

**THE EFFECT OF PRE-PARTUM DIET
ON THE SEVERITY OF POST-PARTUM RUMINAL ACIDOSIS
IN PRIMIPAROUS DAIRY COWS**

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ABSTRACT

Two experiments were conducted. In experiment 1, the objectives were: 1) to develop and evaluate the accuracy and precision of a new continuous ruminal pH measurement system 2) to determine the required frequency for pH electrode standardization and 3) to determine the effect of additional pre-partum concentrate when compared to NRC (2001) recommendations on post-partum ruminal acidosis in primiparous cows. Accuracy and precision of the Lethbridge Research Centre Ruminal pH measurement system (LRCpH) was determined by comparing LRCpH derived values against manual measurement. To determine the required frequency of electrode standardization, three treatments were imposed (24, 48, or 72 h of continuous measurement) and arranged in a repeated 3×3 Latin square design. The LRCpH accurately and precisely measured ruminal pH (repeated measures correlation coefficient = 0.97 and concordance correlation coefficient = 0.97 for 5-min averages). Changes in baseline mV readings for pH readings after 24, 48 or 72 h of ruminal incubation were not significantly different than zero, indicating that daily standardization of new electrodes was not essential. Using the LRCpH to measure ruminal pH overcomes animal mobility restrictions of previous systems.

In experiment 2, the effect of additional concentrate allocation during the pre-partum period was evaluated using 14 ruminally cannulated Holstein heifers. The heifers were assigned to one of two feeding regimes pre-calving: 1) control treatment or 2) an intensive high concentrate feeding treatment (HC). All cows received the same lactation diet post-partum. Ruminal pH was measured continuously from d -5 to d +5, and for 3-consecutive days starting on d +17 ± 1.2, d +37 ± 1.4, and d +58 ± 1.5 relative

to parturition. Feeding additional concentrate pre-partum did not reduce post-partum ruminal acidosis. In fact, animals fed the HC treatment had more daily episodes of acute acidosis and lower dry matter intake and body condition score than animals fed the control treatment. Day relative to parturition affected the occurrence and severity of ruminal acidosis with a dramatic increase in ruminal acidosis after parturition. This study demonstrates that feeding additional concentrate pre-partum did not reduce post-partum acidosis which emphasized the need to develop and implement feeding strategies that reduce this risk.

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LIST OF ABBREVIATIONS

Acute RA	5.2> Ruminal pH
ADF	Acid detergent fiber
BCS	Body condition score
BHBA	β -hydroxy butyric acid
BW	Body weight
CIpH	Continuous indwelling ruminal pH measurement system
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
F:C	Forage to concentrate ratio
HC	High concentrate treatment
LRCpH	Lethbridge Research Center Ruminal pH measurement system
Mild RA	5.8> ruminal pH >5.5
Moderate RA s	5.5> ruminal pH >5.2
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
NE _L	Net energy of lactation
NFC	Non-fibrous carbohydrate
RA	Ruminal acidosis
Total RA	Ruminal pH <5.8
VFA	Volatile fatty acids

1.0 GENERAL INTRODUCTION

The accepted definition of the transition period, as proposed by Grummer (1995), includes the last 3 wk of gestation and the first 3 wk of lactation. The transition period represents a complex phase within the production cycle of lactating dairy cows that is characterized by drastic changes in physiology, metabolism, nutrition, and environment. As a measure of its importance, several reviews have been published describing the challenges and opportunities of feeding cows during the transition period (Bell, 1995; Grummer, 1995; Goff and Horst, 1997; Drackley, 1999; Overton and Waldron, 2004). Successful transition of cows from a non-lactating pregnant state to a non-pregnant, lactating state is essential for optimum animal health, productivity, and farm profitability.

Much of the research conducted during the transition period deals with alleviating the negative energy balance (Dann et al., 1999; Rabelo et al., 2003) that occurs near parturition as nutrient requirements exceed nutrient intake (NRC, 2001). Overlooked in the literature is the effect of the pre-partum diet on the incidence of ruminal acidosis near parturition and in early lactation. Ruminal acidosis is considered to be a major problem in modern dairy production causing losses associated with treatment of sick cows, reduced milk production, and reduced longevity (Garrett et al., 1999). Transition cows may be susceptible to ruminal acidosis because DMI increases following parturition and an abrupt dietary change occurs at parturition, whereby cows are switched from a high forage diet to a high grain diet. Any abrupt dietary change predisposes cows to ruminal acidosis (Nocek, 1997). Introducing grain before calving has previously been shown to increase ruminal papillae surface area (Dirksen et al.,

1985) and adapt the microbial population to a high concentrate diet (NRC, 2001). Both of these mechanisms are thought to reduce the risk of ruminal acidosis. However, ruminal acidosis during the transition period has been overlooked as current instrumentation for continuous ruminal pH measurement requires animals to be tethered therefore, prohibiting use in maternity pens. Thus, the lack of instrumentation has limited the study of ruminal acidosis in transition cows.

The objectives of this study are to: 1) develop and evaluate the accuracy and precision of a continuous ruminal pH measurement system capable of measuring ruminal pH in unrestrained animals, 2) determine the required frequency of electrode standardization, and 3) determine the effect of providing additional concentrate pre-partum on the occurrence and severity of post-partum ruminal acidosis and lactational performance.

2.0 LITERATURE REVIEW

2.1 Ruminal Acidosis

Ruminal acidosis continues to be a problem in modern dairy production. In a surveillance study conducted in the United States, one-third of the herds had a prevalence rate of ruminal acidosis greater than 40%, while the presence of ruminal acidosis during early lactation and mid lactation was 19% and 26%, respectively (Garrett et al., 1999). Furthermore, Krause and Oetzel (2006) reported that the prevalence of ruminal acidosis (ruminal pH < 5.5) throughout d 0 to d 140 days in milk ranged between 12% and 30% (see Figure 2.1). They indicated that the prevalence of ruminal acidosis increased with increasing DMI. The demand for high milk production requires that lactation diets contain high levels of energy. Often, concentrates that are rapidly fermented in the rumen are used to increase the dietary energy content. As a result, there is a balance required between providing concentrate to increase the energy density of the diet and increased risk of ruminal acidosis.

Sub-acute ruminal acidosis (SARA) occurs in dairy cattle when fermentation acid production exceeds buffering mechanisms in the rumen. This situation results from consuming large quantities of concentrate or diets with low structural value (Nocek, 1997; Krause and Oetzel, 2006). The main buffering mechanisms include; absorption of VFA from the rumen (Remond et al., 1996), passage of volatile fatty acids (VFA) out of the rumen (Remond et al., 1996), and salivary secretion of bicarbonate (Allen, 1997). Any accumulation of VFA in the rumen leads to a reduction in ruminal pH (Krause and Oetzel, 2006). Acute acidosis differs from SARA as there is an accumulation of lactic

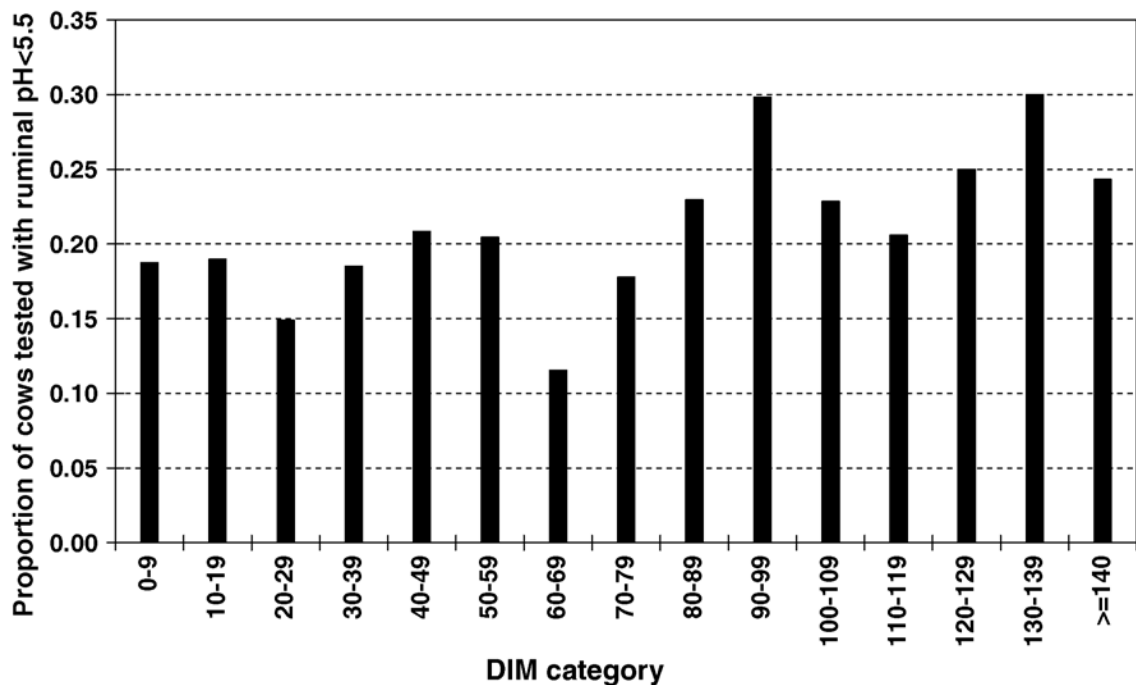


Figure 2.1. Risk of low ruminal pH (<5.5) by days in milk from 662 cows in 55 herds.

Source: Krause and Oetzel, 2006.

acid which is a more potent driver of reduced ruminal pH than VFA (Owens et al., 1998). Lactic acid accumulation rarely occurs in dairy cows and, when lactic acid concentrations do increase, the increase is often short lived (Krause and Oetzel, 2005). Thus, acidosis in dairy cattle is a result of VFA accumulation rather than the reduction of pH associated with lactic acid concentration.

2.1.1 Risk of Acidosis

The risk of acidosis is not equal for all animals. Animals with higher levels of DMI are at a greater risk for acidosis (Nocek, 1997; Stone, 2004) as higher levels of DMI also correspond to a greater VFA production. Maekawa et al. (2002) supported this concept as they suggested that multiparous animals have a higher risk of ruminal acidosis than primiparous cows due to higher levels of DMI. Stone (2004) proposed that cows with a high degree of variability in their pattern of consuming feed throughout the day and between days are also at a high risk of ruminal acidosis. Finally, cows that are not adapted to a diet containing high levels of concentrate are at risk (Nocek, 1997; Stone, 2004). Transition cows may not be adequately adapted to the lactation diet as pre-partum diets often contain high levels of forage but, at parturition an abrupt dietary change occurs whereby animals are switched to a high grain diet. This abrupt dietary change predisposes transition cows to ruminal acidosis (Nocek, 1997). Furthermore, DMI drastically increases following parturition, increasing the risk for ruminal acidosis. Gröhn and Bruss (1990) observed that the greatest number of diagnosed cases of ruminal acidosis occurred in cows during the first few months after calving. This

further confirms that transition animals may be at high risk for ruminal acidosis, although no known studies have been conducted to adequately characterize acidosis in transition cows.

2.1.2 Effects of Acidosis on Digestibility

Extended periods of low pH are toxic to ruminal cellulolytic bacteria (Russell and Wilson, 1996); thus, low ruminal pH has a negative effect on fiber digestibility. However, there is no consensus on the critical ruminal pH threshold, below which fiber digestion is reduced. The critical threshold for individual bacterial species within the rumen may differ, but most studies have examined the effects of pH on the mixed ruminal population. Russell and Wilson (1996) conducted a review of the literature and suggested a threshold of 6.0 for ruminal acidosis, as growth of cellulolytic bacteria was inhibited below this pH. Calsamiglia et al. (2002) proposed a slightly lower threshold based on the effect of pH and pH fluctuations on microbial fermentation and nutrient flow using continuous culture methods. Results show that when pH is maintained at 5.7 there is a reduction in neutral detergent fiber (NDF) digestibility, acid detergent fiber (ADF) digestibility, a reduction in total VFA concentration, and a reduction in crude protein (CP) degradation. However, transient fluctuations from pH 6.4 to pH 5.7 did not decrease digestibility. Results of that study indicate that ruminal bacteria are able to cope with intermittent episodes of low ruminal pH without having an impact on fiber digestibility. Thus, the definition of ruminal acidosis needs to incorporate both a pH threshold and time factor. In 2003, Schwartzkopf-Genswein et al. (2003) suggested a ruminal pH threshold for acidosis of 5.8 for at least 12 h/d. While this recommendation

incorporates a time factor, the recommendation was for beef cattle on a mainly grain-based diet where little forage is included. Dairy rations often contain between 40 and 55% forage and a ruminal pH <5.8 for at least 12 h/d would have negative implications on fiber digestion and, thus nutrient supply. In another study, Krajcarski-Hunt et al. (2002) induced ruminal acidosis in four lactating dairy cows and observed that the NDF and ADF digestibility of corn silage, grass hay, and legume hay were all reduced when ruminal pH was decreased from a mean of 6.4 to 5.7. However, the reduction in digestibility occurred when the time below ruminal pH 6.0 increased by nearly 6 h and the time below ruminal pH 5.6 increased by 2.3 h for the acidosis induction treatment compared to the control treatment. Thus, for dairy cows, the time factor included in the ruminal pH threshold needs to be less than that proposed for beef cattle (Schwartzkopf-Genswein et al., 2003).

2.1.3 Effects of Acidosis on Animal Health

Common effects of ruminal acidosis on animal health include; intermittent diarrhea, erratic appetite, and BW loss. In addition, changes to the ruminal epithelium occur as a result of ruminal acidosis. Ruminal papillae are responsible for absorbing VFA and the growth of ruminal papillae can be improved by feeding additional grain. However, too much grain can result in ruminal acidosis, which negatively affects ruminal papillae. For example, microlesions were found on ruminal papillae in sheep fed wheat grain (McManus et al., 1977). In calves fed high concentrate diets, perakeratotic ruminal papillae and cauliflower shaped papillae were observed (McGavin and Morrill, 1976). Lesions on the ruminal epithelium have been implicated with

systemic bacterial infiltration, which can lead to liver abscesses (Owens, 1998). Perakeratosis also reduces the absorptive capability of the ruminal epithelium for up to six months after the incidence of acidosis (Krehbiel et al., 1995).

Ruminal acidosis increases lipopolysaccharide concentration in ruminal fluid (Gozho et al., 2005). It is speculated that lipopolysaccharides cross the ruminal epithelium and enter portal blood triggering the immune system resulting in the production and release of acute phase proteins (Murata et al., 2004; Gozho et al., 2005). Gozho et al. (2005) conducted an experiment to determine how changes in rumen fluid lipopolysaccharide concentrations affect serum acute phase proteins and observed an increase in serum haptoglobin and serum amyloid-A concentrations. Results from that study indicate that ruminal acidosis increases lipopolysaccharide concentration in the ruminal fluid increasing translocation into the blood resulting in an inflammatory response. Ruminal acidosis has also been implicated as a causative factor for laminitis by increasing histamine concentration in ruminal fluid and serum of cattle (Nocek, 1997; Nordlund et al., 2003).

2.2 Measurement of Ruminal pH

Measurement of ruminal fluid pH is the only reliable and accurate diagnostic test for ruminal acidosis. For this reason, various techniques are available for measuring ruminal pH under both experimental and field conditions. Techniques are broadly classified as spot-sampling methods or continuous methods. Spot sampling methods

include rumenocentesis, oro-ruminal probes, and direct ruminal fluid measurement via a ruminal cannula. Continuous measurement techniques measure ruminal pH values with relatively short intervals between readings usually over several days.

Rumenocentesis has been used to collect ruminal fluid samples for measurement of ruminal pH under both experimental and field conditions (Oetzel and Nordlund, 1998; Garrett et al., 1999; Duffield et al., 2004). Rumenocentesis can be a useful diagnostic technique for ruminal acidosis in commercial settings as it does not require ruminal cannulation and is an accurate means of measuring ruminal pH when compared to oro-ruminal probes (Duffield et al., 2004). When compared to ruminal fluid measurement via a ruminal cannula, rumenocentesis had an accuracy of 81%. However, the usefulness of rumenocentesis in research studies is limited by the risk of peritonitis, even with adequate surgical preparation (Keefe and Ogilvie, 1997; Duffield et al., 2004).

