDF	physically effective neutral detergent fiber
PP	primiparous cows
PREM	enzyme added to the premix, 0.2% of the dietary dry matter
SAS	Statistical Analysis Systems
SUPP	enzyme added to the supplement, 4% of the dietary dry matter
TDN	total digestible nutrients
TMR	total mixed ration
VFA	volatile fatty acids
Yb	ytterbium

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1.0 INTRODUCTION

Forages generally comprise between 40 to 60% of the ration fed to lactating dairy cows in North America. Due to the energy requirements for lactation and the cost associated with growing or purchasing forages there is an increasing trend to feed concentrates. However, unlike concentrates, forages supply structural carbohydrates, which are required to optimize ruminal fermentation, maintain milk fat, and provide physical structure to stimulate chewing and rumination (Mertens, 1997). They also provide a valuable source of energy when digested by the rumen microbes. Due to the higher dry matter intake (DMI) of lactating dairy cows compared to cows fed at maintenance, a greater portion of the potentially digestible nutrients pass through the digestive tract of the lactating cow undigested (Tyrrell and Moe, 1975), leading to inefficiencies and increasing costs. Over the past 10 years, there has been a research initiative directed at using fibrolytic enzyme products to increase feed digestion, and consequently milk production of lactating dairy cows. However, the literature is replete with inconsistency.

The concept of supplementing cattle rations with enzymes is not new. While interest in feed enzymes has increased over the past decade, the possible benefits of using feed enzymes in ruminant diets was first examined in the '60s. Burroughs et al. (1960) was one of the first to show that supplemental enzymes could enhance average daily gain and improve feed conversion of growing beef cattle. However, later studies were unable to reproduce the results; Perry et al. (1966) reported a 6% increase in average daily gain with one enzyme product and an 18% decrease in daily gain using a different enzyme product. Leatherwood et al. (1960) provided an enzyme product in the grain component of the diet and reported no

change in weight gain, however when the enzyme was offered in a capsule weight gain was decreased by 8%. Therefore, earlier studies found that enzymes did have the potential to increase the productivity of ruminants, however the enzyme products were often poorly characterized and methods used to deliver the enzyme products was not always described. It appears that the concept of using feed enzymes in ruminant diets was abandoned until recently due to the variability in response, the high cost of enzymes, and the potential benefits of other new technologies, such as ionophores. These factors make direct comparisons between recent work and earlier studies difficult.

It appears that variability in response continues to limit progress in the field of ruminant feed enzymes. A summary of studies over the past 7 years displays the variability of the response of dairy cows to supplemental feed enzymes (Table 1.1). The average increase in DMI was 1.1 ± 0.8 kg/d, the average increase in milk production was 1.5 ± 1.8 kg/d, and the average increase in digestion was 3.7 ± 3.5% for the 15 studies. Although the majority of these studies report that the main enzyme activities were that of xylanases and cellulases, minor activities can vary substantially because sources of the enzymes are not necessarily the same for any of the products. Not only do these studies differ in the enzyme product used, but also in experimental conditions, diet composition, stage of lactation, and energy status of the cows used in the studies.

Utilizing cows in midlaction, Lewis et al. (1999) reported a 5% increase in milk production, which was accompanied by a 9% increase in DMI. Although dry matter digestibility was decreased by 2%, the amount of DM digested daily was increased by 1.1 kg/d (7%) (Lewis et al., 1999). A second trial, with cows in early lactation confirmed that DMI may increase with enzyme supplementation and that level of supplementation must also

be considered due to the quadratic response in milk production (Lewis et al., 1999). However, Nussio et al. (1997) reported an increase in DMI without an increase in milk production for cows in midlaction and only a trend in increased milk production for cows in early lactation. A summary of these studies demonstrates the necessity to consider the rate of application and the energy balance of the cows receiving the supplement.

Enzyme supplementation did not always increase DMI in studies in which a milk production response was observed. Cows increased milk production by 8% when receiving alfalfa cubes treated with an enzyme supplement (Yang et al., 1999) and increased milk production by 6% when an enzyme product was applied to the concentrate portion of the diet (Yang et al., 2000), without an increase in DMI. Enzymes were effective when applied to either the concentrate or the forage component of the diet. Kung et al. (2000) reported a 7% and 8% increase in milk production depending on the enzyme blend without an increase in DMI, which confirmed previous reports.

The use of fibrolytic enzymes may increase the energy that is available to the cow when a high forage diet is fed. Cows that received a diet consisting of a 55:45 forage to concentrate ratio supplemented with an enzyme product were able to maintain a similar level of production as cows receiving a diet consisting of a 45:55 forage to concentrate ratio without enzyme supplementation (Schingoethe et al., 1999). While the economic implication may imply that feeding of concentrates is a less expensive energy source and easier to handle, the inclusion of a larger proportion of forage in the diet has long-term health benefits to the cow.

Positive responses to enzyme supplementation have occurred when a product was sprayed daily on the forage (Kung et al., 2000) or applied to the concentrate at the time of

manufacturing (Yang et al., 2000). However, when exogenous enzymes were infused directly into the rumen no improvement was observed, even though increases in fibrolytic activities were observed (Hristov et al., 1998a; Hristov et al., 2000). Thus, applying the enzyme product to a component of the feed is necessary, however daily application and application on a large component of the diet is labor intensive and difficult to implement at the feed mill and on the farm. Studies applying enzyme products on small components of the diet are of commercial interest, however effects of applying enzymes to different components are not currently available.

Supplemental feed enzymes have been shown to increase the rate at which feed, especially the slowly degradable fiber portion, is digested in the rumen (Yang et al., 1999; Bowman et al., 2001). Increasing the rate of fermentation creates a higher amount of energy per unit time available to the rumen microbes allowing for their increased growth rate (Sniffen et al., 1983; Hoover and Stokes, 1991). However, this increase in fermentation may also increase the risk of acidosis in dairy cows. Furthermore, applying enzymes directly on the feed may alter the ability of the feed to stimulate chewing, thereby reducing the fiber effectiveness. This reduced effectiveness of the fiber fraction would further increase the risk of acidosis.

It has been accepted that to maintain milk fat percentage a sufficient percentage of the diet is required to be of adequate length to stimulate chewing. Chewing is the primary mode of particle size reduction, which along with ruminal motility and specific gravity of particles, contribute to the rate of passage of digesta from the rumen to the abomasum (Beauchemin, 1991). Dairy cows may chew up to 16 hours in a single day (Mertens, 1997). However, to meet the energy requirements during lactation, concentrates and finely chopped forages are

often used. These ingredients decrease the physical structure of the diet and decrease the total chewing time. Various attempts have been made to quantify the structural fiber requirements of dairy cows. One of the first models proposed was effective NDF (eNDF), which describes the ability of the feed to replace forage such that milk fat percentage remains unchanged. Effective NDF is positively associated with rumen pH (Pitt et al., 1996). Mertens (1997) proposed another model, which describes the physical characteristics of the feed in terms of their ability to stimulate chewing. With this system feed that stimulates maximum chewing receives a physical effectiveness factor (pef) of 1. As chewing time decreases the pef of the feed also decreases. Subsequently, Mertens (1997) proposed using the proportion of feed retained on a 1.18 mm sieve during dry sieving as a means of determining pef because this requires less work than conducting an experiment to measure chewing time. Multipling the pef value by NDF content results in a value that takes into account both the physical structure and the chemical component of the feed. This value has been termed physically effective NDF (peNDF) (Mertens, 1997). While these models are helpful in stimulating further research and discussion on particle size, chewing, and the links to ruminal pH, the models have not been sufficiently validated (NRC, 2001).

Efficiency of chewing and ruminating has been negatively correlated to body size (Bae, 1983). Although eNDF has been used to estimate rumen pH (CPMDairy Version 1.0 and CNCPS Version 4.0), it is generally recognized that this approach is flawed because of the failure to account for differences in rate and extent of ruminal fermentation. Diets in Western Canada are often comprised primarily of barley, which has a faster rate of digestion and depresses rumen pH to a greater extent than corn-based diets (Yang, 1997). There is a

need to characterize feeds that are used in Western Canada and their differing effects upon rumen pH and their ability to effectively stimulate chewing.

Saliva excretion is incessant throughout the day, however salivation rates during chewing episodes are elevated in comparison to times when resting (Bailey, 1961). Saliva is essential in maintaining the buffering capacity and fluidity within the rumen. Few studies have considered salivary secretions in lactating dairy cows and potential parity differences; early studies utilized non-lactating cows or beef cows, which produce less saliva (Bailey and Balch, 1961a; Bailey and Balch, 1961b). There are no studies that have considered the effects of enzyme supplementation on saliva production.

Ruminal pH may be an effective measurement of overall ruminal health and function. Ruminal pH, unlike blood pH, generally fluctuates between 5.2 and 6.8 depending on various factors such as diet and metabolic status. Lactating dairy cows are generally not subjected to clinical acidosis, which has been associated with high concentrate rations fed to feedlot animals leading to a build up of lactic acid and potentially death. Rather, lactating cows may experience reduced ruminal pH for periods during the day, resulting in reduced feed intake, decreased production, decreased digestion, laminitis, and rumen dysfunction (Nocek, 1997). Ruminal pH is a function of the concentration of fermentable carbohydrates in the feed and the rate at which those carbohydrates are digested into volatile fatty acids (VFA). If absorption of VFA across the rumen wall is rapid then build up of VFA concentration in the rumen will not occur and pH remains stable. However, excessive accumulation of VFA leads to a drop in rumen pH (Figure 1.1). Since ruminal pH is closely associated with the fermentation of the diet, an increase in fermentation rate due to inclusion of an enzyme product may depress rumen pH. Furthermore, if fibrolytic enzymes have a pre-ingestive

effect on the diet that alters the physical structure of the diet, then enzymes may reduce the physical effectiveness of the diet, which could depress chewing time. This could lead to subsequent depressions in saliva production and an increased risk of acidosis (Figure 1.1). Thus, due to the interrelatedness of chewing, saliva production, and rumen buffering capacity, differences manifested in any one of these components due to enzyme inclusion in the ration may increase the risk of subclinical acidosis.

1.1 OBJECTIVES

The objectives of this study were to 1) determine the effects of supplementing diets with fibrolytic feed enzymes on dry matter intake, ruminal fermentation, total tract digestion, and milk production of lactating dairy cows, 2) determine whether the portion of the diet treated with fibrolytic enzymes affects the response to fibrolytic enzymes, 3) determine whether supplemental fibrolytic enzymes change the effective fiber content of the diet, and 4) determine if parity of cows affects feeding behavior in a noncompetitive environment.

