EFFECTS OF DIETARY CATION-ANION DIFFERENCE (DCAD) AND NA:K ON DAIRY COWS IN EARLY LACTATION, AND THE INTERACTION OF PARTICLE SIZE REDUCTION VIA MASTICATION AND RUMINATION WITH DIGESTION AND PASSAGE IN CATTLE

BY

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DISSERTATION

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ABSTRACT

Cattle spend many hours per day chewing, either eating or ruminating. Comminution of feed and digesta particles affects the kinetics of digestion and passage, and can also affect voluntary feed intake. These, in turn, determine nutrient availability and productive efficiency. Our objective was to incorporate relevant data into a framework leading towards a dynamic mathematical model for comminution from feed through feces in cattle. Although large particles (i.e., those retained on a screen with 1.18-mm pores) often comprise 80 to 90% of swallowed forage dry matter, they account for about 35% of fecal dry matter. Large particles can be a minority of those in the reticulorumen at any given time; therefore, size is not the only criterion determining passage to the lower gut. Current data support the conclusion that synergism exists between animal and microbial effects; i.e., mastication during eating enhances microbial fermentation, which increases the effectiveness of comminution during rumination. Significant amounts of variation in the particle size distributions of boluses entering the reticulorumen can now be explained from knowledge of feed characteristics. Our understanding of mastication and rumination effects on digestion and passage in cattle is limited because no information is available for mixed diets and few data exist for many common types of forage (none for silages or which address the effects of plant maturity). Data amenable to studying the dynamics of particle size distributions are few and relate to near steady-state conditions; therefore, synergies between mastication, digestion, and rumination under practical conditions remain to be examined.

Six multiparous Holstein cows, fitted with rumen cannulas, averaging 122 ± 31 days in milk were randomly assigned to six treatments allocated in an equiradial (pentagonal) second-order response surface design with a center point to examine the effects of dietary cation-

anion difference (DCAD) and Na:K on lactating dairy cows. Replication of treatments within a 6 x 6 Latin square minimized the potential effects of outliers and allowed a surface covering a 3 × 3 matrix of DCAD and Na:K combinations to be examined. Ranges in DCAD and Na:K were chosen to be equally spaced on logarithmic scales; tripling each time from 0.25 for the former, and 1.5-fold each time from 25 meq/100 g of DM for the latter. The response surface was centered on a molar Na:K of 0.75 (0.60% Na and 1.37% K in DM) and a DCAD of 37.5 meq/100 g of DM. The other 5 treatments were: 1.63, 50.0 (Na:K, DCAD); 0.46, 53.8; 0.25, 35.2; 0.63, 25.1; and 2.00, 31.2. Percentages of Na and K in DM of the TMR for vertices of the pentagon were calculated as 1.05, 1.10; 0.56, 2.08; 0.27, 1.84; 0.44, 1.17; and 0.84, 0.72. Diets were based on corn silage and a corn-based grain mix. The Na:K ratios were varied with $NaHCO_3$ and K_2CO_3. Periods were 14 d. Daily feed intake of each cow was recorded during each period; samples of feed and orts were collected daily. Milk production was measured daily; samples were collected weekly and analyzed for components. Rumen and urine samples were collected and analyzed for pH on the last 3 d of each period. The MIXED procedure of SAS was used for ANOVA. There were no response surface effects of treatment on milk production and components, or DMI (P<0.05). Acetate, propionate, and butyrate concentrations in the rumen were all affected by treatment (P <0.05). There were multiple significant effects on acetate, including an interaction of DCAD and ratio. There were both linear and quadratic effects of ratio on propionate and butyrate. Linear (P <0.05) and quadratic effects (P <0.05) of DCAD on rumen pH were also indicated. A quadratic effect of Na:K (P < 0.01) and interaction of DCAD (P < 0.003) indicated that urine pH was maximal (8.24 or above) at high DCAD and low Na:K. Milk production and components were similar across treatments, but rumen fermentation was affected.

Rumen and urine samples were collected and analyzed for pH on the last 3 d of each period. There was a relationship between pH_6-h and ruminal pH (r^2= 0.64, $P < 0.001$). The relationship between mean ruminal pH and mean urinary pH explained 15% of the variation ($P < 0.022$), but few data were below pH 6. The relationship between mean urinary pH and mean ruminal pH_6-h explained 28% of the variation ($P < 0.001$). Few published data compare ruminal and urinary pH. A relationship between ruminal and urinary pH was measured. More data are necessary to further elucidate this relationship before making determinations of the presence of SARA.

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CHAPTER I

REVIEW OF LITERATURE

Part 1. Mastication and Rumination Effects on Digestion and Passage

Summary

Cattle spend many hours per day chewing, either eating or ruminating. Comminution of feed and digesta particles affects the kinetics of digestion and passage, and can also affect voluntary feed intake. These factors in turn determine nutrient availability and productive efficiency. Our objective was to incorporate relevant data into a framework leading towards a dynamic mathematical model for comminution from feed through feces in cattle. Although large particles (i.e., those retained on a screen with 1.18-mm pores) often comprise 80 to 90% of swallowed forage dry matter, they account for about 35% of fecal dry matter. Large particles can be a minority of those in the reticulorumen at any given time; therefore, size is not the only criterion determining passage to the lower gut. Current data support the conclusion that synergism exists between animal and microbial effects; i.e., mastication during eating enhances microbial fermentation, which increases the effectiveness of comminution during rumination. Significant amounts of variation in the particle size distributions of boluses entering the reticulorumen can now be explained from knowledge of feed characteristics. Our understanding of mastication and rumination effects on digestion and passage in cattle is limited because no information is available for mixed diets and few data exist for many common types of forage (none for silages or which address the effects of plant maturity). Data amenable to studying the dynamics of particle size distributions are few and relate to near steady-state conditions; therefore, synergies between mastication, digestion, and rumination under practical conditions remain to be examined.

Part 2. Dietary Cation-Anion Difference in Lactating Dairy Cows

Introduction

The concept of manipulating acid-base status in livestock in not new; it is well established in non-ruminants (Austic and Patience, 1988). In cattle, much of the work has been done in regard to close up dry cows and the prevention of parturient paresis (Block, 1984; Oetzel et al., 1988). With the accepted use of negative DCAD concentrations in dry cow diets, interest has grown in the optimum DCAD for lactating cows.

Theoretically, DCAD should be high at the onset of lactation, and decrease throughout (Block, 1994). According to NRC (2001), lactating cows should have 29 meq/100g of DM (based on nutrient requirements for a 680 kg cow, BCS 3.0, 35 kg MY, 3.5% fat, 3.0% protein). However, Hu et al. (2007a) examined the relationship in 16 published studies between DMI and DCAD and found that DMI was maximized at 47 meq/100 g of DM.

Numerous studies have investigated the effect of DCAD on production and health of lactating dairy cows. Tucker and Hogue (1990) evaluated the influence of Na, K, and Cl at a constant DCAD and found that DCAD was more important than the individual ions on systemic effects. Sanchez et al. (1994a) investigated potential interrelationships of Na, K, and Cl, especially those between Na and K, and found that they were related to blood acid-base status and mineral concentrations in blood and milk. Sanchez et al. (1994b) built a large database and found interactions between Na, K, and Cl. Responses of DMI and MY to Na or K differed over a range of dietary concentrations of K or Na, Cl and other mineral elements (Sanchez et al. 1994c). Block and Sanchez (2000) fed lactating cows in early lactation one of three diets: a control diet with no added Na or K (DCAD of =18 meq/100g of DM) or two higher DCAD diets (+25 and +52 meq/100 g of DM, manipulated using $NaHCO_3$ or K_2CO_3). Within the higher DCAD diets,

the combination of Na and K resulted in the best response for DMI and milk production, and had the highest blood CO_3^-.

Dry matter intake responses

Tucker et al. (1988) found the greatest DMI in cows fed +20 meq/100 g of DM vs. those with -10 meq/100 g of DM. In another experiment, DMI increased quadratically as DCAD3 (Na+K-Cl) ranged from -12 to 31 meq/100 g of DM (West et al., 1991). West et al. (1992) conducted another trial with heat stressed lactating cows. The diets had $DCAD_3$ of +12, +22, +35, or +46 meq/100 g of DM. Dry matter intake and DMI as a percentage of BW increase linearly with increasing DCAD.

Delaquis and Block (1995) showed that the optimal DCAD for lactating cows may change with stage of lactation. In early and mid-lactation, DMI increased with increasing DCAD, but there was no effect in late lactation. Using empirical modeling techniques, Sanchez and Beede (1996) analyzed data from 10 nutrition experiments investigating macrominerals in the 1980's. They found DMI to be maximal at $DCAD_3$ +38, the best range was between +25 and +50 meq/100 g of DM.

Milk yield and milk composition

If acid base status is disturbed, MY will respond to maintain pH homeostasis. High DCAD diets should counteract acidic conditions with the positive anions Na and K. Tucker et al. (1988) studied lactating cows 3 to 5 months postpartum. Cows fed DCAD3 +20 meq/100 g of DM yielded 9% more milk than -10 meq/100 g of DM. However, typical lactation diets have DCAD of about +20 meq/100 g of DM (Sanchez et al., 2000). West et al. (1991) found in both hot and cool environments, increasing DCAD3 -12 to 31 meq/100 g of DM, MY, 4%FCM and milk protein increased linearly. Delaquis and Block (1995) reported that increasing DCAD (25.8

vs. 5.6 meq/100 g of DM in early; 37.3 vs. 14.0 meq/100 g of DM) increased milk production in early and mid-lactation, but not in late lactation. Milk protein percentage was higher at higher DCAD in early lactation, but milk fat percentage was lower at high DCAD, probably due to high milk production.

Hu et al. (2007a) varied dietary crude protein (CP) percentage and DCAD (-3, 22, or 47 meq/100 g of DM; 16 or 19% CP) in 6 lactating cows in a Latin square design, for 6 weeks. There was a linear effect on DMI, milk fat percentage, 4% fat-corrected milk production, milk true, protein, milk, lactose, and milk solids-not-fat. Milk production itself was unaffected by DCAD. There was no effect of CP % on production measures. Hu et al. (2007b) also conducted a study in early lactation cows (16 Holsteins and 8 Jerseys), and fed diets containing either 22 or 47 meq/100 g of DM with 19% CP. The DCAD did not affect DM intake, milk production, or milk composition. Intake of DM and performance of cows postpartum were not improved when DCAD increased from 22 to 47 mEq/100g of DM, probably because of the high variability of cows in early lactation.

Wildman et al. (2007a) also evaluated effects of dietary CP and DCAD in early lactation cows. Eight primiparous lactating Holstein cows (47 ± 10d in milk) were fed diets providing 15 or 17% CP and DCAD of 25 or 50 meq (100 g of DM. High DCAD improved DMI, MY, and concentrations of milk fat and protein. Wildman et al. (2007b) conducted another study examining DCAD and CP percentage variations in lactating cows under heat stress. Thirty-two cows in late lactation were assigned to one of four treatments were arranged as a 2 × 2 factorial within a randomized complete block design. Diets provided 15 or 17% CP and a DCAD of 25 or 50 meq/100 g of dry matter (DM). A DCAD × CP interaction was detected for MY; MY was less

for high DCAD than for low DCAD for the high-CP diets. The high DCAD was at the high end of the suggested range, and may have been too high for a response in late lactation cows.

While it has been determined that the source of cations (Na or K) has little effect on DMI or MY (West et al., 1992), there is little information on their ratios in dairy rations. Sanchez et al. (1997) compared dietary proportions of $NaHCO_3$, NaCl, and KCl and observed that DMI was influenced by an interaction between Na and K and between Na and Cl. The authors also reported increased 3.5% FCM with higher dietary Na and concluded that interrelationships exist among Na, K, and Cl. Sanchez et al. (1994a) reported that the DMI and MY response to one cation (Na or K) tended to be the greatest when the dietary level of the other cation was low. Wildman et al. (2007c) varied K:Na ratios at two high DCAD (+41 or +58 meq/100 g of DM). The K:Na ratios were 2:1, 3:1, or 4:1 on a as fed basis; treatments were each ratio at both DCAD levels. Dry matter intake was similar across all treatments (22.5 kg/d). There was no DCAD effect on any variables. There was a quadratic effect of K:Na on MY and energy corrected milk. Milk yield was depressed at the 3:1 ratio. This is similar to Sanchez et al. (1994a), where the greatest effect was seen at either high or low Na or K. Hu and Kung (2009) conducted a similar study, but held DCAD constant (33 meq/100 g of DM). The ratios of Na:K was based on a molar value, rather than a percentage. Ratios were 0.21, 0.53 and 1.06 (0.25% Na to 2.0% K; 0.5% Na to 1.6% K; 0.75% Na to 0.20% K). Dry matter intake responded quadratically; it was lowest at the 0.53 ratio. There were no effects on MY or composition.

Rumen parameters

Apper-Bossard et al. (2010) used 6 lactating Holstein cows in a split plot with 3 levels of DCAD in a 3 x 3 Latin square with six diets. The six corn silage based diets varied in DCAD and

concentrate level (low or high). The DCAD ranged from 11 meq/kg DM to 327 meq/kg DM. When cows were fed the high concentrate diet (41% concentrate), mean ruminal pH decreased ($P < 0.01$), ruminal VFA was not affected, molar proportion of acetate tended to decrease ($P < 0.10$) and molar proportion of propionate increased ($P < 0.05$). Increasing DCAD affected mean ruminal pH which tended to be highest at a DCAD of 150 meq/kg DM. Large variations in pH were observed during the feeding cycle, which was expected. Ruminal pH declined more rapidly when cows were fed high concentrate diets, but DCAD had no effect on ruminal pH pattern or acidity. It is worth noting, that DMI was maximized at 24.6 kd/d ($P < 0.05$) at the highest DCAD level, when the high concentrate diet was fed.

Summary

There is interest in the determining an optimum DCAD for lactating dairy cows. Hu et al. (2007a) found DMI is maximized at 47 meq/100g DM. While it has been determined that total DCAD is more important than the cation source, there is interest in the ratio between Na and K. Recent research suggests increasing DCAD primarily with Na and K affects milk yield and energy corrected milk (Wildman et al., 2007c).

Section 3. Subacute ruminal acidosis in dairy cows

Introduction

Ruminal acidosis occurs when diets high in fermentable carbohydrates are fed to ruminants. Ruminants evolved while primarily consuming and digesting forages (Van Soest, 1994); however, high milk production requires that more concentrates, such as ground corn or barley, be fed to provide energy to rumen microbes and their host. Increases of milk yield may

not be advantageous if dry matter intake (DMI), and milk fat yield are reduced, or the incidence of lameness and reproductive loss are increased.

In countries with a quota system, the incidence of acidosis is probably lower than in countries like the US. In the US system, it has been economical to feed large amounts of corn grain to provide the energy to support high milk production. This approach puts US herds at much greater risk of developing rumen acidosis. There are a limited number of field studies in the US documenting the incidence of subacute ruminal acidosis (SARA). A cross-sectional field study (Garrett et al., 1997) of 15 Holstein dairy herds in Wisconsin detected ruminal pH values <5.5 in 19% of cows between 2 and 30 days in milk (DIM) and 26% of cows that were 90 to 120 DIM. In one-third of these herds, >40% of the lactating cows tested had ruminal pH values < 5.5. In a case study conducted on a 500-cow NY farm, Stone (1999) calculated a cost of $400 to $475 lost income per cow per year. This figure was based on an observed decrease in milk production of 3 kg/cow/d and decreased concentrations of milk fat and true protein from 3.7 to 3.4 % and 2.9 to 2.8 %. The cost of lameness, and its effect on reproduction was not estimated, but it was likely larger than the cost of lost milk. It has been estimated that SARA costs the US dairy industry $500 million to $1 billion per year, with a cost per affected cow of $1.12 (Donovan, 1997). This is based on reduced milk production and efficiency of production, premature culling, and death loss (Krause and Oetzel, 2005). Based on these figures, it seems that SARA is the most important nutritional disease of dairy cattle.

Acute acidosis

"Acidosis" is used collectively to describe digestive upset in the rumen and intestines (Owens et al., 1998). Another definition is: a pathological condition resulting from accumulation of acid or depletion of the alkaline reserve (bicarbonate content) in the blood and body tissues,

and characterized by an increase in H ion concentration (decrease in pH) (Blood and Studdert, 1999). Acute acidosis occurs when there is a sudden drop in rumen pH, often following a large intake of very fermentable carbohydrates. As lactic acid concentrations rise, rumen pH drops (Owens et al., 1998). This rise in lactic acid will cause the pH to drop further and, if it falls below 5, death may occur. Cows that have not been adapted to a high grain diet are more susceptible than adapted cows, probably because they have not developed a sizable population of lactic-acid utilizing bacteria, which thrive at a higher pH or because the rumen papillae may be unable to absorb large amounts of acid (Dirksen et al., 1985). Above pH 5.0, *Streptococcus bovis* bacteria are the primary lactate producers (Owens et al., 1998). The fermentation products of *S. bovis* depend on both growth rate and pH; once the pH drops below 5.2, lactate rapidly accumulates which further exacerbates the problem (Russell and Hino, 1985).

The physiological progression during acute ruminal acidosis includes high concentrations of ruminal lactate, rumenitis, ruminal hyperosmololality, dehydration, and systemic acidemia (Owens et al., 1998). Clinical signs of acute acidosis include complete anorexia, abdominal pain, diarrhea, tachycardia, staggering, and death (Rebhun, 1995; Krause and Oetzel, 2006). Treatment protocols are reviewed elsewhere (Radostits et al., 1994; Rebhun, 1995). Cows can survive acute acidosis but could die from rumenitis (Radostits et al., 1994). Generally, acute acidosis is more common in feedlot animals than in dairy cows (Elam, 1976; Owens et al., 1998). The remainder of this section will focus on SARA in dairy cows.

Definition of subacute acidosis

Traditionally, the definition of SARA is based on rumen pH, determined by various methods (Plaizier et al., 2009). Several authors have stated threshold pH levels. Plaizier (2004) used 6.0 as a threshold when using a stomach tube 4-h post feeding. Duffield et al. (2004) found that

ruminal fluid collected from the ventral sac via a cannula and through a stomach tube were 0.33 and 0.35 pH units higher than fluid collected by rumenocentesis. Based on those observations, the authors concluded that pH thresholds for defining SARA should be 5.5, 5.8, and 5.9 when samples are collected by rumenocentesis, through a cannula, and by stomach tube, respectively. Garrett et al. (1999) used a threshold of 5.5 using rumenocentesis. Gozho et al. (2005) used a rumen pH criterion between 5.2 and 5.6 for at least 3 h/day; they found reduced feed intake and increased inflammation were only seen at equal or greater pH depressions.

There is a lack of uniformity of definition; terminology and descriptions of SARA vary widely. Subacute ruminal acidosis (Nordlund et al., 1995; Garrett et al., 1999; and Stock, 2000), chronic rumen acidosis (Slyter, 1976), and subclinical rumen acidosis (Nocek, 1997), have all been used in the literature to define this form of acidosis. A differentiation between subliminal and subclinical (chronic) acidosis has also been made (Owens et al., 1998). "Chronic" probably is not accurate in dairy cows because rumen pH is low during a defined period, usually after feeding or after calving to 5 months into lactation, unlike in beef cattle. Subclinical is not appropriate either because there are clinical signs. Subacute ruminal acidosis (Nordlund et al., 1995; Garrett et al., 1999; and Stock, 2000) seems to be the most appropriate term, because its consequences are clinically detectable (Kleen et al., 2003).

In the past, beef cattle were considered at greater risk than dairy cattle for developing ruminal acidosis. Even though dairy cattle are fed higher forage diets than finishing beef cattle, this difference is offset by their DMI (Krause and Oetzel, 2006). Thus, total non-forage carbohydrates (NFC) intakes are often comparable between beef and dairy cattle. It could be assumed that the prevalence of ruminal acidosis is similar between beef and dairy cattle.

Subacute ruminal acidosis seems to be caused by an increase of total volatile fatty acid (VFA) production. Unlike acute acidosis, lactate does not consistently accumulate in cows afflicted with SARA. In one study, 154 cows from 14 dairy farms in WI had rumen fluid sampled by rumenocentesis (Oetzel et al., 1999). Approximately half of the cows were between 2 and 30 DIM and the other half were at peak intake (90 to 120 DIM). Three cows had ruminal lactate over the "normal" upper concentration of 5 m*M* and none had lactate concentrations over 40 m*M*, the threshold for acute acidosis; however, over 20% of the cows had a rumen pH below 5.5. In another experiment, 8 fistulated cows were fed diets either high (38%) or low (29%) in neutral detergent fiber (NDF), with total starch comprising either 33 to 38% (low NDF) or 24 to 26% (high NDF) of total diet dry matter (Oba and Allen, 2000). Rumen pH was measured continuously using an in-dwelling probe and rumen fluid was sampled every 3 h, during the collection period. Rumen pH was significantly lower and pH 6-h, pH 5.5-h and pH 5-h were all significantly increased in the low NDF diet compared to the high NDF diet. Mean rumen pH for all diets was 5.6 to 5.9, maximum pH was 6.5 to 6.6, and minimum pH was 5.0 to 5.4. Based on mean rumen pH, these cows had episodes of SARA; however, rumen lactate, was never above 1.7 m*M* for any treatment. Other experiments (Mishra et al., 1970) have shown episodes of low pH without an accompanying increase of lactate. It should be noted, however, that there can be transient spikes of lactate, if lactate is measured frequently throughout the day (Kennelly et al., 1999). Two distinct groups of dairy cows are considered at the greatest risk: 1) early lactation cows that are eating energy-rich rations without adaption, resulting in low rumen pH; and 2) mid-lactation cows with high DMI that are very sensitive to sudden changes in diet, either in composition or delivery (Oetzel and Nordlund, 1998).

