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BEHAVIORAL AND PHYSIOLOGICAL ADAPTATIONS ASSOCIATED WITH FEED INTAKE DURING TRANSITIONING CATTLE TO HIGH-GRAIN DIETS

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

By
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ABSTRACT OF DISSERTATION

BEHAVIORAL AND PHYSIOLOGICAL ADAPTATIONS ASSOCIATED WITH FEED INTAKE DURING TRANSITIONING CATTLE TO HIGH-GRAIN DIETS

Transitioning cattle from a high-forage to a high-concentrate diet increases the risk for ruminal acidosis and is often related to decreased feed intake, which compromises animal health and performance. Since control of feed intake and rumen motility are closely related, we hypothesized that a reduction in rumen motility may be associated with a reduction in feed intake during this transition. Computer programs were created to analyze feed disappearance and rumen pressure data for feeding behavior as well as identification and characterization of rumen contractions, respectively. This method enabled timely analysis of large datasets and removed subjectivity associated with manual analysis. In the second part of this series, cattle were moderately transitioned from a 70% to a 90% concentrate diet, and SARA was induced. Although, reductions in feed intake were modest, on day 2 of high-grain feeding, animals slowed feed consumption rate and displayed a reduction in rumen contraction frequency, amplitude, and duration. Next, an abrupt transition from 50% to 90% concentrate was used to induce ruminal acidosis and cause some animals to stop eating. The abrupt increase in dietary concentrate was also associated with reductions in rumen motility. Patterns of ruminal pH, viscosity, and motility changes were related to when cattle reduced feed intake. Endotoxin quantification in blood samples from the ruminal vein, portal vein, and mesenteric artery suggested the point of endotoxin translocation into blood was across the ruminal epithelium. Additionally, the greater the concentration of endotoxin in the plasma, the more likely animals were to go “off-feed.” By understanding the physiological and behavioral mechanisms by which cattle adapt to high-grain diets, we can improve animal health and performance through these diet transitions.

KEYWORDS: acidosis, feeding behavior, motility, rumen environment lipopolysaccharide

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June 21st, 2019

Date

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FREQUENTLY USED ABBREVIATIONS

ADF	Acid detergent fiber
APP	Acute phase proteins
APR	Acute phase response
AR	Acidosis-resistant
AS	Acidosis-susceptible
ATP	Adenosine triphosphate
BW	Body weight
CP	Crude protein
d or D	Day
DM	Dry matter
DMI	Dry matter intake
GIT	Gastrointestinal tract
LPS	Lipopolysaccharide
MA	Mesenteric artery
min	Minute
NDF	Neutral detergent fiber
PV	Portal vein
RpH	Ruminal pH
RV	Ruminal vein
SARA	Subacute ruminal acidosis
SSE	Stratified squamous epithelium
Trum	Ruminal temperature
VFA	Volatile fatty acid

CHAPTER 1: INTRODUCTION

Beef cattle are commonly finished on high-grain or high-concentrate diets in order to maximize energy intake and growth performance. However, diets of receiving cattle prior to feedlot entry are typically forage-based. Therefore, the microbial population is not accustomed to using starches and other rapidly fermentable carbohydrates contained in these high-grain diets. The transition period when cattle are switched from these high-forage to high-grain diets is a critical time in beef cattle husbandry which can lead to several disorders that negatively impact gastrointestinal function and health, such as ruminal acidosis.

Still a common concern in feedlots today despite many prevention strategies, ruminal acidosis, or a depression in ruminal pH, affects animal physiology, behavior, and health. Importantly, animals typically reduce feed intake or display erratic feeding behavior during adaptation to a high-grain diets when experiencing ruminal acidosis, yet the cause of this change in intake and timing of changes are still not fully understood. Additionally, ruminal pH depression alone may not be enough to reduce feed intake or make cattle go “off-feed.” A variety of factors have been suggested to be involved with regulation of feed intake during these dietary transitions such as chemical receptors, reticulorumen motility, energy status, and/or inflammatory responses.

Reticulorumen motility could impact feed intake by altering passage rate. Feed intake regulation and the control of rumen motility appear to have many overlapping mechanisms as well. While reductions in forestomach contraction amplitude and frequency have been noted for animals experiencing severe ruminal acidosis, the effect of chronic ruminal acidosis on reticulorumen motility has yet to be determined.

Bacterial endotoxin, or lipopolysaccharide, in the blood activates an inflammatory response, which could reduce feed intake. The rumen is host for many species of bacteria that have endotoxin as part of their cell wall and concentrations of lipopolysaccharide increase in the rumen when cattle are fed high-grain diets or experience ruminal acidosis. Reduced epithelial barrier function during acidosis may allow endotoxin to translocate across the epithelium and enter the blood, but translocation of endotoxin across the ruminal epithelium, specifically, is contentious.

The goal of this dissertation was to examine the behavior and physiological adaptations that occur when cattle are transitioned to high-grain diets, with particular emphasis on association of these adaptations with changes in feed intake or feeding behavior. We sought to develop a systematic and automated way to evaluate feeding behavior and forestomach motility. Then, these methods were used to determine if reticulorumen motility of cattle was altered during typical dietary transitions and when cattle go “off-feed,” if endotoxin was detectable in ruminal vein blood during these challenges, and if changes were associated with feed intake modifications.

CHAPTER 2: LITERATURE REVIEW

Introduction

Ruminants rely on a symbiotic relationship with rumen microbiota for the ability to survive on structural carbohydrate molecules found in forages. Mammalian enzymes cannot digest structural carbohydrates (e.g. cellulose), but mammals are able to use products from the microbial digestion or fermentation of these compounds. The balance of this rumen ecosystem and the microbiome is important for the nutrition and health of the animal. However, when animals experience a nutritional stress, such as a rapidly fermentable, nonstructural carbohydrate challenge leading to ruminal acidosis, there is a disruption of the rumen microbiome and whole animal homeostasis. Consequently, the cascade of events that occurs following feeding high-grain diets to cattle ultimately influences reticulorumen fermentation and motility, blood acid-base balance, epithelial structure and function, local and systemic inflammation, and feeding behavior.

High-Grain Diets

Beef cattle, dairy steers, sheep, and goats are commonly fattened, or finished, on diets composed mostly of starch or other rapidly fermentable carbohydrates from ingredients, such as cereal grains, to maximize energy intake and improve performance. Researchers, nutritionists, and producers refer to these types of diets as high-grain or high-concentrate diets. A survey of twenty-nine consulting nutritionists in major cattle feeding states of the U.S. found that typical feedlot diets contained between 87 and 100% concentrate on a DM basis, with the average amount of roughage fed for warm and cold seasons at 8.3% and 9%, respectively (Vasconcelos and Galyean, 2007).

Problems Associated with Feeding High-Grain Diets

When cattle or other ruminants consume diets rich in rapidly fermentable carbohydrates, they may experience several disorders including ruminal acidosis, liver abscesses, bloat, laminitis, fatty liver, and displaced abomasum (Nocek, 1997; Andersen, 2003; Ametaj et al., 2005). These disorders negatively influence gastrointestinal function, growth performance, and animal health and welfare, as well as economic profitability (Plaizier et al., 2009). For the purpose of this review, our efforts will focus on problems related to feeding high-grain diets and resulting ruminal acidosis.

Diet Adaptation Methods

Several diet adaptation protocols have been used by the U.S. cattle feedlot industry as cattle are switched from a high-forage to a high-concentrate diet. The diet adaptation period, when transitioning feedlot cattle from a receiving to a finishing diet, is a crucial time where nutritional management can impair or enhance future animal health and performance (Brown et al., 2006). Switching cattle too abruptly to a high-concentrate diet can lead to metabolic disorders, such as bloat and ruminal acidosis, which could negatively influence performance or cause death. However, it is advantageous to get cattle consuming a high-concentrate diet as soon as possible to increase DM and energy intake, ADG, and gain efficiency (Bevans et al., 2005). Thus, it is important that extra care be taken to balance the benefits in gain and efficiency on high-concentrate diets with the increased risk of acidosis if cattle are switched to finishing diets too rapidly.

The goal of the transition period during diet adaptation is for the ruminal microbial populations to adjust and become accustomed to using the rapidly fermentable carbohydrates available in cereal grains without causing health problems (Brown et al.,

2006). Producers want to enhance growth performance without causing ruminal acidosis (Bevans et al., 2005). Since diets of growing or receiving cattle are usually forage-based prior to arrival at the feedlot, the microbial population is not prepared for effective use of starches and other rapidly fermentable carbohydrates. Generally, diet transition models refer to rapid or gradual adaptation protocols. An example of a rapid adaptation protocol would be transitioning from 40 to 90% concentrate in 3 d with one 65% concentrate diet between these levels (Bevans et al., 2005). One method of gradual adaptation involves the use of 2 to 5 step-up diets with intermediate levels of grain, fed on average for 7 d before increasing to the next concentrate level (Vasconcelos and Galyean, 2007). This multiple step-up diet approach was used by about three-fourths of the nutritionists evaluated in 2007. Another method called two-ration blending entails feeding varying proportions of a high- and low-concentrate diet over a period of time. Additionally, a combination of these methods, restricted intake of the final diet, or other methods may be used. Restricted intake of the final finishing diet during diet adaptation of 6 to 9 days resulted in greater DMI variation and lower ADG compared to cattle adapted using a step-up protocol (Perdigao et al., 2017). Most consulting nutritionists from the U.S. recommended using a 21-d adaptation period, regardless of method (Vasconcelos and Galyean, 2007).

Acidosis of the Rumen

Ruminal acidosis remains a common production issue in the beef and dairy industries due to today's energy-intensive feeding protocols (Penner et al., 2010). When fermentation acid is produced at a rate greater than the rate at which acid is buffered and removed from the rumen (via passage and absorption), ruminal acidosis can occur (Allen,

1997). Ruminal acidosis generally refers to a depression in ruminal pH. The level of ruminal acidosis can vary based on the conditions under which the scenario was created and the animal's ability to handle the challenge.

Classification by Severity of Acidosis

The definition of clinical or acute ruminal acidosis varies by publication. The syndrome also used to be called grain engorgement. Generally, a ruminal pH $\leq$ 5.0 - 5.2 and ruminal total lactate between 40 and 90 m*M* or lactic acid between 50 and 150 m*M* are used to diagnose acute ruminal acidosis (Hibbard et al., 1995; Nocek, 1997; Owens et al., 1998; Nagaraja and Titgemeyer, 2007). Animals experiencing acute ruminal acidosis display an obvious illness (Owens et al., 1998). Sheep experiencing acute ruminal acidosis showed decreased plasma or serum Ca, K, and Mg after a carbohydrate challenge (Irwin et al., 1979; Patra et al., 1993). This may be due to increased excretion of minerals in the urine (Harmon and Britton, 1983).

Similar to acute ruminal acidosis, the definition of subacute or subclinical ruminal acidosis (commonly abbreviated as SARA) also varies in the literature, but it generally refers to a condition where there is a reversible pH depression. A ruminal pH below 5.5 (Hibbard et al., 1995), 5.6 (Cooper et al., 1997), 5.8 (Beauchemin et al., 2001), and 6.0 (Krehbiel et al., 1995) have been used as the upper threshold to diagnose SARA. Ruminal pH changes throughout the feeding cycle in a cyclical pattern as VFA's and other fermentation products are produced, absorbed, buffered, and passed. Thus, an animal may have several bouts of low ruminal pH that would be considered subacute. The longer the period of low pH, the more concern for negative effects. There has been research describing the minimum duration that ruminal pH must remain below a threshold to

induce SARA. One study used approximately three hours or more per day below 5.6 to diagnose SARA (Gozho et al., 2005). Conversely, a ruminal pH less than 5.8 for 111 min was considered a mild bout of SARA (Penner et al., 2010). Animals experiencing SARA may not appear to be sick, but may display reductions in feed intake and performance (Owens et al., 1998).

Identification of Acidosis in Practice

The occurrence of SARA is more common than acute acidosis in the feedlot and dairy cattle industries. Also, SARA is a greater issue than acute acidosis on an economic basis (Reid et al., 1957; Dirksen, 1970; Koers et al., 1976). Widespread use of ionophores and adequate adaptation practices have reduced the prevalence of acute acidosis. Detection of acute ruminal acidosis should be noticeable with regular animal checks, yet SARA may go undetected in the feedlot or on the farm because animals do not display obvious signs of illness. Monitoring of ruminal pH to detect animals experiencing or at risk of SARA would be labor intensive, if using rumenocentesis, or require expensive equipment (Danscher et al., 2015). Sampling one time point with rumenocentsis does not provide information about the time ruminal pH was reduced either. Furthermore, ruminal pH depression alone might not always lead to expression of clinical signs of SARA (Khafipour et al., 2009a). To help detect problem animals, researchers have attempted to find alternative variables that could serve as predictors of ruminal pH or serve as inexpensive indicators of SARA.

Data modeling of responses to a carbohydrate challenge suggested serum amylase activity, cholesterol and potassium concentrations, and plasma non-esterified fatty acid concentrations could be useful in distinguishing between steers experiencing SARA or

those not affected (Brown et al., 2000). Likewise, canonical discriminant analysis showed plasma hemoglobin, mean platelet volume, β-hydroxybutyrate, glucose, and reduced hemoglobin were useful for identifying crossbred heifers that were normal, at risk of acidosis, experiencing SARA, or experiencing acute ruminal acidosis (Marchesini et al., 2013). Note that these are not the typical variables (ruminal pH and DMI) used to classify acute or subacute ruminal acidosis.

Methods for Experimentally Induced Acidosis

Research conducted to investigate ruminal acidosis has typically used ruminally cannulated animals to enable strict surveillance of ruminal pH (Nagaraja and Titgemeyer, 2007). Cattle were often fasted before the acidosis challenge in experimentally induced acute and subacute acidosis models to provoke the animals to consume the diet rapidly. Commonly, animals would either be intraruminally dosed with fermentable carbohydrates (examples include various types of processed corn or wheat) or rapidly switched to a high-concentrate diet and allowed to consume *ad libitum* (Nagaraja and Titgemeyer, 2007). Diets that animals were previously adapted to before the carbohydrate challenge has varied from grass hay (Brown et al., 2000) or alfalfa (Harmon et al., 1985) to 90% concentrate (Montaño et al., 1999), depending on the goal of the experiment and severity of response desired. Similar to intraruminal dosing, an oral glucose drench at varying doses per kg of BW was used for induction of different severities of ruminal acidosis (Krehbiel et al., 1995; Penner et al., 2010). Furthermore, studies have employed various methods to induce acidosis by voluntary animal consumption. For example, changing the forage to concentrate ratio from a more forage-based to a more grain-based diet can induce acidosis (Penner et al., 2009b). A rapid grain adaptation (Steele et al.,

2009), as opposed to gradual adaptation, may lead to some cattle developing acidosis. Also, acidosis can be created by short-term feed restriction followed by feeding a high-grain diet (Dohme et al., 2008). However, these two methods of carbohydrate loading, intraruminal dosing (a form of forced animal consumption) and animal consumption, seem to differ in outcomes of development of acidosis. This result may relate to eating rate or buffering from saliva generated with mastication (Nagaraja and Titgemeyer, 2007).

Impact on the Ruminal Environment

Rumen Microbiome

Ruminal microbes ferment dietary carbohydrates to organic acids to generate adenosine triphosphate (ATP) for growth. Intake of energy-dense feed provides ample substrates for fermentation by ruminal microbes that supports rapid growth. For example, shelled corn is approximately 70% starch (DM basis). Microbes are able to quickly break the starch down into glucose units and ferment the glucose to acetate, propionate, butyrate, lactate, methane, and carbon dioxide. Energy, in the form of ATP, generated from the fermentation process facilitates microbial growth. Fiber or cellulose digestion is more complicated and takes the microbes longer to break down, resulting in energy produced at a slower rate and slower growth rates.

During the development of acidosis or as animals are switched to a high-grain diet, the ruminal environment changes dramatically. Changes to microbiota due to excess grain or glucose in the rumen were first described by the father of rumen microbiology, Robert Hungate, and these observations still hold true today (Hungate et al., 1952). Major alterations reported were decreases in number of cellulolytic bacteria, death of protozoa,

and increases in number of gram-positive bacteria. Counts of gram-negative bacteria (GNB) have also been shown to increase with SARA (Zebeli and Metzler-Zebeli, 2012). In general, viable anaerobic bacteria replicate rapidly during high-grain feeding and the onset of ruminal acidosis, which leads to increased numbers (Goad et al., 1998; Nagaraja and Titgemeyer, 2007). While the number of gram-positive and GNB both increase with acidosis, the proportion of gram-negative bacteria decreases. This result is likely due to the differences in structure of the cell wall between these classes of bacteria. Gram-positive bacteria possess a peptidoglycan cell wall as the outer barrier to the environment, which helps it adapt to osmotic and acid stress. Conversely, GNB have inner and outer cell membranes around the cell wall that make these bacteria more chemically resistant due to an additional selectivity barrier. Therefore, GNB are more sensitive to low ruminal pH conditions compared to gram-positive bacteria.

Not only does the number of bacteria increase during acidosis, but the populations and community dynamics are altered. *Streptococcus bovis*, a lactic acid producer, and *Megasphaera elsdenii*, *Selenomonas ruminantium*, and *Prevotella bryantii*, lactate-utilizers, significantly increased as beef steers were adapted from a 20% to 80% grain diet (Fernando et al., 2010). Conversely, two key celluloytic or fiber-digesting bacteria, *Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes*, gradually decreased in the rumen of these animals during adaptation. Cellulolytic bacteria are typically sensitive to low pH, so they die off during acidosis and ruminal fiber-digestion decreases. Thresholds of ruminal pH that indicated SARA and reduced fiber degradation were similar for dairy cows. Using meta-analysis, researchers determined a ruminal pH below 5.8 for more than 5.2 hours/day indicated SARA (Zebeli et al., 2008), while ruminal pH

below 5.8 for more than 5.0 h/d indicated a reduction in fiber degradation (Zebeli et al., 2010b). Therefore, the ability of ruminal microorganisms to ferment and degrade fiber decreases and the amount of lactate produced increases.

Rumen lactate-producers and virulent bacteria outnumber and overpower the lactate-utilizers as the level of acidosis moves from subacute to acute. During severe grain-induced SARA, the rumen was dominated by *S. bovis* and *Escherichia coli* (Khafipour et al., 2009c). The dominate species in mild grain-induced SARA was *M. elsdenii*. Conversely, *Prevotella albensis* was the main species in SARA induced with alfalfa pellets. Dairy cows experiencing SARA had greater numbers of *E. coli* with virulence genes in rumen fluid than cows not experiencing SARA (Khafipour et al., 2011). Additionally, the abundance of *E. coli* was highly correlated with severity of SARA and degree of inflammation in cattle (Khafipour et al., 2009c).

Fermentation of Substrates

In a healthy rumen, the amount of lactate production is balanced with lactate utilization. Therefore, lactate does not accumulate. Lactic acid has a lower pKa than the VFA, so it has a stronger negative effect on ruminal pH. As the ruminal pH decreases and acidosis develops, the ruminal conditions become more ideal for lactate-producers and lactic acid production increases (Kezar and Church, 1979). When there is a corresponding decrease in lactic acid utilization, lactate may accumulate in the rumen. Significant lactate accumulation is not typically observed during SARA, yet it does occur with acute acidosis. Ruminal acidosis used to be called lactic acidosis (Hungate et al., 1952; Dunlop and Hammond, 1965) as researchers believed lactic acid was the causative agent.

Although lactate and VFA are the main products generated during fermentation, other metabolites are also generated during acidosis. Several less common metabolites, such as methylated amines, ethanol, and *N*-nitrosodimethylamine, are produced during periods of stress like feeding high-concentrate diets (Ametaj et al., 2010). These metabolites will be produced in smaller quantities than the typical fermentation products. Pathogenic bacteria may be able to use these metabolites and trigger an inflammatory response (Zebeli and Metzler-Zebeli, 2012), which could result in part of the symptoms of ruminal acidosis.

Ruminal pH

Ruminal pH fluctuates cyclically throughout the feeding cycle creating peak(s) and nadir(s) depending on frequency of feeding. The average ruminal pH of a forage-fed animal is around 6.8 with a range of 6.0 to 7.5. As the proportion of concentrate increases in the diet, the average ruminal pH tends to decrease. Grain-fed cattle typically exhibit a range of ruminal pH between 5.6 and 6.5. However, long periods of low pH can lead to problems for digestion and the animal.

From the definitions, you can see that acidosis is classified by the level of ruminal pH decline. The buildup of lactic acid accounts for the extremely low ruminal pH in acutely acidotic animals. Conversely, ruminal pH reduction during SARA is due to the accumulation of VFA. Lactic acid does not accumulate during SARA because lactate-utilizing bacteria are still active (Goad et al., 1998). Nonpregnant, nonlactating dairy cows fed a high-grain diet (65% grain) displayed a depression of mean, minimum, and maximum daily ruminal pH compared to a period when consuming chopped hay, and cows experienced SARA in the first week of high-grain feeding (Steele et al., 2011a).

Ruminal Osmolality

Osmolality of the ruminal fluid also oscillates in a cyclic manner with changes of pH and concentrations of VFA due to feeding regime (Allen, 1997). Rumen fluid tends to be hypotonic compared to blood (< 280 mosmol/kg) prior to feeding (Warner and Stacy, 1965). After feeding, ruminal osmolality increases with maximum values around 400 mosmol/kg (Warner and Stacy, 1965; Bennink et al., 1978). Researchers found a linear correlation between VFA concentrations and osmotic pressure of rumen fluid (Bennink et al., 1978). Therefore, as the concentration of VFA increases with high-grain feeding and acidosis, ruminal osmolality increases. Osmolality of the rumen changes to a greater extent with acidosis compared to normal conditions than does ruminal pH or hydrogen ion concentration (Owens et al., 1998). Not only does the osmolality of ruminal fluid influence potential difference across the ruminal epithelium (Stacy and Warner, 1972) and thus, absorption of some substrates (Stacy and Warner, 1966; Warner and Stacy, 1968; Tabaru et al., 1990), but high osmolality may also be linked to reduced voluntary feed intake under experimental conditions (Bergen, 1972).

Absorption & Metabolism of VFA

After generation of VFA from fermentation, the VFA may be absorbed into ruminal epithelial cells through passive diffusion of undissociated acid or facilitated diffusion of dissociated VFA via transport proteins (Connor et al., 2010). With pK_a around 4.8, most of the VFAs are in the dissociated state within normal pH ranges of the rumen (5.8-6.8). It was estimated that VFA absorption removes about 53% of protons in the rumen (Allen, 1997). High osmolality during acidosis decreases the rate of acid absorption from the rumen (Tabaru et al., 1990), and thus, VFA and lactate start to

accumulate in the rumen. One mechanism of VFA absorption involves exchanging ionized acids in the rumen for bicarbonate. However, a reduction in absorption rate leads to a decrease in bicarbonate flowing into the rumen, which further diminishes the buffering capacity and decreases the ruminal pH (Owens et al., 1998).

Blood Acid-Base Status

Maintenance of blood acid-base balance is critical for the health and performance of an animal. The relative concentration of acids, bases, and buffers in solution determines the pH of blood, and mammals must maintain blood pH between 7.36 and 7.44 (Houpt, 1989). A blood pH above 7.44 would indicate systemic alkalosis, and a pH below 7.36 would indicate systemic acidosis. Regulation of the pH of body fluids relies primarily on the bicarbonate (HCO_3) buffering system (Owens et al., 1998). Once absorbed from the rumen into epithelial cells, VFA must be in the undissociated form to pass through the basal membrane of the epithelium. As the acids enter the blood, they dissociate into the anion and a hydrogen ion (Huber, 1976). Bicarbonate combines with the hydrogen ion to form carbonic acid (H_2CO_3), and the acid combines with a cation, such as sodium. Carbonic acid then dissociates to release carbon dioxide (CO_2) and water, resulting in a decrease in bicarbonate and increase in CO_2 concentrations in the blood. This change in the ratio of bicarbonate to carbon dioxide would lower blood pH. Normally, there is an excess of base in the blood, but an acid load may overcome the bicarbonate buffering capacity and decrease the base excess (Gianesella et al., 2010).

Changes in fermentation, absorption, and ruminal pH associated with acidosis have potential to affect blood acid-base status. High acid concentrations in the rumen could deplete the bicarbonate buffering system of the blood. In fact, ruminal VFA

concentrations negatively correlate with blood bicarbonate concentrations (Faverdin et al., 1999). However, the influence of ruminal acidosis on acid-base status is still somewhat unclear. While some articles report reduced blood bicarbonate and pH during acute acidosis, others indicate acid-base changes were minimal. For example, after acute ruminal acidosis was induced in cows via oral sucrose dosing, blood pH, bicarbonate, and base excess decreased below the physiological normal range (Indrova et al., 2017). Blood HCO_3^- decreased rapidly after wethers were switched from alfalfa hay to a 65% concentrate diet, which created a subacute ruminal acidotic situation (Huntington et al., 1981). Additionally, rumen pH, blood bicarbonate to carbon dioxide ratio, and blood pH were decreased at the time that steers stopped eating after being switched from alfalfa hay to a high-grain diet (Uhart and Carroll, 1967). Conversely, blood pH and bicarbonate values of steers infused with glucose to simulate acute acidosis were only slightly reduced when ruminal pH was lowest (Harmon et al., 1985). Similarly, during SARA, there were minimal changes in acid-base status of hay-adapated and grain-adapted steers (Goad et al., 1998). These researchers noted that decreases in blood bicarbonate and base excess in cattle following a grain challenge may be due to increased VFA absorption from the rumen during SARA and physiological compensation. When the bicarbonate buffering system becomes exhausted and is no longer able to compensate for the greater VFA absorption, the blood pH may decrease leading to more serious conditions.

Strategies to Prevent Ruminal Acidosis

The best approach for controlling acidosis in production is through prevention. Although the frequency of acute ruminal acidosis is low in today's beef cattle feedlots and dairies, a substantial amount of research has been done on methods to prevent

acidosis, particularly subacute acidosis. General efforts have focused on management practices and methods for controlling ruminal and blood pH, controlling lactate production and utilization, and regulating feed intake and eating patterns.

Feeding management can modulate intake patterns and limit supply of starch to help prevent ruminal acidosis. Diluting a high-grain diet with roughage increases chewing time and saliva production and thereby, decreases eating rate, increases buffer input, and raises ruminal pH (Owens et al., 1998; Galyean and Defoor, 2003). The additional physical fill from the roughage also serves to decrease meal size (Owens et al., 1998), while the scratch factor effect helps to maintain health of ruminal papillae (Bartley et al., 1981; Loerch, 1991). Gradually switching, as opposed to rapidly changing, cattle from a forage diet to a high-grain diet is the most common method of preventing ruminal acidosis in feedlots. Yet, the cost per unit of energy of forage and the slower gain makes a long adaptation uneconomical for producers (Meissner et al., 2010). The chance for ruminal acidosis increases when cattle consume more total feed or eat quickly because these events increase starch delivery to the rumen and decrease pH. Regularity of feed delivery would help prevent animals from overeating and/or increasing eating rate and thus, would inhibit large swings in ruminal pH and decrease the risk of acidosis (Schwartzkopf-Genswein et al., 2004). Use of distilling or brewing byproducts and middlings (ex. wheat midds, gluten feed, distiller's grains), which have had the starch extracted, in place of cereal grains in cattle diets will also help reduce starch intake and control ruminal pH (Owens et al., 1998; Nagaraja and Lechtenberg, 2007a). Similarly, blending grains, such as wheat and barley, that have higher rates of fermentation, with those that have slower rates of fermentation (ex. cracked corn) should decrease the risk of

inducing acidosis (Nagaraja and Lechtenberg, 2007a). Many nutritional and management strategies to prevent acidosis are aimed at ameliorating large depressions in ruminal pH by slowing starch fermentation and limiting starch supply.

Addition of ruminal buffers to the diet helps resist changes in pH by neutralizing acids. Compounds like sodium bicarbonate and potassium bicarbonate ($KHCO_3$) act as buffers by sequestering excess H^+, thereby neutralizing acid (Hernandez et al., 2014). For example, addition of bentonite with dolomite or $KHCO_3$, buffers that were helpful for high-moisture corn diets, to a high-grain diet for steers resulted in a quicker recovery after an acidotic challenge as evidenced by greater blood bicarbonate levels (Horn et al., 1979). *In vitro* supplementation of a high-grain diet with sodium bicarbonate increased final pH and reduced lactate and biogenic amine concentrations, but sodium bicarbonate supplementation was unable to prevent the accumulation of bacterial endotoxin (Mao et al., 2017). Dietary ingredients that increase saliva flow to the rumen also increase buffering capacity through the bicarbonate in saliva and prevent depressions in ruminal pH (Owens et al., 1998). As a result, supplementation of ruminal buffers is an important strategy for preventing ruminal acidosis. Alkalinizing agents, such as magnesium oxide, in the diet increase the pH of ruminal fluid and may also help prevent acidosis. However, some of the effects of acidosis appear to be pH-independent and cannot be resolved by buffers alone (Calsamiglia et al., 2012).

A nutritional management strategy for preventing metabolic acidosis following ruminal acidosis involves altering the dietary cation-anion difference, also known as DCAD. The DCAD is defined as the mEq of Na + K – Cl – S/kg of DM (Apper-Bossard et al., 2010). Cations increase the base load, but anions raise the acid load of the diet

(Owens et al., 1998). Within the gastrointestinal tract, Na and K are usually absorbed in exchange for a proton, while Cl and S are absorbed in exchange for a bicarbonate ion (Apper-Bossard et al., 2010). Thus, the DCAD of the diet has potential to influence blood acid-base status. When you increase the DCAD of the diet, blood pH and blood bicarbonate concentration increases (Peyraud and Apper-Bossard, 2006). Feeding a high DCAD diet increased DMI in cows fed high amounts of rapidly degradable starch likely due to the positive DCAD helping to maintain blood acid-base status (Apper-Bossard et al., 2010). Positive DCAD can also influence ruminal fermentation, increase ruminal pH (Roche et al., 2005), and may have a ruminal buffering effect (Apper-Bossard et al., 2006). However, when the DCAD is too high, it can also lead to an imbalanced acid-base status, leading to changes in urinary pH (Gianesella et al., 2010).

Antibiotics have been widely used in the cattle industry to help prevent ruminal acidosis by controlling microbial populations and altering ruminal fermentation. Tylosin and virginiamycin are two approved antibiotics that inhibit gram-positive, lactate-producing bacteria (Nagaraja et al., 1997) by blocking protein synthesis (Cocito, 1979). These antibiotics help to stabilize fermentation in the rumen and prevent acidosis (Rogers et al., 1995; Coe et al., 1999). For example, cattle could be switched from a forage diet to an all wheat diet without acidosis complications when the cattle were fed virginiamycin (Zorrilla-Rios et al., 1991). Perhaps the most important and widely used antibiotic is an ionophore called monensin, which forms lipid-soluble complexes with cations and affects their transport across cell membranes (Nagaraja and Lechtenberg, 2007a). This compound is antimicrobial because it disrupts sodium-potassium balance and pH in gram-positive bacterial cells, which hinders crucial processes and leads to cell death

(Łowicki and Huczynski, 2013). Thus, monensin alters fermentation by changing the rumen microbiome and fermentation patterns (Nagaraja et al., 1997). However, a unique effect of monensin, unlike that of other antibiotics, is that monensin impacts feed intake. Consistently, monensin lowers feed intake and improves feed efficiency (Nagaraja and Lechtenberg, 2007a). Supplementation of feedlot cattle rapidly switched to high-grain diets with monensin decreased the mean variance in daily intake (Burrin et al., 1988). Inclusion of monensin in the diet of cattle increased number of meals and reduced DMI rate following an acidosis challenge (Erickson et al., 2003). Total daily ruminal contractions tended to be reduced by monensin supplementation, which could have impacted rumination, turnover, and feed intake (Deswysen et al., 1987b). Therefore, monensin and other antibiotics produce a more stable rumen fermentation and reduce the risk of acidosis.

Public concerns about antibiotic resistance have led researchers to examine other feed additives, including dicarboxylic organic acids, for manipulation of the rumen microbiome and prevention of ruminal acidosis (Castillo et al., 2004). Malate and fumarate are the main dicarboxylic acids that have been investigated. As intermediates in the succinate-propionate pathway of bacteria, malate and fumarate stimulate the growth of *S. ruminantium*, a bacteria which uses that pathway, *in vitro* and following a grain challenge, resulting in enhanced utilization of lactate and prevention of a pH decrease (Martin and Streeter, 1995; Castillo et al., 2004). Yet, experimental findings with supplementation of organic acids to prevent ruminal acidosis are conflicting. Malate was ineffective at preventing ruminal acidosis in feedlot studies (Martin et al., 1999), and its

level of supplementation necessary for benefits may be uneconomical (Kung Jr. et al., 1982; Devant et al., 2007).

Digestive functions of the gut can be improved by feeding or dosing with probiotics, also known as direct-fed microbials. Probiotics are live microbial supplements that bolster populations of beneficial bacteria in the GIT microbiome (Hernandez et al., 2014). The goal of probiotics is to stimulate lactate-utilizing bacteria, such as *M. elsdenii* and/or *S. ruminantium*, to decrease lactate concentrations in the rumen, avoid ruminal pH depressions, and help prevent ruminal acidosis (Owens et al., 1998; Hernandez et al., 2014). Drenching of the rumen with *M. elsdenii*, strain 41125, was useful for preventing the depression of pH to acidotic levels, reducing variation in feed intake, and inhibiting the accumulation of lactic acid in cattle and sheep given feedlot diets in experimental situations (Drouillard, 2004; McDaniel, 2009; Meissner et al., 2010). However, the benefits of strain 41125 in large scale, feedlot scenarios still needs to be investigated. Yeasts and fungi, primarily *Saccharomyces cerevisiae* and *Aspergillus oryzae*, respectively, may also aid in prevention of ruminal acidosis by altering rumen motility and reducing lactate production (Desnoyers et al., 2009; Hernandez et al., 2014).

Interestingly, immunization has even been investigated as a way to prevent ruminal acidosis. Rumen pH was greater and L-lactate concentrations were lower in sheep that were vaccinated against *S. bovis* and *Lactobacillus* spp. versus controls (Gill et al., 2000; Calsamiglia et al., 2012). Likewise, ruminal pH was greater and counts of the target bacteria were reduced when cattle were fed polyclonal antibodies against *S. bovis* and *Fusobacterium necrophorum* (DiLorenzo et al., 2006; DiLorenzo et al., 2008). More

research is needed on practicality and economics of using vaccines or antibodies to help prevent ruminal acidosis.

Effects of High-Grain Feeding on the Rumen Epithelium

The rumen is composed of a cornified, non-glandular, multilayered, stratified squamous epithelium (SSE). Proceeding in the direction from the basement membrane to the lumen of the rumen, the layers of the rumen epithelium consist of the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. Ruminal papillae, are finger-like projections of epithelium that function to expand the surface area and increase absorption of VFA.

When ruminants are switched from a high-forage diet to a high-grain diet, the rumen epithelium goes through an adaptation process involving changes in structure and function. A proteomic study using differential in gel electrophoresis (DIGE) demonstrated that after two days of concentrate feeding sixty differentially expressed proteins existed in sheep rumen epithelial tissue between hay-fed and concentrate-fed animals (Bondzio et al., 2011). After six weeks, there were only fourteen differentially expressed proteins, suggesting that the rumen epithelium does adapt during a diet change. Changes of epithelia architecture and metabolism are necessary for the epithelium to be able to handle the greater acid load due to an enhanced rate of fermentation. These data suggested that the ruminal epithelium changes and adapts to high-grain diets.

Structural and Metabolic Changes

Adaptation of the ruminal epithelium to high-grain diets involves changes in structure and cellular maturation. The shape and size of papillae are altered by

mechanical (“scratch factor”) and chemical stimulation. As a result, morphology of rumen papillae varies greatly with diet. Importantly, feeding increasing levels of dietary grain increases papillae size (Goodlad, 1981; Gaebel et al., 1987; Odongo et al., 2006) and increases SSE proliferation (Goodlad, 1981; Shen et al., 2004) and morphogenesis (Steele et al., 2009). These events increase the surface area for VFA absorption.

Furthermore, the layers of the ruminal epithelium change and become less organized as ruminants are fed concentrate-based diets. Feeding a high-grain diet to dairy cows reduced the overall thickness of the ruminal epithelium, as well as depth of the stratum basale, stratum spinosum, and stratum granulosum layers (Steele et al., 2011a). In goats, a high-grain diet fed for seven weeks increased thickness of the stratum corneum and reduced thickness of the granulosum stratum compared to goats fed an all hay diet (Liu et al., 2013). Yet, there was no difference in the thickness of the sum of the spinosum and basale stratum between treatment groups (Liu et al., 2013). The rate of cellular migration increased with high-grain feeding and SARA, resulting in the appearance of undifferentiated cells near the stratum corneum and reduced cellular organization (Steele et al., 2011a).

Parakeratosis or hyperkeratosis often occurs in beef cattle consuming high-grain diets (Nocek, 1997) and is characterized by thickening and excessive sloughing of the stratum corneum and accretion of keratinized, nucleated squamous cells (Bull et al., 1965; Hinders and Owen, 1965). When cattle are experiencing SARA, the rate of cellular aging decreases, which increases the chance ruminal papillae will become parakeratotic, hardened and clump together (Steele et al., 2011a). Ruminal parakeratosis may be an adaptive condition the SSE undergoes to protect against the low ruminal pH and high

acid load due to high-grain feeding by compromising absorption across the ruminal epithelium (Bull et al., 1965; Hinders and Owen, 1965; Penner et al., 2011). Thickness of the stratum corneum in growing goats fed 60% barley grain was about 40% greater compared to goats fed 0 or 30% grain for six weeks (Metzler-Zebeli et al., 2013). Even after a short period of high-grain feeding, sheep had thicker stratum corneum, which was indicative of ruminal parakeratosis (Steele et al., 2012b). The stratum corneum displayed widespread sloughing when dairy cows experienced acute (Steele et al., 2009) or subacute ruminal acidosis (Steele et al., 2011a). Keratinization score of dorsal and ventral rumen were greater, signifying more keratinization, for goats fed 60% grain (Metzler-Zebeli et al., 2013). Another study using goats fed a high-grain diet, showed parakeratosis and significant cellular damage of rumen papillae from goats fed the high-grain diet (Liu et al., 2013).

Exposure of the ruminal epithelium to high acid concentrations for prolonged periods is commonly associated with rumenitis (Fell and Weekes, 1975). Rumenitis is the local inflammation of the rumen wall. Lesions or abscesses in the ruminal epithelium due to acid damage predispose cattle to liver abscesses by allowing bacteria, such as *Fusobacterium necrophorum,* to colonize and penetrate the epithelium (Nagaraja and Lechtenberg, 2007b). Surprisingly, lesion scores were lower, indicating less lesions on papillae, in goats fed 60% grain compared to those fed 0 or 30% (Metzler-Zebeli et al., 2013).

Impact on Barrier Function

The first line of defense for maintaining whole animal homeostasis during a high-grain challenge is the rumen epithelium (Steele et al., 2012b). Luminal contents of the

gastrointestinal tract are essentially extrinsic to the animal; the epithelium must be able to differentiate and absorb usable nutrients but prevent potentially harmful or unwanted compounds from being absorbed (Mani et al., 2012). Barrier function of the rumen epithelium is required for maintenance of electrochemical gradients that are needed for active transport of nutrients. As an important site for absorption of VFA and electrolytes (Na^+, K^+, Mg^+, and Cl^-), the reticulorumen relies heavily on these gradients (Lodemann and Martens, 2006). Rumen epithelium is considered a 'moderately tight' epithelia (Powell, 1981). If the barrier function of the rumen epithelium becomes compromised, it could enable the translocation of harmful metabolites or bacteria from the rumen into systemic circulation via the portal vein (Nagaraja and Titgemeyer, 2007; Plaizier et al., 2009; Zebeli and Metzler-Zebeli, 2012).

Damage to the SSE of the reticulorumen due to low ruminal pH and high rumen osmolality has been associated with a decline in barrier function (Zebeli and Metzler-Zebeli, 2012). High rumen osmolality may lead to swelling of ruminal papillae and eventual rupture (Kleen et al., 2003), which would further compromise the barrier function of the ruminal SSE. Hypertonic ruminal osmotic pressure also induced breakdown of cell junctions in the stratum granulosum (zonula occludens) and increased intracellular spaces in the stratum basale (Gemmell and Stacy, 1973). Large gaps were found between cells of the stratum granulosum, and desmosomes became indistinguishable when dairy cows were fed a high-grain diet, which indicated a reduction in intracellular adhesion and tight junctions (Steele et al., 2011a). These gaps would provide opportunities for microbes and potential pathogens to translocate from the rumen to the blood and cause inflammation. Cellular necrosis in the deeper cell layers,

swelling of mitochondria, and cellular junction erosion has also been detected in rumen epithelial tissue from sheep (Gaebel et al., 1989) and goats (Liu et al., 2013) fed a high-grain diet.

Acidic pH conditions in the rumen that occur during acidosis increase the permeability of the rumen epithelium. For example, permeability of rumen tissue to ^{3}H-mannitol was increased in the presence of lipopolysaccharide (LPS) at acidic pH values between 4.5 and 5.5, but not between 5.5 and 7.4 (Emmanuel et al., 2007). Under normal ruminal pH ranges, the rumen epithelia is impermeable to histamine, another toxic microbial byproduct associated with feeding high-grain diets (Nocek, 1997; Aschenbach et al., 2000). However, absorption of histamine across sheep rumen epithelia increased greatly when the pH declined (Aschenbach et al., 2000). Likewise, permeability of rumen mucosa increased when the mucosal side of an Ussing chamber had a pH of 5.5 (Emmanuel et al., 2007). Reducing the mucosal pH of isolated sheep rumen epithelium to 5.5 led to a reduction in the short-circuit current (I_{sc}) and increased conductance (G_t), which suggested ion transport was reduced and permeability was increased (Gaebel et al., 1989). One group created a mild SARA challenge in sheep with an oral glucose drench and took samples of ruminal epithelia post-slaughter for an *in vitro* experiment (Penner et al., 2010). Baseline measurements of serosal-to-mucosal flux rate of partially ^{3}H-labeled mannitol ($J_{mannitol\text{-}SM,}$ a marker for paracellular permeability), G_t, and I_{sc} were not different between tissues from SARA sheep and control sheep (water drench). Yet, when the mucosal pH of the Ussing chamber was lowered to 5.2, epithelial permeability and conductance increased in the recovery period after the challenge, suggesting epithelial barrier function was reduced at that time (Penner et al., 2010). The authors suggested that

other problems, such as parakeratosis or inflammation, greater acid load severity, or repeated bouts of acid insult, may be necessary to lead to rumen epithelial barrier dysfunction. Therefore, low ruminal pH, and compromised epithelial barrier function, such as that which occurs with acidosis, could lead to a "leaky gut" condition and increased absorption of toxic metabolites produced by microbes (Zebeli and Metzler-Zebeli, 2012).

Localization and expression of tight junctional proteins in rumen SSE is affected by high-grain feeding. Tight junctions (TJ) are important for maintaining the polarity of cells and controlling the permeability of the epithelial barrier (Graham and Simmons, 2005; Penner et al., 2011). Specifically, the tight junctions are key to preventing the translocation of toxins from the rumen. Transmembrane proteins (claudins and occludin) connect neighboring cells together and are linked to plaque proteins (e.g. zona occuldens-1) which connect to the cytoskeleton (Fanning and Anderson, 1998). Ruminal epithelium of hay-fed goats displayed a continuous band of tight junctional proteins stained (claudin-1, claudin-4, and occludin) at the cell borders (Liu et al., 2013). Conversely, goats fed a high-grain diet had irregular and discontinuous staining of claudin-1 and claudin-4 at the cell borders with very little staining of occludin at cell borders and increased staining of occludin in the cytoplasm (Liu et al., 2013). In correspondence with the protein staining, goats fed high-grain had reduced mRNA expression for claudin-4, occuldin, and zona occulden-1 and an increase in expression of claudin-1 in rumen epithelium compared to controls (Liu et al., 2013). Thus, protein expression levels mirrored the mRNA expression patterns.

Alterations in Gene and Protein Expression

Feeding a high-grain diet greatly modifies epithelial gene expression and proteins. A bovine 24 k microarrary revealed 5,200 differentially expressed genes between cows fed a high-concentrate (64% concentrate) and low-concentrate (8% concentrate) diet for approximately four weeks (Taniguchi et al., 2010). For example, expression of the cadherin desmoglein 1 mRNA in rumen papillae was downregulated when dairy cows were fed a high-grain diet compared to a high-forage diet (Steele et al., 2011a). This finding further supports evidence of compromised structural integrity of the rumen epithelium when cattle consume high-grain diets.

Ruminal epithelial growth may be mediated through the regulation of expression of insulin-like growth factor binding proteins (IGFBPs) by ruminal VFA concentrations (Steele et al., 2011a). Insulin-like growth factor binding-protein 5 (IGFBP5) mRNA was upregulated during weeks 1 and 3 of high-grain feeding in non-lactating dairy cows, while IGFBP3 and 6 were downregulated. Since IGFBP5 promotes effects of IGF-1, it could play a role in increasing rumen SSE proliferation. Conversely, IGFBP3 acts in an opposite manner and blocks IGF-1 cellular effects. Because it was downregulated during high-grain feeding, this event could be important for enhancing epithelial proliferation. Butyrate downregulated IGFBP3 in intestinal epithelial cells and blocked apoptosis (Sanderson, 2004). Thus, an increase in the proportion of butyrate in the rumen during high-grain feeding could enhance cellular growth via this mechanism, which is independent of IGF-1 concentrations in blood (Steele et al., 2012a). Insulin-like growth factor binding-protein 6 (IGFBP6) preferentially binds IGF-2, instead of IGF-1, and inhibits growth (Iosef et al., 2010). The upregulation of *IGFBP5* and downregulation of

IGFBP3 were demonstrated in lactating dairy cows fed a high-grain diet also (Steele et al., 2012a). Therefore, these expression patterns could play a role in the ruminal epithelial growth during consumption of high-grain diets.

Carbonic anhydrase 1 is a metabolic protein of rumen SSE involved in a variety of functions, but a critical function during consumption of high-grain diets is maintenance of pH. This enzyme is responsible for the hydration of CO_2 and dehydration of HCO_3^- (Bondzio et al., 2011). Although one method of VFA absorption occurs via exchange for HCO_3^-, carbonic anhydrase 1 protein expression was downregulated in sheep rumen epithelial tissue after six weeks of feeding a concentrate-supplemented diet (Bondzio et al., 2011).

Genes and proteins involved with the regulation of the actin cytoskeleton are influenced by feeding high-grain diets. The actin cytoskeleton controls eukaryotic cell shape and internal organization. Annotation of differentially expressed genes between Holstein cows fed low- or high-concentrate diets showed one of the major pathways influenced was regulation of the actin cytoskeleton (Taniguchi et al., 2010). Maintenance of cell structure is important for the cell to be able to deal with ruminal acidity and increased VFA concentration (Taniguchi et al., 2010). Similarly, proteomic analysis demonstrated that actin-related protein 3 (ARP3), part of a complex that is crucial for the actin cytoskeleton, was upregulated at the translational level after sheep were fed a high-concentrate diet for six weeks (Bondzio et al., 2011). These results suggest alterations in the cytoskeleton and gap junctions might play an important role in structural and morphological modifications to the rumen SSE during high-grain diet adaptation.

Members of the annexin family of proteins, ANXA1 and ANXA5, were also differentially expressed in ovine ruminal epithelial tissue based on concentrate level in the diet (Bondzio et al., 2011). While ANXA1 was upregulated by 2 days and 6 weeks of concentrate feeding compared to hay-fed animals, ANXA5 was dowregulated 2 days after concentrate feeding and was not different at 6 weeks from expression in hay-fed animals. Annexin 1 protein is key to the process of actin remodeling (Xiao et al., 2007), so it could be part of the cytoskeletal changes occurring during high-grain feeding. Additionally, ANXA1 and ANXA5 are able to form ion channels (Isas et al., 2000; Neumann et al., 2000), which could be related to ruminal Ca^{2+} transport (Gerke et al., 2005). Concentrate-fed sheep displayed increased ruminal Ca^{2+} transport compared to hay-fed sheep (Uppal et al., 2003). Cytosolic phospholipase A2 (cPLA2), an important enzyme for signal transduction during inflammation events, can be inhibited by ANXA1 and ANXA5 (Russo-Marie, 1999; Kim et al., 2001). Therefore, the annexin proteins, which exert anti-inflammatory properties, might be involved in the immune response to high-grain diets (Khafipour et al., 2009b; Zebeli et al., 2010a).

Expression of nutrient transporters was altered when ruminants were fed high-grain diets. Some of the monocarboxylate transporter (MCT) family members are important for VFA absorption and transport across the SSE. More specifically, MCT1 is located on the basolateral membrane of ruminal SSE and hindgut epithelium and cotransports VFA, lactate, or ketones with H^+ out of cells into the blood (Kirat et al., 2006; Graham et al., 2007). Researchers believe that an apical MCT, likely MCT4, is responsible for lactate and VFA transport into ruminal epithelial cells (Aschenbach et al., 2009; Aschenbach et al., 2011). The MCT4 protein has also been found at the apical and

basolateral membranes in ruminant hindgut epithelium (Kirat et al., 2007). Monocarboxylate transporter 1 (*MCT1*) mRNA expression in ventral rumen epithelium was upregulated by 45% when goats were fed a diet of 60% ground barley grain for six weeks compared to diets of 0 or 30% grain (Metzler-Zebeli et al., 2013). This response was likely an attempt to reduce the intracellular acid load by greater efflux of acid from the cells and increase available energetic substrates in the blood. Conversely, *MCT4* and sodium-dependent glucose-linked transporter-1 (*SGLT1*) gene expression were downregulated in goats fed the high-grain diet.

Adapting ruminants to a high-grain diet has been associated with changes in genes related to cholesterol homeostasis. One route of VFA metabolism in epithelial cells is through acetyl-CoA and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to cholesterol synthesis. Hypercholesterolemia within cells is associated with alterations in membrane permeability, inflammation, and increased proliferation and migration (Kleemann and Kooistra, 2005). Since these are common issues with ruminal acidosis, genes related to cholesterol homeostasis may be important for regulating this condition. Microarray results and Ingenuity Pathway Analysis demonstrated downregulation of gene expression for enzymes [acetyl-CoA acetyltransferase 2, cytoplasmic 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), HMG-CoA reductase, farnesyl-diphosphate farnesyltransferase 1, farnesyl disphosphate synthase, and lanosterol synthase] involved in the cholesterol biosynthetic pathway in rumen papillae from the first to third week of high-grain feeding in non-lactating dairy cows (Steele et al., 2011b). Similarly, *HMGCS1* expression was downregulated in high-grain fed lactating cows compared to high-forage fed cows during a time when the most severe form of SARA occurred (Steele et al.,

2012a). Sterol regulatory element-binding protein 2 (*SREBP2*) is part of a family of transcription factors that control expression of many of these genes and thus, activation of cholesterol biosynthesis, at the transcriptional level (Steele et al., 2011b; Steele et al., 2012a). Furthermore, two genes in the liver X receptor and retinoid X receptor (LXR/RXR) activation pathway [ATP-binding cassette, subfamily A, member 1 and low-density lipoprotein receptor] regulate efflux and influx of cholesterol from cells, respectively, and were differentially expressed from the first to third week of high-grain feeding (Steele et al., 2011b).

High-Grain Diets and the Inflammatory Response

One physiological response of feeding high-grain diets that has received considerable attention in research is the inflammatory response. Due to the design of the ruminant gastrointestinal tract (i.e., pre-gastric fermentation), ruminants have a greater potential for exposure to toxins than many other animals. Damage to the gut mucosa due to diet and translocation of toxic metabolites because of reduced gut barrier function can lead to local and systemic inflammation, respectively (Horadagoda et al., 1999). For example, feeding dairy cows a high-concentrate diet led to the upregulation of inflammation related genes, such as interleukins-1β, 2, 6, and 8, in ruminal epithelium, suggesting local inflammation had occurred (Zhang et al., 2016). As mentioned previously, high-grain feeding and acidosis can compromise epithelial barrier function of the animal. Experimentally induced leaky gut also caused inflammation in cattle (Kvidera et al., 2017).

Endotoxin/Lipopolysaccharide

While several toxic metabolites are produced in the rumen by microbes during extended bouts of low ruminal pH, endotoxin, also known as lipopolysaccharide (LPS), has received the most attention in research pertaining to high-grain diets and acidosis. Endotoxin is a bioactive, pro-inflammatory molecule contained in the cell walls of all gram-negative bacteria. Concentration of LPS in rumen fluid increases during massive lysis of dead GNB or when free LPS is shed from rapidly growing GNB (Nagaraja et al., 1978b). Baseline concentration of endotoxin in the rumen of cattle derived from several studies was 3.6-3.9 $\log_{10}$ EU/mL (Zebeli et al., 2012).

Since high-grain diets increase the energy available in the rumen and lead to fast growth of bacteria, it is logical that the concentration of LPS in rumen fluid would increase with greater concentrate levels in the diet. In fact, feeding high levels of grain increased the concentration of LPS in the gastrointestinal tract lumen (Emmanuel et al., 2008; Khafipour et al., 2009b). Several other studies have demonstrated an increase in ruminal LPS concentrations when cattle experience acute (Andersen et al., 1994) or subacute acidosis (Gozho et al., 2005; Gozho et al., 2006). Gradual adaptation of Jersey steers from 0 to 61% concentrate (in the form of wheat-barley pellets) diets displayed a quadratic increase for rumen fluid LPS concentration (Gozho et al., 2006). A meta-analysis study using the breakpoint model revealed a diet threshold of 35% concentrate above which rumen endotoxin concentrations in cattle increased linearly with increasing concentrate in the diet (Zebeli et al., 2012).

Researchers hypothesized that LPS from the rumen may translocate across the rumen SSE into the portal circulation during acidosis, which would initiate the acute

phase response leading to systemic inflammation (Dougherty et al., 1975a; Nagaraja et al., 1978a; Andersen et al., 1994; Gozho et al., 2005). *In vitro* studies with isolated epithelia provided support to this hypothesis. Endotoxin was shown to pass through rumen and colonic mucosal tissue from feedlot steers using an Ussing Chamber (Emmanuel et al., 2007). Perfusate pH was held at either 4.5, 5.5, or 6.5 for rumen tissue samples, and LPS translocated to the serosal side under all pH conditions tested. Translocation of LPS was also demonstrated with colonic tissue and pH values of 5.5, 6.5, and 7.4. Yet, the amount of LPS used in this study was at supraphysiological concentrations (500 μg/mL) compared to rumen exposure during SARA (Gozho et al., 2007; Emmanuel et al., 2008).

Evidence of LPS translocation into the blood in the literature is conflicting. Some studies did not detect LPS in peripheral blood under acute acidotic conditions (Andersen and Jarlov, 1990; Andersen et al., 1994). Although grain-induced SARA was related to an increase in ruminal LPS and blood serum amyloid A (SAA), no LPS was detected in peripheral blood (Gozho et al., 2007). Likewise, feeding a 50% grain and alfalfa pellets diet to dairy cows led to low ruminal pH readings and high free rumen LPS concentrations, but LPS and LPS-binding protein concentrations in peripheral blood were not affected (Khafipour et al., 2009a). Thus, these researchers believed that LPS was not translocated into blood and therefore, did not cause inflammation. A couple studies have reported concentrations of LPS in blood by overfeeding grain, which caused acute acidosis (Dougherty et al., 1975a; Aiumlamai et al., 1992). However, only two studies have reported evidence of LPS in peripheral blood of ruminants during SARA (Khafipour et al., 2009b; Liu et al., 2013).

The mechanism(s) and location(s) of LPS translocation are not fully understood. While there was greater concentration of free rumen LPS when SARA was induced with alfalfa pellets than with grain, no LPS was detected in peripheral circulation with alfalfa pellets (Khafipour et al., 2009a; b). These results led researchers to suggest that peripheral LPS does not come from translocation across the rumen (Khafipour et al., 2009a). When rumens of steers were infused with Cr-labeled LPS, there was no evidence of translocation into lymph or portal circulation (Lassman, 1980). Free LPS that travels out of the rumen is detoxified in the duodenum by bile acids (Bertok, 1998). However, portal vein LPS levels were increased when gut permeability was increased by chronic ethanol exposure (Enomoto et al., 2001). Thus, researchers noted peripheral LPS during SARA likely comes from translocation through the simple columnar epithelium of the intestines (Khafipour et al., 2009a).

Several enteric pathogens, including LPS, can reduce epithelial barrier function by altering F-actin and tight junctional proteins (Hecht et al., 1988; Fasano et al., 1991; Philpott et al., 1996; Nusrat et al., 2001; Chin et al., 2002; Scott et al., 2002). *Escherichia coli* LPS (50 μg/mL) applied to the apical side of nontumorigenic epithelial cell monolayers led to apoptosis, interfered with tight junctional zonula occludens-1, and increased epithelial permeability to dextran 3000 (Chin et al., 2006). In this study, LPS initiated disruption of intestinal epithelial barrier function in a caspase-3-dependent manner, which could potentially be a target for therapeutic treatment of microbial-related gastrointestinal conditions, such as inflammatory bowel disease or acidosis. The epithelia of the small and large intestine have very different composition and structure than the rumen epithelium, however, which could affect how much LPS is necessary to

compromise barrier function and the rate of LPS translocation (Graham and Simmons, 2005). Another study provided evidence that the mRNA expression of proinflammatory cytokines (i.e. inflammation) was related to changes in TJ protein expression. These data further suggest that the disruption of ruminal epithelial barrier function may cause a "leaky" gut, which would allow LPS translocation thereby triggering an inflammatory response (Liu et al., 2013). Similarly, the cytokines, tumor necrosis factor-α (TNF-α) and interferon γ (IFN-γ) are known to be able to regulate expression of TJ proteins, so the interaction may be two-fold.

Acute Phase Response

When an event or agent, such as endotoxin, interferes with the homeostasis of an animal, the acute phase response (APR) is initiated. Generally, the APR is a nonspecific attempt to clear the body of the agent(s) that caused the disruption so that homeostasis can be reestablished (Gabay and Kushner, 1999). Therefore, the innate immune system serves to activate an APR and releases acute phase proteins (APP) from the liver and extra hepatic tissues as a protective mechanism. Cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and TNF-α signal hepatocytes to synthesize the APP (Alsemgeest et al., 1996). These APP work to detoxify LPS in the liver so that it does not reach peripheral circulation (Andersen, 2000).

Toll-like receptors (TLR) function as pattern recognition receptors that sense host epimural and exogenous bacteria and bacterial products, such as LPS, and activate an immune response and cytokine production in the animal (Hooper et al., 2012). These receptors (TLR-1 through TLR-10) have been found to be expressed in the rumen epithelium of cattle (Malmuthuge et al., 2012) and are important for homeostasis of host-

microbial interactions in monogastric animals (Hooper et al., 2012). While TLR-2 binds to ligands from gram-positive bacteria (Yoshimura et al., 1999), TLR-4 recognizes ligands from GNB (Takeuchi et al., 1999).