Table 1.1 Dry matter intake (DMI), milk yield, and dry matter digestibility response to various fibrolytic feed enzyme supplements.

Source	Enzyme Characterized	Diet Components	Component Applied and level	DMI Response (kg/d)	Milk Response (kg/d)	Digestion Response (%)
Beauchemin et al., 1999	cellulase, xylanase	barley grain or hulless barley, BS[1], AHL[2]	TMR	-0.7 0.7	0.3 1.5	6.0 6.3
Beauchemin et al., 2000	β-glucanase, xylanase, endocellulase	barley grain, BS, AHL	concentrate (high level) (low level)	 1.2 1.5	 -0.5 -0.5	 0.0 4.0
Higginbotham et al., 1996	cellulase, xylanase	barley grain, corn grain, AH[3], CS[4]	TMR	ND	DIM[6]<40 0.9 DIM>40 0.2	overall 0.9
Kung et al., 2000 (yr 1)	cellulase, xylanase	CS, AH, cornmeal	forages, EA2 EA5	 0.5 -0.2	 2.5 -0.8	 ND[5]
Kung et al., 2000 (yr 2)	cellulase, xylanase	CS, AH, cornmeal	forages EA2 EB1.2	 0.9 0.9	 0.7 2.5	 ND
Lewis et al., 1999 (trial 1)	cellulase, xylanase	barley grain, AH, AHL	forage	1.9	1.3	-1.8
Lewis et al., 1999 (trial 2)	cellulase, xylanase	barley grain, corn grain AH, AHL	forage, low medium high	 1.8 1.8 2.2	 1.2 6.3 1.6	 ND
Nussio et al, 1997	cellulase, xylanase	barley grain, AH	forage, low medium high	 1.5 1.9 2.4	 -2.1 -0.3 0.5	 ND
Rode et al., 1999	cellulase, xylanase	barley grain, CS, AH	concentrate	0.3	3.6	12.0

Table 1.1 (cont).

Source	Enzyme Characterized	Diet Components	Component Applied and level	DMI Response (kg/d)	Milk Response (kg/d)	Digestion Response (%)
Schingoethe et al., 1999	cellulase, xylanase	corn grain, CS, AH	forage,			
			low	0.8	1.1	ND
			medium	-0.3	0.9	
			high	1.7	2.7	
Stokes and Zheng, 1995	cellulase, cellobiase, xylanase	barley grain, hay silage, AH	forage	2.0	4.2	ND
Yang et al., 1999	cellulase, xylanase	barley grain, AH, BS	forage,			OMD[7]
			low	0.3	0.9	2.3
			high	0.3	1.9	4.0
			TMR, high	0.4	1.6	3.3
Yang et al., 2000	cellulase, xylanase	barley grain, AH, CS	TMR	1.0	-0.1	2.8
			concentrate	0.4	2.1	4.2
Zheng and Stokes, 1997	cellulase, cellobiase, xylanase	silage, hay, concentrate	forage	1	2.1	ND

[1] BS = barley silage
[2] AHL = alfalfa silage
[3] AH = alfalfa hay
[4] CS = corn silage
[5] ND = not determined
[6] DIM = days in milk
[7] OMD = organic matter digestibility response

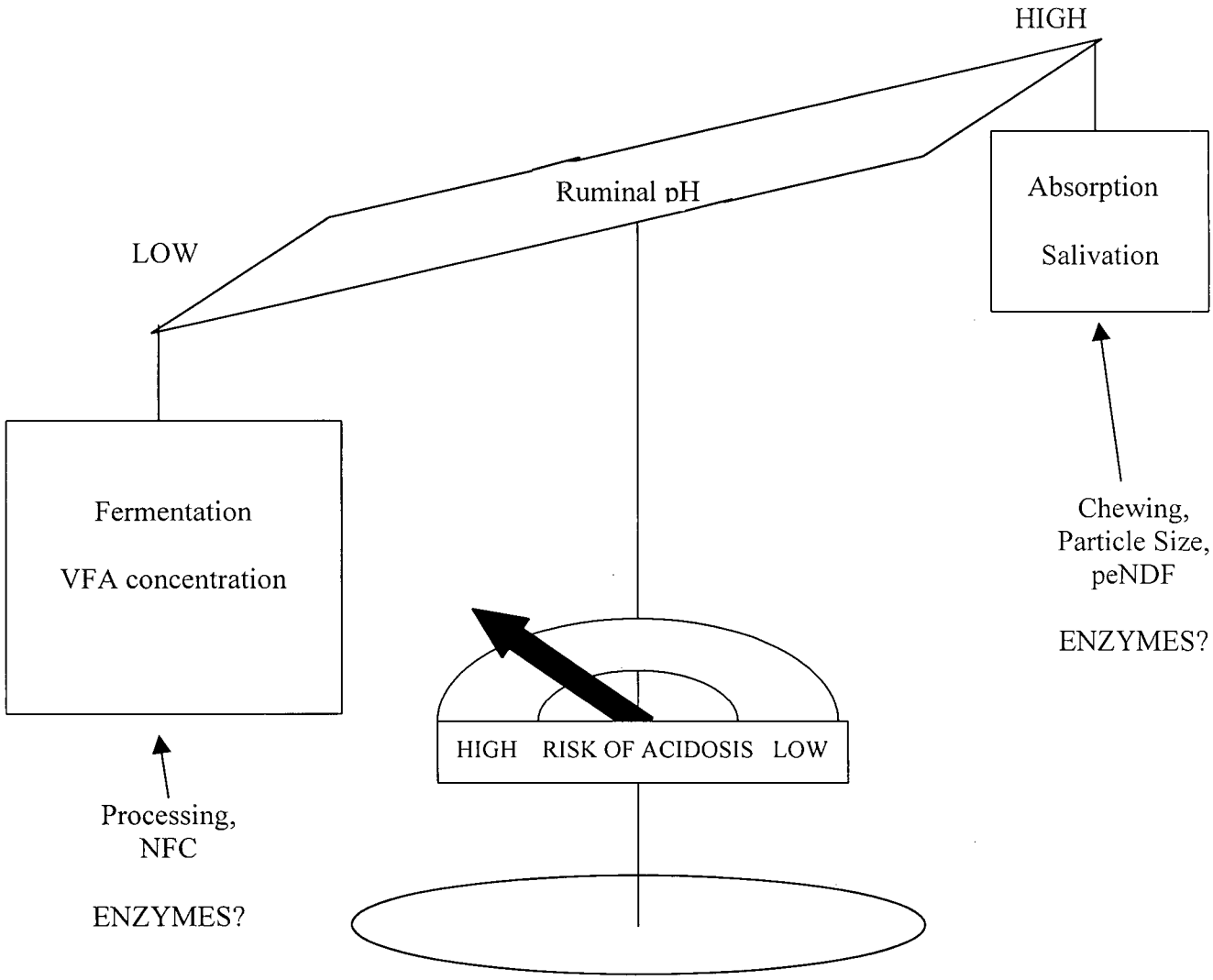

Figure 1.1 Schematic representation of the balance between various factors acting on ruminal pH and the unknown part that fibrolytic enzymes may play.

(peNDF = physically effective NDF, NFC = non-fiber carbohydrates)

2.0 THE EFFECTS OF TREATING DIFFERENT COMPONENTS OF THE DIET WITH A FIBROLYTIC ENZYME ADDITIVE ON THE NUTRIENT DIGESTION BY LACTATING DAIRY COWS

2.1 INTRODUCTION

The use of exogenous enzymes to improve the growth and production of non-ruminants is common; the mode of action is generally to remove anti-nutritional factors thereby increasing the nutritive value of the feed. The rumen contains numerous microbes, which overcome the factors that limit feed utilization in monogastrics. Thus, supplementing the diet of mature ruminants with exogenous enzymes has been met with skepticism. However, studies using various enzyme products fed to ruminants have been recently reported (Beauchemin et al., 1999; Chen et al., 1995; Feng et al., 1996; Lewis et al., 1999; Rode et al., 1999; Yang et al., 2000). Various factors such as enzyme type and preparation, application method, amount of enzyme applied and to what fraction of the diet, and animal differences have lead to inconsistencies and variation of results. For enzyme supplementation of ruminant diets to gain acceptance by the dairy cattle industry variability of results must be reduced and positive responses substantiated.

An increase in milk production was reported when dairy cow diets were supplemented with fibrolytic enzymes (Rode et al., 1999; Yang et al., 2000). However, others did not report increased milk production (Beauchemin et al., 2000; Chen et al., 1995), and milk production was not the only inconsistent variable. Dry matter intake was reported to be both increased (Beauchemin et al., 2000) and unchanged (Beauchemin et al., 1999, Kung

et al., 2000) when enzymes were added to the diet. Use of an enzyme product comprised mainly of xylanase and cellulase was shown to increase digestibility (Rode et al., 1999; Yang et al., 2000). However, when different enzyme formulations were fed (Chen et al., 1995) and when enzymes were applied to the forage portion (Lewis et al., 1999) digestibility was reported as unchanged.

The objectives of this portion of the study were to compare the effects of applying a fibrolytic enzyme product on different components of the total mixed ration on nutrient intake and digestibility, ruminal fermentation, microbial N synthesis, digesta passage kinetics, milk production and composition.

2.2 MATERIALS AND METHODS

2.2.1 Cows and Diets

Eight multiparous lactating Holstein cows were used. Four cows were surgically fitted with ruminal cannulas, measuring 10 cm in diameter that were constructed of soft plastic (Bar Diamond, Parma, ID). Cows averaged 111 ± 32 DIM and 649 ± 52 kg BW.

The design of the experiment was a double 4 x 4 Latin Square with each period lasting 28 d. One of the squares was comprised of cows fitted with ruminal cannulas. Cows received a diet consisting of 45% concentrate and 55% forage (DM basis) (Table 2.1). 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. Dose rate was based on cows consuming 10.7 kg of concentrate and 2.7 kg of supplement, which contained 1 g of enzyme supplement for CONC and SUPP treatments, respectively. The enzyme used was Promote N.E.T.™ (Agribrands International, St. Louis, MO). This commercial enzyme product is characterized with primarily xylanase and cellulase activities.

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 water) was added slowly into a 1-t mixer containing steam-rolled barley and pelleted supplement. For the SUPP treatment, the enzyme solution (93 g/15 L of water) was added to 250 kg of ingredients in the mixer prior to pelleting the supplement. Due to the small volumes that were required for the PREM treatment, the enzyme solution (4 g/16 ml of water) was added to 200 g of wheat bran and mixed using a food processor. Enzyme-feed mixtures were prepared at the beginning of each period.