Physiology of rumen pH

Rumen pH falls below physiological levels when the cow consumes a diet high in energy and low in "structure", e.g. structural fiber. Owens et al. (1998) covered the exact transactions in detail. Basically, each cow has an inherent capacity to buffer and absorb acid, and that capacity determines how far her ruminal pH will fall after a meal containing large amounts of fermentable carbohydrates (Krause and Oetzel, 2006). Rumen pH is the result of the production of VFA by rumen bacteria, water flux across the wall, saliva flow and buffers into the rumen, feed acidity and buffering capacity, and water outflow to the lower tract (Erdman, 1988; Allen, 1997). Rumen pH has also been directly related to rumen VFA concentration (Rumsey et al., 1970). Intake of ruminally fermentable carbohydrates depends on both density of non-structural carbohydrates (NSC) in the diet and total DMI. High DMI have been associated with lower ruminal pH (Oetzel and Nordlund, 1998), and are considered to be a major factor in the incidence of SARA. These data have also been supported clinically (Krause and Oetzel, 2006). Until the third month of lactation, increasing DIM is associated with decreasing ruminal pH. This parallels the normal increase in DMI in early lactation.

During a 24-h period, ruminal pH varies and is driven by the amount of fermentable carbohydrates in each meal. The pH can shift 0.5 to 1.0 pH unit throughout one day, representing a 5- to 10-fold change in H^+ ion concentration. Figure 1.1 illustrates a typical pattern of pH variation during a 24 hour period. Oetzel and Nordlund (1998) found that increasing frequency of feeding (6 times versus 2 times daily) smoothed pH variation, but also led to increased DMI and lower mean ruminal pH.

Since ruminal pH can vary so much after eating, it is difficult to make conclusions about a single ruminal pH value, including under research settings. Woodford and Murphy (1988) fed

diets differing in concentrate and alfalfa physical form and found that while mean rumen pH was similar, the area under a certain pH value differed. Krause et al. (2002) reported that varying forage particle size and level of fermentable carbohydrates in diets of midlactation cows affected area below pH 5.8 more than mean ruminal pH. Continuous measurement by indwelling probes is the best way to evaluate pH changes after eating meals.

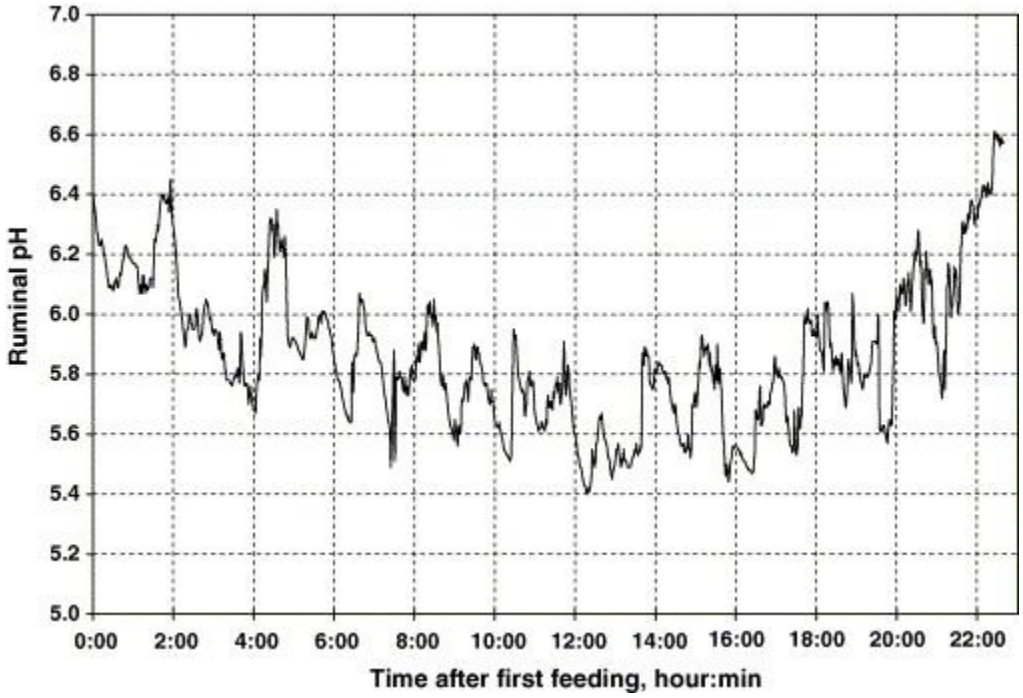

Figure 1.1. Postprandial variation in ruminal pH over a 24-h period. Cows were fed dry, cracked corn grain and finely chopped alfalfa silage twice daily. (Adapted from Krause and Oetzel, 2006).

Intake Regulation

Because acid production varies post feeding, ruminants have developed complex systems to maintain ruminal pH between 5.5 and 7.0. The main mechanism is likely feed intake regulation. Fulton et al. (1979) demonstrated that beef cattle fed high concentrate diets have wide shifts in intake and resultant ruminal pH. Steers averaging 250 kg of body weight (BW) were fed either corn- or wheat-based diets, varying in grain concentration from 35 to 90%. Intake was measured at 2, 4, 6, 8, 12, and 24 h. Wheat-based diets significantly depressed intake more than

corn-based diets. Furthermore, wheat diets resulted in wider fluctuation in rumen pH than corn diets (4.6 to 6.25 vs. 5.27 to 5.97). For both diets, intake was especially depressed after pH fell below 5.6. After a bout of low pH, rumenitis may occur and further depress feed intake.

The ability of the rumen to absorb organic acids also contributes to the stability of rumen pH (Krause and Oetzel, 2006). Volatile fatty acids are passively absorbed across the rumen wall (Bergman, 1990). Absorption is via the rumen papillae, which provide a large surface area for VFA absorption. Ruminal papillae increase in length in cattle fed high-grain diets (Dirksen et al., 1985); this provides increased absorptive capacity, which protects the animal from excess acid accumulation. If rumenitits occurs, this impairs the animal's ability to maintain a stable pH.

As stated earlier, mean ruminal pH values may not be greatly affected by large dietary changes, but nadir pH values are usually greatly affected. Kennelly et al. (1999) fed cows diets containing either 50:50 or 25:75 forage to concentrate ratios. Mean ruminal pH values were not different (6.31 and 6.15), but nadir pH was different (5.9 vs 5.5). Krause and Combs (2003) fed diets differing in forage source and forage particle size. They found that, although mean ruminal pH was similar across diets, finer diets produced significantly lower nadir pH values and longer times below pH 5.8. Similarly, Krause and Combs (2003) reported that replacing alfalfa silage with corn silage did not change mean ruminal pH, but nadir pH and pH 5.8-h were both significantly lower when corn silage was fed.

Endogenous buffering

While the total effect of endogenous buffering on the rumen is small, saliva can mean the difference between health and disease in cows fed highly fermentable rations (Firkins, 1997). Ruminant saliva contains both inorganic and organic buffers. The main inorganic buffer is a bicarbonate phosphate buffer, with the two anions comprising more than 90% of total anion

content from the parotid gland (Bartley, 1976). Owens et al. (1998) estimated that half of the bicarbonate entering the rumen is from salivary sources. The main organic constituents are mucus and urea, and most are secreted from the submaxillary glands. Saliva flow depends on feed intake and diet type. Bailey (1961) estimated saliva flow by cardial outflow to be between 110 to 178 L/d from dry cows eating 5.5 to 7.7 kg of DM/d. Diets varied from all grass hay, mixed hay and grain, fresh grass, and alfalfa silage. In another experiment using lactating cows, intake was between 18.1 and 19.7 kg of DM/d of two diets: 30% hay crop silage and 70% grain, or 40% corn silage and 60% grain (Cassida and Stokes, 1986). Saliva flow was 308 L/d and 284 L/d, respectively. Other factors that may regulate saliva flow are diet DM concentration, forage intake, and forage particle size (Erdman, 1988). It should be noted that low rumimal pH does not trigger saliva flow, but time spent eating, ruminating, and resting were associated with increased saliva flow (Maekawa et al., 2002).

Acid production

Even with the regulation of ruminal pH, excess fermentable carbohydrates will produce more acid than the cow's system can accommodate and ruminal pH drops quickly. Ruminal VFA have a pK_a of about 4.9, and rapidly shift toward protonated (undissociated) form at ruminal pH 5.5 (Figure 1.2). This removes a free hydrogen ion from the ruminal fluid. This also increases VFA absorption across the rumen wall, since VFA are passively absorbed only when they are in undissociated form. However, increased VFA absorption is offset by production of lactate. Lactate has a much lower pK_a of 3.9. When ruminal pH is 5.0, lactate is 5.2 times less dissociated than VFA. While VFA are rapidly absorbed, lactate remains behind and rumen pH decreases (Russell and Hino, 1985).

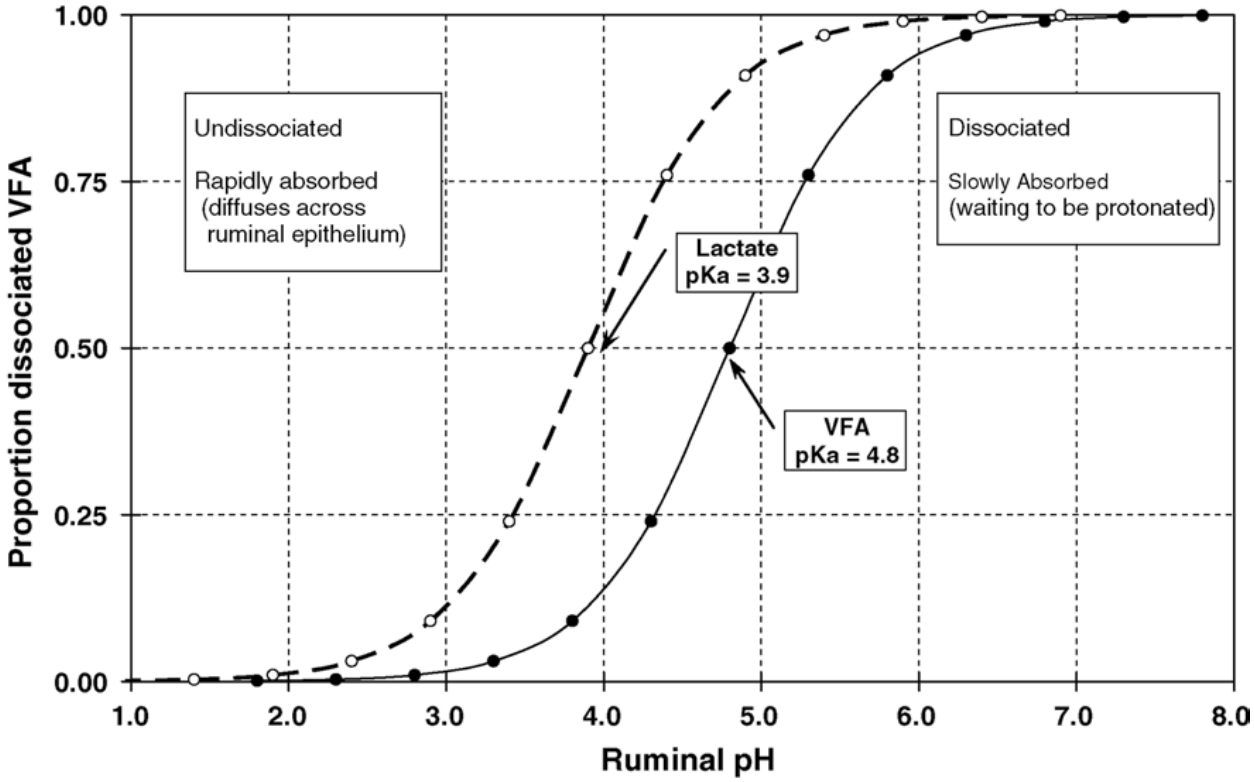

Figure 1.2. Titration curve for the major ruminal VFA (acetate, propionate and butyrate, solid line) and lactate (dashed line). (Adapted from Krause and Oetzel, 2006).

During feed deprivation, ruminal pH can be very high. High pH will inhibit lactate utilizers (Mackie and Gilchrist, 1979). This leaves the rumen ecosystem more susceptible to ruminal acidosis. Not only is microbial balance disrupted, but cattle tend to overeat when feed is re-introduced. This is more evidence that intake cycling could be a more important factor than diet composition itself. Furthermore, pre-existing SARA increases the risk of metabolic acidosis in the event of accidental ingestion of grain, because bacterial populations are unstable and are unable to maintain normal pH.

Metabolic acidosis as a result of SARA

Lactate, especially D-lactate is responsible for the uncompensated metabolic acidosis seen during cases of acute acidosis (Dunlop and Hammond, 1965). As stated earlier, the role of lactate in SARA is probably not as significant as compared to metabolic acidosis; however, because total VFA concentrations are increased, they could be involved in inducing metabolic acidosis. Only acetate reaches the peripheral circulation (Enemark, 2008). Butyrate is converted to hydroxyl-butyrate in the gut epithelium and all the propionate is converted to glucose in the liver (Owens et al., 1998). High concentrations of VFA could cause metabolic acidosis, especially if the rumen wall is compromised. Unlike intestinal epithelial cells, papillae are not protected by mucus from low pH, allowing for damage.

Low body condition is often seen in herds with SARA (Nocek, 1997; Kleen et al., 2003; Enemark, 2008). One theory is that these cows are in chronic metabolic acidosis. There are few data supporting this theory because systemic effects are rarely measured in SARA studies, or not at all in field studies. Twenty Hereford-Angus crossbred steers were fed diets to induce either acute or subacute acidosis, and systemic effects were measured over 14 days (Brown et al., 2000). There was considerable variation between animals in their ability to cope during a carbohydrate challenge. Steers also showed adaptation to the diets. Only mean pH data were shown, but blood pH was significantly lower when rumen pH was lower. Because blood pH is tightly controlled, large changes were not expected in blood pH.

In humans and rats, metabolic acidosis results in increased protein catabolism (Williams et al., 1991; Reaich et al., 1992; Bailey, 1998). There is evidence of this in cattle as well. Twenty lactating cows were fed HCl-treated canola meal to induce chronic metabolic acidosis (Mutsvangwa et al., 2004). Muscle biopsies were obtained on days 1 and 10 for RNA extraction.

The researchers had previously determined that the cows would have metabolic acidosis by day 10, and this was confirmed by significant ($P < 0.05$) reduction in blood pH, bicarbonate, base excess, and urine pH. Muscle RNA analyses revealed that the ubiquitin gene, which codes for "tagging" other proteins for degradation, was up-regulated, indicating possible skeletal muscle wasting.

Acidosis also affects responses to insulin. Jersey cows were fed a high anion diet (low DCAD) to induce metabolic acidosis, and then were subjected to a glucose tolerance test (Bigner et al., 1996). Twenty-one cows had one of 3 treatments: either a high anion diet with a water drench or a bicarbonate drench (to partially alleviate the metabolic acidosis) or a high cation diet with a water drench (control). All cows were dosed with a 50% dextrose solution i.v. and sampled subsequently. Cows on the high anion, water drench treatment had a mean blood pH of 7.32, which was significantly lower than the 7.38 for the high anion, bicarbonate drench treatment ($P < 0.001$). Cows in the high anion, water drench group also had impaired insulin responses compared to glucose infusion control group (65 vs. 84 μIU/ml; $P < 0.001$). These results are most relevant to fresh cows that are at risk for having SARA, ketosis, and possibly metabolic acidosis.

Diagnosing SARA

Diagnosing SARA is difficult because the clinical signs are subtle and also delayed after the onset of acidosis (Kleen et al., 2003; Krause et al., 2006; Enemark, 2008. Therefore, routine monitoring is required and it may also be necessary to use "paraclinical" signs to catch the problem early enough to make changes in management. These signs include erratic feeding pattern, depression in DMI, diarrhea and changes in feces, increased culling, laminitis, and increased incidence of displaced abomasum and bloat.

Feeding pattern

The most consistent symptom of SARA is cyclic feeding pattern (Britton and Stock, 1987). Basically, the cow eats her ration and then refuses further feed because of a drastic fall in pH and increased osmolality of the rumen (Carter and Grovum, 1990). The problem in dairy herds is that cows are typically fed at a bunk in a free-stall situation, so such a pattern is not noticeable. However, rumination time may be easier to measure. If less than 40% of a group of cows are not ruminating at one time, this may be an early indication of SARA (Eastridge, 2000; Maekawa et al., 2002).

Feces

Unless there is a high amount of ruminally undigested starch and excessive hind gut fermentation (Eastridge, 2000), fecal pH is not normally related to ruminal pH (Enemark et al., 2004). However, cows with SARA exhibit bright yellowish feces, with a sweet-sour smell (Kleen et al., 2003). They appear foamy with gas bubbles and contain higher than normal amounts of undigested fiber or grain (Hall, 1999). Because there is an inadequate fiber mat in the rumen, fiber is not retained, and feces will contain 1 to 2 cm particles versus the normal 0.5 cm. Nordlund et al. (1995) reported than herds with loose feces also contained substantial amounts of undigested feed particles. Intermittent diarrhea and the presence of undigested feed particles indicate inadequate digestion and fast passage of feed (Enemark, 2008).

Monitoring SARA

Rumen fluid

Monitoring of rumen fluid is commonly used for diagnosis of acute acidosis; however, it is time consuming and is not part of routine examinations. Rumen pH values obtained via

stomach tube are variable because of saliva contamination, sampling time relative to feeding, and stomach tube placement in the rumen (Duffield et al., 2004; Enemark et al., 2004).

Rumenocentesis is another method of determining ruminal pH. It is generally well accepted by cows. The most used cut off point is ruminal pH of 5.5. According to Garrett et al. (1999), a cow with a pH of less than 5.5 should be considered positive for SARA, and above 5.8 to be negative. Cows in between are considered at risk for developing SARA. Furthermore, Garrett et al. (1999) defined in a group of cows as having SARA when a rumen pH of 5.5 or lower is found in 4 out of 12 cows.

Milk fat

Milk fat percentage is influenced by many factors, including breed, stage of lactation, and ration composition (Grummer, 1991). Low milk fat is often used as an indicator of SARA and also how effective the diet is for chewing (Mertens, 1997). Correlation coefficients between ruminal pH and milk fat concentration in cows over 30 DIM were found to be 0.30 or 0.39, respectively (Allen, 1997; Enemark et al., 2004). In the study of Enemark et al. (2004) the correlation coefficient was found to be negative ($r = -0.06$) in cows under 60 DIM. These are relatively low correlation coefficients, and show that milk fat percentage is not a good indicator of SARA in early lactation.

In the U.S., official fat tests are normally measured once a month. However, monthly measurements will not reveal brief periods of low milk fat. To improve their diagnostic value, it may be more useful to measure milk fat once a week. New technologies will allow for in-line, daily measurements of butterfat (Enemark, 2008). The use of herd or group lactation curves may be useful since can detect sudden one to two percentage points drops in average fat in midlactation cows, that result from insufficient fiber or abrupt ration changes.

It is important to note that milk fat can be affected by other factors in addition to rumen pH. For example, Oba and Allen (2003) increased starch fermentability in a diet to 30% starch resulted in a 15% drop in milk fat, with no change in ruminal pH. Dietary fat also plays an important role in milk fat percentage, especially unsaturated fatty acids. Low milk fat content must take into account the level of dietary lipids and their degree of unsaturation. While it is true that milk fat depression and SARA can occur concurrently, low milk fat syndrome cannot be simply considered an effect of SARA (Kleen et al., 2003).

Blood parameters

Blood gas measurements have not been available for on-farm use, because the sample must be analyzed very quickly on a specific machine. However, Brown et al. (2000) showed decreased blood pH, bicarbonate, and base excess (metabolic acidosis) in beef steers with SARA. This was in agreement with Horn et al. (1979) and Goad et al. (1998). As the use of on farm blood gas determination becomes more available, these parameters will become more useful for diagnosis of SARA. Furthermore, rumenitis probably initiates the production of acute phase proteins such as serum amyloid –A and haptoglobin that can also measured (Gozho et al., 2005, 2006).

Urine parameters

Unlike some other animals, cows have a relatively small lung capacity; therefore, they rely heavily on the kidneys to excrete excess hydrogen ions. Positive relationships have been shown between blood pH and urinary pH and ruminal and urinary pH, respectively (Roby et al., 1987; Fürll, 1993;). However, aciduria can be seen in other conditions, and also in cows fed anionic salts. Cowles et al. (2010) found a significant positive relationship between rumen and

urine pH (r=0.15), but cows were not experiencing SARA. Further research is needed to establish guidelines for use of urine pH as a diagnostic criterion for SARA.

Nutritional management of SARA

Because SARA is primarily a nutritional disease, it is necessary to review approaches that can be taken to prevent and manage SARA. Figure 1.3 summarizes some common issues on commercial dairy farms. Finally, it is important to evaluate the ration the cows actually consume versus the formulated diet.

Exogenous buffering

Fiber and particle size

Diets are formulated based on NDF as a percentage of the ration DM is recommended because of the negative relationship between NDF and energy density of the diet and the positive relationship between NDF and gut fill (Mertens, 1997). However, NDF concentrations alone are not enough because ruminal fermentation is variable (Nocek and Tamminga, 1991). Also, physical characteristics of fiber influence fermentation, animal metabolism, and milk fat content independent of chemical NDF (Mertens, 1997). Therefore, the NRC (2001) recommends that a minimal level of NDF come from forage (0.9% of BW as forage DM). The NRC (2001) and Pitt et al. (1996) also advocate the use of effective NDF (eNDF), which describes the ability of the feed to replace forage but maintain butterfat. Physically effective fiber ($peNDF_{>1.18}$ – the proportion of DM retained on a 1.18-mm screen x dietary NDF) introduced by Mertens (1997). This is a useful tool to estimate chewing, salivation and endogenous buffering (Yang and Beauchemin, 2006; Zebeli et al., 2006).