Alternatively, various oro-ruminal probes are available, notably the one designed by Geishauser (1993). However, ruminal fluid samples collected through oro-ruminal probes are prone to salivary contamination leading to inaccurate ruminal pH values. Furthermore, sampling location within the rumen is unknown (Keefe and Ogilvie, 1997; Duffield et al., 2004). Duffield et al. (2004) recommended discarding the first 200 ml to reduce the potential of salivary contamination. Generally, samples collected and measured using an oro-ruminal probe are 0.2 pH units higher than when samples are collected via a ruminal cannula (Oetzel and Nordlund, 1998). Ruminal pH determined using an oro-ruminal probe and only has an accuracy of 51% (Duffield et al., 2004).

Direct ruminal fluid sampling via a ruminal cannula is still a common method for ruminal pH measurement in controlled research studies (Reis and Combs, 2000; Kononoff et al., 2003; Duffield et al., 2004). The major limitation of spot sampling techniques (rumenocentesis, oro-ruminal probes, direct sampling via a ruminal cannula) is they only indicate ruminal pH at one point in time. Adequate characterization of daily ruminal pH variation requires regular measurement of ruminal pH with short intervals between samples making it too tedious and labour intensive for spot sampling techniques.

Monitoring ruminal pH has been automated with the advent of continuous indwelling pH systems (Dado and Allen, 1993). Continuous indwelling systems have provided comprehensive data improving the characterization of post-feeding ruminal pH variation by collecting more frequent measurements when compared to spot sampling techniques. Increasing the number of measurements collected has increased our understanding of the interactions between diet fermentability, meal size, eating behaviour and ruminal pH (Maekawa et al., 2002; Krause and Combs, 2003). The reported correlation between manual ruminal fluid measurement via a ruminal cannula and continuous indwelling measurement was 0.85 which indicates high precision (Dado and Allen, 1993). Most continuous indwelling pH systems require animals to be tethered and thus, restrict animal mobility and application (Dado and Allen, 1993; Krause and Combs, 2003; Bevans et al., 2005). Recently, several stand-alone systems have been developed (Enemark et al., 2003; Graf et al., 2005). Stand-alone systems continuously measure reticular or ruminal pH without the use of external cables; thereby, allowing the measurement of ruminal pH in grazing or loose-

housed animals. Although stand-alone ruminal pH measurement systems have been developed, development of a new system is warranted as the one designed by Enemark et al. (1997) measures reticular pH and the current study commenced prior to the development of a stand-alone system by Graf et al. (2005). Furthermore, stand-alone ruminal pH measurement systems are in limited use because a thorough validation of these systems is lacking.

2.3 Characteristics of the Transition Period

The transition period begins 3 wk before calving and ends 3 wk after calving (Grummer, 1995). During the transition period, cows undergo changes to endocrine and metabolic status as they prepare for parturition, and the onset of lactation. Nutritional management of transition cows must account for the gradual pre-partum reduction in DMI that occurs at a time when nutrient requirements are increasing (NRC, 2001). Even though DMI rapidly increases immediately after parturition, energy intake is still lower than energy output (Rabelo et al., 2005). Furthermore, an abrupt dietary change occurs at parturition whereby cows are switched from a primarily forage diet to a highly fermentable, energy dense lactation diet. This abrupt dietary change, coupled with increasing DMI post-partum, greatly increases the risk of post-partum ruminal acidosis (Nocek, 1997). To lessen the risk of acidosis, some dairy nutritionists promote the concept of supplying more concentrate pre-partum such that physiological and microbial adaptation of the rumen occurs before parturition. The recommendation is based on the

concept that adapting the ruminal environment will lessen the abrupt changes that occur at calving and, will thereby, reduce the risk of acidosis and other metabolic diseases post-partum.

2.3.1 Current NRC Recommendations

Current recommendations of the NRC (2001) suggest feeding non-lactating, pregnant dairy cows a “close-up” diet (i.e., a diet fed before parturition) that contains a higher nutrient density than the “far-off” diet (i.e., diet fed after dry-off). Increasing the energy density of the close-up diet is designed to overcome the energy deficiency that is caused by decreasing DMI and increasing nutrient requirements as parturition approaches. The increase in dietary energy density is also intended to help promote ruminal adaptation through microbial changes and proliferation of the ruminal papillae (NRC, 2001). As nutrient intake is a function of both DMI and dietary energy density, the NRC (2001) uses the equations derived by Hayirli et al. (2002) to predict daily DMI as a percentage of BW with different equations for heifers and cows. Using these prediction equations, the NRC recommends a close-up diet for heifers and cows that contains 1.62 Mcal NE_L /kg DM compared (DM) to 1.72 Mcal NE_L /kg DM for cows in early lactation. Although heifers have lower DMI and therefore require a diet with a higher energy density than multiparous cows to meet energy requirements, no separate recommendations were made for cows differing in parity. Based on our current knowledge of DMI, a close-up diet containing 1.62 Mcal NE_L /kg DM should on average meet the energy requirements of cows for the majority of the pre-partum period but may not meet the requirements for heifers. Furthermore, the NRC (2001) does not indicate

when to initiate feeding the pre-partum diet. Research is required to more clearly define the nutrient requirements, recommended dietary energy density, and general feeding management strategies for primiparous and multiparous animals to meet energy requirements while reducing metabolic disorders during the transition period.

2.3.2 Effect of Pre-partum Diet on Post-partum Ruminal pH

Few studies have evaluated the effect of pre-partum diet on post-partum ruminal pH. In two studies, pre-partum diet had no effect on post-partum ruminal pH (Dann et al., 1999; Rabelo et al. 2003). However, Andersen et al. (1999) reported that providing pre-partum and post-partum concentrate in one feeding rather than dividing the concentrate into two equal feedings reduced post-partum ruminal pH when all diets were fed in a component feeding system (Figure 2.2). Furthermore, in all known studies, ruminal pH measurements were conducted using a spot sampling technique, which does not provide detailed information on daily ruminal pH variation. Therefore, the effects of the pre-partum diet on the occurrence and severity of ruminal acidosis is not well documented.

2.3.3 Effect of Pre-partum Diet on Ruminal Papillae Adaptation

The positive effect of pre-partum dietary concentrate on ruminal papillae proliferation has been well documented (Galfi et al., 1991). This effect is indirectly mediated through the stimulatory effect of volatile fatty acids (VFA) especially butyrate (Sakata and Tamate, 1978) on papillae growth (Dirksen et al., 1985). However, insulin is the main driver for ruminal papillae proliferation (Sakata et al., 1980).

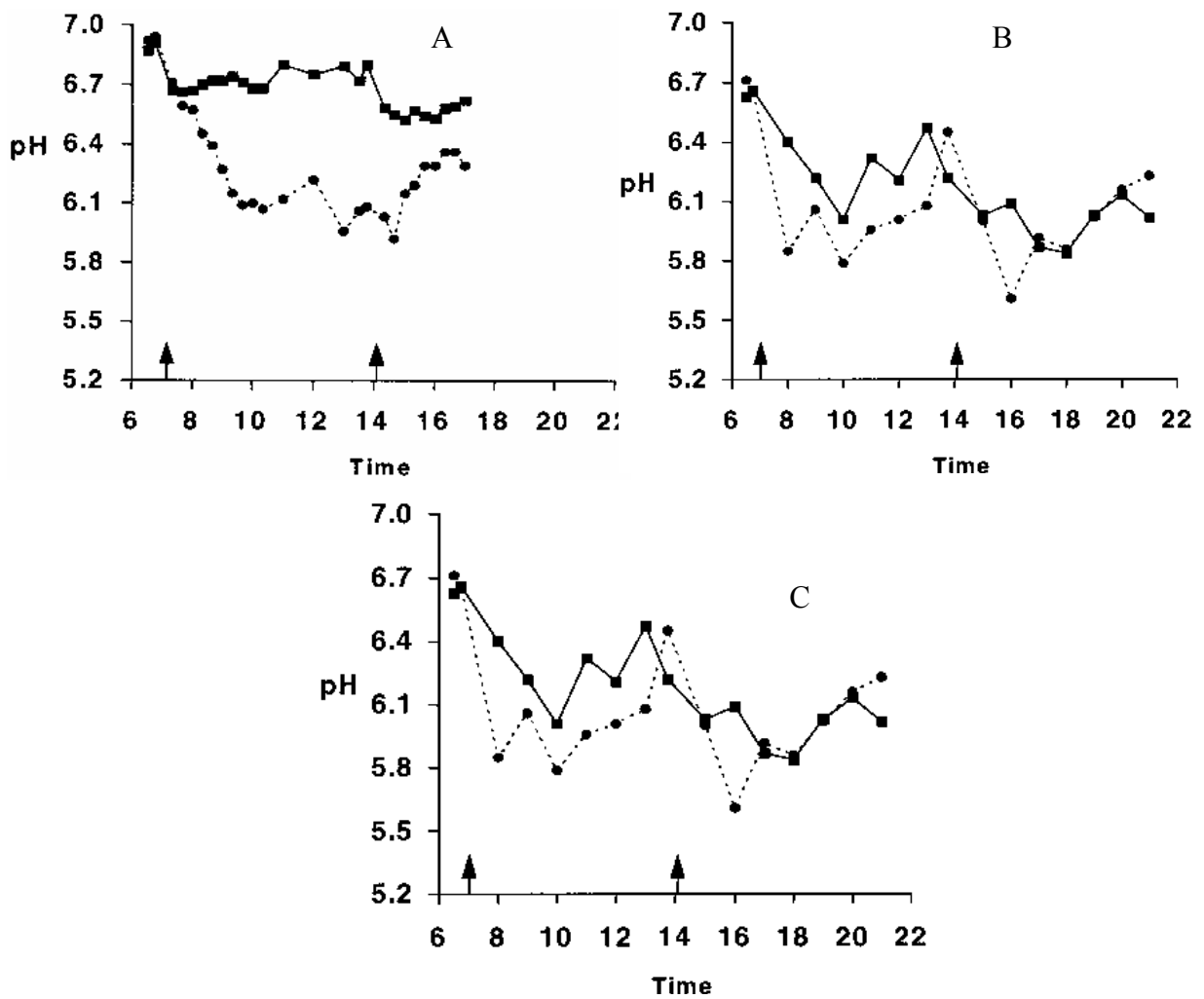


Figure 2.2. Mean ruminal pH on d -10 (A), d +8 (B) and d +28 (C) for cows fed the same amount of concentrate divided in one (dashed line; n=4) or two equal feedings daily (solid line; n=4) daily. Arrows indicate time of feeding. Adapted from: Andersen et al. (1999)

Recommendations for feeding concentrate pre-partum are based on the underlying principle that increased production of VFA would increase growth of ruminal papillae. Understanding the mechanisms responsible for papillae proliferation during the transition period are essential for developing nutritional management strategies that meet the nutritional requirements of transition dairy cows while also promoting papillae proliferation. Dirksen et al. (1985) conducted two experiments to: 1) determine the required time for ruminal papillae adaptation, and 2) investigate the relationship between ruminal papillae structure and absorptive capacity. In study 1, nine ruminally cannulated Holstein-Friesian cows were used starting 8-weeks pre-partum until 8 weeks post-partum. At the beginning of the dry period, the energy content of the diet was drastically reduced and an energy dense lactation ration was fed starting 2-weeks before parturition. Ruminal biopsies were conducted weekly or bi-weekly and analyzed morphometrically and microscopically. Two non-lactating cows were used in second study to evaluate changes in absorptive capacity. Cows were fed a dietary treatment pattern containing low energy for 7-14 wk, high energy for 7-14 wk followed by low energy for 7-14 wk. Absorption experiments were conducted at the end of each treatment. Proliferation of ruminal papillae (experiment 1) increased after the introduction of the energy dense lactation diet but ruminal papillae surface area was not maximized until 6-7 weeks after diet introduction. In the second study, the high energy diet resulted in increased disappearance of ruminally infused VFA when compared to the low energy diets. These results suggested that absorptive capacity of the rumen increases in response to increased papillae size and therefore increased absorptive surface area.

Subsequent studies do not substantiate the conclusions of Dirksen et al. (1985). For example, Andersen et al. (1999) conducted an experiment to determine the effect of dry cow feeding strategy on ruminal epithelium but, observed no differences between cows fed the control diet or cows fed a VFA-loaded diet. Use of a VFA-loaded diet simulated the effects of feeding a high concentrate diet. More recently, Rabelo et al. (2001) fed diets with low- or high-energy density 97 d pre-partum in a Latin square design. Energy density had no effect on ruminal papillae length or width. However, in that study, experimental periods were only 21 d long which may have been too short to detect changes. Reynolds et al. (2004) fed three pre-partum diets: control, control + supplemental barley, and control + supplemental protein (rumen protected soybean protein) and observed no effect of dietary treatment on papillae weight, density or length. Contrary to the findings of Dirksen et al. (1985), additional concentrate and protein decreased mean papillae width and mean papillae surface area (Reynolds et al., 2004). However, the reduction in papillae width did not reduce total surface area when represented as mm^2/cm^2 .

While feeding additional concentrate indirectly initiates papillae proliferation, diets with low energy density and hence a high forage content have been implicated with the regression of ruminal papillae (Dirksen et al., 1985). The main regulatory mechanism for papillae regression is low production of VFA, and therefore, low insulin levels. In addition to low insulin concentrations, high forage diets also have an abrasive nature that increases epithelial cell sloughing. Greenwood et al. (1997) examined the effect of diet abrasion on ruminal papillae using 12 Holstein bull calves assigned to one of three dry feeds differing only in particle size. The dietary abrasive value was

determined by measuring how much paraffin wax was removed from a mixer beater when the diets were mixed for 1.5 h. In calves fed coarse diets (high abrasive value) the ruminal epithelium contained less keratin, and had shorter papillae length than the fine particle size diet. These results indicate that the dietary abrasive value also has an impact on the surface area and keratinization of ruminal papillae. Thus, keratinization of ruminal papillae can be reduced if cows consume an adequate amount of forage. Additional forage may also reduce the risk of ruminal acidosis; however, the inclusion of dietary forage reduces dietary energy density and may limit animal performance.

2.3.4 Effect of Pre-partum Diet on Pre- and Post-partum DMI

Increasing the concentrate proportion of the diet is one method used to improve energy supply during the pre-partum portion of the transition period. Generally, supplying additional concentrate pre-partum increases pre-partum DMI (Keady et al., 2001; Rabelo et al., 2003; Kokkonen et al., 2004). For example, Rabelo et al. (2003) fed diets containing 1.58 or 1.70 Mcal NE_L/kg DM for 28 d before the expected calving date. Animals fed the high energy diet pre-partum consumed 19% more DM pre-partum than animals fed the low energy diet. Furthermore, Hayirli et al. (2002) collected data from nearly 700 Holstein cows in 16 different experiments. Data were used to determine factors affecting DMI during the pre-partum transition period. Significant correlations were reported for DMI and dietary non-fibrous carbohydrate (NFC) concentration ($r = 0.14$) and DMI and neutral detergent fiber (NDF) concentration ($r = -0.12$). Thus, pre-partum cows fed diets with less NDF consumed more DM.

A recent publication does not support the conclusion that increasing the concentrate proportion of the pre-partum diet improves DMI during the pre-partum portion of the transition period (Reynolds et al., 2004). Reynolds et al. (2004) conducted two experiments in which cows were fed a control diet or the control diet supplemented with energy (barley) or energy and protein (soybean meal). Additional energy or protein did not affect DMI, but it is possible that the results may have been different had more barley been fed.

While feeding additional concentrate pre-partum may increase overall pre-partum DMI, there seems to be a greater reduction in DMI around parturition associated with high grain diets. Rabelo et al. (2003) observed a more dramatic drop in DMI for cows fed diets with high energy density when compared to cows fed a diet with low energy density. Furthermore, Hayirli et al. (2002) reported an interaction between time and NDF concentration for DMI, which suggests that pre-partum cows fed diets containing low NDF proportions have a greater reduction in DMI around parturition than cows fed high levels of NDF.

Generally, increased DMI pre-partum as a result of high dietary concentrate does not have any carry over effects into the post-partum period. For example, Halcomb et al. (2001) fed diets with high or low forage content and found no effect of pre-partum diet on post-partum DMI. This finding is in agreement with many other studies (Dann et al., 1999; Keady et al., 2001; Rabelo et al., 2003; Smith et al., 2005).