Lipopolysaccharide-binding protein binds and transports LPS in the blood to help clear it from circulation through transfer to macrophages or lipoproteins (Gallay et al., 1994). As a result, evidence of LPS translocation could be provided by increases in LBP in circulation (Sriskandan and Altmann, 2008). In serum, LBP complexed with LPS facilitates the transfer of LPS to CD14, a glycosylphosphatidylinositol-anchored membrane or soluble protein (Janssens and Beyaert, 2003; Fitzgerald et al., 2004). This new complex binds to and activates TLR-4/myeloid differentiation-2, a receptor complex on the membrane of neutrophils and cells of monocytic lineage that will activate these cells (Fitzgerald et al., 2004). Subsequent signal transduction requires four Toll-interleukin 1 resistance-domain adapter molecules and leads to the activation of transcription factors for nuclear factor κB and production of pro-inflammatory cytokines (Fitzgerald et al., 2004).

Some important APPs in cattle include serum amyloid A (SAA) and haptoglobin (Gozho et al., 2007; Emmanuel et al., 2008; Ametaj et al., 2009; Khafipour et al., 2009b). Serum amyloid A binds to endotoxin in the blood to neutralize it (Levels et al., 2001). Haptoglobin binds free hemoglobin released during hemolysis of erythrocytes and prevents the iron, which is needed for growth and replication, from being used by invading bacteria (Wassell, 2000). Studies have suggested plasma SAA may serve as a better and more sensitive biomarker of inflammation than plasma haptoglobin (Horadagoda et al., 1999; Zebeli et al., 2012).

Feeding a high-grain diet to induce SARA produces an inflammatory response, likely due to the translocation of toxins via the reduced barrier function of the rumen and/or intestinal epithelium. When SARA was experimentally induced, SAA concentrations steadily increased in plasma from 33.6 ± 36.53 to 170.7 ± 36.53 μg/mL, showing a significant difference from hay feeding on d 2, 3, 4, and 5 of feeding wheat-barley pellets (Gozho et al., 2005). Steers had higher serum haptoglobin concentrations on d 3 and 5 of feeding wheat-barley pellets compared to those fed hay.

Haptoglobin was reported to be detectable in cattle only when there was an inflammatory response (Deignan et al., 2000). Feeding a 76% concentrate diet induced SARA and resulted in increased SAA and haptoglobin, suggesting inflammation occurred (Gozho et al., 2006). Grain-induced SARA in mid lactation dairy cows led to an elevated SAA concentration, yet did not affect haptoglobin or other biomarkers of inflammation (Gozho et al., 2007). Additionally, feeding lactating cows 30 or 45% barley grain on a DM basis increased concentrations of APP in plasma, suggesting that there may have been translocation of LPS into the blood (Emmanuel et al., 2008). Expression levels of the proinflammatory cytokines, TNF-α and interferon-γ, mRNA in rumen epithelium increased when goats consumed a high-grain diet (Liu et al., 2013).

Various factors can affect the chance or severity of inflammation from high-grain diets. The risk of systemic inflammation increased linearly when cattle were fed a diet more than 44.1% rapidly fermentable carbohydrates, which was greater than the threshold for endotoxin increase described above (Zebeli et al., 2012). Endotoxin concentrations increased linearly in rumen fluid of cattle when ruminal pH was below 6.0 for more than 95.6 min/d. Similar thresholds were found for increases in plasma SAA and

haptoglobin. These data indicated that cattle may be able to cope with a certain endotoxin load and low ruminal pH before the ruminal epithelium becomes compromised and APR is initiated. Moreover, the toxicity level of endotoxin will affect the ability to cause inflammation in the animal. Endotoxin from *E. coli*, for example, has greater virulence potential and is more toxic than endotoxin from common rumen gram-negative bacteria, such as *M. elsdenii* and *F. succinogenes* (Hurley, 1995; Khafipour et al., 2011).

The development of these inflammatory proteins and activation of an APR can have significant energy and nutrient requirements. Feeding dairy cows in early lactation increasing amounts of barley grain was related to release of LPS and inflammatory proteins, but correlated negatively with feed efficiency and energy balance (Zebeli and Ametaj, 2009). Thus, cattle performance would likely be hindered during an inflammatory response, such as could be induced by feeding high-grain diets (Elsasser et al., 2008).

Feed Intake

The amount of feed an animal consumes and their feeding behavior (i.e., meal size, meal frequency, eating rate, etc.) has a substantial impact on the ruminal environment. Notably, the fermentation profiles, saliva flow, water intake, ruminal passage rates, and ruminal pH patterns are affected by altering feed intake. Understanding how feed intake affects ruminal conditions, metabolism, and whole-animal physiology is important to understanding and solving problems associated with feeding high-grain diets.

Feedback Regulation

Feed intake regulation in animals is a complex system comprised of a multitude of short-term and long-term regulators. Short-term regulation involves control of meal initiation and cessation or hunger and satiety. Whereas, maintenance of body weight or composition is the goal for long-term regulation. Climate, photoperiod, and physiological stage are examples of some factors that would influence long-term feed intake regulation (Forbes and Barrio, 1992). What is interesting about regulation of feed intake is that virtually *all* aspects of the animal and the environment can result in feedback signals at any given time and are translated into usable information by the body. The vast amount of factors that affect intake make it a difficult topic of study.

The gastrointestinal tract is innervated with the vagus nerve and sympathetic nerves, which relay afferent signals from the digestive system to the central nervous system. Specifically, the hypothalamus and nucleus tractus solitarius (NTS) serve as the appetite control centers in the brain. After receiving information from the periphery, the hypothalamus and NTS integrate those signals and "respond" via efferent neurons, leading to an alteration in feed intake (Sartin et al., 2011). Generally, signals from the lateral hypothalamus and dorsomedial hypothalamus are orexigenic (i.e. signal hunger and stimulate feeding), and signals from the ventromedial hypothalamus and paraventricular nucleus are anorexigenic (Sartin et al., 2010; Sartin et al., 2011). Neuropeptide Y (NPY) and agouti-related protein (AGRP) neurons in the arcuate nucleus increase appetite, while proopiomelanocortin (POMC) neurons decrease appetite (Sartin et al., 2010). Although it is commonly stated that animals eat to meet their energy requirements, researchers have yet to discover a receptor in the body for energy (Forbes

and Barrio, 1992). A wide variety of mechanical, chemical, and hormonal factors influence voluntary feed intake of ruminants in combination with each other (Grovum, 1988).

Distention of the reticulorumen is the mechanical or physical factor that limits voluntary dry matter intake in ruminants. As the energy concentration of a diet increases, an animal will commonly increase feed intake in order to try to meet their energy requirements (Montgomery and Baumgardt, 1965) if protein supply is adequate. However, the gut fill or capacity of the reticulorumen can limit the amount of feed the animal can consume (Allen, 1996). Therefore, there is a maximum amount of energy the animal can get from the diet. If the diet has poor digestibility or is high in forage, this may become an issue as the animal may not be able to eat enough to meet energy requirements. Tension receptors, also called mechanoreceptors, in the muscular walls of the reticulorumen sense the stretching of the forestomach from distension and send signals to the central nervous system to stop eating (Leek, 1969). The medial wall of the reticulum and cranial sac contains the most mechanoreceptors, whereas the caudal rumen has few. Distension may be particularly important for regulating feed intake during meals (Ketelaars and Tolkamp, 1996), but it should not be considered alone when attempting to understand feed intake responses (Fisher, 2002). When cattle are consuming a high-grain diet, distention is not a primary regulator of feed intake because the diet contains adequate energy supply.

Products of digestion play important chemostatic roles in feed intake regulation when cattle are fed high-grain diets. Chemical factors, such as cytokines, VFA, osmolality, and other metabolites or nutrients, are known to influence feed intake in

ruminants. Cytokines produced during an immune response serve as chemical controllers of feed intake in ruminants. Peripheral and central administration of two important cytokines, IL-1β and TNFα, lead to a decrease in feed intake by acting indirectly through neural pathways or possibly directly on the brain (Langhans and Hrupka, 1999). In fact, intravenous infusion of a low dose of LPS was associated with a sharp increase in plasma TNFα concentrations and decreased feed intake in cattle (Steiger et al., 1999). Cytokines likely also play important roles in controlling normal feeding, not just during infection or disease (Langhans and Hrupka, 1999). Volatile fatty acids serve as an end-product, negative feedback signal. Therefore, infusions of VFA into the vascular system and rumen depress food intake (Dowden and Jacobson, 1960; Baile and Forbes, 1974). Several studies have also demonstrated that propionate infused into the portal vein of ruminants depressed feed intake to a greater extent than acetate infusion (Baile, 1971; Anil and Forbes, 1980; Elliot et al., 1985). Forestomach epithelial chemical receptors that were activated by VFA infusions have been investigated (Crichlow and Leek, 1981; 1986; Crichlow, 1988). Yet, VFA were often infused or dosed intraruminally as sodium salts (to prevent tissue damage) at supraphysiological levels, so it confounds effects of the VFA themselves and osmotic effects (Forbes and Barrio, 1992). Intraruminal dosing of isosmotic loads of NaCl, polyethylene-glycol (PEG-400; an osmotically active molecule that cannot be absorbed), Na-acetate, and Na-propionate resulted in similar decreases in feed intake of sheep (Grovum and Bignell, 1989; Grovum, 1995). These data suggest that ruminal osmoreceptors help detect increases in rumen fluid osmolality and mediate reductions in feed intake (Carter and Grovum, 1988; Carter and Grovum, 1990; Grovum, 1995). It is critical to consider water intake in these studies and future research

because increased rumen tonicity stimulates drinking, which can dilute infusion effects (Forbes and Barrio, 1992). Additionally, other metabolites and nutrients have been proposed to aid in feed intake regulation in ruminants.

The hepatic oxidation theory states that oxidation of various fuel sources in the liver result in afferent vagal nerve impulses, which are integrated by the NTS and lead to changes in feed intake (Allen et al., 2009; Sartin et al., 2011). This theory assumes that ruminants will increase feed intake when energy consumed per unit of ATP produced in the liver is maximized (Allen et al., 2009). Compounds believed to be controlling feed intake in this manner in ruminants include VFA (Leuvenink et al., 1997; DiCostanzo et al., 1999), amino acids (Kuhara et al., 1991), and lipids (Choi and Palmquist, 1996; Faverdin et al., 1999). Propionate has been a key focus of this work since it is heavily oxidized in the ruminant liver by the tricarboxylic acid cycle (Allen et al., 2009). Therefore, metabolic signals and ruminal tonicity are important chemostatic regulators of feed intake in ruminants.

Hormones synthesized in adipose tissue and the gut can impact feed intake of animals. Leptin, commonly called the satiety hormone, is produced by fat cells (Dyer et al., 1997; Daniel et al., 2003) in response to high energy levels (Kadokawa et al., 2007). Therefore, leptin serves as a signal to inhibit feed intake by crossing the blood-brain barrier (Thomas et al., 2001; Adam et al., 2006) and activating POMC neurons in the hypothalamus that inhibit feeding and inhibiting NPY and AGRP neurons that stimulate feeding (Sartin et al., 2011). Insulin, a hormone produced by the pancreas, has been shown to increase leptin concentrations in blood (Asakuma et al., 2003), so it could indirectly impact feed intake. In contrast to leptin, ghrelin, commonly called the hunger

hormone (Cummings et al., 2001), increases appetite and stimulates feed intake (Howick et al., 2017). Oxyntic glands in the abomasum are the primary site for ghrelin synthesis (Huang et al., 2006), but it is also produced by enteroendocrine cells in the intestines (Date et al., 2000; Sugino et al., 2004). Ghrelin produces an orexigenic response by activating the growth hormone secretagogue receptor subtype 1a in the arcuate nucleus of the hypothalamus (Howick et al., 2017) where the blood-brain barrier is incomplete (Grouselle et al., 2008). Removing rumen contents to decrease rumen fill also resulted in an increase in ghrelin concentrations (Gregorini et al., 2009). In addition, there are many other molecules synthesized by the GIT that produce satiety signals in animals. For example, cholecystokinin (CCK) is a another hormone secreted from enteroendocrine cells in the duodenum and serves as a feed intake suppressor in domestic livestock (Sartin et al., 2011). These hormones signals are integrated by the hypothalamus along with other homeostatic and external factors to regulate feed intake.

Management, feeding practices, and feeding behavior can also have an impact on cattle feed intake, rumination patterns, and risk of acidosis (González et al., 2012). Increasing the frequency of feed delivery from one to four times per day increased feed intake in cattle, which could have partly been due to a reduction in acidosis (Tremere et al., 1968). Another study showed no differences in feed intake, meal size or frequency, and daily mean ruminal pH when the frequency of feeding was increased from one to four times per day in beef cattle (Robles et al., 2007). However, the eating rate and feed consumed within 2 h after the first feeding declined linearly with increasing frequency of feeding (Robles et al., 2007). Feeding behavior of animals can also be influenced by feed bunk management method used by the feedlots. For example, steers fed using a clean

bunk management program consumed less meals than steers fed with a traditional *ad libitum* system (Erickson et al., 2003). The cattle under clean bunk management ate larger meals than the traditional fed steers, but total daily feed intake did not differ. Eating rate was also greater under the clean bunk program. Increasing the eating rate would reduce feed mastication and saliva production, which would reduce buffering capacity of the rumen (González et al., 2012). Because of these behavioral changes, the post-prandial drop in ruminal pH was greater for steers in the clean bunk program, leading to more daily variation in ruminal pH (Erickson et al., 2003). Thus, cattle under clean bunk management could be more likely to experience ruminal acidosis. Day-to-day variations in feed delivered or offered may increase the risk for ruminal acidosis (Schwartzkopf-Genswein et al., 2003; Schwartzkopf-Genswein et al., 2004). Competition at the feed bunk, lameness, and even weather can also alter feeding behavior and therefore, influence ruminal pH and likelihood of animals to experience ruminal acidosis (González et al., 2012).

Effect of High-Grain Diets or Ruminal Acidosis on Feed Intake

When cattle are transitioned to high-grain diets, they are more likely to experience ruminal acidosis and animals often display decreased or erratic feed intake. Increased acid load in the rumen and ruminal pH below approximately 5.5 is linked to a reduction in DMI (Fulton et al., 1979a; Harmon et al., 1985). Acidosis, in both forms, has been characterized by this period of "off-feed" (Fulton et al., 1979b; Kezar and Church, 1979). Researchers have also used the day-to-day variation in feed intake of individual animals as an index of SARA (Britton and Stock, 1987; Britton et al., 1991). Some evidence suggests that animals are most likely to reduce feed intake during adaptation to high-

grain diets when the diet is 70-75% concentrate (Tremere et al., 1968). Furthermore, a reduction in feed intake can directly cause loss of intestinal barrier function and inflammation (Kvidera et al., 2017), which may exacerbate or enable harmful effects of acidosis. However, studies showing feed intake depression during acidosis in cattle have been inconsistent (González et al., 2012). For example, acidosis induced by grain and alfalfa pellets decreased ruminal pH equally, but feed intake was increased with the alfalfa pellets and decreased with the grain (Khafipour et al., 2009a; b). This may be due to the multifaceted acidosis syndrome as well as the complicated network of factors that control feed intake. The mechanisms involved in this reduction in feed intake with diet transitions and SARA have not been fully described and warrant further investigation.

The interrelationship between feeding behavior and rumen acid-base balance are important for understanding changes that occur with feeding high-concentrate diets and acidosis. Synchronization between acid production and elimination (via absorption, passage, or neutralization with buffer) hinges on feeding behavior (González et al., 2012). To begin, meal size influences the amount of organic acids produced in the rumen and therefore, the acid load present. A larger meal, increases the amount of acid produced. Eating rate affects chewing time and feed ensalivation and consequently, buffer addition to the rumen. A faster eating rate reduces chewing time, feed ensalivation, and buffering capacity (Beauchemin et al., 2008). Since high-grain diets have a low percent of forage and small particle sizes, they allow a faster eating rate and result in larger meals (Dado and Allen, 1995; Tolkamp et al., 2002). Pattern of feed intake throughout the day and meal frequency also directly influence the balance of acid production to acid elimination in the rumen. Increasing meal frequency or distributing meals more evenly throughout

the day will lead to a better harmonization of these processes. Thus, animals being transitioned to a more acidogenic diet need to change their feeding behavior by consuming smaller meals more often to help reduce the effects of acidosis (González et al., 2012). Animals undergoing an acidosis challenge reduced meal size and increased meal frequency (DeVries et al., 2009). This response may have been an attempt to stabilize ruminal fermentation patterns throughout the day. When changes in feeding behavior and metabolism overwhelm the capacity of the animal to prevent organic acid accumulation in the rumen, feed intake may be depressed (González et al., 2012). In order to self-regulate, animals must understand the post-ingestive consequences of more fermentable diets (González et al., 2012).

Theories for Reduction in DMI with SARA

Researchers have provided several explanations for a reduction in DMI with SARA, which will be briefly described here. One theory states that a low ruminal pH is sensed by chemoreceptors in the reticulorumen that feedback to the brain to signal a decrease in intake (Forbes and Barrio, 1992). Likewise, accumulation of organic acids, high absorption of VFA, and increased movement of water from the blood to the rumen would increase osmolality in the rumen and blood, which could be detected by osmoreceptors and signal a decrease in feed intake (Carter and Grovum, 1990; Owens et al., 1998). Another theory suggests that the decrease in intake is due to the low ruminal pH reducing forestomach motility and thus, decreasing passage rate (Dougherty et al., 1975b; Slyter, 1976; Forbes and Barrio, 1992). Feed intake may be reduced due to the high concentration of fermentation products available to the liver, leading to high energy status, also known as the hepatic oxidation theory (Allen et al., 2009; Sartin et al., 2011).

Lastly, low ruminal pH may be causing a reduction in DMI by increasing bacterial endotoxins and histamine, thereby, causing an inflammatory response (Kleen et al., 2003; Gonzalez et al., 2008b) and/or decreasing frequency and amplitude of ruminal contractions (Dougherty et al., 1975b; Andersen, 2003; Plaizier et al., 2009). All of these theories are likely correct, at least in part, due to the multidimensional aspects of acidosis syndrome, which makes studying and identifying mechanisms of feed intake regulation during acidosis difficult.

Rumen Motility

Overview of Cattle Forestomach Motility

The stomach of cattle is quadrolocular or composed of four compartments: the rumen, reticulum, omasum, and abomasum (Church, 1976). The reticulorumen (reticulum and rumen) serves as the primary site of microbial fermentation and has complex patterns of motility, which are involved in mixing ingesta (to increase fermentation and absorption), rumination, eructation, and the passage of ruminal contents through the reticulo-omasal orifice. There are five sacs in the rumen separated by pillars: the dorsal sac, dorsal blind sac, ventral blind sac, ventral sac, and cranial sac. Motility patterns result mainly from contractions of the pillars within the reticulorumen. Two muscle layers help coordinate these motility patterns: the deep muscles (which have a circular orientation around the foregut) and the superficial muscles (which run cranial to caudal).

Reticulo-ruminal motility can be separated into different categories based on the cycle of contractions. Primary contractions represent the mixing cycle and occur as a wave of contractions over the foregut, proceeding cranial to caudal. They involve all sacs

of the rumen and always begin with a reticular contraction. An example cycle of a primary contraction would include contractions proceeding in this order: biphasic reticulum contraction → cranial sac → dorsal sac → dorsal blind sac → ventral sac → ventral blind sac → dorsal blind sac → ventral sac. Secondary contractions are the eructation (removal of gas from the rumen) cycle and are independent of a reticular contraction. These contractions tend to occur during or after primary contractions (Ruckebusch and Tomov, 1973) and usually involve contraction of the ventral blind sac → dorsal blind sac → dorsal sac → relaxation of ventral blind sac. The cranial and longitudinal pillars may be involved in secondary cycles, with the cranial pillar remaining partially contracted to prevent digesta from going into the reticulum (Reid and Cornwall, 1959). This cycle serves to push gas to the esophagus and cardial sphincter for eructation. Without secondary cycles to allow the gases produced by microbial fermentation to escape, cattle would experience bloat, a condition which can be life-threatening. The relationship of primary to secondary contractions is generally 1:1 with 1 contraction occurring every minute. Rumination is also another type of motility related to the bovine foregut that occurs before a primary cycle (Church, 1976). Rumination involves a reticular contraction → diaphragm contraction → opening of cardia → antiperistalsis of ingesta up the esophagus → reswallowing of fluid → rechewing feed → and reswallowing feed. As a process of regurgitation, remastication, reinsalivation, and redeglutition, rumination is important for the additional mechanical breakdown of fibrous feedstuffs, reduction of particle size, and buffering of the reticulo-rumen.

The omasum has motility as well, but much less is known about this organ because its anatomical location makes it more difficult to study (Church, 1976). When

the reticulum contracts, the omasal neck contracts, followed by the omasal canal and omasal body. Compared to the reticulo-rumen, these contractions are slower and longer. Pattern of contractility of the omasum proceeds like a wave of contraction which moves slowly over the omasal body (Bueno and Ruckebusch, 1974). For most of the cycle of reticulo-ruminal contractions, the omasal orifice is open. However, after the last contraction of the reticulum, the orifice closes tightly, the pressure in the neck of the omasum drops, and pressure in the omasum increases (Balch et al., 1951). Hence, the omasum has a kind of valve-like action. Also, there is considerable variation in omasal motility between animals.

Normal abomasal motility has not been investigated to a great extent. The fundus region of the abomasum usually does not contract, but the body may have peristaltic waves of contractions. Most of the motility is associated with the distal area of the abomasum or the pyloric antrum (Phillipson, 1970). Generally, it appears that abomasal motility is similar to that of the monogastric stomach.

Mechanism of Control of Rumen Motility

Reticulo-ruminal contractions can also be categorized by their method of regulation (Grovum, 1986). Extrinsic contractions are those which are regulated by the vagus nerve. Conversely, intrinsic contractions are not regulated by the vagus nerve. Motility can be affected by activity of the animal, satiety level, diet, cannulation, and health or disease states (such as acute ruminal acidosis). Neural control of extrinsic gastrointestinal tract motility is regulated by the gastric center of the medulla oblongata in the brain. This area of the brain receives sensory input from various neurons and the hypothalamus and can either stimulate or inhibit gastrointestinal tract motility.

Starting at the point of food entry, buccal receptors in the mouth, stimulated by eating and rumination, communicate with the gastric center to stimulate motility. If the reticulo-rumen becomes severely distended, high-threshold tension receptors in the cardia, reticulum, reticulo-ruminal fold, cranial pillar, and longitudinal pillars can send signals to the gastric center via afferent neurons to decrease motility. Conversely, low-threshold tension receptors in the reticulum, reticulo-ruminal fold, and cranial sac are able to detect mild distension and tell the gastric center to stimulate motility. The presence of free gas in the reticulo-rumen can be detected by stretch (gas) receptors in the cardia and dorsal sac and signal to increase motility. When concentrations of undissociated/protonated VFA increase in the reticulo-rumen, acid receptors in the reticulum and rumen can activate vagal nerve endings and inhibit motility. Likewise, acid receptors in the abomasum tell the gastric center to increase reticulo-ruminal motility when acid builds up due to emptying of abomasal contents. Tension receptors in the abomasum can sense abomasal distension and communicate back to the gastric center to decrease motility. All of these receptors would signal the gastric center via vagal afferent neurons. Then, the gastric center sends efferent motor signals via the vagal nerve to the foregut organs to either stimulate or inhibit motility by alterations in the type, frequency, amplitude, and duration of contractions.

Additionally, foregut motility is also influenced by intrinsic, non-vagal activity. For example, when sheep were subjected to vagotomy, they were able to regain some motility in the reticulo-rumen within 1 d, with strong contractions evident by 1-2 weeks (Gregory, 1982). The myenteric plexus influences the intrinsic cholinergic motility of the reticulo-rumen (as evidenced by inhibition of motility with atropine in vagotomized

sheep), which is mainly regulated by distention of the reticulo-rumen (Gregory, 1984). After vagotomy, the omasum displayed long bursts of slow wave-like activity that was not coordinated with motility of the reticulo-rumen, and atropine did not inhibit omasal motility (Gregory, 1982). Therefore, it is likely that the control of omasal motility does not depend solely on cholinergic control.

Gastrointestinal tract motility is also regulated by hormonal control. Motilin is a produced by EC_2 cells in the gut (small intestine) and brain; it can increase GIT motility and also controls gastric emptying. Substance P is a gut and neuropeptide secreted from EC_1 cells in the gut (reticular groove, reticulum, rumen, omasum, and intestines) and brain (subcortical region and hypothalamus). Since substance P influences blood flow and stimulates smooth muscle contractions, it works to increase motility. Also, vasoactive intestinal peptide (VIP) is produced by D_1 cells in the gut (all segments of the ruminant stomach) and brain (supraoptic nucleus, suprachiasmatic nucleus, and lateral preoptic area) and serves to relax smooth muscle and thereby, inhibit motility.

Thus, the control of bovine foregut motility is highly complex involving an integration of local and central control mechanisms for regulation of motility in the intact animal.

Impact of Acidosis on Ruminal Motility

Acute ruminal acidosis has been associated with a reduction in rumen motility. When the pH of ruminal contents was instantly decreased to 4.0, rumen motility stopped about 2 hours later (Juhasz and Szegedi, 1968). Researchers have observed that amplitude and frequency of rumen contractions gradually decreases to stasis with lactic or acute acidosis (Bruce and Huber, 1973). Experimentally inducing acute acidosis in sheep by

intraruminal carbohydrate challenges [dosing with finely ground wheat (Crichlow, 1989), a sucrose solution (Kezar and Church, 1979), and VFAs (Ash, 1959; Gregory, 1987) has led to inhibition or complete stasis of reticulorumen motility. Studies have demonstrated that forestomach motility is decreased within 4-6 hours, and ruminal stasis or atony may occur between 8-12 hours following carbohydrate dosing (Dirksen, 1970; Crichlow and Chaplin, 1985). Contraction frequency and amplitude of sheep were also reduced by duodenal infusion of lactic acid (Bruce and Huber, 1973). A decrease in ruminal contractions and ruminal stasis may be a protective mechanism the animal has developed to reduce acid absorption and thus, prevent systemic acidosis (Dunlop and Stefaniak, 1965; Ahrens, 1967; Huber, 1976). However, the effect of SARA during typical dietary transitions on rumen motility is still unclear.

These alterations in rumen motility during a diet transition and acidosis are likely due to the regulatory mechanisms mentioned above. For example, switching animals to a higher concentrate diet would probably result in less stimulation of buccal receptors due to finer particles and less rumination, which may reduce motility. Likewise, acid receptors in the rumen would be more stimulated due to the higher fermentation rate of the concentrates and increased acid concentrations, which could also inhibit motility. In the 1970s, the stasis of rumen motility was believed to be due to a blood borne substance (ie. hormone) and not due to local hydrogen ion receptors in the rumen mucosa (Bruce and Huber, 1973; Huber, 1976). Yet, there was some evidence that suggested the central nervous system was involved in the inhibition of reticulorumen motility by undissociated acid in the rumen (Svendsen, 1973). Ruminal hypertonicity (high osmotic pressure) has also been shown to sometimes reduce the frequency of ruminal contractions (Carter and

Grovum, 1990), likely via osmoreceptors in the rumen that also help regulate feed intake. Additionally, some diseases, including endotoxaemia, cause reticuloruminal stasis in cattle (Eades, 1993; 1997). Thus, the possibility exists that during acidosis, the reduced barrier function of the rumen may allow LPS to translocate into venous blood and lead to a series of events that reduce motility of the reticulorumen. If forestomach contractions become reduced, feed intake and behavior of the animal could also be affected. It is still unclear what mechanisms of rumen motility regulation are important during diet transitions and SARA.

Animal Variation in Response to Carbohydrate Challenges

A large amount of animal-to-animal variation exists in response of animals to carbohydrate loading, which makes studying acidosis even more complicated. Many publications have noted the variation in animal responses to rapidly fermentable carbohydrate substrates given to experimental animals on a body weight basis (Huber, 1971; Dougherty et al., 1975b; Suber et al., 1979). Upon evaluating models of experimentally induced acute and subacute acidosis, researchers found considerable variation in the animals' ability to deal with the grain challenge (Brown et al., 2000). For this study, five steers were ruminally dosed with 3% of BW as steam-flaked corn, split into four doses, after one day of feed restriction. One steer was euthanized due to complications from acute ruminal acidosis, but another steer on the same treatment showed no clinical signs. In another study, beef heifers adapted to a 90% concentrate diet from a 40% concentrate diet with both rapid and gradual adaptation protocols demonstrated substantial variation in ability to tolerate the adaptation as evidenced by maintenance of healthy ruminal pH and maintenance of DMI (Bevans et al., 2005).

A large amount of individual variability could be due to differences in absorptive capacity of the rumen epithelium (Zebeli and Metzler-Zebeli, 2012). *In vitro* apical epithelial uptake of acetate and butyrate was greater in rumen tissue from sheep that were more resistant to SARA than susceptible sheep (Penner et al., 2009a). Additionally, sheep that were more resistant to the SARA challenge had greater concentrations of plasma β-hydroxybutyrate than sheep that developed SARA. This suggests that animals with greater absorptive and metabolic capacity of rumen epithelium for these VFAs would be more resistant to SARA (Zebeli and Metzler-Zebeli, 2012).

Furthermore, acidosis-susceptible (AS) and acidosis resistant (AR) cattle may differ in expression of genes involved with intracellular pH regulation. For example, the sodium hydrogen exchangers are prominent in the rumen epithelium. Sodium hydrogen exchanger isoform 3 (*NHE3*), which imports Na^+ from the rumen and exports H^+ to the rumen, had a greater expression in AR steers than AS steers (Schlau et al., 2012). Therefore, AR animals may be accustomed to having a slightly lower ruminal pH and could be less susceptible to acidotic challenges.

Some research has suggested that adaptation of the ruminal microbial populations in grain-adapted animals is responsible for tolerance to grain (Allison et al., 1964). Cattle that differ in susceptibility to SARA also appear to have different rumen microbial communities. Copy number of total bacterial 16S rRNA genes (an indication of bacterial density) in rumen contents and attached to the epithelial surface (epimural) were greater in AS than AR steers (Chen et al., 2012). The authors speculated that the higher bacterial density in AS animals related to a more active bacterial community, greater fermentation as evidenced by higher VFA concentrations, and a lower ruminal pH. Rumen contents

and epimural bacterial diversity were also different between AS and AR cattle. Therefore, differences in epimural bacterial communities within the rumen of animals may play a role in animal variation in response to experimentally-induced acidosis.

Conclusion

Feeding cattle high-grain diets is important for maximizing growth and production. Yet, acidosis that can ensue while transitioning cattle is a multidimensional syndrome, which can affect animal physiology, behavior, and health, leading to decreased productivity. Regulation of feeding behavior and rumen motility appear to be closely related. This may help explain some of the adverse effects of diet transitions and SARA on cattle, as well as why some cattle are able to handle diet transitions to high-grain diets better than others. Additionally, reduced epithelial barrier function during acidosis may enable LPS to translocate across the rumen epithelium and enter the blood, but it has not been determined where exactly LPS translocates from in the gastrointestinal tract. More research is necessary to understand mechanisms of dietary adaptation and inflammation during transitions in an attempt to try to counteract these events and improve animal efficiency.

CHAPTER 3: AUTOMATED SYSTEM FOR CHARACTERIZING SHORT-TERM FEEDING BEHAVIOR AND REAL-TIME FORESTOMACH MOTILITY IN CATTLE

Introduction

Cattle exhibit differing feeding patterns throughout the feeding cycle that can influence the ruminal environment. For example, animals may demonstrate long bouts of eating, followed by long bouts of resting and ruminating, or they may have small meals frequently, with smaller bouts of resting and ruminating. Several health-related (Gonzalez et al., 2008c; Wolfger et al., 2015), environmental (Rittenhouse and Senft, 1982; Hahn, 1995), managerial (Erickson et al., 2003; Schwartzkopf-Genswein et al., 2003; Gonzalez et al., 2008a), and social (Voisinet et al., 1997) factors influence short-term feeding behavior in cattle, which make measuring feeding behavior of interest. However, current systems designed to monitor individual animal feeding behavior only record feeding times (Theurer et al., 2013), are expensive, or do not automate data analysis. Some of these systems are more applicable to a pen-feeding or feedlot situation (Schwartzkopf-Genswein et al., 2011) and may allow for multiple treatments in the same pen. Yet, feeding behavior is also relevant during intensive research studies of individually stalled animals or at facilities without these larger pen-based intake monitoring systems. Evaluation of cattle feeding behavior is important for understanding physiological relationships between feed intake and gastrointestinal function. Therefore, an automated system to monitor and characterize short-term feeding behavior of individually-housed livestock is warranted.

Changes in motility of the reticulo-rumen have been associated with alterations in feed intake and feeding behavior (Church, 1976; Della-Fera and Baile, 1980; Deswysen et al., 1987a; Kaya et al., 1992). Particularly, rumen motility can influence passage rate of contents, and thereby, has potential to alter feed intake. Feed intake also stimulates rumen motility, suggesting the control of these events is interrelated. Patterns of feed intake and forestomach motility of cattle can affect animal health through their influence on ruminal pH. For example, greater ruminal contractions directly increase rate of fermentation. Similarly, larger meals may lead to greater fluctuations in ruminal pH, particularly when cattle are fed high-concentrate diets. Low ruminal pH reduces or is associated with erratic feed intake (Fulton et al., 1979a; Cooper et al., 1999a) and may lead to inhibition of rumen motility (Huber, 1976). Yet, the literature rarely investigates feeding behavior, ruminal motility, and ruminal pH in the same experiment.

The objective of this study was to develop a system to continuously monitor feed intake and rumen motility of individually-housed animals. Specific objectives were to:

(1) Evaluate the use of feed bunk and ruminal cavity instrumentation to measure feed intake and ruminal motility, respectively.
(2) Develop data analysis algorithms to characterize feeding behavior and ruminal contractions.

Materials and Methods

Equipment

Feed Intake System

A welded support bracket was secured to the top metal frame at the front of the animal stalls. At the headspace outside the stall, feed bunks were mounted onto stainless steel, S-beam load cells (LC101-500/LC111-500, Omegadyne, Sunbury, Ohio) and suspended from the bracket approximately 5 cm off the ground using metal chains (Figure 3-1). The two rear sections of bunk, next to the animal pen, was then secured to the front of the animal pen using a short piece of metal chain and double-ended snap to prevent excessive swinging of the bunks during animal interference, such as head-scratching. There were a total of 8 feedbunk-load cells set-up at the facility (University of Kentucky C. Oran Little Research Center, Beef Unit, Versailles, Kentucky). Load cells were connected via 4-conductor shielded cables (PT06F10-6S, Omegadyne) to a data logger (CR1000, Campbell Scientific, Inc., Logan, Utah), which was programmed to record feed weight at 1-min intervals. Based on feeding behavior of cattle, this time interval was believed to be a reliable indicator of short-term feeding behavior (Robles et al., 2007). The load cells were supplied with 13.5 Vdc excitation and produced a nominal 40.5 mV signal at the 227 kg (500 lbs) rated load capacity. The CR1000 analog inputs were set to “AutoRange” when measuring the differential voltage outputs of the load cells. The maximum analog-to-digital conversion range expected based on the loads applied was ±7.5 mV, which provided a resolution of 1.0 μV corresponding to 0.12 kg. Data from the logger were downloaded to a laptop computer after each 24-h feeding cycle via USB connection using logger software (PC200W, Campbell Scientific, Inc.).

The data logger stored data in a .DAT file, which was a comma-delimited text file. Data in the .DAT file was imported into Excel (2016, Microsoft Corporation, Redmond, Washington), split into columns, using the text-to-columns function with comma as the delimiter, and saved as an Excel file (.xlsx) before being imported into MATLAB (R2015b, The Mathworks, Inc., Natick, Massachusetts) for processing and analysis.

Load cells and the feed intake measurement system were evaluated for accuracy by addition of standardized weights (1, 2, 4, 8, 22.7, and 30.7 kg) to the feed bunks and comparison of theoretical weight with CR1000 output weight. On average, the percent error in weight was 1.37 ± 0.89%.

Rumen Motility System

Disposable blood pressure transducers (MLT0670; ADInstruments Inc., Colorado Springs, CO) connected to data acquisition hardware (PowerLab 8/30, ADInstruments, Inc.) through bridge amplifiers (FE221; ADInstruments, Inc.) were used to monitor pressure changes in the rumen and characterize rumen contractions. The pressure monitoring system had a manufacturer specified accuracy of ±0.2 mmHg and a resolution of 0.01 mmHg. Pressure transducers were manually calibrated using a 2-point sample method before each use with a sphygmomanometer (ReliOn® manual blood pressure monitor, Walmart, Inc., Bentonville, AR). LabChart software (ADInstruments, Inc.) incorporated the calibration converting voltage to pressure prior to recording data. A water-filled (2 L; 2 kg) balloon (60.96 cm Tuf-Tex® jumbo balloons, Maple City Rubber Company, Norwalk, OH) attached to a Tygon® catheter (i.d. = 3.2 mm; o.d. = 6.4 mm) with castration bands (Ideal Instruments, Neogen Corporation, Lansing, MI) and plastic hose clamps (i.d. minimum 11.4 mm; i.d. maximum = 13 mm; Cole-Palmer

Instrument Co., Vernon Hills, IL) was inserted into the ventral sac of the rumen of each animal (Figure 3-2A). Balloons were weighed to maintain consistent fill between animals. Balloons were changed if the balloons appeared to leak or were torn resulting in loss of signal. A small hole was made in the plug of the rumen cannula to allow the catheter to pass through. The end of the catheter external to the animal was equipped with a tubing-to-Luer Lock adapter for connection to the pressure transducer stopcock (Figure 3-2B). Water filled the pressure transducer by opening the release valve until water trickled out and no air bubbles were visible in the transducer or catheter.

The data acquisition hardware was directly connected to a laptop computer with a USB connection. Rumen pressure data were recorded continuously using LabChart software at a rate of 4 samples/s, which allowed real-time observation of the pressure signal. The recording file was saved hourly and monitored periodically for problems with equipment or signals. After recording was finished, rumen pressure versus time data were exported from LabChart to Excel. The leading header was deleted from the spreadsheet file, and elapsed seconds of recording were generated in the first column. Periods of poor quality data where equipment issues occurred during recordings, such as a balloon breaking or a cable connection getting wet and no longer working, were manually removed from the spreadsheet and replaced with a value of 0. These zeros were subsequently ignored when processing.

Explanation of Algorithms

Meal Detection

A *LoadCell*.m script was written in MATLAB for meal detection and calculation of meal durations and meal sizes. The script was designed to be run on one 24-h period at

a time in which the animals were fed once daily. No other modification of the import data was necessary unless the user intended to focus on a certain time-period of measurement within that 24 h. The script was designed to handle data from up to eight load cells. Figure 3-3 displays an overview of the *LoadCell*.m algorithm.

The script imported the Excel file containing the load cell data. Script parameters, including the filter order for smoothing and minimum time (min) required between feeding events for them to be considered separate meals (also called the inter-meal interval) were defined. Data from the load cell output file were extracted to sort out timestamp and weight data for each individual load cell. Each individual load cell weight data was separated. Time in elapsed minutes was generated and used for plotting of the data. Weights were smoothed using a one-dimensional n-order median filter, which reduced noise while preserving the sharp transitions in weight at the beginning and end of a meal. The median filter order was manually adjusted to remove noise, any large peaks (high weight values), or significant, rapid changes in weight due to animal interference with the bunk, but not so much as to greatly affect the relative weights recorded that were from feeding.

The difference between two consecutive measurements of the filtered weight data was calculated, thereby generating the first-order derivative of the signal. The differences between measurements were evaluated to determine if there was a change in weight beyond a threshold (i.e. was the slope negative enough to not be due to error in measurement), which distinguished if the animal was eating. Any weight loss from the bunk was assumed to be due to feed consumption. The inter-meal interval (i.e. threshold of time between eating events for them to be considered separate meals) was set to 30

min. Eating events or meals that were within 30 min of the last meal end were combined into a single meal.

The elapsed time, filtered weight data, and beginnings and ends of meals were plotted to help identify any errors in detecting meals (Figure 3-4). The script marked a green “O” where feeding began and a red “X” where feeding stopped for each meal. Results from each animal or load cell were printed on separate figures. The plots provided an opportunity to check the filter order, slope threshold, and meal criterion were appropriate for the data set. For example, if there were still relatively large spikes in the weight data, the order of the filter was increased. Likewise, if the meals did not seem to start and end at the “ramp” of the weight data, the slope threshold was adjusted. Meal durations were calculated in one-minute resolution for each meal by determining the difference of the meal start and meal end elapsed time. Additionally, meal size was estimated for each meal by subtracting the filtered feed bunk weight at the end of the meal from the weight at the start of the meal.

The final two processes of the script served to prepare the resulting analysis for output. Animal identification numbers were associated with each load cell by user prompts. The meal duration and meal size results for all load cells or animals were gathered together, along with the timestamp at which the meals began for each animal and was exported in an Excel spreadsheet. Accuracy of the parameters for the data were evaluated by summing the meal sizes for each animal and comparing the result to manually measured feed and orts.

Ruminal Contractions

The *MotilityPeaks*.m script was prepared in MATLAB for filtering noise from pressure data, detecting contractions, and evaluating contraction amplitude, duration, and frequency. The pressure signals from individual animals were extracted from the channel output and plotted against elapsed time (sec) for visualization. Also, the hour of recording that each reading came from was extracted from the dataset. A one-dimensional n-order median filter smoothed pressure readings, and resulting data were plotted against elapsed time. The order of the median filter and the amplitude threshold were defined as 15 and 4 mmHg, respectively. Figure 3-5 displays an example of the filtered data plotted overtop the raw data for a short period of time.

Ruminal contractions were detected using the *findpeaks* function within the *Signal Processing Toolbox* of MATLAB and filtered pressure data (Figure 3-6). Next, the derivative of the smoothed pressure signal was calculated, filtered to remove noise, and used to determine the duration of each contraction event using start and stop slope thresholds of 0.002 and 0.0005 mmHg/s, respectively. For each peak, the start and stop points of the contraction were found by beginning 3.75 sec (peak offset = 15 samples) in either direction from the peak location and determining where the derivative of the signal surpassed the above thresholds. The peak offset prevented the flat part of the peak being detected as the start or stop of the contraction. Figure 3-7 shows the start, end, and peak of contractions for the same window of time as Figures 3-5 and 3-6 using filtered pressure data. Duration of each contraction was calculated by subtracting the start time from the stop time. An output file was generated which described each contraction event

by listing the peak number, pressure at the peak, amplitude, duration, and hour of recording in which the contraction occurred.

Evaluation of Effectiveness

Meal Detection Script

The *LoadCell*.m script was evaluated using sample data from fourteen days across all eight load cells by manually inspecting the output plot indicating identified meal start and end times in contrast to the filtered weight data. Failure criteria included not identifying that a meal had occurred, not combining successive small meals into a single meal, and identifying a meal that did not occur. During analysis of this sample data, the order of the median filter was set to 28 and the inter-meal interval was set at 30 min.

Ruminal Contractions Script

The *MotilityPeaks*.m script was evaluated using sample data from 24 h of continuous recording from three animals. The script was modified to generate additional figures, which simultaneously plotted the raw data, smoothed data, and contraction start, stop, and peak points for each animal. Upon analyzing the data using the script, random sections of the output plots (100 peaks per animal) were visually inspected to identify and evaluate contraction events. Errors of interest included not identifying a significant contraction that had occurred, identification of a contraction that did not occur, and grossly misrepresenting the start, end, or peak of the contraction.

Experimental Application

All procedures with live animals were approved by the University of Kentucky Institutional Animal Care and Use Committee (#2018-2973). Feed intake and rumen motility systems were used to evaluate short-term feeding behavior and ruminal contractions on two common, grain-based diets for feedlot cattle, approximately 70% concentrate (MED) and 90% concentrate (HIGH), respectively. Eight ruminally cannulated, crossbred beef heifers (BW = 534 ± 23 kg) were housed indoors in individual stalls fitted with the feed intake system at the University of Kentucky, C. Oran Little Research Farm, Beef Unit, Intensive Research building. Animals were fed diets *ad libitum* and had free access to water throughout the experiment. After acclimation (21 d) to the MED diet, rumen motility and feed intake were monitored continuously for 24 h following feeding using the above-described methods. During data collection periods, heifers were haltered and tied to the headspace in front of the bunk and had the stall gate chained back to restrict excessive movement of the animals. However, the cattle were able to lay down, stand up, and exhibit moderate lateral movement. Heifers were given another day on MED following motility recording and then abruptly switched to HIGH. Beginning immediately prior to feeding HIGH, feeding behavior and rumen motility were recorded continuously for 48 h. This abrupt dietary switch was used a model for a subacute ruminal acidosis challenge (defined as ruminal pH below 5.6 for greater than 180 min per day). Samples of diets and orts were collected each day of recording and dried in a 55°C oven for 48 h for determination of dry matter (DM) content and used for manual calculation of dry matter intake (DMI).

All thresholds, peak offset, inter-meal interval, and all other parameter inputs for the scripts used for this experimental application were the same as described above for the evaluation of effectiveness of the scripts. Following download of load cell data from the data logger, the data were analyzed in MATLAB using the meal detection algorithm. A meal was defined as a continuous eating event, and a break of at least 30 min (inter-meal interval) with no weight changes was used to differentiate between meals. The order of the medium filter was set to 30. Number of meals were enumerated, and meal durations and sizes were averaged for each animal and day. Meal sizes of all meals were summed to produce a total daily feed intake for each animal and day for comparison to manually measured as-fed and DMI. For the script-generated DMI, only the DM content of the diet was used to correct as-fed intakes.

Rumen motility data were formatted as described above and analyzed in MATLAB using the rumen contraction algorithm. Any contractions which had a peak pressure, amplitude, duration or width at half-prominence above 100 were removed from the dataset to eliminate outliers that did not make sense biologically. Average contraction amplitude, duration, and peak pressure was determined with *proc MEANS* of SAS 9.4 (SAS Institute Inc., Cary, NC) for each hour and animal for each day.

Statistical Analysis

Day/feeding cycle was considered the treatment (MED, HIGH d1, and HIGH d2). Manually determined daily intakes, daily intakes using summed meal sizes, meal frequency, meal duration, and meal size were analyzed as a randomized block design using *proc MIXED* of SAS 9.4 for the effect of day/treatment. Contraction amplitude, duration, and peak pressure were analyzed using *proc MIXED* for the effect of

day/treatment, hour, and their interaction, in addition to considering hour as a repeated measure. Compound symmetry was used as the covariance structure. In both models, block was considered a random effect. Degrees of freedom were estimated using the Satterthwaite approximation. Means reported are the least square means. Mean comparison was conducted using the least significant difference and $P < 0.05$, if the probability of a greater *F*-statistic was significant for an effect.

Results

Meal Detection

Script Evaluation

A total of 1,003 individual meals were detected from the sample data of eight load cells over fourteen days. The most common error was not combining successive short meals into a single larger meal, which occurred 11 times. Five instances of identifying a meal that did not occur were observed. Two instances of not identifying that a meal occurred, or that the meal size and length were substantially underreported, were also observed. An example of an instance when both the meal size and duration were underreported is shown in Figure 3-8. Combined errors represented approximately 1.8% of the total number of meals detected in the sample data. Given the relatively low error rate for a large sample size, no further adjustments were made to the filtering or threshold settings.

Experimental Results

Results for feed intake and feeding behavior are presented in Table 3-1. Comparison of feeding behavior before the dietary switch (MED) and 2 days

immediately following switching diets (HIGH) using meal detection script results. Manual as-fed intake (kg/d) was affected by treatment ($P = 0.045$); it was reduced on d2 of HIGH feeding, but was not different between MED and HIGH d1. As-fed intake determined by summing the meal sizes from the algorithm output was not different ($P = 0.159$) between treatments and also was between 1.76 and 3.4 kg lower than manually determined as-fed intake. Conversely, treatment tended ($P = 0.097$) to affect manually measured DM intake (kg/d). Manual DM intake was decreased on HIGH d2 compared to HIGH d1, but neither were different from MED. Script-generated DM intake (kg/d), calculated using the script-generated as-fed intake and diet DM, was influenced ($P = 0.029$) by treatment; DM intake on HIGH d1 was greater than MED, but neither treatment was different than HIGH d2. However, script-generated DM intake was between 1.17 and 2.49 kg lower than manually-determined DM intake. Average meal frequency, meal duration, and meal size were not affected ($P > 0.10$) by treatment.

Motility Detection

Script Evaluation

Approximately 6,700 individual contractions were detected from the sample data of three animals over 24 hours. Applying the median filter resulted in a rounding of the contraction peaks. Thus, it was expected and accepted that the smoothing would result in slightly lower peak pressures compared to the raw data. Initially, the order of the median filter was set to 5 in an attempt to minimize peak rounding. However, this resulted in 14 instances alone in the 300 peaks observed where one contraction was identified as 2 peaks and 16.7% of contractions being misrepresented in some manner. Therefore, the order of the filter was increased to 15 and data were reanalyzed. The most common error

in the 300 peaks manually observed was not counting a contraction as a peak because the filtering reduced the amplitude below the threshold, which appeared to occur 6 times. Yet, it was recognized that this was a result of the necessary smoothing that needed to be conducted for adequate analysis. Some odd-shaped contraction waveforms resulted in the start or stop of the contraction (and subsequently, duration) being misrepresented, which occurred 4 times. An example of this error is displayed in Figure 3-9. Only 4% of the observed contractions displayed errors, so no further adjustments were made to the median filter, slope thresholds, or peak offset.

Experimental Results

Ruminal contraction/motility results are shown in Table 3-2. Comparison of rumen motility contractions before the dietary switch (MED) and 2 days immediately following switching diets (HIGH) using motility detection script results. The effect of treatment was significant ($P < 0.05$) for all contraction variables. Amplitude of contractions was greater on MED than on either day on HIGH. Similarly, contraction duration was shorter on HIGH days compared to MED. Duration of contractions was also influenced by hour ($P < 0.01$) as demonstrated in Figure 3-10. After feeding, duration of contractions briefly increased before gradually decreasing. Then, 18 hours after feeding, contraction duration started to increase steadily until the next feeding. There was a significant treatment × hour interaction for contraction frequency, which is depicted in Figure 3-11. There were no differences between treatments for contraction frequency at hours 4, 5, 7, 8, 9, 10, 12, 14, 16, 17, 18, 20, 22, 23, or 24. Contraction frequency was greater for HIGH d1 than MED or HIGH d2 at hour 3, yet HIGH d1 frequency was lower than HIGH d2 at hour 19 and lower than MED at hour 21. HIGH d2 frequency was lower

than MED or HIGH d1 at hour 1 and 15, lower than HIGH d1 at hour 2, 3, and 11, as well as being lower than MED at hour 6 and 13. Contraction peak pressure was greater on HIGH d2 than the other treatments.

Discussion

The purpose of the research was to propose systems for simultaneous monitoring of individual feed intake and rumen motility, as well as algorithms for analyzing data from such systems to characterize feeding behavior and ruminal contractions. This technology would be particularly beneficial in scientific or research settings where animals are housed individually. Ability to gather and easily analyze data pertaining to these events is crucial for understanding interrelationships between animal physiology and behavior. Automated systems for measuring and quantifying feeding behavior and ruminal motility are necessary due to the amount of events each animal exhibits in a feeding cycle. For example, in our experiment using 8 animals recorded for just 3 feeding cycles or days, 268 meals and 50,441 ruminal contractions were detected.

Feeding behavior of cattle has typically been collected by direct observation or time-lapse video recordings (Friend et al., 1977; Vasilatos and Wangsness, 1980). Additionally, before the development of technologies for intake monitoring, feed intake had to be calculated by manually measuring feed provided and refused. These procedures are labor intensive and difficult to conduct for a large number of animals or over an extended period of time, particularly if the researcher is interested in characterization of feed intake for each bunk visit or meal. Thus, several validated measurement systems have used feed disappearance recorded from feed bunks mounted onto load cells to calculate feed intake and/or characterize feeding behavior, such as the Insentec system

(Hokofarm Group B.V., the Netherlands; Tolkamp et al., 2000a; Halli et al., 2015), Pinpointer feeders (Universal Identification Systems Corp., Cookeville, TN; Cole, 1995), Calan gates (American Calan, Northwood, NH; Cole, 1995), Intergado monitoring system (Intergado Ltd., Contagem, Minas Gerais, Brazil; Chizzotti et al., 2015), and others (Bach et al., 2004). The use of radio frequency identification (RFID) tags has also improved the ability to measure feeding behavior on group-housed animals. Some of these RFID systems record bunk visits and location to determine time spent at the bunk and duration of each bunk visit, but do not record feed intake (Sowell et al., 1998; Sowell et al., 1999; DeVries et al., 2003) because bunks are not connected to load cells.

Our system utilized feed disappearance from the bunk to characterize feed intake and feeding behavior. A 1-min interval between weight recordings, such as used with our system, is believed to be a reliable indicator of short-term feeding behavior (Robles et al., 2007). The stored load cell data downloaded from the data logger in this experiment provided a distinct time-series recording where weight of the feed in the bunk decreased in ramps (ie. meals) over time. Feeding behavior of the animals could be assessed by evaluating reduction in weight of feed in the bunks over time. Whereas the previously mentioned systems focus on bunk visits to characterize feeding behavior, our algorithm emphasizes natural hunger-satiety patterns and characterizes feeding behavior by "meals." In addition, meals have been found to be a more biologically relevant unit to characterize short-term feeding behavior than visits (Tolkamp et al., 2000a).

Previous assessment of short-term feeding behavior has used a log-survivorship, log-frequency, or log-normal analysis to split eating events into bouts or meals (Tolkamp et al., 1998; Tolkamp and Kyriazakis, 1999; Tolkamp et al., 2000b) with varying inter-

meal intervals between animals. However, the algorithm described in this article applied a set inter-meal interval criterion to separate eating events into meals across all animals. This interval was determined by manually adjusting the variable in whole minute increments until a value was found which appeared to fit all data sets accurately. Increasing or decreasing the inter-meal interval from the value chosen had little effect on the end feeding behavior results. We believe that the inter-meal interval selected was appropriate for determining overall feeding behavior whilst avoiding over-analyzing the data. In doing so, differences in animal responses detected could be attributable to differences between treatments instead of confounding results due to potential differences between animals.

Based on the evaluation performed for the *LoadCell*.m MATLAB script and due to the relatively small percentage of error, we believed that the script was appropriately detecting meals when there was a reduction in feed from the feed bunk. The script underestimated as-fed intake and DMI by approximately 15%, suggesting this algorithm was not accurate for directly estimating feed and DMI. However, the script was sensitive enough to resolve differences in DMI between treatments that were evident by manual measurement, despite the smoothing used in the algorithm. In another study, animals undergoing an acidosis challenge reduced meal size and increased meal frequency (DeVries et al., 2009). Yet, there were no differences between treatments in this study, which may have been due to the mild severity of the challenge and the large animal-to-animal variation in response to acidosis challenges (Huber, 1971; Brown et al., 2000; Bevans et al., 2005). The results consistently showed biologically acceptable values for feeding behavior across all treatments and variables, when compared to the range of other

reported feeding behavior analyses (Tolkamp et al., 2000a; Robles et al., 2007; Moya et al., 2011; Carlson et al., 2014; Moya et al., 2014; Swanson et al., 2014). Granted, the range of feeding behavior values is highly variable due to differences between rations, feeding conditions and frequency, and management strategies. A comparison and validation of meal patterns and sizes by visual observation would also be beneficial for future work.

Various methods have been reported in the literature for measuring forestomach motility. However, some are invasive, such as electromyography (McLeay and Smith, 2006; Poole et al., 2009), or require extensive manual analysis of pressure recordings (Titchen, 1960; Colvin and Daniels, 1965; Froetschel et al., 1986), which can limit the duration of recording to an amount of data that may be analyzed in an appropriate time-frame. Other technology systems that have been used for motility assessment adapted hardware and software designed for a different type of signal and did not allow for export of time series data (Egert et al., 2014). Several researchers have used a water-filled tube or balloon to record intraruminal pressure within contents and measure forestomach motility (Colvin and Daniels, 1965; Kezar and Church, 1979; McSweeney et al., 1989; Dado and Allen, 1993; Egert et al., 2014). By this method, contractions of the forestomach increase the pressure in the rumen, and changes in pressure relate to rumen motility. Therefore, our balloon and catheter system for recording intraruminal pressure appears to be an appropriate method for collecting motility data. As ruminally-cannulated animals are commonly used in nutritional physiology research on cattle, this system could be used by many researchers without additional training or animal surgery. Motility of the reticulo-rumen recorded by the system described in this paper generated pressure

versus time-series data, which enabled the freedom of choosing a type of analysis. When ruminal contractions occurred, a waveform was evident in which pressure gradually increased and then gradually decreased to relative baseline. Analysis of rumen motility and characterization of ruminal contractions were derived from these data.

Upon evaluating the motility script, ruminal contractions appeared to be accurately detected and few errors were found in the analysis, especially in relation to the number of events or contractions that were detected. Other published studies have reported amplitude of reticuloruminal contractions ranging from about 6 to 20 mmHg (Kezar and Church, 1979; Gregory, 1982; Crichlow, 1989; Egert et al., 2014). The contractions of the ventral sac reported in this article averaged between 9 and 11 mmHg, which places our results within the range of other published studies. Similarly, frequency of reticuloruminal contractions has been reported to range from about 0.75 to 2.85 contractions per minute. Again, the frequencies reported for the three treatments in our experimental application were within the reported range and appear to be biologically relevant. However, it is important to note that the values reported for contraction amplitude and frequency are likely dependent on the method of recording motility, animal management and diet, time after feeding that motility was recorded, and forestomach compartment or sac from which the pressure is measured. Contraction duration has not been commonly reported in the literature, but duration of ventral sac contractions measured using a different recording system and software for analysis was approximately 10 seconds (Egert et al., 2014). Since the average durations of ventral sac contractions from our experiment were approximately 12 seconds, our method provides results that are physiologically acceptable. Pressure at the peak of ruminal contractions is

variable and largely depends on whether the animal is standing or laying (Egert et al., 2014). Therefore, it may serve as an indirect indicator of lying time in cattle. The experimental results would indicate that animals laid down more on the second day on the high grain diet than the other treatments.

Conclusions

This article described systems for monitoring feeding behavior and reticulorumen motility in cattle and detailed algorithms for data analysis of the data received from these systems. Evaluation of the algorithms and how accurately they characterized feeding behavior and rumen motility produced low error rates, leading to successful evaluation of the systems and algorithms. An experiment applying these systems and analyzing the data using the described algorithms produced acceptable results that were close to or within the range of values previously published using less automated methods. As a result, these systems and algorithms may have important applications for ruminant physiology and behavior research in future studies.