Problem	Correction	Effect
Steaming up <4 weeks	Allow for 4–6 weeks stepwise adaptation	Optimal proliferation or rumen mucosa and ruminal microflora
Steaming up is too intensive	Maximum increase of concentrate/day should be 0.25 kg	The rumen environment can absorb/neutralize VFA and lactate
Only one TMR lactation and one TMR dry cow ration	Design rations for group of cows at certain lactation state	Energy content of ration targeted at ruminal mucosa capacity
Errors in nutrient delivery (variation in DM, NE_L and NDF)	Assess and control sources of error (sampling bunker silos, moisture content, accurate weights, bunk sampling)	Ruminal stability (balance between lactogenic and lactolytic bacteria)
Grains to finely ground, steam flaked, extruded or/and wet	Particle size analysis of grains	Less rapid fermentation of grains in the rumen
Diets with high DCAD promoting low rumen pH	Adding buffer to the diet or stimulate chewing and rumination activity (7% fiber particle >3.5 cm), 27–30% NDF (70–80% from forage to ensure adequate eNDF), 35–45% of DM as NFC	Increased ruminal buffer capacity either directly or via increased saliva production
More than 15% long forage particles (promotes sorting)	Analysis of bunk samples along with adequate bunk space	Adequate content of long fibres prevent sorting, and adequate bunk space prevents slug feeding
Over-mixing or over-processing of the TMR (reduced particle size and eNDF	Control mixing time as well as condition of TMR scales	Homogeneous ration providing a stable ruminal environment

Figure 1.3. Commonly occurring feeding and management deficiencies resulting in SARA and suggested corrections (Adapted from Enemark, 2008).

Feed ingredients

Corn silage is probably the most popular forage for dairy cows in the U.S. However, feeding large amounts of corn silage puts cows at a higher risk for SARA when compared to dry hay or hay crop silages. Corn silage varies in digestibility based on genetics, such as BMR corn (Oba and Allen, 1999, 2000), growing and harvest conditions (Bal et al., 1997), and kernel processing (Johnson et al., 1999). Typically, corn silage does not contribute enough long particles in a TMR (totally mixed ration). It is not recommended to chop corn silage too long because fermentation will be impaired and cows will likely sort at the bunk (Kononoff et al., 2003). It is possible to add dry forage to a TMR to increase chewing activity; however, like long corn silage it can be difficult to mix into the TMR so the cows will not sort.

Non-forage fiber can also be a good ingredient choice for dairy cow rations. It can be cost effective and replace some forage in the ration. It has been demonstrated that fibrous by products, such as soy hulls, can replace some of the forage fiber without negatively affected milk production or cow health (Clark and Armentano, 1997). Non-forage fiber sources have low lignin content, which makes them more digestible than forage, and also have higher passage rates. Therefore, they do not stimulate rumination and chewing, and rations containing them may require more total NDF (Pereira et al., 1999).

Penn State Particle Separator and sorting

Particle length distribution of the ration and forages can be easily evaluated on farm using the Penn State Particle Separator (PSPS) (Lammers et al.,1996). Fine rations, or those with less than 7% of particles retained on the top screen of the PSPS puts cows at increased risk of SARA, especially if those diets are low in NDF (Woodford and Murphy, 1988). On the other hand, diets with more than 15% of long particles also put cows at risk for SARA. This can occur when

the long particles are unpalatable and cows sort them and choose shorter particles. Leonardi and Armentano (2003) measured original TMR and orts and found that cows sorted against long particles. The sorting was increased in diets with 400g/kg of alfalfa hay versus those with 200 g/kg (DM basis), and variation between cows was large. It is interesting to note that not all cows sort.

Martin (2000) conducted studies on commercial dairy farms in WI by measuring fed TMR and bunk rations at 6-h intervals. It was shown that before 12 h post feeding, cows were less likely to sort long particles, but after 13 h cows were more likely to sort long particles out. This puts dominant cows at a higher risk for developing SARA, since they are the first at the feed bunk and more likely to sort and select fine particles. The less dominant cows wait and eat a different ration (lower energy) than the rest of the group. This presents a possible scenario that cows at either end of the social spectrum lose body condition and produce poorly. Overcrowding and limiting bunk space to less than 0.45 m per cow will increase these risks. Adding water or a higher quality hay to the TMR may reduce sorting behavior.

Dietary buffering

Forages have inherent buffering or acid-consuming capacities; this is in addition to their role in saliva secretion during eating and rumination (Erdman, 1998). This is a result of the dietary cation-anion difference (DCAD) of the ingredients. In general, based on the work of Jasaitis et al. (1987) and Playne and McDonald (1966), among fresh forages, legumes tend to have a higher buffering capacity than grasses or whole corn plant, and among dry feeds, and cereal grains have lower buffering capacity than hays and protein sources. Diets high in Na and K, relative to Cl and S have higher DCAD concentrations, and tend to support higher ruminal pH and increase DMI and MY (MY) (Block and Sanchez, 2000; Hu et al., 2007a). Optimal DCAD

for early lactation diets is around +400 meq/kg [(Na+K) - (Cl+S)] of DM (Block and Sanchez, 2000; Hu et al., 2007a). Ideal DCAD for mid-lactation diets is between +275 to +400 meq/kg. Because cereal grains and some by-products have a low or negative DCAD, and are also rapidly fermentable, addition of buffers may be necessary to increase the DCAD of a lactation ration.

It was estimated in 2008 that 40.6% of U.S. dairy herds used $NaHCO_3$ as a buffer in lactation rations (Hoard's Dairyman, 2009). Other buffers used include MgO, $KHCO_3$, K_2CO_3, and $Na_3H(CO_3)_2$. The Na and K salts of the bicarbonate or bicarbonate ions, and have pKa around 6.25 (carbonate ions also have a pKa at 10.25). Magnesium oxide acts more like an alkalinizing agent under rumen conditions, since it has no defined pKa (Erdman, 1998). Buffers can be either force fed (i.e., mixed in the TMR) or fed free choice.

Dietary buffers cannot eliminate the causes of ruminal acidosis, but they can help to alleviate the problem. Sodium bicarbonate has been shown to increase DMI, milk production, and milk fat percentage (Erdman, 1988). Buffers show the largest response when fed with corn silage (Erdman, 1988) versus when fed with grass/legume silages (Staples and Lough, 1989). This is probably because of the lower DCAD of corn silage and the increased risk of SARA in cows fed diets high in corn silage. Buffers are also beneficial when added to diets with marginal effective fiber (Krause and Oetzel, 2006; Hu et al., 2007b).

Other feed additives for prevention of SARA

Many dairy producers and nutritionists utilize yeast cultures or direct fed microbials (DFM) in diets. Nocek and Kautz (2006) reviewed DFM in lactating dairy cow diets. They found that three organisms (*Enterococcus faecium*, *Lactobacillus plantarum*, and *Saccharomyces cerevisae*) included in diets at 10^5 cfu/kg reduced diurnal rumen acidity and improved corn silage digestibility. Also, they found that when cows in early lactation were fed DFM (*E. faecium* and

S. cerevisae) they had increased DMI and MY, and enhanced ruminal digestion of forage DM; however, these cows had lower milk fat percentages. Conversely, Beauchemin et al. (2003) fed DFM to feedlot cattle (*S. cerevisae and E. faecium)* and saw no effect in the occurrence of SARA or any significant effects on the site or extent of digestion. The authors concluded that DFM were not beneficial to adapted feedlot cattle. Overall, studies are often conflicting when examining the use of DFM for SARA, they aren't recommended at this time (Enemark, 2008).

Excessive intake of rapidly fermentable CHO

Excessive intake of rapidly fermentable carbohydrates is the major cause of SARA. One major goal of dairy nutrition is to feed as much energy as possible to maximize production, while maintaining a healthy rumen. This creates a tightrope walk for cows, because the effects of feeding too much fermentable carbohydrate (decreased DMI and milk production) are virtually the same as feeding too much fiber. The difference between the two is that slightly overfeeding fermentable carbohydrates causes long-term health problems; whereas slightly underfeeding them will reduce MY, but will not negatively affect health (Krause and Oetzel, 2006).

Therefore, it is important to control the amount of NFC in a ration. The NFC fraction contains organic acids, sugars, starch and soluble fibers like pectin. Hoover and Miller (1995) suggested that when sugar and starch make up most of the NFC, limit the total to 350 to 400 g/kg of diet DM, and between 400 and 500 g/kg when the other carbohydrates predominate. Hall (1999) suggested that within NFC, the components should be 5 g/kg DM sugars, 100 g/kg soluble fibers, and 200 g/kg starch. All of these recommendations assume that effective fiber requirements are being met.

Carbohydrate fermentation varies so much that optimal NFC concentrations will vary as well. It is well documented, and has been reviewed in both dairy and feedlot cattle (Owens et al., 1997; Mills et al., 1999), that both the rate and extent of fermentation is not the same among carbohydrates. According to these reviews, ruminal starch degradability ranges from 30% to 100%. Additionally, source and processing of carbohydrates are also factors. For example, ground high moisture corn reduced rumen pH from 5.99 to 5.85 when it replaced dry, cracked corn (Krause et al., 2002). Yang et al. (2000) found that increasing processing of barley grain reduced ruminal pH linearly in lactating dairy cows. It is useful to measure particle size of grains to assess SARA risk. Total NFC intake is still probably the most important issue, when assessing SARA risk.

Inadequate adaptation

Adaptation to a lactation ration includes microbial changes and lengthening of rumen papillae (Dirksen et al., 1985). For this reason, it has been suggested that grain feeding be increased at the end of the dry period to achieve this, and should decrease the risk of SARA. However, Garrett et al. (1997) saw no effect of dry period diet on ruminal pH in early lactation. In fact, average pH at 15 DIM was higher than at 106 DIM, suggesting that high DMI is a bigger determinant than adaption for the risk of SARA. If cows are fed a TMR after calving, adaptation is probably not an issue in regards to ruminal pH.

Summary

In conclusion, SARA is an important aspect of the health and productivity of dairy cows. As DMI increases, the risk of SARA increases concurrently. Gains in milk production may be offset by losses due to long-term health problems. Ruminants have a complex system to maintain rumen pH, which makes SARA diagnosis difficult and prevention multi-faceted. Adequate

ruminal buffering, by endogenous and dietary buffers, is one aspect of prevention. This can be achieved by feeding a diet balanced in cations to anions ratio and physical fiber that the cows cannot select against. Control of fermentable carbohydrate intake by meeting NDF requirements, proper grain processing, and feedbunk management is also important for SARA prevention.

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CHAPTER II

MODELLING THE DYNAMICS OF COMMINUTION DURING MASTICATION AND RUMINATION, AND THE INTERACTION OF PARTICLE SIZE REDUCTION WITH DIGESTION AND PASSAGE IN CATTLE

Abstract

Cattle spend many hours per day chewing, either eating or ruminating. Comminution of feed and digesta particles affects the kinetics of digestion and passage, and can also affect voluntary feed intake. These, in turn, determine nutrient availability and productive efficiency. Our objective was to incorporate relevant data into a framework leading towards a dynamic mathematical model for comminution from feed through feces in cattle. Although large particles (i.e., those retained on a screen with 1.18-mm pores) often comprise 80 to 90% of swallowed forage dry matter, they account for about 35% of fecal dry matter. Large particles can be a minority of those in the reticulorumen at any given time; therefore, size is not the only criterion determining passage to the lower gut. Current data support the conclusion that synergism exists between animal and microbial effects; i.e., mastication during eating enhances microbial fermentation, which increases the effectiveness of comminution during rumination. Significant amounts of variation in the particle size distributions of boluses entering the reticulorumen can now be explained from knowledge of feed characteristics. Our understanding of mastication and rumination effects on digestion and passage in cattle is limited because no information is available for mixed diets and few data exist for many common types of forage (none for silages or which address the effects of plant maturity). Data amenable to studying the dynamics of particle size distributions are few and relate to near steady-state conditions; therefore, synergies

between mastication, digestion, and rumination under practical conditions remain to be examined.

Introduction

Cattle fed alfalfa hay can spend 70 % (17 h/d) of their time chewing, either eating or ruminating (Jaster & Murphy, 1983). Comminution of feed and digesta particles affects the kinetics of both digestion and passage, and can also affect voluntary feed intake. These, in turn, determine nutrient availability to the animal and productive efficiency. Although large particles (i.e., those retained on a screen with 1.18-mm pores; other thresholds have also been utilized) often comprise 80 to 90% of forage dry matter (DM), they account for only 5 to 10% of fecal DM.

Little agreement exists on which methods to employ for particle size analysis or how to summarize their results. Dry or wet sieving techniques have been most frequently used, sometimes after pretreatment of samples. Size of sample and the sieves used have also varied. Image analysis of particle size distributions has also been proposed but rarely reported. The effects of method, feed, and the interaction of method with feed were significant when nine sieving techniques were compared (Murphy & Zhu, 1997), accounting for 20, 65, and 13% of the variation in median particle size. Deleting data for three techniques reduced the variation that was attributable to method from 20 to 7% and increased the percentage of variation attributable to feed from 65 to 78%; there was little change in the variation associated with the interaction of method with feed. This suggests that compilation of particle size and animal response data across experiments and methods for eventual use in diet formulation is justified. Our objective was to incorporate relevant data into a framework leading towards a dynamic mathematical model for comminution from feed through feces in cattle.

The relationships among digestion, rumination, digesta passage, and particle size are explored first. This is followed by a discussion of two modelling exercises.

Digestion

Although one might surmise that mastication during eating enhances digestion, the effect has been rarely quantified and not always detected. Olubobokun et al. (1990) compared masticated or unmasticated (but chopped) samples of alfalfa, orchardgrass, or bermudagrass hays that were incubated in the rumen for up to 96 h. Less insoluble DM was present in masticated than unmasticated forages ($P < 0.001$); therefore, more DM was soluble in masticated than unmasticated (but chopped) hays. The effects of mastication on DM, crude protein, neutral detergent fiber, and acid detergent fiber digestion were inconsistent, perhaps because unmasticated hays were chopped prior to fermentation. The mean particle sizes of their chopped, unmasticated hays (425 to 860 μm) were much smaller than normally swallowed by cattle eating chopped (3000 to 7000 μm; Kennedy 1985) and long (1400 μm; Jaster & Murphy, 1983) hays.

Two earlier reports indicated that mastication and insalivation increased digestion. In two experiments of Playne et al. (1978), the in situ digestion of hays made from Townsville stylo (*Stylosanthes humilis*), purple-top Chloris grass (*Chloris barbata*) and spear grass (*Heteropogon contortus*) was examined. The three preparations of these tested were hays dried and milled to pass a 1-mm screen, frozen unmilled extrusa from an esophageal fistula, and freeze-dried extrusa milled to pass a 1-mm screen. Feed samples were digested less than samples collected via the esophageal cannula ($P < 0.001$) and, because milling did not increase the digestion of extrusa samples, they also concluded that chewing during eating was sufficient to maximize rates of digestion. It should also be noted that the interaction of sample type and in situ digestion time

was not significant, suggesting that differences in digestion of DM because of mastication were consistent over time.

Bailey (1962) compared the digestion of swallowed grass (brome/orchard) samples to that of similar samples chopped to approximate the size of swallowed grass before in situ ruminal digestion. Digestion of swallowed DM in the first 24 h was greater than that for unswallowed material ($P < 0.01$). Our reanalysis of these data indicated that the fractional degradation rate of swallowed grass was 42% greater than that of unswallowed grass (9.2 vs. 6.4 %/h).

Another perspective on this issue involves the extent to which particle size changes with digestion in the absence of chewing. Murphy & Nicoletti (1984) showed that, after 96 h of in situ digestion of a fraction of coarsely chopped alfalfa hay, 64% of the DM was degraded; however, mean particle size was reduced only 19% (Figure 2.1). McLeod & Minson (1988) estimated that digestion and detrition (rubbing) accounted for 17% of the breakdown of >1180-μm particles to <1180 μm in steers fed leaf or stem fractions of chopped ryegrass or alfalfa hay.

Rumination

Comminution obviously occurs during rumination in cattle; however, few have studied the dynamics of this process or how it relates to the particle size distributions of ingested and excreted material. Kennedy (1985) fed alfalfa, reed canary grass, bromegrass, or red clover to ruminally and esophageally cannulated steers in either chopped or ground and pelleted form. Forages were fed at 95% of voluntary feed consumption at intervals of 2 h; therefore, steady-state conditions were approximated. His results can be recast to enable particle size distribution changes throughout the system to be visualized. Based on the reported distributions of particulate DM on various sieves, $\log_{10}$ means and standard deviations were estimated assuming that they

conformed to a lognormal distribution. These statistics were then used to graph the particle size distributions. For example, Figure 2.2 shows the particle size distributions of masticated material, rumen digesta, and feces across chopped forages. Connected points correspond to observed data; whereas, unconnected symbols on either side of this region depict extrapolated data. As expected, masticated material contained a higher proportion of large particles than rumen digesta, and rumen digesta a higher proportion of large particles than feces. The relatively small size of fecal particles emphasizes the role that rumination plays in increasing the likelihood of passage from the rumen.

Rumination begins with regurgitation of an ‘up’ bolus (Figure 2. 3). Relatively more large particles and fewer mid-sized particles were present in the ‘up’ bolus than in rumen digesta; i.e., some selection of material requiring comminution had occurred. About half of the ‘up’ bolus is immediately reswallowed after regurgitation; it is termed the ‘tail’ bolus and contains mostly small particles that had been in the ‘up’ bolus (Figure 2.4). Material retained in the mouth for rechewing during rumination, then, had an even greater proportion of large particles than was regurgitated in the ‘up’ bolus. Rechewing during rumination greatly reduced the proportion of large particles reswallowed in the ‘down’ boluses. The ‘tail’ and ‘down’ boluses had similar particle size distributions but were much smaller than the ‘up’ bolus. One rumination cycle comminuted essentially all particles in the ‘up’ bolus that were too large to pass from the reticulorumen because the size distributions of reswallowed (‘tail’ and ‘down) boluses were similar to that of feces (Figure 2.5).

Synergy between digestion and rumination

To what extent does digestion enhance rumination and vice versa? Again, few quantitative data are available that directly address the issue. Suzuki et al. (2001) measured the particle size

distributions of ‘up’, ‘tail,’ and ‘down’ boluses during rumination 6 to 12 h, 12 to 18 h, or 18 to 24 h after once daily feeding of either orchardgrass or alfalfa hay to esophageally cannulated steers. Assuming that their sieving data also conformed to a lognormal distribution, particle size distribution statistics were estimated for boluses collected either 6 to 12 h or 18 to 24 h after feeding (Table 2.1). As for the Kennedy (1985) data, the particle size of material retained for rechewing during rumination was considerably larger than that regurgitated in the ‘up’ bolus. That reswallowed after rechewing in the ‘down’ bolus was much smaller than both material retained for rechewing and that regurgitated in the ‘up’ bolus.

The mean particle sizes of boluses and material retained for rechewing were all smaller if collected 18 to 24 h after feeding than if collected 6 to 12 h after feeding; however, forage differences were apparent. For steers fed orchardgrass hay they were 30 to 40 % smaller; whereas, ‘up’ and ‘tail’ boluses of steers fed alfalfa hay were 15 to 20% smaller, and retained material and the down bolus were 50 to 62% smaller. Rechewing also reduced the mean particle size of retained material more 18 to 24 h after feeding than it did 6 to 12 h after feeding; 66 compared to 62% for orchardgrass and 57 compared to 43% for alfalfa. Particle size distributions were also more uniform, i.e., they had smaller $\log_{10}$ standard deviations, 18 to 24 h after feeding than 6 to 12 h after feeding.

Suzuki et al. (2001) also reported two other variables relating to the effect of time after feeding (more digestion) on rumination. Effectiveness of rumination, measured as the percentage of large particles (those >1180 μm) retained for chewing that were reduced to <1180 μm in one rumination cycle, was 32% higher for orchardgrass and 22% higher for alfalfa 18 to 24 h after feeding than 6 to 12 h after feeding (Table 2.2, $P < 0.05$ within forage). Specific fragility, milligrams of large particles (those >1180 μm) reduced to <1180 μm per chew per gram of large

particles retained for chewing, was about 20% higher for both orchardgrass and alfalfa 18 to 24 h after feeding than 6 to 12 h after feeding ($P < 0.05$ within forage). Chai et al. (1984) found that the specific fragility of >3350 μm particles was doubled during rumination at 16 versus 4 h after feeding steers either chopped alfalfa hay or bromegrass. Effectiveness of rumination for these particles was also higher at 16 than at 4 h after feeding for chopped alfalfa hay (76 versus 59%, a 29% increase) or bromegrass (73 versus 56%, a 30% increase). Both measures demonstrate that digestion improved the ability of rumination to comminute large forage particles.

The effect of rumination on digestion was examined in another experiment (Suzuki et al. 2000) where disappearance of neutral detergent fiber in 'up,' 'tail,' and 'down' bolus digesta collected from the steers was measured after 12, 24, and 48 h of incubation in the rumen of sheep. Disappearance of neutral detergent fiber in material retained for rechewing was estimated using 'up' and 'tail' bolus data. Rechewing during rumination increased the disappearance of neutral detergent fiber after 48 h of in situ incubation by 6% for orchardgrass but by 211% for alfalfa (Table 2.3, $P < 0.01$ within forage). Clearly, synergy exists between digestion and rumination in the comminution of digesta particles.

Passage

Although postruminal digestion is considerable, postruminal comminution is considered minimal; therefore, the fecal particle size distribution is taken to approximate that of material passing from the reticulorumen. This assumption is supported by the data of Kennedy (1985) which showed that rumination reduced the particle size of digesta until its distribution approached that of feces. As noted above, large particles are often defined as those retained on a screen with 1180-μm pores but other thresholds have also been utilized. Given that these particle size distributions are effectively continuous, it may not be all that helpful to define a 'critical'

value for passage anyway. In numbers rounded to the closest 5% unit, the chopped forages data of Kennedy (1985) suggest that 35% of fecal dry matter was >1000 μm, 20% was >2000 μm, and 10% was >4000 μm. For comparison, 85, 75, and 60% of particles in masticated boluses of feed were >1000 μm, >2000 μm, and >4000 μm, respectively. About one-third of masticated chopped hay was already of passable size when initially swallowed. When one considers that most feed particles would be even larger than those in masticated boluses, the total amount of comminution required between prehension of feed and the feces explains why cattle spend so much time chewing.

It is also interesting to note that, at least under steady-state conditions, a significant proportion of digesta in the reticulorumen is of passable size (Figure 2.2). Small size is a necessary but not sufficient condition for particle passage; therefore, other factors must also be involved in determining when it occurs. Likely important among these are: fermentation, feed consumption, functional specific gravity, and dilution rate.