2.3.5 Effect of Pre-partum Diet on Energy Balance

One goal of transition feeding programs is to limit the negative energy balance that occurs around parturition. Diets higher in concentrate are inherently higher in energy, and also increase DMI. Because nutrient intake is a function of DMI and dietary nutrient density (NRC, 2001), increasing the concentrate portion of the diet should improve energy supply and reduce the negative energy balance in early lactation. Energy balance is evaluated by measuring energy intake and subtracting energy output (NRC, 2001). Additionally, non-esterified fatty acid (NEFA) concentration, which is an indicator of fat mobilization, is often used to evaluate energy status (Overton and Waldron, 2004).

Although feeding high energy diets post-partum improves the energy status of cows, this is not the case for high energy pre-partum diets (Rabelo et al., 2005). For example, Rabelo et al. (2005) fed diets with low or high energy density and evaluated energy balance using liver triglyceride content, plasma NEFA concentration, and by calculating the difference between energy intake and energy expenditure. Increasing the energy density of the diet did not improve energy balance or liver triglyceride content; however, there was a tendency ($P = 0.10$) for high energy dense diets to decrease NEFA concentration which suggests a reduction in body fat mobilization. As mentioned previously, feeding high energy dense diets pre-partum increases total pre-partum DMI; however, they also result in a greater reduction in DMI near parturition. According to the NRC (2001), nutrient requirements are greatest around parturition. Thus, although cows may be offered a diet containing a high density of energy, the greater reduction in DMI near parturition masks the benefits and does not improve energy balance.

2.3.6 Effect of Pre-partum Diet on Lactational Performance

Milk yield and composition are generally not affected by providing additional concentrate pre-partum (Halcomb et al., 2001; Keady et al., 2001; Rabelo et al., 2003; Smith et al., 2005). Furthermore, increasing the length of time that concentrate (3 wk vs. 6 wk) is fed pre-partum does not improve lactational performance (Mashek and Beede, 2001). Conversely, McNamara et al. (2003) fed three pre-partum diets: 1) grass silage and straw, 2) grass silage alone, or 3) grass silage with 3 kg/d of additional concentrate. Cows fed the pre-partum diet containing silage and straw had lower milk yield than cows fed the silage or silage and concentrate treatments. However, in that study, cows fed the grass silage and straw pre-partum diet had lower pre-partum DMI and thus lower energy intake than cows fed the other treatments. Based on the results from the previous studies, it appears that additional concentrate pre-partum does not improve lactational performance unless dietary energy is limiting.

2.4 Summary

Ruminal acidosis continues to be a problem in modern dairy production as incidence rates range from 12 to 30% with the highest incidences occurring within the first few months of calving. The demand for high milk production requires that lactation diets contain high dietary energy density. Thus, diets are often formulated using rapidly fermentable carbohydrates. Ruminal acidosis reduces ruminal fiber degradation which decreases nutrient supply. Furthermore, animals with ruminal acidosis have variable DMI, lose BCS, and have intermittent diarrhea. Ruminal acidosis

also increases lipopolysaccharide concentration in the rumen resulting in an inflammatory response. Acidosis is also a main risk factor for laminitis and the prevention of acidosis is of great importance for animal health and productivity.

The inclusion of additional concentrate in pre-partum diets is common practice on both North American and European dairy farms. This practice has been designed to increase pre-partum dietary energy intake, adapt ruminal microflora, and stimulate the proliferation of ruminal papillae to better cope with dietary change that occurs at parturition. Proliferation of the ruminal papillae results in increased absorptive surface area of the rumen and has been hypothesized to reduce ruminal acidosis. However, there is a paucity of information on the effect of pre-partum diet on post-partum ruminal acidosis. Animal variability during the transition period and lack of instrumentation has prohibited continuous measurement of ruminal pH in transition dairy cows. In previous studies where ruminal pH was measured, pre-partum diet had no effect on post-partum ruminal pH. However, both studies used occasional spot sampling to measure ruminal pH. Spot sampling techniques do not provide as detailed information as continuous pH measurement and daily variation in ruminal pH would have gone undetected.

Therefore, the objectives of this study were: 1) to develop and evaluate the accuracy and precision of a stand-alone continuous ruminal pH measurement system, 2) determine the required frequency of electrode standardization, and 3) determine the effect of additional pre-partum concentrate on the occurrence and severity of post-partum ruminal acidosis and lactational performance in primiparous Holstein cows.

3.0 AN EVALUATION OF THE ACCURACY AND PRECISION OF A STAND-ALONE SUBMERSIBLE CONTINUOUS RUMINAL PH MEASUREMENT SYSTEM¹.

3.1. Absract

The objectives of this study were to develop and evaluate the accuracy and precision of a new stand-alone submersible continuous ruminal pH measurement system (designated LRCpH) and to compare it to the accuracy and precision of a previously used continuous indwelling ruminal pH system (designated CIpH) and to determine the required frequency for pH electrode standardization. In total, 11 ruminally cannulated cows were used. Mean pH values were measured using one of the continuous pH measurement systems and compared to ruminal pH values obtained using spot samples of ruminal fluid (designated MANpH) obtained at the same time. A correlation coefficient accounting for repeated measures was calculated and results were used to calculate the concordance correlation. The comparison of the LRCpH output to MANpH had higher repeated measures correlation coefficient (0.97 for 5-min average) and concordance correlation coefficient (0.97, for 5-min average) than the comparison of CIpH to MANpH (0.88 and 0.87, repeated measures correlation coefficient and concordance correlation coefficient, respectively). The plotted data from both

¹ A version of this chapter has been published. Penner, G.B., K.A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89: 2132-2140.

continuous measurement systems closely resembled the line $y = x$ indicating both systems were accurate and precise. Changes in baseline mV readings for pH readings after 24, 48 or 72 h of ruminal incubation were not significantly different than zero, indicating that daily standardization of new electrodes was not essential. Results from this study indicate that the LRCpH system can accurately and precisely measure ruminal pH, thus it provides increased opportunity for researchers to measure ruminal pH and the occurrence of ruminal acidosis in unrestrained cattle.

3.2 Introduction

Ruminal acidosis is a common problem in modern ruminant production, particularly in high-producing dairy herds (Nocek, 1997) as the demands for increased milk production have resulted in high grain, low fiber diets being the norm in order to maximize energy intake. Feeding high grain diets can result in a decrease in ruminal pH due to the ruminal accumulation of VFA and, to a smaller extent, lactate, thus resulting in acute or sub-acute ruminal acidosis (Owens et al., 1998). Dairy cows experiencing ruminal acidosis can exhibit a range of clinical symptoms, including; decreased milk production, diarrhea, and laminitis (Underwood, 1992; Nocek, 1997). Measurement of ruminal fluid pH is the only reliable and accurate diagnostic test for ruminal acidosis. For this reason, various techniques are available for measuring ruminal pH under both experimental and field conditions.

Rumenocentesis and oro-ruminal probes have been used to collect ruminal fluid samples for measurement of ruminal pH under both experimental and field conditions (Oetzel and Nordlund, 1998; Garrett et al., 1999; Duffield et al., 2004). However, the usefulness of rumenocentesis in research studies is limited. Furthermore, the risk of peritonitis even with adequate surgical preparation may discourage the use of this technique (Keefe and Ogilvie, 1997; Duffield et al., 2004). Alternatively, various oro-ruminal probes are available, notably the one designed by Geishauser (1993); however, ruminal fluid samples collected through oro-ruminal probes are prone to salivary contamination leading to inaccurate ruminal pH values (Keefe and Ogilvie, 1997; Duffield et al., 2004). Direct ruminal fluid sampling via a ruminal cannula is still a common method for ruminal pH measurement in controlled research studies (Reis and

Combs, 2000; Kononoff et al., 2003; Duffield et al., 2004). However, spot sampling techniques (rumenocentesis, oro-ruminal probes, direct sampling via a ruminal cannula) all have inherent limitations as they only indicate ruminal pH at one point in time. Additionally, characterizing ruminal pH variation over time using spot sampling techniques requires regular sampling with short intervals between samples, thus making it tedious and labour intensive.

Monitoring ruminal pH has been automated with the advent of continuous indwelling pH systems (Dado and Allen, 1993). Continuous indwelling systems have provided data allowing improved characterization of post-feeding ruminal pH variation which has increased our understanding of the interactions between diet fermentability, meal size, eating behavior and ruminal pH (Maekawa et al., 2002; Krause and Combs, 2003). Most continuous indwelling pH systems have inherent limitations as they restrict animal mobility and, thus, application is limited to tethered animals (Dado and Allen, 1993; Krause and Combs, 2003; Bevans et al., 2005). Recently, several stand-alone systems have been developed (Enemark et al., 2003; Graf et al., 2005). These stand-alone systems continuously measure reticular or ruminal pH without the use of external cables; thereby, allowing the measurement of ruminal pH in grazing or loose-housed animals. Although stand-alone ruminal pH measurement systems have been developed, they are in limited use and a thorough validation of these systems is lacking. Therefore, the objectives of the present study were: 1) to develop a stand-alone submersible ruminal pH measurement system for use in cattle and to evaluate its accuracy and precision by comparing its output to pH measurement of ruminal fluid samples collected via a ruminal cannula; 2) to compare the stand-alone system to an older, well-

documented indwelling ruminal pH measurement system; and 3) to determine the required frequency of electrode standardization necessary to minimize changes in baseline mV readings between the start and end of the measurement period.

3.3 Materials and Methods

3.3.1 Design and Evaluation of the Lethbridge Research Centre pH Measurement System (LRCpH)

Three-lactating, primiparous Holstein cows (606 ± 34 kg BW; 25 ± 1 DIM), 6 pregnant Holstein heifers (634 ± 92 kg BW), and 2 Black Angus, non-pregnant heifers (512 ± 7 kg BW) were used in this experiment. All animals were fitted with permanent ruminal cannulae. The 3 lactating Holstein cows were offered ad libitum a diet consisting of (DM basis) barley silage (42%), barley grain (29%), a protein, mineral, and vitamin supplement (16%), and alfalfa hay (4%). The 6 pregnant Holstein heifers were fed ad libitum a diet consisting of (DM basis) barley silage (69%), a protein, mineral and vitamin supplement (17%), grass hay (12%), and barley grain (7%). All diets were fed as a total mixed ration (TMR) at 1330 h daily. The 2 Black Angus heifers were offered ad libitum a diet consisting of (DM basis) barley grain (88%), barley silage (9%), and a supplement containing minerals and vitamins (3%). This TMR was fed at 1630 h daily. The rationale for using 11 experimental animals at different physiological states and fed a wide range of diets was to determine the sensitivity of the LRCpH system under a wide range of pH values and ruminal conditions (i.e., rumen size, ruminal mat structure, etc.). Experimental animals were housed in individual tie stalls at

the Lethbridge Research Centre. In all experiments, experimental animals were cared for according to the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada) and the Lethbridge Research Centre Animal Care Committee approved their use for this study.

The LRCpH system was developed using a data logger (model M1b-pH-1KRTD, Dascor, Escondido, CA), 9-volt battery, and an electrode cable (model S653-ATC-20-BNC, Sensorex, Garden Grove, CA) which were housed in a water tight capsule constructed out of PVC material (Figure 3.1). The pH electrode (model S650-CDHF, Sensorex, Garden Grove, CA) was covered by a 38-mm diameter shroud with four 25-mm holes that was designed to allow particle and liquid passage while preventing the electrode from contacting the ruminal epithelium. Two 900-g weights were fastened to the bottom of the electrode shroud to maintain the electrode in the ventral sac of the rumen. A 30-cm polyester cable was connected to the capsule and the ruminal cannula plug to aid in system location within the rumen and to help maintain the electrode in a vertical position.

With the LRCpH system, ruminal pH was monitored continuously for 24-h during each collection period. Ruminal pH readings were taken every 30 s and readings were averaged over 1- and 5-min intervals, and then stored. A 30-s reading interval was used for the LRCpH system to reduce the amount of memory required for data storage as battery power usage by the data logger increases with memory use. Prior to measuring ruminal pH, readings from the electrodes were recorded in buffers 4 and 7. Data transfer from the data logger to a computer and standardization of the pH

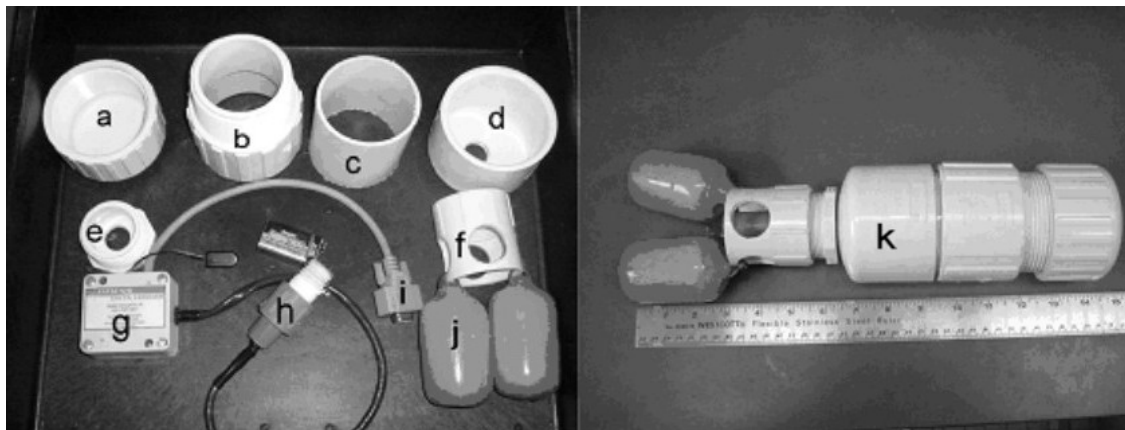


Figure 3.1. Illustration of the Lethbridge Research Centre ruminal pH measurement system (LRCpH) in unassembled and assembled forms. a. threaded cap, b. male adapter, c. pipe, d. slip cap, e. adapter, f. electrode shroud, g. data logger and pH meter, h. electrode sensor cable, i. computer interface cable, j. 900-g weight, k. assembled LRCpH system.

electrodes were conducted daily around 1230 h. Briefly, the LRCpH was removed from the rumen, and the pH electrode was then washed in water at 39°C, and mV readings were recorded in pH 4 and 7 buffer solutions. During this time, pH electrodes and pH buffers were maintained at 39°C until the data were downloaded and mV recordings were taken. The electrodes used have a high thermal mass and maintaining them at 39°C reduces the requirement for temperature compensation. The shift in mV readings from the electrodes between the start and finish standardization was assumed to be linear and was used to convert mV readings to pH units.

For manual pH measurement (MANpH), the ruminal cannula plug was opened and a covered 20-ml container was submersed into the rumen contents. Upon submersion, the LRCpH electrode was located within the rumen and ruminal fluid was allowed to fill the container from the immediate location of the LRCpH electrode. The open end of the container was again covered, removed from the rumen, the ruminal cannula plug was replaced and ruminal fluid pH was immediately measured using a portable pH meter (Model IQ150, IQ Scientific Instruments Inc., San Diego, CA) with a glass electrode (Model PHE-1411, Omega Engineering, Stamford, CT). The portable pH meter was calibrated with a two-point calibration once daily using pH 4 and 7 buffer solutions. Re-calibration occurred if readings obtained with pH buffer solutions were not within 0.02 pH units. The time of ruminal fluid collection was recorded in order to compare the MANpH method to results obtained from corresponding LRCpH measurement. During ruminal fluid collection, positioning of the LRCpH was noted. Ruminal fluid samples were collected on 4 d over a 2-wk duration. On day 1, ruminal fluid samples were collected from the 6 pregnant Holstein heifers every 30 min starting

1 h post-feeding until 3 h post-feeding, and sampling commenced again 8 h post-feeding and ending 10 h post-feeding. Thus, 66 ruminal fluid samples were collected in total on day 1. On day 2, the 3 lactating Holstein cows were sampled every 30 min starting 2.5 h post-feeding and ending 6.5 h post-feeding. In total, 21 ruminal fluid samples were collected. On d 3, ruminal fluid was again collected from the 3 lactating Holstein cows. However, one cow was omitted from this collection period as her ruminal contents were very dry which prohibited adequate LRCpH placement and ruminal pH measurement. Ruminal fluid sampling was started 6 h pre-feeding and ended at 0.5 h pre-feeding with samples collected every 30 min, thus resulting in a total of 24 samples. On d 4, ruminal fluid samples were obtained every 30 min from the 2 Black Angus heifers, starting at 2 h pre-feeding with sampling ending at feeding. Sampling commenced again starting at 2.5 h post-feeding until 5 h post-feeding. In total, 20 ruminal fluid samples were collected. Across all experimental animals, a total of 131 ruminal fluid samples were obtained for determination of ruminal pH.