The enzyme product activities (Table 2.2) were assayed using carboxymethyl cellulose (**CMC**, medium viscosity, Sigma, St. Louis, MO); Avicel PH105 20 μm (FMC Corporation, Philadelphia, PA); oat spelt xylan (Sigma, St. Louis, MO); and wheat arabinoxylan, xyloglucan, and barley β-glucan (Megazyme International Ireland Ltd.,

Wicklow, Ireland) as substrates. Assays were conducted by adding 50 µl of enzyme solution to a tube containing 100 µl of 0.1 M sodium citrate and phosphate buffer (pH 5.0 and 6.0) and 50 µl of 2% substrate. The contents were then incubated at 39°C for 10 min; the reaction was stopped with the addition of Somogyi reagent and boiling. Blanks were also used for corrections. Reducing sugars liberated from the hydrolysis of the various substrates were detected using the Nelson-Somogyi method (Somogyi, 1952). Acetyl-esterase (EC 3.1.1.6) was assayed with p-nitrophenyl substrates obtained from Sigma (St. Louis, MO). The assay was carried out in microtiter plates and consisted of 20 µl of diluted enzyme sample and 80 µl of 1 mM substrate in 0.1 M citrate and PO_4 buffer (pH 5.0 and 6.0). The mixture was incubated at 39°C for up to 30 min; the reaction was then stopped with 100 µl of 0.5 M glycine and NaOH buffer, pH 10.6. Release of p-nitrophenol was measured at 420 nm using a MRX-HD plate reader (Dynatech Laboratories, Inc., Chantilly, VA).

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, except during urine collection and digestibility measurements. Cows were offered feed three times daily at 0800, 1500 and 1800 h and fed for ad libitum intake with 10% of daily feed refused.

The first 11 days of each period were for adaptation, d 12 to 17 were used to determine rate of passage, d 12 to 14 for digestibility measurements, d 14 to 16 for urine collection, d 21 to 28 for milk composition determination and d 26 for rumen fermentation measurements.

Feed offered and refused was measured and recorded daily to determine DMI. Barley silage and alfalfa silage was sampled weekly and DM was determined to adjust diet composition when required. The TMR and ort samples were collected daily for 1 wk coinciding with milk sampling. The TMR and ort samples were dried at 55°C and then ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA) and composited for each cow by period and subsequently analyzed for NDF, ADF, and OM. Milk production was recorded daily and sampled for 1 wk. Individual morning and evening daily milk samples were preserved with potassium dichromate and stored at 4°C until sent to Central Alberta Milk Testing Laboratory (Edmonton, AB, Canada). Milk was analyzed for milk fat, CP, and lactose using an infrared analyzer (Milk-O-Scan 605; Foss Electric, Hillerød, Denmark) (AOAC, 1990).

Cows were weighed at the beginning and end of each period at approximately 0900; these weights were then used to calculate change in BW for each period.

2.2.2 Ruminal Fermentation and Rate of Passage

Ruminal fluid was collected from cannulated cows on d 26 at 0730 and 1300 h. Samples were taken from four locations within the rumen, composited and then squeezed through four layers of cheesecloth with a mesh size of 250 μm. Five milliliters of filtered rumen fluid was added to 1 ml of 25% HPO_3 for VFA determination and 5 ml of filtrate was added to 1 ml of 1% sulfuric acid for NH_3 determination. Samples were stored at -20°C until analysis.

Cr-mordanted NDF was used to measure the rate of passage of particulate matter and Co-EDTA was used as a liquid phase marker. Barley silage was boiled in detergent and

rinsed until the NDF content of the residual fiber exceeded 85%. The resulting fiber was dried at 55°C. Chromium was mordanted to the barley silage fiber and Co-EDTA was prepared according to Udén et al. (1980).

Each cannulated cow received 225 g of Cr-mordanted barley silage and a 300 ml solution containing 15 g of Co-EDTA via the rumen cannula on d 11. Rectal samples of feces were collected at 0, 3, 6, 9, 12, 16, 20, 24, 28, 32, 36, 40, 48, 60, 72, 96 and 120 h after dosing. Samples were oven dried at 55°C and ground to pass a 2-mm screen (standard model 4) and stored until analysis.

Particulate and liquid kinetic parameters were estimated for each cannulated cow for each period from the concentration of Cr and Co, respectively in the feces. A double compartmental model (Grovum and Williams, 1973) was fitted using the non linear regression procedure of SAS (SAS, 1999).

2.2.3 Apparent Digestion

Apparent total tract digestion of nutrients was determined for all cows using $YbCl_3$ (Rhône-Poulenc, Inc., Shelton, CT). Ytterbium solution was incorporated directly into the concentrate at the time of milling. Cows received approximately 1 g of Yb/cow/day throughout the entire study. Fecal samples were taken beginning on d 12 at 0900 h then 3, 6, 9, 12, 16, 20, 24, 28, 32, 36, 40, 48 and 60 h later. Samples were individually dried and ground to pass a 2-mm screen (standard model 4), composited across sampling times for each cow and stored for chemical analysis.

The concentration of Yb was analyzed in feed, orts and feces and then digestibility was calculated using the indicator method (Schneider and Flatt, 1975).

2.2.4 NDF Ruminal Digestibility

Rumen contents were manually emptied on the last day of the period. Total rumen volume was determined by weighing the removed contents. After weighing the entire contents were mixed and subsampled. The DM of the contents was determined by drying at 55°C for 48 h in a fan forced oven. Dried contents were stored for subsequent analysis of indigestible NDF (**INDF**).

Dried rumen contents and TMR were incubated in sacco for 144 h to determine INDF. Bags used for the in sacco digestion were monofilament PeCap® polyester bags (B. & S. H. Thompson, Ville Mont-Royal, QC, Canada) measuring approximately 9.5 x 19.5 cm with a pore size of 51 μm. After removal from the rumen, bags were immediately placed in cold water and further washed in cold water by hand. Bags were dried at 55°C and analyzed for NDF content; amount remaining was referred to as INDF. Digestible NDF (**DNDF**) was calculated as NDF – INDF.

The calculation of ruminal digestibility of NDF was based on the model proposed by Waldo et al. (1972) modified to use NDF rather than cellulose. The basis of this model divides the rumen NDF into two fractions, digestible and indigestible. The rate of potentially digestible NDF digestion within the rumen was calculated as the difference between the total rate of disappearance from the rumen and the rate of passage (Allen and Mertens, 1988). The apparent rumen digestibility of NDF was calculated using the formula:

NDF digestibility = f_d (k_d/(k_d + k_p)) (Allen and Mertens, 1988).

Where f_d is the digestible fraction, k_d is the rate of digestion, and k_p is the rate of passage.

2.2.5 Microbial Nitrogen Synthesis

One kilogram of fresh rumen contents obtained from each cannulated cow during rumen evacuation the last day of each period was blended (Waring Products Division, New Hartford, CT) with 1 liter of 0.9% NaCl for 2 min and then squeezed through 4 layers of cheesecloth. The filtrate was centrifuged (800 x g for 15 min at 4°C) to remove feed particles and then the supernatant was further centrifuged (20,000 x g for 45 min at 4°C) to obtain a bacterial pellet. Bacterial pellets were freeze-dried and ground using a ball mill (Wig-L-Bug; Crescent Dental Mfg. Co., Lyons, IL). The samples were then stored for analysis of purines-N and total-N.

Total output of urine was collected from cannulated animals on d 14 to 16 using a balloon catheter (Rüsch Canada, North York, ON, Canada). Urine was collected in vessels containing sufficient amounts of 4 N H_2SO_4 to maintain pH below 3. Urine volume was measured daily and samples were kept at -20°C until analysis of purine derivatives.

Total purine derivatives (**PD**) excreted (mmol/d) were estimated as the sum of uric acid and allantoin. Excretion of the endogenous PD was a constant at 0.385 mmol/kg $BW^{0.75}$ for individual cows (Chen and Gomes, 1992). Purine absorption of microbial origin (mmol/d) was calculated as:

(total PD excreted – endogenous factor x $BW^{0.75}$)/0.85 (Chen and Gomes, 1992). Synthesis of microbial N within the rumen was calculated as:

(purine absorption x 70)/(purine-N:total-N x 0.83 x 1000) (Chen and Gomes, 1992). The purine-N and total-N were determined from bacterial pellets obtained from individual cows and the average purine N: total N measured in this study was 0.136.

2.2.6 Chemical Analysis

Analytical DM was determined by drying samples at 135°C for 3 h. Oven DM was determined by drying samples at 55°C for 48 h. Organic matter was determined by ashing (AOAC, 1990). The NDF and ADF contents were determined using an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Fairport, NY). Sodium sulphite and heat-stable amylase were used in the analysis of NDF. The N content was determined by flash combustion (Carlo Erba Instruments, Milan, Italy). Contents of Co, Cr and Yb were determined using atomic absorption according to the AOAC procedure (AOAC, 1990). Ruminal VFA were separated and quantified using gas chromatography (5890; Hewlett Packard, Mississauga, ON, Canada) using a 30-m (0.32-mm i.d.) column (Nukol column; Supelco, Oakville, ON, Canada). Rumen ammonia was determined as described by Rhine et al. (1998).

Allantoin in urine was determined by autoanalyzer using the procedure of Pentz (1969) with modifications by Lindberg and Jansson (1989). Uric acid was determined using a commercial kit (Sigma No. 292; Sigma Chem. Co., St. Louis, MO). Purine content in the microbial pellet was determined using the procedure of Zinn and Owens (1986).

2.2.7 Statistical Analysis

Means were calculated for all variables by cow within period. Data were analyzed using the MIXED procedure of SAS (SAS, 1999). Period and cow were considered random effects; diet, parity and cannulation effects were considered fixed. Estimation method was restricted maximum likelihood and the degrees of freedom method was Kenward-Roger (SAS, 1999). Differences were tested using the PDIFF option in SAS (SAS, 1999) using a

protected ($P < 0.10$) LSD test. Differences were declared significant at a $P < 0.05$; and trends were discussed at a $P < 0.15$, unless stated otherwise.