Modelling the dynamics of particle size distributions

Paucity of data severely limits the extent to which the dynamics of particle size distributions between the feed and feces of cattle can be modelled. That said, our first modeling exercise was designed to determine whether the particle size distribution of boluses swallowed during eating could be predicted based on feed characteristics. Secondly, an algebraic model was constructed to compare observed and predicted particle size distributions in the rumen over a 24-h period based on observed particle size distributions of masticated and ruminated boluses, and feces for the data of Kennedy (1985).

Feed to Bolus Entering the Rumen

Twenty publications were compiled (Table 2.4) that reported particle size distribution data for swallowed feed boluses in cattle (Silver 1935; Gill et al. 1966; Pond et al. 1984; Kennedy 1985; Nelson 1988; Shaver et al. 1988; Deswysen et al. 1989; Luginbuhl et al. 1989; Bailey et al. 1990; Fisher et al. 1991; Burns et al. 1992; Mader et al. 1993; Burns et al. 1997; Corley et al. 1997; Reinhardt et al. 1998; Fernandez & Michalet-Doreau 2002; Beauchemin et al. 2003; Fernandez et al. 2004; Ellis et al. 2005; and Boudon et al. 2006). A wide range of diets were represented: grazed forages, long and chopped hays, silages, ground and pelleted forages, and cereal grains in various forms either alone or mixed with forages. Although swallowed feed bolus mean particle size ranged from 552 to 26866 μm, its first and third quartiles were at 1401 and 4350 μm. The highest values reported were likely a function of the sieving method utilized (Murphy & Zhu 1997) and not biologically meaningful. Relatively few data were available about the size and dry matter content of swallowed feed boluses, chews per bolus, or the effects of time within a meal on these variables.

Five of the articles (Shaver et al. 1988; Bailey et al. 1990; Fernandez & Michalet-Doreau 2002; Beauchemin et al. 2003; and Fernandez et al. 2004) provided data suitable to examine the potential relationship between the particle size distribution of boluses swallowed during eating and feed characteristics (Table 2.5). Long, chopped, ground, or pelleted forages; silages, and mixtures of these and cereals were among the dietary treatments. The range, and first and third quartiles (2338 and 4150 μm) for swallowed bolus mean particle size were similar to those of the larger data set; therefore, the subset was representative.

Best subsets regression (MINITAB for Windows 95/NT, Version 12.2, Minitab, Inc., State College, PA) was used. Mallows Cp statistic (i.e., $Cp = SS_{res}/MS_{res} - N + 2p$, where SS_{res} is

the residual sum of squares for the model with p-1 variables, MS_{res} is the residual mean square when using all available variables, N is the number of observations, and p is the number of variables used for the model plus one) was calculated for models containing all possible linear combinations of independent variables. The model with the lowest Cp value approximately equal to p was considered most adequate.

With bolus $\log_{10}$ mean particle size as the dependent variable and feed code (0 = long, 1 = chopped, or 2 = ground), feed $\log_{10}$ mean particle size, feed $\log_{10}$ standard deviation, and DM intake as possible independent variables, the most appropriate model was 4.07 - 0.575*feed code ($P < 0.001$, adj. $R^2 = 0.73$, and Cp = 2.3). For bolus $\log_{10}$ standard deviation as the dependent variable and the same independent variables, the most appropriate model was -0.037 + 1.25*feed $\log_{10}$ standard deviation ($P < 0.01$, adj. $R^2 = 0.27$, and Cp = 1.6).

It is encouraging that significant amounts of variation in the particle size distributions of boluses entering the reticulorumen can be explained from knowledge of feed characteristics; however, practical limitations remain. For example, describing the particle size distributions of forages fed in long form or grazed is problematic. This may not be an intractable problem though because, cows must masticate long and coarsely chopped forages until they reach a swallowable consistency; whereas, small particles in a ground forage do not require mastication to reach swallowable size. Perhaps this is one of the reasons that the categorical variable feed code (0 = long, 1 = chopped, or 2 = ground) could explain most of the variation in bolus mean particle size. Another limitation is the knowledge that bolus particle size distributions change as a meal progresses (Gill et al. 1966; Corley *et al.* 1997) and that these changes may differ with forage (Waghorn *et al.* 1989).

Rumen

Mertens et al. (1984) suggested that particle size comminution and passage from the rumen could be modeled by two basic approaches: treating particles in the rumen as a single distribution continuum varying in mean and shape over time, or compartmentalizing them into two or more pools which act distinctly. Data availability dictated that our approach was essentially a hybrid of these alternatives.

The algebraic model we constructed to compare observed and predicted particle size distributions in the rumen over a 24-h period was based on observed particle size distributions of masticated and ruminated boluses, and feces for the data of Kennedy (1985); however, 39 compartments were utilized. Each compartment represented one of the available 39 U.S. Standard Sieves (National Institute of Standards and Technology) between 38 μm and 25 mm. Rumen digesta (grams of DM) on each of the sieves was first estimated assuming 10 kg of DM in the reticulorumen and the steady-state particle size distribution that Kennedy (1985) reported for chopped forages (after fitting a lognormal distribution to estimate its mean and standard deviation). Size distributions reported for swallowed feed boluses, 'up,' 'tail,' and 'down' rumination boluses and feces were similarly described. Rumination data included 'up,' 'tail,' and 'down' bolus size, and number of cycles observed for individual forages.

After accounting for feed consumption, rumination, and passage in one 24-h period, the predicted particle size distribution averaged across chopped forages for the rumen was compared with the starting steady-state distribution (Figure 2.6). Predicted mean particle size of rumen digesta was 15% larger than the observed value after 24 h (2200 μm instead of 1920 μm) because fewer particles <500 μm, and relatively more intermediate and large particles, were predicted than observed.

Similar calculations were then made for Kennedy's (1985) ground and pelleted forage data (Figure 2.7) which produced similar results. Predicted mean particle size of rumen digesta was again larger than the observed value after 24 h (42%, 920 μm instead of 650 μm) because fewer particles <500 μm, and relatively more intermediate but not large particles, were predicted than observed.

Reasons for these discrepancies are unclear because, with 2-hourly feedings, near steady-state conditions should have minimized potential confounding effects of synergies between digestion and rumination discussed above. Times of collection of masticated feed and rumination boluses, and rumen digesta, between feedings may still have affected results; however, Kennedy (1985) made considerable efforts to avoid them. Frequent feeding reduces the dynamics of most digestion-related variables in ruminants, although hourly-fed sheep apparently revert to the same diurnal rumination pattern exhibited by animals fed once daily (Murphy *et al.* 1983). This pattern is sinusoidal with an early-morning peak and a late-afternoon trough. Assuming collection of ruminated boluses occurred mostly during daytime hours, less rumination per unit of feed consumed during that period could have underestimated the effectiveness of rumination and its generation of small particles.

Discrepancies between observed and model-predicted distributions of particle size in the rumen are more likely related to the suitability of cumulative lognormal distributions for describing the data of Kennedy (1985) or at least estimations of their parameters given available data. Although sieves with 7400, 4000, 3350, 2000, 1000, 710, 500, and 250 μm-pores were used, reported data were for four categories: retained on a 3350-μm or larger sieve; retained on 2000 or 1000-μm sieves; retained on 710, 500, or 250 μm-sieves; or passing a 250 μm-sieve (Kennedy 1985). Statistics describing cumulative lognormal distributions, then, were based on

three points. Although 94 to 99% of the variation was explained by the cumulative lognormal, percentages of particles >3350, >1000, and >250 μm were consistently over-, under-, and overestimated for boluses, rumen digesta, and feces. Chronic underestimation of the percentage of particles passing the 250-μm sieve does not automatically explain discrepancies between observed and model-predicted distributions of particle size in the rumen though because rumen distributions were similarly affected. Data for additional sieves would have allowed more thorough study of these issues.

The combination of particle size distribution data across chopped forages, and ground and pelleted forages, by Kennedy (1985) may have affected our subsequent analyses as well. Variation in rumination effectiveness and specific fragility was within 10% across chopped forages, though, so this does not seem a likely explanation for the discrepancies we observed.

Conclusion

A quantitative understanding of factors involved in comminution, and the kinetics of their interactions, may allow digestion and passage to be manipulated for optimal production. Significant amounts of variation in the particle size distributions of boluses entering the reticulorumen can now be explained from knowledge of feed characteristics. Current limits to our understanding of mastication and rumination effects on digestion and passage in cattle are that no information is available for mixed diets and few data exist for many common types of forage (none for silages or which address the effects of plant maturity). Current limits to our understanding of the dynamics of particle size distributions are that only the data of Kennedy (1985) are amenable to such analyses and these data relate to near steady-state conditions; therefore, synergies between mastication, digestion, and rumination under practical conditions remain to be examined.

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TABLES and FIGURES

Table 2.1. Particle size distribution statistics estimated for boluses collected by Suzuki et al. (2001) either 6 to 12 h or 18 to 24 h after feeding steers either orchardgrass or alfalfa hay.

	Orchardgrass hay		Alfalfa hay	
	6 to12 h	18 to 24 h	6 to 12 h	18 to 24 h
Mean particle size, Log_{10} μm (μm)				
'Up'	3.05 (1127)	2.90 (796)	3.09 (1225)	3.02 (1039)
'Tail'	2.85 (705)	2.65 (445)	2.91 (817)	2.82 (662)
Retained	3.16 (1442)	3.00 (1001)	3.40 (2545)	3.10 (1266)
'Down'	2.74 (555)	2.53 (336)	3.16 (1460)	2.74 (549)
Log_{10} SD				
'Up'	0.79	0.66	0.58	0.54
'Tail'	0.76	0.67	0.76	0.44
Retained	0.79	0.62	0.72	0.53
'Down'	0.49	0.42	0.44	0.35

Table 2.2. Effect of time after feeding either orchardgrass or alfalfa hay on the rumination of boluses by steers (Suzuki et al. 2001; $P < 0.05$ within hay).

	Orchardgrass hay		Alfalfa hay	
	6 to12 h	18 to 24 h	6 to 12 h	18 to 24 h
Effectiveness,[1] %	62.1	82.0	54.3	66.0
Specific fragility[2]	12.1	14.2	11.0	13.2

[1]Large particles (>1180 μm) reduced to <1180 μm in one rumination cycle as a percentage of large particles retained for chewing.
[2]Large particles (>1180 μm, in milligrams) reduced to <1180 μm per chew per gram of large particles retained for chewing.

Table 2.3. In situ disappearance after 48 h of incubation for neutral detergent fiber in material retained for rechewing during rumination and 'down' boluses from steers fed either orchardgrass or alfalfa hay (Suzuki et al. 2001; $P < 0.01$ within hay).

Orchardgrass hay		Alfalfa hay	
Retained	Down	Retained	Down
46.5	49.4	8.1	25.2

Table 2.4. Univariate statistics for the entire swallowed feed bolus dataset (Silver 1935; Gill et al. 1966; Pond et al. 1984; Kennedy 1985; Nelson 1988; Shaver et al. 1988; Deswysen et al. 1989; Luginbuhl et al. 1989; Bailey et al. 1990; Fisher et al. 1991; Burns et al. 1992; Mader et al. 1993; Burns et al. 1997; Corley et al. 1997; Reinhardt et al. 1998; Fernandez & Michalet-Doreau 2002; Beauchemin et al. 2003; Fernandez et al. 2004; Ellis et al. 2005; and Boudon et al. 2006).

Variable	n	Mean	SD	Minimum	Maximum
Body size, kg	81	492	140	267	886
Dry matter intake,					
kg	47	12.0	6.2	3.1	23.9
% of body size	47	2.01	0.74	0.78	3.7
NDF, %	22	50.1	11.0	36.0	73.9
Feed,					
log_{10} mean	30	3.67	0.37	2.80	4.43
mean, μm	30	6674	6526	631	26866
log_{10} SD	26	0.57	0.18	0.32	1.00
Bolus,					
g	26	139	28	93	191
% DM	16	16.5	3.6	11.9	25.4
g of DM	12	22.8	6.4	12.0	30.0
log_{10} mean	100	3.39	0.30	2.74	4.43
mean, μm	100	3208	2513	552	26742
log_{10} SD	63	0.62	0.36	0.27	2.11
Chews per bolus	12	30	9	17	43
Time into meal, min	12	35	27	0	70

Table 2.5. Univariate statistics for the swallowed feed bolus data subset (Shaver et al. 1988; Bailey et al. 1990; Fernandez & Michalet-Doreau 2002; Beauchemin et al. 2003; and Fernandez et al. 2004).

Variable	n	Mean	SD	Minimum	Maximum
DM intake, kg	25	16.5	4.6	11.4	23.9
Feed					
code[1]	25	1.08	0.40	0	2
$\log_{10}$ mean	25	3.68	0.40	2.80	4.43
$\log_{10}$ SD	25	0.58	0.18	0.32	1.00
Bolus					
$\log_{10}$ mean	25	3.45	0.27	2.74	4.09
$\log_{10}$ SD	25	0.69	0.41	0.33	2.11

[1]0 = long, 1 = chopped, and 2 = ground.

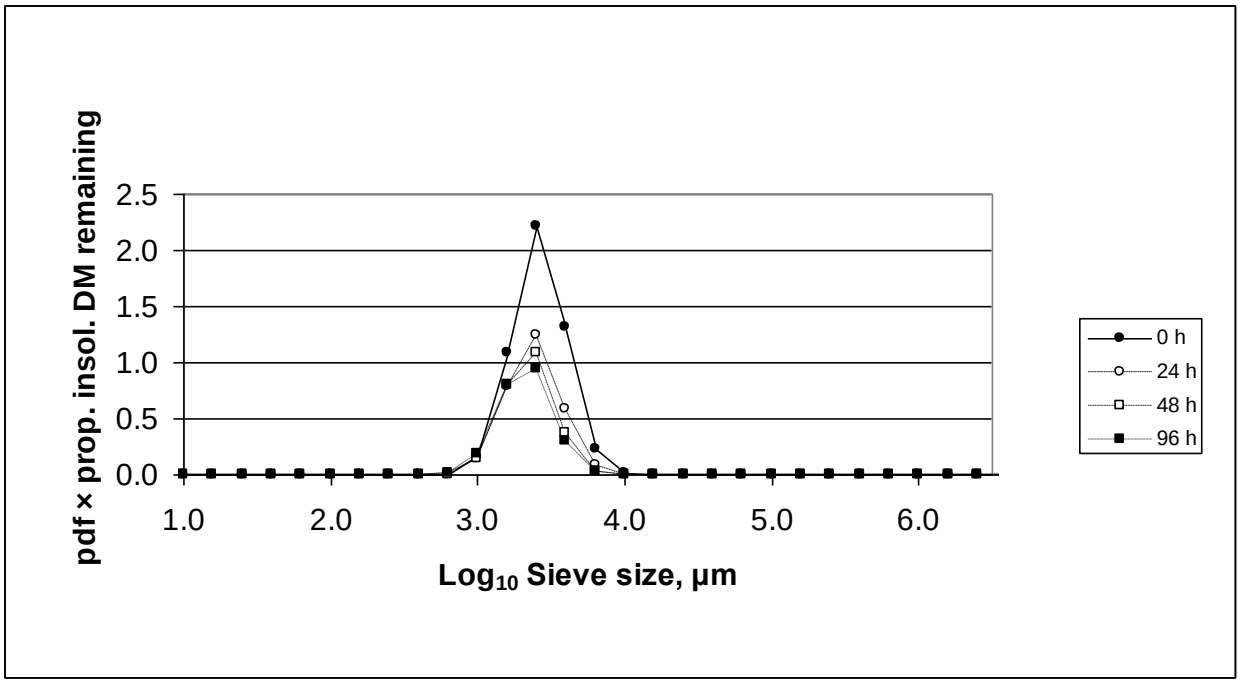

Figure 2.1. Particle size distributions of a fraction of chopped alfalfa hay (collected between sieves with pore sizes of 2360 and 1700 µm) before, and after various times of, in situ incubation Adapted from Murphy & Nicoletti (1984).

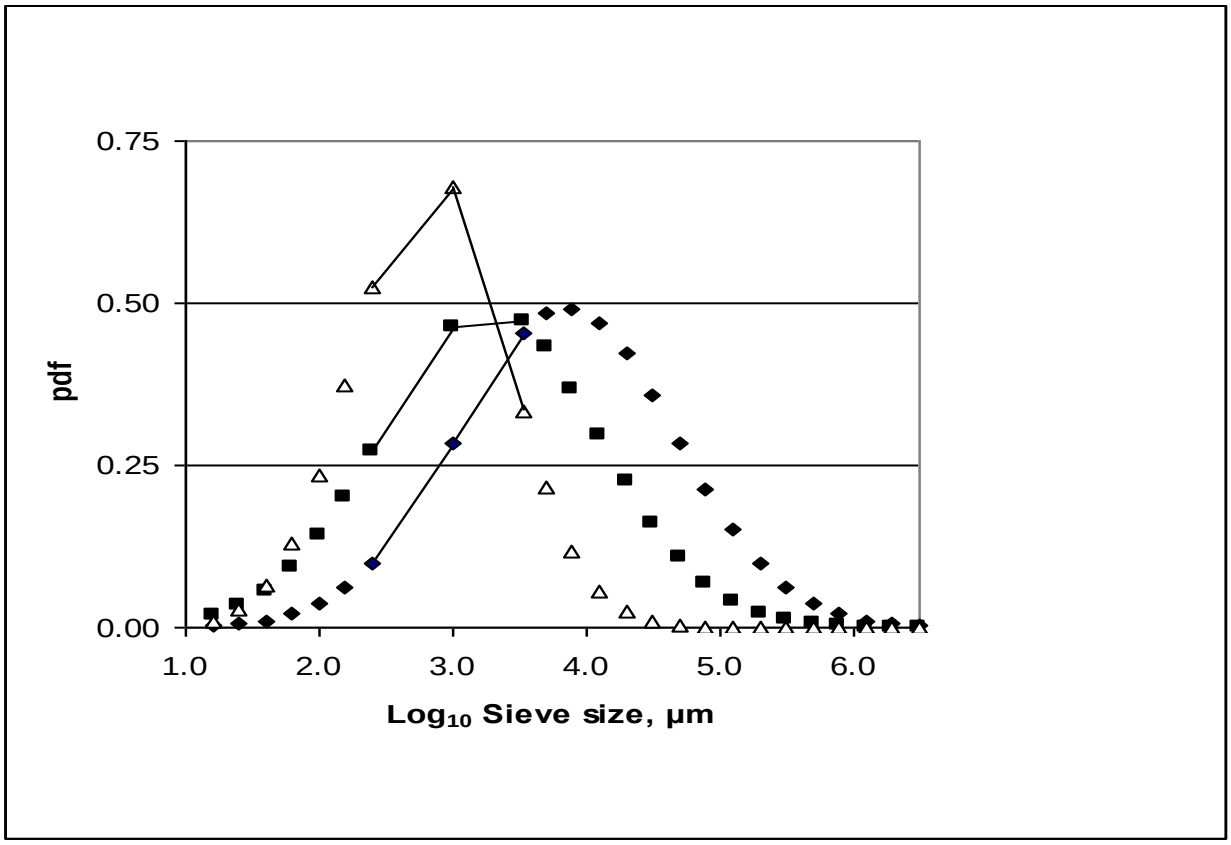

Figure 2.2. Particle size distributions of swallowed masticate (♦), rumen digesta (■), and feces (Δ) in steers fed various chopped forages. Adapted from Kennedy (1985). Connected points denote the range of observed data; whereas, unconnected points were estimated assuming that the observed data conformed to a lognormal distribution.

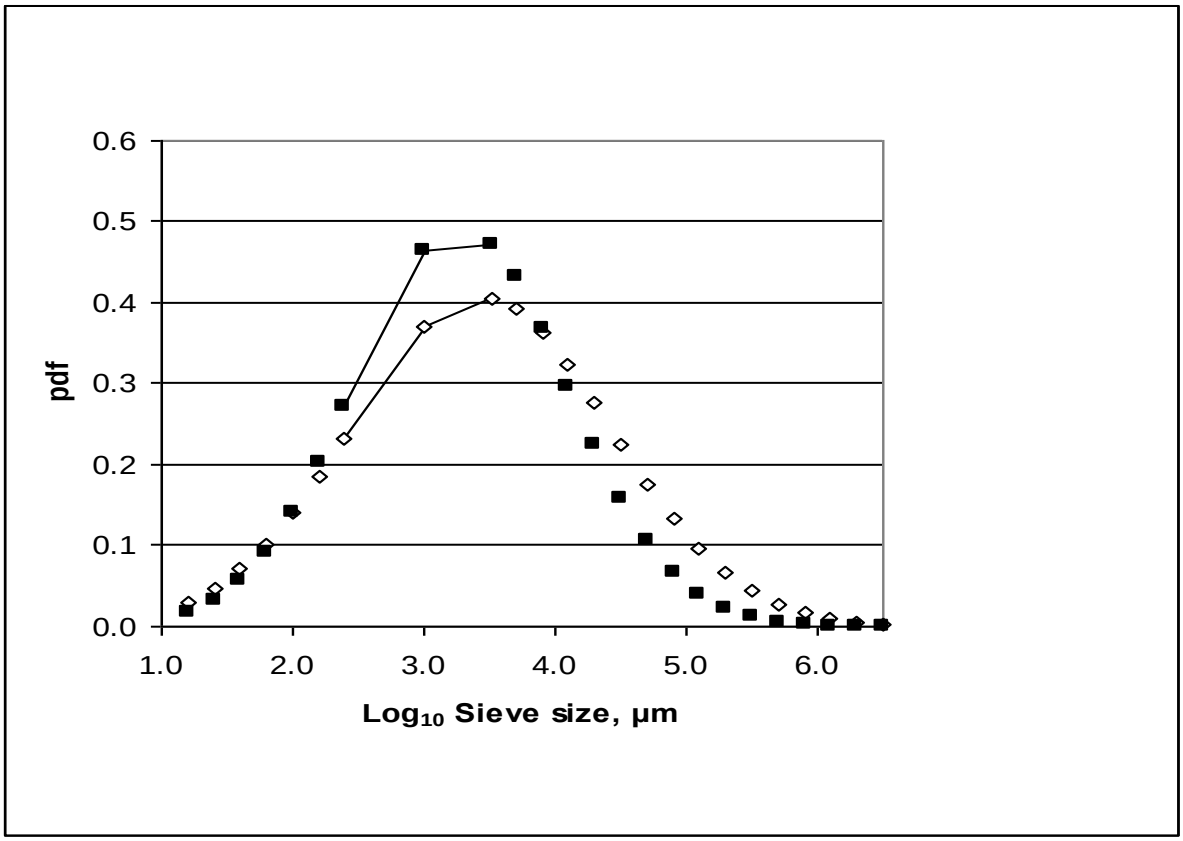

Figure 2.3. Particle size distribution of the 'up' bolus regurgitated at the start of rumination (◊) compared to that of rumen digesta (■) in steers fed various chopped forages. Adapted from Kennedy (1985). Connected points denote the range of observed data; whereas, unconnected points were estimated assuming that the observed data conformed to a lognormal distribution.