Tables and Figures

Table 3-1. Comparison of feeding behavior before the dietary switch (MED) and 2 days immediately following switching diets (HIGH) using meal detection script results

Variable	Dietary Treatment			SEM[3]	*P*-value[4]
	MED[1]	HIGH[2] d1	HIGH d2		
Manual as-fed intake, kg/d	19.60[a]	18.98[a]	15.80[b]	1.52	0.0449
Manual dry matter intake, kg/d	10.91[ab]	12.35[a]	10.22[b]	0.67	0.0965
Script-generated as-fed intake, kg/d	16.20	15.96	14.04	2.50	0.1593
Script-generated dry matter intake, kg/d	8.42[b]	10.35[a]	9.05[ab]	0.67	0.0288
Meal frequency, meals/d	11.1	11.8	13.0	1.22	0.1810
Meal duration, min	17.98	19.43	15.67	1.70	0.3062
Meal size, g (as-fed)	1463.9	1560.5	1264.2	208.1	0.5013

[1] MED = 70% concentrate diet
[2] HIGH = 90% concentrate diet
[3] SEM = Standard error of the mean
[4] Data were analyzed using proc MIXED of SAS 9.4 (SAS Institute Inc., Cary, NC) for the effect of treatment.
[a-b] Means within a row without common superscripts differ ($P < 0.05$)

Table 3-2. Comparison of rumen motility contractions before the dietary switch (MED) and 2 days immediately following switching diets (HIGH) using motility detection script results

Contraction Variable	Dietary Treatment			SEM[3]	*P*-value[4]		
	MED[1]	HIGH[3] d1	HIGH d2		Trt	Hour	Trt* Hour
Amplitude, mmHg	10.61[a]	9.33[b]	9.28[b]	0.38	<0.01	0.632	0.113
Duration, s	12.20[a]	11.94[b]	11.91[b]	0.21	<0.01	<0.01	0.661
Frequency, contractions/min	1.72[a]	1.67[a]	1.55[b]	0.16	<0.01	<0.01	<0.01
Peak pressure, mmHg	22.42[b]	22.26[b]	24.60[a]	1.49	0.049	0.264	0.453

[1] MED = 70% concentrate diet
[2] HIGH = 90% concentrate diet
[3] SEM = Standard error of the mean
[4] Data were analyzed using proc MIXED of SAS 9.4 (SAS Institute Inc., Cary, NC) for the effect of treatment, hour, and their interaction, using hour as a repeated measure.
[a-b] Means within a row without common superscripts differ ($P < 0.05$)

Figure 3-1. Example set-up of a feed bunk suspended from the S-beam load cell mounted onto the support bracket in front of an animal's pen.

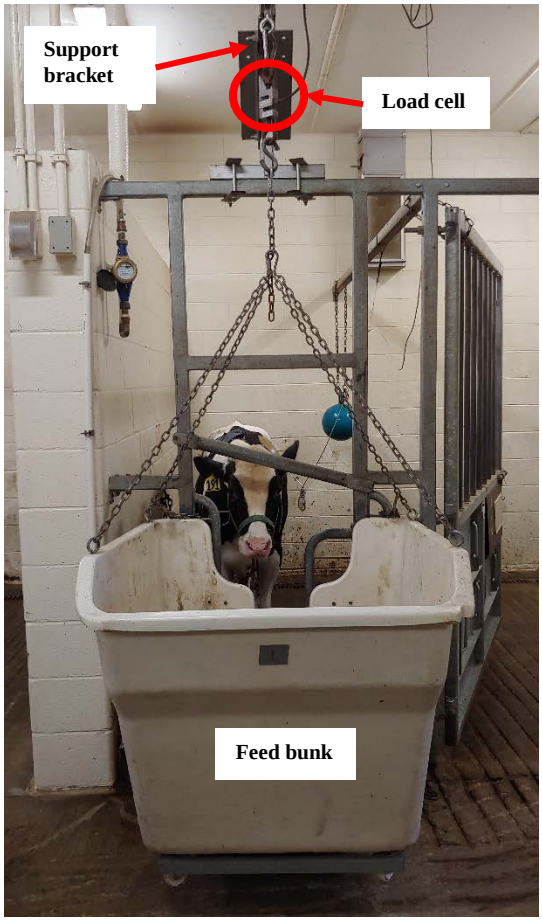


Figure 3-2. A) A 2-L water-filled balloon attached to a catheter using castration bands placed over top of the balloon on the tubing between tubing cuffs and secured with plastic hose clamps. B) Display of how the balloon catheter exits the rumen and the pressure transducer is secured to the rumen cannula. Zip ties were used to make sure connections between the catheter and transducer remained tight, to stabilize the stopcock so that it remained open to allow passage of water from the catheter to the transducer, and to attach the pressure transducer to the rumen cannula.

Figure 3-3. Flow diagram illustrating the process of feeding behavior analysis using MATLAB "*LoadCell*.m" script.

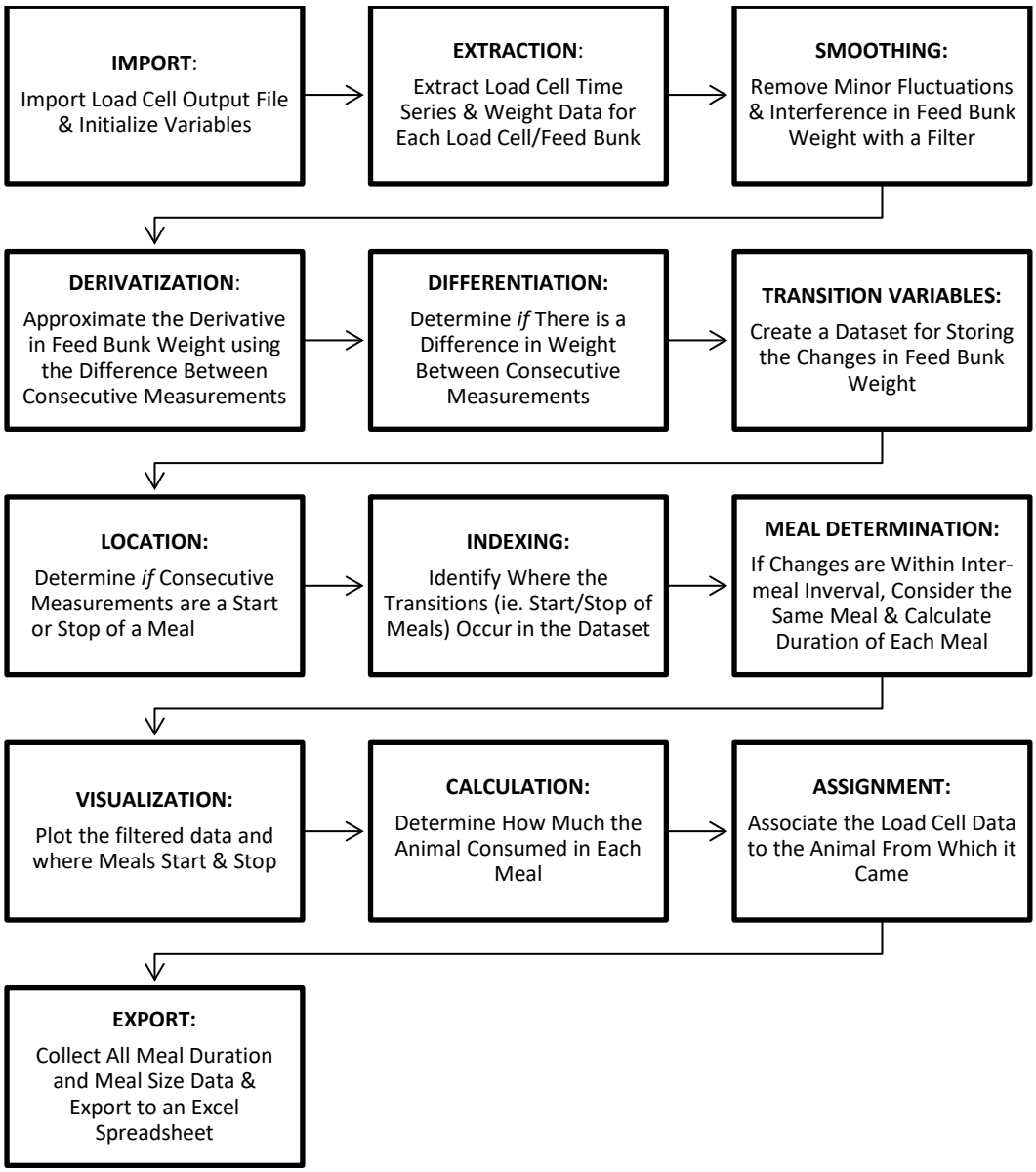


Figure 3-4. Example load cell data with meal start and end times shown.

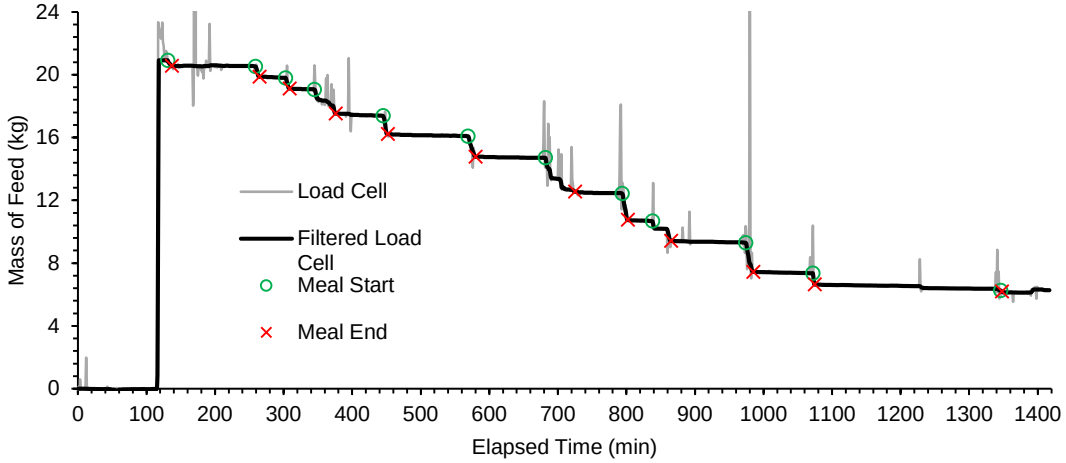


Figure 3-5. Comparison of raw and filtered data from rumen motility recordings for a short period of time.

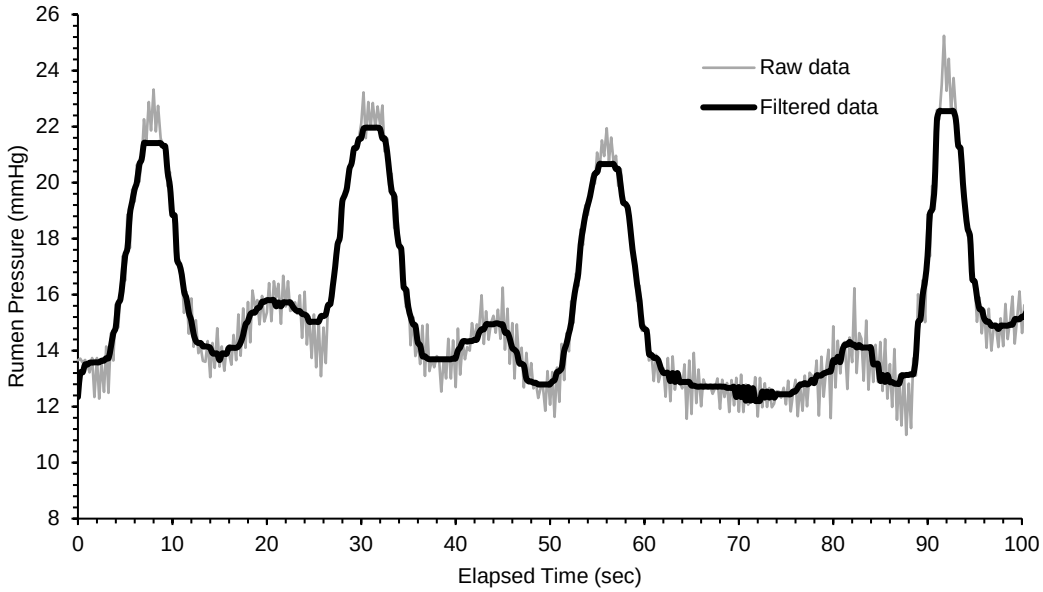


Figure 3-6. Analysis of sample rumen motility data using the MATLAB “findpeaks” function (figure exported from MATLAB). Prominence was used as the contraction amplitude. However, width (half-prominence) was not used in any calculations for contraction characteristics.

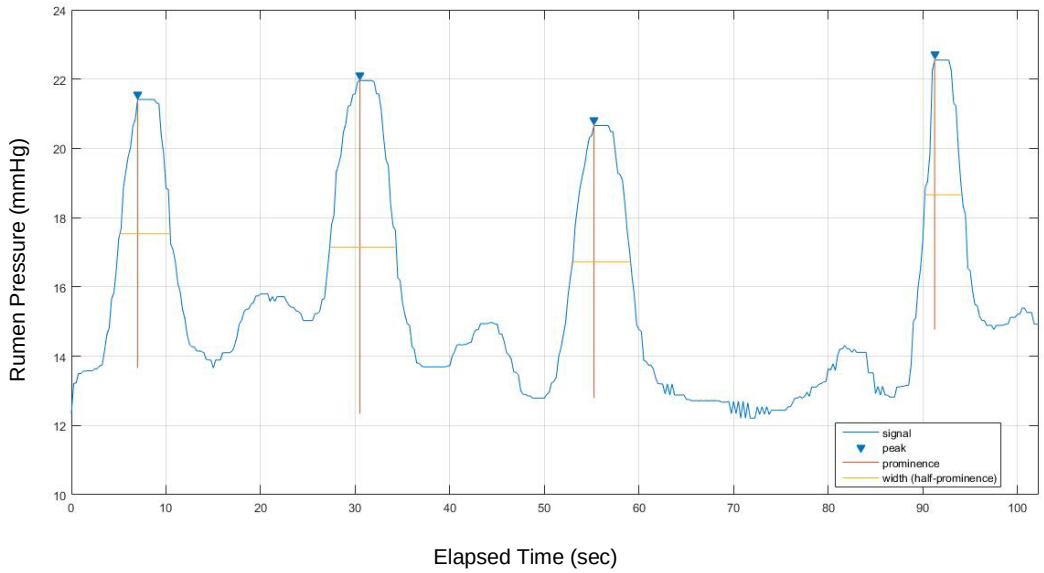


Figure 3-7. Identification of rumen contraction start, end, and peak values from sample data. The contraction start and end points were determined by calculating the first derivative of the filtered data and determining when the derivative surpassed start and stop thresholds. Contraction peak values were obtained from MATLAB “findpeaks” function.

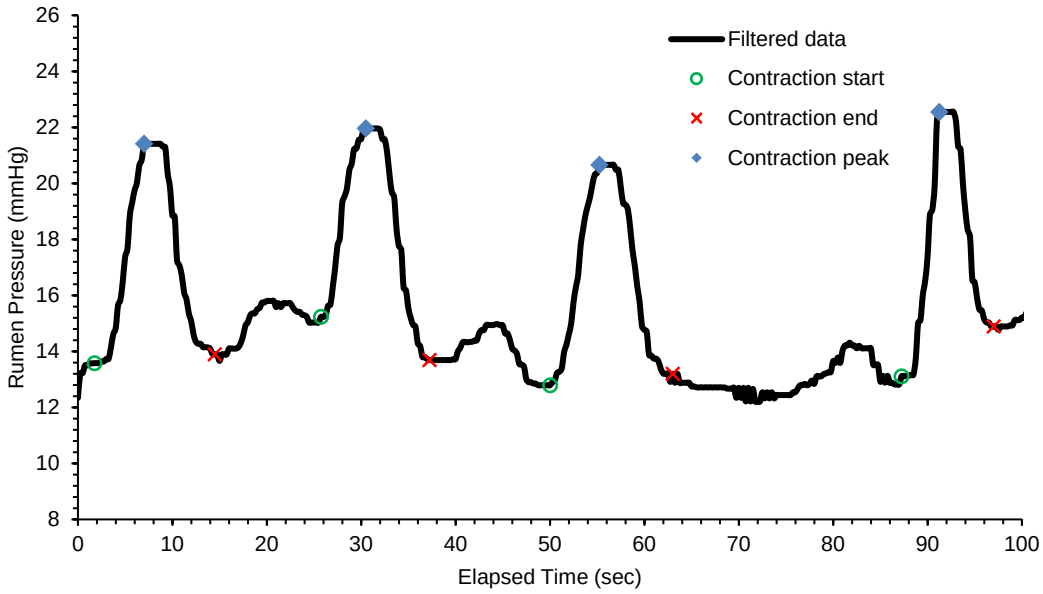


Figure 3-8. Example error of a meal size and length being underreported. Both the start and the end of the meal were incorrectly identified due to a more gradual change in slope until the thresholds were reached.

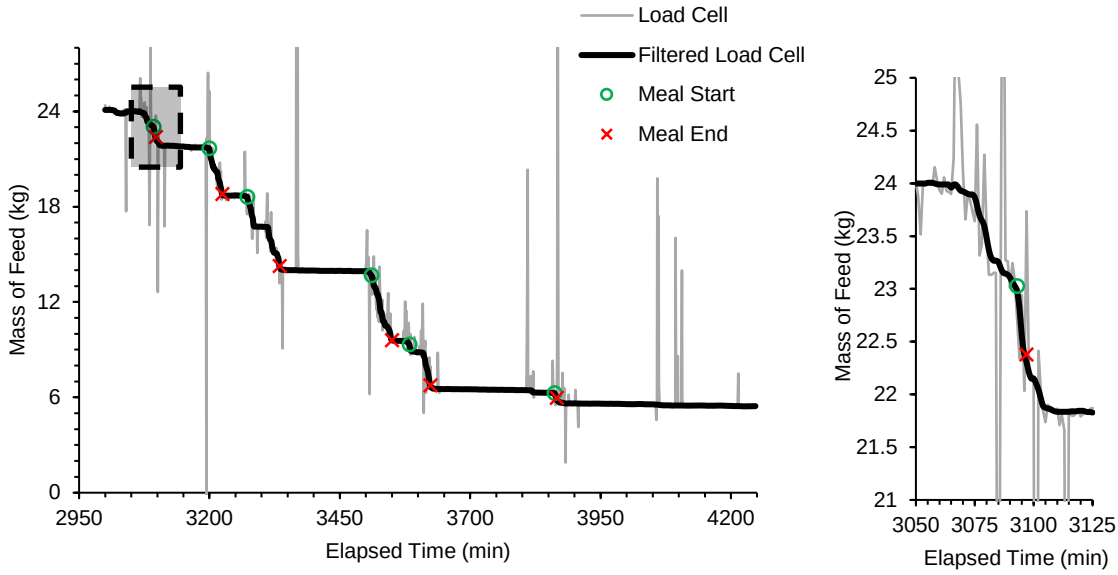


Figure 3-9. Example error of a contraction stop and duration being misrepresented due to the odd-shape of the contraction waveform.

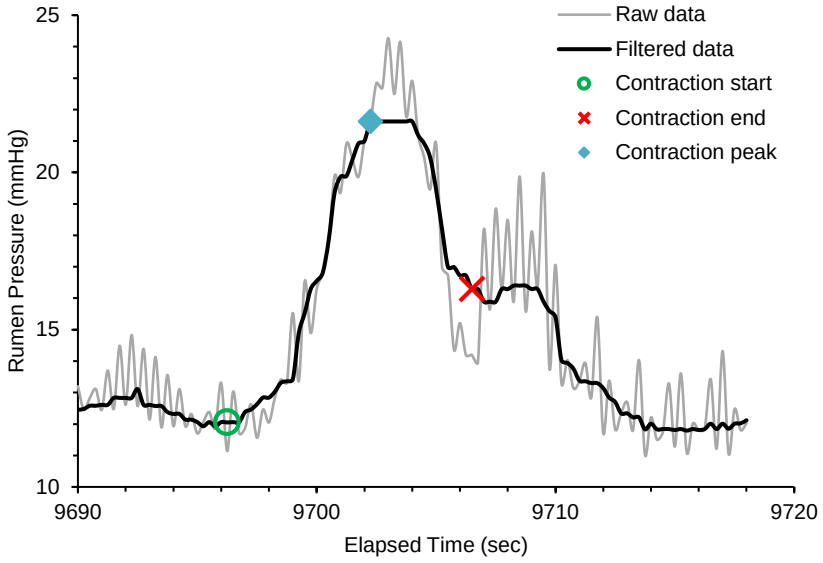


Figure 3-10. Duration of ruminal contraction throughout the feeding cycle as determined by analysis using the ruminal contractions algorithm. Contraction duration was calculated by subtracting the end time of the contraction from the start time. Error bars represent the standard error of the mean contraction duration.

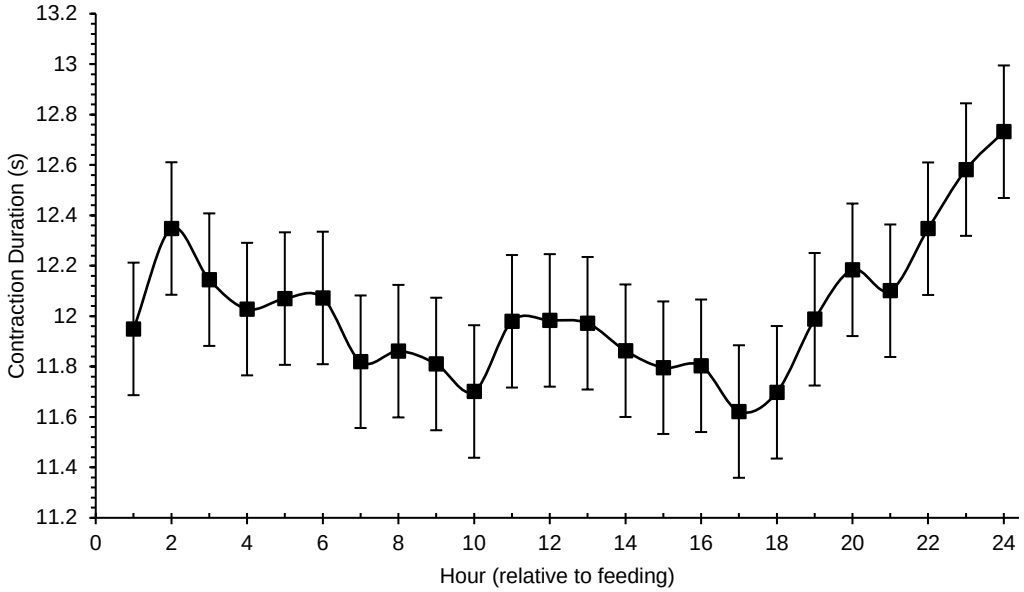
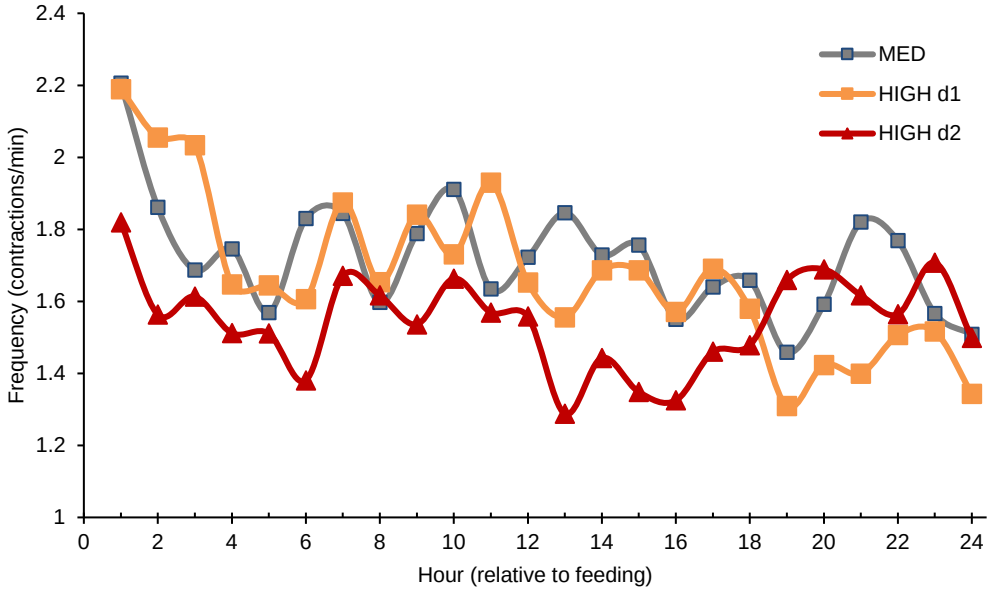


Figure 3-11. Ruminal contraction frequency throughout the feeding cycle determined from number of contraction peaks identified using the ruminal contraction algorithm. Rumen motility was measured using a water-filled balloon inserted into the ventral sac of the rumen connected to a pressure transducer and signal integration system. Pooled SEM = 0.197.



CHAPTER 4: A MODERATE TRANSITION OF BEEF HEIFERS TO A 90% CONCENTRATE DIET AS A MODEL FOR SUBACUTE RUMINAL ACIDOSIS INDUCED ALTERATIONS IN FEEDING BEHAVIOR, RUMEN ENVIRONMENT, RETICULORUMEN MOTILITY, AND BLOOD ACID-BASE STATUS

Introduction

The transition period when cattle are acclimated from a high-forage diet to a high-grain, rapidly fermentable diet increases the risk for ruminal acidosis (Bevans et al., 2005; Brown et al., 2006). If ruminal acidosis occurs, cattle typically decrease feed intake, exhibit erratic feeding behavior (Fulton et al., 1979a; Cooper et al., 1999b), or may go "off-feed" (Fulton et al., 1979b; Kezar and Church, 1979). Ultimately, these changes result in lower average daily gains (Koers et al., 1976; Owens et al., 1998) and significant losses for producers. Yet, relationships between ruminal acidosis and feed intake variation or potential mechanisms for these alterations have not been fully described.

One aspect of rumen physiology that has received little attention in cattle fed high concentrate diets is rumen motility. Since rumen motility can influence digesta passage out of the rumen, it has potential to alter feed intake. Several overlapping factors of regulation suggest that control of motility and feed intake are related (Bruce and Huber, 1973; Church, 1976; Grovum, 1986; Kaya et al., 1992). Ruminal stasis and reductions in contraction amplitude and frequency have been demonstrated with acute acidosis (Juhász and Szegedi, 1968; Bruce and Huber, 1973; Cebrat, 1979; Kezar and Church, 1979), but rumen motility during typical diet transitions, which induce SARA, has not be investigated. Rumen motility directly influences rate of fermentation, and reduced motility may serve as a protective mechanism to prevent further reductions in ruminal

pH. There is no evidence in the literature to relate rumen motility with ruminal pH and voluntary feed intake in cattle other than acute acidosis causing ruminal stasis. Feeding behavior and reticulorumen motility may directly affect animal health and productivity by influencing the rumen environment, specifically rumen acid-base balance.

The objectives of this experiment were to 1) characterize a moderate transition of beef cattle to a high-grain diet in regards to feeding behavior, liquid passage rate, ruminal pH, rumen fluid VFA, ruminal temperature, rumen motility, and blood acid-base status, 2) determine if this typical dietary transition and associated SARA would impact rumen motility, and 3) evaluate the relationships between feeding behavior, ruminal pH, and rumen motility.

Materials and Methods

All procedures used in this experiment involving heifers were approved by the University of Kentucky Institutional Animal Care and Use Committee (2018-2973) and conducted at the University of Kentucky C. Oran Little Research Center, Beef Unit, in Versailles, KY.

Animals and Experimental Design

Eight ruminally-cannulated Angus crossbred beef heifers (BW = 534 ± 23 kg) were adapted to a 70% concentrate, high-moisture corn-based diet (T70; Table 4-1) and remained on this diet for 14 d prior to the initiation of the trial. For logistical reasons and due to frequency of sampling, the experiment was conducted in 3 blocks of experimental animals (block 1, n = 3; block 2, n = 3, and block 3, n = 2). At the beginning of the trial, animals were weighed, randomly assigned to blocks, and one block was moved indoors

for housing in individual stalls. Heifers were accustomed to being housed indoors from previous experiences, but they were given a 6 d acclimation period in indoor housing while being fed ad libitum the transition diet, T70, before experimentation began (total adaptation period was 14 d). All animals had ad libitum access to water. Heifers were fed T70 for 3 (d -2 through 0) additional days to allow for baseline measurements on the T70 diet.

On d 1, all animals were switched to a 90% concentrate, high-moisture corn-based diet (H90; Table 4-1) offered ad libitum for 4 days (d 1-4). Data were collected throughout the experiment with sampling occurring for different physiological measurements at various time points as discussed below. The process was repeated for the second and third blocks of animals as described above.

Feeding Behavior

Feeding occurred at 0900, and feed samples were collected daily, composited for each diet, and analyzed for nutrient composition by wet chemistry through Dairy One Forage Laboratory (DM: AOAC method 930.15; ash: AOAC method 942.05; crude protein: AOAC method 990.03; fat: AOAC method 2003.05; ADF: ANKOM Technology method 5; aNDF: ANKOM Technology method 6; lignin: ANKOM Technology method 9; minerals: samples digested using CEM Microwave Accelerated Reaction System with MarsXpress Temperature Control and analyzed by ICP using a radial spectrometer). Orts were collected daily at 0800, weighed, and recorded from the previous day in order to adjust feed amounts and maintain at least 5% excess throughout the experiment as well as calculate individual feed intake. Water consumption was measured using flow meters and recorded daily at the time of feeding to determine daily water intake. On d -2, -1, 1, and

2, frequency of meals (meals/d) and average meal size and duration were calculated for each animal on each day by inputing feed disappearance data from feed bunks mounted onto load cells (LC101-500/LC111-500, Omegadyne, Sunbury, OH) which record weight at 1-min intervals into a meal detection algorithm written in MATLAB R2015b (The MathWorks, Inc., Natick, MA) as outlined previously (Dissertation Chapter 3). A meal was defined as a continuous feeding event, where a break of at least 30 min with no weight changes was used to differentiate between meals. Additionally, the time for consumption of 25%, 50%, 75%, and 100% of daily intake was calculated by inputting feed disappearance data into GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA) and modeling using the exponential – one phase decay model with automatic outlier elimination.

Rumen Fluid Sample Collection and Analyses

Rumen fluid samples were collected via a suction strainer from the ventral sac. A slit in the cannula plug was created, which allowed passage of the strainer, to ease the stress of multiple cannula plug removals on the animals and to avoid possible disruption of the ruminal environment. Approximately 100 mL of rumen fluid was collected from each animal immediately before feeding (0 h) and 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 54, 60 & 66 h after feeding on d -2 during 70% concentrate diet feeding and at the same times relative to feeding on d 1 during 90% concentrate diet feeding. A 15-mL sample of rumen fluid from each animal was transferred to screw-top conical vials and centrifuged for 5 min at 2000 × *g*. Duplicate 1-mL samples of supernatant rumen fluid from each animal and time point were processed for VFA analysis by being placed into microcentrifuge tubes, combined with 100 μL 85 mM 2-ethylbutyrate internal standard,

capped, and mixed for approximately 2 seconds using a vortex. Next, 100 μL 50% meta-phosphoric acid were added, tubes were recapped, mixed for approximately 5 seconds using a vortex, and frozen (-4°C) to allow for protein precipitation. Tubes were thawed, centrifuged at 20,000 × *g* for 20 min, and the supernatant was transferred to GC injection vials and capped. Gas chromatography with a flame ionization detector (Agilent HP6890 Plus GC with Agilent 7683 Series Injector and Auto Sampler; Agilent Technologies, Santa Clara, CA) and a Supelco 25326 Nukol fused silica capillary column (15 m × 0.53 mm × 0.5 μM film thickness; Sigma/Supelco, Bellefonte, PA) were used to determine VFA concentrations in the rumen fluid samples. Analysis involved injection of 0.2 μL of each sample in duplicate at 110°C with a 2:1 split, a 1-min hold, temperature increase at 5°C/min to 125°C for 2 min, and the set point for inlet and injector at 260°C.

Ruminal pH & Temperature Measurements

Ruminal pH (RpH) was monitored (readings every 1 min) using rumen data loggers (SRL-T9, DASCOR, Inc., Escondido, CA) for 48 h beginning immediately prior to feeding on d -2 and d 1. A data logger and lithium ion battery sealed in a watertight capsule was equipped with a pH electrode exposed to the rumen contents but unable to contact the ruminal epithelium (Penner et al., 2006). This device was inserted into the ventral sac of the rumen through the cannula before feeding on d -2 and 1. The pH electrodes were calibrated before and after each measurement period using pH buffers 7 and 4. Ruminal pH was measured and recorded continuously from 0 to 24 h relative to feeding the T70 diet on d -2 and -1 as well as 0 to 24 h relative to feeding the H90 diet on d 1 and 2. When not in use, the pH electrodes were stored in a storage solution (DASCOR, Inc.). Data were stored in the data logger and downloaded after each use.

From these data, minimum RpH, mean RpH, maximum RpH, duration RpH < 5.8 (min/d), duration RpH < 5.6 (min/d), and duration RpH < 5.5 (min/d) were determined and summarized for each animal and each 24-h period or day. Furthermore, mean RpH was calculated for each hour on each day.

The data logger was also equipped with a built-in sensor to measure ruminal temperature (Trum) at the same time pH was recorded (Mohammed et al., 2014). Minimum Trum, mean Trum, and maximum Trum were determined for each animal on each day. Additionally, mean Trum was calculated for each hour on each day.

Rumen Fill and Liquid Dilution Rate Estimates

Prior to feeding on d -3 and 4, the rumens of heifers were completely manually evacuated to determine rumen fill. Contents for each animal were weighed, thoroughly mixed by hand, and subsampled in triplicate for DM analysis (dried at 55°C for 48 h). Remaining contents were then immediately returned to the animal. Dry contents of the rumen were determined by multiplying the weight of wet contents by the average DM percentage of rumen contents divided by 100.

On d -2 and d 1, animals were intraruminally pulse-dosed with 500 mL Cr:EDTA solution (53 m*M* Cr, adjusted to pH 6.7) immediately before feeding at 0855 to evaluate liquid passage from the rumen. The Cr:EDTA solution was spread throughout the rumen by injection into various areas. Approximately 100 mL of rumen fluid was collected via suction strainer from the ventral sac at 0 (before feeding, before Cr:EDTA dosing), 1, 2, 4, 8, 12, 18, and 24 h post-dosing. Ten mL samples of fluid from each animal and time point were placed in a conical vial and frozen (-4°C). Samples for the 4, 8, 12, and 24 h time periods were taken from the 100 mL collected for VFA analysis mentioned above to

prevent excessive removal of rumen fluid. After thawing, samples were centrifuged at 20,000 × g for 30 min. One mL of supernatant was transferred to glass test tubes and diluted using nanopure water at a ratio of 1:4 for analysis. Chromium concentrations for each sample were determined in triplicate using atomic absorption analysis (AAnalyst 200, PerkinElmer Inc., Waltham, MA) at a wavelength of 357.87 nm. If sample responses were out of the range of the standard curve, a portion of the sample was diluted until within the concentration range and reanalyzed. Liquid volume of the rumen and passage rate variables were estimated by linear regression of the natural logarithm of Cr concentration against sampling time using GraphPad Prizm 7 (GraphPad Software, Inc., La Jolla, CA). Rate of outflow was determined by the following equation:

$$|P| = \frac{Cr_d}{Cr_z}\ (FDR)$$

Where P represented liquid passage rate (L/h), Cr_d represented the amount of Cr dosed (mg), Cr_z represented the concentration of Cr at time zero (mg/L), and FDR represented the fractional dilution rate of Cr (slope or /h). Ruminal liquid dilution rate (%/h) was calculated as the absolute value of FDR*100. Retention time (RT) was calculated as the absolute value of 1/FDR. Liquid half-life in the rumen was calculated as the absolute value of natural log of 2/FDR. Rumen liquid volume (L) was determined by dividing the amount of Cr dosed (Cr_d) by the amount of Cr present at time zero (Cr_z).

Motility Measurements

Rumen motility was measured continuously for 48 h beginning immediately prior to feeding on d -2 and d 1. Disposable blood pressure transducers (MLT0670; ADInstruments Inc., Colorado Springs, CO) connected to a PowerLab 8/30

(ADInstruments, Inc.) through bridge amplifiers (FE221; ADInstruments, Inc.) were used to monitor pressure changes in the rumen and characterize rumen contractions. A water-filled (2 L) balloon attached to a Tygon catheter (i.d. = 3.2 mm; o.d. = 6.4 mm) was inserted into the ventral sac of the rumen on d -2 and d 1 prior to feeding. Balloons were weighed to maintain consistent fill between animals and were changed if the balloon broke or leaked. A small hole was made in the plug of the rumen cannula to allow the balloon catheter to pass through. The end of the catheter external to the animal was equipped with a tubing-luer lock adapter for connection to the pressure transducer stopcock. Data were recorded using LabChart software (ADInstruments, Inc.) and imported into MATLAB for smoothing and analysis.

Analysis of rumen contractions was conducted as described previously (Chapter 3) with modifications. Briefly, pressure versus time data were imported into MATLAB and filtered using a one-dimensional median filter (order = 15). The “findpeaks” function of MATLAB was used to detect contraction peaks through the duration of the recording. Peak prominence was used as the contraction amplitude, and the amplitude threshold was set at 4 mmHg. The start and end of each contraction was determined by scanning the derivative of the pressure signal and finding the time where the derivative of the pressure signal fell below the slope threshold of 0.05 on either side of the peak. A peak offset of 10 was used to prevent the flat portion at the peak from being detected as the beginning or end. Then, the duration of each contraction was calculated by taking the difference of the start and stop times. Contractions that had amplitudes (mmHg) or durations (s) greater than 100 were removed due to inconsistency with biological conditions. Means for contraction amplitude and duration were generated for each animal, day (Avg. of T70

days, d1 on H90, and d2 on H90), and hour using SAS. Contraction frequency (contractions/min) was calculated as the number of peaks detected each hour divided by 60 min. Statistical analysis was performed on these means.

Blood Samples and Measurements

The jugular vein of the heifers was catheterized (BD Angiocath venous catheter, 14 g, 5.25") on d -3 and an I.V. catheter extension set was attached. Before sample collection, 10 mL of blood and heparinized saline was extracted and discarded. An approximately 1 mL blood sample from each animal and time point were then collected into a sterile, heparinized syringe, capped, and placed on ice, for no more than 30 min, until analysis in duplicate for pH, pCO_2, pO_2, Na^+, K^+, and Ca^{2+} via a blood gas analyzer (GEM Premier 3000, Instrument Laboratory, Bedford, MA). After collection, catheters were filled with approximately 5 mL heparinized saline (20 U heparin/mL) to prevent clotting. Blood HCO_3^- was calculated from the pH and pCO_2 using a pK of 6.1 for the bicarbonate buffering system and the following equation, derived from the Henderson-Hasselbach equation:

$$HCO_3^- = 0.03 \times pCO_2 \times 10^{(pH-6.1)}.$$

Base excess (BE) was determined using the following equation:

$$BE = (0.02786 \times pCO_2 \times 10^{(pH-6.1)}) + (13.77 \times pH) - 124.58.$$

Statistical Analysis

Animal was considered the experimental unit. Effects were considered significant at $P < 0.05$, and effects with $0.05 < P < 0.1$ were considered to show a tendency. The lsmeans were calculated for each analysis and compared when significant effects were present using the least significant difference (LSD) test. All data were analyzed using

proc MIXED of SAS 9.4 as a randomized complete block with repeated measures, where block was always considered a random effect. An autoregressive covariance structure was used for repeated measures models, unless otherwise noted. Since timing of events was one of the critical inquiries of this study, the treatment was time relative to the high-grain diet/SARA challenge. Thus, most variables were compared between the average of days on T70 (to establish a baseline) and each day on the H90 diet (d1 H90 and d2 H90) making day (relative to transition) the only fixed effect in the model, and day was considered the repeated variable.

Additionally, mean ruminal pH and temperature for each hour and day were analyzed using proc MIXED of SAS as described above with modifications; fixed effects included in the model were day, hour, and the interaction, and day*hour was used as the repeated variable. Linear, quadratic, and cubic regressions were conducted using proc REG of SAS on the lsmeans for mean ruminal pH and temperature when effect of hour was significant to determine goodness of fit.

Rumen motility variables were analyzed as described above for ruminal pH, however, using a compound symmetry covariance structure. When an interaction was present, regressions (linear, quadratic, and cubic) were conducted on hourly lsmeans for each day to determine the nature of the response by day.

Mean VFA concentrations in rumen fluid and blood variables were analyzed for fixed effects of diet (T70 and H90), hour (0-66h) and the interaction, with diet*hour as the repeated variable.

Prior to analysis of rumen liquid dilution and passage related variables, four extreme observations were removed from this data set (one observation each for RT,

liquid half-life, rumen liquid volume, and liquid flow rate) because they were driving non-normality for these variables. Similarly, prior to analysis of rumen motility variables, extreme observations for each variable were removed in an effort to correct non-normality if they were greater than the third quartile + interquartile range or below the first quartile – interquartile range.

In order to ascertain relationships between feed intake, ruminal pH, and motility variables, daily mean values for variables in the above categories were used for multiple linear regression. Possible regressors included DMI, water intake, meal duration, meal size (g), meal frequency, minimum ruminal pH, mean ruminal pH, maximum ruminal pH, time ruminal pH < 5.8, time ruminal pH < 5.6, time ruminal pH < 5.5, mean ruminal temperature, contraction amplitude, contraction duration, and contraction frequency. When evaluating an intake and feeding behavior variable, all other intake or feeding behavior variables were removed from the model. Similar conditions were used for motility variables and ruminal variables. The REG procedure of SAS was used with backward elimination variable selection, which removed the variable in the model with the largest *p*-value at each step until all variables remaining in the model were significant at $\alpha = 0.05$.

Results

During this moderate transition from a 70% to a 90% concentrate, high-grain diet, DMI of heifers tended (*P*=0.087; Figure 4-1A) to be influenced by day relative to H90 feeding. Heifers tended to increase DMI on the first day of H90 feeding compared to T70 and then returned to T70 levels on the second day. Water intake was affected (*P*=0.008; Figure 4-1B) by day relative to H90 feeding, where water intake increased on the first

day of H90 feeding compared to T70. Total water intake tended (P=0.10) to be different between days, such that water intake on the first day of H90 was greater than on the second day of H90 feeding. However, average meal duration, size, and frequency were not affected by day (Table 4-2). Feeding behavior analysis resulted in significant effects of day for time to consume 25% (P=0.007), 50% (P=0.009), and 100% (P=0.036) of daily intake and tended to be significant for time to consume 75% (P=0.068) of daily intake (Table 4-2). The time it took heifers to consume 25%, 50%, and 100% of their daily intake was greater on d 2 of H90 feeding compared to T70 or d1 H90, meaning consumption rate was reduced on d 2.

Acetate, propionate, butyrate, valerate, total VFA, and butyrate proportion of total VFA were affected by time (P<0.001; Table 4-3) and displayed temporal patterns relative to feeding at 0, 24, and 48 h. There was also a significant effect of diet ($P\leq$0.001; Table 4-3) for propionate and total VFA, where concentrations were greater on H90 compared to T70. Additionally, there was a diet*time interaction for isobutyrate (P=0.034) and isovalerate (P=0.006) and tended to be an interaction for acetate (P=0.071; Table 4-3). Isobutyrate concentration at 4 and 8 h after feeding was greater during H90 than T70, whereas concentration at 66 h after feeding was greater during T70 compared to H90 (Figure 4-2A). Isovalerate concentrations were reduced on H90 compared to T70 at 30, 36, 54, 60, and 66 h post-feeding (Figure 4-2B). Interactions between diet and time also existed for acetate proportion (P<0.001), propionate proportion (P=0.037), and the acetate:propionate ratio (P=0.020). Acetate proportion of total VFA during H90 was reduced compared to T70 from 20 h through 66h (Figure 4-3A). Propionate proportion of total VFA during H90 was greater than T70 at 30, 48, 54, 60, and 66 h (Figure 4-3B). In

accordance with these results, the acetate:propionate ratio differed between diets at 4, 48, 54, 60, and 66 h, where the ratio was reduced during H90 compared to T70 at 48-66h (Figure 4-3C).

Ruminal pH minimum (P=0.003), mean (P<0.001), and maximum (P=0.007) were significantly influenced by day relative to H90 feeding (Table 4-4). Minimum and mean RpH were greater on T70 compared to either day on H90, yet maximum RpH decreased successively with each day on H90. The amount of time RpH was below thresholds of 5.8 (P<0.001), 5.6 (P<0.001), and 5.5 (P=0.002) were all affected by day, where amount of time below the thresholds increased successively with each day on H90 (Table 4-4). Minimum, mean, and maximum ruminal temperature were not affected by day. However, mean ruminal temperature tended (P=0.068) to have a day*hour interaction. Both mean RpH (Figure 4-4A) and Trum (Figure 4-4B) displayed an overall effect of hour or time (P<0.001) across both diets with quadratic responses (mean RpH: R^2=0.856; mean Trum: R^2=0.931). Mean RpH decreased to about 12 h after feeding and then increased (Figure 4-4A). Conversely, mean Trum increased until about 16 h after feeding and then decreased (Figure 4-4B).

The effect of diet was not significant for any rumen fill or rumen liquid dilution and passage rate related variables (Table 4-5).

Results from analysis of rumen motility variables are shown in Table 4-6. Reticulorumen contraction amplitude was reduced (P<0.001) on d 1 and d 2 of feeding the H90 diet compared to feeding the T70 diet (Figure 4-5). Contraction duration was reduced (P<0.001) on d 1 of H90 feeding compared to T70 or d 2 H90 (Figure 4-6A). Additionally, hour relative to feeding influenced (P<0.001) duration of contractions, with

a cubic (P<0.001; R^2=0.663) response through the 24-h feeding cycle (Figure 4-6B). There was a day*hour interaction (P<0.001) for frequency of reticulorumen contractions (Figure 4-7). The frequency response over time during T70 feeding was cubic (P<0.001; R^2=0.546). The best-fitting regression model for d 1 H90 was a linear model (P<0.001; R^2=0.620), because although the quadratic and cubic models were significant, the quadratic and cubic variables were not significantly different from zero, suggesting those models overfit the data. No regression model was significant for d 2 H90, but there were several time points that d 2 H90 was less than both T70 and d 1 H90 (1 and 2 h), less than T70 (6, 13, 15, and 16 h), and less than d 1 H90 (3, 7, and 9 h). Furthermore, d 2 H90 contraction frequency was greater than that of T70 at 23 h. Pressure at the peak of motility contractions was affected by day (P=0.009) of transition, where peak pressure on d 2 of H90 feeding was greater than that on T70 (Figure 4-8).

For jugular blood acid-base and electrolyte status, there were no diet*time interactions (Table 4-7). Blood pH tended to be influenced by time (P=0.063). The partial pressure of CO_2 was affected by time (P<0.001), where the response decreased after feeding and then increased slightly before each feeding at 24 and 48 h (Figure 4-9A). Blood HCO_3 concentrations were reduced (P=0.030) during H90 feeding and also affected by time (P<0.001), following a similar pattern as pCO_2 (Figure 4-9B). Similarly, base excess in blood was lower (P=0.024) on the H90 diet and impacted by time (P<0.001), displaying temporal patterns similar to pCO_2 and HCO_3 (Figure 4-9C). Conversely, ionized Na concentration in blood was greater (P<0.001) on the H90 diet and affected by time (P=0.013; Figure 4-10A). Ionized K concentrations were only affected by time (P<0.001; Figure 4-10B).

Multiple linear regression conducted using backwards elimination variable selection resulted in significant models for all dependent variables chosen, except DMI, some of which resulted in a simple linear model with only one variable remaining in the model (Table 4-8, Table 4-9, and Table 4-10). Mean ruminal pH and time below ruminal pH thresholds were the most common dependent variables regressing intake and feeding behavior, but reticuloruminal contraction frequency also helped moderately explain ($R^2 \approx$ 0.4-0.5) these variables (Table 4-8). Meal characteristic and ruminal pH variables were common regressors for motility dependent variables (Table 4-9). In fact, approximately 72% of the variation in contraction frequency could be explained by meal duration, meal size, mean RpH, and time that RpH < 5.5. Water intake and meal characteristics were significant drivers of ruminal pH related dependent variables (Table 4-10). Additionally, contraction frequency was also a significant regressor for time that RpH < 5.6 and 5.5. Although it resulted in a significant linear regression with contraction duration, only about 20% of the variation in mean Trum could be explained using this model. Thus, some of these models should be used with caution when used for predicting values of dependent variables.

Discussion

The aim of the present study was to interrelate feeding behavior, rumen variables and motility, along with acid-base status during a moderate transition of beef cattle to a high-grain diet. Both acute and subacute acidosis may be characterized by a period of "off-feed" (Fulton et al., 1979b; Kezar and Church, 1979), but less is known in regards to physiological changes associated with a mild or more typical dietary transition. While the impact of severe or acute ruminal acidosis had been demonstrated to strongly inhibit

(Cebrat, 1979; Kezar and Church, 1979) or completely abolish rumen motility (Juhasz and Szegedi, 1968; Bruce and Huber, 1973), the effect of a subacute condition on rumen motility was yet to be determined before this study.

During this experiment, there was a tendency for heifers to increase DMI on the first day of high-grain feeding (Figure 4-1A). Despite a return to baseline (T70) levels on the second day of high-grain feeding, the animals did not appear to reduce feed intake or go "off-feed" but rather decreased intake compared to the first day which is typical for stepwise transitions. It was logical that the animals consumed more water on the first day of high-grain feeding (Figure 4-1B) when they tended to consume more DM. This extra water may also have helped to alleviate some of the negative consequences of a greater DMI of a more rapidly fermentable diet by moderating ruminal pH, but this did not occur on the second day of high-grain feeding as water intake (including from feed) was lower indicating DMI was the primary driver for water intake (Figure 4-1C).

Feeding behavior can greatly impact rumen acid-base balance through its influence on fermentable substrate present in the rumen. For example, larger meals and faster consumption rates increase the acid production. If feeding behavior and rumen metabolism overwhelm the capacity of the animal to prevent acid accumulation in the rumen, ruminal pH would be depressed and feed intake may decrease (González et al., 2012). In the current study, there were no effects of transitioning cattle from the 70% concentrate transition diet to the 90% high-grain diet on meal duration, size, or frequency (Table 4-2). Conversely, another study had demonstrated that animals undergoing an acidosis challenge reduced meal size and increased meal frequency (DeVries et al., 2009). Meal size did appear to decrease as cattle were transitioned, but this was not

significant ($P \approx 0.14$; Table 4-2). The biggest impact on feeding behavior occurred on the second day of high-grain feeding with an increase in time to consume feed. Thus, because heifers tended to consume more feed on the first day of high-grain feeding but they did so in the same amount of time, they had a faster consumption rate on this first day of high-grain feeding compared to 70% concentrate feeding. This could have contributed to unfavorable conditions for ruminal acid-base balance and caused a reduction in ruminal pH, which led to animals reducing consumption rate (or increasing the time to consume feed proportions) on the second day of high-grain feeding, such as was seen in this study.

Volatile fatty acid concentrations in rumen fluid changed over time in patterns related to feeding, which have been previously demonstrated. As is typical for when cattle are switched to a diet with more grain, propionate and total VFA concentrations were greater on the diet containing more concentrate (H90; Table 4-3). This suggested that there was a greater acid load in the rumen on the higher concentrate diet, but ruminal total VFA concentrations increased only 10%.

The goal of this study to introduce a mild SARA was achieved as evidenced by the time below ruminal pH thresholds. One study used a ruminal pH below 5.6 for 180 min or more per day to diagnose SARA (Gozho et al., 2005), while another classified a ruminal pH less than 5.8 for approximately 100 min as a mild bout of SARA (Penner et al., 2010). In the current study, time that ruminal pH was below 5.6 was much less than 180 min on the 70% diet (Table 4-4). However, switching animals from a 70% to a 90% concentrate diet progressively increased the amount of time that ruminal pH was below 5.6, greater than 500 min/d, for the first two days on the high-grain diet. Meanwhile, the ruminal pH did not drop excessively low to indicate acute acidosis.

The patterns of ruminal pH and temperature over time were as expected for a once daily feeding, ad libitum bunk management scenario. Under these conditions, ruminal pH decreased from microbial fermentation of substrates immediately after feeding until about half-way through the feeding cycle, then returned to baseline levels before the next feeding (Figure 4-4A). Consequently, the heat of fermentation generated after feeding (Blaxter, 1962) likely resulted in a slight rise in ruminal temperature and then subsequent decrease after fermentation substrates available in the rumen began to diminish (Figure 4-4B). Despite having ad libitum access to feed, ruminal pH and temperature patterns mirrored that of animals fed once daily. Research has suggested that ad libitum feed bunk management is advantageous over alternative strategies because restricted feeding or clean bunk management can lead to animals consuming a few large meals during the day at a faster rate, which resulted in greater post-prandial reductions in ruminal pH (Erickson et al., 2003; Schwartzkopf-Genswein et al., 2003). Frequency of feeding has been shown to influence feed intake, eating rate, rumination patterns, and post-prandial patterns of ruminal pH (Robles et al., 2007; González et al., 2012). For example, if the animals in this study were fed twice daily instead of once, two low periods for ruminal pH would have been expected during the 24-h feeding cycle instead of only one. Increasing feeding frequency may lead to more stable ruminal pH and decreased acidosis problems by encouraging more meals, a more even distribution of feed intake over time, smaller meal sizes (particularly of meals immediately following feed delivery), and better synchronization of acid production, elimination, and neutralization with salivary buffers from rumination (Soto-Navarro et al., 2000; González et al., 2012).

Although cattle did not stop eating during this dietary transition which induced SARA, reticulorumen motility was, in fact, altered. Specifically, amplitude of contractions was reduced by about 10% on both the first and second day of high-grain feeding compared to on the 70% diet, and duration of contractions was reduced by about 2% on the first day of high-grain feeding but returned to normal on the second. These reductions occurred despite no obvious change in rumen fill within these first two days (personal observational data, ruminal fill, and liquid volumes). Both the first and second days of high-grain feeding indicated that animals were experiencing SARA. The frequency of reticulorumen contractions over time was influenced by day, where frequency declined linearly over time on the first day of high-grain feeding and was less than other days on the second day at several time points, which suggested a sustained reduction. Particularly, d2 H90 lacked the greater contraction frequency values immediately around feeding as seen in other days. Thus, the appearance of SARA coincided with a decrease in rumen motility, despite the small magnitude of changes. It is still unclear, however, which regulation pathway(s) for rumen motility (i.e. buccal receptors, acid receptors, osmoreceptors, central nervous system, or an inflammatory response from bacterial endotoxin) is/are responsible for the alterations (Svendsen, 1973; Huber, 1976; Grovum, 1986; Carter and Grovum, 1990; Andersen, 2003). Contraction duration was also influenced by time, which agreed with a previously published study (Egert et al., 2014) and displayed similar post-prandial patterns. The increase in contraction peak pressure on the second day of high-grain feeding could have been due to an alteration in lying behavior, where lying increased baseline pressure in the rumen and subsequently, peak pressure. Since the animals were experiencing long periods of low

ruminal pH by the second day after the dietary switch, they could have laid down more (not determined) often to ruminate. The instance of lying increasing pressure in the rumen was also observed in Egert et al. (2014).

Despite alterations in rumen motility, ruminal liquid dilution and flow rate were not affected within the first few days of switching to the high-grain diet (Table 4-5). However, it is unclear if particulate or fiber passage rate was affected. Due to the small average particle size and highly fermentable ingredients of these diets, it would be surprising if particulate passage was reduced, unless motility was completely abolished. Evidence in the literature suggested that there was a close, positive relationship between frequency of reticular contractions and rumen fluid liquid turnover rates when comparing diets of no hay, low hay or high hay content (Sissons et al., 1984). In contrast, another study demonstrated that duration of reticular contractions was the influential variable of ruminal motility that can affect fractional passage rates of ruminal fluid and particulate matter (Okine et al., 1989). However, this study used a 100% forage-based diet of ground bromegrass and alfalfa hays. As both frequency and duration of reticulorumen contractions were altered in the current experiment, perhaps the lack of effect on ruminal liquid dilution rate could be due to the low forage/highly fermentable ingredients of this diet.

Bicarbonate serves as the primary buffering system of bodily fluids, such as blood. Animals were able to maintain blood pH within normal ranges (Houpt, 1989) during the dietary transition from the 70% to 90% concentrate diet, suggesting bicarbonate was able to compensate for any potential changes in blood pH (Owens et al., 1998). However, the transition did impact other measures of metabolic acid-base status as

evidenced by a reduction in base excess and bicarbonate; however, the magnitude of change was small (Table 4-7). In this case, the base excess was technically a base deficit due to the negative values, which signified that the animals were experiencing metabolic acidosis on both diets, with a more severe metabolic acidosis on the high-grain diet. Base excess of blood is reduced when bicarbonate leaves the blood. Since the high-grain diet indicated greater concentrations of VFA in the rumen fluid and lower ruminal pH, the reduction in base excess and blood bicarbonate may have been due to greater movement of bicarbonate across ruminal epithelium into the rumen in exchange for ionized VFA (Stevens, 1970) or to help maintain healthy ruminal pH. Theoretically, if there was a reduction in bicarbonate concentration in the blood without a coinciding reduction in pCO_2, the blood pH should have decreased (Higgins, 2008). Yet, no differences were detected between diets for blood pH or pCO_2. It is important to note that low bicarbonate concentrations can disturb central nervous system function, even when blood pH is not affected (Owens et al., 1998). Because rumen motility is controlled in part by the central nervous system and vagus nerve (Grovum, 1986), acid-base status may have contributed to the reduction in rumen motility.

Electrolyte balance is also important for homeostasis and many bodily functions. Sodium, for example, is the major cation in extracellular fluid and helps maintain osmotic balance of cells. In this study, blood sodium concentrations increased when animals were transitioned to the high-grain diet (Table 4-7). The sodium hydrogen exchanger isoform 3 present in the rumen epithelium imports Na^+ from the rumen in exchange for a H^+ from the blood (Schlau et al., 2012). Thus, the increase in Na in the blood may have been in

part due to an upregulation of this exchanger to try to maintain appropriate blood pH and would help explain the reduction in ruminal pH seen on the high-grain diet.

Multiple regression analysis confirmed that feeding behavior, ruminal pH, and ruminal motility variables are intricately related. Whether the parameter estimates were positive or negative can give some insight into the direction of the relationships between these variables. For example, DMI, as a percentage of the 70% diet intake, of cattle could be moderately explained by mean ruminal pH and the time ruminal pH was below 5.8, such that as the mean ruminal pH or length of time that pH was below that threshold increased, the DMI was reduced. A similar situation existed for the change in DMI in kg from the 70% diet intake (Table 4-8). Likewise, meal duration and meal size could be moderately estimated by the time that ruminal pH was below 5.8 and 5.5 along with ruminal contraction frequency. Increases in contraction frequency and ruminal pH below 5.5 were associated with increases in these variables, while increases in the time ruminal pH was below 5.8 was associated with decreases in these variables. Perhaps the opposite nature of the association for the two thresholds in this case was due to the time ruminal pH was below 5.8 being more an indicator of SARA, whereas time ruminal pH was below 5.5 may be more of an indicator of acute ruminal acidosis. Therefore, it would seem logical that if animals had a large meal size, they may experience more periods of low, acute-like ruminal pH (<5.5). Yet, if ruminal pH was modestly reduced (<5.8), the animals would be under a subacute situation and may reduce meal size. It is important to not use these regressions to determine cause and effect because it cannot be determined from these equations which event caused what response; it can only be used to report that there is a relationship between the variables and the strength of those relationships.