Paired data for ruminal pH from the LRCpH (1- and 5-min averages) and MANpH were analyzed using the Proc Mixed procedure of SAS (version 9.13, SAS Institute Inc., Cary, NC) with repeated measures to calculate the correlation coefficient (Hamlett et al., 2004). The correlation coefficient was then used to calculate the concordance correlation (Lin, 1989; Lin, 1992). Also used in this calculation were the overall mean and variance for each ruminal pH measurement method. The correlation coefficient calculated to account for repeated measures was used to determine precision by determining the deviation of the data from the best-fit linear line. The concordance correlation coefficient was used to determine accuracy by determining how much the

best-fit line deviated from the line $y = x$. The data from the experimental method (LRCpH, 1- and 5-min averages) were plotted on the y-axis and the data obtained from MANpH were plotted on the x-axis. Data were also analyzed separately for two cows (one observed to have very dry and very liquid ruminal contents) using the same procedures as above. This was conducted to determine if consistency of ruminal contents (due to DM content) had an affect on accuracy and precision.

3.3.2 Evaluation of a Continuous Indwelling Ruminal pH Measurement System (CIpH)

The 3-cannulated, lactating primiparous Holstein cows used in the LRCpH experiment were also used in this experiment. These cows were selected for this experiment because a wide range of ruminal pH values were measured in these animals during the previous experiment. Diets and feeding management were the same as in the LRCpH experiment.

The design and use of the CIpH system has been previously documented (see Maekawa et al., 2002; Beauchemin and Yang, 2005; Bevans et al., 2005). Briefly, the CIpH system was composed of an industrial microprocessor-based pH controller (model PHCN-37, Omega Engineering, Stamford, CT). The pH electrodes (S650-CDHF, Sensorex, Garden Grove, CA) were connected to the pH controller with a 9-m cable (PHEH-65-30-ATC, Omega Engineering, Stamford, CT) suspended above the cows. The cable passed through a ruminal cannula plug and extended approximately 50-cm into the rumen. The cable was protected from the ruminal environment with a plastic hose. A shroud was constructed around the pH electrode with four 25-mm holes which

allowed material to percolate through but prevented the electrode from contacting the ruminal epithelium. Two 900-g weights were attached to the electrode shroud to maintain positioning within the ventral sac.

Continuous measurements of ruminal pH were collected over 2 consecutive 24-h collection periods using the CIpH. Ruminal pH was measured every 5 s. For the CIpH system, the 5-s ruminal pH readings were averaged over 5-min intervals and recorded by a data logger (model CR10, Campbell Scientific, Logan, UT). Averaging the 5-s pH readings over 5-min intervals has previously been reported in our laboratory (Beauchemin and Yang, 2005). The 5-min averages corresponded to actual ruminal fluid sampling times used for MANpH (i.e. when the rumen cannula plug was open) and were used to determine the relationship between CIpH and MANpH. In this experiment, pH electrodes were standardized as described for the LRCpH system in the previous experiment.

For manual pH measurement, ruminal fluid samples were collected using procedures already described in the previous experiment. During ruminal fluid collection, positioning of the CIpH was noted. Ruminal fluid samples were collected at 30-min intervals over 48 h. In the first 24 h, ruminal fluid samples were collected starting at 5.5 h pre-feeding ending at 3.5 h post-feeding, and re-commencing at 7 h post-feeding and ending 10 h post-feeding. During the second 24-h interval, ruminal fluid samples were collected starting at 4.5 h pre-feeding and ending at 1 h pre-feeding. Thus, 32 ruminal fluid samples were collected from each of the 3 cows resulting in a total of 96 data pairs.

Paired data for ruminal pH from CIpH (5-min averages) and MANpH were analyzed using the Proc Mixed procedure of SAS (version 9.13, SAS Institute Inc., Cary, NC) with repeated measures to calculate the correlation coefficient (Hamlett et al., 2004). The correlation coefficient was then used to calculate the concordance correlation (Lin, 1989; Lin, 1992). Also used in this calculation were the overall mean and variance for each ruminal pH measurement method. The correlation coefficient calculated to account for repeated measures was used to determine precision by determining the deviation of the data from the best-fit linear line. The concordance correlation coefficient was used to determine accuracy by determining how much the best-fit line deviated from the line $y = x$. The data from the experimental method (CIpH, 5-min averages) were plotted on the y-axis and the data obtained from MANpH were plotted on the x-axis.

3.3.3 Frequency of Electrode Standardization

The 6-cannulated, pregnant Holstein heifers used in LRCpH experiment were selected for use in this experiment. Animals were housed individually in tie-stalls. Diets and feeding management were the same as in the LRCpH experiment. Continuous ruminal pH measurement was conducted using the LRCpH system as already described. At the beginning of the experiment, 6 new pH electrodes (model S650CD-HF, Sensorex, Garden Grove, CA) were installed. In this experiment, pH electrodes were assigned to a random sequence of three treatments: baseline mV readings after 24 (**R24**), 48 (**R48**) or 72 h (**R72**) in the rumen. Millivolt readings in pH 4 and 7 buffer solutions for each pH electrode were recorded immediately before pH electrodes were placed into the rumens

of experimental animals. Corresponding pH electrodes were removed from the rumen at 24, 48 or 72 h, and mV readings in pH 4 and 7 buffer solutions were again recorded for each pH electrode. Baseline reading difference was defined as the difference in mV readings between the start and end of each treatment duration.

To evaluate the frequency required for electrode standardization, the change in baseline mV readings between the start and end of each treatment duration was calculated for each electrode. These data were analyzed as a double 3×3 Latin square design using the Proc Mixed procedure of SAS. The model included the fixed effect of treatment with period and electrode considered random effects. Differences among treatments were compared using Fisher's protected LSD test adjusted with the Tukey-Kramer option. Differences were considered significant at $P < 0.05$.

3.4 Results and Discussion

3.4.1 Comparison of the LRCpH and CIpH Continuous pH Measurement Systems to MANpH

The primary objective of the present study was to develop a stand-alone submersible ruminal pH measurement system for use in unrestrained cattle and to evaluate its accuracy and precision by comparing its output to pH measurement of ruminal fluid samples collected via a ruminal cannula. Currently, several other direct methods, including oro-ruminal probes and rumenocentesis, are in use for the measurement of ruminal pH; however, the major drawback of these techniques is that they involve spot sampling of ruminal fluid at various intervals and do not yield

comprehensive data on post-feeding profiles of ruminal pH. Dado and Allen (1993) originally developed a system for performing continuous measurements of ruminal pH; however, the major drawback of this system is that the indwelling pH electrode is directly connected by a cable to a pH transmitter or computer located next to the cow, thus animal mobility is restricted and application is limited to tethered animals. The LRCpH system developed in this study does not require animals to be tethered and provides the capability to measure comprehensive ruminal pH data.

Relationships between the LRCpH and ClpH continuous ruminal pH measurement systems and manual sampling via the ruminal cannula are shown in Table 3.1. Because the ClpH averaged ruminal pH measurements over 5-min intervals, the LRCpH ruminal pH measurements were also averaged over 5-min intervals. This allowed direct comparison of the two systems. In addition, ruminal pH measurements from the LRCpH were averaged over 1-min intervals to determine if averaging readings over a shortened interval affects accuracy and precision. Overall, mean ruminal pH values obtained using the LRCpH (1- and 5-min averages) and the ClpH systems were numerically lower (0.03 and 0.05 pH units, respectively) when compared to mean ruminal pH values obtained using the MANpH method (Table 3.1). In other studies, mean ruminal pH values were 0.11 pH units (Dado and Allen, 1993) and 0.06 to 0.18 pH units (Graf et al., 2005) lower when measured using an indwelling pH electrode system that continuously monitored ruminal pH compared to direct measurement in ruminal fluid samples obtained via ruminal cannula. With manual sampling of ruminal fluid through the ruminal cannula, there is usually a delay until ruminal fluid pH is actually measured. Smith (1941) postulated that this delay might allow the escape of

Table 3.1. Relationship between continuous ruminal pH measurement and manual ruminal pH measurement using two different continuous ruminal pH measurement systems.

Variable	LRCpH ¹ vs. MANpH ²		CIpH ⁵ vs. MANpH
	1-min ³	5-min ⁴	
Number of data pair	131	131	96
Mean pH (MANpH)	6.14	6.14	6.14
Variance	0.171	0.171	0.126
Mean pH (continuous pH system)	6.11	6.11	6.09
Variance	0.188	0.194	0.111
Correlation coefficient ⁶	0.98	0.97	0.88
Concordance correlation	0.96	0.97	0.87
Location shift ⁷	0.07	0.09	0.16
Scale shift ⁸	0.95	0.93	1.04

¹Lethbridge Research Centre ruminal pH measurement system.

²Manual pH measurement of rumen fluid samples obtained via a rumen cannula.

³LRCpH values were averaged over 1-min intervals corresponding to MANpH.

⁴LRCpH values were averaged over 5-min intervals corresponding to MANpH.

⁵Continuous indwelling ruminal pH measurement system.

⁶Correlation coefficient accounting for repeated measures as described by Hamlett et al. (2004).

⁷Would equal 0 if $y = x$.

⁸Would equal 1 if $y = x$.

CO₂ from ruminal fluid samples, thus elevating ruminal fluid pH. In the current study, continuous ruminal pH measurement occurred while the ruminal cannula plug was open. Thus, the release of carbon dioxide and disruption of the ruminal mat during manual sampling may have increased the values of the continuous measurements. In the current study, the effects of opening the ruminal cannula plug on ruminal fluid pH were not of concern, because this study did not intend to characterize ruminal pH as a function of diet. Rather, the objective was to evaluate the accuracy and precision of two continuous ruminal pH measurement systems by comparing the output to MANpH. This study confirms results from other laboratories (Dado and Allen, 1993; Graf et al., 2005) indicating that mean pH from manual sampling methods are higher than mean pH values obtained from continuous pH systems although our mean differences were lower than previously reported values. However, caution should be used when evaluating mean ruminal pH differences between two systems because means indicate very little about the relationship between the two methods.

Averaging the LRCpH output over 1- or 5-min intervals did not affect mean pH (Table 3.1) and, consequently, the correlation coefficient that accounted for repeated measures and concordance correlation coefficients were similar between the LRCpH and MANpH when LRCpH output was averaged over 1- or 5-min. The LRCpH (1- and 5-min averages) correlation coefficients that accounted for repeated measures (0.98 and 0.97, respectively) were numerically higher than that calculated for the CIpH (0.88) (Table 3.1). The concordance correlation coefficient was numerically higher for the LRCpH when compared to the CIpH. Dado and Allen (1993) previously reported a Pearson correlation coefficient of 0.85 for the relationship between a continuous

indwelling ruminal pH measurement system and manual ruminal pH measurement. The Pearson correlation coefficient indicates how closely the results are related between two methods but does not indicate how the plotted data deviates from the line $y = x$ (Lin, 1992). Thus, in our study, a correlation coefficient that accounted for repeated measures was used to replace the Pearson correlation coefficient and was also used to calculate the concordance correlation. The repeated measures correlation is used to evaluate precision as it determines the deviation of the data set from the best-fit linear line while the concordance correlation is used to evaluate accuracy by determining the deviation of the best-fit linear line to the line $y = x$.

No other studies known to the author have reported the correlation coefficient, accounting for repeated measures, or the concordance correlation coefficient when evaluating the accuracy and precision of a continuous ruminal pH measurement system. The correlation coefficient results from this study suggest that the LRCpH more closely reflected the MANpH results than did the CIpH, thus indicating higher accuracy and precision. The improvement in accuracy and precision can be attributed to the design of the system and not the pH recording interval since the LRCpH had similar correlation coefficients and concordance correlation coefficients regardless of whether the data were averaged over 1- or 5-min intervals corresponding to ruminal fluid collection time for MANpH (Table 3.1).

The location shift for the relationship between the LRCpH and MANpH (0.07 and 0.09 for the 1- and 5-min averages, respectively) was lower than that of the CIpH and MANpH (0.16). The line $y = x$ would have a location shift of zero and the location shift indicates how the y-intercept of the plotted data differs from the y-intercept of the line $y = x$.

= x. Thus, for both systems the y-intercept was less than zero indicating that MANpH results are slightly higher than results from the LRCpH or CIPh. The scale shift was similar for the relationship between the LRCpH and MANpH and between the CIPh and MANpH; however, the shift occurred in opposite directions. The scale shift indicates a discrepancy in slope between the plotted data and the line $y = x$. The plotted data for the CIPh vs. MANpH and LRCpH (1- and 5-min averages) vs. MANpH, appear in Figures 3.2, 3.3, and 3.4 respectively.

3.4.2 Other General Observations.

It was noted during manual ruminal fluid collection that the LRCpH did not migrate within the rumen to the same extent as the CIPh likely because of the design elements; rigid PVC encapsulation, flexible cable fastening to the rumen cannula plug and heavier total weight (two 900-g weights + data logger + PVC capsule). Ruminal conditions can affect the accuracy and precision of the data collected from continuous pH measurement systems as the pH electrodes are designed for submersible application and require movement of liquid over the sensor for reliable measurement. The accuracy and precision observed were numerically reduced when ruminal pH was measured in an animal observed to have relatively dry ruminal contents when compared to measurement in an animal observed to have relatively fluid ruminal contents. The correlation coefficient which accounted for repeated measures, and concordance correlation coefficient were 0.75 and 0.74, respectively, in the animal with dry ruminal contents, and were 0.96 and 0.96, respectively, in the animal with more fluid ruminal contents. This indicates that the system may function less well when used in animals with a lower

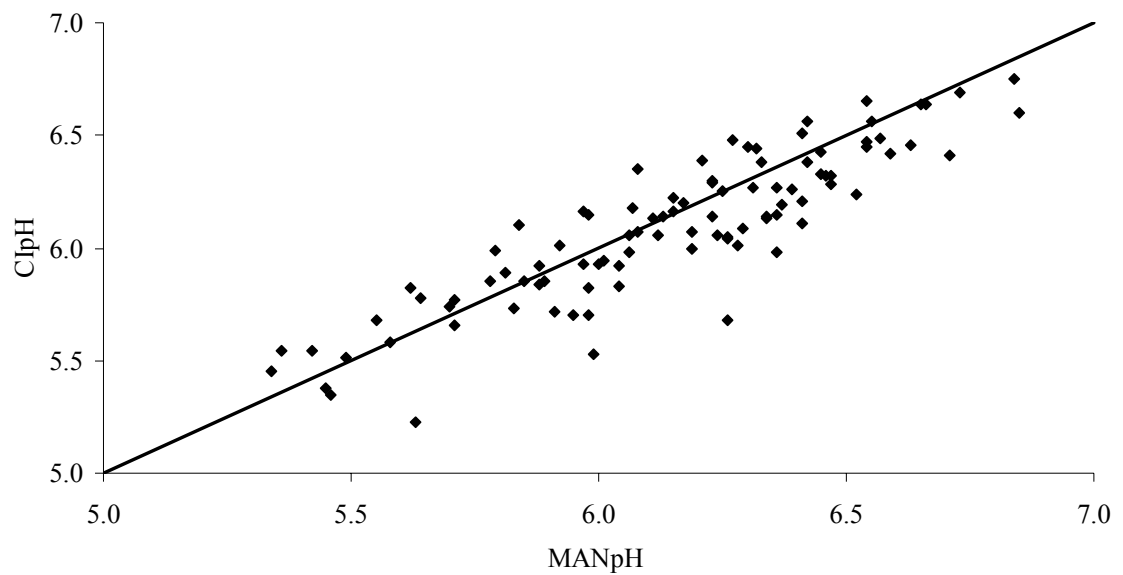


Figure 3.2. Relationship between ruminal pH determined using ruminal fluid obtained via a ruminal cannula (MANpH) and using a continuous indwelling pH measurement system (CIpH). The solid line represents the line $y = x$.