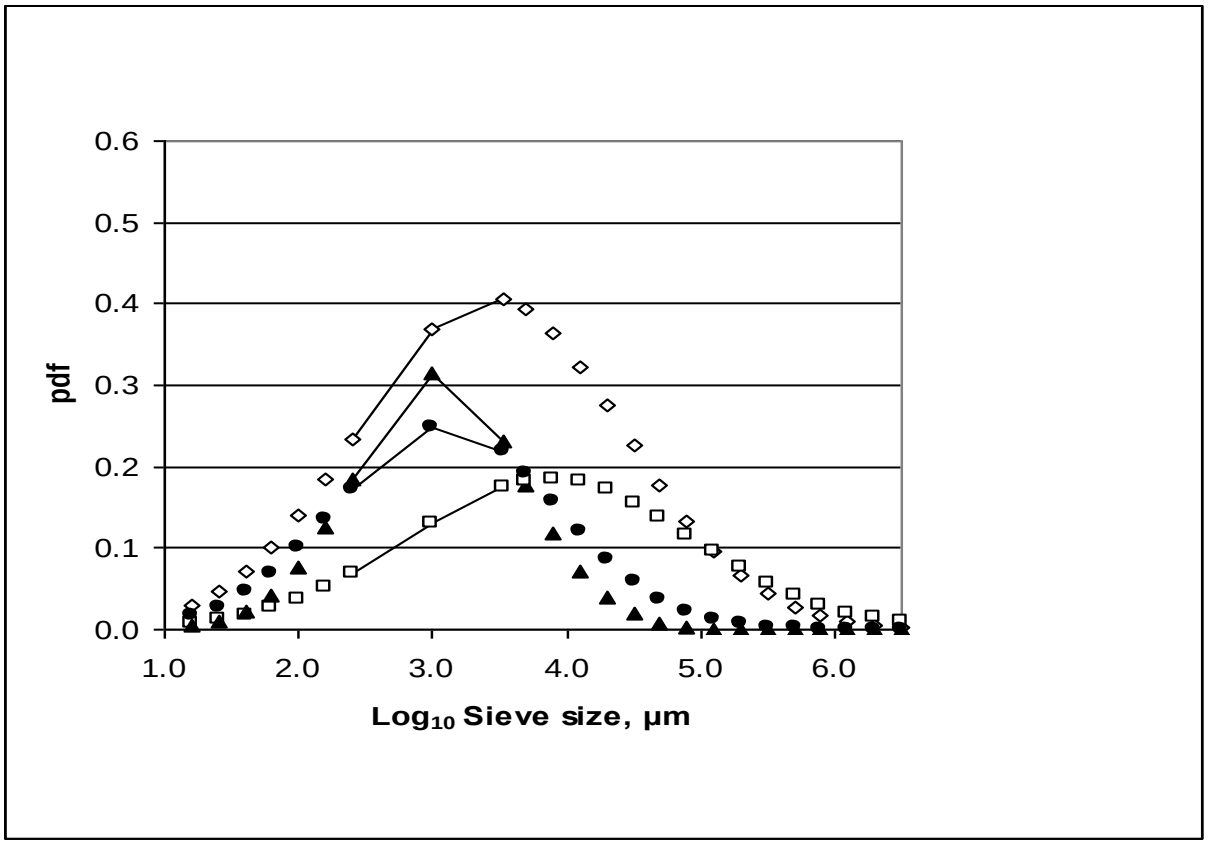

Figure 2.4. Particle size distributions of the 'up' bolus regurgitated at the start of rumination (◊); of the reswallowed 'tail' bolus just after regurgitation during rumination (●), but before rechewing; of material retained in the mouth for rechewing (□); and of the 'down' bolus reswallowed after rechewing during rumination (▲) in steers fed various chopped forages. Adapted from Kennedy (1985). Connected points denote the range of observed data; whereas, unconnected points were estimated assuming that the observed data conformed to a lognormal distribution.

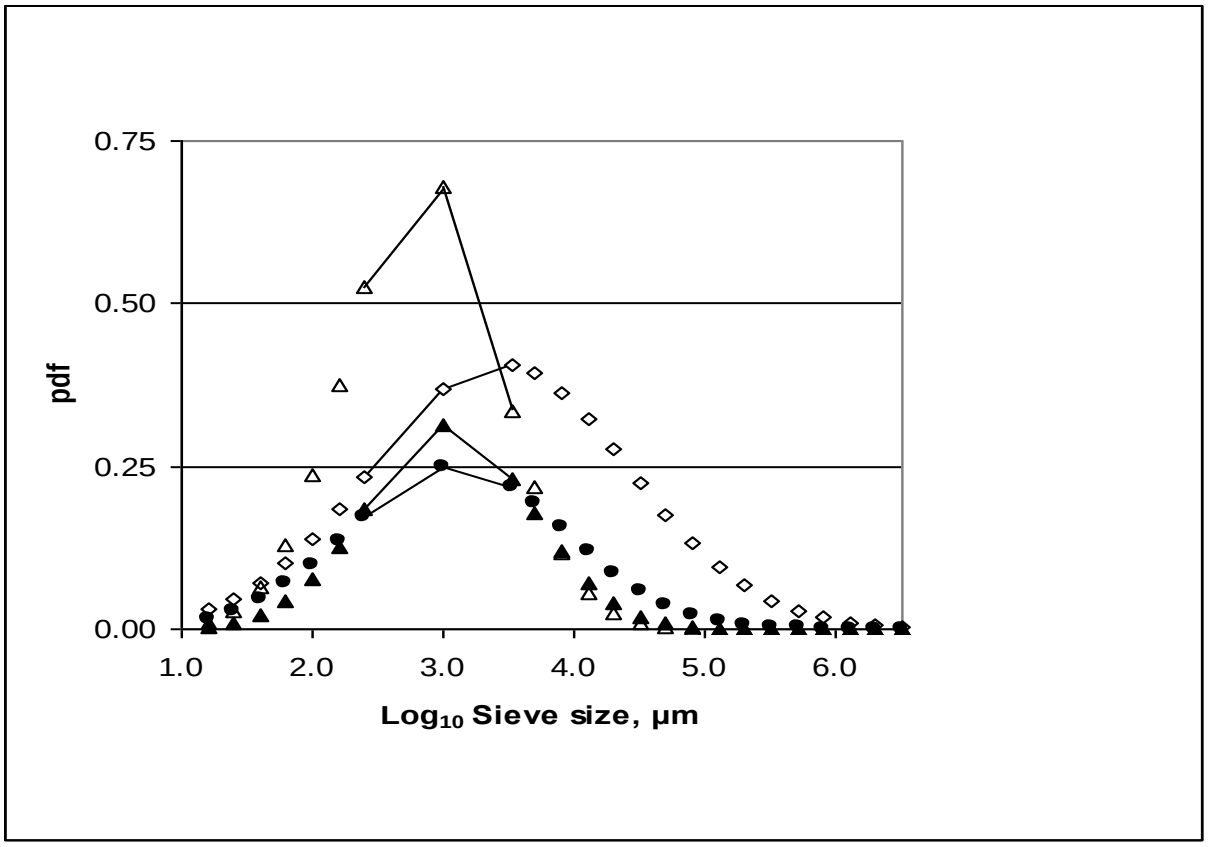

Figure 2.5. Particle size distributions of the 'up' bolus regurgitated at the start of rumination (◊), of the 'tail' bolus reswallowed just after regurgitation (●) during rumination, of the 'down' bolus reswallowed after rechewing (▲), and of feces (Δ) in steers fed various chopped forages. Adapted from Kennedy (1985). Connected points denote the range of observed data; whereas, unconnected points were estimated assuming that the observed data conformed to a lognormal distribution.

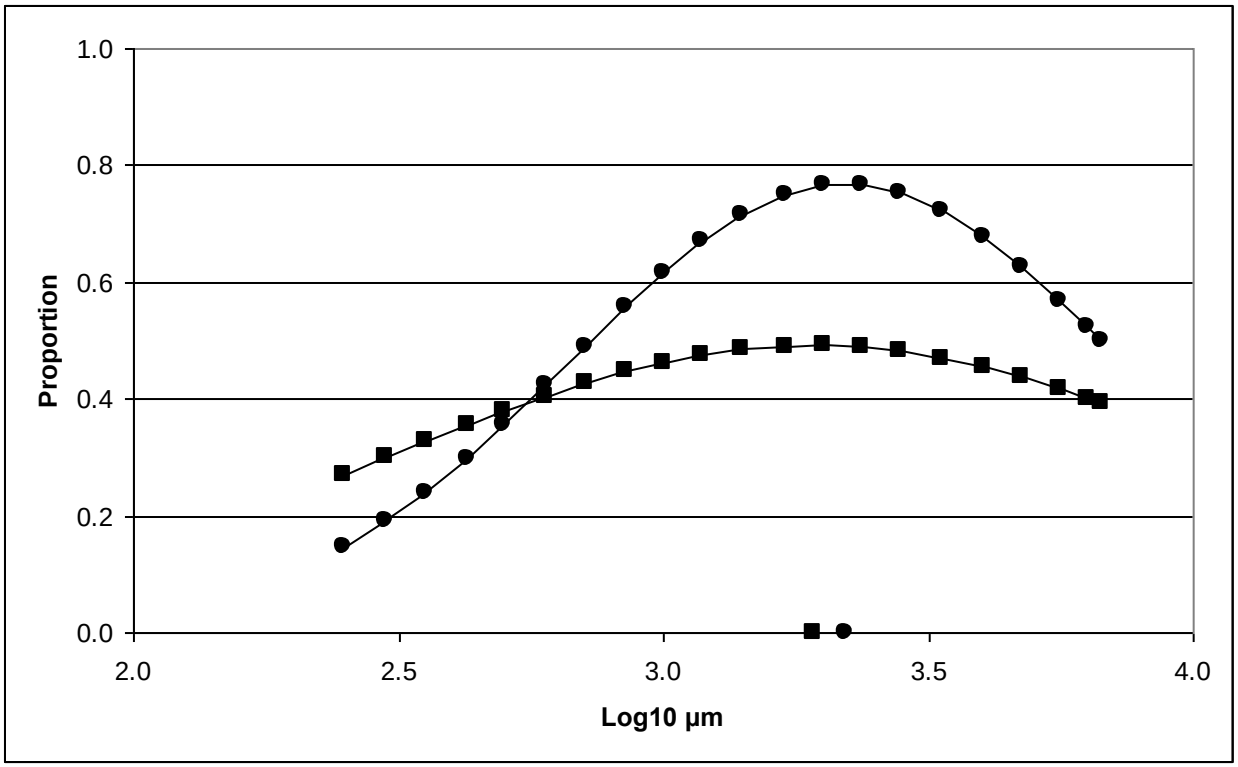

Figure 2.6. Starting steady-state particle size distribution in the rumen (■) compared to that predicted after accounting for feed consumption, rumination, and passage in one 24-h period (●) averaged across chopped forages. Starting mean particle size in the rumen and that predicted after the 24-hour period are indicated on the abscissa. Adapted from and calculated based on data of Kennedy (1985).

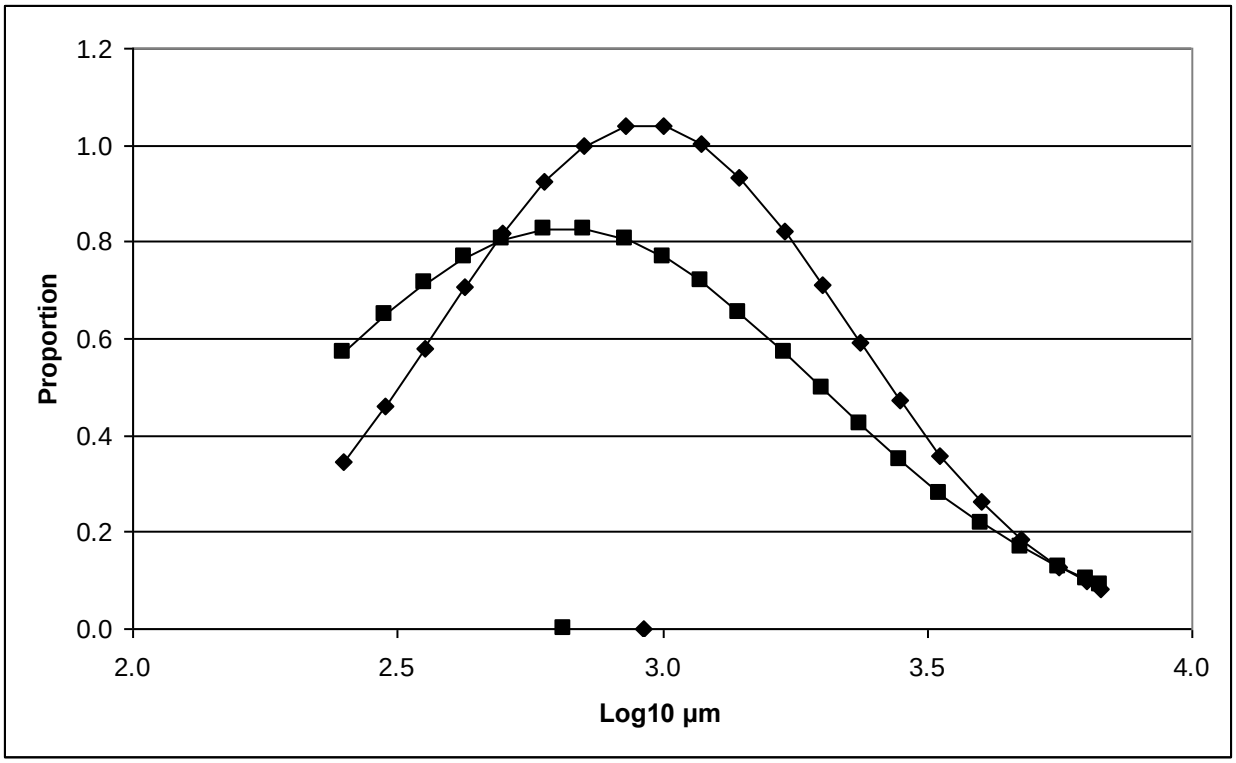

Figure 2.7. Starting steady-state particle size distribution in the rumen (■) compared to that predicted after accounting for feed consumption, rumination, and passage in one 24-h period (●) averaged across ground and pelleted forages. Starting mean particle size in the rumen and that predicted after the 24-hour period are indicated on the abscissa. Adapted from and calculated based on data of Kennedy (1985).

CHAPTER III

EFFECTS OF VARYING DIETARY CATION-ANION DIFFERENCE AND MOLAR SODIUM TO POTASSIUM RATIO IN LACTATING COW DIETS ON PRODUCTION AND FERMENTATION

Abstract

Six multiparous Holstein cows, fitted with rumen cannulas, averaging 122 ± 31 days in milk were randomly assigned to six treatments allocated in an equiradial (pentagonal) second-order response surface design with a center point to examine the effects of dietary cation-anion difference (DCAD) and Na:K on lactating dairy cows. Replication of treatments within a 6 x 6 Latin square minimized the potential effects of outliers and allowed a surface covering a 3 × 3 matrix of DCAD and Na:K combinations to be examined. Ranges in DCAD and Na:K were chosen to be equally spaced on logarithmic scales; tripling each time from 0.25 for the former, and 1.5-fold each time from 25 meq/100 g of DM for the latter. The response surface was centered on a molar Na:K of 0.75 (0.60% Na and 1.37% K in DM) and a DCAD of 37.5 meq/100 g of DM. The other 5 treatments were: 1.63, 50.0 (Na:K, DCAD); 0.46, 53.8; 0.25, 35.2; 0.63, 25.1; and 2.00, 31.2. Percentages of Na and K in DM of the TMR for vertices of the pentagon were calculated as 1.05, 1.10; 0.56, 2.08; 0.27, 1.84; 0.44, 1.17; and 0.84, 0.72. Diets were based on corn silage and a corn-based grain mix. The Na:K ratios were varied with $NaHCO_3$ and K_2CO_3. Periods were 14 d. Daily feed intake of each cow was recorded during each period; samples of feed and orts were collected daily. Milk production was measured daily; samples were collected weekly and analyzed for components. Rumen and urine samples were collected and analyzed for pH on the last 3 d of each period. The MIXED procedure of SAS was

used for ANOVA. There were no response surface effects of treatment on milk production and components, or DMI (P<0.05). Acetate, propionate, and butyrate concentrations in the rumen were all affected by treatment (P <0.05). There were multiple significant effects on acetate, including an interaction of DCAD and ratio. There were both linear and quadratic effects of ratio on propionate and butyrate. Linear (P <0.05) and quadratic effects (P <0.05) of DCAD on rumen pH were also indicated. A quadratic effect of Na:K (P < 0.01) and interaction of DCAD (P < 0.003) indicated that urine pH was maximal (8.24 or above) at high DCAD and low Na:K. Milk production and components were similar across treatments, but rumen fermentation was affected.

Introduction

Dietary cation anion difference (DCAD) is a well-accepted concept in dry cow nutrition. Negative DCAD diets reduce the incidence of periparturient paresis (Block, 1984; Oetzel et al., 1988). However, recently there has been interest in defining optimum DCAD levels in lactating cows. Hu and Murphy (2004) conducted a meta-analysis of multiple macromineral studies and found that DMI (dry matter intake) peaked at 40 mEq/100 g of DM, and milk yield (MY) was highest at 34 mEq/100 g of DM. Based on a similar analysis, Sanchez and Beede (1996) found that DMI and MY was maximized at 38 mEq/100 g of DM, over a range of 25 to 50 mEq/100 g of DM. Apper-Bossard et al. (2006) reported that a high DCAD diet increased DMI in cows fed diets high in rapidly degraded starch, but not in cows fed diets with lower amounts of starch. Apper-Bossard et al. (2010) found a similar effect: cows fed high DCAD diets with high starch had higher DMI than those fed lower starch diets. In contrast, Wildman et al. (2007a) saw no effects on DMI at 41 or 58 mEq/100 g of DM.

Sodium and K cations are important for acid-base equilibrium, osmotic balance, and

kidney function. The most common example is the Na -K pump, which requires ATP and maintains high K and low Na intracellularly. There are limited data about Na:K in diets for lactating dairy cows. Results of research focusing on the source of ions vary. No differences in DMI or MY were observed when either K or Na was used as a source of cations (West et al., 1992). Tucker et al. (1988) concluded that the overall DCAD was more important than individual ions. Both of these studies examined source of ions; there are few experiments in which ratios of dietary K to Na have been varied in lactating cow rations. Sanchez et al. (1997) found that DMI was influenced by an interaction between Na and K, and Na and Cl. There was an increase in 3.5% FCM with higher dietary Na and it was concluded that interrelationships exist among Na, K, and Cl. In another experiment, DMI and MY responses tended to be greater when one cation (Na or K) was high and dietary concentration of the other cation was low (Sanchez et al., 1994). Wildman et al. (2007a) reported a quadratic effect from altering dietary K:Na on MY, but DMI was unaffected. Hu and Kung (2009) found a quadratic effect on DMI with the lowest DMI at a Na:K of 0.53, compared with ratios of 0.21 and 1.06. The objective of this experiment was to determine the effects of varying both DCAD and molar Na:K on DMI, MY, rumen fermentation, and blood metabolites.

Materials and Methods

This experiment was conducted at the University of Illinois dairy research farm, Urbana, between June and September 2007. All experimental protocols used in the trial were approved by the University of Illinois Animal Care and Use Committee.

Experimental Design and Cows

The trial was conducted as an equiradial (pentagonal) second-order response surface

design with a center point (Papas et al., 1984, St. Pierre and Weiss, 2009) to examine the effects of DCAD and Na:K on dairy cows in mid lactation. Six multiparous cows (122±30 DIM) were randomly assigned to 1 of 6 treatments. Replication of treatments within a 6 x 6 Latin square minimized the potential effects of outliers and allowed a surface covering a 3 × 3 matrix of Na:K and DCAD combinations to be examined while avoiding an impractical 9 × 9 Latin square. Periods were 14 d in length.

The two main effects for this experiment were level of DCAD and Na:K. Ranges in Na:K and DCAD were chosen to be equally spaced on logarithmic scales; tripling each time from 0.25 for the former, and 1.5-fold each time from 25 meq/100 g DM for the latter. The response surface was centered on a Na:K of 0.50 (0.60% Na and 1.37% K in DM) and a DCAD of 37.5 meq/100 g of DM. For comparison, current NRC (2001) recommendations for Na, K, Cl, and S in the diet (0.23, 1.04, 0.26, and 0.20%, respectively) of a mature 680 kg Holstein cow producing 35 kg of milk with 3.5% fat result in a a DCAD of 16.8 meq/100 g of DM and a molar Na:K of 0.38. The other 5 treatments (vertices of the pentagon) were: 27.01, 0.95 (DCAD; Na:K); 56.25, 0.50; 42.50, 0.18; 27.01, 0.26; and 42.50, 1.42.

Each cow was fitted with a rumen cannula. They were housed in tie stalls indoors except for milking and during an exercise period on a dirt lot between the a.m. milking and feeding. Feed offered was adjusted daily and 110% of consumption the previous day (as-fed basis), and provided at 1100 and 1630 h. Water was available for ad libitum consumption. Cows were milked twice daily at approximately 0600 and 1500 h.

The TMR ingredients are shown in Table 3.1. Dietary cation-anion difference and Na:K was varied using $NaHCO_3$ and K_2CO_3 in the concentrate mixes (Table 3.2).