Regression analysis showed that decreases in amplitude of reticulorumen contractions were also associated with reductions in minimum ruminal pH (Table 4-9). Frequency of ruminal contractions produced the most accurate prediction equation of all the variables tested with an R^2=0.72 using meal duration, meal size, mean ruminal pH, and time ruminal pH was below 5.5 as regressors. Reductions in contraction frequency were associated with increases in meal duration, mean ruminal pH, and/or time ruminal pH was below 5.5, as well as decreases in meal size. These data agreed with literature that showed rumen contraction amplitude and frequency were reduced during acute acidosis (Bruce and Huber, 1973). Not surprisingly, water intake was the primary regressor for minimum, mean, and maximum ruminal pH variables (Table 4-10). Intake of water can greatly affect ruminal temperature and pH. However, for the time below threshold levels, meal duration and contraction frequency appeared more influential (larger absolute value for parameter estimate) than water intake. For example, a one min change in meal duration would have changed the time ruminal pH was below 5.8 by approximately 770 min, whereas a one L change in water consumption would have only changed the time ruminal pH was below 5.8 by about 26 min. Greater length of time ruminal pH was below 5.6 and 5.5 (i.e. the animal was experiencing longer bouts of SARA) was associated with a reduction in contraction frequency or motility. Therefore, this further emphasizes the finding that SARA was associated with a change in reticulorumen motility.

Conclusion

This study demonstrated that rumen motility was reduced by a SARA challenge experienced during a typical feedlot dietary transition. However, animals did not stop

eating, suggesting rumen motility was not impacted enough to slow passage rate and influence voluntary DMI. While meal size, duration, and frequency were not changed during the transition, consumption rate of feed was increased on the first day of high-grain feeding and likely influenced the depression in ruminal pH. Overall, feeding behavior, ruminal pH, and ruminal motility variables were clearly interrelated. Further research needs to be conducted to ascertain mechanisms of rumen motility regulation that are important during SARA or periods where animals reduce feed intake during dietary transitions.

Tables and Figures

Table 4-1. Composition of diets and ingredients.

Item	Diet	
	T70[1]	H90[2]
Ingredient, % DM basis		
Corn silage	54.00	18.00
High moisture corn	37.48	74.95
Soybean meal	5.69	4.00
Urea	0.55	0.50
Tallow	0.30	0.30
Limestone	1.10	1.40
Trace Mineral Premix[3]	0.45	0.45
Vitamin A, D, & E Premix[4]	0.15	0.15
Sodium sulfate	0.16	-
Potassium sulfate	0.12	0.25
Nutrient Composition, % DM basis		
Crude Protein	12.6	11.2
ADF	14.6	6.4
aNDF	22.1	9.6
TDN	75.0	83.0
Calcium	0.66	0.52
Phosphorus	0.40	0.37
Magnesium	0.15	0.12
Potassium	0.89	0.70
Sodium	0.236	0.177

[1] T70: Transition diet; 70% concentrate
[2] H90: High-grain finishing diet; 90% concentrate
[3] Contained 56.34% Cl, 36.53% Na, 1.2% S, 0.06% Ca, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, and 5520.2 ppm Zn on a DM basis.
[4] Composed of vitamin A acetate (1,814,368 IU/kg), D-activated animal sterol (source of vitamin D3; 362,874 IU/kg), vitamin E supplement (227 IU/kg), roughage products, calcium carbonate, and mineral oil.

Table 4-2. Feeding behavior for beef heifers during transitioning to a high-grain, finishing diet

Item	Treatment			SEM[3]	*P*-value
	T70[1]	d1 H90[2]	d 2 H90		
Meal Characteristics					
Duration, min	18.51	16.78	15.69	1.53	0.372
Size[3], g	1525	1299	1264	140.3	0.143
Frequency, meals/d	13.0	12.3	12.6	0.7	0.524
Consumption Rate Modeling (amount of daily intake)					
Time to consume 25%, min	185.6[b]	222.8[b]	434.9[a]	48.5	0.007
Time to consume 50%, min	465.4[b]	485.2[b]	763.0[a]	64.3	0.009
Time to consume 75%, min	871.3	933.1	1216.3	89.9	0.068
Time to consume 100%[4], min	1454[b]	1450[b]	1854[a]	140.3	0.036

[1] T70: Average of d -2 and -1 when on 70% concentrate transition diet
[2] H90: High-grain finishing diet; 90% concentrate
[3] SEM represents a pooled SEM across all days
[a,b] Means within a row without a common superscript letter are significantly different.
[4] Statistical analysis was conducted on transformed data due to non-normality. Means presented are back-transformed means.

Table 4-3. Volatile fatty acid (VFA) concentrations in rumen fluid from beef heifers during transitioning to a high-grain, finishing diet

Item	Diet		SEM	*P*-value		
	T70[1]	H90[2]		Diet	Time[3]	Diet*Time
Volatile fatty acid, m*M*						
Acetate	54.51	57.01	3.55	0.106	<0.001	0.071
Propionate	33.64	41.67	4.61	0.001	<0.001	0.840
Butyrate	10.24	11.85	3.15	0.542	<0.001	0.457
Isobutyrate	0.903	0.913	0.12	0.897	0.001	0.034
Isovalerate	2.41	2.04	0.18	0.110	<0.001	0.006
Valerate	2.86	3.19	0.39	0.152	<0.001	0.601
Total VFA	104.6	116.7	9.11	<0.001	<0.001	0.611
Proportion of total VFA						
Acetate	0.526	0.493	0.019	0.004	<0.001	<0.001
Propionate	0.319	0.352	0.021	0.081	<0.001	0.037
Butyrate	0.095	0.100	0.019	0.729	<0.001	0.350
Acetate:Propionate	1.701	1.578	0.216	0.407	<0.001	0.020

[1] T70: 70% concentrate transition diet
[2] H90: High-grain finishing diet; 90% concentrate
[3] VFA were evaluated at 0, 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 54, 60 & 66 h after feeding

Table 4-4. Ruminal pH and temperature for heifers during transitioning to a high-grain, finishing diet

Item	Treatment			SEM	*P*-value		
	T70[1]	d 1 H90[2]	d 2 H90		Day	Hour	Day*Hour
Ruminal pH							
Minimum	5.51[a]	5.17[b]	5.06[b]	0.083	0.003	-	-
Mean	6.16[a]	5.76[b]	5.59[b]	0.099	<0.001	<0.001	0.635
Maximum	6.88[a]	6.65[b]	6.41[c]	0.092	0.007	-	-
Time below 5.8, min	159[c]	756[b]	1024[a]	129[3]	<0.001	-	-
Time below 5.6, min	47[c]	557[b]	862[a]	139[3]	<0.001	-	-
Time below 5.5, min	22[c]	478[b]	745[a]	3.62[3]	0.002	-	-
Ruminal Temperature							
Minimum	38.9	38.2	39.1	0.39	0.141	-	-
Mean	40.3	40.4	40.3	0.13	0.888	<0.001	0.068
Maximum	41.0	41.1	40.9	0.13	0.371	-	-

[1] T70: Average of d -2 and -1 when on 70% concentrate transition diet
[2] H90: High-grain finishing diet; 90% concentrate
[3] SEM represents pooled standard error of the means
[a,b,c] Means within a row without a common superscript letter are significantly different
- Indicates effect was not evaluated for that variable

Table 4-5. Rumen fill and liquid dilution rate estimates for cattle consuming a 70% or 90% concentrate-based diet

Item	Diet		SEM	*P*-Value
	T70[1]	H90[2]		
Rumen content dry matter, %	14.2	15.5	0.65	0.245
Wet contents, kg	55.6	52.2	2.3	0.196
Wet contents, g/kg BW	104.0	97.9	5.0	0.216
Dry contents, kg	7.9	8.1	0.5	0.824
Dry contents, kg/kg BW	14.8	15.2	1.0	0.790
Fractional dilution rate (FDR)	-0.057	-0.054	0.0055	0.556
Liquid dilution rate, %/h	5.68	5.38	0.55	0.556
Mean retention time, h	18.38	18.09	1.4[3]	0.857
Half life, h	12.74	12.53	0.98[3]	0.846
Rumen liquid volume, L	77.15	74.76	4.51[3]	0.256
Liquid flow rate, L/h	4.33	4.26	0.25[3]	0.878

[1] T70: Transition diet; 70% concentrate
[2] H90: High-grain finishing diet; 90% concentrate
[3] SEM represents pooled standard error of the means

Table 4-6. ANOVA analysis and regressions for rumen motility variables

Item	*P*-value			Linear		Quadratic		Cubic	
	Day	Hour	Day*Hour	*P*-value	Adj. r^2	*P*-value	Adj. r^2	*P*-value	Adj. r^2
Amplitude	<0.001	0.217	0.354	-	-	-	-	-	-
Duration	<0.001	<0.001	0.781	0.524	-0.03	<0.001	0.490	<0.001	0.612
Frequency	<0.001	<0.001	<0.001	-	-	-	-	-	-
T70[1]	-	-	-	0.002	0.318	0.01	0.296	0.001	0.478
d1 H90[2,3]	-	-	-	<0.001	0.603	<0.001	0.598	<0.001	0.605
d2 H90	-	-	-	0.531	-0.027	0.408	-0.006	0.471	-0.017
Peak Pressure	0.009	0.610	0.978	-	-	-	-	-	-

[1] T70: average on transition diet; 70% concentrate
[2] H90: High-grain finishing diet; 90% concentrate
[3] Despite significant quadratic and cubic models for d1 H90 contraction frequency, the quadratic and cubic variables were not significantly different from zero, suggesting these models were overfitting the data.

Table 4-7. Jugular blood acid-base and electrolyte status of beef heifers during a moderate transition to a finishing diet

Item	Diet		SEM[3]	*P*-value		
	T70[1]	H90[2]		Diet	Time	Diet*Time
Blood acid-base						
pH	7.414	7.407	0.005	0.134	0.063	0.710
pCO_2, mmHg	35.08	33.92	0.53	0.120	<0.001	0.576
pO_2, mmHg	45.42	46.65	1.7	0.311	0.231	0.458
HCO_3^-, mmol/L	21.56	20.50	0.40	0.030	<0.001	0.915
Base Excess, mmol/L	-2.49	-3.56	0.42	0.024	<0.001	0.934
Electrolyte						
Ionized Na, mmol/L	141.9	143.1	0.15	<0.001	0.013	0.562
Ionized K, mmol/L	3.28	3.24	0.032	0.283	<0.001	0.152
Ionized Ca, mmol/L	1.02	1.01	0.027	0.246	0.541	0.665

[1] T70: Transition diet; 70% concentrate
[2] H90: High-grain finishing diet; 90% concentrate
[3] SEM represents pooled standard error of the means

Table 4-8. Multiple regression analysis of intake and feeding behavior variables to determine relationships between intake, ruminal, and motility variables and predictability of those variables.

Dependent Variable	Variables remaining in model	Model *P*-value	r^2	Adj. r^2	Parameter estimate	SE
DMI, kg	None	-	-	-	-	-
DMI, % of T70[1]	Mean RpH[2]	0.026	0.430	0.343	-147.32	49.03
	RpH < 5.8				-0.109	0.04
Change in DMI from T70, kg	Mean RpH	0.030	0.418	0.329	-16.41	5.47
	RpH < 5.8				-0.012	0.005
Water, L	RpH < 5.5	0.002	0.447	0.394	0.013	0.003
	Contraction frequency				16.06	6.67
Meal duration, min	RpH < 5.8	0.003	0.510	0.433	-0.0019	0.00073
	RpH < 5.5				0.0018	0.00075
	Contraction frequency				0.692	0.265
Meal size, g	RpH < 5.8	0.001	0.561	0.491	-0.931	0.29
	RpH < 5.5				0.803	0.32
	Contraction frequency				823.02	257.4
Meal frequency, meals/d	RpH < 5.6	0.014	0.436	0.342	0.010	0.0045
	RpH < 5.5				-0.011	0.0046
	Mean Trum[3]				0.634	0.226

[1] T70: Transition diet; 70% concentrate; average value for DMI on this diet
[2] RpH: ruminal pH
[3] Trum: ruminal temperature

Table 4-9. Multiple regression analysis of rumen motility variables to determine relationships between intake, ruminal, and motility variables and predictability of those variables.

Dependent Variable	Variables remaining in model	Model *P*-value	r^2	Adj. r^2	Parameter estimate	SE
Amplitude, mmHg	Minimum RpH[1]	0.005	0.309	0.277	2.06	0.66
Duration, s	Meal duration	0.028	0.389	0.288	-24.83	11.19
	Meal size, g				0.025	0.011
	Mean Trum[2]				0.073	0.025
Frequency, contractions/min	Meal duration	<0.001	0.720	0.658	-19.62	9.33
	Meal size, g				0.020	0.009
	Mean RpH				-0.97	0.223
	RpH < 5.5				-0.00093	0.0002

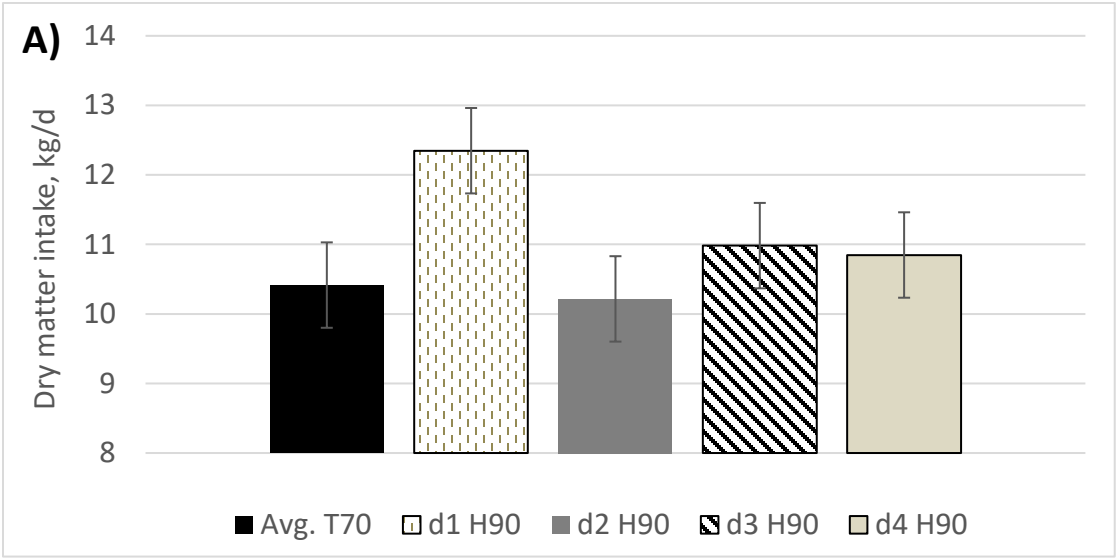
[1] RpH: ruminal pH
[2] Trum: ruminal temperature

Table 4-10. Multiple regression analysis of ruminal pH and temperature variables to determine relationships between intake, ruminal, and motility variables and predictability of those variables.

Dependent Variable	Variables remaining in model	Model *P*-value	r^2	Adj. r^2	Parameter estimate	SE
Minimum RpH[1]	Water	0.006	0.405	0.346	-0.018	0.0063
	Meal size, g				0.00038	0.00016
Mean RpH	Water	0.001	0.487	0.436	-0.026	0.0076
	Meal size, g				0.00051	0.00019
Maximum RpH	Water	0.006	0.300	0.269	-0.0203	0.0066
Time RpH < 5.8, min	Water	<0.001	0.504	0.454	26.55	8.98
	Meal duration				-771.53	229.45
Time RpH < 5.6, min	Water	<0.001	0.512	0.466	33.19	8.69
	Contraction frequency				-1032.5	324
Time RpH < 5.5, min	Water	<0.001	0.537	0.493	33.74	8.35
	Contraction frequency				-1033.5	311.2
Mean Trum[2], °C	Contraction duration	0.033	0.200	0.162	2.89	1.26

[1] RpH: ruminal pH
[2] Trum: ruminal temperature

Figure 4-1. Dry matter (A; *P*=0.087), water (B; *P*=0.008), and total water (including from feed; C; *P*=0.10) intake of beef heifers during a moderate transition from a 70% (T70) to a 90% concentrate (H90) diet. The T70 bar represents the average of 4 days on T70 (d -3 through 0), immediately prior to switching to the H90 diet. Columns without a common letter differed (*P*<0.05).



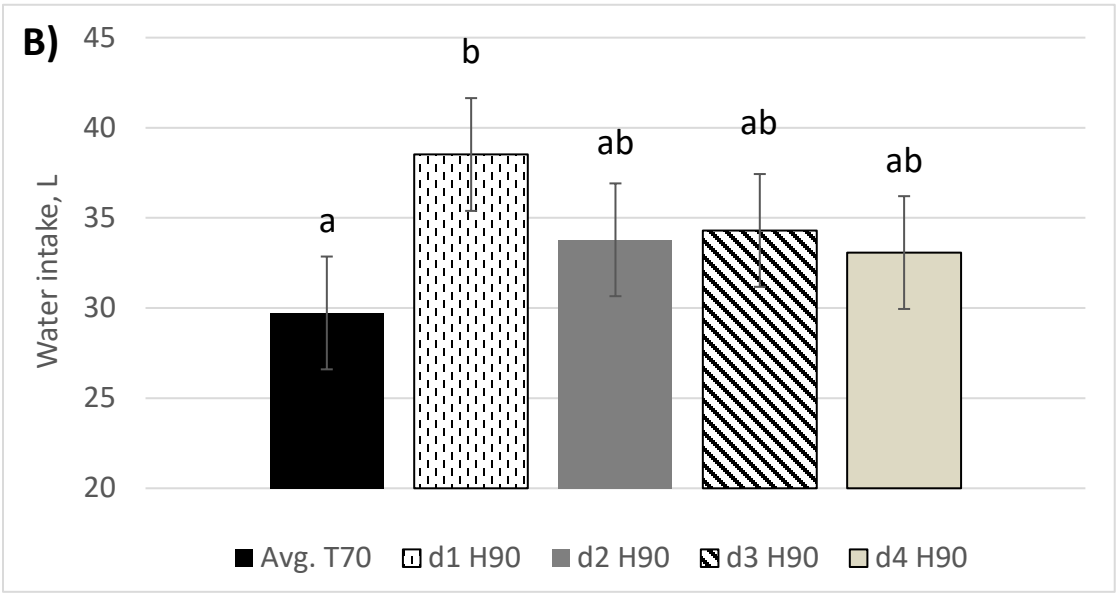


Figure 4-1 continued.

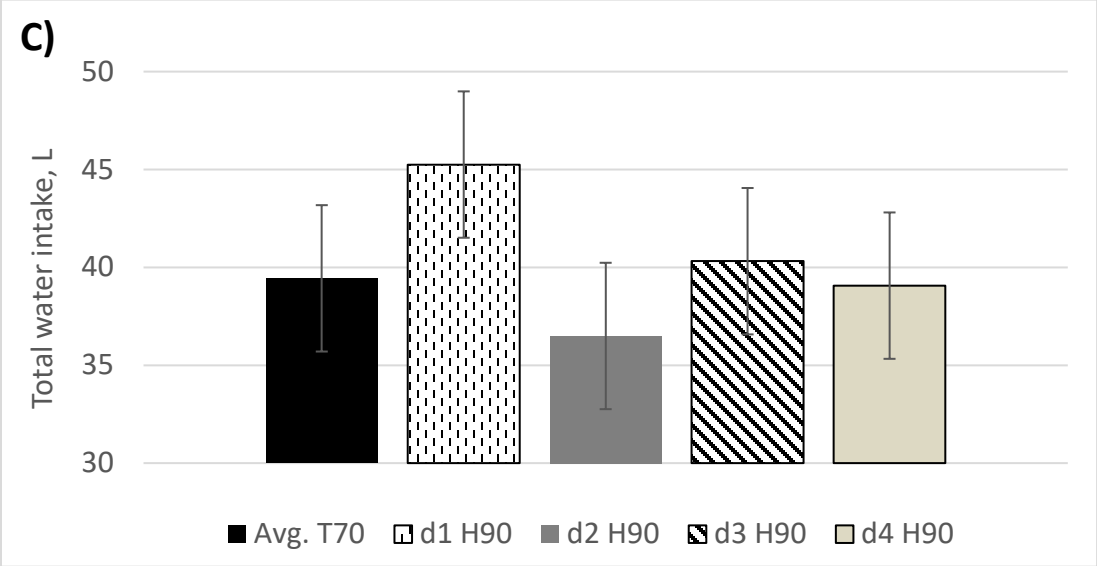


Figure 4-2. Concentrations of isobutyrate (A) and isovalerate (B) in rumen fluid at a given time point relative to feeding differed between diets (diet*time interaction). Times marked with an asterisk (*) denote that T70 and H90 differed (P<0.05) at that time point.

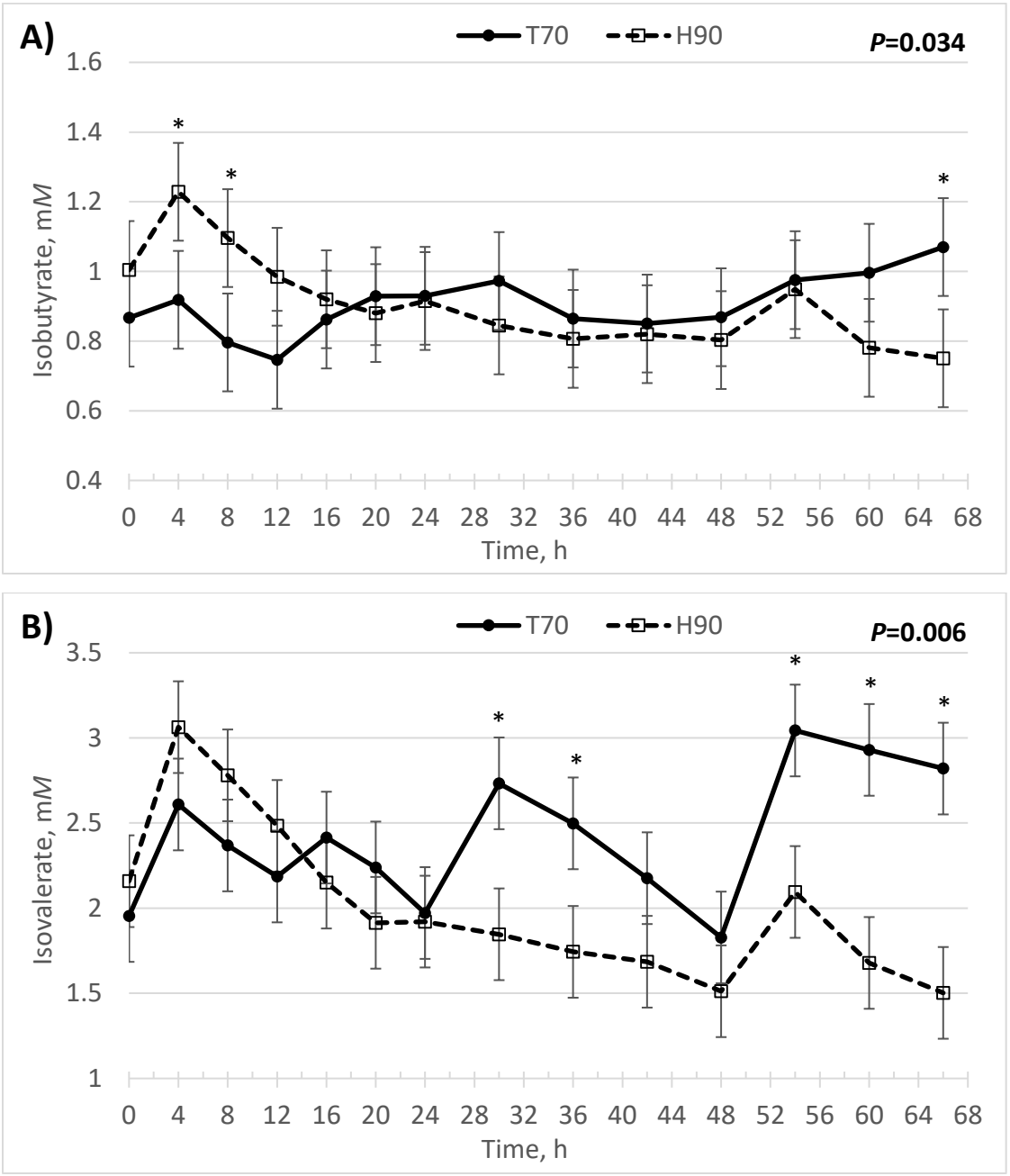
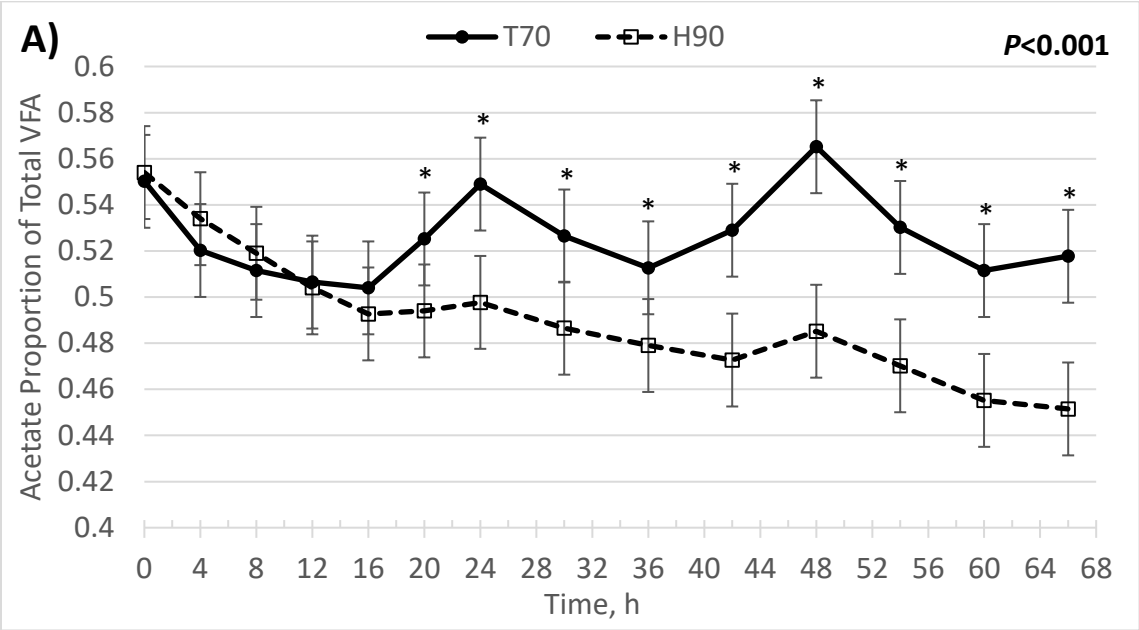


Figure 4-3. Proportion of total VFA in rumen fluid represented by acetate (A), propionate (B), as well as the acetate:propionate ratio (C) at a particular time point relative to feeding was dependent on diet (diet*time interaction). Times marked with an asterisk (*) denote that T70 and H90 differed (P<0.05) at that time point.



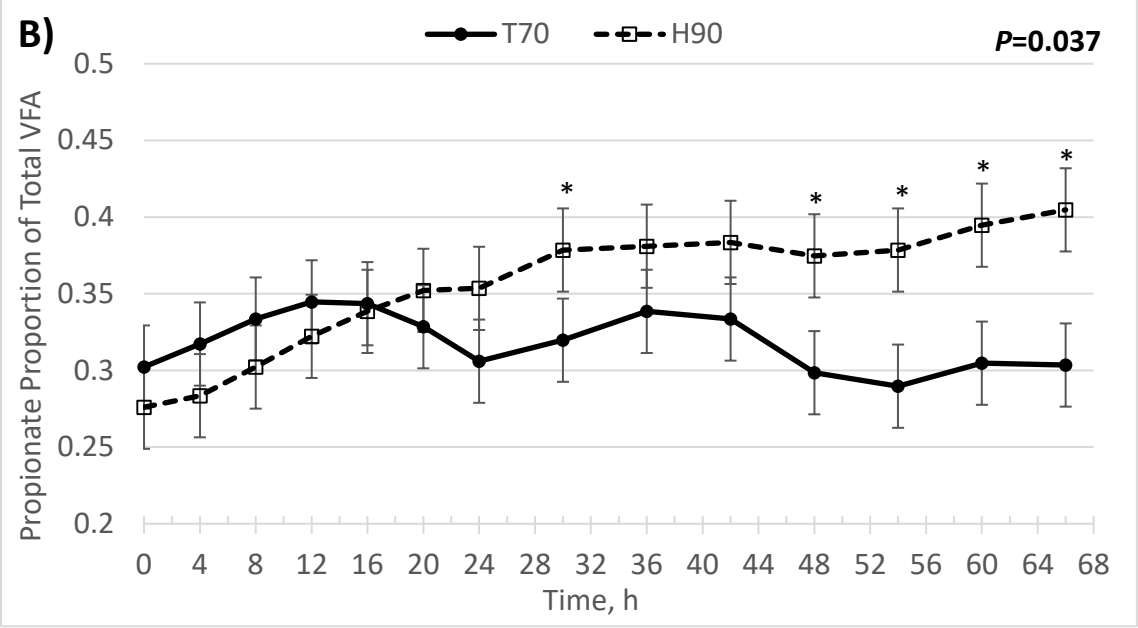


Figure 4-3 continued.

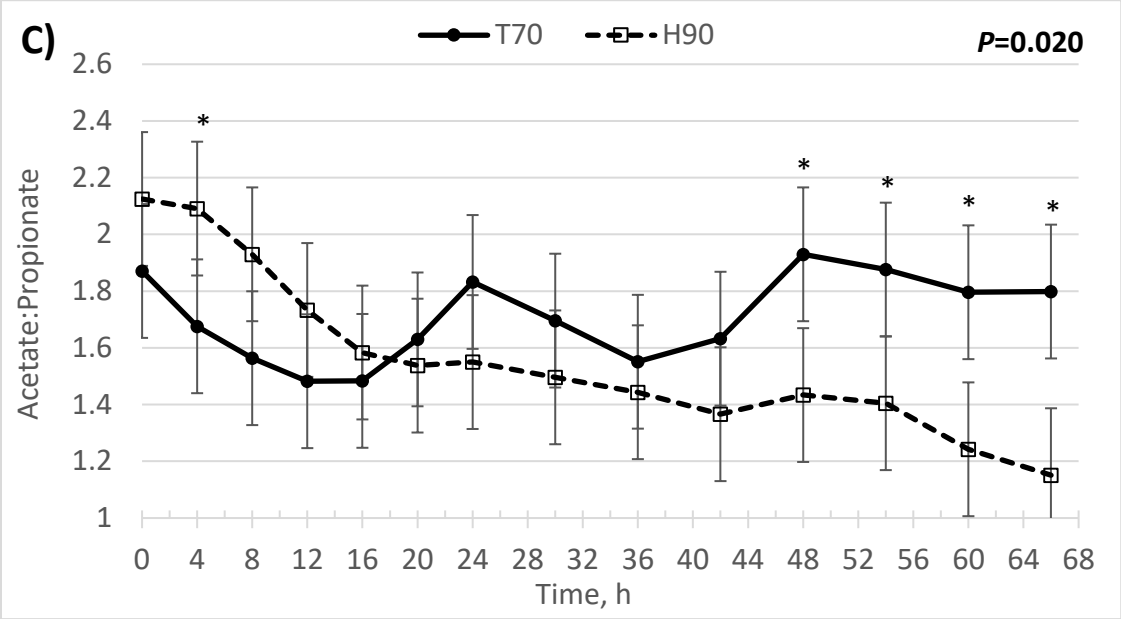


Figure 4-4. Mean ruminal pH (A) and temperature (B) varied by hour relative to feeding. A) Across the 24-hour feeding cycle, mean ruminal pH decreased and then increased resulting in a significant quadratic (P<0.001) response. B) Inversely, mean ruminal temperature increased then decreased over the feeding cycle, which also resulted in a significant quadratic (P<0.001) response.

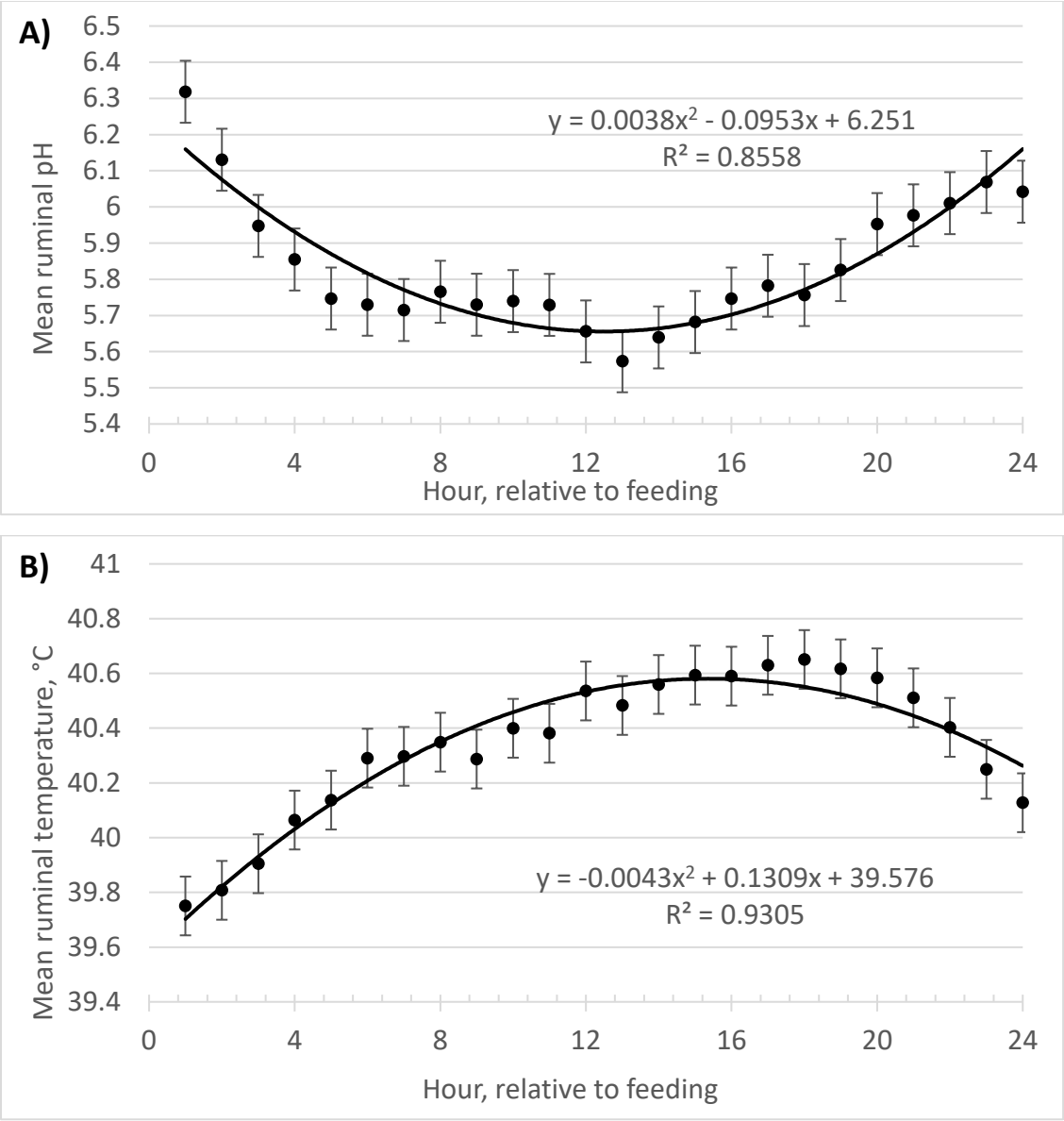


Figure 4-5. Reticulorumen contraction amplitude in cattle during a moderate transition from a 70% concentrate diet (T70) to a high-grain finishing diet (H90). Amplitude was reduced (P<0.001) on both days during H90 feeding compared to during T70 feeding. Columns without a common letter differed (P<0.05).

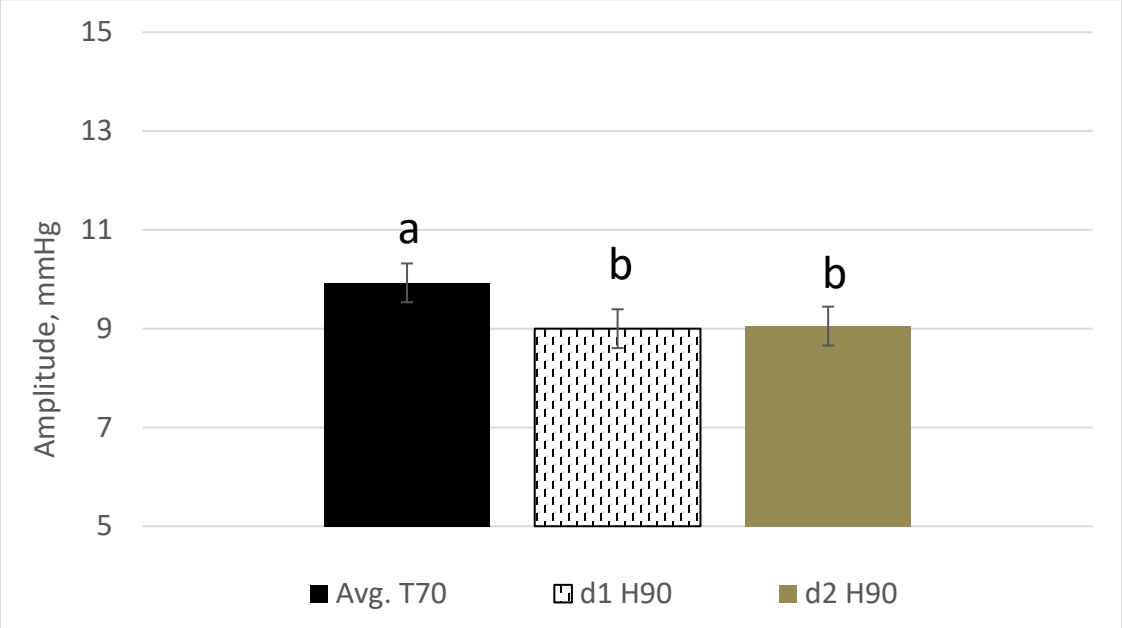


Figure 4-6. Cattle reticulorumen contraction duration during a moderate transition to a high-grain finishing diet. A) Day influenced (P<0.0001) contraction duration where contraction duration was reduced on the first day of high-grain (d1 H90) feeding compared to on a 70% concentrate diet (Avg. T70) or the second day of high-grain feeding (d2 H90). Columns without a common letter differed (P<0.05). B) Duration of contractions was affected (P<0.0001) by hour after feeding, where in general, the response was cubic (P<0.001; r^2=0.663) throughout the feeding cycle. Error bars represent the average SEM across all hours.

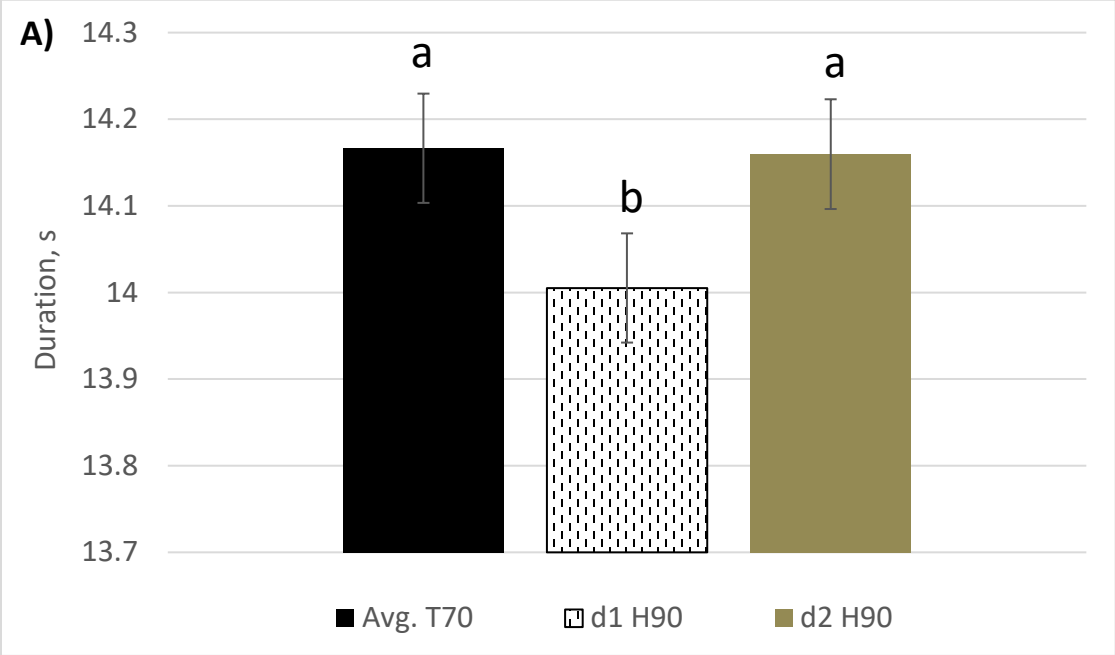


Figure 4-6 continued.

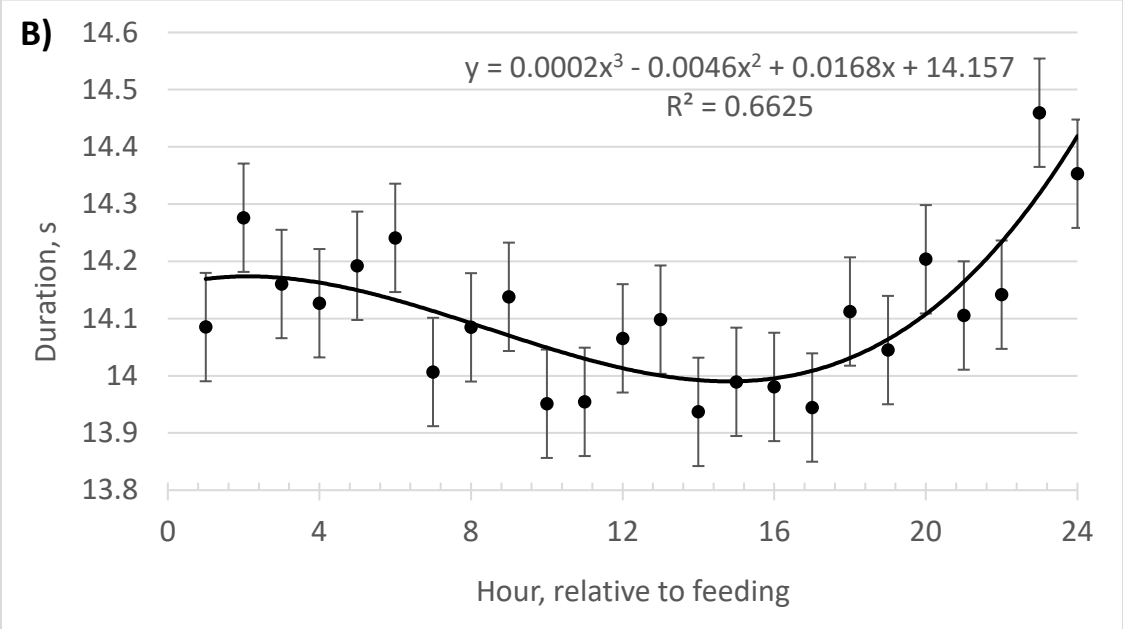


Figure 4-7. Frequency of reticulorumen contractions in cattle during a moderate transition from a 70% concentrate transition diet (T70) to a 90%, high-grain finishing diet (H90). The effect of hour was influenced by day ($P<0.001$). Generally, contraction frequency was reduced for several hours after feeding on d2 H90 compared to those hours on previous days.

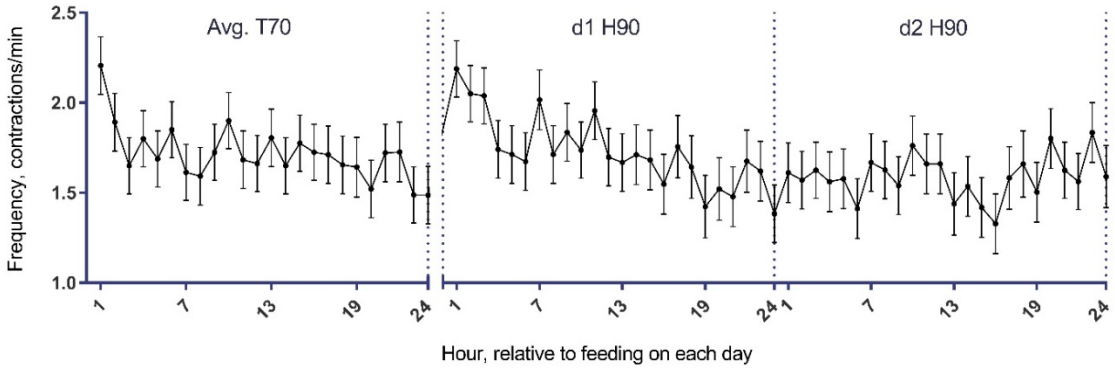


Figure 4-8. Pressure at the peak of reticulorumen contractions in cattle during a moderate transition from a 70% concentrate diet (T70) to a 90%, high-grain diet (H90). Peak pressure was affected (P=0.009) by day of transition, where peak pressure increased on d 2 of feeding H90 compared to feeding T70. Columns without a common letter differed (P<0.05).

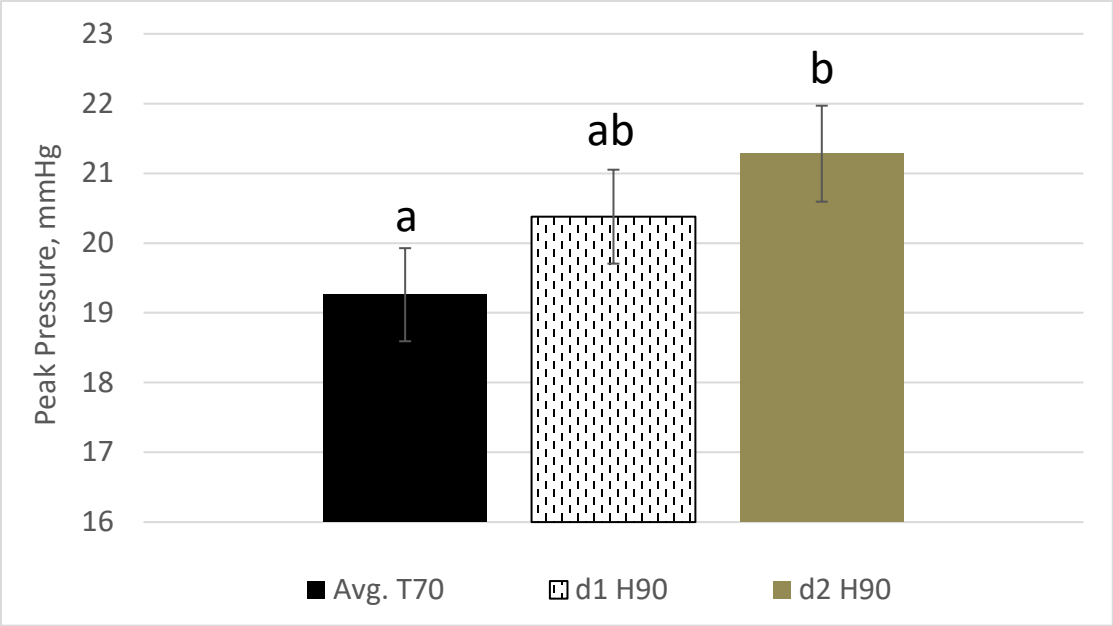


Figure 4-9. Jugular blood partial pressure of CO_2 (A), bicarbonate (B), and base excess (C) were affected (P<0.001) by time. Error bars present the average SEM across all hours.

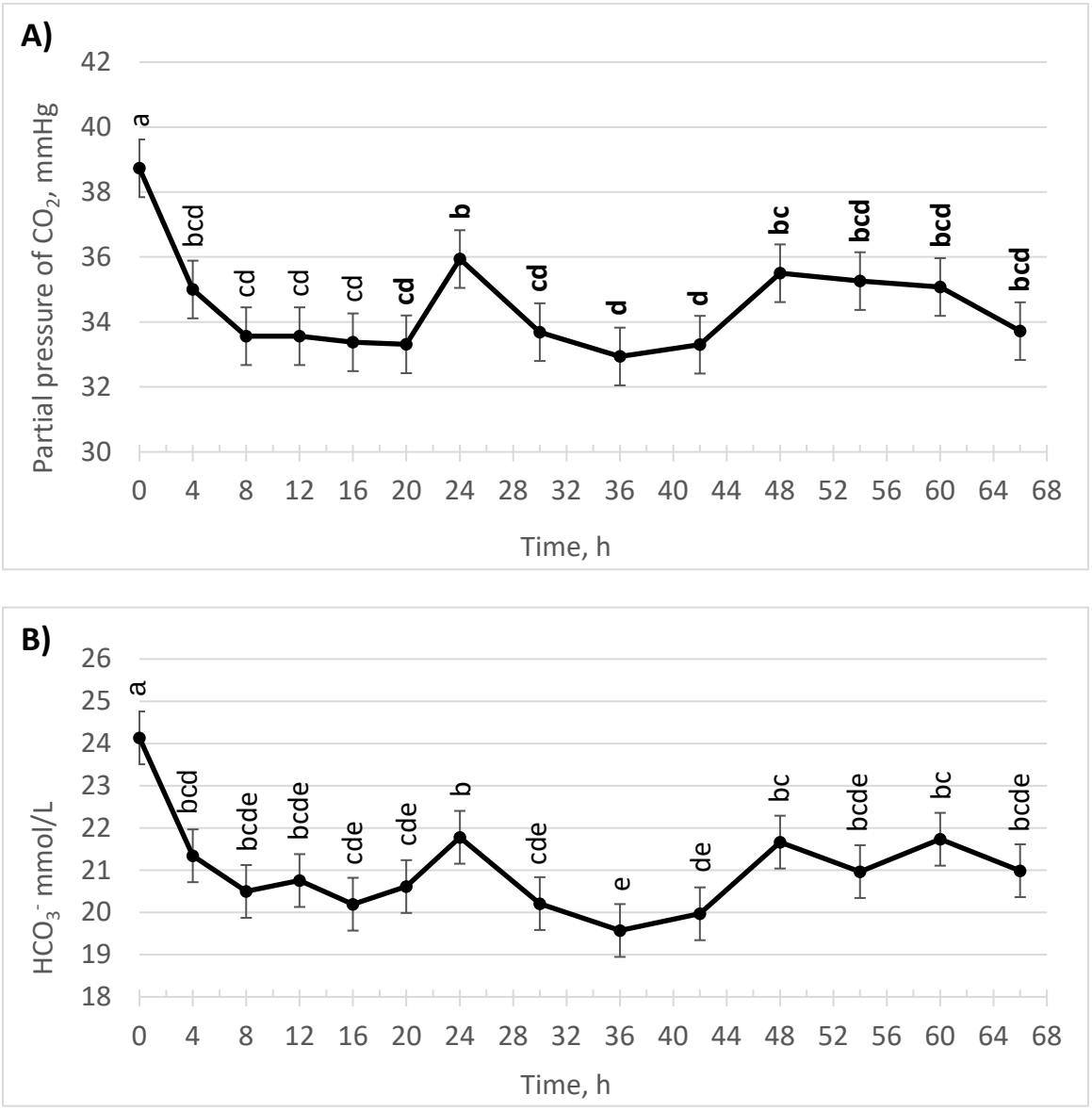


Figure 4-9 continued.

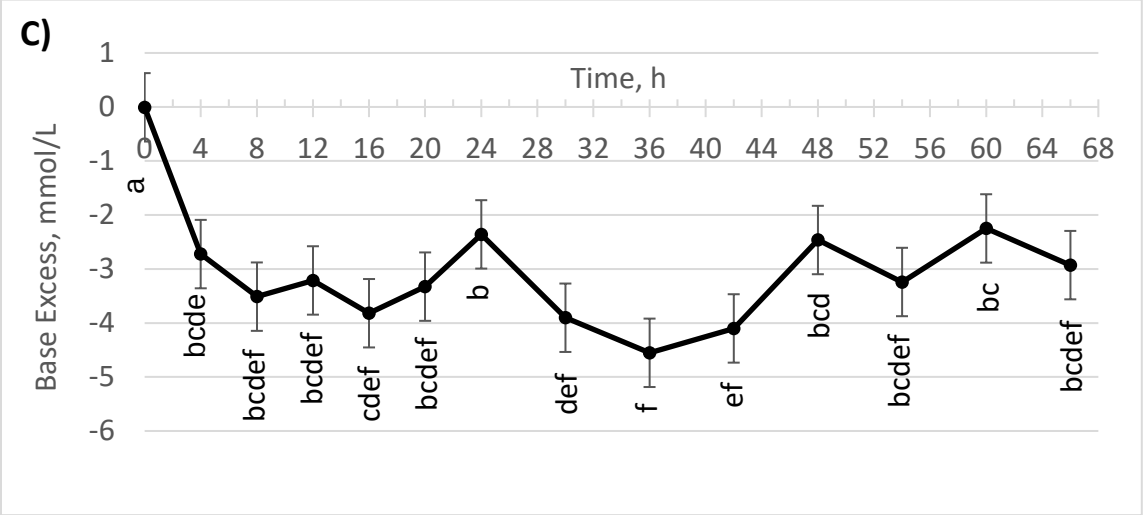
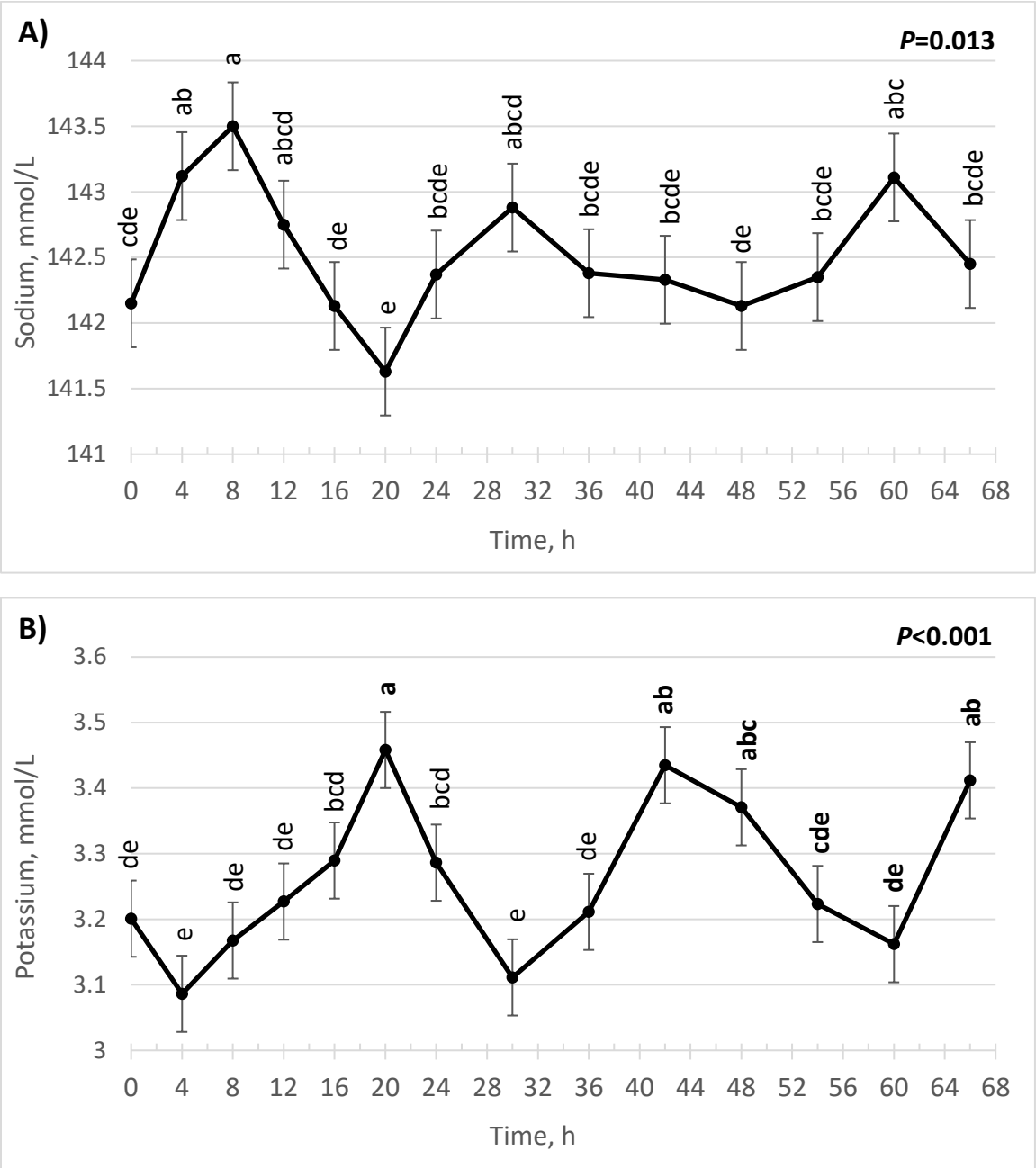


Figure 4-10. Jugular blood ionized sodium (A) and ionized potassium (B) were affected by time. Error bars present the average pooled SEM across all hours. Means without a common letter differed (P<0.05).



CHAPTER 5: EVALUATION OF CHANGES IN FEEDING BEHAVIOR, RUMINAL ENVIRONMENT, AND RUMEN MOTILITY ASSOCIATED WITH REDUCTIONS IN FEED INTAKE FOLLOWING A LARGE INCREASE IN DIETARY CONCENTRATE

Introduction

During the transition to a high-grain diet, cattle may experience periods of subacute ruminal acidosis (SARA) and demonstrate erratic feeding behavior, or go "off-feed." This period of "off-feed" has been noted under both acute and subacute acidotic conditions (Fulton et al., 1979a; Kezar and Church, 1979). Although reductions in DMI have been used to diagnose SARA (Kleen et al., 2003), effects of SARA on DMI are inconsistent. Furthermore, some cattle appear to be highly affected during a dietary transition, whereas others are not (Brown et al., 2000; Bevans et al., 2005). Feeding behavior, such as meal size and frequency, may be altered by increasing concentrate in the diet, even if total DMI is not affected (Li et al., 2012), and can influence ruminal pH and the rumen environment.

Rumen motility can influence digesta passage, content mixing, and presentation of substrate to the absorptive surface of the rumen, and therefore, it has potential to alter feed intake and VFA absorption. Little is known about motility during the transition to high-grain diets and periods of ruminal acidosis. Previous research from our laboratory demonstrated that rumen motility was reduced when cattle were transitioned from a 70% to a 90% concentrate diet (Dissertation Chapter 4). Yet, the effects of a more abrupt transition (increase in concentrate content of the diet) where cattle are given *ab libitum* access to feed on rumen motility has not been investigated.

As cattle are switched to a high-grain diet, notable changes occur in the color and consistency of rumen fluid. Rate of VFA absorption from the rumen has been shown to

decrease during acidosis, which could be due to high osmolality from accumulation of VFA (Tabaru et al., 1990) and/or slow diffusion of substrates through rumen fluid. Viscosity of rumen fluid may play a key role in quality of mixing, movement of VFA through the fluid, and presentation of VFA to the ruminal epithelium for absorption, thereby influencing ruminal pH. However, there is no evidence in the literature that relates rumen motility with ruminal pH, rumen fluid viscosity and altered feed intake in cattle.