2.3 RESULTS

2.3.1 Milk Production, Composition and Dry Matter Intake

Cows receiving the CONC treatment produced the most milk; significantly more than cows receiving the PREM treatment, however enzyme treatments were similar to CTRL (Table 2.3). Although not significant, there was a trend for cows receiving the CONC treatment to increase 4% FCM by 3% when compared to CTRL and SUPP treatments, and by 5% over the PREM treatments. Use of an enzyme supplement tended to increase both fat and protein percentage and decrease lactose percentage in milk, however only the PREM treatment was significantly different from CTRL.

Dry matter and other nutrient intakes were not significantly altered by inclusion of the enzyme product (Table 2.4). There was a significant interaction between the main effects of treatment and cannulation for dry matter intake. Cannulated cows receiving the CONC treatment decreased DMI in comparison to cannulated cows receiving CTRL, while their intact counterparts increased DMI while receiving the CONC treatment compared to CTRL (Figure 2.1). Cannulated cows receiving the SUPP and PREM treatments increased DMI compared to the CTRL. Dry matter intake for intact cows increased for SUPP and decreased for PREM in comparison to intact cows receiving the CTRL treatment.

2.3.2 Total Tract Digestibility and Ruminal NDF Digestibility

Inclusion of the enzyme product into the TMR exhibited a trend towards increased digestion in comparison to the control. A trend in increased total tract digestion of OM and DM with the use of this enzyme product was observed. However, cows receiving the CONC treatment significantly increased OM and DM digestion compared to cows receiving the CTRL ration (Table 2.4). Total tract digestion of NDF and ADF was also significantly increased comparing the CONC to the CTRL treatment; while SUPP and PREM did not differ increase fiber digestion compared to CTRL. The CONC treatment increased ruminal digestibility of NDF by 5 percentage units when compared to the CTRL treatment, although this increase was not significant (Table 2.5). Rumen pool size of various constituents was unchanged.

2.3.3 Ruminal Fermentation

Ruminal fermentation measurements taken pre-feeding showed no treatment differences (Table 2.6). However, CONC treatment exhibited a trend in elevated ammonia nitrogen in pre-feeding samples in comparison to other treatments. The SUPP and PREM treatments had differing propionate concentrations post-feeding, but neither treatment differed from the control. The differences in propionate concentration led to differences in the acetate to propionate ratio between these two treatments. Cows receiving PREM showed a decrease in acetate to propionate ratio in comparison to all other treatments. There was a trend in post-feeding, ammonia nitrogen concentrations in the rumen were increased when enzyme was added to the diets.

2.3.4 Rate of Passage

Supplementation with this enzyme product had little effect on the rumen fluid outflow or digesta passage from the rumen (Table 2.7).

2.3.5 Microbial Nitrogen Synthesis

Purine derivatives excreted in urine were similar for all treatments (Table 2.8). Cows receiving the PREM treatments excreted the highest amount of purine derivatives. Cows fed PREM produced significantly more microbial nitrogen than did cows receiving the SUPP treatment, and a similar amount to cows receiving the CTRL and CONC treatments. Although not significant, CONC and PREM treatments increased microbial nitrogen production by 6% and 9%, respectively, compared to the CTRL.

2.4 DISCUSSION

The results from this study indicate that the concentrate component of the diet to which, this enzyme product is applied plays a key role in determining whether digestion increases. Increases in OM digestibility over the control in this study ranged from 1% to 10%, depending upon the component of the diet to which the enzyme was applied.

Rode et al. (1999) reported a 10% increase in DMD for cows in early lactation when enzyme was added to the concentrate, while Yang et al. (2000) reported a 5% increase in OM digestibility, when enzymes were applied to the concentrate portion of a TMR. Others using different enzyme formulations applied enzymes to sorghum grain (Chen et al., 1995) or directly to the forage portion (Lewis et al., 1999) and reported no increase in digestibility.

The application of the enzyme product prior to pelleting, as was the case for the SUPP treatment, may have hindered the efficacy of the product. The pelleting process involves elevated temperatures (94 °C) that may reduce the activity of the enzymes, thereby delivering insufficient activity levels when fed to the animal. Applying the enzyme product to the supplement after the pelleting process may be a viable solution for future studies. Alternatively, decreased efficacy of the SUPP treatment may indicate that enzymes are more effective when applied to a larger portion of the diet.

While optimal enzyme application rate was not considered in this study, previous reports have shown enzyme supplementation produces a quadratic response; as level of enzyme applied was increased benefits were negated (Lewis et al., 1999; Beauchemin et al., 2000). This may explain the decreased response when enzymes were applied to SUPP or PREM. The 200 g of premix contained the same level of enzyme as 2.1 kg of supplement and 10.6 kg of concentrate for the SUPP and CONC treatments, respectively. Thus, the premix added to the ration was highly concentrated in comparison to other treatments. This may have resulted in microenvironments within the rumen comprised of elevated enzyme concentrations, these acting in a similar manner to over supplementation with the enzyme product. Beauchemin et al. (1999) postulated that applying enzymes to feed creates a 'slow release' mechanism releasing the enzyme into the rumen fluid as feed is digested. The decreased particle size to which the enzyme was applied for the PREM may have allowed rapid passage out of the rumen, lessening the enzyme effect for cows fed PREM.

This enzyme product's main influence is on fiber digestion. The portion of the ration treated with enzyme for CONC was larger, in comparison to SUPP and PREM, which may

have maximized the dispersion of the enzyme within the rumen and increased the likelihood of the exogenous enzyme being beneficial.

Previous studies using enzyme supplementation have reported increased total tract NDF digestion. However few studies have investigated whether this response was due to an increase in ruminal digestion, lower tract digestion or a combination of both. Yang et al. (1999) showed a significant increase in total tract NDF digestion and an increase in the percent of NDF digested within the rumen. Another study reported a 2.9-percentage unit increase ($P < 0.07$) in NDF total tract digestion using an enzyme product with barley based diets; however the ruminal digestion of NDF decreased by 1.6-percentage units (Beauchemin et al., 1999). The current study showed an increase in total tract NDF digestibility and a numerical increase in ruminal NDF digestibility for the CONC treatment. For the cannulated cows the proportion of total NDF that was digested within the rumen increased to 62% for cows fed CONC from 60% for cows fed CTRL. These results imply that the enzyme effect is mainly in the rumen, however activity in the lower digestive tract is still present. Previously it was thought that exogenous enzyme sources were susceptible to rumen microorganisms and gastrointestinal degradation. This notion has recently been refuted and it is now known that some exogenous enzyme sources have the ability to withstand both ruminal and intestinal degradation (Morgavi et al., 2001), although resistance to proteolysis is not the same for all enzyme products (Hristov et al., 1998a; Hristov et al., 1998b).

There are few studies in which cannulated and intact animals are studied simultaneously and the interaction between diet and cannulated effects for DMI were unanticipated (Figure 2.1). Cannulated cows decreased DMI when receiving CONC, however due to the increase in total tract digestibility, intake of DDM was increased for cows

fed CONC compared to cows fed CTRL. It is hypothesized that due to production differences between cannulated and intact cows additional nutrients available due to enzyme supplementation were in excess of requirements and cannulated cows receiving the CONC treatment subsequently lowered DMI. Intake of DDM for cows fed PREM was similar to cows fed CTRL, indicating comparable total tract digestion.

Milk production and composition were reported, but were not the main focus of this study due to the limited number of cows used. Other researchers feeding various enzyme products have shown both an increase (Rode et al., 1999; Yang et al., 2000) and no change (Beauchemin et al., 2000; Chen et al., 1995) in milk production. In the present study, the trend for an increase in 4% FCM for cows fed CONC was attributed to the increase in diet digestibility because DMI was not affected by diet. Milk production followed the same trend as intake of DDM. This experiment confirmed previous studies that showed enzyme supplementation did not increase DMI (Beauchemin et al., 1999; Hristov et al., 1998a; Kung et al., 2000). However, the effects of enzyme supplementation on DMI may depend on the specific formulation evaluated, as other studies using different enzyme products fed to beef steers (Feng et al., 1996) or dairy cows (Beauchemin et al., 2000) have shown an increase in DMI.

The trend of increased fat and/or protein percentage due to fibrolytic enzyme supplementation has been previously reported (Luchini et al., 1997; Nussio et al., 1997; Schingoethe et al., 1999). In contrast, Kung et al. (2000) showed similar fat and protein percentage with enzyme supplementation, while Beauchemin and co-workers reported an increase in protein percentage with no difference in daily production of fat or protein (Beauchemin et al., 2000). To date, most studies that reported an increase in milk production

due to enzyme supplementation observed either a decrease or no effect on fat and protein percentage (Kung et al., 2000; Rode et al., 1999; Yang et al., 1999; Yang et al., 2000). In our study, it is hypothesized that the increased intake of digestible energy due to enzyme supplementation (CONC) that was not used for milk production was allocated to milk fat, milk protein, and body reserves. This is also supported by the increase in BW for cows fed CONC compared to cows fed CTRL.

Though no difference was seen for digesta or passage kinetics in this study others have reported trends of increased digesta passage for dairy cows (Beauchemin et al., 1999) and significantly increased digesta passage for beef steers (Feng et al., 1996) due to enzyme supplementation. However, fibrolytic enzyme supplements generally have been shown not to alter passage of either ruminal fluid or particulate material (Hristov et al., 2000; Lewis et al., 1996; Yang et al., 1999).

The decrease in propionate, subsequently leading to an increase in the acetate to propionate ratio was unexpected for the PREM treatment. Previous studies providing enzyme supplementation have reported no effect (Beauchemin et al., 1999; Kung et al., 2000) to slight differences in molar ratios (Hristov et al., 2000), even when the total VFA concentration was increased (Lewis et al., 1996). Increase in the acetate to propionate ratio may be attributed to elevated ruminal fiber digestion for the PREM treatment, although significant differences were not observed using the current methodology. Differences in ammonia N concentrations pre- and post-feeding were expected and are explained by decreased synchrony between protein and energy.

Two main factors can affect rumen microbial yield; rate of outflow from the rumen and amount of OM fermented within the rumen. Because rumen flow kinetics were not

affected by enzyme supplementation, this is not likely to account for increased microbial yield due to enzyme supplementation. The trend of increased microbial nitrogen synthesis is attributed to increases in ruminal digestion and overall rumen efficiency.