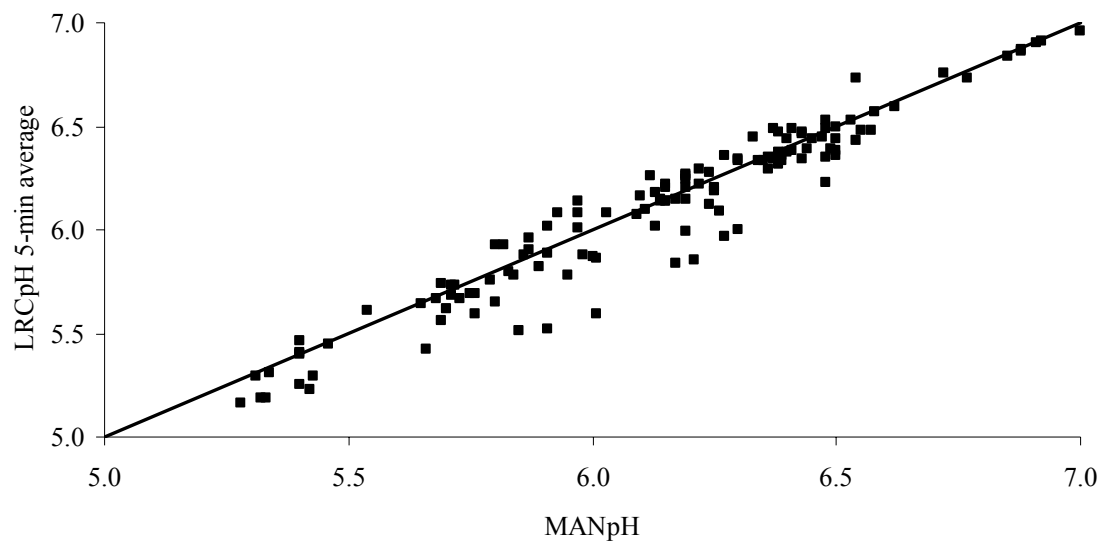


Figure 3.3. Relationship between ruminal pH determined using ruminal fluid obtained via a ruminal cannula (MANpH) and a submersible continuous ruminal pH measurement system (LRCpH) when output was averaged over 5-min intervals. The solid line represents the line $y = x$.

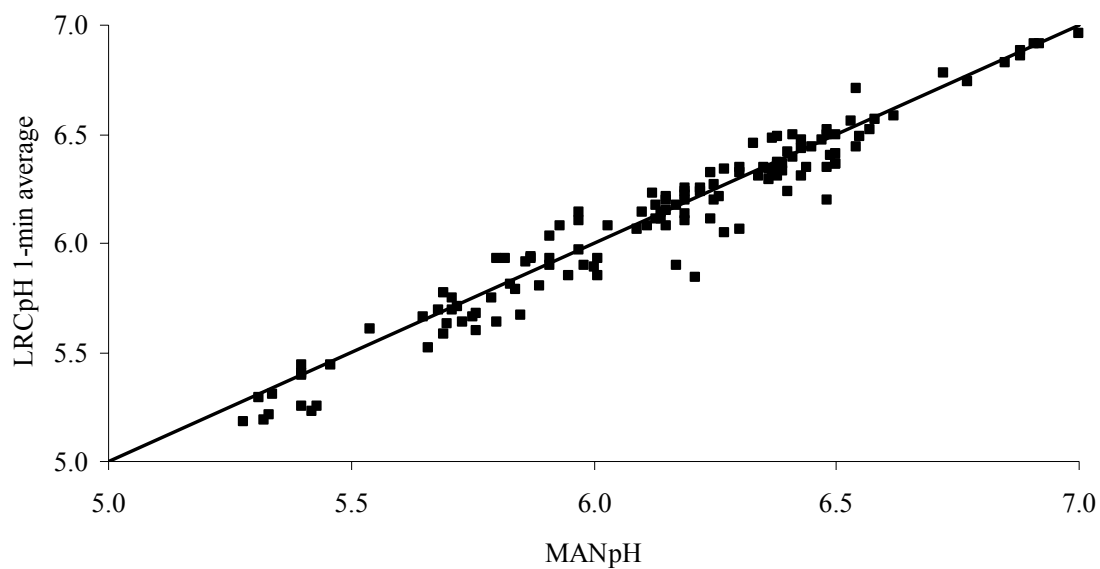


Figure 3.4. Relationship between ruminal pH determined using ruminal fluid obtained via a ruminal cannula (MANpH) and a submersible continuous ruminal pH measurement system (LRCpH) when output was averaged over 1-min intervals. The solid line represents the line $y = x$

proportion of ruminal fluid. However, results generated from the entire data set indicate that there were no significant differences in accuracy and precision among animals ($P = 0.12$) or time relative to feeding ($P = 0.25$).

3.4.3 Frequency of Electrode Standardization

No electrode failure occurred in the current study. Mean baseline mV readings in pH 4 ($P = 0.94$) and 7 ($P = 0.23$) buffer solutions were not different for pH electrodes after 24, 48 or 72 h of ruminal incubation (Table 3.2). Furthermore, the changes in baseline mV readings for all treatments did not differ ($P > 0.05$) from zero (Table 3.2). However, electrode drift for individual probes did not always occur in the same direction for any of the treatment durations (data not shown). Previously, Nocek et al. (2002) noted a requirement for electrode standardization; however, that study only examined the effect of probe cleansing and standardization on pH readings after 4, 6, 8, 12, and 24 h in the rumen. Enemark et al. (2003) maintained electrodes in the reticulum for 8 days with minimal electrode drift; however, only two electrodes were used. Results from the current study suggest that new electrodes can be maintained in the rumen for at least 72 h without having a significant impact on mV readings. Using mV readings to calculate pH values indicated that the mean error which occurred by not recalibrating electrodes and not correcting data for changes in baseline mV readings within a 72-h period was 0.03 pH units. The maximum possible error found in this study between consecutive standardizations was 0.13, 0.18, and 0.10 pH units for R24, R48, and R72, respectively, within a pH range of 4.5 to 7.0. Based on the results of this study, there is no requirement for daily removal and standardization of new electrodes;

Table 3.2. Effect of electrode standardization frequency on electrode readings in pH buffers 4 and 7.

Variable	Treatment			SEM	P value
	R24 ¹	R48 ²	R72 ³		
Mean baseline reading difference in pH buffer 4, mV	2.70	4.38	4.67	4.37	0.94
Difference from zero in pH buffer 4, P value	0.55	0.33	0.30		
Mean baseline reading difference in pH buffer 7, mV	-8.40	4.90	3.78	5.83	0.23
Difference from zero in pH buffer 7, P value	0.17	0.41	0.53		

¹Electrodes maintained in the rumen for 24 h.

²Electrodes maintained in the rumen for 48 h.

³Electrodes maintained in the rumen for 72 h.

however, there may be a requirement for regular inspection of electrode function as malfunction would result in a loss of data. Unlike the CIPh, the LRCpH does not have a visual display indicating mV or pH readings and electrode failure would not be diagnosed until the time of electrode standardization. More research is required to determine how electrode usage over time impacts baseline mV reading stability between standardizations. It can be concluded that daily standardization of new electrodes is not required and the duration between consecutive standardizations could be extended to 72 h.

3.5 Conclusion

Indwelling systems for continually monitoring ruminal pH provide an accurate and precise means of measuring changes in pH over time. A new submersible system called the LRCpH that can be used in feedlots, free-stall dairies, or in grazing applications was developed and shown to be highly accurate and precise. Based on results of this study, new electrodes can be continuously maintained in the rumen for at least 72 h without adverse effects on measurement accuracy. The LRCpH provides an increased opportunity for researchers to accurately and precisely measure ruminal pH and the occurrence of ruminal acidosis in unrestrained cattle.

4.0 THE SEVERITY OF RUMINAL ACIDOSIS IN PRIMIPAROUS HOLSTEIN COWS DURING THE PERIPARTURIENT PERIOD²

4.1 Abstract

The objectives of this study were to determine the effect of providing additional pre-partum concentrate on the occurrence and severity of ruminal acidosis (RA) and lactational performance during the periparturient period in primiparous cows. The hypothesis was that providing additional concentrate pre-partum would reduce post-partum RA. Fourteen ruminally-cannulated Holstein heifers were paired by expected calving date and body condition score. The heifers were assigned to one of two pre-partum feeding regimens: 1) control treatment 2) a high concentrate (HC) feeding program. All cows received the same lactation diet post-partum. Ruminal pH was measured continuously from d -5 to d +5, and for 3-consecutive days starting on d +17 \pm 1.2, d +37 \pm 1.4, and d +58 \pm 1.5 relative to parturition using an indwelling ruminal pH system. Ruminal acidosis was considered to occur when ruminal pH was <5.8 (total acidosis). Total acidosis was partitioned into: 1) mild RA (5.8 > ruminal pH > 5.5), 2) moderate RA (5.5 > ruminal pH > 5.2), and 3) acute acidosis (ruminal pH < 5.2). Feeding additional concentrate pre-partum did not reduce post-partum RA. In fact, cows fed the HC treatment had more daily episodes of acute acidosis than cows fed the control

² A version of this chapter has been accepted for publication. Penner, G.B., K.A.

Beauchemin, and T. Mutsvangwa. 2006. The severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. J. Dairy Sci. (in press).

treatment. Day relative to parturition affected the occurrence and severity of RA; RA increased following parturition and was sustained thereafter. The dry matter intake and BCS were lower for cows fed the HC treatment compared to cows fed the control treatment, but lactational performance was not affected. The results of this study indicated that feeding additional concentrate pre-partum did not reduce post-partum RA. Furthermore, the incidence and severity of RA increased immediately post-partum which emphasized the need to develop and implement feeding strategies that reduce this risk.

4.2 Introduction

Transition cows undergo drastic metabolic and hormonal changes in response to increased nutrient demands of the growing fetus, parturition, and the onset of lactation (Grummer, 1995; Dann et al., 1999; Drackley, 1999). The majority of these changes increase the cow's susceptibility to metabolic disorders immediately before and after parturition (Drackley, 1999). In first lactation heifers, body growth also competes for nutrients (NRC, 2001). As parturition approaches, DMI decreases (Hayirli et al., 2003) but nutrient requirements increase (NRC, 2001). To account for the reduction in DMI as parturition approaches, various nutritional strategies have been developed. One particular strategy is to increase the dietary energy density by including higher proportions of concentrate (McNamara et al., 2003; Rabelo et al., 2003). However, feeding a diet with low NDF content often results in a greater magnitude of DMI depression near parturition (Hayirli et al. 2003; Rabelo et al. 2003). Positive benefits of supplying more concentrate pre-partum include improved growth of ruminal papillae

(Dirksen et al. 1985) and changes in ruminal microflora (Tajima et al., 2000) such that the rumen is better adapted to the energy dense diet fed post-partum (NRC, 2001). Post-partum diets often contain high levels of fermentable carbohydrate and low levels of fiber in order to maximize energy intake. Large changes in dietary composition, which occur at parturition, increase the risk of RA (Nocek, 1997). This problem may be exacerbated in primiparous cows when compared to multiparous cows as primiparous cows have not had previous exposure to a highly fermentable lactation diet. Krause and Oetzel (2006) investigated the incidence of ruminal acidosis (RA) post-partum using clinical observation data and reported that primiparous cows had a higher risk of RA than multiparous cows.

Gröhn and Bruss (1990) conducted a large epidemiological study (> 60 000 cows) and found that the greatest number of cases of RA were diagnosed during the first few months after calving. While Gröhn and Bruss (1990) have characterized the occurrence of RA throughout the production cycle, only one spot sample of ruminal fluid was collected for ruminal pH measurement and pH paper was used as an indicator of low ruminal pH. As a result, only qualitative assessments were made based on irregular sampling, thus many cases of sub-acute RA would have gone undetected. Furthermore, Gröhn and Bruss (1990) did not report any dietary information. In contrast, Krause and Oetzel (2006) conducted a review of sub-acute RA, and reported that the risk for RA increases with increasing days in milk corresponding to increasing DMI.

In order to understand ruminal pH dynamics during the periparturient period, it is important to comprehensively measure ruminal pH daily and relate the results to dietary treatment. Previously, the lack of equipment available for continuous ruminal pH measurement in unrestrained cows limited measurements in transition cows. However, Penner et al. (2006) developed a new system capable of continuously measuring ruminal pH in unrestrained cattle that can characterize ruminal pH in applications where continuous measurement was previously not feasible. The objective of this study was to determine the effect of additional pre-partum concentrate allocation on post-partum RA and lactational performance. The hypothesis was that the occurrence and severity of RA would increase post-partum, but feeding additional concentrate pre-partum would ameliorate post-partum RA.

4.3 Materials and Methods

4.3.1 Experimental Design and Animals

Eighteen Holstein heifers were used in a randomized complete block design with two dietary treatments. Heifers, rather than multiparous cows, were used in this study because there are few published studies that have examined feeding strategies to transition heifers from a pregnant non-lactating state to a non-pregnant lactating state. Furthermore, the inclusion of parity as an additional factor in the experiment would have reduced the statistical power had an interaction between diet and parity occurred. The experiment began in March 2005 and ended in November 2005. Individual cows were monitored from 60 d before the expected calving date until 60 d after calving. Heifers

were fitted with a ruminal cannula (Bar Diamond, Parma, ID) between 70 and 100 d in gestation and were housed at the Lethbridge Research Center Dairy and Metabolism Facility (Lethbridge, AB). Cows were weighed on two consecutive days and BCS was determined (Wildman et al., 1982) prior to the start of the study, before each diet change, and on $d +17 \pm 1.2$, $d +37 \pm 1.4$, and $d +58 \pm 1.5$ post-calving. After parturition, cows were milked twice daily at 0630 and 1630 h and milk yield was recorded. Mean and standard deviation values for BW, BCS (5-point scale), and DMI at the start of the study ($d -58 \pm 4.7$ relative to parturition) were 633 ± 52.0 kg, 3.5 ± 0.23 , and 12.0 ± 1.44 kg/d respectively. All procedures were approved by the Lethbridge Research Center Animal Care Committee and were in accordance with the guidelines of the Canadian Council of Animal Care (Ottawa, ON).

4.3.2 Experimental Treatments

Heifers were paired by expected calving date and BCS and assigned to: 1) control treatment consisting of a far-off diet (forage:concentrate, F:C = 81:19) fed from $d -60$ to $d -25$ and a close-up diet (F:C = 54:46) fed from $d -24$ until parturition; or 2) a high concentrate (HC) feeding program consisting of 4 pre-partum diets, HC-1 (F:C = 68:32) fed from $d -60$ to $d -43$, HC-2 (F:C = 60:40) fed from $d -42$ to $d -25$, HC-3 (F:C = 52:48) fed from $d -24$ to $d -13$, and HC-4 (F:C = 47:53) fed from $d -12$ until parturition. All cows received the same lactation diet post-partum.

Diets HC-1 and HC-2 were formulated to have CP contents similar to the far-off control diet while diets HC-3 and HC-4 were formulated to have CP contents similar to the close-up control diet. The F:C ratio of the HC diets was altered by decreasing the

proportion of barley silage and grass hay (ratio of barley silage to grass hay was maintained) and increasing the proportion of non-forage fiber sources (wheat middlings, dried beet pulp, and dried distiller's grains) and barley grain. The concentrate ingredients were combined and pelleted. Diets were formulated using the Cornell-Penn-Miner System (CPM Dairy, Version 3.0.4a, University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY and William H. Miner Agricultural Research Institute, Chazy, NY). The lactation diet was formulated to supply adequate metabolizable energy and metabolizable protein for a 650 kg cow producing 35 kg/d of milk containing 3.5% fat and 3.2% protein. Ingredient composition of diets is given in Table 4.1. Weekly samples of the barley silage were collected and the DM content was determined by oven drying at 55°C for 48 h. Diets were adjusted over the course of the study to maintain the specified F:C ratio on a DM basis.

Cows were fed ad libitum (10% orts) and fresh feed was offered in equal proportions twice daily at 1330 and 1800 h. The weight of feed offered and refused was recorded daily throughout the duration of the study. Milk was sampled on 2 consecutive days starting on $d +3 \pm 0.4$, $d +16 \pm 1.1$, $d +36 \pm 1.4$, and $d +59 \pm 1.3$ relative to parturition. Samples of the TMR were collected once weekly with one sub-sample used for particle size measurement using the Penn State Particle Size Separator (PSPS) (Lammers et al., 1996; Kononoff et al., 2003). The second sub-sample of TMR was analyzed for DM content and stored for later chemical analysis. Daily ort samples were collected and a weekly composite was analyzed for DM.