The objectives of this experiment were to 1) investigate what behavior and physiological parameters could be associated with the timing of when animals go "off-feed", 2) determine if an abrupt transition by increasing concentrate in the diet would affect rumen motility, and 3) evaluate rumen fluid viscosity as cattle are adapted to a high-grain diet.

Materials and Methods

All procedures used in this experiment involving heifers were approved by the University of Kentucky Institutional Animal Care and Use Committee (2018-2973) and conducted at the University of Kentucky C. Oran Little Research Center, Beef Unit, in Versailles, KY.

Animals and Experimental Design

Ten Holstein steers (pre-surgery BW ± SEM = 278 ± 10.3 kg) were fitted with rumen cannula (#2C rumen cannula, Bar Diamond Inc., Parma, ID). Steers were withheld from food (24 h) and water (12 h) prior to surgery. Steers were blocked by weight prior to surgery to determine pairs for surgeries and sampling.

The cannulated steers were adapted to a diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement (MIX; Table 5-1) prior to the initiation of the trial. Steers were given at least a 21 d acclimation period and fed ad libitum the MIX diet. Animals (pre-sampling BW ± SEM = 304 ± 13 kg) were fed MIX for three additional days (d -2 to 0) to allow for baseline measurements conducted primarily on d -1 (also called MIX day). All animals were then abruptly changed to a 90% concentrate, high-moisture corn-based diet (HG; Table 5-1) offered *ad libitum*. Measurements were taken on d 1-3 of feeding HG (HGd1, HGd2, and HGd3, respectively). Thus, each block (n=7) included measurements on the baseline MIX diet and after switching to the HG diet. Data were collected throughout the experiment at various time points as discussed below. All animals had *ad libitum* access to water throughout the experiment.

Ruminal pH was monitored manually using a benchtop pH meter (S220 SevenCompact™ pH/Ion meter, Mettler Toledo, LLC, Columbus, OH) at rumen fluid collection times (see below). If rumen fluid measured pH of 4.2 or lower, the rumen of affected animal(s) was evacuated immediately and inoculated with rumen contents from a donor animal, ending the sampling from that animal.

Feeding Behavior

Feeding occurred at 0900, and feed samples were collected daily, composited for each diet, and analyzed for DM content (55°C for 48 h; used for DMI adjustment) and nutrient composition by wet chemistry through Dairy One Forage Laboratory (DM: AOAC method 930.15; ash: AOAC method 942.05; crude protein: AOAC method 990.03; fat: AOAC method 2003.05; ADF: ANKOM Technology method 5; aNDF: ANKOM Technology method 6). Orts were collected daily at 0830, weighed, and

recorded from the previous day in order to adjust feed amounts and maintain at least 5% excess throughout the experiment as well as calculate individual feed intake. Water consumption was measured using flow meters and recorded at 0, 4, 8, 12, and 24 h relative to feeding on sampling days to determine water intake. Total water intake (L/d) was determined by summing the measured water intake and amount of water in feed consumed (based on DM content of feed; assuming 1 kg = 1 L). Frequency of meals (meals/d) and average meal size and duration was analyzed (MATLAB R2015b, The MathWorks, Inc., Natick, MA) for each animal on each day by feed disappearance from feed bunks mounted onto load cells (LC101-500/LC111-500, Omegadyne, Sunbury, OH) which record bunk weight at 1-min intervals using an algorithm previously described (Dissertation Chapter 3). A meal was defined as a continuous feeding event. A break of at least 30 min with no weight changes was used to differentiate between meals. A median filter (n=20) was applied to the data before meal detection to remove noise created from animals moving the suspended feed bunk. The time for consumption of 75% of daily intake was calculated using one-phase decay exponential regression with automatic outlier determination (GraphPad Prism 7, GraphPad Software, Inc., La Jolla, CA).

Animals were also scored based on their susceptibility to go “off-feed” during the transition by their DMI on HGd2, where animals that consumed more than 4 kg of DM received a score of 1 (least susceptible), 1-4 kg DM received a score of 2 (moderately susceptible), 0-1 kg DM received a score of 3 (highly susceptible), and animals for which the experiment had to be stopped early due to low ruminal pH received a score of 4 (most susceptible). This score was only used as a means of categorizing animals for multiple regression, as discussed below.

Rumen Fluid Sample Collection and Analyses

Rumen fluid samples were collected via a suction strainer from the ventral sac. A slit in the cannula plug was created to allow passage of the strainer and prevent any effect of multiple plug removals on the animals' responses. Approximately 50 mL of rumen fluid was collected from each animal into screw-top conical vials immediately before feeding (0 h) and 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 18, 20, and 24 h after feeding during baseline measurements (d -1) and at the same times, relative to feeding of the HG diet on d 1-3. Samples were transported to the laboratory immediately measured manually for pH using a benchtop meter (S220 SevenCompact™ pH/Ion meter, Mettler Toledo, LLC, Columbus, OH; used for monitoring animal condition and was separate from continuous pH recording discussed below), and then placed on ice. A portion of the rumen fluid from each animal and time point was strained through a fine mesh, stainless steel strainer (item # MS2K-3S; Winco, DWL International Trading, LLC, Lodi, NJ), collected into 15 mL conical vials, frozen at -20°C, and saved for subsequent viscosity analysis. Remaining rumen fluid was centrifuged for 5 min at 2000 × *g*. Duplicate 1-mL samples of supernatant rumen fluid from each animal and time point were processed for VFA analysis.

Processing for VFA analysis involved combining 1 mL supernatant sample and 100 μL 85 mM 2-ethylbutyrate internal standard into microcentrifuge tubes, capping, and mixing for approximately 2 seconds using a vortex. Next, 100 μL 50% meta-phosphoric acid was added, tubes were recapped, mixed for approximately 5 seconds using a vortex, and frozen (-20°C) to allow for protein precipitation. Tubes were thawed, centrifuged at 20,000 × *g* for 20 min, and supernatant was transferred to GC injection vials and capped.

Gas chromatography with a flame ionization detector (Agilent HP6890 Plus GC with Agilent 7683 Series Injector and Auto Sampler; Agilent Technologies, Santa Clara, CA) and a fused silica capillary column (Supelco 25326 Nukol; 15 m × 0.53 mm × 0.5 µM film thickness; Sigma/Supelco, Bellefonte, PA) was used to determine VFA concentrations in the rumen fluid samples. Analysis involved injection of 0.2 µL of each sample in duplicate at 110°C with a 2:1 split, a 1-min hold, temperature increase at 5°C/min to 125°C for 2 min, and the set point for inlet and injector at 260°C.

For viscosity analysis, the 15 mL samples of rumen fluid in conical vials were thawed and vortexed for at least 10 s to thoroughly mix any substances that precipitated during freezing. A 40 mm diameter, parallel plate (stainless steel Peltier plate) rheometer (Discovery HR-2 hybrid rheometer, TA Instruments, New Castle, DE) and TRIOS Software (TA Instruments) were used to measure the viscosity of rumen fluid between shear rates of 20 and 200 s^{-1} using a 4 point method, where viscosity was averaged over 30 seconds at each shear rate. Samples were pre-warmed for 5 min in a 39°C water bath, analyzed at 39°C in duplicate, and a table of shear rate, shear stress, and viscosities over the 4 shear rates was exported from TRIOS into Microsoft Excel. Apparent viscosity was calculated by averaging the measured viscosity of the 4 shear rates. According to the power law model of the rheological properties of fluids:

$$\sigma = K\dot{\gamma}^{n},$$

where σ = shear stress, K = consistency coefficient, $\dot{\gamma}$ = shear rate, and n = power law index. Taking the natural log of both sides of the equation resulted in the equation:

$$\ln \sigma = \ln K + n \ln \dot{\gamma}.$$

A graph of ln(shear stress) versus ln(shear rate) was generated. Thus, the power law index of the sample was equal to the slope of a linear trendline and the consistency coefficient equaled $e^{(y\ intercept)}$. Analysis was only accepted if the R^2 value of the regression was greater than or equal to 0.99.

Liquid Dilution Rate, Volatile Fatty Acid Absorption, and Saliva Production Estimates

On d -1, 1, 2, and 3, animals were intraruminally pulse-dosed with 500 mL Cr:EDTA/*n*-valeric acid solution (53 m*M* Cr, 20 m*M* valeric acid, adjusted to pH 6.0) immediately before feeding at 0900 to evaluate liquid passage rate through the rumen and estimate VFA absorption. Approximately 50 mL of rumen fluid was collected via suction strainer from the ventral sac at 0 (before feeding, before Cr:EDTA/*n*-valeric acid dosing), 0.5, 1, 2, 4, 8, 12, 18, 20, and 24 h post-dosing. Samples of fluid from each animal and time point were strained and placed in a conical vial and frozen (-4°C). Samples for common time periods were taken from the samples collected for VFA analysis mentioned above to prevent excessive removal of rumen fluid. The same 15-mL frozen sample was used to take samples for Cr and viscosity analysis. After thawing, a portion of the sample was transferred to a microcentrifuge tube and centrifuged at 20,000 × *g* for 30 min. The supernatant was then diluted 5-fold with nanopure water. Chromium concentrations for each sample were determined in duplicate using atomic absorption analysis (AAnalyst 200, PerkinElmer Inc., Waltham, MA) and a wavelength of 357.87 nm. If samples responses were out of the range of the standard curve, a portion of the sample was diluted until within the concentration range. Baseline concentrations of Cr (0 h) were used to correct the concentrations measured at each individual time point. Concentration of Cr after dosing and fractional clearance rate of Cr were determined by calculation of the

exponential decay rate for Cr using the proc NLIN procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) and the following equation:

$$C_t = C_0 \times e^{-k \times t},$$

where C_t represented concentration at a given time, C_0 represented the concentration at time 0 h, k represented the fractional rate of clearance (also known as fractional dilution rate), and t represented time in hours (Allen et al., 2000; Resende Junior et al., 2006; Penner et al., 2009b). Liquid half-life in the rumen was calculated as the absolute value of 0.693/k. Rumen liquid volume (L) was determined by dividing the amount of Cr dosed by the amount of Cr present at time zero (C_0). Liquid flow rate (L/h) was calculated as k*rumen liquid volume.

Valerate concentrations were determine by gas chromatography as described above. As with Cr, concentration of valerate at 0 h were used to correct concentrations measured at each individual time point. Exponential decay of valerate over time was determined using proc NLIN of SAS and the above equation, where k represented the fractional clearance rate (Kc) of valerate. Fractional clearance rate (Kc) of valerate included both absorption and passage. Thus, fractional absorption rate (Ka) of valerate was determined by subtracting the rate of Cr clearance (assumed to equal valerate passage rate, Kp) from the rate of valerate clearance (Kc). It was also assumed that rate of *n*-valeric acid absorption rate was similar to the rates of other VFA absorption (Allen et al., 2000).

Endogenous contributions to ruminal fluid outflow were estimated by subtracting total water consumed (L/d) from ruminal fluid outflow (L/d; obtained by multiplying the liquid flow rate by 24 h/d). Net transruminal flux was assumed to be minimal, so this

endogenous contribution was considered saliva production (Jacques et al., 1989). Salivary flow was calculated by dividing the saliva production by 24 h.

Motility Measurements and Lying Behavior Estimates

A water-filled (2 L) balloon attached to a Tygon catheter (i.d. = 3.2 mm; o.d. = 6.4 mm) was inserted into the cranial sac of the rumen on d -2 prior to feeding. Balloons were weighed to maintain consistent fill between animals. Balloons were changed and replaced after 24 h of use or if equipment failure occurred. A small hole was made in the plug of the rumen cannula to allow the catheter to pass through. Disposable blood pressure transducers (MLT0670; ADInstruments Inc., Colorado Springs, CO) connected to a PowerLab 8/30 (ADInstruments, Inc.) through bridge amplifiers (FE221; ADInstruments, Inc.) were used to monitor pressure changes in the rumen and characterize rumen contractions. The end of the catheter external to the animal was equipped with a tubing to luer lock adapter for connection to the pressure transducer stopcock. Rumen motility was measured continuously (2 samples/sec) for 24 h beginning immediately prior to feeding on d -1 and for 72 h after feeding on d 1. Animals were tied in their stalls with access to water and their feed bunk during recordings to prevent complications with equipment connections.

Data was recorded using LabChart software (ADInstruments, Inc.), saved every 6 h, raw data were exported from LabChart, and imported into MATLAB for smoothing (median filter; order = 10) and analysis of contractions using the “findpeaks” function of the MATLAB Signal Processing Toolbox, as previously described (Chapter 4). A minimum amplitude of 3 mmHg was used in the algorithm to identify contractions peaks. Post-processing was conducted in SAS, whereby contractions having an amplitude or

duration greater than 30 mmHg or 30 sec, respectively, were removed from the dataset to eliminate appropriate outliers, such as those created when the animal laid down, causing a spike in pressure not associated with a forestomach contraction. The MEANS procedure of SAS was used to calculate number of contractions detected each hour and generate mean contraction peak pressure, amplitude, and duration for each hour. Then, the number of contractions detected per hour was divided by 60 min to determine contraction frequency (contractions/min). Additionally, the hourly means were categorized as being in either period 1 (1-6 h post-feeding), 2 (7-12 h post-feeding), 3 (13-18 h post-feeding), or 4 (19-24 h post-feeding) of the feeding cycle.

When animals laid down, the baseline pressure reading suddenly and greatly increased and would be sustained until the animal stood back up. With this observation, these periods of increased pressure were used to estimate lying behavior of cattle during pressure recordings on MIX, HGd1, HGd2, and HGd3, or until the experiment was stopped. Post-experimentation, each recording was observed in LabChart at 5:1 horizontal scaling and scrolled through to find lying bouts. Using the Data Pad feature of LabChart, the start time, end time, and duration of each lying bout was estimated by selection of the increased pressure section. Total lying time for each animal and day was determined by summing all lying bouts. The MEANS procedure of SAS was used to determine number of lying bouts and average lying bout duration for each day relative to HG feeding.

Ruminal pH & Temperature Measurements

In addition to the spot rumen fluid sampling to monitor ruminal pH manually, ruminal pH (RpH) was monitored continuously (readings every 1 min) using rumen data

loggers (SRL-T9, DASCOR, Inc., Escondido, CA) for 24 h beginning immediately prior to feeding on d -1 and 72 h from feeding on d 1. A data logger and lithium ion battery sealed in a watertight capsule was equipped with a pH electrode exposed to the rumen contents but unable to contact the ruminal epithelium (Penner et al., 2006). This device was inserted into the ventral sac of the rumen through the cannula before feeding on d -1 and 1. A new probe was inserted every 24 h after insertion on d 1 to ensure the probe was working properly throughout the high-grain feeding. The pH electrodes were calibrated before and after each measurement period using pH buffers 7 and 4. When not in use, the pH electrodes were stored in a storage solution (DASCOR, Inc.). Readings were stored in the data logger and downloaded from the data logger after each use. From these data, minimum RpH, mean RpH, maximum RpH, duration RpH < 5.8 (min/d), duration RpH < 5.5 (min/d), and duration RpH < 5.0 (min/d) were determined and summarized for each animal, day, and period (as described above for motility variables). Mean RpH was also determined for each animal, day, and hour.

The rumen data logger (SRL-T9) was also equipped with a built-in sensor to measure ruminal temperature (Trum) at the same time pH was recorded (Mohammed et al., 2014). Minimum Trum, mean Trum, and maximum Trum were determined for each animal on each day and period. Mean Trum was also determined for each animal, day, and hour.

Statistical Analysis

The treatment was the high-grain diet challenge (transition from MIX to HG) with interest in the timing of changes. All data were analyzed using proc MIXED procedure of SAS 9.4 as a randomized complete block with repeated measures using block as a

random variable. An autoregressive covariance structure was used for most repeated measures models, with exceptions for intake and feeding behavior (heterogeneous compound symmetry), liquid passage (heterogeneous autoregressive), motility and viscosity (compound symmetry). These other structures were used because the autoregressive structure had errors or the chosen structure lowered the Bayesian information criterion (BIC). Fixed effect included in all models was day relative to HG feeding (MIX, HGd1, HGd2, and HGd3). When appropriate for some variables, the fixed effects of hour or period and the interaction with day were also included in the model. Effects were considered significant at $P < 0.05$, and effects with $0.05 < P < 0.1$ were considered to show a tendency. The lsmeans were calculated for each analysis and compared when significant effects were present using the least significant difference (LSD) test. The sampling protocol had to be stopped early on the second day of HG feeding (HGd2) for two animals because the RpH dropped too low (MIX: n = 10; HGd1: n = 10; HGd2: n = 8; HGd3: n = 8).

Results

Dry matter intake was influenced (P=0.0002) by day relative to the dietary switch. Animals increased DMI on HGd1 compared to the MIX diet (Figure 5-1A). Then, DM intake was reduced on HGd2 and HGd3 compared to both MIX and HGd1. Water intake was also affected by day (P=0.0083), where total water intake was reduced on HGd2 compared to previous days, but was not different from HGd3 (Figure 5-1B).

Feeding behavior of cattle during the abrupt dietary increase in concentrate was impacted by day. In particular, meal size was not different between MIX and HGd1, but was lower on HGd2 and HGd3 (P=0.0053; Table 5-2). Meal duration was also influenced

by day (P<0.0001), where duration was not different between MIX and HGd1, but was lowest on HGd2 and intermediate on HGd3 (Table 5-2). However, meal frequency was not affected by day (P=0.2764). Consumption rate modeling showed that the time for animals to consume 75% of their daily DMI was reduced on HGd1 compared to MIX, suggesting animals increased consumption rate on HGd1 (P=0.047; Table 5-2). Time to consume 75% of DMI on HGd2 was not different from any other day, and had returned to MIX baseline levels on HGd3.

Ruminal concentrations of all measured individual VFA and total VFA displayed day*hour interactions (P<0.01; Table 5-3; Figure 5-2). The percentage of total VFA represented by acetate and propionate also showed day*hour interactions (P<0.02; Figure 5-3A and B), while percentage of total VFA represented by butyrate tended to have a day*hour interaction, with significant day (P=0.0016) and hour (P=0.0022) effects (Figure 5-3C and D).

Rumen fluid apparent viscosity showed a day*hour interaction (P<0.0001; Figure 5-4). Viscosity was unchanged throughout the feeding cycle on MIX, but increased greatly between 6 and 12 h post-feeding on HGd1, evidenced by the greater viscosity at 12 h post-feeding on HGd1 than all previous time points. Viscosity remained elevated through HGd2 but progressively decreased and by HGd3, viscosity levels were returned to baseline MIX levels. The power law index of the rumen fluid also displayed a day*hour interaction (P=0.0273; Figure 5-5). Once again, by 12 h post-feeding on HGd1, power law index was reduced and remained reduced through 6 h post-feeding on HGd2, but returned to baseline levels by 12 h post-feeding HGd2 for the remainder of the experiment.

Rumen fractional clearance rate of Cr tended (P=0.0624) to be affected by day with greater rates on HGd3 (Table 5-4). Half-life of Cr in the rumen tended (P=0.0946) to be altered by day, with greater values on HGd2 (Table 5-4). Rumen liquid volume was reduced on HGd3 compared to all other days (P=0.0094; Table 5-4). Liquid flow rate was not affected by day (P=0.4074). Clearance rate of VFA from the rumen was reduced by HGd2 after the dietary switch (compared to MIX and HGd1), and on HGd3 clearance rate of VFA was only reduced compared to MIX (P=0.0045). Positive clearance values signify net loss or removal of VFA from the rumen, whereas negative clearance rates denote net accumulation of VFA in the rumen. In other words, negative clearance rates suggest no net loss of VFA from the rumen by passage or absorption, but VFA production was exceeding passage and absorption. Absorption rate of VFA from the rumen was not changed on HGd1 compared to MIX, but was reduced on HGd2 and HGd3 compared to MIX (P=0.0058). Additionally, saliva production estimates differed between days (P=0.039; Figure 5-6), where production was greater on HGd3 than HGd1 or HGd2.

When hourly means of reticulorumen motility variables were analyzed for the effects of day, hour and the interaction, only day was significant for contraction amplitude (P<0.0001) and duration (P<0.0001), but a day*hour interaction existed for contraction frequency. Amplitude was reduced on HGd1 compared to MIX and further reduced on HGd2 and HGd3 compared to HGd1 (Figure 5-7A). Duration of contractions was not changed on HGd1, but was greater on HGd2 compared to all other days (Figure 5-7B). Contraction frequency over time showed a positive quadratic response on MIX and HGd1 that were similar (P<0.0001), negative quadratic response on HGd2

(P<0.0001) below MIX and HGd1 levels, and a positive linear response on HGd3 (P<0.01; Figure 5-7C). When hourly motility means were analyzed for the effects of day, period, and the interaction, all reticulorumen motility variables displayed a day*period interaction (P<0.05; Table 5-5). Reticulorumen contraction amplitude began to be reduced on HGd1, particularly towards the end of the feeding cycle, was significantly reduced in period 1-3 of HGd2 compared to MIX, and remained reduced on all periods of HGd3 (P=0.0402). Contraction duration began to increase on HGd2, particularly period 3 and 4, but was mostly returned to baseline levels on HGd3 (P=0.0038). On MIX and HGd1, contraction frequency showed similar patterns (P<0.0001). However, on HGd2 and HGd3, frequency was reduced compared to previous days, with a low reached in period 4 of HGd2. Contraction peak pressure displayed similar patterns throughout the feeding cycle on MIX and HGd1 with an increase in peak pressure after feeding through period 3, after which it began to return to initial levels. Yet, peak pressure decreased across the feeding cycle on HGd2, then showed a similar pattern, but with greater peak pressures, for HGd3 as MIX and HGd1.

Lying bout duration was influenced by day (P=0.0133; Table 5-5). Animals appeared to have average lying duration that was greater on MIX than the first three days on HG. Additionally, lying bout frequency tended (P=0.0784) to be greater on HGd1 than HGd2 (Table 5-5). Total lying time was not significantly altered by day.

Minimum RpH as well as duration RpH< 5.8, 5.5, and 5.0 were influenced by day (P<0.01; Table 5-6). The minimum RpH was reduced on HGd1, HGd2, and HGd3 compared to MIX (P<0.0001). Duration RpH < 5.8 and 5.5 was greater on HGd1, HGd2, and HGd3 compared to MIX (P=0.0031 and 0.0179, respectively). For HGd1 and HGd2,

duration RpH < 5.0 increased from that on MIX, but was returned to MIX levels on HGd3 (P=0.0148). Mean RpH displayed a day*hour interaction (P=0.0001), where significant quadratic responses (P<0.01) were found for MIX, HGd1, and HGd3 and a positive linear response (P<0.01) was found for HGd2. When RpH variables were analyzed for the effects of day, period, and the interaction, all showed a day*period interaction ($P \leq 0.0011$; Figure 5-9, Figure 5-10).

Minimum Trum was reduced on HGd2 compared to previous days (P=0.0363). Mean Trum showed a day (P=0.0308) and hour (P<0.0001) effect. Mean Trum was less on HGd2 than HGd3. When analyzed for the effects of day, period, and the interaction, minimum Trum showed a significant interaction (P=0.0298) while mean and maximum Trum had significant day and period effects (alternative Table 5-6). Mean Trum was progressively increased from period 1 through 3, but period 4 was not different from 2 or 3. Maximum Trum was greater on all three days of HG feeding compared to MIX.

Discussion

This study aimed to investigate the relationships between physiological parameters and timing of when animals go “off-feed” during an abrupt increase in dietary concentrate. Reductions in feed intake, variable DMI, and/or erratic feeding patterns have been reported when ruminal pH was low, animals are transitioned to a high-grain diet, or when animals undergo an acidosis challenge (Dirksen, 1970; Fulton et al., 1979a; Cooper et al., 1999a). However, cattle appear to vary greatly in their ability to tolerate ingested grain or their susceptibility to experience ruminal acidosis and go “off-feed” during these events (Dougherty et al., 1975b; Brown et al., 2000; Bevans et al., 2005; Brown et al., 2006). Therefore, the results of the study attempted to evaluate if the timing of changes in

a variety of physiological variables were associated with when animals reduced feed intake or the extent to which they went “off-feed.”

In this experiment, steers increased DMI on the first day of high-grain feeding and reduced intake on the second day of high-grain feeding, which is typical when concentrate percentage increases in the diet and animals are allowed *ad libitum* access to feed. Since diets with greater percentages of concentrate have smaller particle sizes with greater density, animals are able to eat them faster (Beauchemin et al., 2008), as evidenced by the 50% reduction in time to consume 75% of their DMI. Then, the post-ingestive consequences of the greater intake take hold at the end of the first day and could be associated with the reduction in feed intake on the second day. Some animals completely or nearly completely avoided feed on the second day of high-grain feeding, which demonstrated that the abrupt dietary switch from 50% concentrate to 90% concentrate was sufficient to cause some animals to go “off-feed.” However, as mentioned previously, there was a great diversity in individual animal tolerance.

Animals varied greatly in ability to tolerate the grain challenge. A greater susceptibility score denoted that animal went “off-feed” to a greater extent. Three steers had susceptibility scores of 1 (low), whose DMI on HGd1 ranged from 78-150% of their MIX DMI. The animal who consumed 78% of its previous DMI was the only animal that appeared to self-regulate and consume less DMI on the first day of high-grain feeding. Nevertheless, some researchers believe that animals need to have experienced the post-ingestive consequences of diets that are more fermentable in order to be able to do this voluntarily to avoid a ‘lethal meal’ (González et al., 2012). One steer had a susceptibility score of 2 and consumed about 115% of their MIX DMI on HGd1. Four steers had a

score of 3, and they consumed between 132% and 260% of their MIX DMI on HGd1. Despite one animal consuming 260% of their MIX DMI on HGd1 and going completely "off-feed" on HGd2, this animal did not experience excessively low ruminal pH. This suggested that animal may have compensated for the increased acid load by other means, such as a greater absorptive capacity of ruminal epithelium (Penner et al., 2009a; Zebeli and Metzler-Zebeli, 2012), greater expression of genes in the rumen involved in maintenance of intracellular pH (Schlau et al., 2012), or a more well-adapted ruminal microbial community (Allison et al., 1964; Chen et al., 2012). Two steers had a score of 4 and consumed 123-159% of their MIX DMI on HGd1. Thus, there appeared to be no clear correlation between DMI consumed on HGd1 and susceptibility of the animal to go "off-feed" during the dietary transition; other mechanisms must be involved with helping the animal deal with the acidosis challenge.

Feeding behavior is a key determinant of rumen acid-base balance due to its implications on synchronization of acid production and elimination. Larger meals, which often occur for animals consuming high-grain diets with small particle sizes, and faster consumption rates increase the acid load in the rumen and may overwhelm the capacity for ruminal metabolism and pH regulation leading to accumulation of acid in the rumen, reduction in ruminal pH, and potential reductions in feed intake (Dado and Allen, 1995; Tolkamp et al., 2002). In this study, meal size was not increased on the first day of high-grain feeding, but animals did consume their daily DMI faster on this day than on the MIX diet. Faster consumption would also increase the acid challenge for the animals. Conversely, smaller, more frequent meals help to stabilize ruminal pH and reduce the risk of ruminal acidosis (González et al., 2012). In a previous study, animals undergoing an

acidotic challenge reduced meal size and also increased meal frequency (DeVries et al., 2009). Meal size and duration were reduced on the second day of high-grain feeding in this experiment, which is when animals consumed less DM than previous days, but meal frequency was not affected. Overall, the greater DMI and faster consumption rate on the first day of high-grain feeding, might have resulted in post-ingestive consequences that led animals to reduce intake, meal size, and meal duration on the second day of high-grain feeding. Along with these changes in meal characteristics that would help synchronize acid production and elimination, the greater saliva production on HGd3 would have aided in buffering the rumen and may have facilitated animal recovery from the acidotic insult.

On the first day of high-grain feeding, total VFA concentrations in the rumen were greater than all other days for a significant portion of the day (6-18h post-feeding). This suggested that there was a greater acid load in the rumen at this time. Yet, saliva production, which would help eliminate acid through neutralization with bicarbonate, was not different from MIX on this day. Ruminal pH data showed reduced pH on HGd1 and increased time pH was below thresholds and changes were sustained through HGd3. Together, these data suggested the experiment was successful at experimentally inducing ruminal acidosis. Previously, some studies have used a ruminal pH below 5.6 for more than 180 min or below 5.8 for about 100 min to diagnose SARA (Gozho et al., 2005; Penner et al., 2010). On all three days of feeding the high-grain diet, the duration that ruminal pH was below 5.5 was well above 180 min, indicating that the challenge was at least a severe SARA challenge. Since ruminal pH was below 5.0 for more than 100 min on HGd1 and HGd2, one may conclude that the abrupt dietary switch imposed in this

study was actually an acute acidotic challenge. Interestingly, despite ruminal pH being below 5.0 for approximately 200 min on the first day of high-grain feeding (including a significant portion during period 3), animals did not appear to reduce feed intake until the second day, suggesting ruminal pH was not solely responsible for reducing feed intake. Previous research in dairy cows (Khafipour et al., 2009a; b) and beef cattle (Faleiro et al., 2011) also came to the conclusion that ruminal pH was not responsible for the reduction in feed intake. For example, while two acidosis challenges with either grain or alfalfa pellets both reduced ruminal pH, the grain-induced acidosis reduced feed intake, but the alfalfa-induced acidosis actually increased intake (Khafipour et al., 2009a; b).

Around the same time on the first day of high-grain feeding that the reduction in mean ruminal pH was first evident (period 2 or 7-12 h post-feeding), rumen fluid viscosity was greatly increased (12 h post-feeding). In fact, the main period that ruminal pH was reduced and viscosity was increased were very similar. Viscosity was increased from 12 h post-feeding on the first day through 12 h post-feeding on the second day of high-grain feeding, while ruminal pH was reduced from period 2 on the first day through period 3 on the second day of high-grain feeding. This finding suggested that rumen fluid viscosity and maintenance of ruminal pH are associated, as hypothesized. Power law index, which was below 1 for the rumen fluid samples, revealed that rumen fluid behaves as a pseudoplastic or shear thinning fluid, which means viscosity would decrease as mixing increases. This property may be beneficial during this transition since the rumen fluid had a greater viscosity and would then need less force for the same extent of mixing.

Reticulorumen motility is responsible for the mixing of rumen fluid and contents (Waghorn and Reid, 1983). Our previous study, which induced SARA, observed a reduction in reticulorumen contraction amplitude, duration, and frequency. In the current study using a more severe acidotic model, reticulorumen motility was also altered, but in a different manner. While amplitude and frequency was reduced during the dietary transition, contraction duration actually increased slightly on the second day of high-grain feeding. The reason for an increase in contraction duration is unknown and warrants further investigation. Previous research has reported inhibition (Cebrat, 1979; Kezar and Church, 1979) or complete abolishment (Juhasz and Szegedi, 1968) of rumen motility with severe or acute acidosis, so the reduction in contraction amplitude and frequency were expected. Motility changes occurred primarily on the second day of high-grain feeding, which was after important changes in ruminal pH. Thus, the reduction in ruminal pH may have at least in part been associated with or led to reductions in rumen motility. Some research has suggested that factors, such as bacterial endotoxins or histamine, arising during low ruminal pH periods could reduce contraction amplitude and frequency (Dougherty et al., 1975b; Andersen, 2003; Plaizier et al., 2009). Yet, the exact mechanism (such as buccal receptors, acid receptors, osmoreceptors, central nervous system, or the acute phase response) responsible for altering rumen motility during dietary transitions and ruminal acidosis is still unknown. Furthermore, the reduced motility could resemble a lower shear rate. Despite the reduction in shear rate, the reduced power law index could help maintain mixing of the greater viscosity rumen fluid.

Lying behavior of cattle was also altered by the abrupt increase from 50% concentrate to 90% concentrate in this study. In our previous studies, we noted that lying

increased baseline ruminal pressure (Egert et al., 2014), thus creating a relationship between lying behavior and contraction peak pressures, which we used to quantify lying behavior in this experiment. Based on this relationship, the contraction peak pressures suggested that animals laid down less on the second day and more on the third day of high-grain feeding compared to the MIX diet. Total lying time tended to be lower on the second day of high-grain feeding, which would agree with the lower peak pressure seen on this day. Steers laid down for longer periods of time on average on the 50% concentrate diet compared to the high-grain diet, which would be expected for a more forage-based diet that fills up the rumen faster and requires longer periods of rumination.

The reduction in rumen motility seen in this study may have been associated with the tendencies for alterations in ruminal liquid retention time and half-life, which could have suggested slowed passage. Other research has demonstrated close, positive relationships between frequency and duration of reticular contractions with ruminal liquid passage rates (Sissons et al., 1984; Okine et al., 1989). However, liquid flow rate was not affected by day and fractional liquid dilution rate only tended to be different between days. Thus, perhaps the reduction in motility was not large enough to slow liquid passage in this study.

Estimates of VFA absorption rate suggested that absorption was reduced on the second and third day of high-grain feeding compared to the 50% concentrate diet. Research has shown that a linear correlation existed between VFA concentrations and osmotic pressure of rumen fluid (Bennink et al., 1978). Additionally, high osmolality in the rumen during acidosis decreased the rate of acid absorption (Tabaru et al., 1990). Ruminal osmoreceptors can also detect increases in rumen fluid osmolality and mediate

reductions in feed intake (Carter and Grovum, 1988; Carter and Grovum, 1990). While ruminal osmolality was not measured in this experiment, the switch to the high-grain diet did lead to the animals experiencing ruminal acidosis and increased VFA concentration. This would suggest greater ruminal osmolality and may help explain the reduced VFA absorption rate seen in this study. The greater viscosity of rumen fluid observed in this study could slow the movement of absorptive substrates and VFA through the rumen fluid and to the ruminal epithelium, which could also have been related to the reduced VFA absorption. A decreased VFA absorption rate would lead to an accumulation of VFA in the rumen and associated reduction in ruminal pH, such as described for the current study. Furthermore, since one mechanism of VFA absorption from the rumen involves exchange of an ionized acid for a bicarbonate, reduced VFA absorption by this mechanism would also reduce bicarbonate flow into the rumen and buffering capacity (Owens et al., 1998).

Conclusions

This study investigated the relationships between physiological parameters and the timing of when animals go “off-feed” during an abrupt dietary increase in concentrate. The grain engorgement model switching cattle from 50% to 90% concentrate was successful at inducing ruminal acidosis and did reduce reticulorumen motility, albeit minor in magnitude. Patterns of ruminal pH, viscosity, and motility changes were related to when cattle reduced feed intake. Furthermore, this study determined that rumen fluid viscosity increased during an abrupt increase in dietary concentrate and was associated with periods of reduced ruminal pH, motility, and VFA absorption.

Tables and Figures

Table 5-1. Composition of diets and ingredients.

Item	Diet	
	MIX	HG
Ingredient, % DM basis		
Fescue, K31 Hay	50	-
Corn silage	-	18.00
Cracked corn	37.4	-
High-moisture corn	-	74.95
Soybean meal	-	4.00
Dried distiller grains	4.6	-
Corn gluten feed	6.1	-
Urea	0.46	0.50
Tallow	0.46	0.30
Limestone	1.05	1.40
Trace mineral premix[1]	0.6	0.45
Magnesium oxide	0.03	-
Potassium oxide	0.11	-
Salt	0.18	-
Vitamin A, D, & E Premix[2]	-	0.15
Potassium sulfate	-	0.25
Nutrient Composition, DM basis		
Crude Protein, %	12.5	11.6
ADF, %	24.8	6.4
aNDF, %	47.2	15.1
TDN, %	67	77
Calcium, %	0.27	0.44
Phosphorus, %	0.41	0.36
Magnesium, %	0.17	0.12
Potassium, %	1.45	0.71
Sodium, %	0.211	0.144
Sulfur, %	0.16	0.15
Iron, ppm	175	105
Zinc, ppm	66	43
Copper, ppm	8	9
Manganese, ppm	43	25
Molybdenum, ppm	0.9	0.5

[1] Contained 56.34% Cl, 36.53% Na, 1.2% S, 0.06% Ca, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, and 5520.2 ppm Zn on a DM basis.

[2] Composed of vitamin A acetate (1,814,368 IU/kg), D-activated animal sterol (source of vitamin D3; 362,874 IU/kg), vitamin E supplement (227 IU/kg), roughage products, calcium carbonate, and mineral oil.

Table 5-2. Feeding behavior of cattle as they were abruptly switched from a hay and grain mixed diet (MIX) to a 90% concentrate, high-grain (HG) diet.

Item	Day				Pooled SEM[5]	*P*-value
	MIX[1]	HGd1[2]	HGd2[3]	HGd3[4]		
Meal Characteristics						
Size, g	1817[a]	1706[a]	667[b]	857[b]	199	0.0053
Duration, min	40.9[a]	30.5[a]	8.1[c]	15.2[b]	3.1	<0.0001
Frequency, meals/d	7.50	9.00	5.58	9.34	1.18	0.2764
Time to consume 75% of DMI, min	620[a]	294[b]	407[ab]	813[a]	111	0.047

[1]MIX: diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement
[2]HGd1: first day of feeding a 90% high-moisture corn based, finishing diet
[3]HGd2: second day of feeding a 90% high-moisture corn based, finishing diet
[4]HGd3: third day of feeding a 90% high-moisture corn based, finishing diet
[5]SEM represents a pooled SEM across all days
[a,b,c] Means within a row without a common superscript letter are significantly different.

Table 5-3. Ruminal volatile fatty acid (VFA) concentrations in cattle that where abruptly switched from a hay-grain mix (MIX) diet to a high-grain (HG) diet.

Item	*P*-value			Linear		Quadratic		Cubic	
	Day	Hour	Day* Hour	*P*-value	Adj. r^2	*P*-value	Adj. r^2	*P*-value	Adj. r^2
Acetate	<0.0001	0.0048	0.0013						
MIX[1]				0.0414	0.2652	0.0033	0.6178	0.0082*	0.6174
HGd1[2]				0.0994*	0.1572	<0.0001	0.9084	<0.0001	0.9671
HGd2[3]				0.0009	0.6184	0.0044*	0.5950	0.0129*	0.5753
HGd3[4]				0.1322*	0.1205	0.2118*	0.1202	0.0594	0.3928
Propionate	0.0013	0.0006	<0.0001						
MIX				0.0803*	0.1843	0.0008	0.7119	<0.0001	0.9102
HGd1				0.7205*	-0.0777	0.0005	0.7347	<0.0001	0.9902
HGd2				0.8310*	-0.0862	0.0094	0.5279	0.0194*	0.5333
HGd3				<0.0001	0.8657	<0.0001*	0.8630	0.0001*	0.8480
Butyrate	0.0002	<0.0001	0.0004						
MIX				0.2655*	0.0304	<0.0001	0.8100	<0.0001	0.9026
HGd1				0.0220	0.3372	<0.0001	0.9535	<0.0001*	0.9489
HGd2				0.0002	0.7006	0.0010*	0.6996	0.0012*	0.7534
HGd3				0.0841*	0.1784	0.0062	0.5661	0.0024	0.7124
Valerate	<0.0001	<0.0001	0.0063						
MIX				0.0013	0.5928	0.0015*	0.6736	0.0007	0.7794
HGd1				0.6063*	-0.0637	0.0104	0.5182	<0.0001	0.9112
HGd2				0.0003	0.6820	0.0021*	0.6503	0.0084*	0.6156
HGd3				0.0067	0.4569	0.0058*	0.5720	0.0043*	0.6701
Total VFA	0.0002	0.0002	0.0008						
MIX				0.0471	0.2497	0.0008	0.7141	0.0006	0.7921
HGd1				0.1521*	0.1023	<0.0001	0.9096	<0.0001	0.9803
HGd2				0.0020	0.5607	0.0091*	0.5317	0.0075*	0.6250
HGd3				0.0061	0.4656	0.0078*	0.5456	0.0042	0.6720
Percentage of Total VFA									
Acetate	0.0001	0.1150	0.0164						
MIX				0.1328*	0.1200	0.0028	0.6291	0.0001	0.8482
HGd1				0.8557*	-0.0875	<0.0001	0.8325	<0.0001	0.9333
HGd2				0.0003	0.6903	<0.0001	0.9517	<0.0001*	0.9543
HGd3				<0.0001	0.9253	<0.0001*	0.9316	<0.0001*	0.9264
Propionate	0.0045	0.2260	<0.0001						
MIX				0.1199*	0.1331	0.0022	0.6459	<0.0001	0.8931
HGd1				0.0021	0.5533	0.0016	0.6691	<0.0001	0.9066
HGd2				0.0008	0.6262	<0.0001	0.8332	0.0003*	0.8172
HGd3				0.0040	0.5036	0.0005	0.7391	0.0002	0.8369
Butyrate	0.0016	0.0022	0.0604	0.0416	0.2647	<0.0001	0.9242	<0.0001*	0.9176

[1]MIX: diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement
[2]HGd1: first day of feeding a 90% high-moisture corn based, finishing diet
[3]HGd2: second day of feeding a 90% high-moisture corn based, finishing diet
[4]HGd3: third day of feeding a 90% high-moisture corn based, finishing diet
* Highest order term for this regression model was not significantly different from zero

Table 5-4. Rumen liquid dilution or passage rate (Kp) and volatile fatty acid (VFA) clearance (Kc) and absorption rate (Ka) estimates from Cr and valerate disappearance from the rumen for cattle through the transition as they were abruptly switched from a 50% chopped hay and 50% concentrate supplement (MIX) to a 90% concentrate finishing diet (HG).

Item	Day				Pooled SEM[5]	*P*-Value
	MIX[1]	HGd1[2]	HGd2[3]	HGd3[4]		
Fractional dilution rate, %/h (FDR; Kp)[6]	7.27	8.10	7.54	11.67	1.36	0.0624
Cr half-life, h	9.93	9.02	16.36	7.55	1.95	0.0946
Rumen liquid volume, L	41.67[a]	39.76[a]	41.15[a]	32.49[b]	2.45	0.0094
Liquid flow rate, L/h	3.00	3.15	2.71	3.81	0.381	0.4074
VFA clearance rate, %/h (Kc)[7]	8.35[a]	3.46[ab]	-6.49[c]	-0.62[bc]	2.6	0.0045
VFA absorption rate, %/h (Ka)[8]	1.20[a]	-4.96[ab]	-11.44[b]	-12.74[b]	2.8	0.0058
Total VFA absorption, mol/h[9]	2.79	-10.15	-36.21	-27.12	10.2	0.0576

[1]MIX: diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement
[2]HGd1: first day of feeding a 90% high-moisture corn based, finishing diet
[3]HGd2: second day of feeding a 90% high-moisture corn based, finishing diet
[4]HGd3: third day of feeding a 90% high-moisture corn based, finishing diet
[5]SEM represents a pooled SEM across all days
[6] Fractional dilution rate of liquid in the rumen was calculated by exponential decay of Cr
[7]VFA clearance rate was calculated by exponential decay of valerate
[8]VFA absorption rate was calculated by subtracting the rumen fractional dilution rate (assumed to equal valerate passage rate; Kp) from the rumen VFA clearance rate (Kc)
[9]Total VFA concentration at 0 h (mol/L) × rumen liquid volume (L) × VFA absorption rate (%/h)
[a,b,c] Means within a row without a common superscript letter are significantly different.

Table 5-5. Reticulorumen motility contraction characteristics and lying behavior as cattle are abruptly switched from a 50% chopped hay and 50% concentrate supplement (MIX) diet to a 90% concentrate finishing diet (HG).

Item	Day				Pooled	*P*-value		
	MIX[1]	HGd1[2]	HGd2[3]	HGd3[4]	SEM[5]	Day	Period	Day*Period
Amplitude, mmHg	7.80	7.10	6.74	6.62	0.47	<0.0001	0.4679	0.0402
Duration, s	15.1	14.9	15.9	15.2	0.20	<0.0001	0.0123	0.0038
Frequency, contractions/min	1.39	1.40	0.89	0.82	0.07	<0.0001	<0.0001	<0.0001
Peak pressure, mmHg	6.12	8.39	5.87	12.14	2.36	<0.0001	<0.0001	0.0030
Lying frequency, bouts/d	10.2	13.2	9.8	11.1	1.7	0.0784	-	-
Lying duration, min/bout	66.6[a]	44.4[b]	48.8[b]	54.7[b]	4.8	0.0133	-	-
Total lying time, min	653.4	567.0	458.8	572.8	83.7	0.2213	-	-

[1]MIX: diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement
[2]HGd1: first day of feeding a 90% high-moisture corn based, finishing diet
[3]HGd2: second day of feeding a 90% high-moisture corn based, finishing diet
[4]HGd3: third day of feeding a 90% high-moisture corn based, finishing diet
[5]SEM represents a pooled SEM across all days
[a,b] Means within a row without a common superscript letter are significantly different.

Table 5-6. Ruminal pH and temperature during an abrupt transition from a hay-grain mixed (MIX) diet to a high-grain, finishing (HG) diet.

Item	Day				SEM[5]	*P*-value		
	MIX[1]	HGd1[2]	HGd2[3]	HGd3[4]		Day	Hour	Day*Hour
Ruminal pH								
Minimum	6.38[a]	5.04[b]	5.01[b]	5.30[b]	0.157	<0.0001	-	-
Mean	6.82	5.80	5.96	6.12	0.137	<0.0001	<0.0001	0.0001
Maximum	7.19	6.93	7.11	7.11	0.109	0.355	-	-
Duration < 5.8, min	<1[b]	617.6[a]	508.6[a]	495.6[a]	117	0.0031	-	-
Duration < 5.5, min	<1[b]	426.2[a]	318.7[a]	288.8[a]	95	0.0179	-	-
Duration < 5.0, min	<1[b]	205.6[a]	141.4[a]	33.9[b]	60	0.0148	-	-
Ruminal temperature								
Minimum, °C	37.6[a]	36.7[a]	34.3[b]	36.0[ab]	0.78	0.0363	-	-
Mean, °C	39.3[ab]	39.4[ab]	39.0[b]	39.6[a]	0.16	0.0308	<0.0001	0.9355
Maximum, °C	40.1	40.3	39.8	40.3	0.24	0.2249	-	-

[1]MIX: diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement
[2]HGd1: first day of feeding a 90% high-moisture corn based, finishing diet
[3]HGd2: second day of feeding a 90% high-moisture corn based, finishing diet
[4]HGd3: third day of feeding a 90% high-moisture corn based, finishing diet
[5]SEM represents a pooled SEM across all days
[a,b] Means within a row without a common superscript letter are significantly different

Figure 5-1. Feed and total water intake as cattle were underwent a large increase in dietary concentrate from 50% (MIX) to a 90% concentrate, high-grain (HG) diet. A) Dry matter intake was altered (*P*=0.0002) by day relative to the switch. Cattle increased DMI on the first day of high-grain feeding (HGd1) compared to MIX, but then reduced DMI on the second (HGd2) and third (HGd3) days of high-grain feeding compared to previous days. B) Total water intake (including from feed) was influenced (*P*=0.0083) by day relative to the abrupt switch to the high-grain diet, where consumption was reduced on HGd2 compared to previous days.

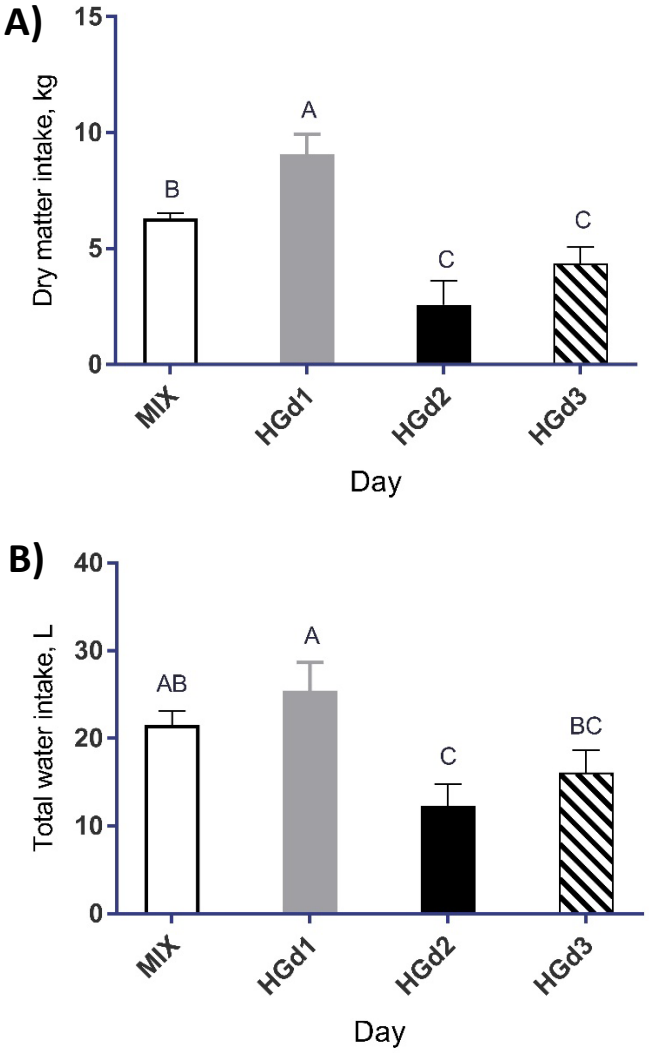


Figure 5-2. Volatile fatty acid (VFA) concentrations in rumen fluid displayed day*hour interactions (P<0.01). A) Acetate concentration was greater on HGd1 than all other days from 8 h to 20h post-feeding. B) Propionate concentration was greater on HGd1 than all other days from 4 h to 10 h post-feeding. C) Butyrate concentration was greater on HGd1 than all other days from 8 h to 18 h post-feeding. D) Total VFA concentration was greater on HGd1 than all other days from 6 h to 18 h post-feeding.

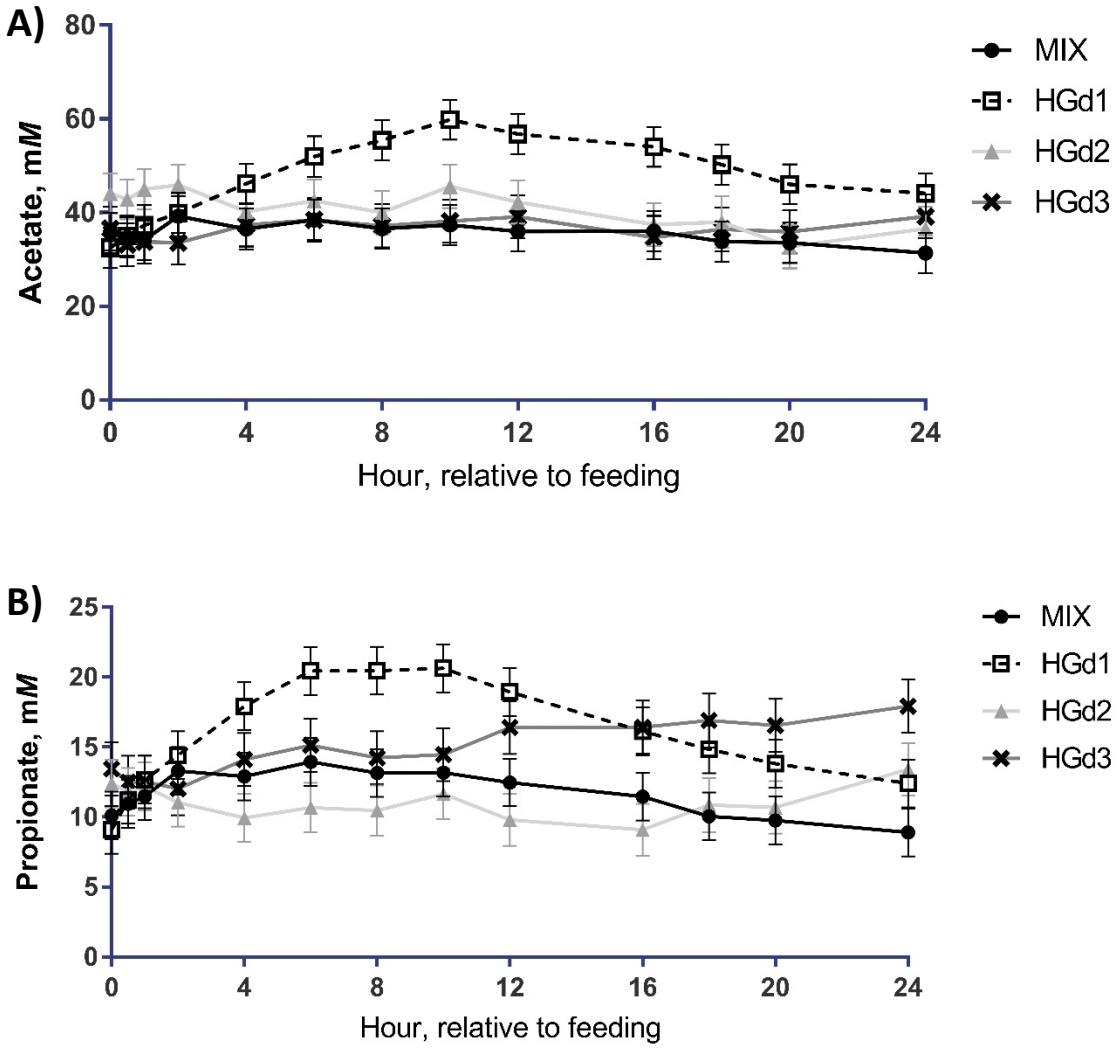


Figure 5-2 continued.

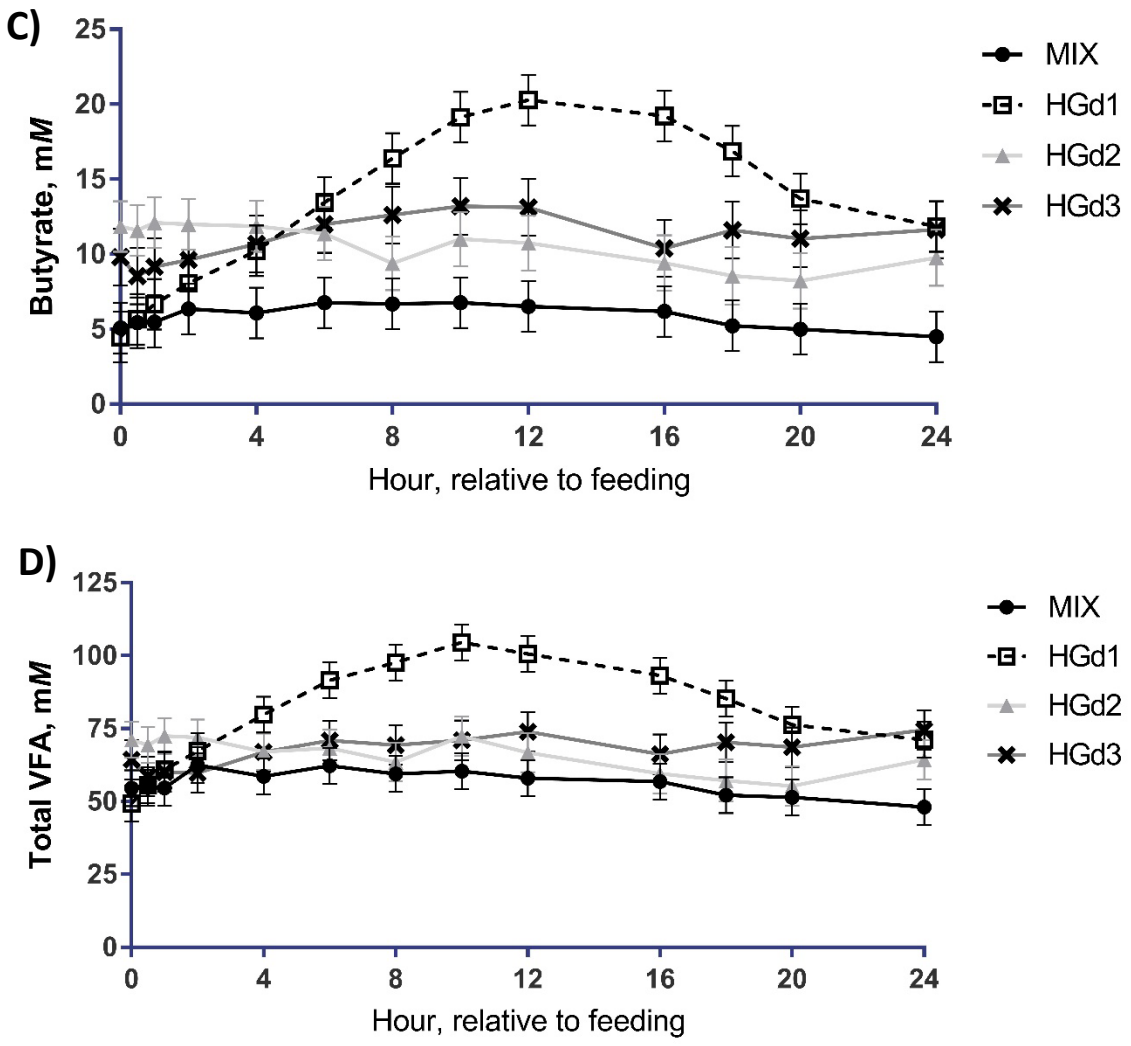


Figure 5-3. A) The percentage of total VFA represented by A) acetate and B) propionate displayed day*hour interactions (P<0.02). Acetate percentage was reduced around feeding (0 h) on HGd3 compared to all other days. Propionate percentage was reduced on HGd2 from 0 to 12 h following feeding compared to all other days. Percentage of total VFA represented by butyrate had significant effects of C) day (P=0.0016) and D) hour (P=0.0022). Percentage of butyrate was greater on all three days of HG feeding compared to MIX and showed a quadratic (*P*<0.0001) response by hour.