2.5 CONCLUSION

The component of the diet to which a fibrolytic enzyme product is applied must be considered to achieve the maximum benefit. Cows in midlactation fed the diet to which an enzyme product was applied to 45% of the TMR showed a trend towards increased milk production. This increase in production is attributed to an increase in digestibility and intake of digestible DM, not to increased DM intake. Only the CONC treatments containing fibrolytic enzymes significantly increased total tract digestibility of OM, NDF, and ADF. Thus, cows fed the diet in which the enzyme was applied to a reduced component of the diet did not show the same increased digestibility when compared to cows fed the control. It appears that this enzyme product influences digestion in both the rumen and lower tract. Future studies are required to understand the mode of action of fibrolytic exogenous enzymes. Studies focusing on method of application, component of diet that the enzyme is applied, and mode of action for increased digestion would greatly advance the understanding of exogenous enzymes for ruminants.

Table 2.1 Ingredients and chemical composition of the diet.

Item	Diet
Ingredient	(% of DM)
Barley Silage	36.9
Alfalfa Silage	18.2
Barley Grain, steam rolled	33.4
Barley Grain, ground*	0.4
Soybean Meal*	1.4
Blood Meal*	1.4
Corn Gluten Meal*	6.3
Dicalcium Phosphate*	0.93
Beet Molasses*	0.24
Vitamins and Minerals[1]*	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

[1] Contained 2,000 IU of vitamin A/g, 200 IU of vitamin D/g and 20 IU of vitamin E/g. Contained 58.8% NaCl, 2.0% $ZnSO_4$, 2.4% $MnSO_4 \cdot 4H_2O$, 0.8% $CuSO_4 \cdot H_2O$, 90 mg/kg $CoSO_4 \cdot 5H_20$, 88 mg/kg Na_2SeO_3, 16% Dynamate (Pitman Moore, Inc., Mundelein, IL; 22% S, 18% K, and 11% Mg) and 120 mg/kg of ethylenediamine dihydroiodide.

* Ingredients which were in the pelleted supplement.

Table 2.2 Polysaccharidase and glycosidase activities of enzyme preparation[1].

Substrate or activity	Enzyme (lot 1)		Enzyme (lot 2)	
	pH 5	pH 6	pH 5	pH 6
CMC	510	414	463	381
Avicel	241	181	300	177
Arabinoxylan	241	181	299	177
β-Glucan	932	689	754	623
Xylan	1307	1230	1313	1069
Xyloglucan	280	258	266	258
Acetyl esterase	55	84	53	82

[1]Polysacchridase activities are expressed as nmol glucose equivalents liberated from the substrate/min/mg protein and glucosidase activities as nmol of nitrophenol released from the p-nitrophenyl substrate/min/mg protein.

Table 2.3 Milk production and composition for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments[1]				SE
	CTRL	CONC	SUPP	PREM	
Milk production, kg/d					
Actual	29.4 [a,b]	30.0 [a]	28.8 [a,b]	27.9 [b]	2.2
4% FCM	28.9	29.8	28.9	28.4	1.9
Milk composition, %					
Fat	3.91 [a]	4.01 [a,b]	4.06 [a,b]	4.18 [b]	0.17
Protein	3.59 [a]	3.61 [a,b]	3.62 [a,b]	3.68 [b]	0.11
Lactose	4.51 [a]	4.49 [a,b]	4.48 [a,b]	4.45 [b]	0.04
Milk composition, kg/d					
Fat	1.14	1.19	1.16	1.15	0.07
Protein	1.05	1.07	1.03	1.02	0.05
Lactose	1.33 [a]	1.34 [a]	1.29 [a,b]	1.24 [b]	0.09
Milk/kg DMI	1.25	1.26	1.20	1.18	0.08
BW change, kg/d	0.49	0.83	0.36	0.68	0.32

[1] CTRL = 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).

[a,b] Means in the same row with different superscripts differ ($P < 0.10$).

Table 2.4 Nutrient intake and total tract digestibility for cows fed diets containing enzyme treatments applied to different components of a TMR.

	Treatments[1]				
Item	CTRL	CONC	SUPP	PREM	SE
Intake, kg/d					
DM	23.6	23.7	24.0	23.7	0.5
DDM	15.4 [a]	17.2 [b]	16.8 [a,b]	15.6 [a,b]	0.6
OM	21.8	21.9	22.0	21.7	0.4
NDF	7.1	7.0	7.0	7.3	0.2
Digestibility, %					
DM	65.1 [a]	72.6 [b]	70.1 [a,b]	65.9 [a]	2.4
OM	66.6 [a]	73.8 [b]	71.4 [a,b]	67.2 [a]	2.3
NDF	44.3 [a]	55.6 [b]	50.6 [a,b]	46.9 [a,b]	4.0
ADF	43.6 [a]	55.6 [b]	50.2 [a,b]	47.4 [a,b]	4.1

[1] CTRL = 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).

[a,b] Means in the same row with different superscripts differ ($P < 0.10$).

Table 2.5 Ruminal pool size and ruminal NDF digestibility for cows fed diets containing enzyme treatments applied to different components of a TMR.

	Treatments[1]				
Item	CTRL	CONC	SUPP	PREM	SE
Whole rumen contents					
Wet, kg	90.2	89.2	92.9	93.5	3.8
DM, kg	13.8	13.3	14.6	14.6	0.7
NDF, kg	7.7	7.6	8.1	8.1	0.4
INDF,[2] kg	5.6	5.6	5.7	6.0	0.3
Ruminal digestibility					
NDF, %	29.7	34.8	30.0	32.6	3.4

[1] CTRL = 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).

[2] INDF = indigestible neutral detergent fiber.

Table 2.6 Ruminal fermentation for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments[1]				SE
	CTRL	CONC	SUPP	PREM	
Pre-feeding (0 h)					
VFA, mM	118.3	122.5	121.1	118.6	7.2
VFA, mol/100 mol					
Acetate (A)	64.9	63.6	63.9	65.3	1.1
Propionate (P)	19.7	19.6	21.4	17.9	1.5
Butyrate	11.1	12.2	10.7	12.6	0.7
A:P	3.35	3.27	3.10	3.68	0.28
NH3 N, mg/L	104.5	140.7	104.5	125.3	14.7
Post-feeding (5 h)					
VFA, mM	122.7	126.9	116.3	128.5	8.3
VFA, mol/100 mol					
Acetate (A)	63.8	64.2	63.0	64.9	1.6
Propionate (P)	20.9 [a,b]	20.3 [a,b]	22.2 [a]	18.7 [b]	1.9
Butyrate	11.1	11.5	10.9	12.5	0.6
A:P	3.17 [a]	3.23 [a,b]	2.98 [a]	3.53 [b]	0.36
NH3 N, mg/L	56.2	70.4	70.7	77.1	13.0

[1] CTRL = 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).

[a,b] Means in the same row with different superscripts differ ($P < 0.10$).

Table 2.7 Digesta and ruminal fluid passage kinetics for cows fed diets containing enzyme treatments applied to different components of a TMR.

Item	Treatments[1]				SE
	CTRL	CONC	SUPP	PREM	
Liquid					
LORR, [2]%/h	12.1	10.1	10.4	9.9	2.2
RRT, [3] h	9.5	11.5	10.4	10.7	1.8
TRT, [4] h	24.2	22.9	20.9	21.7	1.6
Particle					
PORR, [5] %/h	4.8	5.1	4.8	5.0	0.6
RRT, h	21.8	19.9	23.8	20.4	3.2
TRT, h	47.8	49.2	50.0	46.1	3.2

[1] CTRL = 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).

[2] Liquid outflow rate from the reticulorumen.

[3] Rumen retention time.

[4] Total tract retention time.

[5] Particle outflow rate from the reticulorumen.

Table 2.8 Urinary purine derivatives and microbial nitrogen synthesis for cows fed diets containing enzyme treatments applied to different components of a TMR.

	Treatments[1]				
Item	CTRL	CONC	SUPP	PREM	SE
uric acid, mmol/d	23.5	25.2	24.9	29.6	4.2
allantoin, mmol/d	309	305	293	322	17
total PD,[2] mmol/d	332	331	318	352	19
microbial N, g/d	196[a,b]	209[a,b]	185[a]	216[b]	12

[1] CTRL = 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).

[a,b] Means in the same row with different subscripts differ ($P < 0.10$).

[2] PD = purine derivatives excreted in urine.

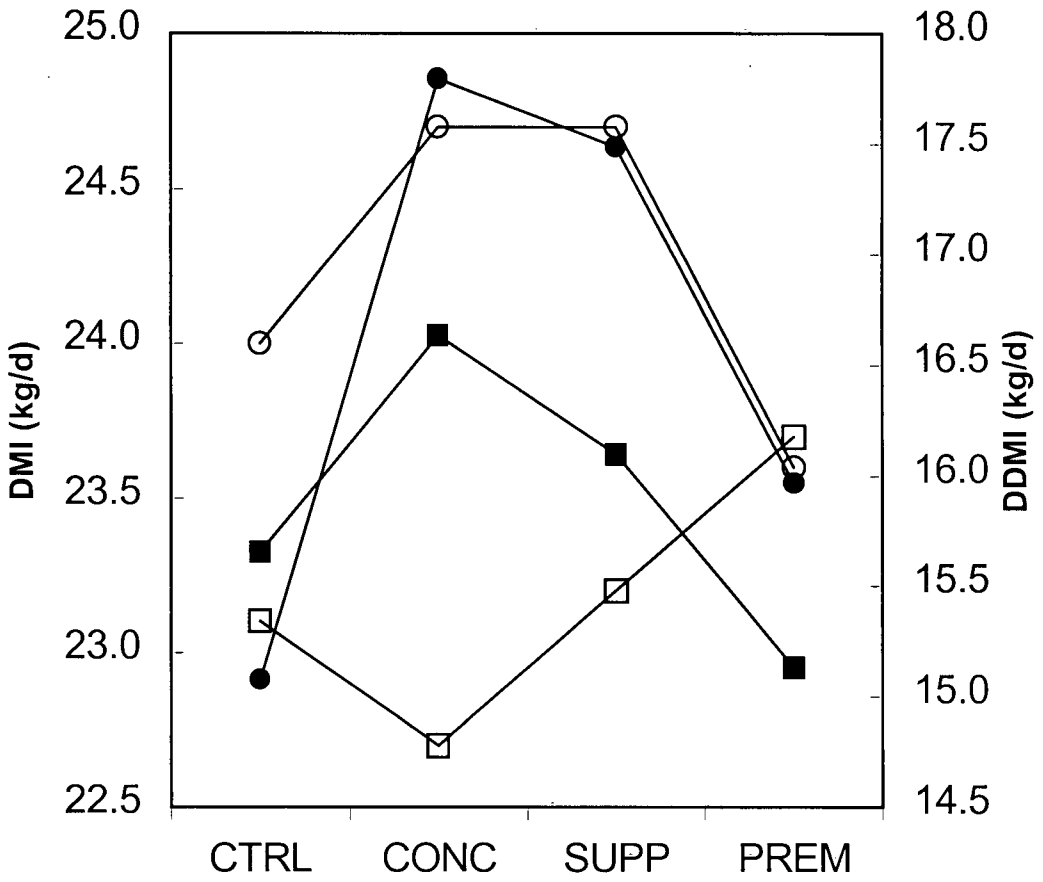

Figure 2.1 Interaction between cannulation and enzyme treatment effects for DMI. Dry matter intake for cannulated (□) (N=4) and intact (○) (N=4) cows (SE=0.63) and digestible dry matter intake (DDMI) for cannulated (■) and intact (●) cows (SE=0.86). CTRL = control, CONC = enzyme applied to concentrate (45% of TMR), SUPP = enzyme applied to supplement (4% of TMR), PREM = enzyme applied to premix (0.2% of TMR).