Sample Collection and Analysis

Feed and Orts. Feed intake of each cow was recorded daily; samples of feed and orts were collected daily. Orts from each cow were weighed before the a.m. feeding. Feed and ort samples were dried in a forced-air oven at 55°C until constant weights were obtained. Weekly samples of ingredients and TMR were stored at -15°C until the end of the experiment, and then composited and pooled for later analysis. Nutrient contents of ingredients and TMR were analyzed by wet chemistry for DM, CP, ADF, NDF, starch and minerals (Dairy One Forage Laboratory, Ithaca, NY). Also, energy concentration was calculated (Dairy One Forage Laboratory, Ithaca, NY). The nutrient composition presented in Table 3.1 was calculated from the analyses of composited TMR samples by period.

Rumen and Urine. Kinetics of ruminal pH, total VFA, and NH_3 content were monitored every 4 h on the last 3 d of each period, arranged to provide resulting in a sample for each hour of the day. Rumen fluid (200 ml) was sampled from several locations in the rumen via the rumen cannula, using a suction pump and a rigid plastic tube. At each sampling time, pH as measured immediately. Samples were strained through 6 layers of cheesecloth and centrifuged at 12,000 x *g* for 15 min. Aliquots were then acidified to pH 2 with 50% sulfuric acid and frozen at -20°C for VFA and NH_3 analysis. At each time a rumen sample was obtained, urination was induced by perineal stimulation. A 50-ml sample of midstream urine was taken for immediate pH determination. Volatile fatty acids were determined using a gas chromatograph. Ruminal NH_3 was determined spectrophotometrically .

Blood. On the last day of each period, immediately before the a.m. feeding, 5 mL of jugular venous blood was collected anaerobically with a plastic syringe containing lithium heparin, capped, placed on crushed ice, and analyzed for pH, partial pressure of CO_2 (pCO_2),

partial pressure for O_2 (pO_2), HCO^{3-}, and base excess in a blood gas analyzer (Rapidlab 850 system, Bayer Diagnostics, Tarrytown, NY) within 2 h. Simultaneously, Na^+, K^+, Cl^-, and Ca^{2+} were determined using an ion-selective electrode, and anion gap was calculated by using the blood gas analyzer (Rapidlab 850 system, Bayer Diagnostics).

Milk Production and BW. Milk production was measured at 0600 and 1500 h daily. Milk samples were taken weekly. Samples were collected from consecutive p.m. and a.m. milkings, composited based on p.m. and a.m. production, and analyzed for milk fat, true protein, lactose, SNF, SCC, and urea N using a Milkoscan System 4000 (Foss North American, Eden Prairie, MN) by an infrared method (Dairy Lab Services, Dubuque, IA). Body weights were measured at both the start and end of the trial, and once weekly. They were averaged by period for statistical analysis.

Statistical Analysis

Dry matter intake and milk production were reduced to daily means for each cow, milk composition data were reduced to weekly means and BW data were reduced to period means. Data were analyzed using the MIXED procedure (SAS Institute, 2008) according to the model for a Latin square design:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

Where μ = overall mean; C_i = effect of cow i (i = 1, 2, 3, 4, 5, 6); P_j = effect of period j (j = 1, 2, 3, 4, 5, 6); T_k = effect of treatment k (k = 1, 2, 3, 4, 5, 6); and e_{ijk} = residual error associated with each $Y_{ijk.}$

Significance was defined as $P \leq 0.05$; whereas $P \leq 0.05 < P \leq 0.10$ was considered to a trend. Single degree-of-freedom contrasts were constructed to test linear effect of DCAD, linear effect of Na:K ratio, quadratic effect of DCAD, quadratic effect of Na:K ratio, and the interaction of linear effect of DCAD and Na:K ratio.

Results and discussion

Environmental Conditions

Environmental conditions during this study are shown in Table 3-3. Mean maximum and minimum dry bulb temperatures averaged 30.7 and 18.0 °C and were similar throughout the experiment. When daily mean THI exceeds 22.2, Holstein cows become less productive (Johnson, 1987). The daily mean THI for this experiment was 24.6, exceeding that limit.

BW, DMI, and Milk Yield and Composition

Dry matter intake and BW were not affected by treatment. Milk yield and composition were also not affected by treatment, except for milk urea N (Table 3.4). Milk urea N peaked 13.1 mg of N/dl at a DCAD of 37.5 meq/100 g of DM. Milk protein percentage was also numerically highest at this DCAD level.

O'Connor et al. (1988) did not observe any differences in DMI or MY of mid-lactation Holstein cows fed 2 concentrations of Na (0.24 vs. 0.62%) or K (1.14 vs. 1.59%). Hu and Kung (2009) observed a negative quadratic response in DMI at a Na:K of 0.53, but no milk response was detected. This is in contrast to Wildman et al. (2007a), who observed no differences in DMI but saw quadratic responses in MY and energy corrected milk (ECM). At Na:K ratio of 1:3, MY and ECM decreased from 27.7 kg/d at Na:K 1:2 to 26.2 kg/d, then increased to 28.1 kg/d. In this experiment no effect of DCAD was observed on DMI or MY.

In regards to DCAD, Hu and Murphy (2004) conducted a meta-analysis and reported peak DMI at 40 meq/100 g of DM (2004). In another experiment, Hu et al. (2007), observed a linear increase in DMI as DCAD increased from -3 to 47 mEq/100 g of DM. Wildman et al. (2007b), reported significant effects of DCAD on DMI, MY, and ECM; when DCAD was increased from 25 to 50 mEq/100 g of DM. Milk fat percentage was positively affected by increasing DCAD, from 2.44% to 2.92%. Apper-Bossard et al. (2010) also found a linear effect of DCAD, DMI was highest (24.0 kg/d) at 30.6 mEq/100 g. The 4% FCM also increased as DCAD increased, to a maximum of 37.8 kg/d.

In the present experiment, cows were undoubtedly heat stressed, with a mean THI of 24.6 (Table 3-3). Cows had low DMI, averaging 20.6 kg/d, and had milk fat depression with an average milk fat percentage of 2.78. Based on least squares means, all treatments resulted in inversion of milk fat and milk protein (Table 3.4). These results are similar to those of Wildman et al. (2007b), except we observed no significant effects of DCAD or Na:K on production.

Rumen Fermentation and Urine

Least squares means of ruminal fermentation parameters are presented in Table 3.5. Rumen fermentation was affected by treatment (Table 3.6). There were multiple significant effects of treatment on acetate, including an interaction of DCAD and Na:K ratio (Figure 3.1). Acetate peaked at 64 m*M* when DCAD was highest and at the median Na:K. In this case, Na:K ratio had more of an effect at a higher DCAD than when DCAD was lower.

There was both a linear and quadratic effect of Na:K ratio on propionate (Figure 3.2). Propionate was maximal at a low DCAD and a median Na:K ratio. As DCAD decreases, so does ruminal buffering capacity. Similar to propionate, there were both linear and quadratic effects of

Na:K ratio on butyrate; it was highest (12 m*M*) at a low DCAD and a high ratio (Figure 3-3). Again, ruminal pH tends to be lower at a lower DCAD.

While data exist regarding DCAD and fermentation, there is very limited information on fermentation changes in response to Na:K. Generally, as DCAD increases, rumen pH increases (Tucker et al., 1988; Apper-Bossard et al., 2010). In the same study, DCAD had no effect on VFA patterns. Wildman et al. (2007b) observed no effects on pH or fermentation patterns with a DCAD of 25 mEq to 50 mEq/100 g of DM.

There were significant quadratic effects of DCAD in addition to a linear effect of Na:K on ruminal NH_3 (Figure 3.4). This figure indicates that ruminal NH_3 was minimal (3.4 mg/dl or below) when DCAD was high and Na:K was low. Ruminal NH_3 concentration increased (towards 5.4 mg/dl) as both DCAD decreased and Na:K increased. Apper Bossard et al. (2010) observed a significant quadratic interaction of low DCAD (16 mEq/100 g of DM) and high concentrate (40%) on ruminal ammonia concentration (8.0 mg/dl) which was the lowest of all combinations. There were no effects of DCAD on ruminal ammonia.

There was a quadratic effect on rumen pH (Figure 3.5), with the lowest pH at the median DCAD. There was a both a quadratic effect of Na:K ratio, and an interaction of DCAD and Na:K ratio on ruminal pH_6-h (area below pH6) (Mackie and Gilchrist, 1979). There were 2 peaks; one with median DCAD and high Na:K ratio, and one when both DCAD and Na:K were low (Figure 3.6). By increasing DCAD ruminal pH was above pH 6 longer. In addition, if Na:K was too high or too low, rumen pH was depressed.

There were multiple effects on urine pH, including linear and quadratic effects of Na:K, and an interaction of DCAD and Na:K (Figure 3.7). Urine pH was maximized at a high DCAD and a median to low Na:K. This was expected since urine pH is very responsive to changes in

DCAD in both dry (Block, 1984; Charbonneau et al., 2006) and lactating cows (Hu et al., 2007; Apper-Bossard et al., 2010).

Jugular Venous Blood

Least squares means of jugular venous blood parameters are presented in Table 3.7. Jugular venous Na and K were not altered; this in agreement with Tucker et al. (1988) and Hu and Kung (2009). Both experiments found serum Na and K were not affected by the concentrations of these minerals in the diet. Like Na and K, jugular venous Cl and Mg were also unaffected. Jugular venous HCO_3^- was not affected in the present study, which is in agreement with both Wildman et al. (2007a) and Hu and Kung (2009). The absence of a DCAD effect on DMI and jugular venous bicarbonate suggests that all DCAD levels provided sufficient metabolic buffering for the cow.

There were significant effects on pO_2, Ca^{2+} and creatinine (Table 3.7). Figure 3.8 illustrates jugular venous pO_2 which was highest at a median DCAD and a high Na:K. Jugular venous Ca^{2+} was maximized at a low DCAD and a high Na:K (Figure 3.9). This is consistent with feeding a low DCAD diet in the close-up dry period to increase plasma Ca and decrease incidence of hypocalcemia post-parturtition (Oetzel et al., 1988; Charbonneau et al., 2006).

Jugular venous creatinine was highest at a low DCAD and high Na:K (Figure 3.10). Hu and Kung (2009) observed a quadratic effect of Na:K on coccygeal venous creatinine with an Na:K of 0.53 having 0.80 mg/dL creatinine, compared to 0.73 mg/dL at a Na:K of0.21, and 0.79mg/dL at a Na:K 1.06. Hu et al. (2007) observed an interaction between CP and DCAD; jugular venous creatinine was highest with a DCAD of -3 mEq/100 g of DM and a 16% CP diet. In the same study, urinary creatinine decreased linearly as DCAD increased. Urinary creatinine

was not measured in the current study.

Conclusion

Cows were fed diets containing several combinations of DCAD and Na:K ratios. Dry matter intake, and milk production and composition were not affected by treatment. Heat stress likely negatively affected the cows' performance. Ruminal fermentation was affected by both DCAD and Na:K ratio. Urine pH was maximized when diets had high DCAD and low a Na:K ratio.

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TABLES and FIGURES

Table 3.1. Ingredients and composition of experimental diets differing in DCAD[1] (37.5, 27.0, 56.2, 42.5, 27.0, and 42.5) and molar ratio of Na:K (0.50, 0.95, 0.50, 0.18, 0.26, and 1.42).

Item	Treatment					
DCAD	37.5[2]	27.01	56.25	42.50	27.01	42.50
Na:K	0.50[2]	0.95	0.50	0.18	0.26	1.42
Ingredient, % of DM						
Corn silage[3]	46.50	46.18	45.75	46.30	46.92	46.92
Wet brewers grains[4]	13.41	13.32	13.20	13.36	13.53	13.53
Whole cottonseed[5]	5.36	5.32	5.27	5.34	5.41	5.41
Wheat straw[6]	3.35	3.32	3.29	3.33	3.38	3.38
Concentrate	31.38	31.85	32.48	31.67	30.77	30.77
Composition[7]						
DM, %	48.3	49.0	47.7	49.0	48.4	46.9
CP, %	17.4	16.7	18.0	18.0	17.7	17.1
Fat%	4.9	4.8	4.9	4.9	5.0	4.9
NE_L, Mcal/kg	1.653	1.676	1.653	1.675	1.653	1.632
ADF, %	20.3	19.1	19.2	18.4	18.7	22.7
NDF, %	33.1	31.5	31.0	31.6	33.8	36.6
Starch, %	31.2	32.6	31.0	31.6	33.8	36.6
Ca, %	0.54	0.57	0.56	0.58	0.82	0.65
P, %	0.38	0.40	0.37	0.37	0.38	0.36
Mg, %	0.25	0.25	0.23	0.24	0.26	0.25
Na, %	0.474	0.605	0.607	0.244	0.215	0.928
K, %	1.54	0.99	2.05	1.98	1.5	1.01
Cl, %	0.39	0.41	0.37	0.37	0.36	0.36
S, %	0.22	0.22	0.22	0.22	0.22	0.22
Na:K	0.50	0.95	0.50	0.18	0.26	1.42
DCAD, meq/100 g of DM	35.4	26.4	54.8	37.2	23.9	42.4

[1]DCAD in milliequivalents of ($Na^+ + K^+ - Cl^- - S^=$) per 100 g of DM.
[2]Center point of pentagon.
[3]Contained 38.8% DM, 9.3% CP, 21.2% ADF, 37.4% NDF, and 40.8% starch on a DM basis.
[4]Contained 27.0% DM, 34.0% CP, 24.0% ADF, and 49.0% NDF on a DM basis.
[5]Contained 90.1% DM, 22.5% CP, 41.4% ADF, and 55.7% NDF on a DM basis.
[6]Contained 94.0% DM, 5.7% CP, 65.6% ADF, and 82.2% NDF on a DM basis.
[7]Composition data from TMR samples composited across experiment.

Table 3.2. Composition of experimental diet concentrate mixes differing in DCAD[1] (37.5, 27.0, 56.2, 42.5, 27.0, and 42.5) and molar ratio of Na:K (0.50, 0.95, 0.50, 0.18, 0.26, and 1.42).

Ingredient	%, DM basis					
	Treatment					
DCAD	37.5[2]	27.0	56.2	42.5	27.0	42.5
Na:K	0.50[2]	0.95	0.50	0.18	0.26	1.42
Ground corn	54.04	53.13	51.41	53.28	55.78	55.62
Distillers dried grains with solubles	18.32	18.01	17.42	18.06	18.91	18.85
Soybean meal, 48% CP	15.19	14.93	14.45	14.97	15.68	15.63
Limestone	3.36	3.30	3.19	3.31	3.46	3.45
NaCl	1.14	1.12	1.08	1.12	1.17	1.17
Trace mineral premix[3]	0.57	0.56	0.54	0.56	0.59	0.59
Magnesium oxide	0.28	0.28	0.27	0.28	0.29	0.29
Urea	0.57	0.28	0.27	0.28	0.29	0.29
$NaHCO_3$	2.84	7.83	4.06	0.00	0.00	4.10
K_2CO_3	3.98	0.56	7.31	8.13	3.82	0.00

[1]DCAD in milliequivalents of ($Na^+ + K^+ - Cl^- - S^=$) per 100 g of DM.

[2] Center point of pentagon.

[3]Contained 10% S, 7.5% K, 5% Mg, 3% Mn, 2% Fe, 5,000 mg/kg Cu, 250 mg/kg I, 150 mg/kg Se, 40 mg/kg Co, 2,200,000 IU/kg
vitamin A, 660,000 mg/kg vitamin D, and 80,000 mg/kg vitamin E.

Table 3.3. Environmental conditions for the experiment.

	Week												
Item	1	2	3	4	5	6	7	8	9	10	11	12	Mean ± SD
Maximum temperature, °C	33.5	29.4	27.8	31.8	28.5	28.3	30.1	32.0	33.0	29.8	32.2	32.0	30.7 ± 2.0
Minimum temperature, °C	19.2	16.7	14.9	18.4	15.6	14.6	17.8	22.9	20.6	19.9	18.8	16.1	18.0 ± 2.5
Maximum RH,[1] %	76.8	91.7	88.7	92.3	90.8	87.3	88.5	87.8	89.6	91.1	91.2	83.7	88.3 ± 4.3
Minimum RH, %	30.2	52.5	49.9	48.1	37.9	46.2	50.7	50.9	42.2	55.8	39.8	27.8	44.3 ± 8.9
THI[2]	25.1	24.4	22.9	26.2	22.0	22.8	24.9	26.7	26.5	25.3	25.5	23.1	24.6 ± 1.6

[1] RH = Relative humidity.
[2] THI = Temperature-humidity index = Tdb x 0.35 + Twb x 0.65 in Celsius; Bianca (1962).

Table 3.4. Least squares means for production measures for cows fed diets differing in DCAD[1] (37.5, 27.0, 56.2, 42.5, 27.0, and 42.5) and molar ratio of Na:K (0.50, 0.95, 0.50, 0.18, 0.26, and 1.42).

		Treatment						
	DCAD	37.5[2]	27.0	56.2	42.5	27.0	42.5	
Variable	Na:K	0.50[2]	0.95	0.50	0.18	0.26	1.42	SEM[3]
DMI, kg/d		20.1	21.2	20.5	19.3	21.3	21.2	0.6
BW, kg		673	691	691	687	967	685	5.0
BCS		2.72	2.77	2.72	2.69	2.79	2.74	0.04
Milk								
Yield, kg/d		28.9	30.1	29.1	28.5	30.4	30.4	1.0
3.5% FCM, kg/d[4]		25.3	26.3	26.4	25.6	25.8	26.7	1.3
Fat, %		2.79	2.77	2.94	2.93	2.53	2.74	0.16
Fat, kg/d		0.79	0.82	0.85	0.82	0.78	0.83	0.06
Protein, %		3.21	3.13	3.11	3.20	3.02	3.12	0.13
Protein, kg/d		0.92	0.93	0.89	0.88	0.93	0.95	0.05
Lactose, %		4.65	4.73	4.62	4.75	4.39	4.71	0.16
Lactose, kg/d		1.42	1.50	1.49	1.51	1.41	1.49	0.06
SNF, %		5.58	5.65	5.53	5.67	5.24	5.62	0.19
SNF, kg/d		1.71	1.79	1.79	1.81	1.69	1.78	0.08
MUN[5] mg of N/dL		13.1	11.9	11.4	12.3	10.5	11.8	0.56
SCC, ×1,000/mL		392	388	287	214	155	428	123.1

[1] DCAD in milliequivalents of ($Na^+ + K^+ - Cl^- - S^=$) per 100 g of DM.

[2] Center point of response surface.

[3] Standard error of the mean; because of missing data, the largest value is presented.

[4] FCM = 0.3246 × (kg of milk) × 12.86 (kg of fat) + 7.04 × (kg of protein); Tyrell and Reid (1965).

Table 3.5. Least squares means of ruminal fermentation parameters for cows fed diets differing in DCAD[1] (37.5, 27.0, 56.2, 42.5, 27.0, and 42.5) and molar ratio of Na:K (0.50, 0.95, 0.50, 0.18, 0.26, and 1.42).

	Treatment						
	DCAD	37.5[2]	27.0	56.2	42.5	27.0	42.5	
Variable	Na:K	0.50[2]	0.95	0.50	0.18	0.26	1.42	SEM[3]
Ruminal pH		6.22	6.14	6.33	6.24	6.19	6.28	0.12
Acetate, m*M*		45.7	60.0	60.6	51.4	54.0	49.5	3.40
Propionate, m*M*		26.3	30.5	24.6	25.6	24.9	26.3	2.72
Butyrate, m*M*		9.5	11.1	9.6	10.3	9.8	10.1	0.71
Total VFA, m*M*		86.4	97.9	91.9	87.1	84.5	82.2	5.1
NH_3, mg/L		6.57	6.49	4.54	5.33	4.23	5.82	0.76

[1] DCAD in milliequivalents of ($Na^+ + K^+ - Cl^- - S^=$) per 100 g of DM.

[2] Center point of response surface.

[3]Standard error of the mean; because of missing data, the largest value is presented.

Table 3.6. Effects on cows fed diets differing in DCAD[1] (37.5, 27.0, 56.2, 42.5, 27.0, and 42.5) and molar ratio of Na:K (0.50, 0.95, 0.50, 0.18, 0.26, and 1.42).

Dependent Variable	Intercept	DCAD	Ratio	DCAD x DCAD	Ratio X Ratio	DCAD x Ratio
Acetate, m*M*	569.7 (272.1)	-309.3 (150.3)	-75.2 (28.4)	46.5 (20.7)	9.2 (2.7)	24.8 (7.8)
Propionate, m*M*	19.0 (14.5)	NS	11.85 (3.44)	NS	8.21 (2.30)	NS
Butyrate, m*M*	9.8 (3.6)	NS	2.97 (0.84)	NS	1.88 (0.56)	NS
Ruminal NH_3, mg/L	-135.5 (43.6)	77.6 (24.2)	0.74 (0.28)	-10.7 (3.3)	NS	NS
Ruminal pH	14.9 (3.8)	-4.38 (2.06)	0.84 (0.42)	0.651 (0.286)	NS	-0.24 (0.11)
Ruminal pH_6h	-2.022 (275.53)	NS	NS	NS	0.84 (0.37)	2.35 (1.11)
Urine pH	8.8 (0.44)	NS	1.56 (0.51)	NS	-1.22 (0.04)	-0.48 (0.13)
MUN, mg of N/dL	-105.2 (53.7)	62.22 (29.7)	NS	NS	NS	NS
pO_2, mmHg	-212.3 (117.6)	129.9 (64.5)	-40.2 (15.1)	-17.1 (8.8)	NS	11.3 (4.2)
$Ca^{2+,}$ mg/dL	13.9 (1.1)	-0.95 (0.3)	3.11 (1.21)	NS	NS	-0.82 (0.33)
Creatinine, mg/dL	6.18 (8.9)	NS	2.77 (1.04)	NS	NS	-0.74 (0.29)

[1]DCAD in milliequivalents of ($Na^+ + K^+ - Cl^- - S^=$) per 100 g of DM.

[2]The full model included linear and quadratic effects of DCAD and Na:K and the 2-way interaction. Reduced models included effects that were significant ($P < 0.05$). Values in parentheses are SE of the coefficient.

