DEFINING THE RISK, PREVALENCE, AND PATHOLOGICAL THRESHOLD OF LOW RUMINAL pH IN FEEDLOT CATTLE

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ABSTRACT

The diet transition phase is thought to be the highest risk period for development of low ruminal pH, while pathology associated with low reticulo-ruminal pH (**RRpH**) induced ruminal acidosis (**RA**) is often found at slaughter, months after the diet transition. Two experiments were conducted to 1) determine the risk of low RRpH during the transition phase and 2) explore the association of rumen fermentation and acute phase protein response during finishing with pathology identified post mortem. In experiment 1, RRpH was measured in 32 mixed breed steers (n = 16) and heifers (n = 16) housed in commercial feedlot pens with 227 ±13 and 249 ± 6 hd/pen cohort steers and heifers, respectively. Cattle were transitioned from a diet containing 46.5% forage and 53.5% concentrate to a diet containing 9.5% forage and 90.5% concentrate dry matter (DM) basis) over 40 d. In addition, wheat replaced barley as the grain source during the dietary transition. Both mean and minimum RRpH decreased as the proportion of concentrate in the diet increased. The area (duration × severity) that RRpH was < 5.6, duration that RRpH was <5.6, and the number of cattle experiencing a bout of low RRpH (pH < 5.6 for > 180 min), increased with increasing concentrate. Despite having a high risk for low RRpH, most cattle had only 1-3 bouts of low RRpH during the diet transition, and extent was mild. Steers had greater dry matter intake (DMI), lower RRpH, and greater standard deviation of RRpH than heifers, suggesting that susceptibility to RA may differ between steers and heifers. In experiment 2, ruminal pH, short-chain fatty acid concentrations and serum acute phase proteins were measured in 28 cannulated steers during the final 5 wk of finishing when fed a diet containing 5% forage and 95% concentrate (DM basis). Rumen and livers were examined and pathology scores were determined at slaughter. There was no difference in minimum pH, mean pH, or duration that ruminal pH was < 5.5 between steers with or without pathology. However, steers with pathology spent more time with ruminal pH < 5.2 and tended to spend more time with ruminal pH < 5.8. Acetate concentration tended to be greater in steers with pathology than without pathology. Serum amyloid A was greater and haptoglobin tended to be greater in steers with pathology than those without. Overall, liver and rumen pathology was associated with a greater duration that ruminal pH is < 5.2 and a chronic systemic acute phase protein response. In summary, feedlot cattle experience low RRpH during dietary transition and that the risk increases with increasing levels of concentrate. However, during the dietary transition the extent of low RRpH was mild. During the last 5 wk of finishing, the duration that ruminal pH was < 5.2 and the plasma concentration of serum amyloid A, were

associated with greater rumen and liver pathology scores, suggesting that low ruminal pH occurring during the latter part of finishing may have an impact on risk for rumenitis and liver abscesses.

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"Education is the kindling of a flame, not the filling of a vessel" - Socrates

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
d	Day
DE	Digestible Energy
DMI	Dry matter intake
DOF	Days on feed
F:C	Forage to concentrate ratio
G:F	Gain to feed ratio
h	Hour
Hp	Haptoglobin
Hb	Hemoglobin
LBP	Lipopolysaccharide binding protein
LPS	Lipopolysaccharide
$NaHCO_3$	Sodium Bicarbonate
NDF	Neutral detergent fiber
MGO	Magnesium oxide
P3	Distal Phalange, coffin bone
PAP	Polyclonal antibody
peNDF	Physically effective Neutral detergent fiber
SAA	Serum amyloid A
SARA	Subacute ruminal acidosis
Se	Sensitivity
SB	Sodium bicarbonate
SCFA	Short chain fatty acid
TCJ	Tight cell junction
VM	Vancomycin
wk	Week