3.0 EFFECTS OF FIBROLYTIC ENZYMES ON FEEDING BEHAVIOR, SALIVA PRODUCTION AND RUMINAL PH OF LACTATING DAIRY COWS

3.1 INTRODUCTION

Concentrates and finely chopped forages are often used in the diets of lactating dairy cows. These feeds are characterized by reduced particle size, which improves intake and could influence rate of fermentation. However, a decrease in the physical structure of the diet reduces its ability to stimulate chewing (Mertens, 1997). A variety of models have been proposed to characterize the physical and chemical properties of feed. However, these models lack information on the physical effectiveness of barley silage, which is commonly fed in Western Canada.

Fibrolytic enzymes have the capacity to degrade fiber components and because they are usually applied to the diet prior to ingestion, pre-ingestive effects may occur. Beauchemin et al. (2000) hypothesized that some feed enzymes might erode the fiber structure prior to ingestion, thereby increasing intake. This collapse of the plant structure could decrease the effective physical fiber of the diet, thereby decreasing both chewing time and saliva output. The results may be, depressed rumen pH and increased 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 non-lactating beef cattle (Bailey, 1961). As well, differences in saliva production between parity groups have been reported (Maekawa et al., in press), with multiparous cows having a higher salivation rate compared to primiparous cows. Saliva

production increases as eating and ruminating time increases (Beauchemin, 1991). Alterations in mechanical processing (Beauchemin and Rode, 1994) and chemical properties (Beauchemin and Buchanan-Smith, 1989) of feed can significantly alter chewing behavior, and consequently 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., 2000), but ruminal pH has been lowered (Lewis et al., 1996) or unchanged (Yang et al., 1999).

The objectives of this portion of the study were to investigate the effects of enzyme supplementation on the eating behavior of lactating dairy cows, effective fiber content of the diet and the relationship between saliva production and ruminal pH.

3.2 MATERIALS AND METHODS

3.2.1 Cows and Diets

Four multiparous and four primiparous 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 BW at the start of the experiment, respectively.

The design of the experiment was a double 4 x 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) (Table 2.1). The diet was formulated as previously described. 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) CTRL, 2) CONC, 3) SUPP, and 4) PREM as previously described. The enzyme used contained the same activities as described earlier.

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 for 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 days of each period were for adaptation; d 12 to 17 were previously described to be used for digestibility, rate of passage, and urine collection, d 19 was used to determine saliva produced while eating, d 21 to 23 for electronic measurement of rumen pH, feeding behavior and ruminating time, and d 28 for saliva produced while resting.

Feed offered and refused was measured and recorded daily to determine DMI. Barley silage and alfalfa silage was sampled weekly and DM was determined to adjust diet composition when required.

3.2.2 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. Digesta was held in closed plastic bags that were submerged in warm water to minimize any change in temperature. Once contents were removed the animals were allowed to eat uninterrupted for 5 min prior to 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 two minutes 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). Minimums of three samples were taken while the cow was eating. If fewer samples were obtained then any previous samples were discarded and the collection was repeated at a later date. Cows 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 sub-sampled. 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:

saliva (ml) = weight of bolus (g) – weight of feed as fed (g).

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 was calculated as the salivation rate (ml/min) multiplied by the time (min) spent eating each day. It was assumed that the salivation rate was similar for eating and ruminating (Bailey and Balch, 1961a; Seth et al., 1974). Total daily saliva produced during rumination was calculated as the time (min) spent ruminating each day multiplied by the salivation rate during eating.

3.2.3 Resting Saliva Production

Rumen contents were completely removed for measurement of production of saliva while resting. 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 any 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.

3.2.4 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 locate itself in the reticulum. Probes were weighed down and 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 prior to insertion into the rumen; pH

probes were removed and calibrated every 24 h thereafter. Ruminal 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 percent 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 repeated at a later time during the period.

3.2.5 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 prior to using the leather halters for data collection; thus allowing 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.

3.2.6 Meal Duration and Eating Behavior

Eating behavior was monitored for 48 h, during which time 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 using Collect software (Labtronics, Inc., Guelph, ONT, CAN). 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 DM intake 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, B – 1800 to 0800, and C – 0800 to 1500) 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).

3.2.7 Statistical Analysis

Means were calculated for all variables by cow within period. Data were analyzed using the MIXED procedure of SAS (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.

3.3 RESULTS

3.3.1 Eating Behavior

Chewing behavior variables were not significantly affected by enzyme treatment (Table 3.1), except for the number of ruminating episodes, which was significantly reduced for cows receiving SUPP and PREM treatments compared to cows receiving the CTRL and CONC treatments. Enzyme supplementation did not alter the amount of DM consumed within a feeding episode, rate of feed intake or duration of a feeding episode (Table 3.2). However, a trend ($P < 0.06$) for lower total feed consumption within C block 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 3.1). Furthermore, multiparous cows spent the same amount of time eating as primiparous cows.

In contrast, multiparous cows tend to spend a greater portion of the day ruminating ($P = 0.09$) and the duration of a single ruminating episode was longer ($P < 0.05$) when compared to primiparous cows. A significant difference was observed between parities for the rate at which feed was ingested (Table 3.2). Primiparous cows consumed feed more slowly than multiparous cows. Multiparous cows 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 ($P = 0.07$). Differences for the first two meals within feeding block B account for over 76% of the total difference in DMI between parities (Figure 3.1). 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 B block, with 83 versus 67 g DM/min, and C block, with 83 versus 57 g 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.

3.3.2 Eating and Resting Salivation

There was a trend for an increase in eating salivation rate ($P = 0.07$) with enzyme supplementation when compared to the CTRL (Table 3.3). Total daily saliva production was 5.7 to 16.5% higher for cows receiving enzyme treatments compared with CTRL ($P = 0.03$; Table 3.4).

Salivation rates (ml/min) while eating ($P = 0.15$) and resting ($P = 0.10$) tended to be higher for multiparous cows than for primiparous cows (Table 3.3). Consequently, total daily saliva output was higher ($P = 0.05$) for multiparous cows compared with primiparous cows (Table 3.4). 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.

3.3.3 Ruminal pH Measurements

Ruminal pH measurements were lower than expected across all diets (Table 3.5). 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 (pH x 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 3.5). 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 (Fig. 2).

3.4 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 alter the ability of the feed to promote chewing. A similar trend was not seen in this study, indicating that any possible alterations to the feed prior to 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 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 the DMI is controlled by numerous interacting factors including physical factors in feed that limit intake (Allen, 1996), as well as the absorption of nutrients which 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 production is remote; rather these two factors likely act together.

Multiparous cows were more efficient than primiparous cows while ruminating. Using a variety of ruminant species varying in size, Bae et al. (1983) reported that chewing efficiency increased as 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 single 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 d) 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 DM/min compared with 69.5 g DM/min for primiparous cows. Feeding rates for the current study also shows a difference between parity groups; multiparous cows ingested 12.5 g DM more a 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 influenced 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 ranging 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 compared to Maekawa et al. (in press) who reported resting salivation rates of 114 and 88 ml/min for multiparous and primiparous cows, respectively.

Total daily saliva output has been estimated at between 98 to 190, accounting for 90% of the fluid added to the rumen daily (Bailey, 1961). These values were obtained from beef or non-lactating 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 L/d (Maekawa et al., in press) 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. 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. (in press) 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 comprised 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 (Beauchemin et al., 2000; Yang et al., 1999), 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. Cows receiving CTRL and CONC diets 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 manual sampling due to loss of CO_2 (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.

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 celluloytic 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 supplement in the current study had the lowest ruminal pH. The cows receiving the CONC supplement also had the highest total tract fiber digestibility and ruminal NDF digestion across all treatment. 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 appears to be times throughout the day that the acid load in the rumen is larger for multiparous cows compared with primiparous cows (Figure. 3.2). There is large individual cow variation when measuring ruminal pH, thus requiring a substantial numbers of animals to obtain significant differences. More work is required to determine the effects such differences in rumen pH may cause.

3.5 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 effects on digestibility, enzyme supplementation and method of application had no effect on ruminal pH measurements when compared to the 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. Multiparous cows may be at a higher risk for ruminal acidosis than primiparous cows.

Table 3.1 Chewing behavior profiles of multiparous and primiparous lactating cows receiving an enzyme supplement.

	Enzyme[1]					Parity[2]		
Item	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 ($P = 0.09$).

[1] CTRL = 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).

[2] MP = multiparous, PP = primiparous

Table 3.2 Eating behavior profiles of multiparous and primiparous lactating cows receiving an enzyme supplement.

	Enzyme[1]					Parity[2]		
Item	CTRL	CONC	SUPP	PREM	SE	MP	PP	SE
DMI, kg								
A block, (1500 to 1800)	6.39	6.45	6.46	6.40	0.42	6.62	6.23	0.42
B block, (1800 to 0800)**	9.49	9.75	9.13	9.73	0.51	10.46	8.59	0.64
C block, (0800 to 1500)	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	25.9	24.1	27.1	25.8	1.7	24.8	26.7	1.6

[a,b] Parity means in the same row with different superscripts differ ($P < 0.05$).

[1] CTRL = 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).

[2] MP = multiparous, PP = primiparous

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

Table 3.3 Eating and resting salivation of primiparous and multiparous lactating cows receiving an enzyme supplement.

	Enzyme[1]					Parity[2]		
Item	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

[a] Contrast comparison CTRL versus CONC, SUPP, PREM ($P = 0.07$).

[1] CTRL = 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)

[2] MP = multiparous, PP = primiparous

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

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

Table 3.4 Total daily saliva output for primiparous and multiparous lactating cows receiving an enzyme supplement.