Table 4.1. Ingredient composition of the experimental diets (DM basis).

	Treatment ¹						Lactation diet
	Control		HC				
	Far-off	Close-up	HC-1	HC-2	HC-3	HC-4	
Composition, % DM							
Forages							
Barley silage	69.1	47.0	58.8	51.8	45.6	40.6	42.2
Alfalfa hay	-	7.2	-	-	-	-	4.4
Grass hay	11.7	-	8.7	7.6	6.0	6.0	-
Concentrate							
Barley grain	8.8	13.6	17.1	18.0	21.2	22.3	30.9
Canola meal	5.3	4.5	5.5	4.1	5.8	5.5	1.8
Corn gluten meal	1.6	3.6	-	-	-	-	2.9
Distillers' grain	-	-	0.4	0.8	6.4	4.9	-
Wheat middlings	-	-	4.2	8.0	5.1	7.8	-
Beet pulp	-	13.6	4.2	8.0	5.1	7.8	2.2
Calcium carbonate	0.9	1.8	0.8	0.8	0.8	0.8	0.6
Calcium diphosphate	0.6	0.7	-	-	-	-	0.7
Sodium bicarbonate	-	-	-	-	-	-	0.6
Magnesium oxide	-	0.4	-	-	-	-	0.1
Megalac ²	-	-	-	-	-	-	2.0
Beet molasses	1.3	0.9	-	-	-	-	1.7
Canola oil	0.2	0.5	0.1	0.8	0.8	0.1	0.5
Nutrichlor ³	-	5.9	-	-	3.2	4.0	-
Vitamin-mineral mix ⁴	0.4	0.3	0.2	0.2	0.2	0.2	0.67
SoyPass ⁵	-	-	-	-	-	-	8.8

¹Control treatment: far-off diet was fed from -60 to -25 d and the close-up diet was fed from -24 d until parturition. Step treatment: step 1 was fed from d -60 to -43, step 2 from d -42 to -25, step 3 from d -24 to -13, and step 4 from d -12 until parturition. Both treatments received the same diet post-partum.

²Megalac calcium salts of palm oil (Church and Dwight Co., Inc., Princeton, NJ).

³Nutri-tech Solutions, Abbotsford, B.C. Canada

⁴Supplied 0.81% Ca, 0.41% P, 0.23%Mg, 1.2% K, 0.25% S, 0.30% Na, 1.0% Cl, 145 ppm Fe, 60 ppm Zn, 18 ppm Cu, 53 ppm Mn, 0.3 ppm Se, 0.6 ppm Co, 0.3 ppm I, 8.3 KIU/kg Vitamin A, 1.3 KIU/kg vitamin D, and 62.7 IU/kg Vitamin E on a DM basis.

⁵LignoTech USA Inc. Rothchild, WI.

The weekly TMR samples were composited by 3-mo periods and the composite samples were analyzed for chemical composition. The analytical DM content was determined by oven drying at 135°C for 2 h (AOAC, 1990). Ash content was determined by combustion at 550°C for 6 h. The OM content was calculated as the difference between DM and ash. The nitrogen content for CP calculation ($CP = N \times 6.25$) was determined by flash combustion (Carlo Erba Instruments, Milan, Italy). The NDF and ADF contents were determined sequentially using a digestion technique (model A200, ANKOM Technology Corp., Fairport, NY) that included amylase and sodium sulphite for NDF as described by Van Soest et al. (1991).

4.3.2 Ruminal Measurements

Ruminal VFA were measured in samples of ruminal fluid collected daily from d -5 to d +5 and on two consecutive days starting on d +17 \pm 1.2, d +37 \pm 1.4, and d +58 \pm 1.5 relative to parturition. Collection occurred at 1630 h, which corresponded to 3 h post-feeding. In total, approximately 750 ml of ruminal fluid was obtained from three different locations in the rumen: the reticulum, the ventral sac, and the interface between the fluid phase and ruminal mat. Samples were immediately strained through perforated material (PEETEX, pore size = 355 μ m, Sefar Canada Inc., Scarborough, ON, Canada) and 10 ml of strained ruminal fluid was added to 2 ml of 25% metaphosphoric acid. Samples were stored at -20°C for later analysis. Ruminal VFA were separated and quantified by gas chromatography (Varian 3700; Varian Specialties Ltd., Brockville,

ON) using a 15-m (0.53-mm i.d.) fused silica column (DB-FFAP column; J and W Scientific, Folsom, CA).

Ruminal pH was continuously measured from d -5 to d +5 \pm 0.0, and for 3 consecutive days starting on d +17 \pm 1.2, d +37 \pm 1.4, and d +58 \pm 1.5 relative to parturition using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA, USA) as described by Penner et al. (2006). Briefly, the system consisted of a watertight capsule containing a pH electrode and data acquisition device that was placed into the rumen of the cow. The pH electrode (model S650-CDHF, Sensorex, Garden Grove, CA) was covered by a shroud that allowed particle and liquid passage but kept the pH electrode from contacting the surface of the ruminal epithelium. The capsule was attached to the ruminal cannula plug to aid in system location within the rumen and to help maintain the electrode in a vertical position. Two 900-g weights were fastened to the bottom of the electrode shroud to maintain the electrode in the ventral sac of the rumen. Readings in pH buffers 4 and 7 were recorded prior to placing the LRCpH system in the rumen. Ruminal pH readings were taken every 30 s and stored by the data logger (model M1b-pH-1KRTD, Dascor, Escondido, CA, USA). After about 23.5 h of continuous pH measurement, the LRCpH was removed from the rumen, washed in 39°C water and mV readings were recorded in pH buffers 4 and 7. Electrodes and buffers were maintained at 39°C until the data were downloaded. The shift in mV readings from the electrodes between the start and finish standardization was assumed to be linear and was used to convert the mV data to pH units. The daily ruminal pH data were averaged for each minute and summarized daily

as minimum pH, mean pH, and maximum pH. The occurrence and extent of acidosis was determined from the pH records using 3 pH thresholds: 5.8, 5.5, and 5.2. Acidosis was considered to occur when ruminal pH was < 5.8 (total acidosis). The pH profiles were further characterized as mild RA when $5.8 > \text{ruminal pH} > 5.5$, moderate RA when $5.5 > \text{ruminal pH} > 5.2$, and acute acidosis when ruminal pH < 5.2 . The thresholds 5.8 and 5.5 were assigned as they have previously been used in our laboratory (Maekawa et al., 2003; Beauchemin and Yang, 2005) or defined by others (Nocek, 1997). The duration (h/d) and total area (pH \times min) that pH was below each threshold was calculated. In addition, the number of daily episodes of each category of acidosis was noted. A daily episode was defined to begin when ruminal pH was below the pre-defined threshold and ended when ruminal pH met or exceeded the threshold.

4.3.3 Milk Sampling and Analysis

Milk was sampled on 2 consecutive days starting on $d +3 \pm 0.4$, $d +16 \pm 1.1$, $d +36 \pm 1.4$, and $d +59 \pm 1.3$ relative to parturition to determine milk composition. Milk samples were preserved with potassium dichromate and stored at 4°C until sent to the Central Alberta Milk Testing Laboratory (Edmonton, Alberta, Canada). Milk was analyzed for fat, CP, and lactose using an infrared analyzer (Milk-O-Scan 605; Foss Electric, Hillerød, Denmark). Milk composition was corrected for differences in milk volume between a.m. and p.m. milking.

4.3.4 Blood Sampling and Analysis

Blood was sampled from the jugular vein at 0900 h on d -49 ± 4.9 , d -14 ± 4.1 , d -7 ± 1.2 , d $+7 \pm 0.4$, d $+19 \pm 1.1$, and $+61 \pm 1.3$ relative to parturition to assess glucose, insulin, NEFA, and BHBA concentrations. For plasma, blood was collected into a 7-ml vacutainer containing lithium heparin (Fisher Scientific Company, Nepean, ON). Blood samples were centrifuged at $3000 \times g$ for 25 min immediately after sampling. A sub-sample of plasma was analyzed immediately for glucose (IDEX Laboratories Inc. Westbrook, Maine, USA). A second sub-sample of plasma was stored at -20°C for analysis of insulin. Plasma was iodinated using the chloramine-T-method as described by Greenwood et al. (1963). Crystalline bovine insulin (Lilly Research Laboratories, Indianapolis, IN, USA; lot no. 615-70N-80) was used as a standard. Iodinated samples were analyzed using a double antibody radio immunoassay (Brockman, 1979). For serum, blood was collected into a 7-ml vacutainer tube containing silica gel (Fisher Scientific Company, Nepean, ON). Serum samples were chilled on ice for 30 min and centrifuged at $3000 \times g$ for 25 min. Samples were then stored at -20°C and were sent to the Animal Health Laboratory (Guelph, ON, Canada) for analysis of NEFA and BHBA.

4.3.5 Statistical Analysis

Four cows were removed from the study as they calved early in relation to their expected calving date. This resulted in seven cows per treatment completing the study. Data were summarized for each cow by measurement period. For DMI, only the data for d -5 to d $+5 \pm 0.0$, and for 3 consecutive days starting on d $+17 \pm 1.2$, d $+37 \pm 1.4$,

and $d + 58 \pm 1.5$ were used as these measurements corresponded to ruminal pH, ruminal VFA, BW, BCS and milk yield and composition measurements. For DMI, BW, BCS, milk yield and composition, ruminal pH, and ruminal VFA concentrations, statistical analysis was performed using the PROC MIXED procedure of SAS (version 9.13, SAS Institute Inc., Cary, NC) accounting for repeated measures with the random effect of cow and the fixed effect of treatment. Day within measurement period and measurement period were considered to be the repeated measures. Various variance-covariance error structures were used depending on which error structure produced the lowest Akaike's information criterion and Bayesian information criterion values for each variable. For blood metabolite and hormone concentration, data were analyzed using the PROC MIXED procedure of SAS (version 9.13, SAS Institute Inc., Cary, NC) accounting for repeated measures. Cow was considered the random effect with the fixed effect of treatment. Day of sampling was the repeated measure. All data were analyzed for the main effect of treatment, period, and treatment \times period and are presented as pre- and post-partum means. For ruminal pH, data were also analyzed using the PROC MIXED procedure of SAS (version 9.13, SAS Institute Inc., Cary, NC) using measurement period as a regression variable to determine how day relative to parturition affected ruminal acidosis. Both linear and quadratic relationships were tested. Differences were considered significant when $P < 0.05$ and trends are discussed when $P < 0.10$.

4.4 Results and Discussion

4.4.1 Experimental Diets

Two pre-partum feeding regimes were used in this study. The control treatment was designed to reflect the NRC (2001) recommendations for transitioning a heifer from a non-lactating pregnant state to a non-pregnant lactating state. In contrast, the HC treatment was designed to provide additional energy pre-partum by increasing the proportion of concentrate. The pelleted concentrate used for the HC treatment included steam rolled barley grain and non-forage fiber sources such as wheat middlings, dried beet pulp, and dry corn distillers' grains (Table 4.1). The non-forage fiber sources were used to increase the rapidly digestible NFC fraction, without providing excessive starch (Table 4.2). The close-up diet in the control treatment had a NEL content which was slightly (0.04 Mcal/kg) lower than the NRC (2001) recommendations. For the HC treatment, the diets fed during the close-up period (i.e., diets HC-3 and HC-4) provided NRC recommended levels of NEL (Mcal/kg). Thus, the objective of supplying more dietary energy for the HC treatment was achieved. Despite widely differing F:C ratios the combined amount of feed retained on the PSPS sieves with 1.18-mm, 8-mm and 19-mm openings exceeded 90% for all diets (Table 4.2). This occurred as much of the concentrate was retained on the 1.18-mm and 8-mm sieves; however, this was not expected to compromise the objectives since all animals received the same lactation diet post-partum. The lactation diet supplied 29.4% total NDF, 20.4% NDF from forage sources, and 43.2% NFC. Thus, the NRC (2001) recommendations of a minimum of

Table 4.2. Nutrient composition and particle size distribution of the experimental diets.

Item	Treatment ¹						Lactation diet
	Control		HC				
	Far-off	Close-up	HC-1	HC-2	HC-3	HC-4	
Nutrient composition							
DM, %	50.4	56.5	59.3	59.2	63.2	63.7	57.5
OM, % of DM	91.3	91.2	92.3	92.6	92.6	92.3	91.5
CP, % of DM	14.0	16.7	14.9	14.9	17.8	16.4	17.3
NDF, % of DM	36.4	31.3	33.2	33.7	30.6	30.4	29.4
ADF, % of DM	19.2	18.3	16.9	16.9	15.6	15.5	15.6
NFC ² , % DM	39.6	41.8	43.3	42.4	42.7	44.9	43.4
Predicted NE _L ³ , Mcal/kg	1.41	1.50	1.53	1.55	1.58	1.59	1.72
Particle size, ⁴ % DM							
> 19 mm	23.35	12.52	17.91	14.74	11.62	9.85	10.57
> 8 mm	36.46	47.11	47.24	51.86	55.65	65.93	40.72
> 1.18 mm	35.68	36.27	30.79	29.49	27.95	21.05	42.38
< 1.18	4.52	4.10	4.06	3.92	4.79	3.17	6.33

¹Control treatment: far-off diet was fed from -60 to -25 d and the close-up diet was fed from -24 d until parturition. High concentrate (HC) treatment: HC-1 was fed from d -60 to -43, HC-2 from d -42 to -25, HC-3 from d -24 to -13, and HC-4 from d -12 until parturition. Both treatments received the same diet post-partum.

²Average values for ether extract and NDIN were used (NRC, 2001).

³Calculated using equations from NRC (2001).

⁴Determined using the Penn State Particle Size Separator (Kononoff et al., 2003).

25% total NDF, 19% NDF from forage sources, and a maximum of 44% NFC was met, and consequently the chemical and physical fiber supplied by the lactation diet was considered above the requirement to prevent ruminal acidosis (NRC, 2001; Zebeli et al., 2006).

4.4.2 Ruminal Fermentation Characteristics

Contrary to the hypothesis, feeding additional concentrate pre-partum did not reduce post-partum RA (Table 4.3). Previously, others have demonstrated that feeding concentrate pre-partum promoted ruminal papillae proliferation with increases in length, width, and surface area (Dirksen et al., 1985). Thus the hypothesis that increases in papillae surface area due to additional concentrate feeding pre-partum would increase the absorptive capacity for VFA absorption, and thereby reduce RA. However, the results do not support this hypothesis. To the authors knowledge, this is the first study that has comprehensively measured ruminal pH and the incidence of acidosis during the periparturient period. Alternatively, spot sampling techniques have been used to measure the effect of pre-partum dietary treatment on ruminal pH (Dann et al., 1999; Rabelo et al., 2003); however, spot sampling techniques require a considerable amount of labor to collect ruminal pH measurements and therefore, are not taken frequently enough to adequately describe ruminal pH variation over an extended period of time. In the current study, ruminal pH was measured over several consecutive days at various times relative to parturition using continuous ruminal pH measurement (Penner et al., 2006) to provide comprehensive characterization of ruminal pH during the periparturient period and into early lactation.

In the current study, cows fed the HC treatment pre-partum tended to have lower pre-partum minimum pH ($P = 0.06$; Table 4.3). Krause and Oetzel (2006) suggested that measurement of minimum ruminal pH values may improve the probability of detecting treatment differences over mean ruminal pH values when continuous pH measurement is used. Cows fed the HC treatment also had a tendency for longer duration ($P = 0.09$) and area ($P = 0.09$) of total acidosis and greater area of total acidosis, than cows fed the control treatment. This resulted in cows on the HC treatment spending approximately 1 h/d more than cows on the control treatment with ruminal pH < 5.8 . An interaction ($P = 0.01$) was detected for the number of daily episodes of severe RA. Cows fed the HC treatment had a greater number of daily episodes of mild RA before calving, but fewer episodes after calving than the control treatment. There were no differences for the duration or total area of mild RA. There was a tendency ($P = 0.09$) for cows fed the HC treatment to have a greater duration of moderate RA than cows fed the control treatment indicating increased severity of ruminal acidosis. Acute acidosis rarely occurred pre-partum. However, cows fed additional concentrate pre-partum had a greater increase post-partum for the number and duration of daily episodes of acute acidosis when compared to the control treatment resulting in significant treatment \times period interactions ($P = 0.02$ and 0.01 respectively). The results of the current study are not supported by previous studies where cows were fed additional dietary energy pre-partum. For example, Rabelo et al. (2003) used rumenocentesis to collect ruminal fluid samples in cows fed pre-partum diets containing high or low

Table 4.3. The effect of pre-partum dietary treatment on ruminal acidosis.