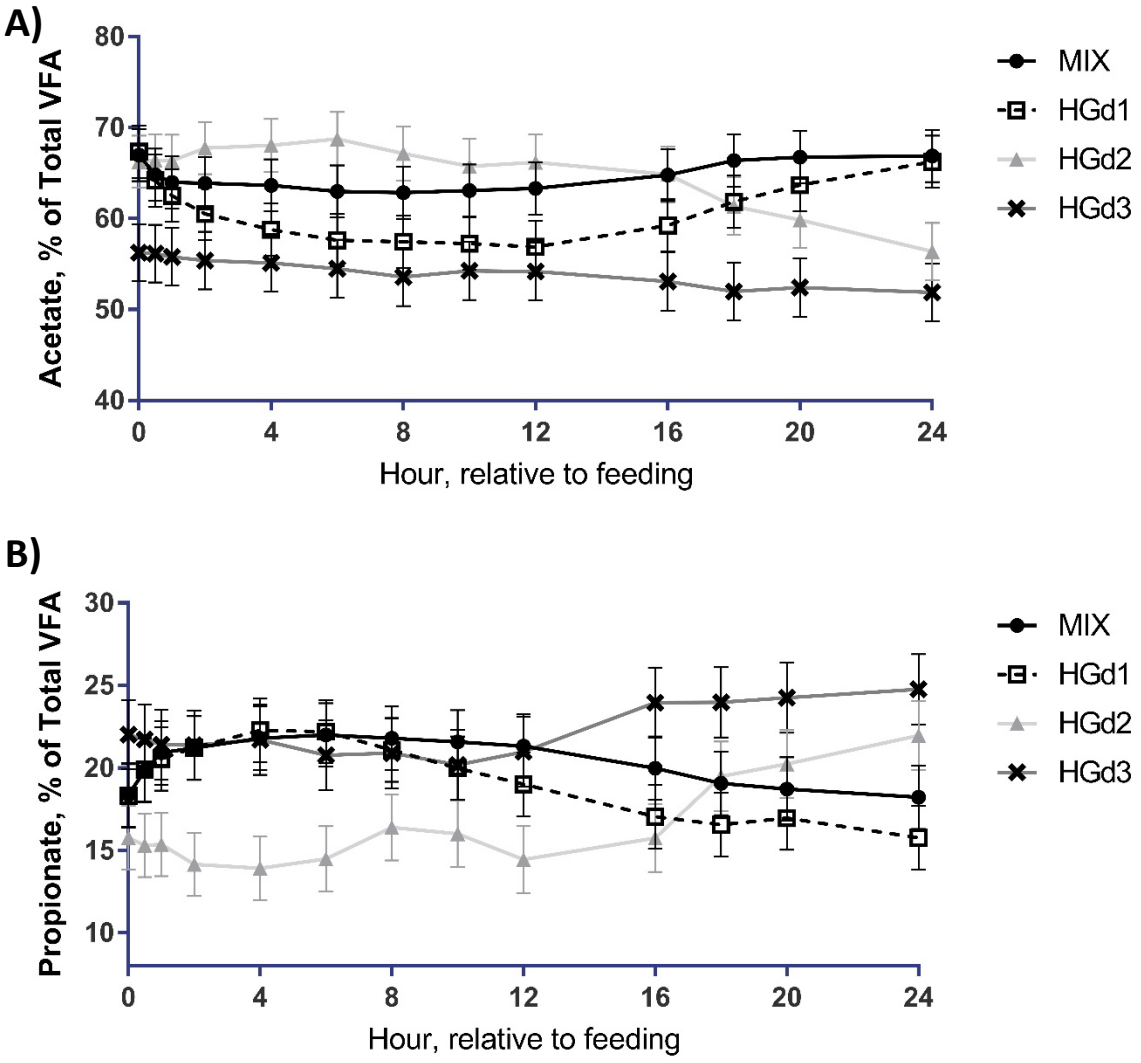


Figure 5-3 continued.

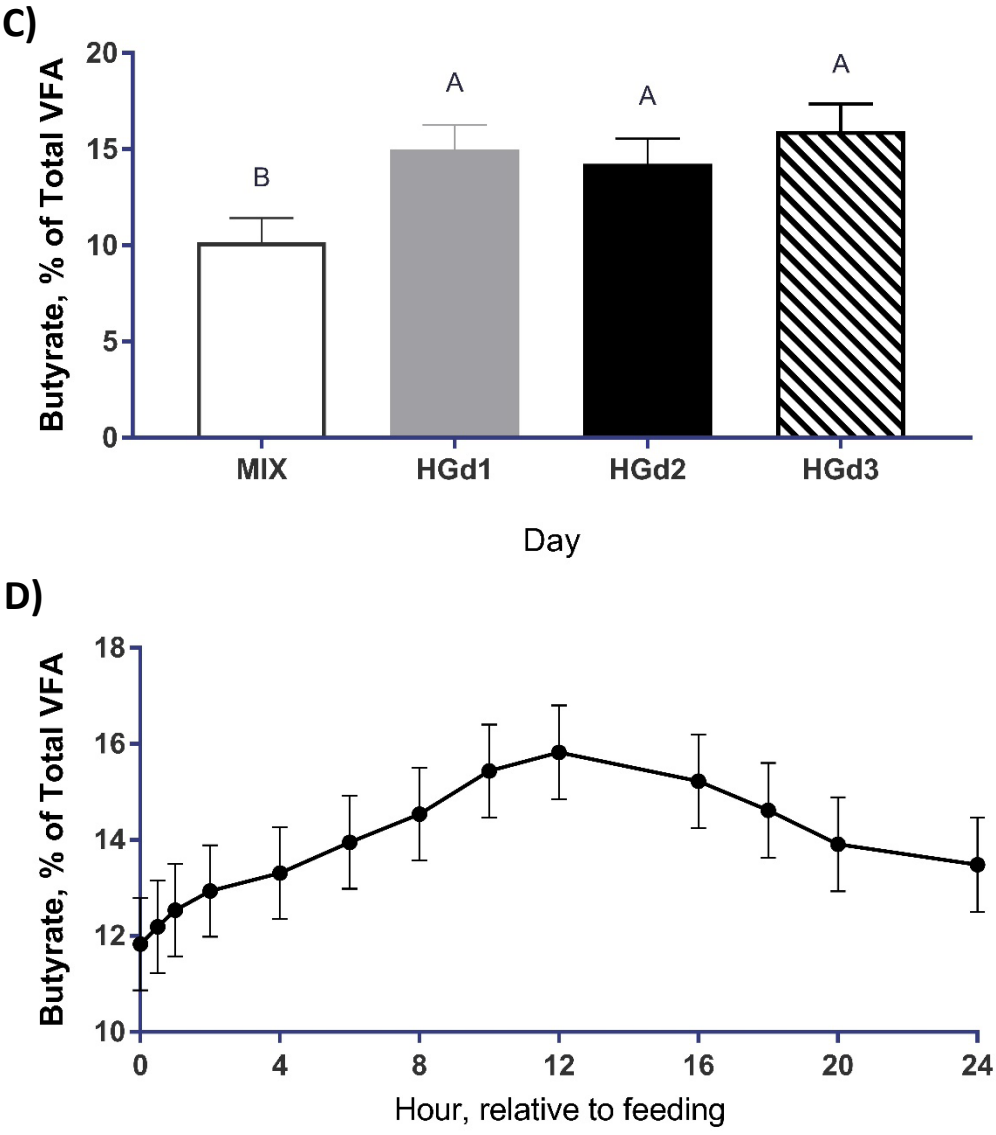


Figure 5-4. Rumen fluid viscosity displayed a day*hour interaction (P<0.01). Rumen fluid viscosity of steers abruptly switched to a high-grain diet increased greatly by 12 h after feeding on the first day of high-grain feeding (HGd1) compared to the 50% concentrate diet (MIX) and remained elevated throughout the second day of high-grain feeding (HGd2).

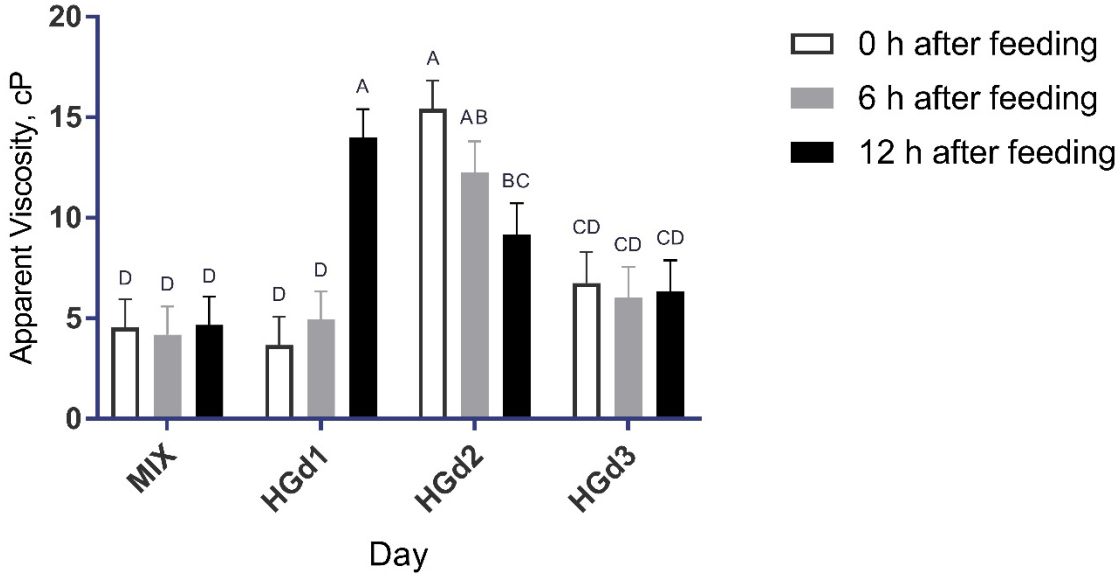


Figure 5-5. The power law index for rumen fluid displayed a day*hour interaction (P<0.01), where the index was reduced from 12 h after feeding on the first day of high-grain feeding (HGd1) through 6 h after feeding on the second day of high-grain feeding (HGd2).

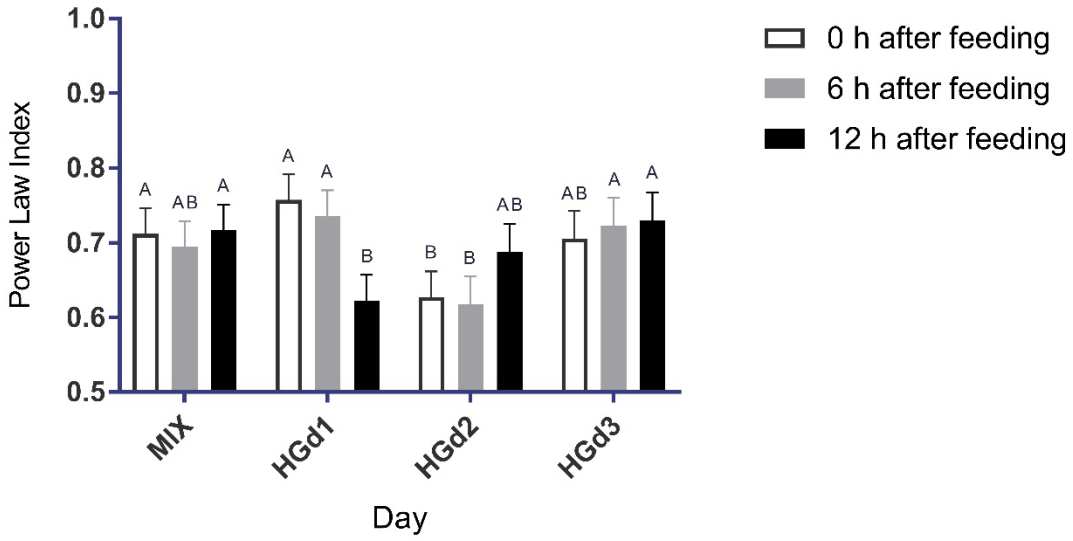


Figure 5-6. Saliva production was influenced by day (P=0.039), where saliva production was greater on the third day of high-grain feeding (HGd3) compared to the first or second day of high-grain feeding (HGd1 and HGd2, respectively).

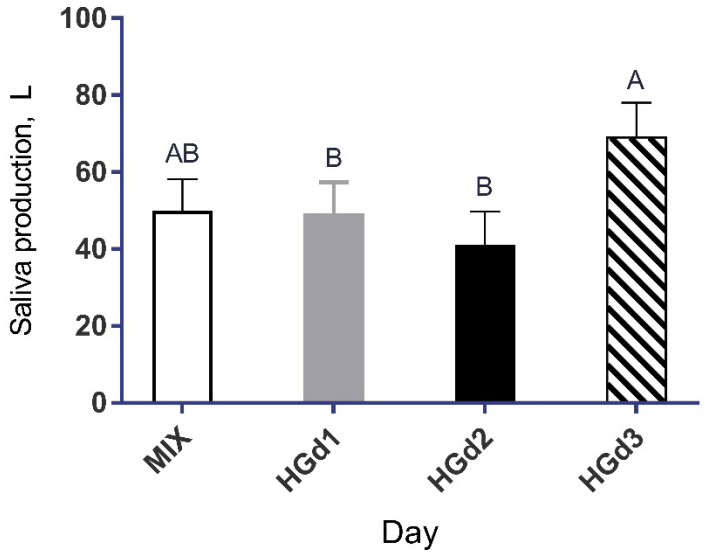


Figure 5-7. Changes in reticulorumen motility following abruptly switching cattle from a 50% concentrate to a 90% concentrate high-grain diet. A) Reticulorumen contraction amplitude was reduced (P<0.0001) by the first day of high-grain feeding (HGd1) and was further reduced on day 2 (HGd2) and day 3 (HGd3) of high-grain feeding. B) Reticulorumen contraction duration was greater (P<0.0001) on the second day of high-grain (HGd2) feeding compared to all other days. C) Reticulorumen contraction frequency displayed a day*hour interaction (P<0.0001). On the hay-grain mixed diet (MIX), high-grain diet day 1 (HGd1), and high-grain diet day 2 (HGd2) the responses were quadratic (P<0.0001) throughout the feeding cycle, whereas contraction frequency increased linearly (P<0.01) on high-grain day 3 (HGd3).

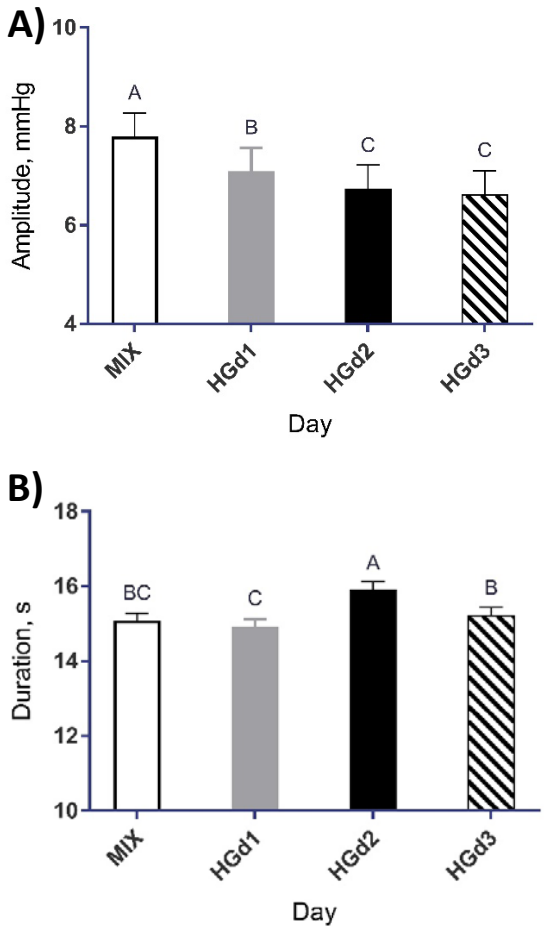


Figure 5-7 continued.

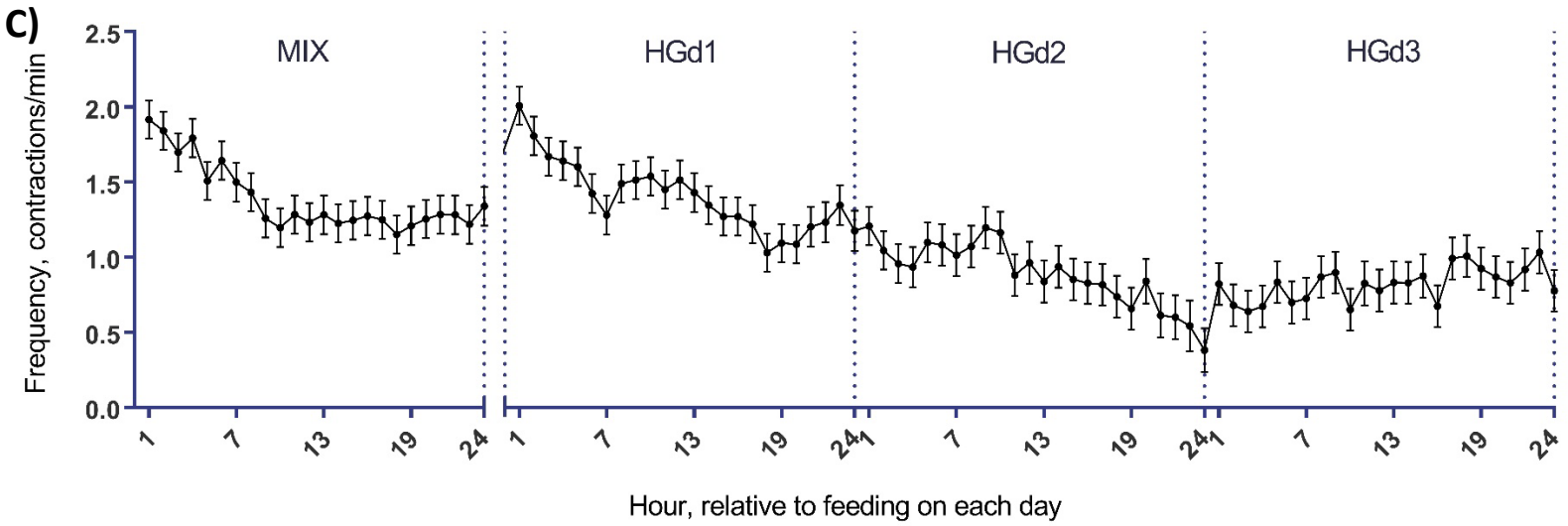


Figure 5-8. Ruminal pH mean showed a day*hour interaction (P=0.0001). Quadratic responses (P<0.01) over hour relative to feeding were found for the 50% concentrate diet (MIX), first day of 90% concentrate, high-grain feeding (HGd1), and third day of high-grain feeding (HGd3) and a positive linear response (P<0.01) was found for the second day of high-grain feeding (HGd2).

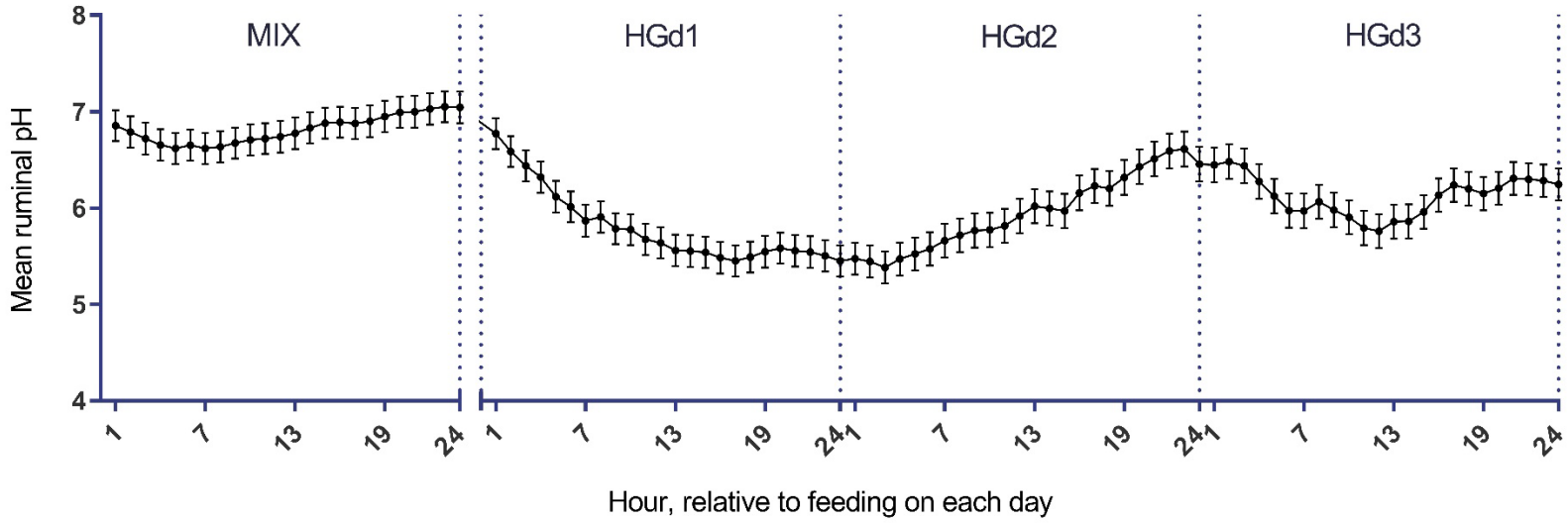


Figure 5-9. Ruminal pH changes as cattle were abruptly switched from a 50% to a 90% concentrate diet. Ruminal pH minimum, mean, and maximum showed day*period interactions (P<0.001). A) Minimum ruminal pH was reduced during period 1 of the first day of high-grain feeding (HGd1) and remained reduced for the remainder of the experiment. B) Mean ruminal pH was decreased during period 1 of HGd1 and was further reduced in later periods. On the second day of high-grain feeding (HGd2), mean ruminal pH increase progressively. While still reduced compared to the 50% concentrate diet (MIX), mean ruminal pH showed a similar pattern as MIX on HGd3. C) Maximum ruminal pH showed a similar pattern across days as minimum and mean ruminal pH.

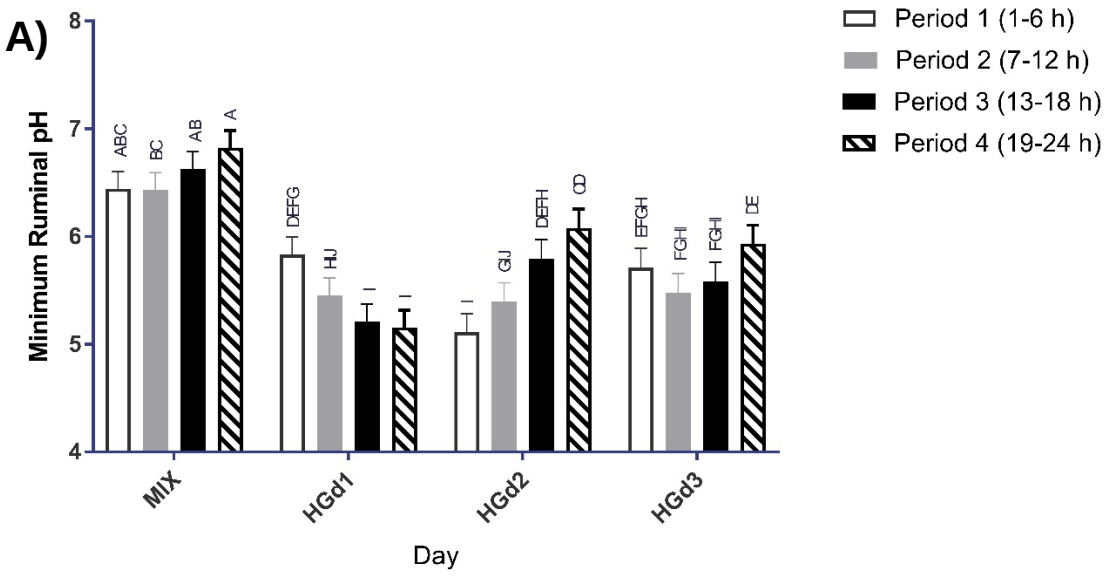


Figure 5-9 continued.

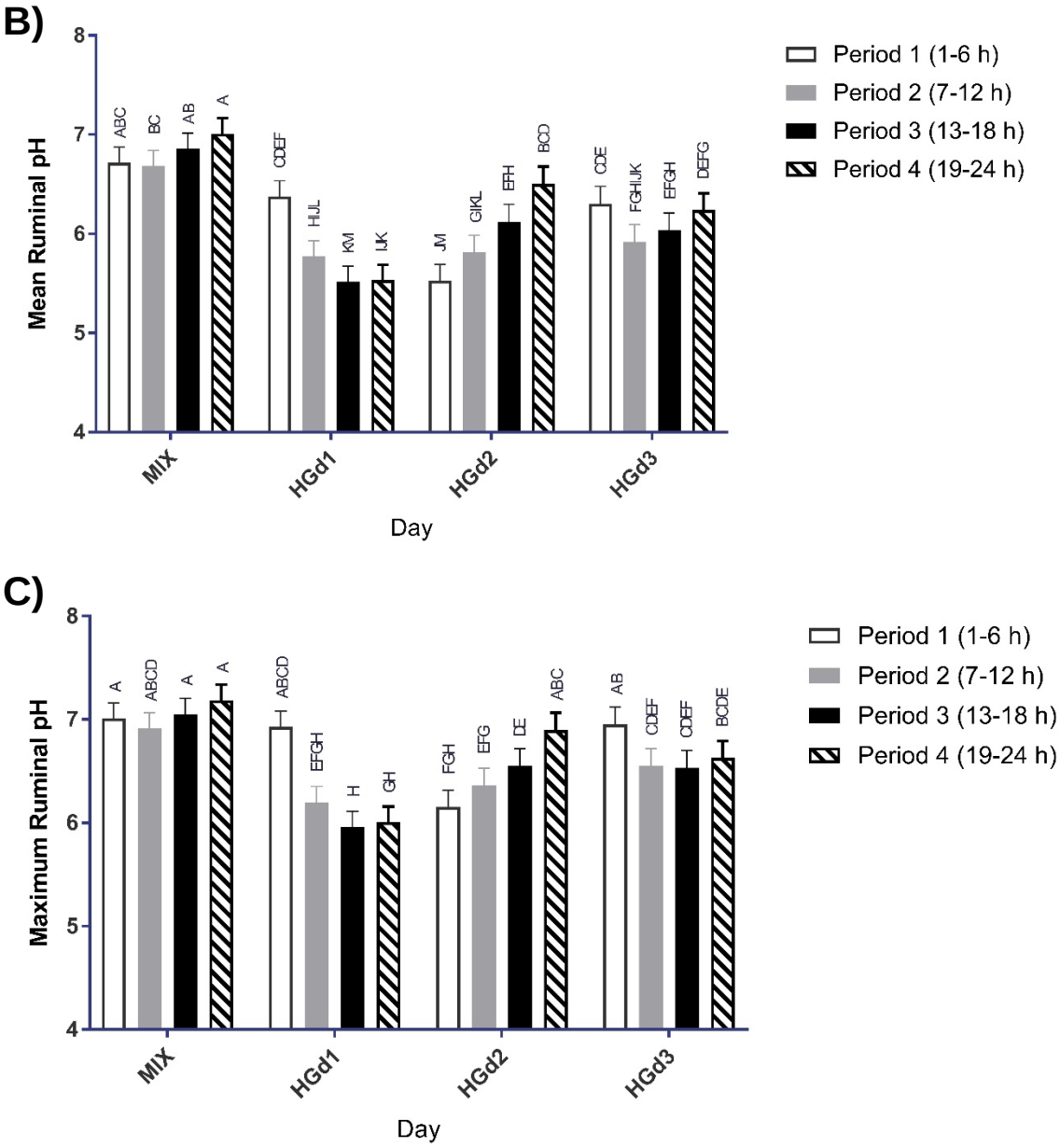


Figure 5-10. Changes of duration that ruminal pH was below thresholds as cattle were abruptly switched from a 50% to 90% concentrate diet. Duration that ruminal pH was below 5.8, 5.5, and 5.0 showed day*period interactions (P<0.01). A) Duration that ruminal pH was below 5.8 increased greatly by period 2 of the first day of high-grain feeding (HGd1), then decreased across the second day of high-grain feeding (HGd2), and yet, remained elevated compared to the 50% diet on the third day of high-grain feeding (HGd3). B) The duration that ruminal pH was below 5.5 increased by period 2 on HGd1, returned to MIX durations in period 4 of HGd2, and then increased by period 2 again on HGd3. C) Duration that ruminal pH was below 5.0 increased in period 3 of HGd1, but returned to baseline levels in period 2 of HGd2.

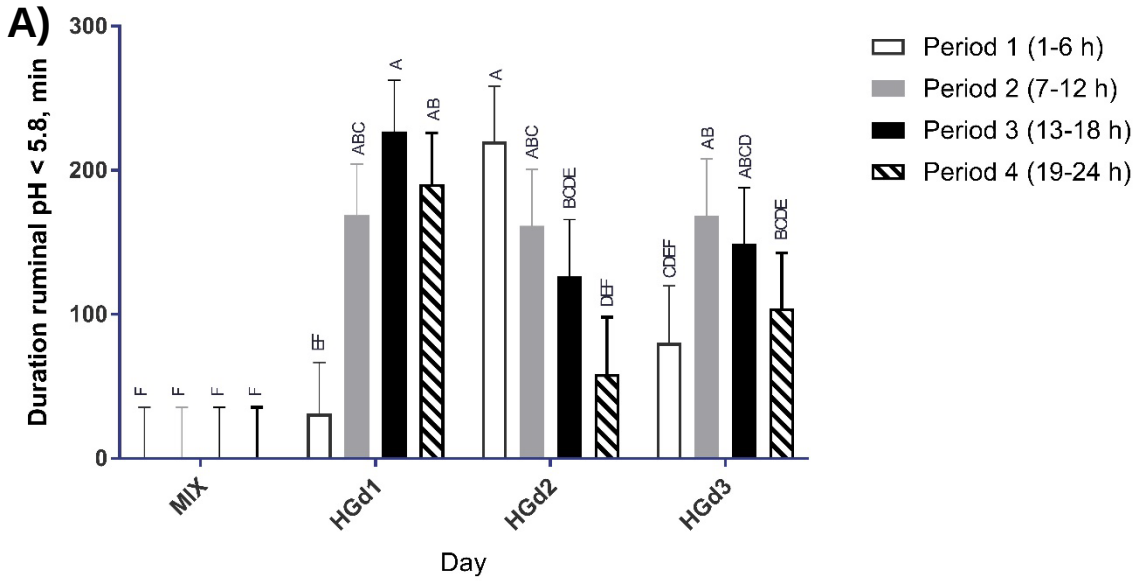
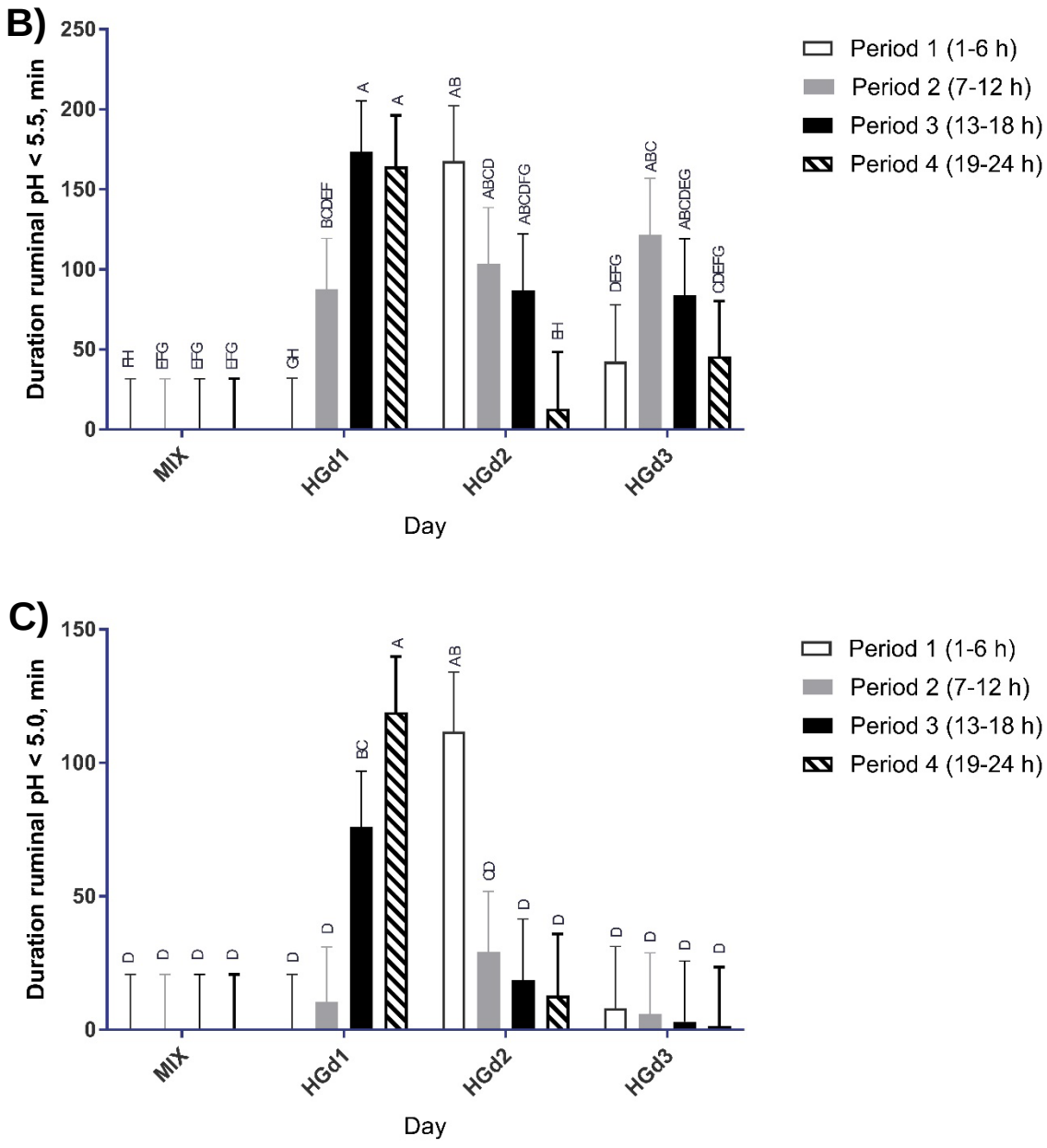


Figure 5-10 continued.



CHAPTER 6: IMPACT OF A LARGE INCREASE IN DIETARY CONCENTRATE ON BLOOD ACID-BASE STATUS AND PLASMA LIPOPOLYSACCHARIDE CONCENTRATIONS AND ASSOCIATED REDUCTION IN FEED INTAKE OF CATTLE

Introduction

During the transition to a high-grain diet, cattle may experience periods of subacute ruminal acidosis (SARA) and demonstrate erratic feeding behavior, or go "off-feed." This period of "off-feed" has been noted under both acute and subacute acidotic conditions (Fulton et al., 1979a; Kezar and Church, 1979). Although reductions in DMI have been used to diagnose SARA (Kleen et al., 2003), effects of SARA on DMI are inconsistent. Furthermore, some cattle appear to be highly affected during a dietary transition, whereas others are not (Brown et al., 2000; Bevans et al., 2005).

Communication between the immune system and central nervous system could also be important for a reduction in feed intake during diet transitions. Cattle can experience inflammation, elicited by bacterial endotoxin or lipopolysaccharide (LPS), as they are transitioned to high-grain diets (Gozho et al., 2005; Gozho et al., 2006; 2007; Khafipour et al., 2009b; Zebeli and Metzler-Zebeli, 2012). Although LPS increases in the rumen of cattle during transition to high-grain diets, evidence of LPS in the blood has been conflicting (Gozho et al., 2007; Khafipour et al., 2009a; b; Liu et al., 2013). Some researchers believe that LPS in peripheral blood during SARA comes from translocation from the intestines and therefore, does not pass the stratified squamous ruminal epithelium (Khafipour et al., 2009a). However, translocation of LPS across the rumen epithelium has been shown *in vitro* (Emmanuel et al., 2007). Although LPS has been detected in peripheral and portal blood samples taken during SARA, this result cannot determine the location of LPS passage within the gastrointestinal tract.

The objectives of this experiment were to 1) investigate what physiological parameters, specifically related to blood variables, could be associated with the timing of when animals go “off-feed” and 2) determine if LPS moves across the rumen epithelium to blood.

Materials and Methods

All procedures used in this experiment involving heifers were approved by the University of Kentucky Institutional Animal Care and Use Committee (2018-2973) and conducted at the University of Kentucky C. Oran Little Research Center, Beef Unit, in Versailles, KY.

Animals and Experiment Design

Animal feeding and management was conducted as described above (Chapter 5). Ten Holstein steers (pre-surgery BW ± SEM = 278 ± 10.3 kg) were fitted with permanent indwelling catheters in the mesenteric artery (MA) (Huntington et al., 1989), portal vein (PV), and (Silicone Tubing 1.02 mm ID, 2.16 mm OD, Helix Medical Inc., Carpinteria, CA) the right ruminal vein (RV) (Kristensen and Harmon, 2004) and fitted with a rumen cannula (#2C rumen cannula, Bar Diamond Inc., Parma, ID). Steers were withheld from food (48 h) and water (24 h) prior to catheterization surgery. A jugular catheter (Abbocath AB453534, 14 gauge catheter, 17 gauge needle, 5.5”) was first placed and then tied in place with 2 braunamid sutures placed cutaneously and filled with heparinized saline to ensure patentcy. Anesthesia was induced by i.v. administration of 0.11 mg/kg xylazine followed by 1.0 mg/kg ketamine, and the steers were intubated and placed on the surgery table in left lateral recumbency. Steers received Lactated Ringers

i.v. during surgery (6-10 mL/kg BW/h). The surgical site was prepared for sterile surgery by scrubbing with Hibiclens (3 times) and rinsing with sterile water. The final scrub was performed wearing sterile gloves, rinsing with sterile water, and finally spraying with alcohol and iodine. All surgical personnel wore caps, masks, sterile gowns and gloves, and the steer was draped with sterile drapes. Catheter patency was maintained by weekly aspiration of catheter fluid, flushing with saline, and filling with saline containing 100 U/mL of heparin and 0.1 % benzyl alcohol. Animals were allowed to recover for two weeks prior to rumen cannulation surgery and given at least two weeks for recovery following cannulation surgery before experimentation began. Animals were paired by weight to determine animals for surgeries, using the heaviest animals first. Surgeries on sets of animals were staggered in an effort to minimize time between surgeries and experimentation for each block. Steers (pre-sampling BW ± SEM = 304 ± 13 kg) were adapted to a diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement (MIX; Table 5-1). Baseline measurements on MIX were taken, and then animals were abruptly switched to a 90%, high-moisture corn based diet (HG; Table 5-1) offered *ad libitum*. Measurements were taken on d 1-3 of feeding HG (HGd1, HGd2, and HGd3, respectively).

Blood Sampling and Analysis

Blood (approximately 10 mL from each vessel) was collected from the MA, PV, and RV of each animal into sterile, heparinized syringes and transferred to screw-top conical vials immediately before feeding (0 h) and 2, 4, 6, 8, 10, 12, 16, and 24 h after feeding during MIX baseline measurements (d -1) and at the same times, relative to feeding of the HG diet, on d 1-3. Catheters were inserted into the jugular vein on d -2 for

a representative systemic blood sample in case MA catheters had already stopped working or failed during sampling. Before sample collection, approximately 8 mL of blood and heparinized saline was extracted and discarded. A 10 mL-sample of blood from each vessel and animal was then collected into heparinized syringes, transferred to a screw-top vial, and put on ice until transport to the laboratory for processing. Additionally, 1 mL samples of blood from each vessel were collected via sterile, heparinized syringes, capped, placed on ice, for no more than 30 min, until analysis in duplicate for pH, pCO_2, and pO_2 via a blood gas analyzer (GEM Premier 3000, Instrument Laboratory, Bedford, MA). Blood HCO_3^- and base excess were calculated as previously described (Chapter 4). After collection, catheters were refilled with 4 mL heparinized saline to prevent clotting. Plasma was obtained by centrifuging at 5000 × g for 30 min, separated into 2-mL aliquots, and stored at -20°C for later analyses. Concentration of LPS in plasma was measured by a chromogenic end-point *Limulus* amebocyte lysate assay (Pierce™ Chromogenic Endotoxin Quant Kit, catalog #s: A39552 and A39553, Thermo Fisher Scientific, Waltham, MA). Prior to the assay, aliquot plasma samples were thawed, diluted to 1:100, heated at 75°C for 15 min on a heating block, and cooled before analysis according to the manufacturers' instructions. Prior to statistical analysis, each sample was categorized by level of LPS (Below = below detection limit of assay or 0-1 EU/mL; Low = 1-2.99 EU/mL; Medium = 3-5.99 EU/mL; High = 6-10 EU/mL; Above = greater than 10 EU/mL). The PV and RV venous-arterial difference for each animal and time point available was calculated by subtracting the concentration of the systemic sample at that time from the concentration of the venous sample.

Statistical Analysis

The treatment was the high-grain diet challenge (transition from MIX to HG) with interest in the timing of changes. All data, except LPS levels in blood, were analyzed using proc MIXED procedure of SAS 9.4 as a randomized complete block with repeated measures using block as a random variable. Fixed effect included in all models was day relative to HG feeding (MIX, HGd1, HGd2, and HGd3). When appropriate for some variables, the fixed effects of hour or period and the interaction with day were also included in the model. Effects were considered significant at $P < 0.05$, and effects with $0.05 < P < 0.1$ were considered to show a tendency. The lsmeans were calculated for each analysis and compared when significant effects were present using the least significant difference (LSD) test.

Lipopolysaccharide in the blood was analyzed multiple ways. Firstly, data from all vessels were analyzed using proc MIXED as a randomized complete block design using block as a random variable and vessel type, day, and vessel*day as fixed effects. The remaining analyses were conducted separately for each type of vessel. Concentration of LPS was analyzed using proc MIXED as a randomized complete block with repeated measures using block as a random variable. Fixed effects included in the model were day, hour, and the interaction. The repeated variable was hour, the subject was animal, and a compound symmetry covariance structure was used. Additionally, the level of LPS was analyzed using proc GLIMMIX of SAS as a randomized complete block, using block as a random variable and day, hour, and the interaction as fixed effects. When the effect of day was or tended to be significant, frequency of samples in each level was determined

for each day and used for regression analysis using proc REG of SAS for linear and quadratic models.

In order to investigate relationships between the physiological variables measured and animal susceptibility to go “off-feed” during this dietary transition, daily mean values for variables measured in the previous experiment (Chapter 5) as well as blood variables measured in this experiment were used in proc CORR of SAS and multiple linear regression. Variables of interest and possible regressors included: susceptibility score, DMI, water intake, total water intake, meal frequency, meal duration, meal size, fractional liquid dilution rate, VFA absorption rate, saliva production, mean RpH, duration RpH < 5.5, duration RpH < 5.0, mean Trum, contraction amplitude, contraction duration, contraction frequency, lying bouts, lying duration, rumen fluid viscosity, systemic pH, systemic HCO_3, PV pH, pV HCO_3, RV pH, RV HCO_3, systemic LPS, PV LPS, and RV LPS. Only variables which were significantly correlated with the variable of interest were used as regressors for multiple linear regression. However, when evaluating a variable, all other variables of the same category (or those that it had been calculated from or used to calculate) were removed from the model, even if significantly correlated. For example, water intake and meal characteristics would not be used in multiple regression for DMI, even though they were significantly correlated. The REG procedure of SAS was used with backward elimination variable selection, which removed the variable in the model with the largest *p*-value at each step until all variables remaining in the model were significant at $\alpha = 0.05$.

Results

Blood vessel catheter patency was difficult to maintain. Ultimately, blood sampling occurred from all ten animals for systemic (MA: n=6; jugular vein: n=4) samples, nine animals for PV samples, and four animals for RV samples.

Systemic blood pH, bicarbonate and base excess exhibited day*hour interactions (Table 6-1; Figure 6-1). The partial pressure of CO_2 in systemic blood was reduced on HGd2 compared to previous days (P=0.0352) and also influenced by hour (P<0.0001). All PV blood variables, except partial pressure of O_2, displayed significant day*hour interactions (Table 6-1; Figure 6-2). Partial pressure of O_2 in PV blood was affected by day (P=0.006), where it was reduced on HGd1 and HGd2 compared to MIX, and was affected by hour (P<0.0001). Ruminal vein blood partial pressures of CO_2 and O_2 were influenced by day (P=0.0151 and 0.0434, respectively; Table 6-1), where CO_2 pressure was lower on HGd1, HGd2, and HGd3 and O_2 pressure was greater on HGd1 compared to MIX. Day tended (P=0.0751) to impact bicarbonate concentration in RV blood, with lower concentrations on HGd2. Hour affected RV partial pressure of O_2 (P=0.0463), bicarbonate (P=0.0027), and base excess (P=0.0094), while it tended to influence partial pressure of CO_2 and hematocrit.

Analysis of LPS concentrations across all vessels demonstrated a tendency (P=0.0763) for a vessel effect, where RV LPS was greatest (Systemic = 3.94 $\pm$ 0.6; PV = 3.14 $\pm$ 0.65; RV = 5.54 $\pm$ 0.93). There was also a significant day (P=0.0355) effect in which LPS concentration was greater on all HG days compared to MIX. Systemic blood vessel LPS concentration in plasma was not impacted by day or hour, but LPS level was influenced by day (P=0.0034; Table 6-2). The number of plasma samples with detectable

levels of LPS increased with time on the HG diet as evidenced by the fact the number of samples below detectable limits decreased linearly (P=0.0241; Figure 6-3A). Portal vein plasma LPS concentration and V-A difference were not influenced by day or hour. However, LPS level in PV plasma tended (P=0.0833) to be impacted by day, where once again, the number of samples below detectable limits decreased linearly (P<0.0001) over successive days (Figure 6-3B). Concentration of LPS in RV plasma tended (P=0.0665) to be affected by day, where concentrations tended to be higher on HGd3 compared to other days. Additionally, the V-A difference for RV plasma samples were impacted by day (P=0.0294), where V-A difference was greater on HGd3 than MIX of HGd2, suggesting concentrations were higher in the RV than systemic blood on HGd3.

The Pearson correlation coefficients helped to focus the list of possible regressors, so that the model for multiple regression was of full rank (i.e. it did not have an infinite number of least squares solutions for the estimates). Multiple linear regression conducted by backwards elimination variable selection resulted in significant models for all dependent variables chosen, yet many models led to simple linear models with only one variable remaining in the model (Table 6-3, Table 6-4, Table 6-5, Table 6-6, Table 6-7, Table 6-8). Intake and feeding behavior variables most frequently had motility and PV variables as significant regressors (Table 6-3). Meal frequency had the greatest R-squared for intake and feeding variables, suggesting the regressors could explain about 61% of variation in meal frequency (Table 6-3). Susceptibility score was positively, linearly correlated (P=0.0111) with duration RpH < 5.0, which was the only remaining regressor (Table 6-3). Rumen fluid viscosity was linearly related (P=0.0069) to contraction duration, where increases in contraction duration were associated with increases in

viscosity (Table 6-4). Bicarbonate in blood pools were important regressors for liquid dilution rate, VFA absorption rate, and saliva production (Table 6-4). Reticulorumen motility and lying behavior variables commonly had RV variables as regressors (Table 6-5). Approximately 71% of the variation in contraction frequency could be explained by DMI and RV pH (Table 6-5). All ruminal pH variables resulted in simple linear models with only one regressor (Table 6-6). Saliva production and DMI were common regressors for blood acid-base variables (Table 6-7). About 66% of variation in PV bicarbonate could be explained by DMI, saliva production, and duration RpH < 5.5 (Table 6-7). Systemic LPS concentration was positively related (P=0.0433) to susceptibility score (Table 6-8). Concentration of LPS in blood pools helped explain several variables (Table 6-3, Table 6-4, Table 6-5, and Table 6-7). For example, contraction amplitude and RV LPS were negatively related (P=0.3678; Table 6-5 and Table 6-8). Some of the regression models resulted in low R-squared values, and these should be used with caution for predicting values for dependent variables.

Discussion

This study aimed to investigate the relationships between blood acid-base and endotoxin concentrations and timing of when animals go “off-feed” during an abrupt increase in dietary concentrate. In addition, it was intended to determine associations between all of the physiological variables measured in the previous experiment to evaluate the complex relationships involved during periods when animals reduce feed intake during dietary transitions.

In addition to serving as a key buffering system in the rumen, bicarbonate is the primary acid-base buffering system in the blood. Blood bicarbonate concentrations have

been negatively correlated with ruminal VFA concentrations (Faverdin et al., 1999). The high ruminal VFA concentrations (Chapter 5) that occurred after switching to the high-grain diet in this study could potentially deplete the bicarbonate buffering system of the blood by increasing movement of bicarbonate into the rumen in exchange for ionized VFA (Stevens, 1970) or to help maintain adequate ruminal pH. Changes in blood bicarbonate and pH due to ruminal acidosis have been reported (Huntington et al., 1981; Indrova et al., 2017), but were sometimes small in magnitude (Harmon et al., 1985; Goad et al., 1998). Systemic blood pH was very close to ideal (7.4) during the dietary switch, suggesting the system was able to compensate. Bicarbonate concentrations in systemic blood were greater on the first day of high-grain feeding from about 6 h to 10 h post-feeding and then lower for several hours after feeding on the second and third day of high-grain feeding compared to other days. Thus, the period with reduced bicarbonate concentration was associated with a time when most animals were “off-feed.” One may speculate that because animals were not consuming feed, they were chewing less, and thereby producing less saliva, which resulted in a smaller bicarbonate pool. Yet, the exact timing or cause and effect of these events cannot be determined from the current study. Blood bicarbonate to carbon dioxide ratio and blood pH were also decreased when steers stopped eating after a switch from alfalfa hay to a high-grain diet (Uhart and Carroll, 1967). Low bicarbonate concentrations can disrupt central nervous system function, even if blood pH is not affected (Owens et al., 1998). Therefore, reductions in feed intake and rumen motility observed in this study (Chapter 5), which are controlled in part by the central nervous system and vagus nerve (Grovum, 1986; Sartin et al., 2010; Sartin et al., 2011), could have been associated with this reduction in bicarbonate. Additionally, the

partial pressure of carbon dioxide in PV was also reduced for several hours after feeding on the second and third day of high-grain feeding. Comparison of pH and bicarbonate for systemic and PV blood, showed that systemic pH and bicarbonate were still reduced on HGd3 when feed intake started to return to normal, yet PV pH and bicarbonate appeared to increase the latter half of HGd2. This suggested that the systemic blood pH and bicarbonate was not a driver for the transitory reduction and recovery of feed intake, but that PV pH and bicarbonate may be a potential driver.

Regardless of diet, RV blood pH was lower than portal and systemic blood pH and at levels that would be considered acidotic for systemic blood. The relatively lower pH of RV blood compared to PV and systemic blood is likely due to the vast amount of VFA that are absorbed from the rumen, which increase the acid load of the blood and reduce pH. Portal vein blood is a mixture of blood from the rumen as well as blood from the intestines, so it would be diluted RV blood since there are comparatively not as many VFA or acids absorbed through the intestines. Bicarbonate tended to be impacted by day, and partial pressure of carbon dioxide was decreased by the high-grain diet. The lowest bicarbonate tended to be on the second day of high-grain feeding, which could have been associated with why animals had reduced feed intake on this day. There is a high demand for bicarbonate in the rumen to help absorb VFA and buffer ruminal contents. One mechanism of VFA absorption from the rumen involves exchanging an ionized acid from the rumen for bicarbonate from the blood (Owens et al., 1998). As ruminal VFA absorption rate estimates suggested VFA absorption rate was reduced in this study (Chapter 5), this could have impacted bicarbonate concentrations. Since the ruminal epithelium is composed of highly active cells, CO_2 produced from cellular aerobic

metabolism must be exported from cells to the blood. There was greater partial pressure of CO_2 in RV compared to systemic or PV blood, which could be indicative of the cellular respiration capacity of ruminal epithelial cells.

Bacterial endotoxin, also known as LPS, has been one of the factors associated with the negative effects of feeding high-grain diets and ruminal acidosis. As part of the cell wall of gram-negative bacteria, LPS is abundant in the rumen and increased in the gastrointestinal lumen when ruminants are fed high-grain diets (Emmanuel et al., 2008; Khafipour et al., 2009b; Zebeli et al., 2012). In this study, LPS concentrations did not increase in systemic plasma or portal vein plasma. However, it is important to note that there was a wide range of LPS concentrations obtained, which inflated the standard errors and could have attributed to the lack of effects and ruminal vein observations were quite limited because of the loss of catheter patency. Despite this lack of effect for actual concentrations in these blood pools, when LPS was categorized by five levels, there was a significant effect of day for systemic plasma and tended to be significant for portal vein plasma. To further explain, the number of samples with detectable levels of LPS increased with days on the high-grain diet. Interestingly, RV plasma LPS concentrations tended to increase on the high-grain diet, particularly by the third day. Ultimately, the LPS can trigger an inflammatory response once it is in the blood, which could affect reticulorumen motility as well as feed intake. Due to the presence of LPS detected in blood pools during this experiment, it is likely that animals were under some degree of inflammatory response. However, blood cytokines were not measured for this study.

Reports of LPS translocation into the blood are conflicting; while some studies observed increases in LPS in peripheral blood with acute acidosis (Dougherty et al.,

1975a; Aiumlamai et al., 1992) and SARA (Khafipour et al., 2009b; Liu et al., 2013), other studies found no changes (Gozho et al., 2007; Khafipour et al., 2009a). The point of LPS entry into the blood has also been a point of conflicting evidence in the literature (Emmanuel et al., 2007; Khafipour et al., 2009a). Therefore, another objective of this experiment was to try to deduce the point of translocation of LPS into the blood. The V-A difference for RV plasma LPS was positive and significantly increased by the third day of high-grain feeding. This suggested that concentrations were higher in the RV than systemic blood on this day, which was likely due to translocation of LPS from the rumen into the RV. Although these data do not distinctly prove translocation across the ruminal epithelium, they do support the possibility. This translocation appeared to not occur until the third day of high-grain feeding when animals began to recover from the acidosis challenge, indicating translocation across the ruminal epithelium may not be a large driver for the depression in feed intake seen on the second day of high-grain feeding. Greater V-A difference for RV plasma LPS suggested that rumen epithelial integrity and barrier function was compromised during the abrupt transition from a 50% concentrate to 90% concentrate diet. This would agree with previously reported findings of a decline in barrier function of the reticulorumen epithelium during acidosis, low ruminal pH, high ruminal osmolality, or when animals were fed high-grain diets (Gaebel et al., 1989; Emmanuel et al., 2007; Penner et al., 2010; Steele et al., 2011a; Zebeli et al., 2012; Liu et al., 2013).

Multiple regression analysis enabled the deduction of important relationships between these physiological variables that changed as cattle were switched to the high-grain diet. Many of the relationships observed have been previously reported, such as

DMI and reticulorumen contraction frequency were positively related. For example, contraction frequency was greater when animals were consuming feed compared to resting periods (Church, 1976; Waghorn and Reid, 1983; Grovum, 1986). Because animals that consumed more feed, generally spent more time eating, this relationship is logical. Also, mean RpH was positively linearly related to lying duration, meaning animals that had higher ruminal pH, would have greater average lying duration. Theoretically, this makes sense because a higher pH would typically mean the animal was on a more forage-based diet, which would require more rumination and would likely be associated with longer periods spent lying down and ruminating. The fact that susceptibility score was positively linearly correlated with duration that ruminal pH was below 5.0 and systemic LPS, separately, made sense with the scoring system. Animals which had longer durations with ruminal pH below 5.0 suggested more severe ruminal acidosis, or greater concentrations of LPS suggested more chance for an inflammatory response. These animals were more likely to go “off-feed”, thus, having a greater susceptibility score. These data suggested that the animals that were more tolerant to the grain challenge may have had less epithelial barrier damage (allowed less LPS to get to the blood) or were somehow equipped to maintain a healthier ruminal pH. Other relationships were more novel. As an example, rumen fluid viscosity was positively related to contraction duration. In this experiment, contraction duration increased with high-grain feeding along with apparent viscosity. Whether the increased contraction duration was an attempt to counteract the reduced mixing from greater viscosity or the viscosity increased to try to reduce mixing/slow fermentation despite increased contraction duration in order to maintain a healthier ruminal pH, is yet to be determined.

Other research has reported reductions in motility or reticuloruminal stasis in cattle with endotoxemia (Eades, 1993; 1997), or LPS in the blood. Results of correlation and regression analysis showed that ruminal vein LPS, not systemic or portal vein, was the variable most associated with contraction amplitude. As ruminal vein LPS concentrations increased, contraction amplitude was reduced, suggesting the inflammatory response induced from LPS translocated across the ruminal epithelium may be a key driver for the reduction in rumen motility during an acidotic challenge. Ruminal vein LPS was also the only remaining regressor for systemic pH and portal vein pH, indicating relationships between immune response elicitors and pH balance.

Not surprisingly, saliva production (Chapter 5) helped explain bicarbonate concentrations in all three blood pools analyzed. Saliva has a high concentration of bicarbonate and phosphate and is very important for rumen function and buffering, particularly neutralization of acids (Bailey and Balch, 1961; Erdman, 1988). Contrary to what would be expected, systemic LPS concentration decreased as susceptibility score increased (i.e. the animal reduced intake to a greater extent). Yet, the low R-squared for this model and large range in systemic LPS concentrations indicated this relationship may not be accurate. Overall, it was evident that the feeding behavior, ruminal pH, reticulorumen motility, blood acid-base, and blood LPS variables were intimately related.

Conclusions

This study investigated the relationships between physiological variables and the timing of when animals go “off-feed” during an abrupt dietary increase in concentrate. The control of feed intake and relationships with the feeding behavior, ruminal pH, motility, blood acid-base, and plasma LPS variables appeared to be highly complex. The

V-A difference for ruminal vein plasma concentrations suggested that LPS was likely translocated across the ruminal epithelium, but it may not be a driver for the reduction of feed intake since it occurred after the large decrease in feed intake. Further research is needed to focus specifically on the various aspects of physiological changes associated with when animals go “off-feed” in order to potentially find a way to amend the problem.

Tables and Figures

Table 6-1. Blood acid-base status of cattle during an abrupt, large concentrate increase in order to transition to a finishing diet

Item	Day				SEM[5]	*P*-value		
	MIX[1]	HGd1[2]	HGd2[3]	HGd3[4]		Day	Hour	Day*Hour
Systemic Vessel[6]								
pH	7.409	7.419	7.399	7.400	0.0087	0.0017	0.0007	0.0054
pCO_2, mmHg	47.68^{a}	47.50^{ab}	45.82^{c}	46.01^{bc}	0.5487	0.0352	<0.0001	0.5812
HCO_3^-, mmol/L	29.18	29.75	27.48	27.71	0.5515	<0.0001	<0.0001	0.0276
Base Excess, mmol/L	4.54	5.20	2.82	3.04	0.6117	<0.0001	<0.0001	0.0105
Hematocrit, %	26.39	26.41	26.60	26.22	0.7018	0.9028	0.1034	0.1405
Portal Vein								
pH	7.311	7.314	7.318	7.327	0.0047	0.1132	<0.0001	0.0086
pCO_2, mmHg	61.36	61.03	56.29	56.41	1.1045	<0.0001	<0.0001	0.0014
pO_2, mmHg	47.60^{b}	51.05^{a}	50.12^{a}	49.21^{ab}	1.3243	0.0060	<0.0001	0.0955
HCO_3^-, mmol/L	29.96	30.03	27.94	28.56	0.5306	<0.0001	<0.0001	0.0004
Base Excess, mmol/L	3.923	4.041	2.14	2.856	0.511	0.0004	<0.0001	0.0003
Hematocrit, %	25.80	26.22	26.51	25.99	0.689	0.7381	0.0433	0.0061
Ruminal Vein								
pH	7.174	7.224	7.242	7.247	0.039	0.1206	0.4094	0.2357
pCO_2, mmHg	79.11^{a}	70.28^{b}	65.24^{b}	64.94^{b}	6.52	0.0151	0.0884	0.8757
pO_2, mmHg	56.45^{b}	63.40^{a}	60.32^{ab}	59.36^{ab}	4.46	0.0434	0.0463	0.4861
HCO_3^-, mmol/L	27.66	27.79	26.20	27.00	0.634	0.0751	0.0027	0.1045
Base Excess, mmol/L	-0.107	0.693	-0.585	0.299	0.696	0.3761	0.0094	0.1189
Hematocrit, %	26.21	26.45	25.86	25.43	0.647	0.4746	0.0992	0.5620

[1]MIX: diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement
[2]HGd1: first day of feeding a 90% high-moisture corn based, finishing diet
[3]HGd2: second day of feeding a 90% high-moisture corn based, finishing diet
[4]HGd3: third day of feeding a 90% high-moisture corn based, finishing diet
[5]SEM represents a pooled SEM across all days
[6]Systemic blood samples were collected from either the jugular vein (n=4) or mesenteric artery (n=6)
[a,b,c] Means within a row without a common superscript differ

Table 6-2. Plasma lipopolysaccharide concentrations in blood pools and venous-arterial differences as cattle were abruptly switched from a 50% hay, 50% concentrate mixed (MIX) diet to a 90% concentrate, high-grain, finishing (HG).