[3]NS=Not significant

Table 3.7. Least squares means for jugular venous blood acid-base measures and mineral concentrations for cows fed diets differing in DCAD[1] (37.5, 27.0, 56.2, 42.5, 27.0, and 42.5) and molar ratio of Na:K (0.50, 0.95, 0.50, 0.18, 0.26, and 1.42).

	Treatment						
	DCAD	37.5[2]	27.0	56.2	42.5	27.0	42.5	
Variable	Na:K	0.50[2]	0.95	0.50	0.18	0.26	1.42	SEM[3]
pH		7.415	7.412	7.415	7.407	7.385	7.409	0.009
pCO^2, mmHg		41.3	44.2	42.3	43.8	43.5	44.2	1.6
pO2, mmHg		33.8	33.5	29.3	29.1	33.1	29.5	1.6
HCO_3^-, meq/L		23.8	24.9	24.6	24.6	23.5	25.2	1.2
Base excess, m*M*		2.17	3.72	2.50	3.04	0.81	3.28	1.11
Na^+, mEq/L		137.3	138.1	137.7	137.7	137.6	136.9	1.0
K^+, mEq/L		3.77	3.95	3.89	3.91	3.74	4.02	0.17
Cl^-, mEq/L		100.4	99.5	100.1	100.2	100.3	99.2	0.7
Ca^{2+}, mg/dL		10.2	10.3	10.1	10.2	10.1	10.7	0.2
BCAD[4], mEq/L		40.4	42.7	41.8	41.9	41.1	41.6	0.9
Anion[5] gap, mEq/L		16.9	17.2	17.1	16.2	17.6	16.8	0.5
Urea N, mg/dL		11.4	11.3	11.3	10.7	11.2	11.7	0.6
Creatinine, mg/dL		0.77	0.78	0.78	0.70	0.60	1.01	0.12

[1]DCAD in milliequivalents of ($Na^+ + K^+ - Cl^- - S^=$) per 100 g of DM.
[2]Center point of the response surface.
[3]Standard error of the mean; because of missing data, the largest value is presented .
[4]BCAD = jugular venous blood cation-anion difference (Na + K – Cl).
[5]Anion gap = jugular venous blood $Na^+ - Cl^- - HCO_3^-$.

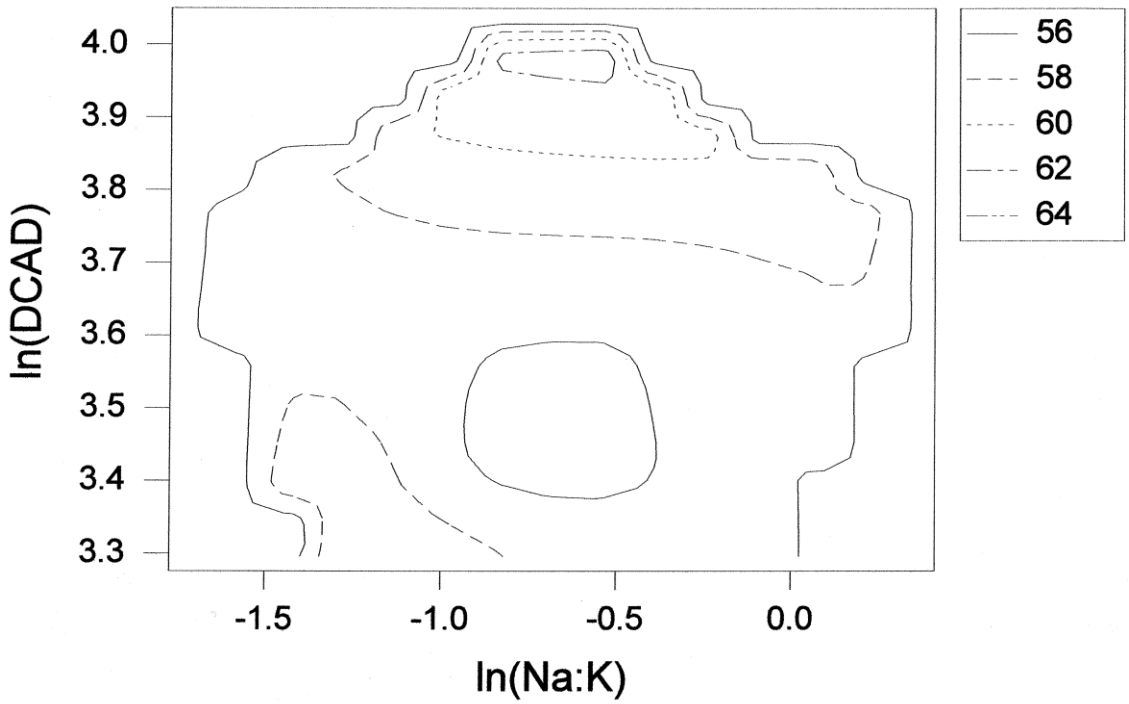

Figure 3.1. The response surface shown is for ruminal acetate concentration in millimoles per liter. The response surface equation is Acetate = 569.7 - 309.3 × DCAD – 75.2 × Ratio + 46.5 × $DCAD^2$ + 9.2 × $Ratio^2$ + 24.8 × DCAD × Ratio.

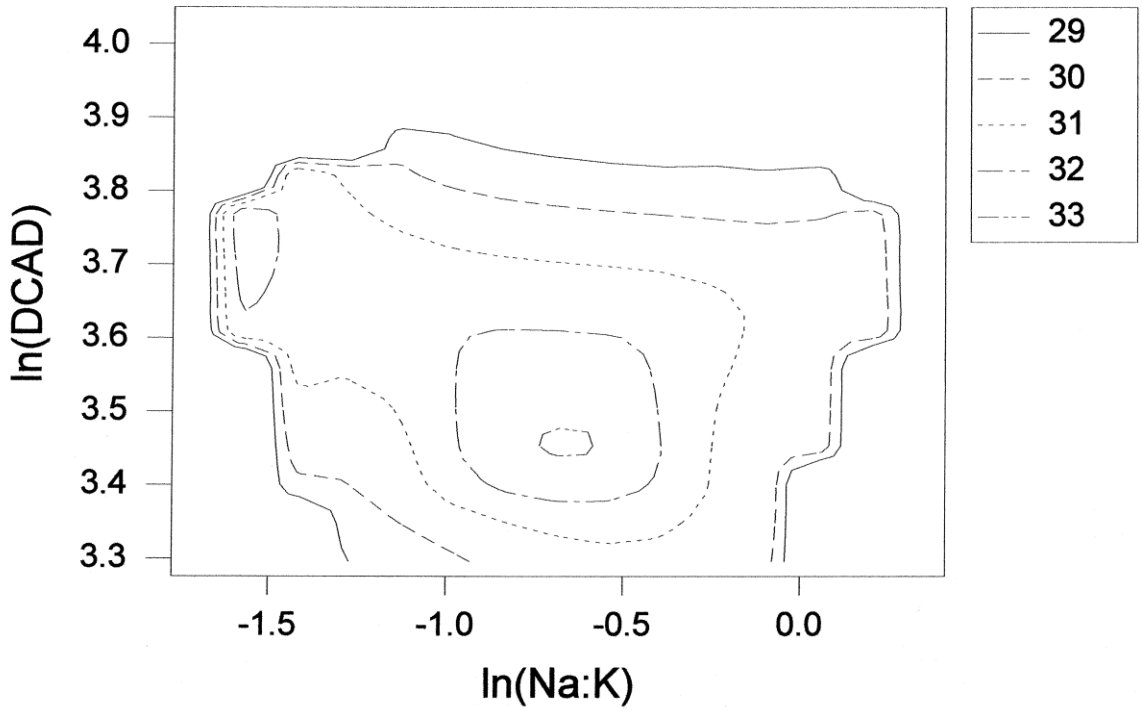

Figure 3.2. The response surface shown is for ruminal propionate concentration in millimoles per liter. The response surface equation is Propionate = 19.0 +11.85 × Ratio + 8.21 × Ratio^2 + 24.8 × DCAD × Ratio.

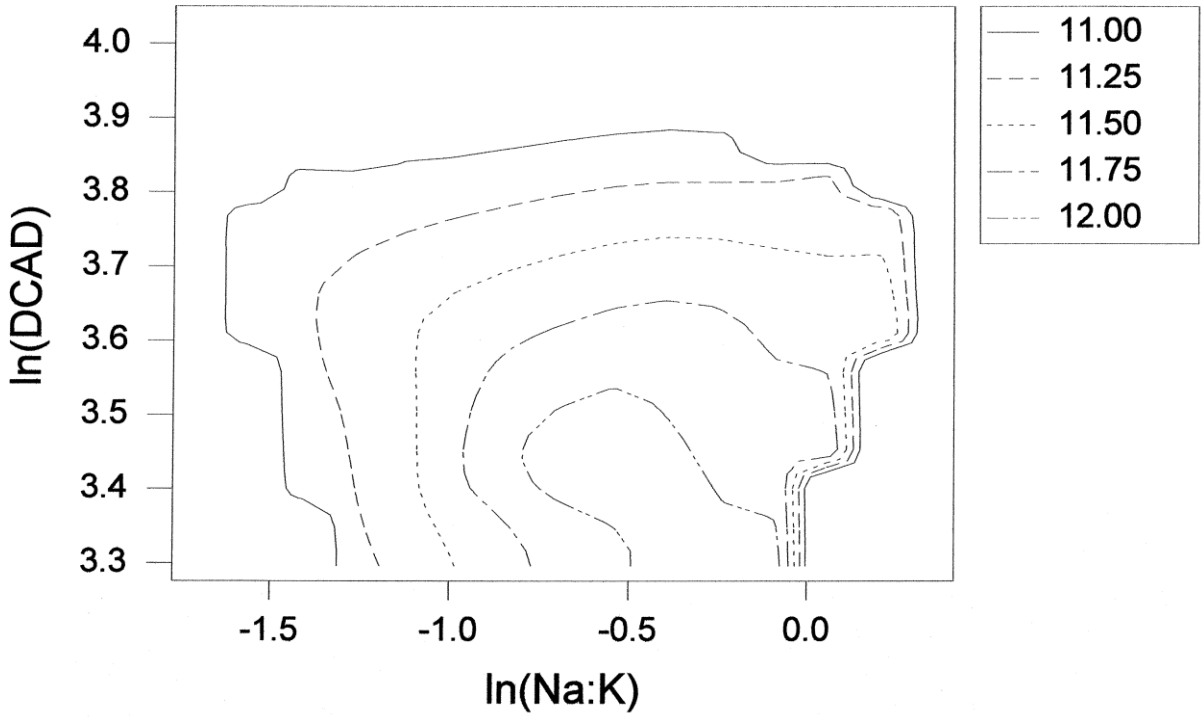

Figure 3.3. The response surface shown for ruminal butyrate concentration in millimoles per liter. The response surface equation is Butyrate = 9.8 + 2.97 × Ratio + 1.88 × Ratio^2.

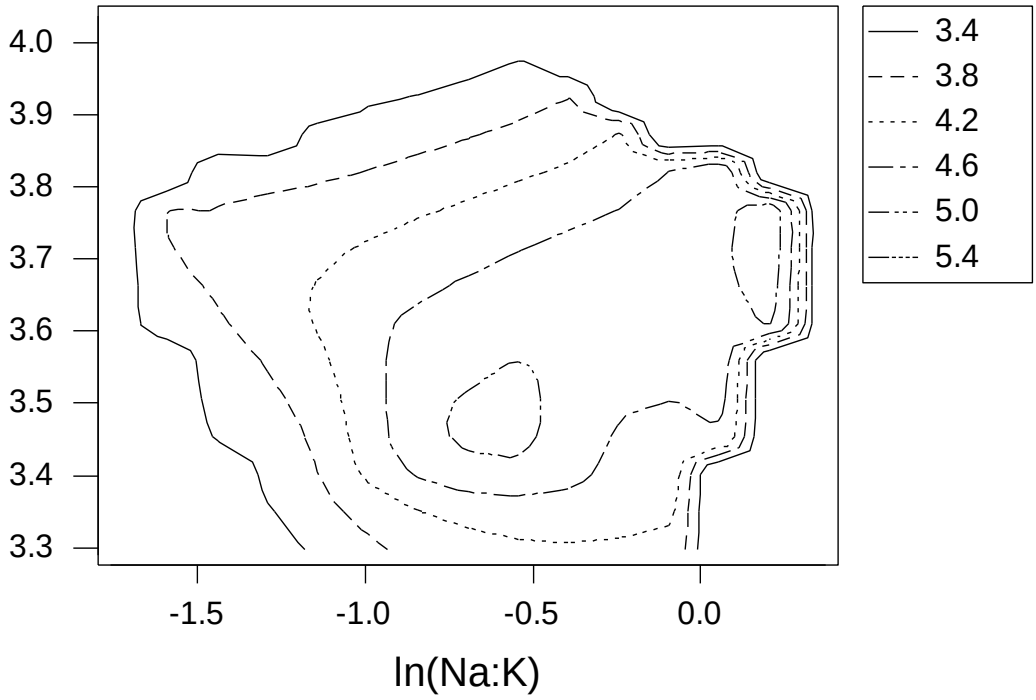

Figure 3.4. The response surface shown is for ruminal NH_3 concentration in millimoles per liter. The response surface equation is $NH_3 = -135.5 + 77.6 \times DCAD + 0.74 \times Ratio - 10.7 \times DCAD^2$.

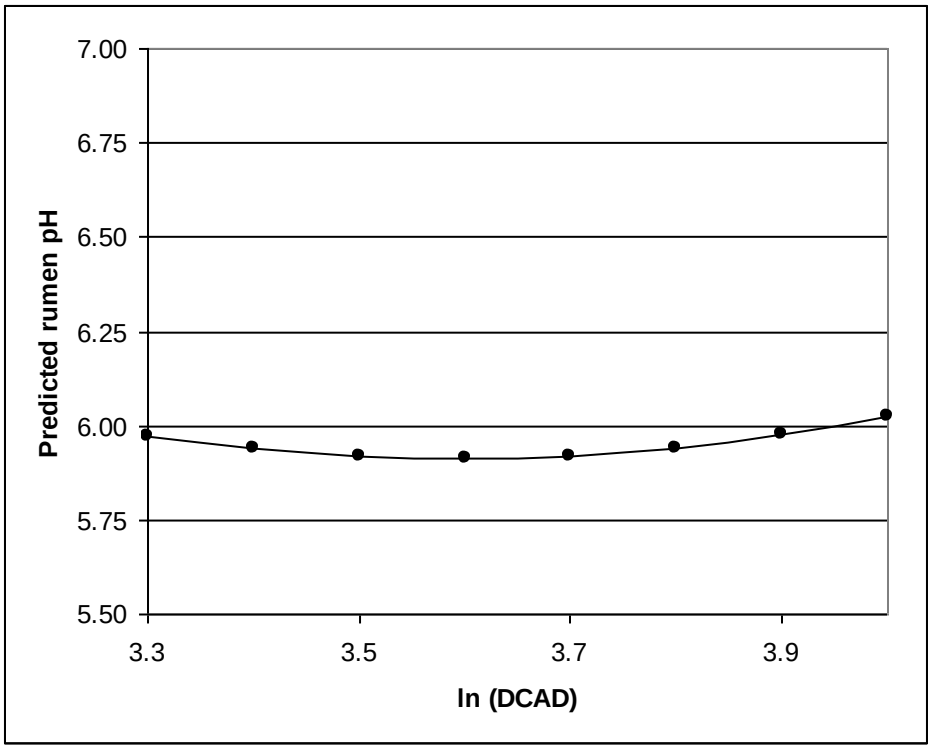

Figure 3.5. The response surface shown is for ruminal pH. The response surface equation is pH = 14.9 – 4.38 × DCAD + 0.84 × Ratio + 0.65 × DCAD + 2.35 × DCAD × Ratio.

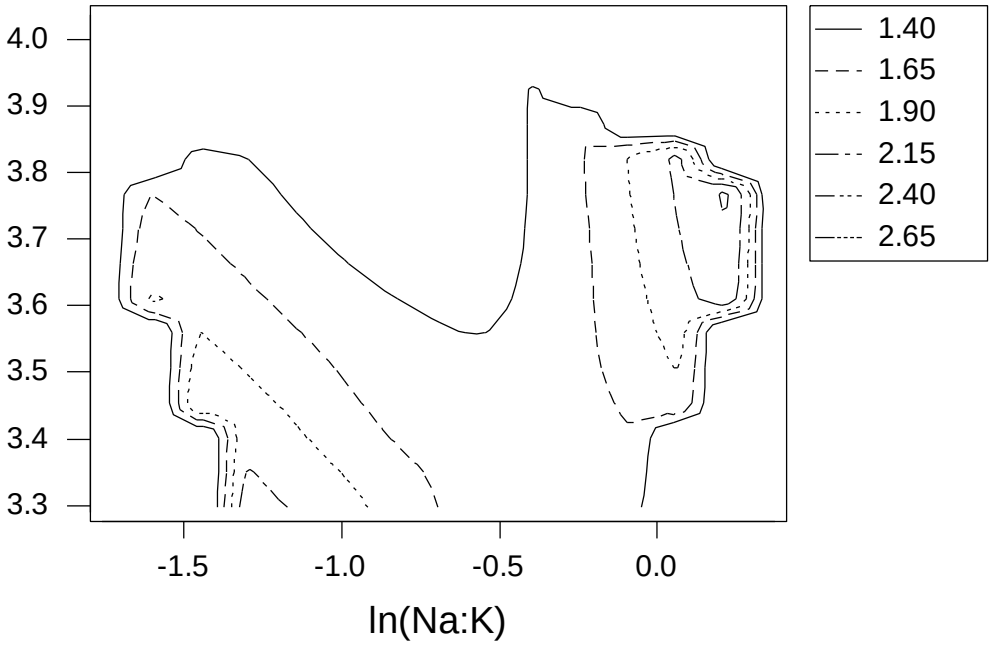

Figure 3.6. The response surface shown is for ruminal pH_6-h (area below pH 6). The response surface equation is $pH_6\text{-h} = -2.022 + 0.84 \times \text{Ratio}^2 + 2.35 \times \text{DCAD} \times \text{Ratio}$.

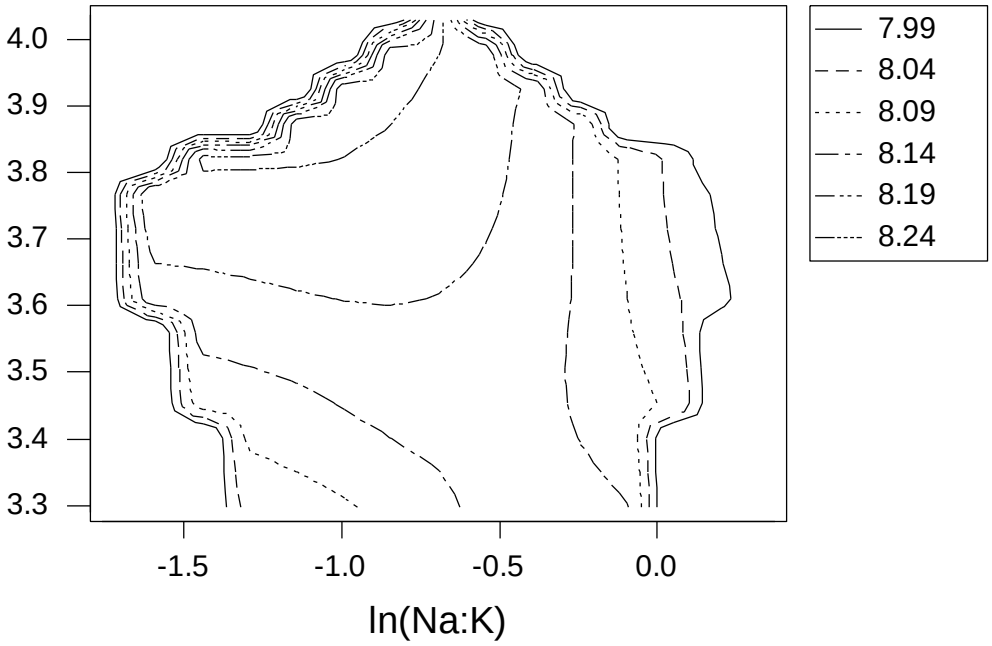

Figure 3.7. The response surface shown is for urine pH. The response surface equation is Urine pH = 8.8 + 2.97 × Ratio - 1.22 × Ratio^2 – 0.48 × DCAD × Ratio.

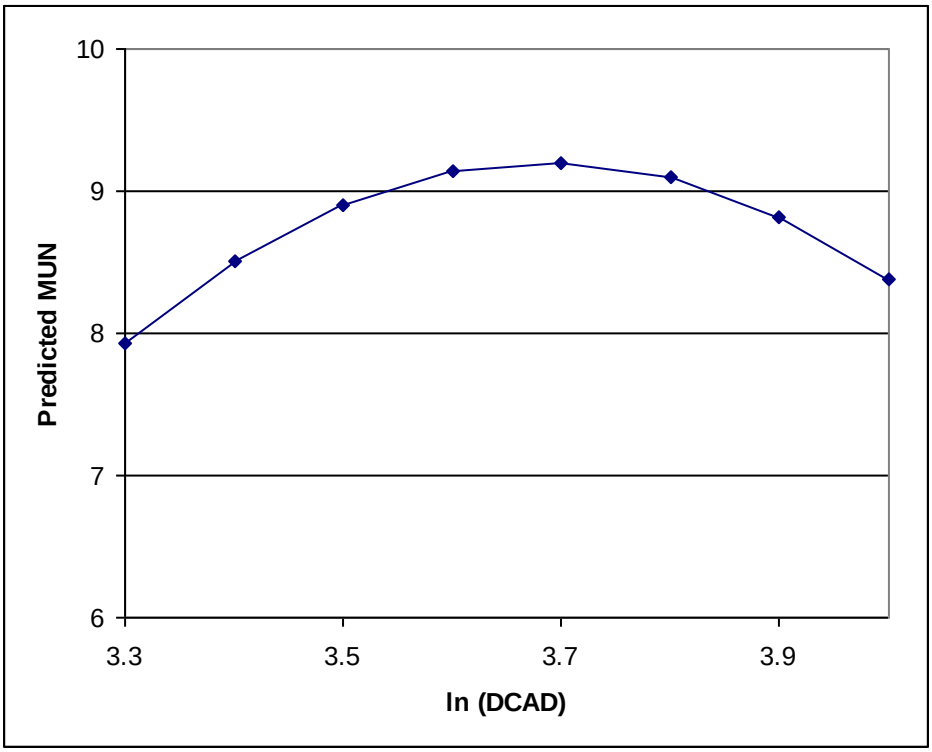

Figure 3.8. The response surface shown is the mean milk urea nitrogen concentration. The response surface equation is MUN = -105.2 + 62.22 × DCAD.