	Enzyme[1]					Parity[2]		
Saliva	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

[a] Contrast comparison CTRL versus CONC, SUPP, PREM and parity means in the same row differ ($P < 0.05$).

[1] CTRL = 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)

[2] MP = multiparous, PP = primiparous

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

Table 3.5 Ruminal pH profiles for primiparous and multiparous lactating cows receiving an enzyme supplement.

	Enzyme[1]					Parity[2]		
pH variables	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 x h/d	133	132	135	135	2	135	132	2
Area under pH 5.5, pH x h/d	2.1 [ab]	2.7 [a]	1.4 [b]	1.3 [b]	0.5	2.3	1.5	0.4
Time under 5.5, h/d	11.1	12.8	9.1	8.8	1.8	11.9	9.1	2.0
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 x h/d	6.5	7.5	5.4	5.0	0.9	6.8	5.4	1.0
Time under 5.8, h/d	17.3	18.1	16.7	15.8	1.7	17.6	16.3	2.1
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 ($P < 0.05$).

[1] CTRL = 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)

[2] MP = multiparous, PP = primiparous

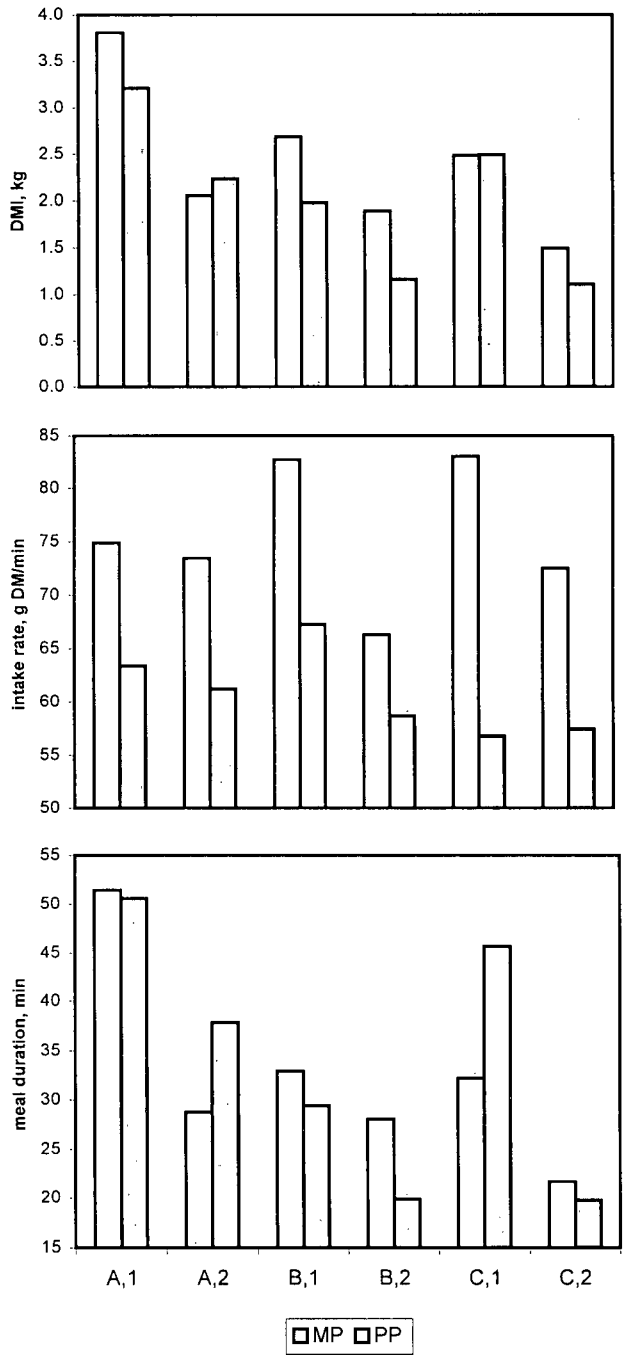

Figure 3.1 DMI, intake rate, and duration for multiparous (MP) and primiparous (PP) cows. Individual daily meals are: A = block A-1500 to1800, B = block B-1800 to 0800, C = block C-0800 to 1500, 1 = first meal, 2 = second meal.

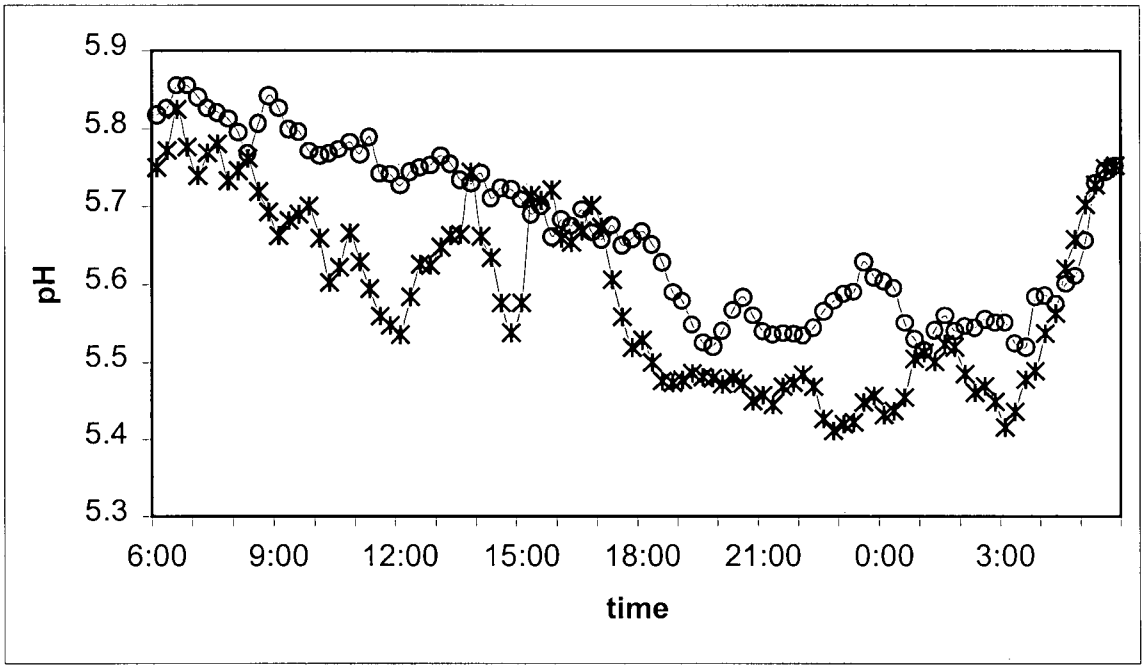

Figure 3.2 Ruminal pH measurements of multiparous and primiparous cows.

Multiparous cows = ✳ and Primiparous cows = O.

4.0 GENERAL DISCUSSION

The use of supplemental fibrolytic enzymes in the diet of dairy cows offers tremendous potential in terms of increasing the energy available for milk production. This study illustrates the advantages in digestion that may be conferred upon lactating dairy cows when fed diets supplemented with fibrolytic enzymes. It also shows that simply providing the enzyme to the cow is not sufficient to elicit a positive response in digestion. The results clearly indicate that applying this enzyme product on the entire concentrate improved dry matter digestion by 12% in comparison to control. It is interesting to note that when the enzyme was applied to only 4% of the TMR, total tract dry matter digestion was improved by 8%. This implies that the fibrolytic enzyme enhanced digestion of a larger portion of the TMR than that to which it was applied.

Although the increase in digestibility when the enzyme was applied to only 4% of the TMR was less than that obtained compared when enzyme was added to 45% of the TMR, it is unclear why the difference occurred. The difference may not be due to the reduction in the surface area to which the enzyme was applied; rather it may be the result of elevated temperatures during the pelleting process. However, application of the enzyme onto only 0.2% of the diet had no effect on total tract dry matter digestibility. The lack of response due to this treatment may be that the small particle size of the premix hastened the passage of the enzyme into the lower tract reducing the residence time in the rumen. Alternatively the relatively high concentration of enzyme on the premix may have created an over supplementation effect, which has been shown to negate the positive effects of enzyme supplementation (Lewis et al., 1999).

The energy demands that are placed on the lactating dairy cow during early and mid lactation are often difficult for the cow to meet. Moreover, peak intake may lag behind peak milk production by up to 6 weeks (NRC, 2001). To deal with the increase in dry matter consumption the passage rate of feed from the rumen to the abomasum is increased, however this increase in passage rate deceases the residence time of feed in the rumen and ruminal digestion decreases. Enzymes increase the rate of digestion, and not necessarily the extent of digestion (Bowman et al., 2001). Thus, enzyme supplementation may allow increased digestion of the fraction that may have otherwise passed from the rumen undigested (Figure 4.1).

The increase in digestion improves the energy status of the cow. Therefore, animals in negative energy balance should benefit most from enzyme supplementation. Energy that is available to the cow is partitioned into maintenance, lactation, and growth requirements. Animals were in mid to late lactation in the current study and were able to meet all energy requirements, resulting in a positive energy balance. An estimation of total energy outputs, including energy required for maintenance, lactation, and growth, was calculated. Assuming that cows in early lactation would partition excess energy to milk production after maintenance requirements were meet, the total amount of energy available to produce milk was calculated for cows in early lactation. It was assumed that milk composition would remain unchanged. Cows receiving the CONC treatment had sufficient energy to produce 36 kg of milk each day, which was a 9% (3 kg/d) increase in daily milk production compared with cows receiving the control treatment.

Fibrolytic enzymes have the capacity to enhance fiber digestion. Questions regarding mode of action and the potential for pre-ingestive effects on feeds have not been adequately

addressed. Possible pre-ingestive effects have been suggested when a different enzyme product was applied to feed (Beauchemin, 2000). In the current study, enzyme supplementation of feed did not change feeding or ruminating behavior of cows, indicating no alterations to the physical structure of the feed. Thus, this enzyme product had no pre-treatment effects that would alter the physical effectiveness of the ration in relation to chewing behavior.

Since pre-ingestive effects were non-existant, applying exogenous enzymes to feed does not appear to be a feed pretreatment. Rather, adding enzyme to feed is a vehicle for delivery of the product to the rumen. The question is then one of optimizing the delivery of such a product. Maximizing the surface area to which the enzyme is applied appears to be important. It may be that applying the enzyme onto the feed imparts a degree of stability, thereby increasing the residence time in the rumen and decreasing the likelihood of inactivation by proteolysis (Morgavi et al., 2001, Wang et al., 2001).