Item	Day				SEM[5]	*P*-value		
	MIX[1]	HGd1[2]	HGd2[3]	HGd3[4]		Day	Hour	Day*Hour
Systemic Vessel[6]								
LPS, EU/mL	0.74	2.12	2.29	1.31	1.58	0.7092	0.3896	0.9993
Portal Vein								
LPS, EU/mL	1.54	3.39	4.65	3.16	1.12	0.262	0.6395	0.5168
V-A difference, EU/mL	-1.23	-0.34	-2.55	1.40	1.54	0.3785	0.133	0.4069
LPS Level						0.0833	0.944	0.957
Ruminal Vein								
LPS, EU/mL	2.55	5.44	4.94	9.48	1.72	0.0665	0.9315	0.2076
V-A difference, EU/mL	0.12[b]	2.60[ab]	-3.65[b]	6.80*[a]	2.6	0.0294	0.9204	0.5305

[1] MIX: diet composed of 50% chopped tall fescue K31 hay and 50% concentrate supplement
[2] HGd1: first day of feeding a 90% high-moisture corn based, finishing diet
[3] HGd2: second day of feeding a 90% high-moisture corn based, finishing diet
[4] HGd3: third day of feeding a 90% high-moisture corn based, finishing diet
[5] SEM represents a pooled SEM across all days
[6] Systemic blood samples were collected from either the jugular vein (n=4) or mesenteric artery (n=6)
* V-A difference mean was different from zero ($P<0.05$)
[a,b] Means within a row without a common superscript differ

Table 6-3. Multiple regression analysis of intake and feeding behavior variables to determine relationships with other variables.

Dependent Variable	Variable(s) remaining in model	Model *P*-value	R-squared	Parameter estimate	SE
DMI	Contraction frequency	<0.0001	0.3794	5.47	1.2
Water intake	PV[1] LPS[2]	0.0165	0.1771	-1.04	0.4
Total water intake	Duration RpH[3] < 5.0	0.0069	0.3474	0.016	0.0075
	Contraction frequency			7.75	3.71
	PV LPS			-0.900	0.433
Meal duration	Contraction duration	<0.0001	0.5792	-10.57	2.78
	Lying duration			0.433	0.125
	PV HCO_3			3.47	1.33
Meal size	Contraction duration	<0.0001	0.5586	-296.46	129.4
	Contraction frequency			873.80	270.4
	PV LPS			-62.27	28.4
Meal Frequency	Fractional liquid dilution rate	<0.0001	0.6098	106.63	24.1
	Saliva production			-0.0999	0.034
	PV HCO_3			0.859	0.31
Susceptibility score[4]	Duration RpH < 5.0	0.0111	0.1749	0.0025	0.0009

[1] PV: portal vein
[2] LPS: lipopolysaccharide
[3]RpH: ruminal pH
[4] Scores were based on the animal's DM intake on the second day of high-grain feeding: 1 (low susceptibility) = consumed 4 or more kg DM; 2 = 1-4 kg DM consumed; 3 = 0-1 kg DM consumed; and 4 (high susceptibility) = experiment was stopped due to low ruminal pH

Table 6-4. Multiple regression analysis of rumen fluid viscosity, ruminal passage rate, VFA absorption rate, and saliva production variables to determine relationships with other variables.

Dependent Variable	Variable(s) remaining in model	Model *P*-value	R-squared	Parameter estimate	SE
Rumen fluid viscosity	Contraction duration	0.0069	0.1959	2.29	0.80
Fractional liquid dilution rate	Systemic[1] HCO_3	0.0059	0.6983	-0.021	0.009
	PV[2] HCO_3			0.031	0.010
	RV[3] LPS[4]			0.0037	0.001
VFA absorption rate	PV pH	0.0119	0.2545	-3.64	1.3
Saliva production	RV HCO_3	0.0136	0.4103	7.25	2.5

[1] Systemic samples were taken from the jugular vein (n=4) or mesenteric artery (n=6).
[2] PV: portal vein
[3] RV: ruminal vein
[4] LPS: lipopolysaccharide

Table 6-5. Multiple regression analysis of reticulorumen motility and lying behavior variables to determine relationships with other variables.

Dependent Variable	Variable(s) remaining in model	Model *P*-value	R-squared	Parameter estimate	SE
Contraction amplitude	RV[1] LPS[2]	0.0215	0.3678	-0.149	0.06
Contraction duration	Rumen fluid viscosity RV pH	0.0030	0.6526	0.115 6.18	0.04 1.9
Contraction frequency	DMI RV pH	0.0011	0.7089	0.084 -2.45	0.02 0.86
Lying bouts	RV pH	0.0477	0.2884	-30.0	13.6
Lying duration	Meal duration Mean RpH[3]	0.0030	0.2971	0.304 10.81	0.15 4.6

[1] RV: ruminal vein
[2] LPS: lipopolysaccharide
[3] RpH: ruminal pH

Table 6-6. Multiple regression analysis of ruminal pH and temperature variables to determine relationships with other variables.

Dependent Variable	Variable(s) remaining in model	Model *P*-value	R-squared	Parameter estimate	SE
Mean RpH[1]	Lying duration	0.0058	0.2034	0.015	0.005
Duration RpH < 5.5	PV[2] HCO_3	0.0157	0.1794	-80.35	31.4
Duration RpH < 5.0	Susceptibility score[3]	0.0111	0.1794	69.48	25.9
Mean Trum[4]	Meal frequency Contraction amplitude	0.0014	0.3293	0.066 -0.12	0.02 0.05

[1] RpH: ruminal pH
[2] PV: portal vein
[3] Scores were based on the animal's DM intake on the second day of high-grain feeding: 1 (low susceptibility) = consumed 4 or more kg DM; 2 = 1-4 kg DM consumed; 3 = 0-1 kg DM consumed; and 4 (high susceptibility) = experiment had to be stopped early due to low ruminal pH
[4] Trum: ruminal temperature

Table 6-7. Multiple regression analysis of blood acid-base variables to determine relationships with other variables.

Dependent Variable	Variable(s) remaining in model	Model *P*-value	R-squared	Parameter estimate	SE
Systemic[1] pH	RV[2] LPS[3]	0.0295	0.3370	0.00034	0.001
Systemic HCO_3	DMI	<0.0001	0.4856	0.304	0.07
	Saliva production			0.021	0.009
PV[4] pH	RV LPS	0.0484	0.2868	0.002	0.0009
PV HCO_3	DMI	<0.0001	0.6625	0.271	0.05
	Saliva production			0.02	0.007
	Duration RpH[5] < 5.5			-0.0018	0.0006
RV pH	Contraction frequency	0.0064	0.6010	-0.11	0.04
	Lying bouts			-0.0088	0.003
RV HCO_3	DMI	0.0060	0.6058	0.198	0.08
	Saliva production			0.046	0.017

[1] Systemic samples were taken from the jugular vein (n=4) or mesenteric artery (n=6).
[2] RV: ruminal vein
[3] LPS: lipopolysaccharide
[4] PV: portal vein
[5] RpH: ruminal pH

Table 6-8. Multiple regression analysis of plasma lipopolysaccharide (LPS) variables to determine relationships with other variables.

Dependent Variable	Variable(s) remaining in model	Model *P*-value	R-squared	Parameter estimate	SE
Systemic[1] LPS	Susceptibility Score[2]	0.0433	0.1147	-1.17	0.56
PV[3] LPS	Water intake	0.0165	0.1771	-0.17	0.07
RV[4] LPS	Contraction amplitude	0.0215	0.3678	-2.48	0.94

[1] Systemic samples were taken from the jugular vein (n=4) or mesenteric artery (n=6).
[2] Scores were based on the animal's DM intake on the second day of high-grain feeding: 1 (low susceptibility) = consumed 4 or more kg DM; 2 = 1-4 kg DM consumed; 3 = 0-1 kg DM consumed; and 4 (high susceptibility) = experiment had to be stopped early due to low ruminal pH
[3] PV: portal vein
[4] RV: ruminal vein

Figure 6-1. Systemic blood vessel blood-acid base changes as cattle were abruptly switched from a 50% to 90% concentrate, high-grain diet. Systemic vessel pH and bicarbonate showed day*hour interactions (P=0.0054 and *P*=0.0276, respectively).

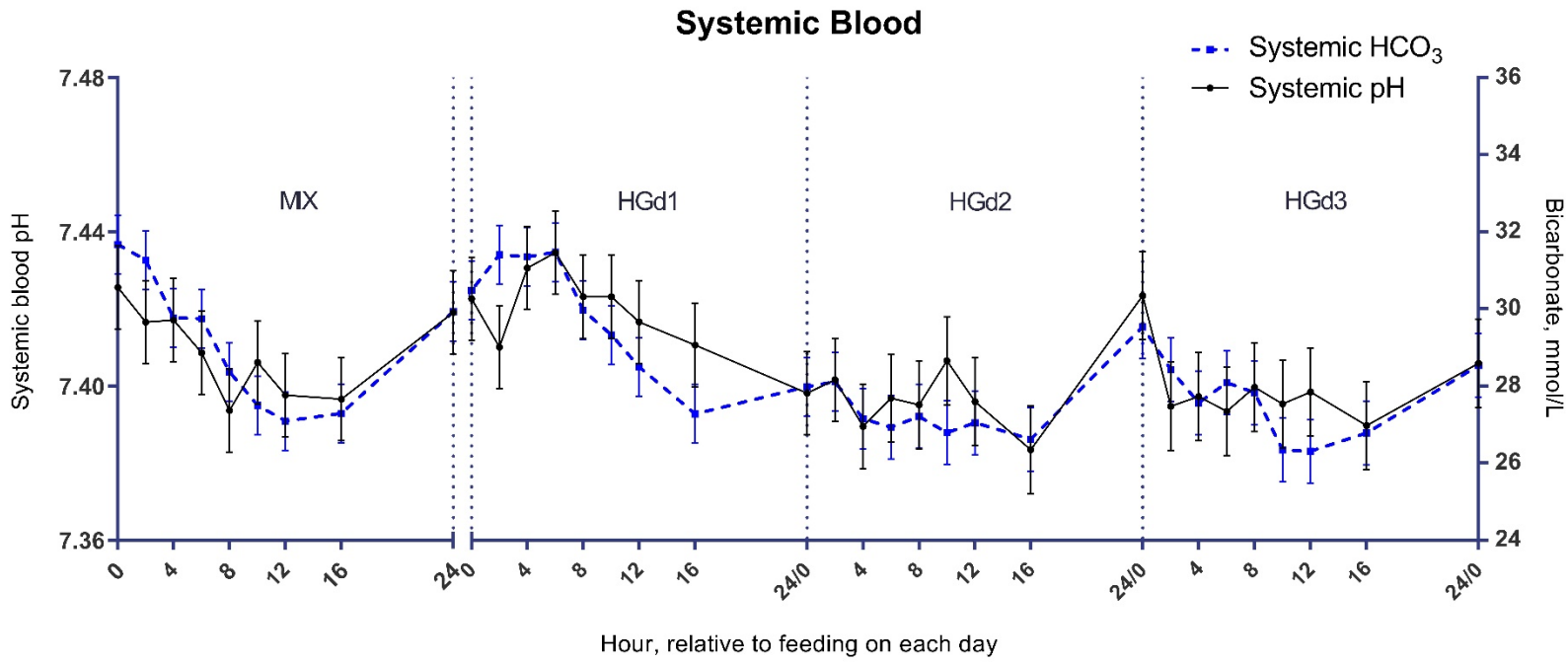


Figure 6-2. Portal vein blood acid-base changes as cattle abruptly switched from a 50% to 90% concentrate, high-grain diet. Portal vein blood pH and bicarbonate concentrations displayed day*hour interactions (P=0.0086 and P=0.0004), respectively).

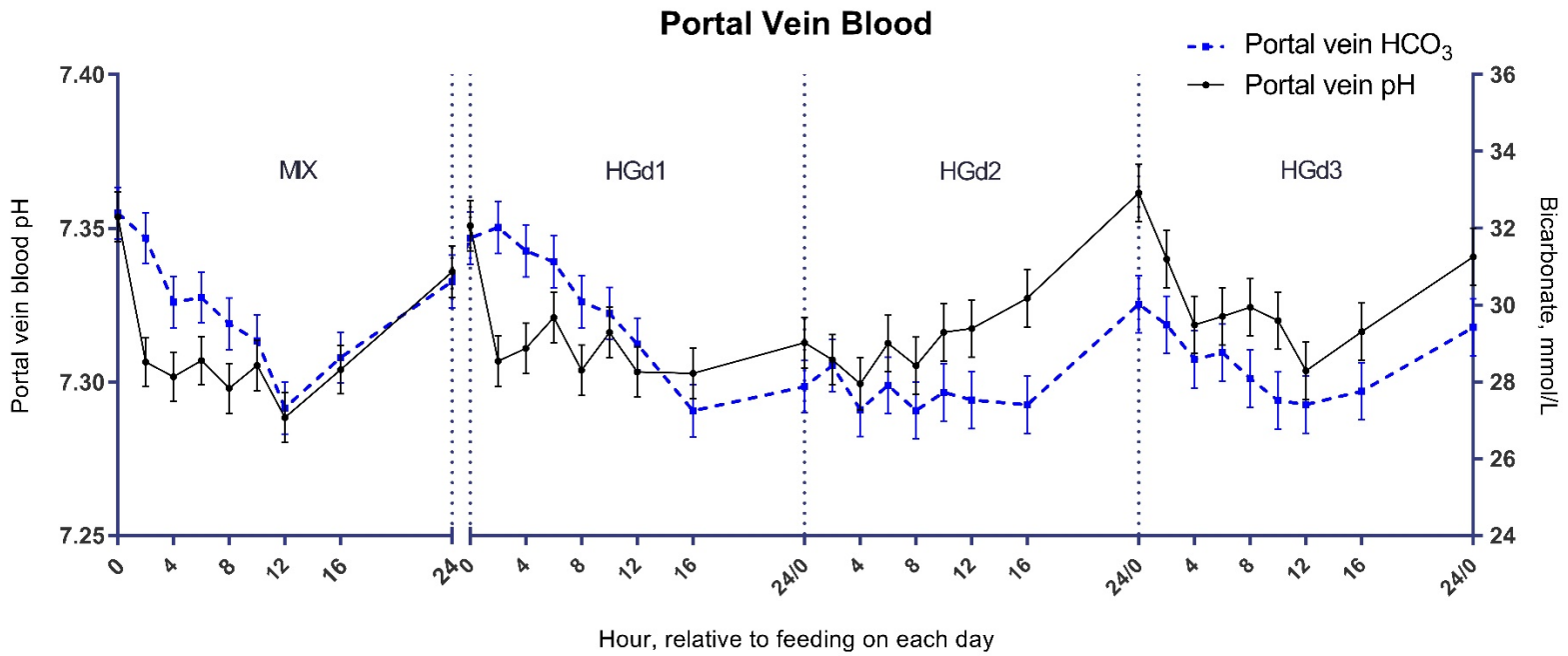
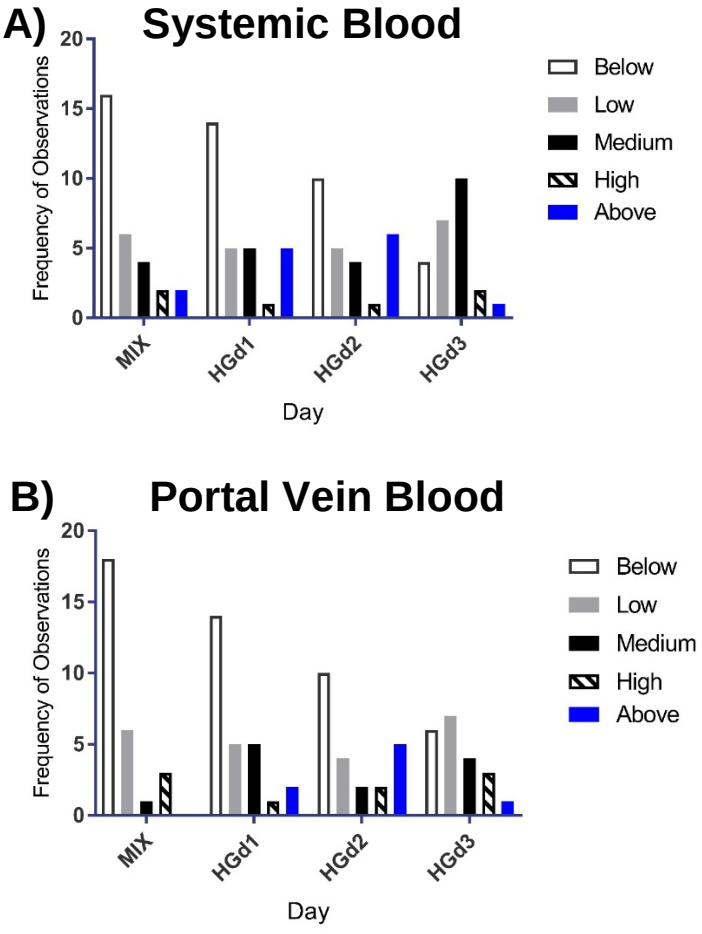


Figure 6-3. Level of lipopolysaccharide in systemic and portal vein blood samples. A) Systemic blood vessel lipopolysaccharide level in cattle was influenced (*P*=0.0034) by day relative to the abrupt dietary switch from a 50% to a 90% concentrate diet (Hour: *P*=0.1514; Day*Hour: *P*=0.352). The frequency of observations for samples that were below detectable levels was reduced linearly (*P*=0.0241), meaning more samples had detectable levels, with increasing time on the high-grain diet. B) Portal vein plasma lipopolysaccharide levels tended (P=0.0833) to be influenced by day relative to the abrupt dietary transition, where the number of samples below detectable limits decreased linearly (*P*<0.0001) over successive days following the dietary switch.



CHAPTER 7: SUMMARY AND CONCLUSIONS

Finishing beef cattle on high-concentrate diets is typical for the feedlot industry, which helps to maximize productivity and profitability. Yet, the period when cattle are transitioned from a high-forage to a high-concentrate diet can increase the risk for ruminal acidosis, along with other gastrointestinal disorders, and may be associated with reductions in feed intake or erratic feeding behavior. Animal responses to high-concentrate challenges or dietary transitions are also highly variable; some animals appear better equipped to adjust to the new fermentation substrates without complications compared to others. The reasons for feed intake reductions and varying animal susceptibility for adverse intake or health effects with transitions to high-grain diets are still not fully understood. Particularly, the goal of this dissertation was to investigate relationships between behavior and physiological adaptations of cattle during transitioning to high-concentrate diets with emphasis on effects on forestomach motility and endotoxin translocation.

To study this dietary transition period in cattle, two measurement systems and their corresponding algorithms for analysis were developed and validated for recording and characterizing short-term feeding behavior and reticulorumen motility. The low error rates found in validation and response values congruent with published literature suggested these systems were accurate. The automated nature of these systems, along with the ability to analyze large amounts of data, were significant improvements from other methods and make them vital to research efforts.

While acute ruminal acidosis had been previously reported to reduce reticulorumen motility (Juhász and Szegedi, 1968; Bruce and Huber, 1973; Cebrat, 1979; Kezar and Church, 1979) and led to a reduction in feed intake (Dirksen, 1970; Fulton et al., 1979a; b), the effects of subacute ruminal acidosis on reticulorumen motility had not been determined, despite some studies indicating reductions in feed intake. The second part of this series of experiments showed that transitioning cattle from an approximately 70% concentrate to 90% concentrate diet which induced a mild subacute ruminal acidosis reduced reticulorumen motility, altered consumption rate without affecting meal characteristics, and confirmed that feeding behavior, ruminal pH, and rumen motility variables were intricately related. Although reticulorumen motility was reduced, it did not slow passage rate and therefore, may not have been responsible for the moderate decreases in DMI seen on the second day of high-grain feeding compared to the first day.

In the next experiment of these series, an abrupt increase in dietary concentrate from 50% to 90% concentrate was used to induce a more severe ruminal acidosis and cause some animals to go “off-feed.” Steers exhibited greater DMI and faster consumption rate on the first day of high-grain feeding, which may have resulted in post-ingestive consequences that led animals to reduce intake, meal size, and meal duration on the second day of high-grain feeding. As has been reported before in the literature, there was great variation between animal susceptibility to go “off-feed.” Furthermore, reticulorumen contraction amplitude and frequency were also reduced, while rumen fluid viscosity was increased, which could have reduced presentation of VFA to the absorptive surface and subsequent absorption rate, as suggested by absorption rate estimates.

Blood acid-base status and inflammatory response during a nutritional challenge, such as this abrupt transition from the 50% to 90% concentrate diet, can also be related to when changes in feed intake occurred. Reductions in systemic and portal vein bicarbonate were associated with the second day of high-grain feeding when cattle were "off-feed." Additionally, plasma samples had more detectable levels of lipopolysaccharide with increasing days on feed, which would induce an inflammatory response. Concentrations of lipopolysaccharide within the ruminal vein were greater than systemic plasma suggesting that translocation likely occurred across the ruminal epithelium, for which previous evidence was conflicting.

In conclusion, it was clear that the relationships between changes in feeding behavior, reticulorumen motility, rumen fluid viscosity, blood acid-base balance, and blood lipopolysaccharide were linked with when cattle reduced feed intake. These associations appeared to be convoluted, which greatly complicates the challenge of determining why animals went "off-feed" during these dietary transitions. A multi-faceted approach to alleviate the intake reduction associated with transitioning cattle to high-grain diets and subsequent potential ruminal acidosis is necessary. By increasing understanding of the physiological and behavioral mechanisms by which cattle adapt to high-grain diets, we could improve animal health and performance through these diet transitions.

APPENDICES

Appendix A: Preliminary dietary step-up experiment

Introduction

In order to increase growth performance and efficiency, cattle are often finished on high-grain diets. It has been reported that incidence, prevalence, and severity of ruminal acidosis was greatest towards the end of the finishing phase (Castillo-Lopez et al., 2014). However, the transitioning period when cattle are acclimated from a forage diet to a high-concentrate, rapidly fermentable diet increases the risk for ruminal acidosis (Bevans et al., 2005; Brown et al., 2006) and disrupts the microbial population (Goad et al., 1998; Tajima et al., 2001). As the proportion of concentrate in the diet increases, volatile fatty acid (VFA) production in the forestomach increases, which decreases the pH of ruminal contents. Starch intake has been positively associated with maximum and mean ruminal temperature, and the maximum ruminal temperature was negatively related to minimum ruminal pH (Mohammed et al., 2014). If ruminal acidosis occurs, cattle typically decrease feed intake which, along with decreased absorption, results in lower average daily gains (Koers et al., 1976; Owens et al., 1998). As a result, ruminal acidosis leads to significant losses for the producer. Yet, the cause and effect relationship of ruminal acidosis and feed intake variation has not been determined (Cooper et al., 1999).

Type and processing of grain could also influence extent and rate of ruminal fermentation and ruminal pH, which may alter feed intake. For example, grain processing which decreases particle size or gelatinizes starch granules should increase starch degradation in the rumen and VFA production, enhancing the risk for acidosis (Stock et al., 1987). High-moisture corn (HMC), a common component of feed for

finishing cattle, is fermented more rapidly than dry rolled corn (DRC), making it more likely to induce ruminal acidosis (Benton et al., 2005; Nagaraja and Lechtenberg, 2007).

The objectives of this experiment were to 1) characterize how transitioning cattle to a high-grain diet affects feed intake, ruminal pH and VFA concentrations, ruminal temperature, rumen motility and 2) determine a diet (DRM versus HMC) and dietary transition period likely to induce ruminal acidosis.

Materials and Methods

Animals and Management

Eight ruminally-cannulated Holstein steers (BW = 559 ± 35 kg) were maintained on corn silage in outdoor, partially covered barns prior to the initiation of the experiment. At the beginning of the experiment, animals were weighed, paired by weight into 4 blocks, and moved indoors for housing at 22°C in individual 3 m × 3 m stalls with ad libitum access to water. Steers were accustomed to housing indoors from previous experiences, but they were given a 7 d acclimation period on the starter diet before experimentation begins. Then, experimentation started with all steers fed a starter diet (CS; approximately 50% concentrate; Phase 1; Table A-1) composed primarily of corn silage at 2.5 x NEm for 7 days. Steers from each block were randomly assigned and transitioned to one of two high-grain diet treatments: dry-rolled corn (DRC) or high-moisture corn (HMC). A supplement was prepared for each diet to meet protein, mineral, and vitamin requirements (NRC, 2000). Each transition diet, either a DRC 70% concentrate (T-DRC) or HMC 70% concentrate (T-HMC) diet (Phase 2), was fed for 7 days (Table A-1; Figure A-1). The final diets, either a DRC 90% concentrate (F-DRC) or HMC 90% concentrate (F-HMC), however, were fed for 21 d (Phase 3 and 4; Table A-1;

Figure A-1). Orts were collected daily, weighed, and recorded from the previous day before feeding in order to adjust feed amounts and maintain *ad libitum* feeding throughout the experiment. Additional data was collected throughout the experiment with sampling occurring for different variables at various time points (Figure A-1).

Motility Measurements

Rumen motility was measured for 24 hr periods beginning on d 2, 5, and 7 of each diet beginning at 0900 and additionally, twice at the end of feeding the finishing diet (experimental d 2, 5, 7, 9, 12, 14, 16, 19, 21, 33, and 35). A wireless telemetry system (emkaPACK4G telemetry system, emka TECHNOLOGIES USA, Falls Church, Virginia) was used to monitor pressure changes in the rumen and characterize rumen contractions as described by Egert et al. (2014) with modifications. Briefly, a water filled (2L) balloon attached to a Tygon catheter (i.d. = 3.2 mm; o.d. = 6.4 mm) was inserted into the ventral sac of the rumen. Balloons were weighed to maintain consistent fill between animals and days. The balloons remained in the rumen, even when motility was not being measured, to help eliminate alterations in motility due to the added weight of the water-filled balloon in the rumen on sampling days. Balloons were changed on d 2, 8, 15, 22, and 32, or as needed. A small hole was made in the plug of the rumen cannula to allow the catheter to pass through. The end of the catheter external to the animal was equipped with a shut-off female quick coupling (collection 1; KENT Systems, LLC., Loveland, CO) to prevent water leakage when not connected to the pressure transducer system. Bovine backpacks (BBPs) were made using a 200-round ammo pack and a strap (composed of a buckle, about 90 cm of 5 cm wide nylon webbing, about a 90 cm elastic insert riveted to the nylon, then 120 cm of nylon webbing) with 3 eyelets where the

webbing would sit on the top of the back of the animals. A 17.2 cm x 7.6 cm snap-lid plastic container (Snapware, World Kitchen, Rosemont, IL), with a hole burned through one side for insertion of a stainless steel male luer lock bulkhead adapter with luer lock side on the outside of the container, housed the pressure transducer and transmitter and was inserted into the ammo pack. Cheesecloth was placed on the bottom of the container to prevent excessive movement. A female luer lock to 2.4 cm barb adapter connected the pressure transducer to a 5.5 cm piece of silicone tubing (i.d. = 2.4 mm; o.d. = 4.0 mm) attached to the barb of the bulkhead adapter. The transducer was taped to the side of the container to prevent the tubing from kinking. A piece of about 20 cm piece of Tygon tubing (i.d. = 3.2 mm; o.d. = 6.4 mm) was connected to the bulkhead adapter in the plastic container with a luer lock to hose adapter (female luer thread style 200 series barb, 3.2 mm i.d. tubing; Cole-Parmer Instruments, Vernon Hills, IL) through a hole in the bottom of the ammo pack. A collection 1 triggering open-flow rotating male quick coupling on the other end of the 20 cm Tygon tubing allowed passage of water from the balloon and catheter to the pressure transducer for measurement of ruminal contractions. The ammo pack was attached to the strap using zip ties placed through the eyelets in the straps. The BBPs were then be placed over the animals back, and the strap was secured tightly around the animal with the buckle. The buckle was secured to the cannula with a zip tie by making a small hole in the cannula flange at least 2.5 cm from the edge of the cannula. Transmitters connected to the transducers sent wireless signals to a receiver which were displayed and recorded on a laptop.

Following data analysis using the rhythmic analyzer, values for contraction base, peak, amplitude, frequency, time to peak (TTP), relaxation time (RT), duration, and area

under the curve for each animal were averaged for each day using the proc MEANS procedure of SAS (SAS 9.3, SAS Inst. Inc., Cary, NC).

Ruminal Measurements

Ruminal pH (RpH) was monitored (readings every 1 min) for 24 hr using rumen data loggers (SRL-T9, DASCOR, Inc., Escondido, CA) on the same days which motility was measured. This device was inserted into the ventral sac of the rumen through the cannula before feeding. The pH electrodeswere calibrated before and after each measurement period using pH buffers 7 and 4. Data loggers were attached to the catheter by the top of the balloon with a zip-tie to aid in locating them in the rumen. When not in use, the pH electrodes were stored in a storage solution (DASCOR, Inc.). Readings were stored in the data logger and downloaded from data logger after each use. From these data, minimum RpH, mean RpH, maximum RpH, duration RpH < 5.8, and duration RpH < 5.5 were determined for each animal on each day.

The data logger was also equipped with a built-in sensor to measure ruminal temperature (Trum) at the same time pH was recorded (Mohammed et al., 2014). Minimum Trum, mean Trum, and maximum Trum were determined for each animal on each day.

Rumen Fluid Sample Collection and VFA Analysis

Approximately 100 mL of rumen fluid was collected from each animal immediately before feeding and 4, 8, and 12 hr after feeding on motility sampling days using a suction strainer. A 15 mL sample of rumen fluid from each animal was transferred to screw-top conical vials and centrifuged for 5 min at 2000 × g. Duplicate 1-mL samples of supernatant rumen fluid from each animal and time point was placed into

microcentrifuge tubes, combined with 100 µL 85 mM 2-ethylbutyrate internal standard, capped, and mixed for approximately 2 seconds using a vortex. Next, 100 µL 50% meta-phosphoric acid was added, tubes were recapped, mixed for approximately 5 seconds using a vortex, and frozen overnight (-4°C) to allow for protein precipitation. Tubes were thawed, centrifuged at 20,000 × g for 20 min, and supernatant were transferred to GC injection vials and capped. Gas chromatography with a flame ionization detector (Agilent HP6890 Plus GC with Agilent 7683 Series Injector and Auto Sampler; Agilent Technologies, Santa Clara, CA) and a Supelco 25326 Nukol fused silica capillary column (15 m × 0.53 mm × 0.5 µM film thickness; Sigma/Supelco, Bellefonte, PA) was used to determine VFA concentrations in the rumen fluid samples. Analysis involved injection of 0.2 µL of each sample in duplicate at 110°C with a 2:1 split, a 1-min hold, temperature increase at 5°C/min to 125°C for 2 min, and the setpoint for inlet and injector at 260°C.

Statistical Analysis

Data were analyzed using a randomized complete block design. Blocking criterion was body weight, placing animals into 2 dietary treatments (4 blocks of 2 steers). Diets were randomly assigned within each block. Concentrations of VFA were analyzed using proc MIXED of SAS for randomized complete block design with a split-split plot, with treatment (DRC or HMC) as the whole plot factor, phase (1, 2, 3, or 4) as the sub-plot factor, and time (hour relative to feed) as the sub-sub plot factor. All other variables were analyzed as an RBD split plot.

Conclusions

- DMI was variable through diet transitions → major fluctuations and decrease between d 9 and 21 (70% to 90% transition)
- Some differences between DRC and HMC, with HMC having…
    - Reduced DMI in phase 3
    - Reduced water consumption phases 2-4
    - Longer duration RpH < 5.5
    - Reduced mean and max Trum in phase 3
- Unexpected results for motility variables => no effect on motility in phases 1-3 and greater motility in the finishing phase (highest concentrate level)
- VFA patterns as expected – concentrations increased with time and shifted to greater proportion of propionate production with greater concentrate level

Tables and Figures

Table A-1. Preliminary experiment composition of diets and ingredients

Diet Ingredient	%, DM basis				
	CS	T-DRC[1]	T-HMC[2]	F-DRC	F-HMC
Corn silage	90.00	54.00	54.00	18.00	18.00
Dry rolled corn	-	37.48	-	74.95	-
High moisture corn	-	-	37.48	-	74.95
Soybean meal	7.38	5.69	5.69	4.00	4.00
Urea	0.60	0.55	0.55	0.50	0.50
Tallow	0.30	0.30	0.30	0.30	0.30
Limestone	0.80	1.10	1.10	1.40	1.40
Trace mineral premix[3]	0.45	0.45	0.45	0.45	0.45
Vitamin A, D, & E premix[4]	0.15	0.15	0.15	0.15	0.15
Sodium sulfate	0.32	0.16	0.16	-	-
Potassium sulfate	-	0.12	0.12	0.25	0.25

[1] DRC: dry-rolled corn
[2] HMC: high-moisture corn
[3] Contained 56.34% Cl, 36.53% Na, 1.2% S, 0.06% Ca, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, and 5520.2 ppm Zn on a DM basis.
[4] Composed of vitamin A acetate (1,814,368 IU/kg), D-activated animal sterol (source of vitamin D3; 362,874 IU/kg), vitamin E supplement (227 IU/kg), roughage products, calcium carbonate, and mineral oil.

Table A-2. Volatile fatty acid concentrations during the dietary transition of steers from 50% to 90% concentrate diets using an intermediate 70% diet. No treatment effects or interactions were detected.

Item	Phase					P-Value	
	All	1	2	3	4	Phase	Time
Total VFA, m*M*		87.9	100.0	102.8	122.0	<0.01	<0.01
0 h	88.0						
4 h	101.0						
8 h	107.0						
12 h	116.6						
Acetate, m*M*		53.3	58.5	52.5	63.5	<0.01	<0.01
0 h	50.2						
4 h	56.2						
8 h	58.7						
12 h	62.7						
Propionate, m*M*		17.3	22.1	34.3	41.5	<0.01	<0.01
0 h	22.8						
4 h	27.8						
8 h	30.4						
12 h	34.1						
Butyrate, m*M*		13.1	15.1	12.1	12.0	0.166	<0.01
0 h	11.2						
4 h	12.6						
8 h	13.4						
12 h	15.1						
Acetate:Propionate		3.2	2.9	2.3	1.8	<0.01	<0.01
0 h	2.8						
4 h	2.6						
8 h	2.5						
12 h	2.3						

Figure A-1. Preliminary experiment dietary transition and sampling timeline

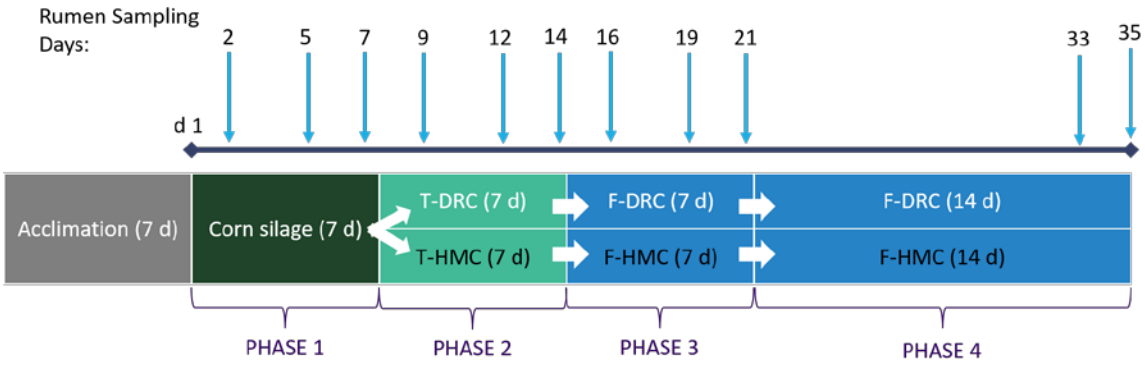


Figure A-2. Dry matter intake of steers throughout the experiment on sampling days only comparing dry-rolled corn (DRC) and high-moisture corn (HMC) treatments. The effect of treatment depended on phase (*P*=0.0008). Intake was lower for HMC treated steers in period 3 (i.e. the beginning of the finishing diet after animals were switched from 70% to 90%).

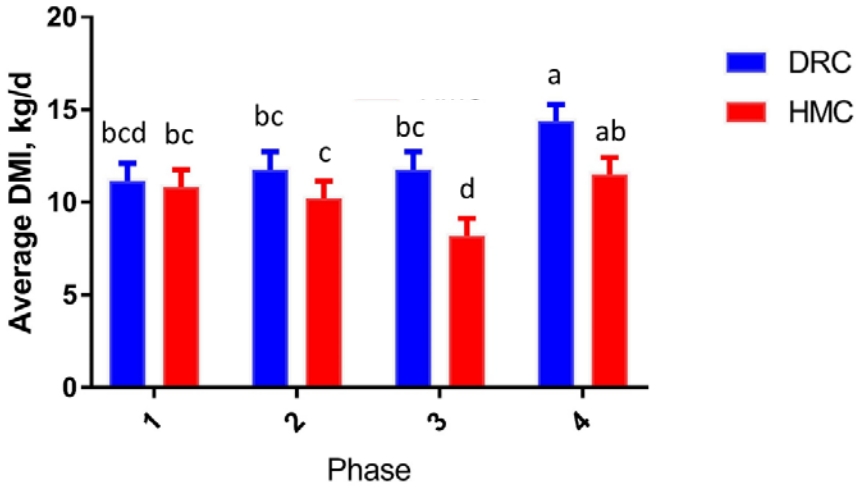


Figure A-3. Water intake varied by treatment (P=0.04) and phase (P<0.0001) separately. Water intake was lower on HMC compared to DRC.

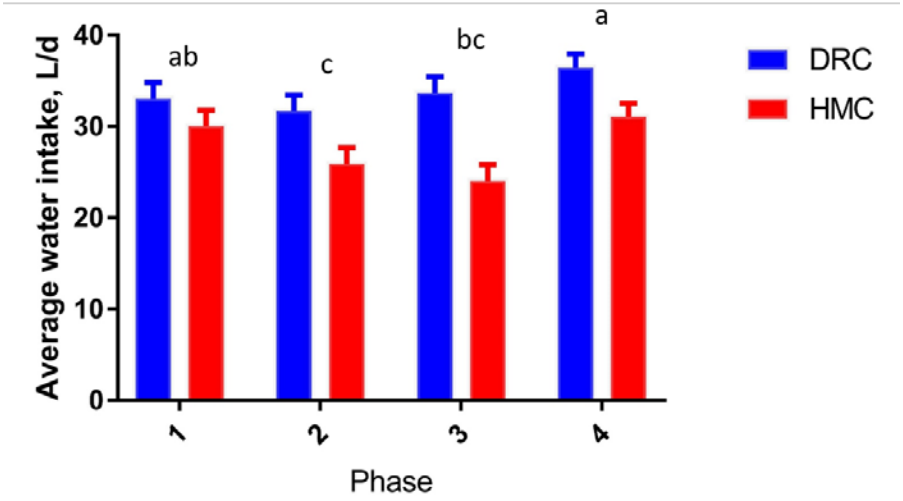


Figure A-4. Mean ruminal pH decreased with each step in diet transition (Phase: P<0.0001).

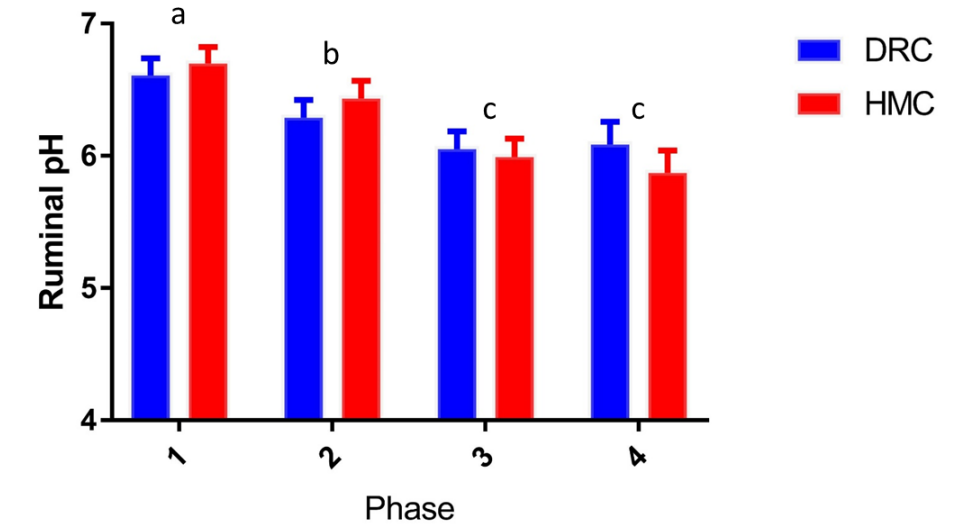


Figure A-5. Minimum ruminal pH was reduced as concentrate level increased in the diet (Phase: $P<0.0001$).

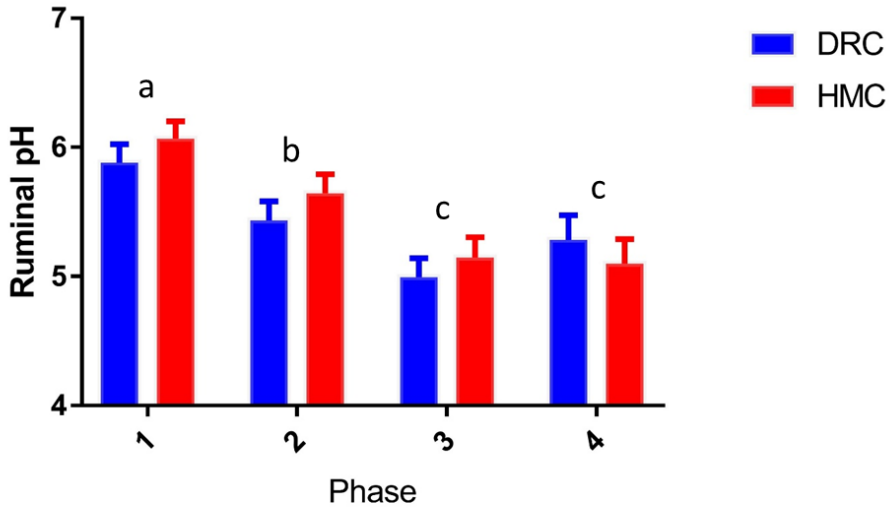


Figure A-6. Increasing concentrate level in the diet increased duration ruminal pH was below 5.8 ($P<0.0001$).

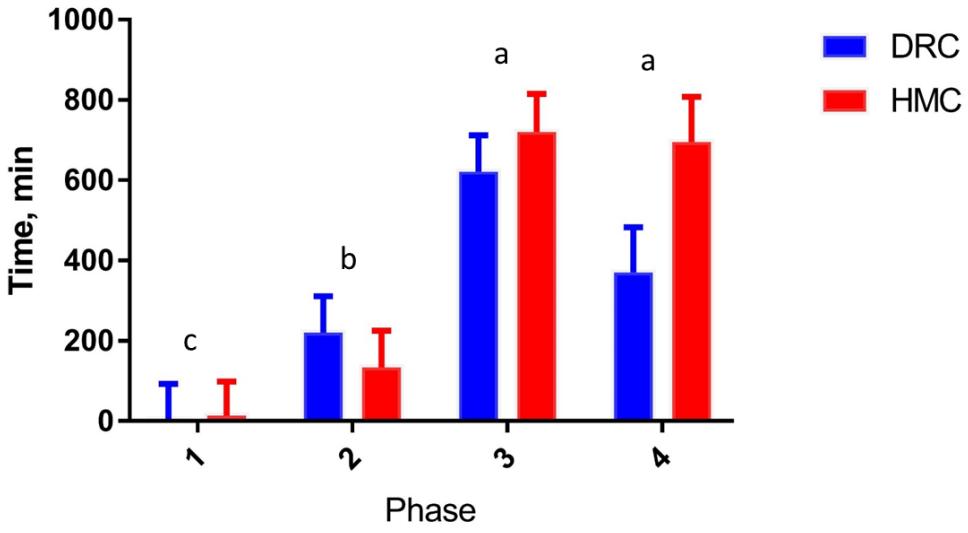


Figure A-7. Steers fed HMC had a ruminal pH below 5.5 for more time than DRC-fed steers at the end of the finishing period (*P*=0.0402).

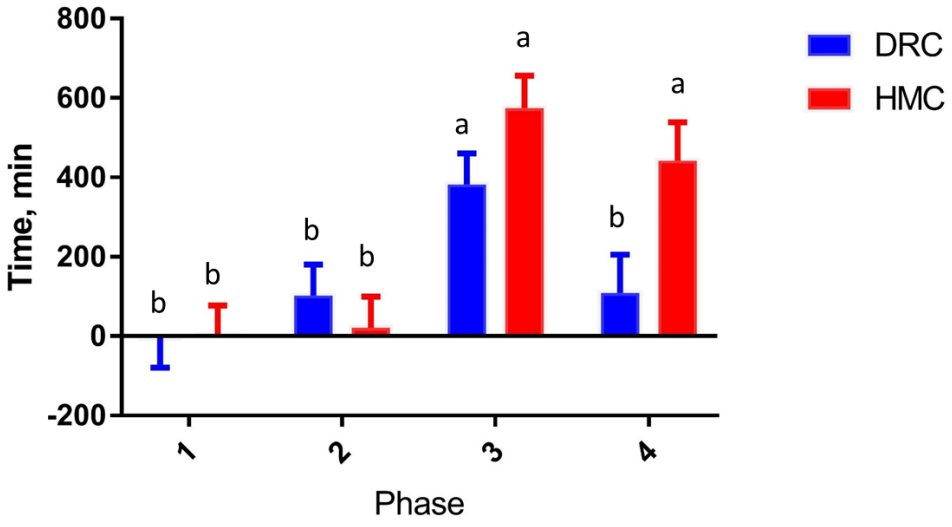


Figure A-8. Mean ruminal temp. tended to be lower for HMC-fed steers in the beginning of the finishing phase (Phase: *P*=0.0124; Treatment*Phase: *P*=0.0635).

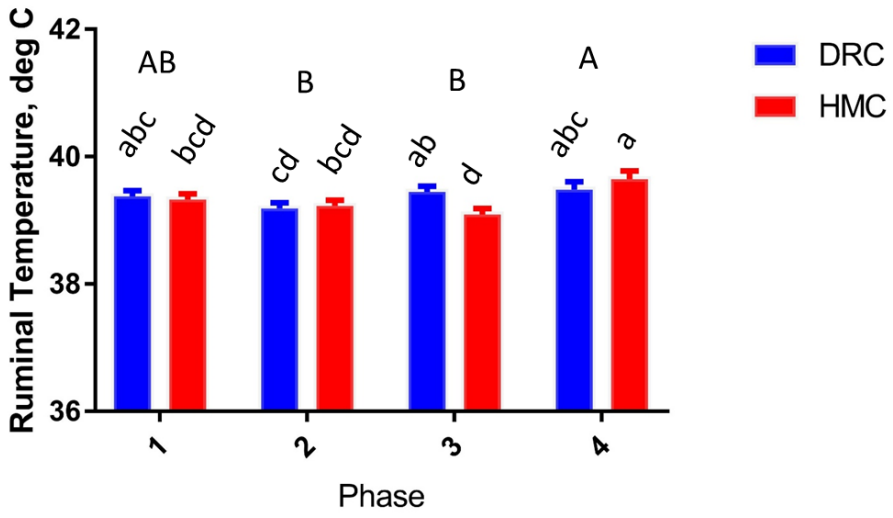


Figure A-9. Minimum ruminal temperature was not affected by treatment or phase.

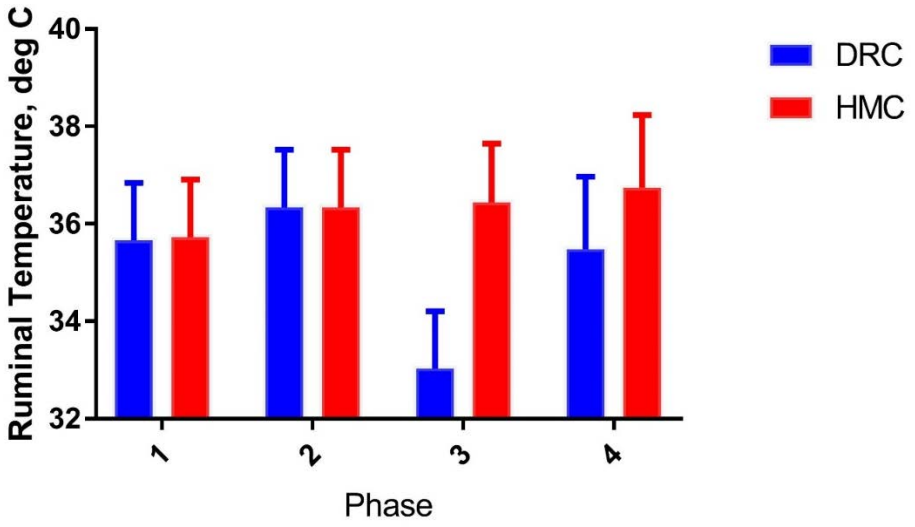


Figure A-10. Maximum ruminal temperature was lower for HMC steers in the beginning of the finishing phase (*P*=0.0453).

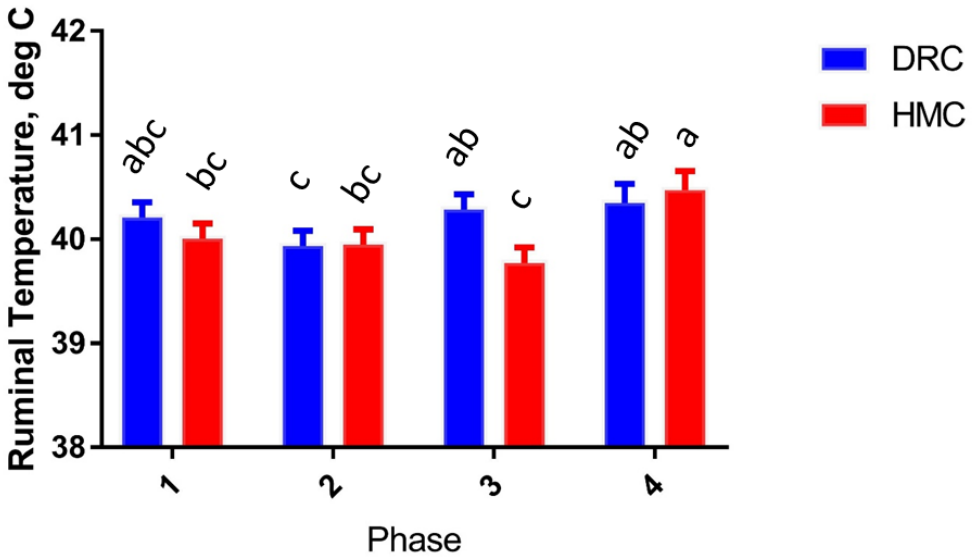


Figure A-11. Contraction base (A) and peak (B) tended to be greater at the end of the finishing phase (P=0.0772 and P=0.0573, respectively).

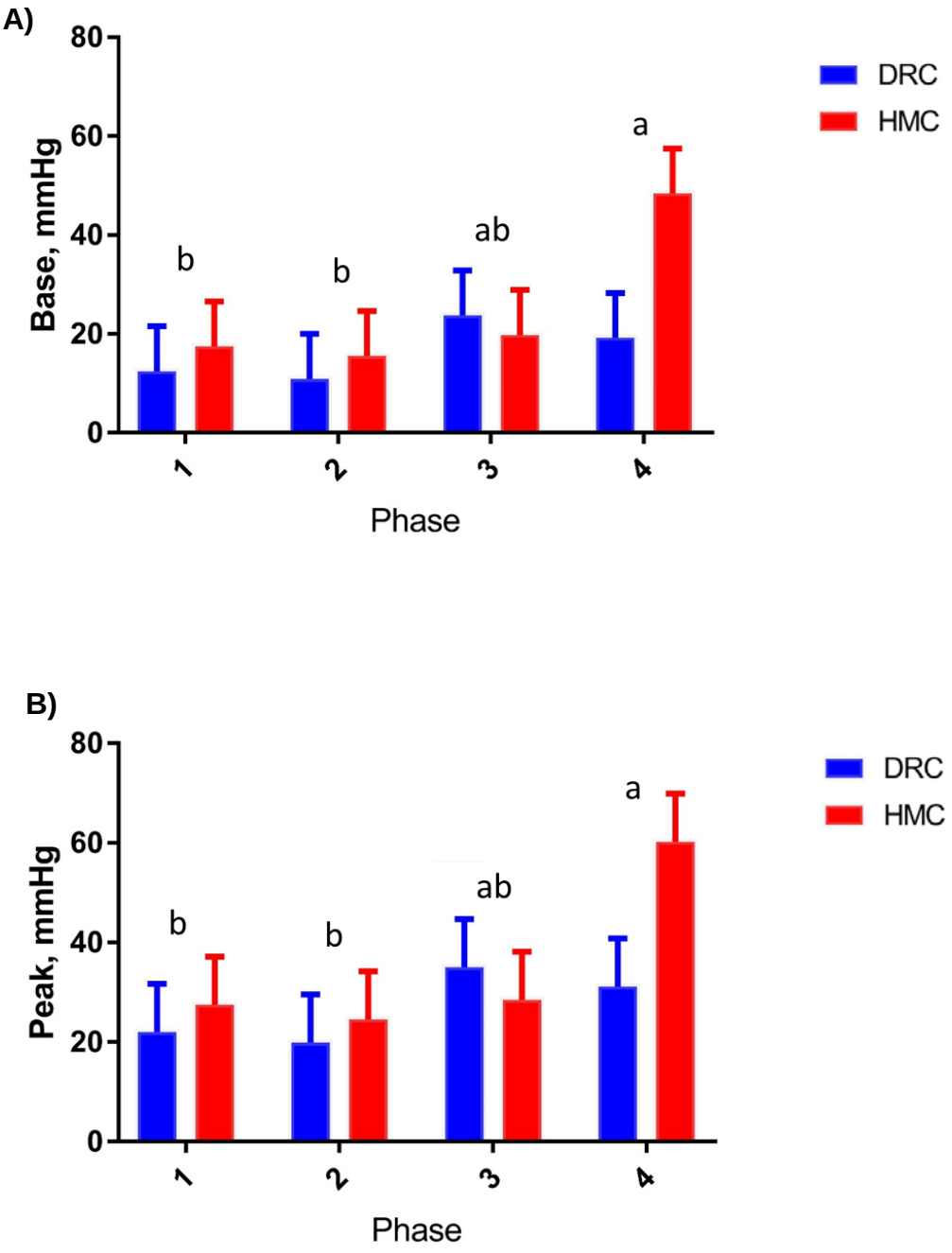


Figure A-12. Contraction amplitude (A) and frequency (B) was greater at the end of the finishing phase (*P*=0.0205).

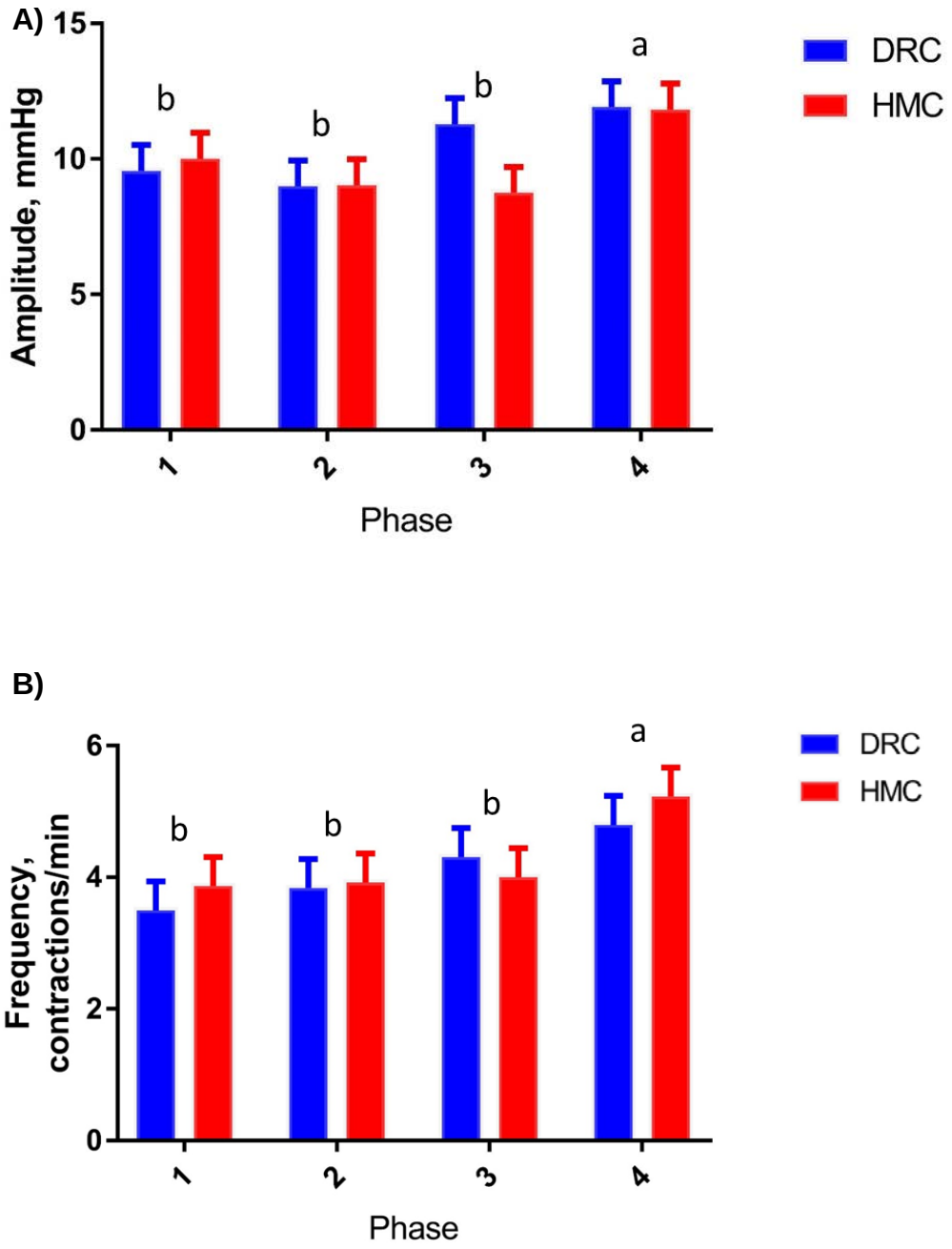


Figure A-13. Contraction time to peak was greater on the starter diet compared to all other phases (*P*=0.0088*).*

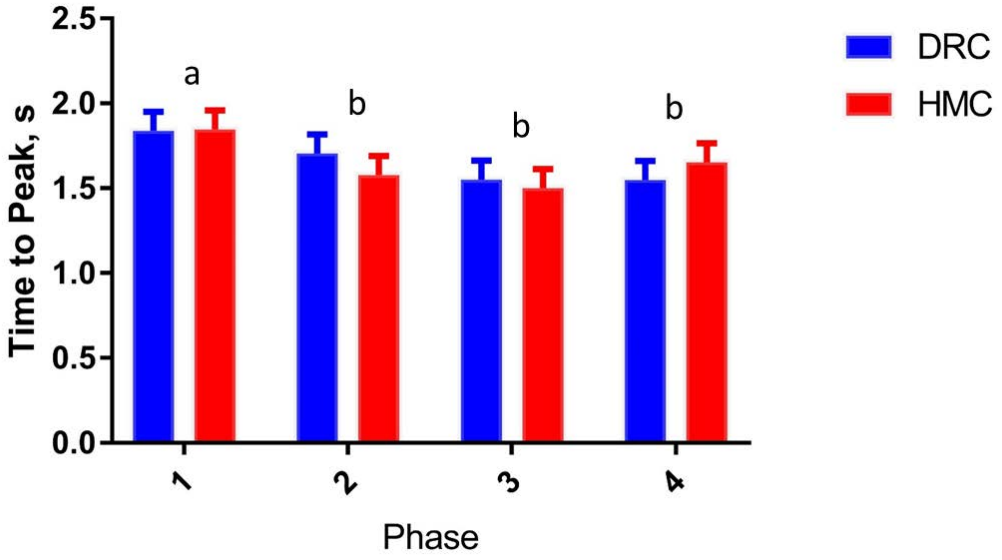
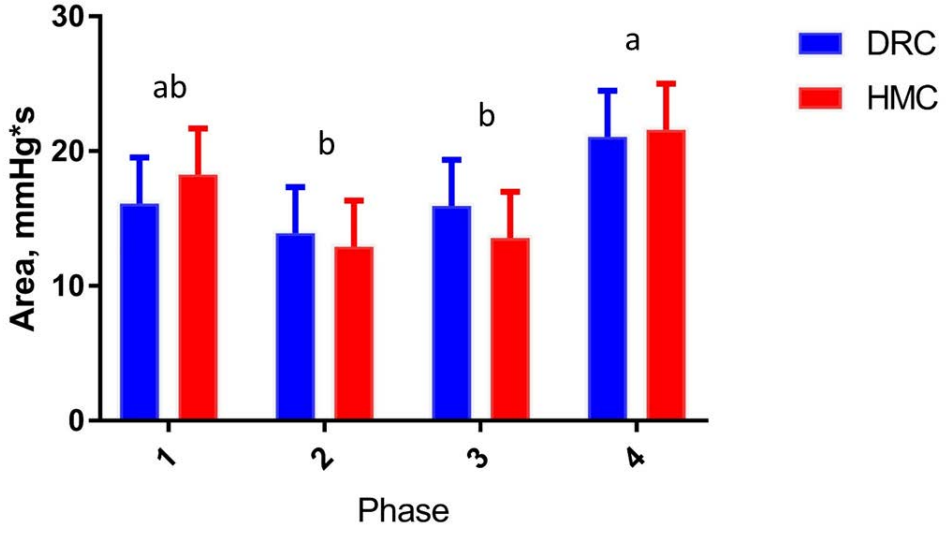


Figure A-14. Contraction area under the curve was greater at the end of the finishing phase compared to phase 2 or 3 (*P*=0.0542).