	Treatment ¹				SEM		<i>P</i> value	
	Control		HC		Pre-partum	Post-partum	Diet	Period
	Pre-partum	Post-partum	Pre-partum	Post-partum				
Ruminal pH								
Minimum	5.80	5.37	5.67	5.36	0.04	0.03	0.06	<0.01
Mean	6.33	5.99	6.31	5.96	0.03	0.03	0.39	<0.01
Maximum	6.86	6.65	6.88	6.59	0.03	0.02	0.51	<0.01
Total RA								
Duration, h/d	0.7	7.1	1.5	8.3	0.4	0.7	0.09	<0.01
Area, pH × min	571.86	2229.1	960.3	4294.4	332.4	965.1	0.09	<0.01
Mild RA								
Daily episodes ²	2.0	11.3	4.9	10.2	0.8	0.8	0.23	<0.01
Duration, h/d	0.5	4.2	1.2	4.5	0.2	0.4	0.14	<0.01
Area, pH × min	570.2	2196.4	957.7	4241.3	331.2	958.1	0.09	<0.01
Severe RA								
Daily episodes	0.5	5.2	0.7	5.6	0.3	0.7	0.54	<0.01
Duration, h/d	0.2	2.3	0.3	2.7	0.1	0.3	0.40	<0.01
Area, pH × min	1.6	28.9	2.6	42.5	1.3	6.8	0.14	<0.01
Acute RA								
Daily episodes ³	0.0	0.9	0.1	2.4	0.07	0.4	0.01	<0.01
Duration, h/d ⁴	0.0	0.6	0.0	1.1	0.00	0.01	<0.01	<0.01
Area, pH × min	0.01	3.8	0.01	10.6	0.001	2.9	0.10	<0.01

¹Control treatment: far-off diet was fed from -60 to -25 d and the close-up diet was fed from -24 d until parturition. High concentrate (HC) treatment: HC-1 was fed from d -60 to -43, HC-2 from d -42 to -25, HC-3 from d -24 to -13, and HC-4 from d -12 until parturition. Both treatments received the same diet post-partum.

²Significant treatment × period interaction (*P* = 0.01)

³Significant treatment × period interaction (*P* = 0.02)

⁴Significant treatment × period interaction (*P* < 0.01)

energy densities and observed no effect of pre-partum diet on post-partum ruminal pH. Dann et al. (1999) also reported no effect of pre-partum dietary treatment on post-partum ruminal pH. However, in the previous studies, spot sampling techniques were used and ruminal fluid was only collected post-partum once (Rabelo et al., 2003) or over a 24-h period with minimum sampling intervals of 2 h (Dann et al., 1999). Thus, differing results between the current study and previous studies may be related to the method and frequency of ruminal pH measurement.

As expected, the incidence and severity of RA tended to be higher post-partum ($P = 0.09$) than pre-partum as indicated by the area and duration of total acidosis (Table 4.3). Furthermore, RA was affected by day relative to parturition (Table 4.4). In fact, dietary change at parturition induced RA to a similar extent as previous studies (Keunen et al., 2002; Osbourne et al., 2004; Gozho et al., 2006). The RA observed postpartum was not, however, as severe as would be expected had cows been subjected to a grain challenge typically used to induce severe RA (Krause and Oetzel, 2005). In the current study, pre-partum cows (d -5 to d-1 relative to parturition, Table 4.4) had ruminal pH below 5.5 for 1.2 h/d but the duration increased to 3.8 h/d below ruminal pH 5.5 following parturition (d +1 to +5 relative to parturition; Table 4.4). The drastic increase in RA in the current study occurred even though DMI was much lower (pooled treatment means = 11.7 kg/d during the first 5-d post-partum) than in grain induced sub-acute RA studies (Keunen et al., 2002; Osbourne et al., 2004; Krause and Oetzel, 2005; Gozho et al., 2006). Furthermore, RA occurred even though the lactation diet was considered to supply adequate concentrations of NDF of sufficient particle length.

Minimum, mean, and maximum pH decreased (quadratic effect; $P < 0.01$) at d +1 to 5, with the decrease sustained on d +17, +37 and +58 after which ruminal pH measurements were terminated when compared to d -5 to -1. The duration (quadratic effect; $P < 0.01$) and area (linear effect; $P < 0.01$) of total acidosis (pH < 5.8) increased following parturition with the duration of total acidosis decreasing from d +17 to d +58. In all parameters measured, the severity of RA increased following parturition. The increased severity of RA was associated with dietary change at parturition and increasing post-partum DMI. These results support previous results by Gröhn and Bruss (1990) where they reported the greatest number of cases of RA within the first few months after parturition. Furthermore, Krause and Oetzel (2006) reported that the occurrence of RA increased with increasing DIM up to 3 mo; however, the current study does not indicate a worsening of RA with increasing DIM up to 2 mo. The severity of RA as indicated by time spent below ruminal pH 5.8 observed in the current study was similar to previously reported values for primiparous cows in mid-lactation fed barley-based diets (Maekawa et al., 2002).

Variation in ruminal pH values can indicate the relative risk of acidosis (Bevans et al., 2005); however, high variation can also mask treatment differences. In the current study, substantial variation (as indicated by high SEM) was associated with daily ruminal pH parameters near parturition. Furthermore, the current study indicates that animal variability is higher around parturition than later in lactation suggesting an increased risk for acidosis during this period. For example, from Table 4.4, the SEM for the area of total acidosis for the first five days before until 5 days after parturition accounted for 25% of the mean, compared to approximately 20% of the mean for the

Table 4.4. The effect of day relative to parturition on ruminal acidosis.

Variable	Day relative to parturition					<i>P</i> value	
	-5 to -1	+1 to +5	+17	+37	+58	Linear	Quadratic
Minimum pH	5.74 ^a	5.38 ^b	5.37 ^b	5.32 ^b	5.37 ^b	<0.01	<0.01
SEM	0.02	0.04	0.04	0.04	0.04		
Mean pH	6.32 ^a	5.96 ^b	5.95 ^b	5.96 ^b	6.03 ^b	<0.01	<0.01
SEM	0.02	0.04	0.04	0.04	0.04		
Maximum pH	6.87 ^a	6.62 ^b	6.63 ^b	6.59 ^b	6.62 ^b	<0.01	<0.01
SEM	0.02	0.04	0.03	0.03	0.04		
Total RA							
Duration, h/d	1.1 ^c	7.3 ^{ab}	9.0 ^a	8.3 ^{ab}	6.1 ^b	<0.01	<0.01
SEM	0.3	0.9	1.0	1.2	0.9		
Area, pH × min	766.1 ^b	9221.5 ^a	139.9 ^c	146.4 ^c	99.7 ^c	<0.01	0.87
SEM	235.1	1788.2	20.6	27.1	23.8		
Mild RA							
Daily episodes ¹	3.4 ^b	9.5 ^a	10.5 ^a	12.5 ^a	11.2 ^a	<0.01	<0.01
SEM	0.5	1.0	1.0	1.1	1.3		
Duration, h/d	0.9 ^c	3.6 ^b	5.4 ^a	4.8 ^{ab}	3.9 ^b	<0.01	<0.01
SEM	0.2	0.4	0.6	0.7	0.5		
Area, pH × min	764.0 ^b	9163.0 ^a	109.2 ^c	103.4 ^c	70.7 ^c	<0.01	0.91
SEM	234.2	1773.1	15.0	17.0	13.6		
Moderate RA							
Daily episodes	0.6 ^c	4.1 ^b	7.5 ^a	6.3 ^{ab}	4.6 ^{ab}	<0.01	<0.01
SEM	0.2	0.7	1.3	1.0	0.9		
Duration, h/d	0.3 ^c	2.4 ^{ab}	3.2 ^a	2.7 ^{ab}	1.7 ^b	<0.01	<0.01
SEM	0.1	0.5	0.5	0.5	0.4		
Area, pH × min	2.1 ^b	44.7 ^a	28.9 ^a	38.4 ^a	24.5 ^a	<0.01	<0.01
SEM	0.9	10.8	5.8	9.7	8.6		
Acute RA							
Daily episodes	0.1 ^b	1.8 ^a	1.0 ^a	2.1 ^a	1.4 ^{ab}	<0.01	0.16
SEM	0.1	1.8	1.0	2.1	1.0		
Duration, h/d	0.0 ^c	1.4 ^a	0.4 ^{bc}	0.9 ^{ab}	0.6 ^{bc}	0.05	0.28
SEM	0.0	0.5	0.2	0.3	0.3		
Area ² , pH × min	0.0 ^e	13.8 ^a	1.7 ^d	4.7 ^b	4.4 ^c	0.01	0.76
SEM	0.0	5.7	0.8	1.6	2.3		

¹Significant treatment × period interaction ($P = 0.04$).²Significant treatment × period interaction ($P < 0.01$).

average of d +17, +37, and +58. Measurements conducted near calving are inherently fraught with a high degree of variability (Drackley, 1999), hindering the probability of detecting differences between treatments. Furthermore, a high degree of animal variability is also common when measuring ruminal pH during dietary adaptation (Bevans et al., 2005) further reducing the probability of detecting treatment differences. In addition to variation between treatments, variability within treatment was large for all ruminal pH variables, especially the area of total acidosis. It remains puzzling why some cows within a treatment are better able to cope with diet change than others, but these differences among cows may be related to sorting of feed, number of meals, eating rate within a meal, and salivation rate, among other factors.

Pre-partum dietary treatment did not affect total ruminal VFA concentration or individual VFA molar proportions with the exception of butyrate (Table 4.5). However, total VFA concentration increased after parturition when compared to pre-partum ($P < 0.01$). Rabelo et al. (2003) observed no effect of dietary treatment on total ruminal VFA concentration or individual VFA molar proportions when additional concentrate was fed pre-partum (Rabelo et al., 2003). A significant interaction was observed between treatment and period (pre- and post-partum) for the molar proportion of butyrate ($P = 0.02$). This was a result of the HC treatment having higher molar proportions of butyrate pre-partum but similar concentrations post-partum. Pre-partum diets for the HC treatment were formulated to increase ruminal production of butyrate as butyrate has been implicated as a driver of ruminal papillae proliferation (Dirksen et al., 1985). Lactate in the rumen was measured in 50 ruminal fluid samples around

Table 4.5. The effect of pre-partum dietary treatment on ruminal VFA profiles in primiparous Holstein cows.

Variable	Treatment ¹				SEM		<i>P</i> value	
	Control		HC		Pre-partum	Post-partum	Treatment	Period
	Pre-partum	Post-partum	Pre-partum	Post-partum				
Total VFA, mmol/L	108.2	117.3	107.0	122.3	2.3	1.5	0.31	<0.01
Acetate:propionate	3.67	2.68	3.47	2.75	0.07	0.07	0.34	<0.01
Acetate, mmol/100 mol	66.36	61.15	64.97	61.57	0.28	0.43	0.18	<0.01
Propionate, mmol/100 mol	18.24	23.69	18.92	23.49	0.28	0.51	0.57	<0.01
Butyrate ² , mmol/100 mol	10.79	10.78	11.66	10.66	0.22	0.20	0.08	0.02

¹Control treatment: far-off diet was fed from -60 to -25 d and the close-up diet was fed from -24 d until parturition. High concentrate (HC) treatment: HC-1 was fed from d -60 to -43, HC-2 from d -42 to -25, HC-3 from d -24 to -13, and HC-4 from d -12 until parturition. Both treatments received the same diet post-partum.

²Significant treatment \times period interaction ($P=0.02$)

parturition. The concentration of lactate was undetectable in 48 samples and below 2 mmol in the remaining samples. In the current study, lactate was not a contributor to ruminal pH depression.

4.4.3 Dry Matter Intake, BW, BCS, and Lactational Performance

Cows fed the HC treatment had lower DMI ($P = 0.01$) than the control treatment during the last 5-d of gestation but post-partum DMI was similar (Table 4.6 and Figure 4.1). Similar post-partum DMI was a result of cows on the HC treatment having a tendency for a higher rate ($P = 0.09$) of DMI increase during the first 21 d post-partum (173%) ($P = 0.09$) when compared to the control treatment (139%). Drastic changes in DMI corresponding to increases in dietary concentrate predispose cows to RA (Nocek, 1997). Thus, strategies that minimize the pre-partum reduction in DMI and regulate the rate of DMI increase post-partum may reduce RA post-partum.

Others have reported higher pre-partum DMI and similar post-partum DMI for cows fed additional concentrate pre-partum (Halcomb et al., 2001; Rabelo et al., 2003) compared to cows fed lower levels of dietary concentrate pre-partum. However, the previously reported studies measured DMI continuously for 28 d before parturition, whereas the current study only focused on DMI for last 5 d before parturition and at various times post-partum. It is speculated that increased episodes of pre-partum RA contributed to the reduction in DMI for the HC treatment when compared to the control treatment. Previously, Brown et al. (2000) reported reductions in DMI when steers were challenged with either a sub-acute or acute acidosis treatment which supports observed data in the current study.

Table 4.6. The effect of pre-partum dietary treatment on DMI, BW, BCS, milk production, and milk composition in periparturient primiparous Holstein cows.

Variable	Treatment ¹				SEM		<i>P</i> value	
	Control		HC		Pre-partum	Post-partum	Treatment	Period
	Pre-partum	Post-partum	Pre-partum	Post-partum				
DMI, kg/d	10.0	14.6	8.2	14.1	0.4	0.3	<0.01	<0.01
BW, kg	684	584	692	580	16	6	0.80	<0.01
BCS	3.8	2.9	3.8	2.8	0.1	0.04	0.27	<0.01
Milk yield, kg/d	-	30.43	-	29.01	-	1.13	0.38	-
Fat yield, kg/d	-	1.15	-	1.11	-	0.52	0.59	-
CP yield, kg/d	-	0.92	-	0.91	-	0.03	0.87	-
Fat, %	-	3.92	-	3.91	-	0.21	0.98	-
CP, %	-	3.08	-	3.19	-	0.07	0.29	-
Lactose, %	-	4.59	-	4.68	-	0.06	0.34	-

¹Control treatment: far-off diet was fed from -60 to -25 d and the close-up diet was fed from -24 d until parturition. High concentrate (HC) treatment: HC-1 was fed from d -60 to -43, HC-2 from d -42 to -25, HC-3 from d -24 to -13, and HC-4 from d -12 until parturition. Both treatments received the same diet post-partum.

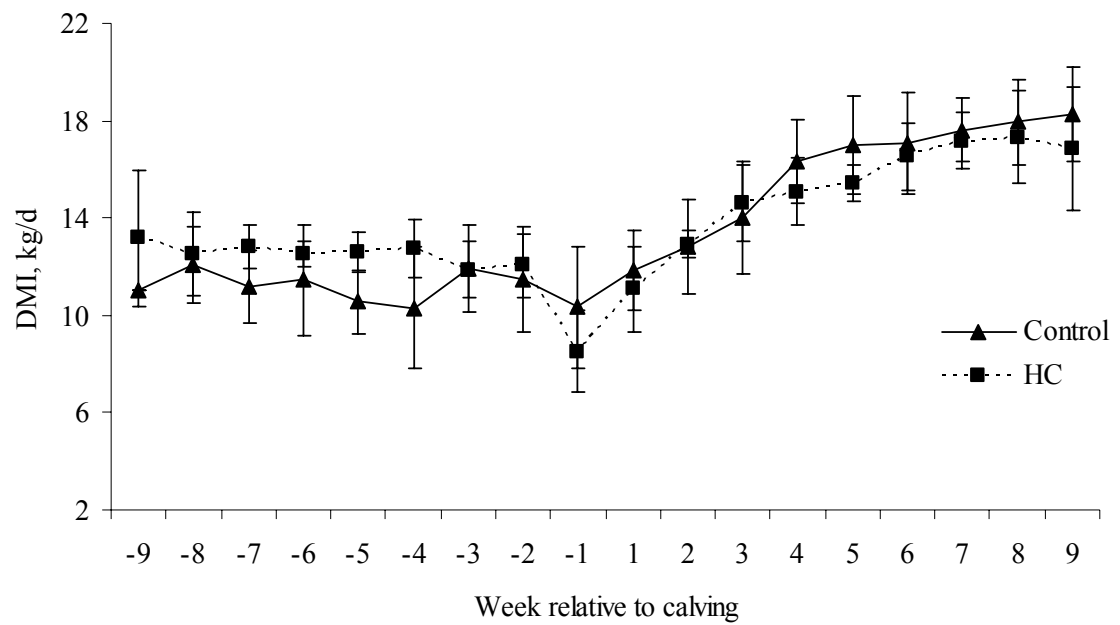


Figure 4.1. Weekly DMI for periparturient primiparous Holstein cows fed the control (n = 7) or an intensive pre-partum concentrate feeding program (HC; n = 7).