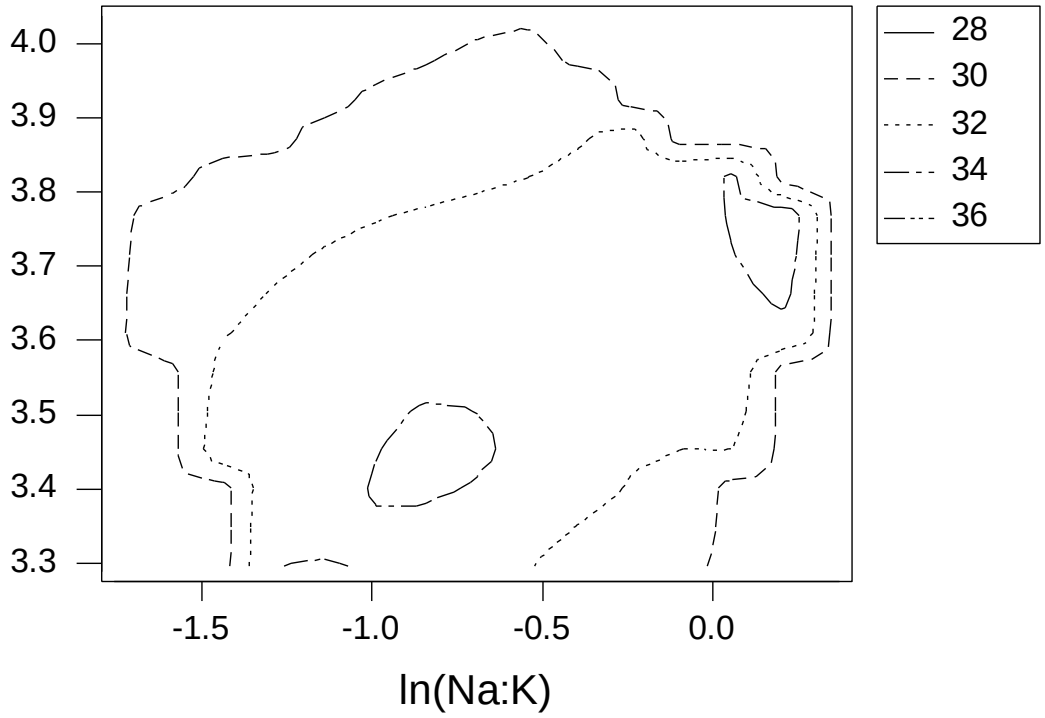

Figure 3.9. The response surface shown is for jugular venous pO_2 in millileters of Hg. The response surface equation is $pO_2 = -212.3 + 129.9 \times DCAD - 40.2 \times Ratio - 17.1 \times DCAD^2 + 11.3 \times DCAD \times Ratio$.

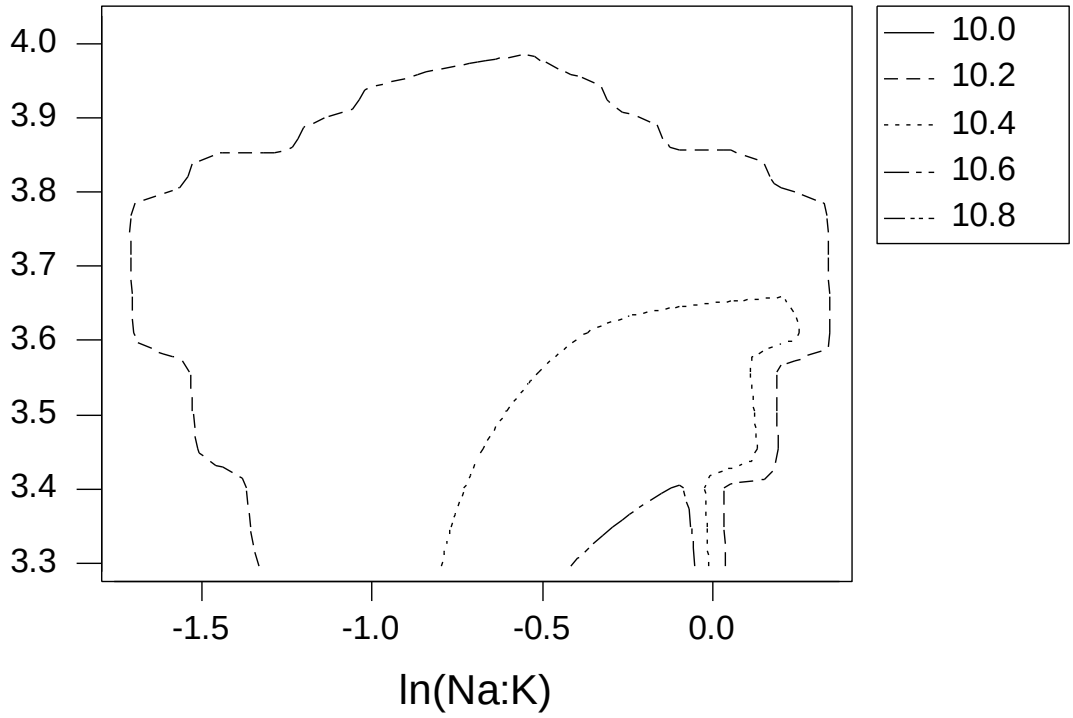

Figure 3.10. The response surface shown is for jugular venous Ca. The response surface equation is Ca in milligrams per deciliter = 13.9 – 0.95 × DCAD + 3.11 × Ratio – 0.82 × DCAD × Ratio.

CHAPTER IV

RELATIONSHIPS BETWEEN RUMINAL pH AND URINARY pH IN LACTATING DAIRY COWS FED DIETS VARYING IN DIETARY CATION-ANION DIFFERENCE AND MOLAR RATIO OF SODIUM TO POTASSIUM

ABSTRACT

Six multiparous Holstein cows, fitted with rumen cannulas, averaging 122 ± 31 days in milk were randomly assigned to six treatments varying in dietary cation-anion difference (DCAD)and molar Na:K allocated in an equiradial (pentagonal) second-order response surface design with a center point to examine potential relationships between ruminal and urinary pH in lactating dairy cows. Replication of treatments within a 6 x 6 Latin square minimized the potential effects of outliers. Diets were based on corn silage and corn-based grain mixes. Periods were 14 d. Daily feed intake of each cow was recorded during each period; samples of feed and orts were collected daily. Milk production was measured daily; samples were collected weekly and analyzed for components. Rumen and urine samples were collected and analyzed for pH on the last 3 d of each period. There was a relationship between pH_6 -h and ruminal pH (r^2= 0.64, $P < 0.001$). The relationship between mean ruminal pH and mean urinary pH explained 15% of the variation ($P < 0.022$), but few data were below pH 6. The relationship between mean urinary pH and mean ruminal pH_6-h explained 28% of the variation ($P < 0.001$). Few published data compare ruminal and urinary pH. A relationship between ruminal and urinary pH was measured. More data are necessary to further elucidate this relationship before making determinations of the presence of SARA.

INTRODUCTION

Ruminal acidosis occurs when diets high in fermentable carbohydrates are fed to ruminants. Traditionally, the definition of subacute rumen acidiosis (**SARA**) is based on rumen pH, determined by various methods (Plaizier et al., 2009). Subacute ruminal acidosis seems to be caused by an increase of total volatile fatty acid (**VFA**) production. There are a limited number of field studies in the US documenting the incidence of SARA. A cross-sectional field study (Garrett et al., 1997) of 15 Holstein dairy herds in Wisconsin detected ruminal pH values <5.5 in 19% of cows between 2 and 30 days in milk (**DIM**) and 26% of cows that were 90 to 120 DIM. In one-third of these herds, >40% of the lactating cows tested had ruminal pH values <5.5. In a case study conducted on a 500-cow NY farm, Stone (1999) calculated the a case of SARA cost $400 to $475 lost income per cow per year. This figure was based on an observed decrease in milk production of 3 kg/cow/d, and decreased milk fat and true protein from 3.7 to 3.4 % and 2.9 to 2.8 %.

Measurement of rumen fluid pH is commonly used for diagnosis of acute acidosis; however, it is time consuming and is not part of routine examinations. Rumen pH values obtained via stomach tube are variable due to saliva contamination, sampling time in relation to feeding, and stomach tube placement in the rumen (Duffield et al., 2004; Enemark et al., 2004). Rumenocentesis is another method of determining ruminal pH. It is generally well accepted by cows. The most used cut off point is ruminal pH of 5.5. According to Garrett et al. (1999), a cow with a pH of less than 5.5 should be considered positive for SARA, and above 5.8 to be negative. Cows with a ruminal pH between 5.5 and 5.8 are considered at risk for developing SARA. Furthermore, Garrett et al. (1999) defined in a group of cows as having SARA when a rumen pH

of 5.5 or lower is found in 4 out of 12 cows. While rumenocentesis is considered to be reliable, it is expensive and relatively invasive.

We hypothesized that, in cows experiencing SARA, a positive relationship could be determined between ruminal and urine pH. Ruminants have a relatively small lung capacity; acid elimination via the kidneys is important. During SARA, renal excretion of H^+ is increased (Enemark, 2008). Positive relationships have been shown between rumen and urine pH (Fürll, 1993; Enemark, 2008). Trained personnel can obtain a urine sample by perineal stimulation, which is not invasive compared to rumenocentesis.

The objectives of this experiment were investigate the relationship between ruminal and urinary pH, and to describe a potential non-invasive method of diagnosing SARA using urinary pH.

MATERIALS AND METHODS

Design and Diets. This experiment was completed concurrently with the experiment discussed in Chapter 3. Briefly, six treatments were allocated in an equiradial (pentagonal) second-order response surface design with a center point. Six multiparous cows (122±30 DIM) were randomly assigned to 1 of 6 treatments varying in DCAD and molar Na:K ratio. Periods were 14 d in length. Cows were fed a corn silage and corn grain-based diet which can be found in Chapter 3 (Table 3.1).

Each cow was fitted with a rumen cannula. They were housed in tie stalls indoors except during milking and during an exercise period on a dirt lot between a.m. milking and feeding. Feed offered was adjusted daily and 110% of consumption the previous day (as-fed basis) was provided at 1100 and 1630 h. Water was available for ad libitum consumption. Cows were milked twice daily at approximately 0600 and 1500 h.

Rumen and Urine. Kinetics of ruminal pH and urine content were monitored every 4 h on the last 3 d of each period, arranged to provide resulting in a sample for each hour of the day. Rumen fluid (200 ml) was sampled from several locations in the rumen via the rumen cannula, using a suction pump and a rigid plastic tube. At each sampling time, pH was measured immediately. Samples were strained through 6 layers of cheesecloth and centrifuged at 12,000 x *g* for 15 min. Aliquots were then acidified to pH 2 with 50% sulfuric acid and frozen at -20°C for later analysis. At each time a rumen sample was obtained, urination was induced by perineal stimulation. A 50-ml sample of midstream urine was taken for immediate pH determination.

Statistics. Data were analyzed by regression analysis with the REG procedure in SAS (SAS, 2008).

RESULTS AND DISCUSSION

Mean ruminal pH for the entire experiment is shown in Figure 4.1. The shaded area indicates rumen pH_6-h. Mackie and Gilchrist (1979) suggested that time and extent to which rumen pH remained below a certain critical pH is an important determinant of bacterial growth rate. Further studies have shown cellulolytic bacteria are especially sensitive to pH below 6 (Russell and Dombrowski, 1980; Erfle et al., 1982). In addition to negatively impacting cellulolytic bacterial growth, as ruminal pH drops below 6 protonated VFA are absorbed more rapidly across the rumen wall (Gäbel et al., 1989). Based on this information and the guidelines of Garrett et al. (1999), cows in the present study did not experience SARA.

Figure 4.2 illustrates the relationship between pH_6-h and mean ruminal pH (r^2= 0.64, P < 0.001). The relationship between mean ruminal pH and mean urinary pH is shown in Figure 4.3. Only 15% (P < 0.02) of the variation was explained, but few data were below pH 6. Figure 4.4

shows the relationship between mean ruminal pH_6-h. urinary pH. In this case, 28% of the variation was explained ($P < 0.001$). Few published data on urinary pH and rumen pH_6-h compare ruminal and urinary pH.

In the literature, there are mixed results regarding the use of urine pH as an indicator of rumen pH. Enemark (2008) reported in a review that in an unpublished study, he found a correlation of $r = 0.28$ (n = 323, $P < 0.01$) between rumen and urine pH. In contrast, Kricziokat et al. (2009) found a weaker relationship between rumen and urinary pH ($r = 0.19$, $P < 0.001$). However, the latter used stomach tubes on 348 heifers and cows to collect rumen samples and the experiment was under field conditions. Workers in Iran (Tajik et al., 2009) collected ruminal fluid from 32 cows from 10 dairies by rumenocentesis and also collected urine samples from the same cows. Unfortunately, only *P*-values are reported and it is not clear how comparisons were made.

CONCLUSION

In conclusion, there appears to be a relationship between ruminal and urinary pH. Rumen pH_6-h had a stronger relationship to urinary pH than ruminal pH. However, more data, especially from cows experiencing SARA are needed to develop diagnostic criteria for SARA.

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FIGURES

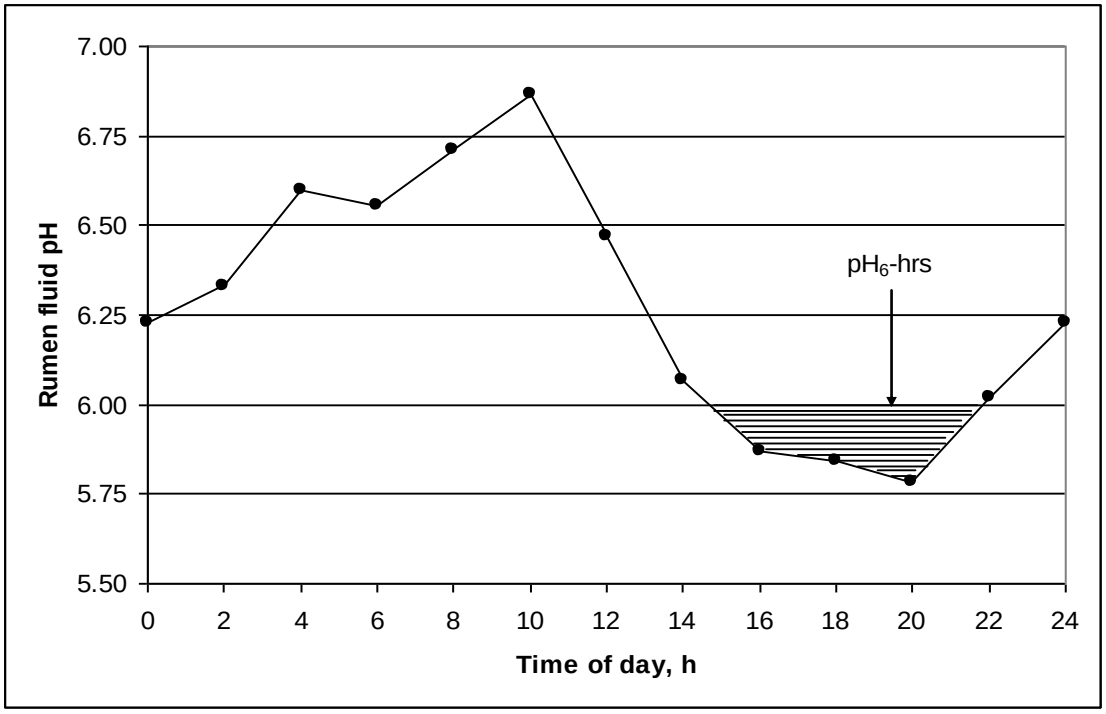

Figure 4.1. Mean ruminal pH over time for the experiment illustrating pH6-h.

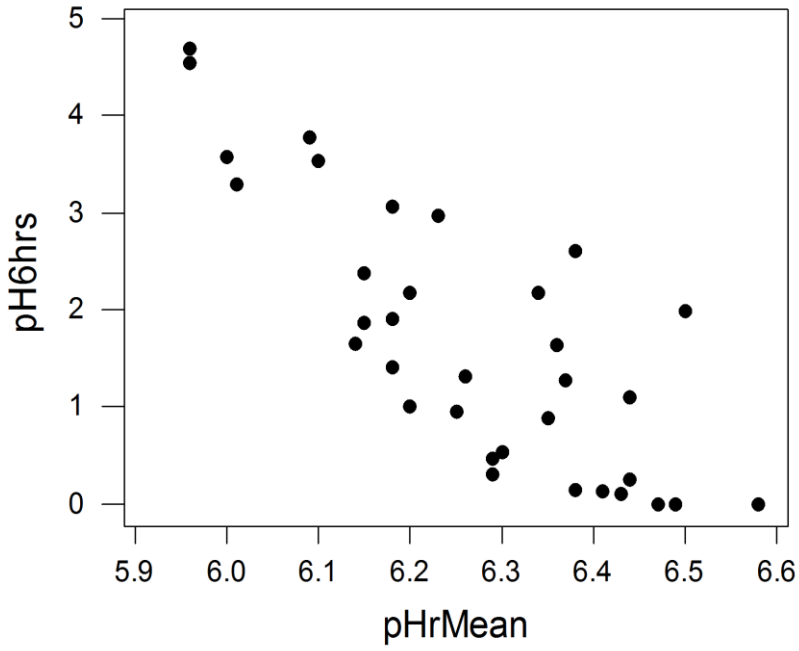

Figure 4.2. pH6-h = 44.0 - 6.75 mean ruminal pH; r^2= 0.64 $P < 0.001$.

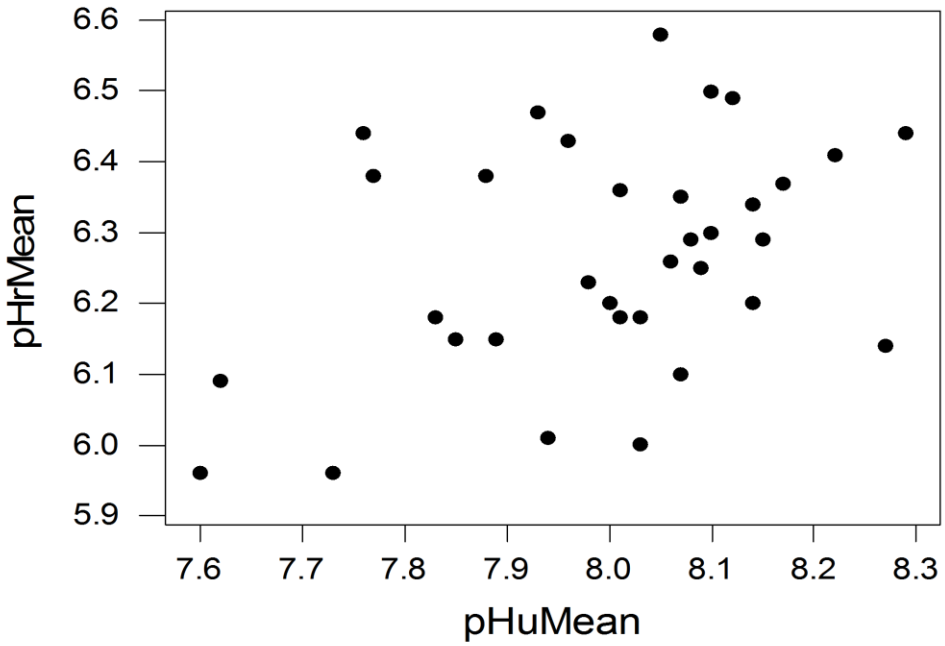

Figure 4.3. Mean ruminal pH = 3.25 + 0.377 mean urinary pH; r^2= 0.15, $P < 0.02$.

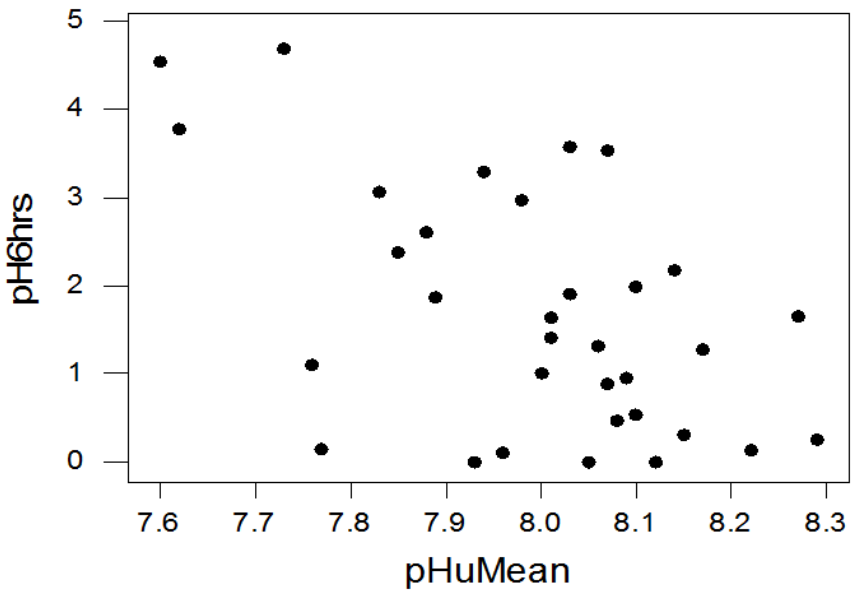

Figure 4.4. Ruminal pH6-h = 35.7 – 4.25 mean urinary pH; r^2= 0.28; *P* < 0.001.