Appendix B: MATLAB meal detection and characterization script

```
%*********************************************************************
%* Title: LoadCell.m                                                 *
%* Author: Michael P. Sama & Amanda M. Egert-McLean                  *
%* Date: 10/25/2018                                                  *
%* Description: This program takes the raw load cell file in Excel   *
%*   format and reports the start and duration of each meal. The raw *
%*   data is filtered using a median filter and meals that are close *
%*   together are considered a single meal.                          *
%* Prerequisites: None, but variables n and m should be adjusted     *
%*********************************************************************

clear;  %clear all variables
clc; %clear the command window

n = 20; %order of median filter, adjust as needed
m = 30; %difference between start of Meal and end of last Meal for
combining into one Meal
[~,~,DATA] = xlsread('Insert file name here'); %read in the load cell
data file

%extract individual load cell data
LC1_LBS_AVG = cell2mat(DATA(5:length(DATA),13));
LC2_LBS_AVG = cell2mat(DATA(5:length(DATA),14));
LC3_LBS_AVG = cell2mat(DATA(5:length(DATA),15));
LC4_LBS_AVG = cell2mat(DATA(5:length(DATA),16));
LC5_LBS_AVG = cell2mat(DATA(5:length(DATA),17));
LC6_LBS_AVG = cell2mat(DATA(5:length(DATA),18));
LC7_LBS_AVG = cell2mat(DATA(5:length(DATA),19));
LC8_LBS_AVG = cell2mat(DATA(5:length(DATA),20));

TS = DATA(5:length(DATA),1); %extract the timestamps

ElapsedTime = (0:1:length(LC1_LBS_AVG)-1)'; %x-axis variable for
elapsed time

% plot raw data
figure(10)
subplot(4,2,1)
plot(ElapsedTime,LC1_LBS_AVG);
title('LC1')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,2)
plot(ElapsedTime,LC2_LBS_AVG);
title('LC2')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,3)
plot(ElapsedTime,LC3_LBS_AVG);
title('LC3')
```

```
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,4)
plot(ElapsedTime,LC4_LBS_AVG);
title('LC4')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,5)
plot(ElapsedTime,LC5_LBS_AVG);
title('LC5')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,6)
plot(ElapsedTime,LC6_LBS_AVG);
title('LC6')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,7)
plot(ElapsedTime,LC7_LBS_AVG);
title('LC7')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,8)
plot(ElapsedTime,LC8_LBS_AVG);
title('LC8')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')

%filter all load cell data using an order-n one-dimensional median
filter
LC1_LBS_AVG_F = medfilt1(LC1_LBS_AVG,n);
LC2_LBS_AVG_F = medfilt1(LC2_LBS_AVG,n);
LC3_LBS_AVG_F = medfilt1(LC3_LBS_AVG,n);
LC4_LBS_AVG_F = medfilt1(LC4_LBS_AVG,n);
LC5_LBS_AVG_F = medfilt1(LC5_LBS_AVG,n);
LC6_LBS_AVG_F = medfilt1(LC6_LBS_AVG,n);
LC7_LBS_AVG_F = medfilt1(LC7_LBS_AVG,n);
LC8_LBS_AVG_F = medfilt1(LC8_LBS_AVG,n);

F1 = length(LC1_LBS_AVG_F);
F2 = length(LC2_LBS_AVG_F);
F3 = length(LC3_LBS_AVG_F);
F4 = length(LC4_LBS_AVG_F);
F5 = length(LC5_LBS_AVG_F);
F6 = length(LC6_LBS_AVG_F);
F7 = length(LC7_LBS_AVG_F);
F8 = length(LC8_LBS_AVG_F);

DATA_OUT = cell.empty();
DATA_OUT(1,:) =
{'LC1','','LC2','','LC3','','LC4','','LC5','','LC6','','LC7','','LC8','
'};
DATA_OUT(2,:) =
{'Weight','','Weight','','Weight','','Weight','','Weight','','Weight','
','Weight','','Weight',''};
```

```
DATA_OUT(3:F1+2,1) = num2cell(LC1_LBS_AVG_F(1:length(LC1_LBS_AVG_F)));
DATA_OUT(3:F2+2,3) = num2cell(LC2_LBS_AVG_F(1:length(LC2_LBS_AVG_F)));
DATA_OUT(3:F3+2,5) = num2cell(LC3_LBS_AVG_F(1:length(LC3_LBS_AVG_F)));
DATA_OUT(3:F4+2,7) = num2cell(LC4_LBS_AVG_F(1:length(LC4_LBS_AVG_F)));
DATA_OUT(3:F5+2,9) = num2cell(LC5_LBS_AVG_F(1:length(LC5_LBS_AVG_F)));
DATA_OUT(3:F6+2,11) = num2cell(LC6_LBS_AVG_F(1:length(LC6_LBS_AVG_F)));
DATA_OUT(3:F7+2,13) = num2cell(LC7_LBS_AVG_F(1:length(LC7_LBS_AVG_F)));
DATA_OUT(3:F8+2,15) = num2cell(LC8_LBS_AVG_F(1:length(LC8_LBS_AVG_F)));

[~,~,temp] = xlsread('Filtered.xlsx');
if ~isempty(temp)
    xlswrite('Filtered.xlsx',zeros(size(temp))*nan);
end
xlswrite('Filtered.xlsx',DATA_OUT);

%calculate the first derivative as the difference between two
measurements
LC1_LBS_AVG_D = diff(LC1_LBS_AVG_F);
LC2_LBS_AVG_D = diff(LC2_LBS_AVG_F);
LC3_LBS_AVG_D = diff(LC3_LBS_AVG_F);
LC4_LBS_AVG_D = diff(LC4_LBS_AVG_F);
LC5_LBS_AVG_D = diff(LC5_LBS_AVG_F);
LC6_LBS_AVG_D = diff(LC6_LBS_AVG_F);
LC7_LBS_AVG_D = diff(LC7_LBS_AVG_F);
LC8_LBS_AVG_D = diff(LC8_LBS_AVG_F);

%calculate a binary threshold to differentiate slope
BinaryLC1 = LC1_LBS_AVG_D < -0.1;
BinaryLC2 = LC2_LBS_AVG_D < -0.1;
BinaryLC3 = LC3_LBS_AVG_D < -0.1;
BinaryLC4 = LC4_LBS_AVG_D < -0.1;
BinaryLC5 = LC5_LBS_AVG_D < -0.1;
BinaryLC6 = LC6_LBS_AVG_D < -0.1;
BinaryLC7 = LC7_LBS_AVG_D < -0.1;
BinaryLC8 = LC8_LBS_AVG_D < -0.1;

%variables for storing low-to-high and high-to-low transitions
LH1 = zeros(1,length(BinaryLC1));
HL1 = zeros(1,length(BinaryLC1));
LH2 = zeros(1,length(BinaryLC2));
HL2 = zeros(1,length(BinaryLC2));
LH3 = zeros(1,length(BinaryLC3));
HL3 = zeros(1,length(BinaryLC3));
LH4 = zeros(1,length(BinaryLC4));
HL4 = zeros(1,length(BinaryLC4));
LH5 = zeros(1,length(BinaryLC5));
HL5 = zeros(1,length(BinaryLC5));
LH6 = zeros(1,length(BinaryLC6));
HL6 = zeros(1,length(BinaryLC6));
LH7 = zeros(1,length(BinaryLC7));
HL7 = zeros(1,length(BinaryLC7));
LH8 = zeros(1,length(BinaryLC8));
HL8 = zeros(1,length(BinaryLC8));
```

```
%determine the low-to-high and high-to-low transitions
for i = 1:length(BinaryLC1)-1
    if ((BinaryLC1(i) == 0) && (BinaryLC1(i+1) == 1))
        LH1(i) = 1;
    elseif ((BinaryLC1(i) == 1) && (BinaryLC1(i+1) == 0))
        HL1(i) = 1;
    end
    if ((BinaryLC2(i) == 0) && (BinaryLC2(i+1) == 1))
        LH2(i) = 1;
    elseif ((BinaryLC2(i) == 1) && (BinaryLC2(i+1) == 0))
        HL2(i) = 1;
    end
    if ((BinaryLC3(i) == 0) && (BinaryLC3(i+1) == 1))
        LH3(i) = 1;
    elseif ((BinaryLC3(i) == 1) && (BinaryLC3(i+1) == 0))
        HL3(i) = 1;
    end
    if ((BinaryLC4(i) == 0) && (BinaryLC4(i+1) == 1))
        LH4(i) = 1;
    elseif ((BinaryLC4(i) == 1) && (BinaryLC4(i+1) == 0))
        HL4(i) = 1;
    end
    if ((BinaryLC5(i) == 0) && (BinaryLC5(i+1) == 1))
        LH5(i) = 1;
    elseif ((BinaryLC5(i) == 1) && (BinaryLC5(i+1) == 0))
        HL5(i) = 1;
    end
    if ((BinaryLC6(i) == 0) && (BinaryLC6(i+1) == 1))
        LH6(i) = 1;
    elseif ((BinaryLC6(i) == 1) && (BinaryLC6(i+1) == 0))
        HL6(i) = 1;
    end
    if ((BinaryLC7(i) == 0) && (BinaryLC7(i+1) == 1))
        LH7(i) = 1;
    elseif ((BinaryLC7(i) == 1) && (BinaryLC7(i+1) == 0))
        HL7(i) = 1;
    end
    if ((BinaryLC8(i) == 0) && (BinaryLC8(i+1) == 1))
        LH8(i) = 1;
    elseif ((BinaryLC8(i) == 1) && (BinaryLC8(i+1) == 0))
        HL8(i) = 1;
    end
end

%find the indices of all transitions and calculate Meal duration
LH1i = find(LH1); %find the indices of all low-to-high transitions
HL1i = find(HL1)+1; %find the indices of all high-to-low transitions
and shift by 1
LH2i = find(LH2); %find the indices of all low-to-high transitions
HL2i = find(HL2)+1; %find the indices of all high-to-low transitions
and shift by 1
LH3i = find(LH3); %find the indices of all low-to-high transitions
HL3i = find(HL3)+1; %find the indices of all high-to-low transitions
and shift by 1
LH4i = find(LH4); %find the indices of all low-to-high transitions
```

```
HL4i = find(HL4)+1; %find the indices of all high-to-low transitions
and shift by 1
LH5i = find(LH5); %find the indices of all low-to-high transitions
HL5i = find(HL5)+1; %find the indices of all high-to-low transitions
and shift by 1
LH6i = find(LH6); %find the indices of all low-to-high transitions
HL6i = find(HL6)+1; %find the indices of all high-to-low transitions
and shift by 1
LH7i = find(LH7); %find the indices of all low-to-high transitions
HL7i = find(HL7)+1; %find the indices of all high-to-low transitions
and shift by 1
LH8i = find(LH8); %find the indices of all low-to-high transitions
HL8i = find(HL8)+1; %find the indices of all high-to-low transitions
and shift by 1

%combine feeding events that are less than m minutes apart
j1 = 0; %iterator variable
j2 = 0; %iterator variable
j3 = 0; %iterator variable
j4 = 0; %iterator variable
j5 = 0; %iterator variable
j6 = 0; %iterator variable
j7 = 0; %iterator variable
j8 = 0; %iterator variable

for i = 2:length(LH1i) %iterate through all but start of first Meal
    if LH1i(i) < (HL1i(i-1) + m) %if start is within m minutes of last
end
        j1 = j1+ 1; %increment the iterator
        LH1r(j1) = i;   %store the index of the start to remove
        HL1r(j1) = i-1; %store the index of the end to remove
    end
end
for i = 2:length(LH2i) %iterate through all but start of first Meal
    if LH2i(i) < (HL2i(i-1) + m) %if start is within m minutes of last
end
        j2 = j2+ 1; %increment the iterator
        LH2r(j2) = i;   %store the index of the start to remove
        HL2r(j2) = i-1; %store the index of the end to remove
    end
end
for i = 2:length(LH3i) %iterate through all but start of first Meal
    if LH3i(i) < (HL3i(i-1) + m) %if start is within m minutes of last
end
        j3 = j3+ 1; %increment the iterator
        LH3r(j3) = i;   %store the index of the start to remove
        HL3r(j3) = i-1; %store the index of the end to remove
    end
end
for i = 2:length(LH4i) %iterate through all but start of first Meal
    if LH4i(i) < (HL4i(i-1) + m) %if start is within m minutes of last
end
        j4 = j4+ 1; %increment the iterator
        LH4r(j4) = i;   %store the index of the start to remove
        HL4r(j4) = i-1; %store the index of the end to remove
```

```
    end
end
for i = 2:length(LH5i) %iterate through all but start of first Meal
    if LH5i(i) < (HL5i(i-1) + m) %if start is within m minutes of last
end
        j5 = j5+ 1; %increment the iterator
        LH5r(j5) = i;   %store the index of the start to remove
        HL5r(j5) = i-1; %store the index of the end to remove
    end
end
for i = 2:length(LH6i) %iterate through all but start of first Meal
    if LH6i(i) < (HL6i(i-1) + m) %if start is within m minutes of last
end
        j6 = j6+ 1; %increment the iterator
        LH6r(j6) = i;   %store the index of the start to remove
        HL6r(j6) = i-1; %store the index of the end to remove
    end
end
for i = 2:length(LH7i) %iterate through all but start of first Meal
    if LH7i(i) < (HL7i(i-1) + m) %if start is within m minutes of last
end
        j7 = j7+ 1; %increment the iterator
        LH7r(j7) = i;   %store the index of the start to remove
        HL7r(j7) = i-1; %store the index of the end to remove
    end
end
for i = 2:length(LH8i) %iterate through all but start of first Meal
    if LH8i(i) < (HL8i(i-1) + m) %if start is within m minutes of last
end
        j8 = j8+ 1; %increment the iterator
        LH8r(j8) = i;   %store the index of the start to remove
        HL8r(j8) = i-1; %store the index of the end to remove
    end
end


LH1i(LH1r) = [];  %remove the intermediate Meal start
HL1i(HL1r) = [];  %remove the intermediate Meal end
LHL1 = HL1i-LH1i(1:length(HL1i)); %calculate the elapsed time (min) for
each Meal
LH2i(LH2r) = [];  %remove the intermediate Meal start
HL2i(HL2r) = [];  %remove the intermediate Meal end
LHL2 = HL2i-LH2i(1:length(HL2i)); %calculate the elapsed time (min) for
each Meal
LH3i(LH3r) = [];  %remove the intermediate Meal start
HL3i(HL3r) = [];  %remove the intermediate Meal end
LHL3 = HL3i-LH3i(1:length(HL3i)); %calculate the elapsed time (min) for
each Meal
LH4i(LH4r) = [];  %remove the intermediate Meal start
HL4i(HL4r) = [];  %remove the intermediate Meal end
LHL4 = HL4i-LH4i(1:length(HL4i)); %calculate the elapsed time (min) for
each Meal
LH5i(LH5r) = [];  %remove the intermediate Meal start
HL5i(HL5r) = [];  %remove the intermediate Meal end
```

```
LHL5 = HL5i-LH5i(1:length(HL5i)); %calculate the elapsed time (min) for
each Meal
LH6i(LH6r) = [];  %remove the intermediate Meal start
HL6i(HL6r) = [];  %remove the intermediate Meal end
LHL6 = HL6i-LH6i(1:length(HL6i)); %calculate the elapsed time (min) for
each Meal
LH7i(LH7r) = [];  %remove the intermediate Meal start
HL7i(HL7r) = [];  %remove the intermediate Meal end
LHL7 = HL7i-LH7i(1:length(HL7i)); %calculate the elapsed time (min) for
each Meal
LH8i(LH8r) = [];  %remove the intermediate Meal start
HL8i(HL8r) = [];  %remove the intermediate Meal end
LHL8 = HL8i-LH8i(1:length(HL8i)); %calculate the elapsed time (min) for
each Meal

%plot filtered data and Meal start/stop points
figure(1)
subplot(4,2,1)
plot(ElapsedTime,LC1_LBS_AVG_F, LH1i, LC1_LBS_AVG_F(LH1i),'go',HL1i,
LC1_LBS_AVG_F(HL1i),'rx');
title('LC1')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,2)
plot(ElapsedTime,LC2_LBS_AVG_F, LH2i, LC2_LBS_AVG_F(LH2i),'go',HL2i,
LC2_LBS_AVG_F(HL2i),'rx');
title('LC2')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,3)
plot(ElapsedTime,LC3_LBS_AVG_F, LH3i, LC3_LBS_AVG_F(LH3i),'go',HL3i,
LC3_LBS_AVG_F(HL3i),'rx');
title('LC3')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,4)
plot(ElapsedTime,LC4_LBS_AVG_F, LH4i, LC4_LBS_AVG_F(LH4i),'go',HL4i,
LC4_LBS_AVG_F(HL4i),'rx');
title('LC4')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,5)
plot(ElapsedTime,LC5_LBS_AVG_F, LH5i, LC5_LBS_AVG_F(LH5i),'go',HL5i,
LC5_LBS_AVG_F(HL5i),'rx');
title('LC5')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,6)
plot(ElapsedTime,LC6_LBS_AVG_F, LH6i, LC6_LBS_AVG_F(LH6i),'go',HL6i,
LC6_LBS_AVG_F(HL6i),'rx');
title('LC6')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,7)
```

```
plot(ElapsedTime,LC7_LBS_AVG_F, LH7i, LC7_LBS_AVG_F(LH7i),'go',HL7i,
LC7_LBS_AVG_F(HL7i),'rx');
title('LC7')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')
subplot(4,2,8)
plot(ElapsedTime,LC8_LBS_AVG_F, LH8i, LC8_LBS_AVG_F(LH8i),'go',HL8i,
LC8_LBS_AVG_F(HL8i),'rx');
title('LC8')
xlabel('Elapsed Time (min)')
ylabel('Weight (lbs)')

% Assigns animal numbers to load cells
anim1 = input('What animal used load cell #1?  ');
anim2 = input('What animal used load cell #2?  ');
anim3 = input('What animal used load cell #3?  ');
anim4 = input('What animal used load cell #4?  ');
anim5 = input('What animal used load cell #5?  ');
anim6 = input('What animal used load cell #6?  ');
anim7 = input('What animal used load cell #7?  ');
anim8 = input('What animal used load cell #8?  ');

%Calculate meal sizes
S1 = LC1_LBS_AVG_F(LH1i(1:length(HL1i))) - LC1_LBS_AVG_F(HL1i);
S2 = LC2_LBS_AVG_F(LH2i(1:length(HL2i))) - LC2_LBS_AVG_F(HL2i);
S3 = LC3_LBS_AVG_F(LH3i(1:length(HL3i))) - LC3_LBS_AVG_F(HL3i);
S4 = LC4_LBS_AVG_F(LH4i(1:length(HL4i))) - LC4_LBS_AVG_F(HL4i);
S5 = LC5_LBS_AVG_F(LH5i(1:length(HL5i))) - LC5_LBS_AVG_F(HL5i);
S6 = LC6_LBS_AVG_F(LH6i(1:length(HL6i))) - LC6_LBS_AVG_F(HL6i);
S7 = LC7_LBS_AVG_F(LH7i(1:length(HL7i))) - LC7_LBS_AVG_F(HL7i);
S8 = LC8_LBS_AVG_F(LH8i(1:length(HL8i))) - LC8_LBS_AVG_F(HL8i);

%compile add processed data into a single spreadsheet and export
N1 = length(HL1i);
N2 = length(HL2i);
N3 = length(HL3i);
N4 = length(HL4i);
N5 = length(HL5i);
N6 = length(HL6i);
N7 = length(HL7i);
N8 = length(HL8i);
% DATA_OUT = num2cell(zeros(N+2,16));
DATA_OUT = cell.empty();
DATA_OUT(1,:) =
{'LC1',anim1,'','LC2',anim2,'','LC3',anim3,'','LC4',anim4,'','LC5',anim
5,'','LC6',anim6,'','LC7',anim7,'','LC8',anim8,''};
DATA_OUT(2,:) = {'Timestamp','Meal Duration (min)','Meal Size
(lbs)','Timestamp','Meal Duration (min)','Meal Size
(lbs)','Timestamp','Meal Duration (min)','Meal Size
(lbs)','Timestamp','Meal Duration (min)','Meal Size
(lbs)','Timestamp','Meal Duration (min)','Meal Size
(lbs)','Timestamp','Meal Duration (min)','Meal Size
(lbs)','Timestamp','Meal Duration (min)','Meal Size (lbs)'};
DATA_OUT(3:N1+2,1) = TS(LH1i(1:length(HL1i)));
```

```
DATA_OUT(3:(N1+2),2) = num2cell(LHL1);
DATA_OUT(3:(N1+2),3) = num2cell(S1);
DATA_OUT(3:N2+2,4) = TS(LH2i(1:length(HL2i)));
DATA_OUT(3:N2+2,5) = num2cell(LHL2);
DATA_OUT(3:(N2+2),6) = num2cell(S2);
DATA_OUT(3:N3+2,7) = TS(LH3i(1:length(HL3i)));
DATA_OUT(3:N3+2,8) = num2cell(LHL3);
DATA_OUT(3:(N3+2),9) = num2cell(S3);
DATA_OUT(3:N4+2,10) = TS(LH4i(1:length(HL4i)));
DATA_OUT(3:N4+2,11) = num2cell(LHL4);
DATA_OUT(3:(N4+2),12) = num2cell(S4);
DATA_OUT(3:N5+2,13) = TS(LH5i(1:length(HL5i)));
DATA_OUT(3:N5+2,14) = num2cell(LHL5);
DATA_OUT(3:(N5+2),15) = num2cell(S5);
DATA_OUT(3:N6+2,16) = TS(LH6i(1:length(HL6i)));
DATA_OUT(3:N6+2,17) = num2cell(LHL6);
DATA_OUT(3:(N6+2),18) = num2cell(S6);
DATA_OUT(3:N7+2,19) = TS(LH7i(1:length(HL7i)));
DATA_OUT(3:N7+2,20) = num2cell(LHL7);
DATA_OUT(3:(N7+2),21) = num2cell(S7);
DATA_OUT(3:N8+2,22) = TS(LH8i(1:length(HL8i)));
DATA_OUT(3:N8+2,23) = num2cell(LHL8);
DATA_OUT(3:(N8+2),24) = num2cell(S8);



[~,~,temp] = xlsread('Results.xlsx');
if ~isempty(temp)
    xlswrite('Results.xlsx',zeros(size(temp))*nan);
end
xlswrite('Results.xlsx',DATA_OUT);
```

Appendix C: MATLAB rumen motility contraction detection and characterization script

```
%*********************************************************************
%* Title: MotilityPeaks.m                                            *
%* Author: Amanda M. Egert-McLean & Michael P. Sama                  *
%* Date: 03/20/2018                                                  *
%* Description: This program takes the processed pressure versus time *
%*   data from an Excel spreadsheet exported from LabChart files,    *
%*   filters the data, and performs a peak analysis to measure peak  *
%*   amplitude, duration, and frequency.                             *
%* Prerequisites: None, but variables aT and n should be adjusted    *
%*********************************************************************

clear;  %clear all variables
clc; %clear the command window

aT = 3; %amplitude threshold
n = 10; %order of median filter, adjust as needed

[~,~,DATA] = xlsread('Insert file name here'); %read in the motility
data file

%extract individual animal motility pressure data from channels
Ch1 = cell2mat(DATA(2:length(DATA),4));
Ch2 = cell2mat(DATA(2:length(DATA),5));
Ch3 = cell2mat(DATA(2:length(DATA),6));

%extract additional data needed from the input file
% Sec = cell2mat(DATA(2:length(DATA),1)); %extract the elapsed seconds
from the recording
ElapsedTime1 = (0:1:length(Ch1)-1)./4'; %x-axis variable for elapsed
time %MPS:changed the formula to keep the dimensions of ElapsedTime and
motility data the same
ElapsedTime2 = (0:1:length(Ch2)-1)./4'; %x-axis variable for elapsed
time %MPS:changed the formula to keep the dimensions of ElapsedTime and
motility data the same
ElapsedTime3 = (0:1:length(Ch3)-1)./4'; %x-axis variable for elapsed
time %MPS:changed the formula to keep the dimensions of ElapsedTime and
motility data the same
Hour = cell2mat(DATA(2:length(DATA),7)); %extract the hour variable for
averaging peaks within each hour later

% %plot raw data
figure(20)
subplot(1,3,1)
plot(ElapsedTime1,Ch1);
title('Channel 1')
xlabel('Elapsed Time (sec)')
ylabel('Pressure (mmHg)')
```

```
subplot(1,3,2)
plot(ElapsedTime2,Ch2);
title('Channel 2')
xlabel('Elapsed Time (sec)')
ylabel('Pressure (mmHg)')
subplot(1,3,3)
plot(ElapsedTime3,Ch1);
title('Channel 3')
xlabel('Elapsed Time (sec)')
ylabel('Pressure (mmHg)')

% filter all motility data using an order-n one-dimensional median
filter
Ch1_F = medfilt1(Ch1,n);
Ch2_F = medfilt1(Ch2,n);
Ch3_F = medfilt1(Ch3,n);

% plot filtered data
figure(1)
plot(ElapsedTime1,Ch1_F);
title('Channel 1')
xlabel('Elapsed Time (sec)')
ylabel('Pressure (mmHg)')

figure(2)
plot(ElapsedTime2,Ch2_F);
title('Channel 2')
xlabel('Elapsed Time (sec)')
ylabel('Pressure (mmHg)')

figure(3)
plot(ElapsedTime3,Ch3_F);
title('Channel 3')
xlabel('Elapsed Time (sec)')
ylabel('Pressure (mmHg)')

%%Use the findpeaks function to detect peaks with a specific minimum
%%height and output the peak value, location, half-width, and
prominence of each -
%% Graphical findpeaks example - switch to [pks,locs,w,p] =
findpeaks(...) to save the values to variables and remove
'Anotate','extents' when not plotting.
figure(4)
title('Channel 1 Analysis')
findpeaks(Ch1_F,ElapsedTime1,'Annotate','extents','MinPeakHeight',1,'Mi
nPeakWidth',1,'MinPeakProminence',aT)
axis([0,max(ElapsedTime1),0,max(Ch1_F)]);
[pks1,locs1,halfwidth1,amp1]=findpeaks(Ch1_F,ElapsedTime1,'MinPeakHeigh
t',1,'MinPeakWidth',1,'MinPeakProminence',aT);

figure(5)
title('Channel 2 Analysis')
findpeaks(Ch2_F,ElapsedTime2,'Annotate','extents','MinPeakHeight',1,'Mi
nPeakWidth',1,'MinPeakProminence',aT)
```

```
axis([0,max(ElapsedTime2),0,max(Ch2_F)]);
[pks2,locs2,halfwidth2,amp2]=findpeaks(Ch2_F,ElapsedTime2,'MinPeakHeigh
t',1,'MinPeakWidth',1,'MinPeakProminence',aT);

figure(6)
title('Channel 3 Analysis')
findpeaks(Ch3_F,ElapsedTime3,'Annotate','extents','MinPeakHeight',1,'Mi
nPeakWidth',1,'MinPeakProminence',aT)
axis([0,max(ElapsedTime3),0,max(Ch3_F)]);
[pks3,locs3,halfwidth3,amp3]=findpeaks(Ch3_F,ElapsedTime3,'MinPeakHeigh
t',1,'MinPeakWidth',1,'MinPeakProminence',aT);

%% Caclulate the duration of each peak
dCh1_F = diff(Ch1_F);   %compute the first derivative of the pressure
signal
dCh1_F(length(Ch1_F)) = 0;  %add a value of zero to the end to keep the
array length the same
dCh1_F = medfilt1(dCh1_F,n);    %filter the derivative result to remove
noise

figure(7)   %create a new figure for plotting the derivative
plot(ElapsedTime1,dCh1_F); %plot the derivative of the pressure signal
versus elapsed time

locs1i = locs1*4;   %create an integer array of the peak locations
(rather than in time)

start = zeros(size(locs1i));    %create an array to store the time at
the beginning of a contraction surrounding each peak
stop = zeros(size(locs1i));     %create an array to store the time at
the end of a contraction surrounding each peak

start_slope_threshold = 0.002; %sets the threshold in terms of the
derivative of pressure that defines when a contraction starts and
stops. lowering this value will produce longer durations, increasing it
will produce shorter durations.
stop_slope_threshold = 0.005; %sets the threshold in terms of the
derivative of pressure that defines when a contraction stops. lowering
this value will produce longer durations, increasing it will produce
shorter durations.
peak_offset = 25; %sets the starting point in either direction from the
peak when searching for the beginnning and end. This prevents the flat
portion at the peak from being detected as the start or stop time

for i = 1:length(locs1i) %for each peak, find the beginning and end by
searching from the peak location until the derivative of pressure falls
below the threshold
    j = locs1i(i)-peak_offset;
    if j < 0
         j=1;
    end
    while(j>1)
       j=j-1;
       if dCh1_F(j) < start_slope_threshold
```

```
            break
        end
    end
    start(i) = j/4;
    j = locs1i(i)+peak_offset;
    while(j<length(ElapsedTime1))
        j=j+1;
        if dCh1_F(j) > -stop_slope_threshold
            break
        end
    end
    stop(i) = j/4;
end

duration1 = stop-start;  %calculate the duration of each contraction

figure(1)
hold on
plot(locs1,pks1,'kD',start,Ch1_F(start*4),'gO',stop,Ch1_F(stop*4),'rX')
;    %add markers showing peak, start, and stop locations for each
contraction
hold off

for i = 1:length(locs1)
    for j = 1:24
        if ((j-1)*3600) <= locs1(1,i) & locs1(1,i) < (j*3600)
            hour1(1,i)=j;
        elseif locs1(1,i) > 86400;
            hour1(1,i)=24;
        end
    end

end



dCh2_F = diff(Ch2_F);   %compute the first derivative of the pressure
signal
dCh2_F(length(Ch2_F)) = 0;  %add a value of zero to the end to keep the
array length the same
dCh2_F = medfilt1(dCh2_F,n);    %filter the derivative result to remove
noise

figure(9)   %create a new figure for plotting the derivative
plot(ElapsedTime2,dCh2_F); %plot the derivative of the pressure signal
versus elapsed time

locs2i = locs2*4;   %create an integer array of the peak locations
(rather than in time)

start2 = zeros(size(locs2i));    %create an array to store the time at
the beeginning of a contraction surrounding each peak
stop2 = zeros(size(locs2i));     %create an array to store the time at
the end of a contraction surrounding each peak
```

```
for i = 1:length(locs2i) %for each peak, find the beginning and end by
searching from the peak location until the derivative of pressure falls
below the threshold
    j = locs2i(i)-peak_offset;
    if j < 0
         j=1;
    end
    while(j>1)
       j=j-1;
       if dCh2_F(j) < start_slope_threshold
          break
       end
    end
    start2(i) = j/4;
    j = locs2i(i)+peak_offset;
    while(j<length(ElapsedTime2))
       j=j+1;
       if dCh2_F(j) > -stop_slope_threshold
          break
       end
    end
    stop2(i) = j/4;
end

duration2 = stop2-start2;  %calculate the duration of each contraction

figure(2)
hold on
plot(locs2,pks2,'kD',start2,Ch2_F(start2*4),'gO',stop2,Ch2_F(stop2*4),'
rX');    %add markers showing peak, start, and stop locations for each
contraction
hold off

for i = 1:length(locs2)
    for j = 1:24
        if ((j-1)*3600) <= locs2(1,i) & locs2(1,i) < (j*3600)
            hour2(1,i)=j;
        elseif locs2(1,i) > 86400;
            hour2(1,i)=24;
        end
    end

end

dCh3_F = diff(Ch3_F);   %compute the first derivative of the pressure
signal
dCh3_F(length(Ch3_F)) = 0;  %add a value of zero to the end to keep the
array length the same
dCh3_F = medfilt1(dCh3_F,n);    %filter the derivative result to remove
noise

figure(11)   %create a new figure for plotting the derivative
```

```
plot(ElapsedTime3,dCh3_F); %plot the derivative of the pressure signal
versus elapsed time

locs3i = locs3*4;   %create an integer array of the peak locations
(rather than in time)

start3 = zeros(size(locs3i));    %create an array to store the time at
the beeginning of a contraction surrounding each peak
stop3 = zeros(size(locs3i));     %create an array to store the time at
the end of a contraction surrounding each peak

for i = 1:length(locs3i) %for each peak, find the beginning and end by
searching from the peak location until the derivative of pressure falls
below the threshold
    j = locs3i(i)-peak_offset;
    if j < 0
         j=1;
    end
    while(j>1)
       j=j-1;
       if dCh3_F(j) < start_slope_threshold
          break
       end
    end
    start3(i) = j/4;
    j = locs3i(i)+peak_offset;
    while(j<length(ElapsedTime3))
       j=j+1;
       if dCh3_F(j) > -stop_slope_threshold
          break
       end
    end
    stop3(i) = j/4;
end

duration3 = stop3-start3;  %calculate the duration of each contraction

figure(3)
hold on
plot(locs3,pks3,'kD',start3,Ch3_F(start3*4),'gO',stop3,Ch3_F(stop3*4),'
rX');    %add markers showing peak, start, and stop locations for each
contraction
hold off

for i = 1:length(locs3)
    for j = 1:24
        if ((j-1)*3600) <= locs3(1,i) & locs3(1,i) < (j*3600)
            hour3(1,i)=j;
        elseif locs3(1,i) > 86400;
            hour3(1,i)=24;
        end
    end

end
```

```
%%Compile analysis results and export as an excel spreadsheet
N1 = length(pks1);
N2 = length(pks2);
N3 = length(pks3);

% Assigns animal numbers to LabChart channels
anim1 = input('What animal used channel #1?  ');
anim2 = input('What animal used channel #2?  ');
anim3 = input('What animal used Channel #3?  ');

DATA_OUT = cell.empty();
DATA_OUT(1,:) = {'Channel 1',anim1,'','','','','Channel
2',anim2,'','','','','Channel 3',anim3,'','','',''};
DATA_OUT(2,:) = {'Peak Num','Peak
Pressure','Amplitude','Width(FWHM)','Duration', 'Hour','Peak Num','Peak
Pressure','Amplitude','Width(FWHM)','Duration', 'Hour','Peak Num','Peak
Pressure','Amplitude','Width(FWHM)','Duration', 'Hour'};


    DATA_OUT(3:N1+2,1) = num2cell(1:length(pks1),1);
    DATA_OUT(3:N1+2,2) = num2cell(pks1(1:length(pks1),1));
    DATA_OUT(3:N1+2,3) = num2cell(amp1(1:length(amp1),1));
    DATA_OUT(3:N1+2,4) = num2cell(halfwidth1(1,1:length(halfwidth1)));
    DATA_OUT(3:N1+2,5) = num2cell(duration1(1,1:length(duration1)));
    DATA_OUT(3:N1+2,6) = num2cell(hour1(1,1:length(hour1)));

    DATA_OUT(3:N2+2,7) = num2cell(1:length(pks2),1);
    DATA_OUT(3:N2+2,8) = num2cell(pks2(1:length(pks2),1));
    DATA_OUT(3:N2+2,9) = num2cell(amp2(1:length(amp2),1));
    DATA_OUT(3:N2+2,10) = num2cell(halfwidth2(1,1:length(halfwidth2)));
    DATA_OUT(3:N2+2,11) = num2cell(duration2(1,1:length(duration2)));
    DATA_OUT(3:N2+2,12) = num2cell(hour2(1,1:length(hour2)));

    DATA_OUT(3:N3+2,13) = num2cell(1:length(pks3),1);
    DATA_OUT(3:N3+2,14) = num2cell(pks3(1:length(pks3),1));
    DATA_OUT(3:N3+2,15) = num2cell(amp3(1:length(amp3),1));
    DATA_OUT(3:N3+2,16) = num2cell(halfwidth3(1,1:length(halfwidth3)));
    DATA_OUT(3:N3+2,17) = num2cell(duration3(1,1:length(duration3)));
    DATA_OUT(3:N3+2,18) = num2cell(hour3(1,1:length(hour3)));

[~,~,temp] = xlsread('MotilityResults.xlsx');
if ~isempty(temp)
    xlswrite('MotilityResults.xlsx',zeros(size(temp))*nan);
end
xlswrite('MotilityResults.xlsx',DATA_OUT);
```

Appendix D: Rumen score results after switching animals from a 70% to 90% concentrate diet

Immediately upon evisceration post-slaughter on d 5, visceral organs were transported to the animal lab in W.P. Garrigus Building for processing. The reticulorumen was separated from the rest of the digestive tract and emptied of digestive contents. Rumen mucosa was inspected for gross pathological lesions and evaluated using the following scoring system as outlined by Rezac et al. (2014): normal (epithelium appears healthy with thick, lush papillae and no signs of inflammation), mild (sections of the ruminal mucosal surface with short [relative to normal] or denuded papillae), and severe (active rumenitis lesions: ulcerations characterized by irregularly circular, depressed, red spots or healed ulcerations characterized by puckered scars devoid of papillae). Two scientists scored each rumen to ensure proper characterization.

Table D-1. Rumen scores and characteristics

Animal	Block	Score	Notes
B77	1	Normal[1]	high keritanization
B97	1	Mild[2]	small burn spot; one potential scar
B112	1	Normal	some keritanization
B104	2	Mild	keritanization, burn spot, grey color
B114	2	Mild	very little keritanization, large burn spot, black color
B114-2	2	Mild	shortened papillae, burn spot, no keritanization visible, black color
B13	3	Was not notified when animals were sent to the meat's lab	
B109	3		

[1] Normal: epithelium appears healthy with thick, lush papillae and no signs of inflammation
[2] Mild: sections of the ruminal mucosal surface with short (relative to normal) or denuded papillae

Appendix E: Effect of dietary citrulline on beef cattle performance and carcass characteristics through the transition period and finishing

Introduction

Transitioning cattle to high-grain, finishing diets increases the risk for ruminal acidosis (Bevans et al., 2005; Brown et al., 2006) and associated loss of epithelial barrier function (Penner et al., 2011; Steele et al., 2011a; Zebeli and Metzler-Zebeli, 2012). Damage to the gut epithelia during this time can lead to translocation of toxic metabolites, such as lipopolysaccharide, resulting in local (Zhang et al., 2016) and systemic (Gozho et al., 2005; Gozho et al., 2006) inflammation (Horadagoda et al., 1999; Zebeli et al., 2012). Feeding beef cattle backgrounding and finishing diets led to inflammation, as evidenced by increases in plasma acute phase proteins (Ametaj et al., 2009). In addition, inflammatory responses stimulated by lipopolysaccharide have been shown to alter feeding behavior (Zebeli and Metzler-Zebeli, 2012) and reduce DMI in dairy cows (Waldron et al., 2003). The transition phase when feedlot cattle are switched to high-grain diets appears to be a critical period where supporting immune function could be beneficial to cattle growth and performance.

Arginine is a vital component of inflammation and immune response, as well as for several metabolic pathways (Satriano, 2004; Wu, 2013). During the early phase response to an inflammatory insult, arginine is converted to nitric oxide (NO) by inducible nitric oxide synthase (iNOS). Nitric oxide can have cytotoxic antimicrobial activity towards some pathogens and thereby, provide some protection to mammalian tissues (De Groote and Fang, 1995). Arginine is also a precursor for the synthesis of

polyamines, proline, and agmatine, which serve as key immune modulators (Zhao et al., 2018). The pro-proliferative effects of polyamines and role of proline in the extracellular matrix make them key components of the repair phase of the inflammatory response (Satriano, 2004). Agmatine helps regulate the generation of NO and intracellular polyamines levels (Satriano, 2004; Zhao et al., 2018). Arginine supplementation may be beneficial during situations that would cause an inflammatory response. During inflammatory conditions, arginine consumption is increased but de novo and exogenous supplies are reduced due to use for various metabolic and immune functions (Luiking et al., 2009; Wijnands et al., 2015), thus creating an arginine deficiency (Zhao et al., 2018). However, exogenous L-arginine administration reduced the production of pro-inflammatory cytokines in rats (Mohamed et al., 2015). Supplementation or intravenous infusion of arginine to weaned pigs (Zhu et al., 2013), broiler chickens (Tan et al., 2014), fish (Jiang et al., 2015), and mice (Calkins et al., 2001) has been shown to have positive effects on the immune response. In addition, jugular arginine infusion to dairy cows prevented the decrease in DMI induced by LPS administration (Zhao et al., 2018). Therefore, increased arginine available during conditions which may trigger an inflammatory response, such as during transitioning to a high-grain diet, could help improve cattle performance.

Originally, all free amino acids were believed to be extensively degraded in the rumen by microbes. Recently, however, researchers discovered that citrulline can act as a rumen-protected arginine supplement. When adult steers were fed an L-citrulline supplement, L-citrulline concentrations in rumen fluid did not change within 4 h post-feeding, and an in vitro experiment demonstrated little degradation of L-citrulline by

rumen microbes within 4 h of incubation (Gilbreath et al., 2017). Citrulline can be converted to arginine in virtually all cell types, including enterocytes, adipocytes, endothelial cells, macrophages, monocytes, and neurons (Wu and Morris, 1998). Oral administration of citrulline to sheep increased plasma citrulline and arginine levels (Gilbreath et al., 2018). Thus, citrulline should be able to escape the rumen and be available to extrahepatic tissues for biosynthesis of arginine.

The objectives of this experiment were to determine if citrulline supplemented in the diet of beef cattle would increase blood arginine levels, evaluate the effects of citrulline supplementation on blood cytokine concentrations, and compare performance of cattle who were supplemented with citrulline to those given monensin and tylosin. We hypothesized that feeding cattle citrulline through the dietary transition to a high-grain diet would improve performance; we expected cattle supplemented with citrulline would have greater blood arginine concentrations and lower cytokine concentrations compared to those not supplemented with citrulline and that monensin and tylosin supplementation would impact these responses.

Materials and Methods

Animals and Experimental Design

The experiment was conducted as a randomized complete block design with a 2 x 2 factorial arrangement of treatments. One hundred twenty Angus-crossbred steers, from the University of Kentucky beef herd (n=48; BW=307.3 ± 0.5 kg) and purchased (n=72; BW= 373.7 ± 0.5 kg), were removed from pasture (E+ and non-toxic treatments) and held in holding pens overnight before weighing to obtain a shrunk weight on d -6. Animals were blocked by source, previous endophyte treatment, and then shrunk weight

to randomly allocate animals to pens in 2 barns at the facility. Pens were blocked across barn and location within barn. Then, treatments were randomly assigned to pens. All animals received a corn silage based diet for 7 d (Exp. D -6 to 0). Prior to beginning the study, animals were weighed on 2 consecutive days (Exp. D -1 and 0). Pens received one of 4 treatments: 1) control (CTRL; no monensin or tylosin, no citrulline), 2) monensin + tylosin, no citrulline (MT), 3) no monensin or tylosin with 45 g citrulline/hd/d (CIT), or 4) monensin + tylosin with 45 g citrulline/hd/d (MT+CIT). Animals were housed 3 animals per pen, 10 pens per treatment in an exterior, partially-covered barn at the University of Kentucky C. Oran Little Research Center in Versailles, KY. Pens were concrete floored and the third closest to the bunk was cleaned and bedded weekly with sawdust.

All animals underwent a step-up transition protocol to a finishing ration over 35 d. Four transition diets (T1-T4) were utilized, each being fed for 7 days. Two supplements were used, one with monensin + tylosin and one without monensin + tylosin. Supplements were mixed into rations at 10% of each diet DM. Pens receiving CIT and MT+CIT had their feed top-dressed with 330 g of a cracked corn and L-citrulline mixture to provide approximately 45 g citrulline/hd/d. Top-dress mixture was 43.775% L-citrulline (DM basis) or 40.9% (as-fed basis). Pens receiving CTRL and MT had their feed top-dressed with 330 g of a cracked corn and urea mixture to provide an equivalent amount of N. Mixture was 22.647% urea (DM basis) or 65.8% (as-fed basis). Pens were fed *ad libitum* once daily. Bunk calls were made daily to adjust feed amounts and maintain *ad libitum* intake. Feed samples were collected weekly for determination of diet DM. Orts were weighed weekly, composited by treatment, and a subsample was saved

for DM analysis to calculate daily DMI. Following the 28 d step-up transition, all animals were fed a finishing ration (FIN) containing treatments for 7 days and then weighed on D 35. Then, all animals received a common diet, without citrulline but containing monensin and tylosin, until reaching finishing weight. Cattle were weighed every 28, 21, or 14 d after D 35 until they reached finishing weight.

Blood Sampling and Analysis

Jugular vein blood samples from each animal were collected into plasma (10 mL; BD Vacutainer EDTA tubes) and serum (10 mL; BD Vacutainer serum blood collection tubes) tubes immediately before the step-up (D 0) and the last day of the treatments (D 35). Plasma tubes were placed on ice until transport to the laboratory where they were centrifuged at 1500 x g for 10 min at 4°C. Serum tubes were allowed to clot for approximately 60 min and then centrifuged at 1500 x g for 10 min at 4°C. Supernatant from serum and plasma tubes was frozen in 2 mL aliquots and stored at -4°C until analysis.

We had intended to analyze plasma for arginine, citrulline, and serum amyloid A (SAA), as well as analyze serum for haptoglobin.

Harvest

Animals were shipped in 4 truck loads on 3 dates (December 18th, January 8th, and January 22nd) to a commercial slaughter facility in Illinois. Carcass data were obtained for each animal from the facility. Final body weight was determined by multiplying the farm live weight on the day of shipping by 0.97 (pencil shrink). This final body weight was used to determine dressing yield based on hot carcass weights. Livers were scored by a trained professional according to the following system: A- = 1-2 small abscesses or

abscess scars present; A = 2-4 well-organized abscesses present, generally under 1 inch in diameter; A+ = 1 or more large active abscesses present along with inflammation of liver tissue surrounding the abscess (Brown et al., 1975).

Statistical Analysis

Performance data were analyzed using proc MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) as a randomized complete block design with repeated measures for the fixed effects of MT, CIT, and the interaction, with block considered a random variables. The repeated variable was day and an autoregressive covariance structure was used. The experimental unit was pen with animal as a replicate within pen. Carcass data were analyzed using proc MIXED of SAS as a randomized complete block design for the fixed effects of MT, CIT, and the interaction, with block as a random variable.

Results

Two cattle were removed from the study and not included in analysis due to lameness issues. Citrulline treated cattle had lower DMI from D0-D140 and the entire experiment (transition and finishing; D0-D154) and tended to have lower DMI from D0-D91 and D0-D119 than those not given citrulline (Table E-3). Average daily gain for citrulline treated cattle was lower from D0-D91 and tended to be lower D0-D63 and D0-D119 compared to those not given citrulline (Table E-4). Steers given monensin + tylosin were consumed less DM (Table E-3) during the transition period (D0-D35) and had greater average daily gains from D0-D63 and D0-D154, which was associated with greater feed efficiency (lower feed:gain) in the middle of the finishing period (Table E-4). Cattle treated with monensin + tylosin during the transition period had greater final body weights, hot carcass weights, and ribeye area (Table E-5). Dressing yield displayed an

interaction of MT and CIT, where dressing yield was greater in MT steers compared to CTRL steers (Table E-5). Citrulline-treated cattle tended to have a greater percentage of cattle grade USDA choice, but this appeared to be related to a numerical reduction in percent grading USDA prime (Table E-5).

Conclusions

- Citrulline supplementation at a dose of approximately 45 g/hd/d during a 35-d transition period to a high-grain diet did not appear to be beneficial to beef cattle performance or carcass characteristics.

Tables and Figures

Table E-1. Composition of diets and ingredients

Diet Ingredient	%, DM basis				
	T1[a]	T2[b]	T3[c]	T4[d]	FIN[e]
Corn silage	70	50	35	25	10
Dried distiller grains	10	20	25	25	25
Cracked corn	10	20	30	40	27.5
High-moisture corn	-	-	-	-	27.5
Treatment supplement[1]	10	10	10	10	10

[a] T1: transition diet #1
[b] T2: transition diet #2
[c] T3: transition diet #3
[d] T4: transition diet #4
[e] FIN: finishing diet
[1] See Table E-2 for composition of treatment supplements

Table E-2. Composition of supplements for inclusion or exclusion of monensin and tylosin from the diet

Ingredient	%, DM basis	
	No MT[1]	MT[2]
Ground corn	69.49	69.236
Vitamin A, D, & E premix[3]	0.219	0.219
Trace mineral premix[4]	5.006	5.004
Limestone	19.213	19.207
Choice white grease	2.505	2.504
Urea	3.568	3.567
Rumensin-90	-	0.175
Tylan-40	-	0.088

[1] No MT = supplement used for treatments where no monensin + tylsoin is provided in diet (CTRL & CIT)
[2] MT = supplement used for treatments where monensin + tylosin are provided in the diet (MT & MT+CIT)
[3] Composed of vitamin A acetate (1,814,368 IU/kg), D-activated animal sterol (source of vitamin D3; 362,874 IU/kg), vitamin E supplement (227 IU/kg), roughage products, calcium carbonate, and mineral oil.
[4] Contained 56.34% Cl, 36.53% Na, 1.2% S, 0.06% Ca, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, and 5520.2 ppm Zn on a DM basis.

Table E-3. Weights and dry matter intakes of beef steers that were given supplements containing or not containing citrulline (CIT) as well as monensin and tylosin (MT) during the transition period (D0-D35).

Item	Treatment				SEM	*P*-values		
	No MT		MT			MT	CIT	MT*CIT
	CTRL (No CIT)	CIT	MT (No CIT)	MT+CIT				
Weight, kg								
D0	344.31	346.56	348.53	349.56	20.25	0.3296	0.6559	0.8682
D35	403.83	407.54	406.94	405.84	22.83	0.8863	0.7914	0.6264
D63	469.72	468.95	485.14	476.61	21.46	0.0257	0.3527	0.4371
D91	548.41	542.84	561.23	550.92	20.2	0.0756	0.1726	0.6799
D119	612.81	573.34	595.22	597.17	35.16	0.8749	0.3469	0.2998
D140	621.51	575.26	595.66	595.68	35.89	0.9254	0.4285	0.4283
D154	619.67	554.9	590.28	585	42.2	0.9933	0.4177	0.4903
Dry Matter Intake, kg/hd/d								
D0-D35 (during treatments)	9.607	9.513	9.113	8.781	0.51	0.0456	0.475	0.689
D0-D63	10.326	10.225	10.268	9.805	0.42	0.33	0.2518	0.4593
D0-D91	11.214	11.004	11.289	10.716	0.35	0.6262	0.08	0.4082
D0-D119	11.661	11.343	11.632	11.182	0.3	0.6316	0.0591	0.7388
D0-D140	11.61	11.22	11.684	11.066	0.2	0.8392	0.0161	0.5652
D0-D154	11.667	11.183	11.977	11.017	0.23	0.7545	0.005	0.3049

Table E-4. Average daily gains and feed efficiency of beef steers throughout the finishing period that were given supplements containing or not containing citrulline (CIT) as well as monensin and tylosin (MT) during the transition period (D0-D35).

Item	Treatment				SEM	*P*-values		
	No MT		MT			MT	CIT	MT*CIT
	CTRL (No CIT)	CIT	MT (No CIT)	MT+CIT				
Average Daily Gain, kg/d								
D0-D35 (during treatments)	1.679	1.745	1.652	1.608	0.133	0.3435	0.8982	0.5236
D0-D63	1.984	1.943	2.164	2.017	0.055	0.0169	0.0714	0.3009
D0-D91	2.241	2.157	2.332	2.213	0.044	0.1036	0.0273	0.6926
D0-D119	2.252	2.165	2.277	2.217	0.046	0.3125	0.0589	0.7213
D0-D140	2.124	2.078	2.183	2.123	0.064	0.1623	0.1529	0.8387
D0-D154	2.073	1.977	2.142	2.127	0.1	0.0464	0.2883	0.434
Feed:Gain								
D0-D35 (during treatments)	5.827	5.498	5.628	5.666	0.28	0.9458	0.5248	0.4234
D0-D63	5.199	5.278	4.769	4.864	0.16	0.0008	0.4531	0.9447
D0-D91	5.008	5.119	4.863	4.836	0.16	0.0366	0.6714	0.4869
D0-D119	5.178	5.257	5.126	5.044	0.13	0.2079	0.9885	0.4406
D0-D140	5.483	5.426	5.369	5.234	0.17	0.1484	0.3606	0.7046
D0-D154	5.648	5.737	5.62	5.19	0.26	0.0951	0.309	0.1296

Table E-5. Carcass characteristics after finishing for beef steers that were given supplements containing or not containing citrulline (CIT) as well as monensin and tylosin (MT) during the transition period (D0-D35).

Item	Treatment				SEM	*P*-values		
	No MT		MT					
	CTRL (No CIT)	CIT	MT (No CIT)	MT+CIT		MT	CIT	MT*CIT
Final BW, kg	630	621.8	638	635.8	9.8	0.046	0.3351	0.572
Hot carcass weight, kg	388.56	387.63	397.98	394.16	4.83	0.019	0.4666	0.6578
Dressing yield, %	61.69[b]	62.36[ab]	62.42[a]	62.02[ab]	0.36	0.441	0.5847	0.0353
USDA yield grade	3.23	3.17	3.08	3.17	0.21	0.5102	0.9477	0.5158
Yield grade 5, %	< 1	3.33	3.33	3.33	2.96	0.5595	0.5595	0.5595
Yield grade 4, %	36.67	30	21.67	30	10.42	0.2719	0.9021	0.2716
Yield grade 3, %	50	46.66	55	46.67	8.11	0.7599	0.4773	0.7601
Yield grade 2, %	13.33	20	20	20	10.57	0.6494	0.6494	0.6494
Ribeye area, cm^2	83.16	84.06	86.64	84.97	2.13	0.034	0.6982	0.2062
Marbling[1]	536.45	510.33	527.91	523.25	17.52	0.9015	0.3864	0.5447
12^{th} rib backfat thickness, cm	1.61	1.54	1.59	1.58	0.12	0.8938	0.6174	0.7389
KPH[2], %	1.97	1.96	1.94	1.92	0.04	0.2807	0.5817	0.8622
USDA Prime, %	28.33	16.67	23.33	13.33	7.25	0.5695	0.1448	0.9091
USDA Choice, %	71.67	83.34	70	86.67	7.82	0.9159	0.0795	0.7514
USDA Select, %	0	0	6.67	0	2.22	0.1434	0.1434	0.1434
Liver Score 0[3], %	86.67	83.33	83.33	90	6.27	0.7717	0.7719	0.3867
Liver Score A-[3], %	13.33	16.67	16.67	10	6.27	0.7717	0.7719	0.3867

[1] Marbling score 600-699 = moderate

[2] KPH = kidney, pelvic, and heart fat was calculated, not an actual measurement

[3] Liver score was based on the following system: 0 = no abscesses; A- = 1-2 small abscesses or abscess scars

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PEER-REVIEWED PUBLICATIONS:

Journal Articles

Egert-Mclean, A. M., M. P. Sama, J. L. Klotz, K. R. McLeod, N. B. Kristensen, and D. L. Harmon. 2018. Automated system for characterizing short-term feeding behavior and real-time forestomach motility in cattle. (Submitted to Computers and Electronics in Agriculture).

Klotz, J. L., G. E. Aiken, **A. M. Egert-McLean**, F. N. Schrick, N. Chattopadhyay, and D. L. Harmon. 2018. Effects of grazing different ergovaline concentrations on vasoactivity of bovine lateral saphenous vein. J. Anim. Sci. 96:3022-3030. DOI: 10.1093/jas/sky163

Egert, A. M., D. Kim, F. N. Schrick, D. L. Harmon, and J. L. Klotz. 2014. Dietary exposure to ergot alkaloids decreases contractility of bovine mesenteric vasculature. J. Anim. Sci. 92:1768-1799.

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Abstracts

Egert-McLean, A. M. and D. L. Harmon. 2018. Characterization of feeding behavior, ruminal motility, and rumen environment of beef heifers during a moderate transition to a 90% concentrate diet. J. Anim. Sci. 96 (E-Suppl. 2) Accepted.

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