Feeding additional concentrate pre-partum did not improve milk yield or alter milk composition. Others have found similar results. For example, Rabelo et al. (2003) fed cows a low energy or high energy pre-partum diet and did not observe any pre-partum dietary effects on milk yield, component yield or milk composition. Alternatively, McNamara et al. (2003) fed three different pre-partum diets varying in F:C ratio and energy density and observed that diets higher in energy density and lower in F:C ratio resulted in increased milk yield. However, it is noteworthy that unlike in the current study, McNamara et al. (2003) fed transition diets containing higher levels of forage.

4.4.4 Blood Metabolites and Hormones

There were no effects of dietary treatment on glucose, BHBA, NEFA, or insulin concentrations ($P > 0.05$; Figures 4.2 and 4.3). Other studies have reported that pre-partum treatment did not affect post-partum glucose or insulin concentration (Dann et al., 1999; Rabelo et al., 2005). However, Dann et al. (1999) found a decrease in pre-partum NEFA and BHBA concentrations when diets contained higher levels of ruminally degradable carbohydrate. The lack of a treatment effect for NEFA in the current study suggests that feeding additional concentrate pre-partum did not improve energy balance or reduce adipose tissue mobilization around parturition.

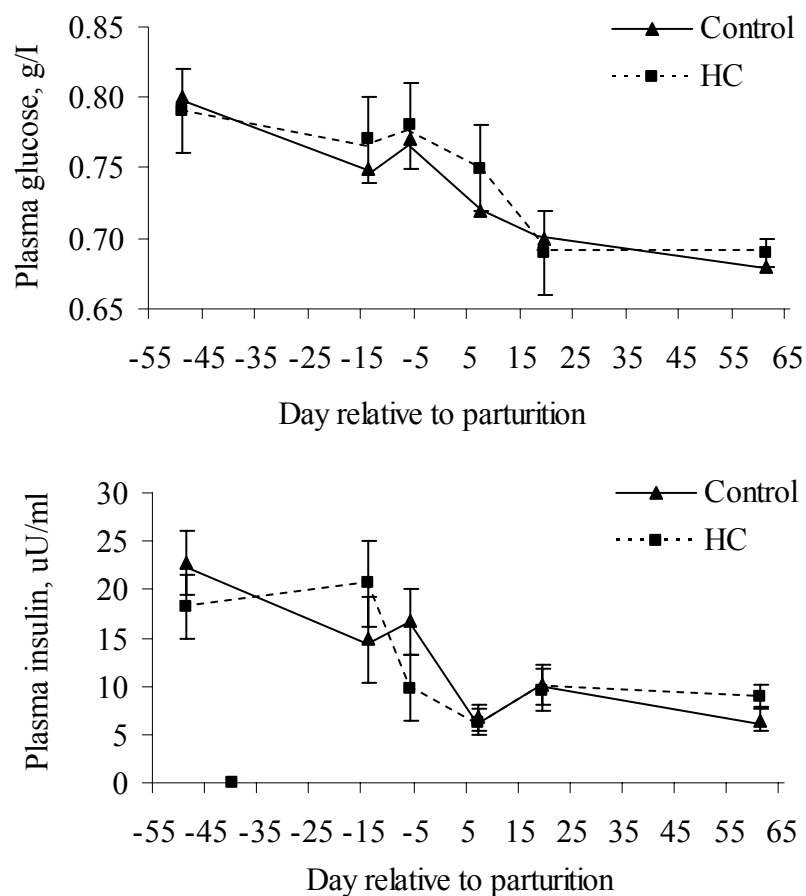


Figure 4.2. Plasma glucose and insulin concentrations in periparturient primiparous Holstein cows fed the control or an intensive pre-partum concentrate feeding program (HC).

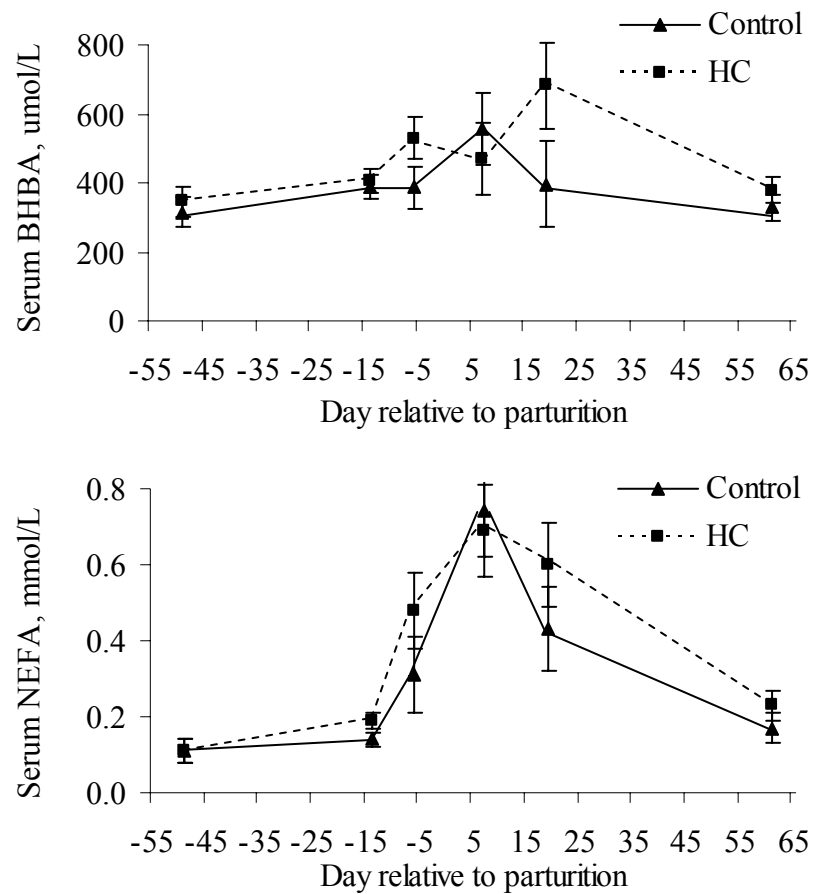


Figure 4.3. Serum NEFA and BHBA concentrations in periparturient primiparous Holstein cows fed the control or an intensive pre-partum concentrate feeding program (HC).

4.5 Conclusion

Feeding additional concentrate pre-partum did not reduce the risk of post-partum RA in primiparous Holstein cows. Furthermore, the occurrence and severity of RA increased immediately post-partum, regardless of pre-partum diet. Post-partum acidosis may have negative implications on animal health, fiber digestion, and nutrient supply, thus exacerbating the effects of the negative energy balance in early lactation cows. Results of this study emphasize the importance of developing new feeding strategies that reduce the risk of RA near parturition.

5.0 GENERAL DISCUSSION

5.1 Development of a Technique to Measure Ruminal pH in Transition Cows

The first objective of this study was to develop a continuous pH measurement system capable of measuring ruminal pH in unrestrained cattle. It was also desirable to know the required frequency for electrode standardization. The LRCpH was developed and it accurately and precisely measured ruminal pH for up to three consecutive days without a requirement for electrode standardization. Furthermore, the LRCpH was shown to have higher accuracy and precision than an existing continuous measurement system (CIpH). The development of the LRCpH overcomes limitations of previous indwelling ruminal pH measurement systems that require animals to be tethered. Thus, continuous ruminal pH measurement can now be conducted in grazing, feedlot, or pen-housed cows without daily electrode standardization. Furthermore, the use of the LRCpH system provides the opportunity to incorporate ruminal pH data with diet fermentability and animal behavior when cows are housed in group settings.

5.2 The Severity of Ruminal Acidosis in Primiparous Holstein Cows During the Periparturient Period

To my knowledge, this is the first study that continuously measured ruminal pH during the periparturient period to determine the effect of pre-partum dietary treatment on post-partum ruminal acidosis and animal performance. To accomplish the objective of this study, ruminal pH was measured using the LRCpH system in primiparous cows during the periparturient period. I hypothesized that the occurrence and severity of

ruminal acidosis would increase post-partum as a result of dietary change, but, that providing additional concentrate pre-partum would promote microbial adaptation and ruminal papillae proliferation, thereby reducing ruminal acidosis post-partum. However, the results of this study do not support my original hypothesis. This study showed that additional concentrate allocation pre-partum does not reduce the occurrence or severity of post-partum ruminal acidosis.

Results from the current study demonstrate that feeding additional concentrate pre-partum did not reduce post-partum ruminal acidosis. Others have reported similar results. For example, Rabelo et al. (2003) used rumenocentesis to measure ruminal pH in cows fed a low or high energy diet pre-partum and a low or high energy diet post-partum. They reported no effect of pre-partum dietary treatment on ruminal pH. In contrast, pH was affected by post-partum diet; cows fed the high energy diet post-partum had lower ruminal pH than cows fed the low energy diet. The results of the current study confirm the observations of Rabelo et al. (2003) and suggest that pre-partum dietary treatment has little effect on post-partum ruminal pH. Therefore, emphasis should be placed on nutritional management during the post-partum portion of the transition period in order to reduce ruminal acidosis while optimizing energy intake.

The second part of the original hypothesis deals with the concept of ruminal adaptation via stimulation of ruminal papillae proliferation. Although “lead feeding” is a common commercial practice (Kokkonen et al., 2004) and is thought to pre-adapt the ruminal environment (both microbial and physiological) to the lactation diet thus reducing ruminal acidosis (NRC, 2001), little data lends scientific support to this practice. In the current study, there was no reduction in post-partum ruminal acidosis

between treatments. Furthermore, there was no effect of pre-partum dietary treatment on papillae length, width, or surface area (data not presented). The lack of response in papillae proliferation may be a causative factor for the lack of treatment effects on ruminal acidosis, as papillae are responsible for the majority of VFA absorption (Dirksen et al., 1997).

In the current study, there was an attempt to condition cows fed the higher concentrate treatment to the lactation diet using stepwise increases in the concentrate portion of the diet. In the process, I focused on the F:C ratio rather than dietary ingredients. For example, the F:C ratio in the high concentrate treatment was adjusted by including more barley grain, wheat middlings, dry distillers' grains, and beet pulp while reducing the amount of barley silage and grass hay. At parturition, cows on the high concentrate treatment did not experience a change in the F:C ratio of their diet, but they did experience a dietary change in the ingredient composition of the diet. The lactation diet included ingredients such as alfalfa hay and corn gluten meal that were not incorporated into the pre-partum diet. Furthermore, the lactation diet did not include wheat middlings or dry distillers' grains. Thus, while the cows were adapted to a high concentrate diet, it is possible that cows were not adapted to a high concentrate diet with the same ingredients as used in the lactation diet. The implication of this inconsistency may have been a failure of the microbial population to readily adapt to the lactation diet. Previously published studies (Tajima et al., 2000; Tajima et al., 2001) have adapted animals to high concentrate diets using intermediate diets similar in ingredient composition while measuring changes in microbial populations. Thus, the use of

different dietary ingredients for the pre-partum diets and post-partum diet may have resulted in increased ruminal acidosis as a result of dietary ingredient changes at parturition.

The third reason for a lack of treatment response was high variation within and between treatments. For example, in the present study the mean square error for the duration of total acidosis was 0.66. Power analysis was used to determine the probability of detecting significant differences ($P < 0.05$) between the observed means. This resulted in a power of 0.51 indicating detection of significant differences only 49% of the time. To achieve an 80% probability of detecting differences with the observed means and variation, 10 cows per treatment are required. Because the author is not aware of any other studies that have measured ruminal pH during the periparturient period, no preliminary data on ruminal pH variation near parturition was previously available. This study suggests that a minimum of 10 cows per treatment is required for the detection of differences between treatments during the periparturient period for ruminal pH parameters. Others have reported high variation for continuous ruminal pH parameters during diet adaptation (Bevans et al., 2005). Bevans et al. (2005) used variation as an indicator of the risk of acidosis. In the current study, large increases in variation (SEM) associated with ruminal pH occurred immediately after parturition. This indicates that animal variability and the risk of acidosis are higher around calving than later in lactation. For example, the SEM for the area of total acidosis for the first five days before parturition accounted for 30% of the mean, compared to approximately

20% of the mean post-partum. Measurements conducted during the transition period have a high degree of variability (Drackley, 1999) hindering the probability of detecting differences between treatments.

This study evaluated the occurrence and severity of post-partum acidosis as affected by pre-partum dietary treatment. While acidosis is recognized to be an important problem in the dairy industry, few studies have evaluated the occurrence of post-partum ruminal acidosis. In the current study, ruminal acidosis drastically increased after parturition. In fact, for the first five days post-partum the occurrence of daily ruminal acidosis (ruminal pH <5.8) was 93%. This indicates that nearly all cows experienced some type of ruminal acidosis during each of the first five days after parturition. The practical implication of this finding for dairy producers is that it may be beneficial to use a post-partum diet that is of moderate fermentability for the first week after calving when the risk of acidosis is high; however, limiting dietary fermentability reduces energy supply and may reduce lactational performance.

The criteria used to define SARA are not consistent in the literature. Nocek (1997) suggested that the critical pH threshold for SARA is <5.5 and <5.0 for acute acidosis. However, based on the negative effects of pH on fiber digestion, the critical threshold to evaluate SARA may need to be higher than that suggested by Nocek (1997). For example, little growth of cellulolytic bacteria occurs in the rumen at pH <6.0 (Russell and Wilson, 1996), and consequently, DM degradation decreases (Mould et al. 1983). Calsamiglia et al. (2002) reported that prolonged exposure of rumen microbes to a pH of 5.7 has a negative impact on fiber digestion. Because the effects of ruminal pH on ruminal fiber digestion become increasingly severe as pH decreases,

three ruminal pH threshold values were used to characterize acidosis: mild SARA ($5.8 > \text{pH} > 5.5$), severe SARA ($5.5 > \text{pH} > 5.2$), and acute acidosis ($\text{pH} < 5.2$). Partitioning total acidosis into three categories allowed the characterization of acidosis based on severity, thus improving the understanding of ruminal dynamics. For example, without this characterization, it would have been impossible to detect the interaction between treatment and period for the area of acute acidosis.

5.3 Future Research

1. The LRCpH system could be used in group-housed animals and integrated with animal behaviour measurements to better understand the relationship between diet fermentability, feeding behaviour, and ruminal pH. Group housing incorporates the aspects of animal interaction and competition and more closely reflects conditions in commercial production.
2. Further research is needed to design new transition feeding strategies that reduce the occurrence and severity of post-partum ruminal acidosis. This should include further examination of the relationship between pre-partum diet, ruminal acidosis, and ruminal papillae characteristics.
3. Subsequent studies are needed to determine the effect of pre-partum dietary treatment on post-partum ruminal acidosis in multiparous animals. Multiparous cows are different than primiparous cows as they have higher DMI that may predispose them to ruminal acidosis (Nocek, 1997). However, multiparous animals have had previous exposure to high concentrate diets and may have more developed papillae than primiparous animals at parturition.

4. Studies evaluating the effect of pre- and post-partum dietary treatment on the incidence of ruminal acidosis should also incorporate the use of similar dietary ingredients for both the pre- and post-partum diets in order to allow for microbial adaptation.

6.0 GENERAL CONCLUSION

Results from the current study demonstrate that the LRCpH can accurately and precisely measure ruminal pH continuously for up to 72 consecutive hours. Using the LRCpH provides the opportunity to measure ruminal pH without requiring animals to be tethered. The LRCpH was used to determine the effect of additional pre-partum concentrate allocation on post-partum ruminal acidosis. Post-partum ruminal acidosis was not affected by pre-partum dietary treatment but, ruminal acidosis increased drastically after parturition. The results of this study show that ruminal acidosis is common immediately post-partum and in early lactation emphasizing the importance of developing feeding strategies that reduce the risk of ruminal acidosis near parturition.

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