The concept that dairy cows require feed of sufficient size and structure to promote chewing and maintain rumen function is well established. However, quantifying 'sufficient' fiber has been an open debate for some time and continues to be an area of active discussion. The recently revised NRC (2001) recognized that dietary NDF alone was not sufficient, rather minimum forage NDF and maximum nonfiber carbohydrates (NFC) recommendations are also included. The concepts of effective NDF (eNDF) and physically effective NDF (peNDF) provide a framework which attempt to address the physical characteristics and chemical properties of the feed in terms of the response they illicit in the animal (Mertens, 1997). Based on the chewing activity observed in this study, it appears that exogenous fibrolytic enzymes do not decrease eNDF or peNDF of the diet.

While ruminal pH is known to be an important aspect of overall rumen function, the ability to accurately predict and model this aspect of the ruminal environment is limited. A variety of methods have been used to measure ruminal pH; currently it is only through the use of ruminally cannulated animals that ruminal pH can be studied in real time. Variation in ruminal pH can vary greatly during the day and between different animals (Krause et al., 1998) on the same ration (Figure 4.2). Diet formulation software (CPMDairy, Version 1.0) predicted a ruminal pH of 6.21 for the ration, while the actual pH measured in this study was 5.62. The likely reason for this discrepancy is the use of rolled barley in the diet, which is highly fermentable (Yang et al., 1997). The model predicts rumen pH based on eNDF and does not account for variation in fermentability of the carbohydrate fraction. Future research needs to improve the accuracy of predicting rumen pH by accounting for fermentation of the diet.

It can be postulated that if fibrolytic enzymes increase digestibility, then a subsequent increase in VFA concentration would occur, which could lead to a build-up of acids within the rumen, and consequently a decrease in rumen pH. However, the concentration of VFA in the rumen was not altered through the use of enzymes and, although the CONC treatment significantly increased total tract digestion, it did not significantly decrease ruminal pH. The increase in buffering capacity is attributed to the concomitant increase in saliva production when enzymes were applied to the diet. It is unlikely that the enzyme product itself caused the increase in salivation or that an alteration to the physical structure of the feed due to enzyme supplementation increased saliva production. It seems likely that the increase in fermentable products produced during digestion resulted in the increase in salivary secretion.

This implies that lactating dairy cows may alter saliva production to aid in the buffering of VFA to prevent their accumulation when VFA absorption is maximized.

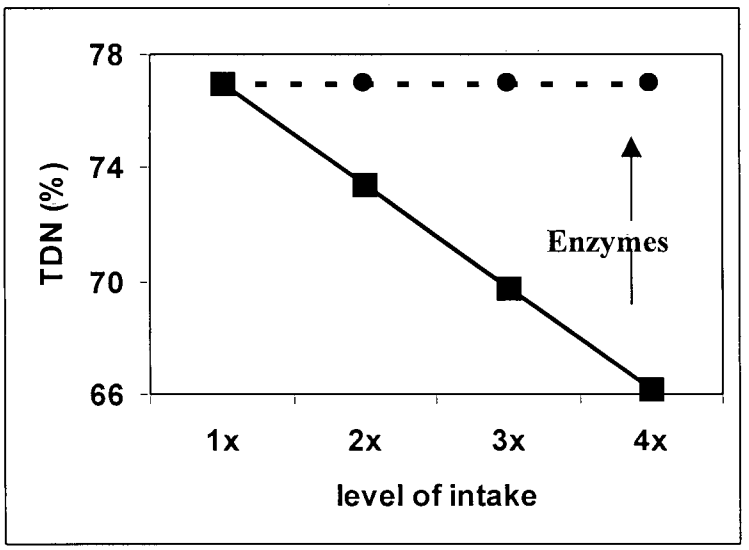

Figure 4.1 Effect of level of intake above maintenance and resulting TDN.

Potential TDN of diet (●) Discounted TDN of diet due to elevated intake (■). Discounted values obtained from NRC (2001).

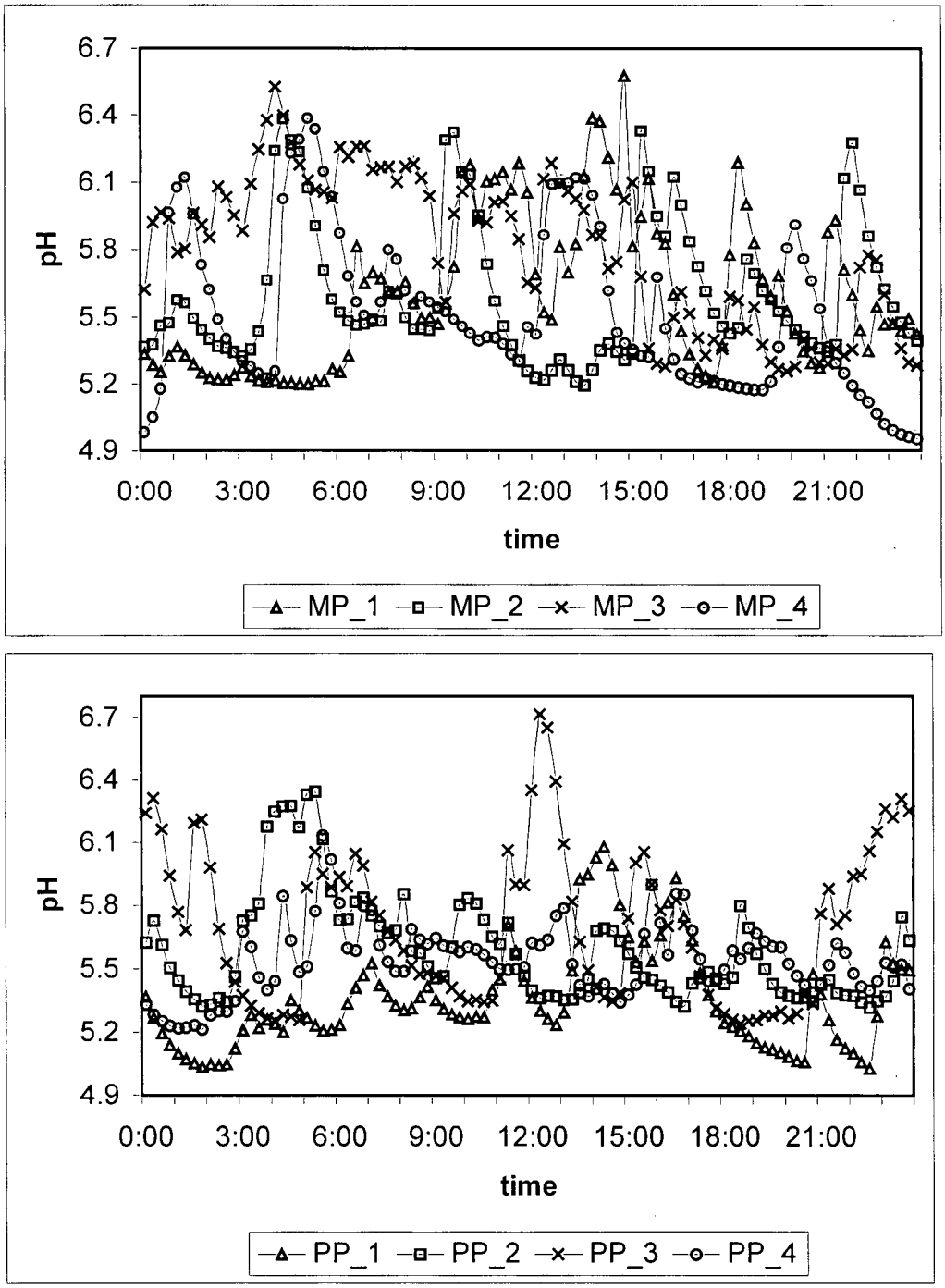

Figure 4.2 Variation in ruminal pH of multiparous and primiparous cows over 24 h. All cows receiving the control diet, multiparous cows 1 to 4 (MP_1 to MP_4) and primiparous cows 1 to 4 (PP_1 to PP_4).

5.0 GENERAL CONCLUSIONS

Fibrolytic enzymes enhanced digestion of feed by lactating dairy cows. However, the magnitude of the increase in digestion depended upon the component of the diet to which the enzyme was added. Applying the enzyme product on a large portion of the diet was most effective and increased total tract digestion by 12%. When the enzyme was applied to 4% of the diet, digestibility increased by 8% when compared to the control. Applying the enzyme product on a very small portion (0.2%) resulted in no increase. Therefore, this enzyme product may be applied to as little as 4% of the diet, however to maximize digestion, application on a larger portion is recommended. Application of the enzyme prior to pelleting may reduce efficiency of the product, thus enzyme should be added after pelleting. Future studies are required to determine the mechanism whereby enzymes added to the diet of dairy cows increases digestibility.

Despite improvements in feed digestion, the milk production response to enzyme supplementation was minimal, which was attributed to the positive energy status of cows. However, the calculated energy balance revealed that cows receiving the CONC treatment had a larger surplus of energy once maintenance requirements were met, which if partitioned to production could increase daily milk output by 9% compared to cows not receiving the enzyme product. Cows in early lactation that are in a negative energy balance would likely benefit most from receiving an enzyme supplement.

Ruminal pH was not significantly depressed, nor was VFA concentration increased, when diets were supplemented with exogenous enzyme despite an increase in DM digestion. The lack of effect of supplemental feed enzymes on ruminal pH can be attributed to the

higher saliva secretion of cows receiving a diet supplemented with enzymes. Higher saliva secretion would have increased the buffering capacity within the rumen and may have compensated for increased ruminal fermentation due to increased digestibility of feed.

The enzyme product used did not alter the physical effectiveness of the feed to stimulate chewing. The absence of any pre-ingestive effects confirms that this enzyme product's main influence is within the rumen environment and possibly to a lesser extent in the lower digestive tract. Future studies are required to address the biochemical and microbiological action of exogenous enzymes within the digestive tract. Applying the enzyme product onto the feed prior to feeding may create a stable feed-enzyme complex, which acts as a vehicle to deliver the product into the digestive tract of the cow.

Parity differences exist in feeding behavior of lactating dairy cows. Multiparous cows consume feed at a faster rate than primiparous cows. To process the additional DM consumed, rumination time increases followed by an increase in saliva secretion. Despite the increase in saliva production, multiparous cows tend to have a lower rumen pH than primiparous cows, which may increase the risk of ruminal acidosis. Further research is needed to determine the full ramifications of low rumen pH on production and health of the cow.

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