1.0 GENERAL INTRODUCTION

In an effort to improve gain-to-feed (**G:F**) and production economics, North American feedlot cattle are generally transitioned from primarily forage based diets to highly fermentable (finishing) diets containing upwards of 85% concentrate (Vasconcelos and Galyean, 2007). These highly fermentable diets lead to increased organic acid production by rumen microbes, which, although beneficial energetically, can lead to conditions of low ruminal pH when the rate of production exceeds the rate of acid removal from the rumen (Penner et al., 2009a). This condition, known as ruminal acidosis occurs as a continuum, ranging from acute (pH < 5.2) to subacute (SARA, pH 5.2 to 5.6). As such, symptoms range from clinical illness to mild reductions in performance (Golder et al., 2014; Castillo-Lopez et al., 2014). Ruminal acidosis is also associated with rumenitis (Steele et al., 2009), liver abscesses (Nagaraja and Lechtenberg. 2007), and laminitis (Noeck et al., 1997). Unfortunately, clinical signs are often non-specific, and pathology is not detected until after slaughter, making diagnosis and treatment of ruminal acidosis difficult in commercial settings. For this reason, much work has been conducted to identify the risk factors and to develop management strategies to prevent ruminal acidosis from occurring. Traditionally, the diet transition period was thought to be a high-risk period for ruminal acidosis and, as such, management strategies have been developed and adopted including a common gradual dietary step-up approach (Samuelson et al., 2016). Despite this, recent reports of rumen and liver pathology in North American cattle at slaughter (Rezac et al., 2014) suggest that ruminal acidosis is not completely mitigated by current management practices. Moreover, a recent study completed in a small-scale research facility reported the finishing period to have greater risk for ruminal acidosis than the diet transition period (Castillo-Lopez et al., 2014). Due to the performance and animal welfare implications of ruminal acidosis, it is important to identify and manage the greatest risk periods, as well as to define the relationship between ruminal pH and the health and performance of cattle. Therefore, the aim of this thesis was to 1) determine the prevalence of ruminal acidosis during dietary transition in a large scale commercial setting and 2) relate rumen and liver pathology observed at slaughter to ruminal pH, ruminal short-chain fatty acids, and systemic acute phase proteins in cattle observed during the finishing period.

2.0 LITERATURE REVIEW

2.1 The Ruminal Environment and General Characteristics of Ruminants

The ability of ruminants to subsist solely on forage emphasizes the importance of the populations of bacteria, protozoa, and fungi living within the anaerobic environment of the reticulo-rumen. In a symbiotic relationship, the ruminal microbes utilize the ingested feed for growth and reproduction, producing short chain fatty acids (**SCFA**) as byproducts. The SCFA are then absorbed from the rumen into portal circulation and used as an energy source for the ruminant. The microbes themselves also provide a valuable source of vitamins and essential amino acids for the ruminant, as they flow out of the rumen and are digested in the small intestine (Sniffen and Robinson, 1987).

An important outcome of microbial fermentation in the reticulo-rumen is the release of short-chain fatty acids. Short-chain fatty acids serve as the primary energy source for ruminants with the dominant SCFA, in terms of relative abundance, being acetate, propionate, and butyrate. In dairy cattle on a low roughage diet, Sutton et al. (2003) reported net production rates of 49.0, 36.2 and 4.8 mol/d of acetic, propionic and butyric acid, respectively. It has been suggested that 30% of the acetate, 55% of the propionate, and 90 % of the butyrate are utilized by gut tissue (Britton and Krehbiel, 1993). Butyrate is extensively catabolized by the rumen epithelium with much of the energy released being used by the reticulo-ruminal epithelium via β-oxidation (Krehbiel et al., 1992) or released as β-hydroxybutyrate. In contrast, acetate is largely released by the reticulo-ruminal epithelium with recovery estimates ranging from 45 % (Britton and Krehbiel, 1993) to over 100% (Kristensen and Harmon, 2004). Acetate is used as a source of energy by visceral tissues, the mammary gland, and adipose tissue for fatty acid synthesis. While both acetate and butyrate are lipogenic, propionate is an important gluconeogenic substrate. In ruminants, hepatic uptake of propionate has been shown to change depending on physiological state, but ranges from 60 to 74% (Yost et al., 1977; Aschenbach et al., 2010). The features of anaerobic fermentation and the use of SCFA as an energy source and glucose precursor are distinguishing features for ruminants.

In conventional dairy and beef production systems in North America, cattle are often fed highly fermentable diets to meet demands for growth and lactation. Such diets have been reported to alter the microbial community structure (Petri et al., 2013) favoring microbes with amylolytic

(starch digesting) activity. Part of the response for a shift in the microbial community structure is likely related to altered substrate availability; increased starch at the expense of structural carbohydrates. When reticulo-ruminal pH < 6.0 (Russel and Wilson, 1996), the growth and reproduction of the cellulolytic (fiber digesting) bacteria is limited as these bacteria are less able to regulate their intracellular pH when exposed to low pH environments. Consequently, the digestibility of NDF and ADF decreases (Brink and Steele, 1985). Concurrent with diets with a high fermentability is the proliferation of acid-tolerant amylolytic bacteria and lactate utilizing bacteria. The change in the microbial structure leads to an increase in propionate, butyrate, and the overall SCFA concentration which, although beneficial energetically, has a depressive effect on reticulo-ruminal pH and can increase the risk for ruminal acidosis.

2.2 Acute and Subacute Ruminal Acidosis

When the production of SCFA and, in some cases, lactate overwhelms the rate of removal, RRpH decreases. Acute ruminal acidosis is a severe digestive disorder that is defined to occur when ruminal pH is < 5.0 (Nagaraja and Lechtenberg, 2007). With acute ruminal acidosis, the osmolality of the reticulo-ruminal contents increases above blood osmolality of 286 to 310 mOsm/L (Owens et al., 1998), which can lead to rumen epithelial damage, dehydration, hypovolemia, and in severe cases, death. These cases typically occur sporadically, with prevalence reported to be 0.3% in dairy herds and likely account for 3 to 7% of all feedlot morbidities (Beauchemin and McAllister, 2006). Although not well documented, acute acidosis may be linked to an inciting cause such as over mixing, improper feed delivery, or grain engorgement (Garry and McConnel, 2009). It should be noted that acute acidosis is not exclusive to feedlot cattle and recent cases have been reported for cattle in extended whole-plant corn grazing systems (Jose, 2015). While severe in nature, if caught early, treatment can be successful and may include rumen lavage, transfaunation, and other supportive care such as intravenous fluid and antibiotic administration.

Subacute ruminal acidosis (**SARA**), on the other hand, is more insidious in nature. Traditionally, it has been described to occur when ruminal pH is between 5.2 and 5.8; however, the use of indwelling pH measurement devices have allowed researchers to refine the definition of SARA and more accurately determine prevalence and severity (Penner et al., 2007; Penner et al., 2009). As such, SARA is now more commonly reported as duration (of time spent below a pH threshold), frequency of bouts (a bout equating to ≥ 180 minutes below a pH value), or as area (a

function of severity and duration) below a threshold pH value. Unfortunately, there is still debate within the literature over the correct pH threshold, with some groups using a pH of 5.5 (Castillo-Lopez et al., 2014), pH 5.6 (Bevans et al., 2005; Gozho et al., 2005) and pH 5.8 (Dohme et al., 2008) as a cut-off value for SARA. In dairy cattle a threshold of pH <5.6 for >180 minutes has been associated with an acute phase protein (**APP)** response, yet to date there has not been any studies investigating which SARA definition correlates best with clinical signs of SARA in beef cattle. It must also be considered that critical thresholds for reduced performance likely differ when comparing beef and dairy systems. For example, a reduction in fiber digestion due to SARA (Allen, 2000) is far more significant in a dairy ration than in a feedlot ration, where fiber inclusion is typically low (Samuelson et al., 2016) and it could be argued that fiber contributes very little to the DE of the diet. As such, the performance of feedlot cattle may be less sensitive to low ruminal pH than dairy cattle. In turn, the impact of SARA on the performance of feedlot cattle may be due to its sequelae; rumenitis and liver abscesses. Therefore, a different pH threshold for SARA may be warranted in each industry.

Difficulty in measuring ruminal pH prevents SARA from being diagnosed routinely in commercial settings. In non-cannulated cattle, ruminal pH measurement can be accomplished via rumenocentesis or stomach tube. When compared to in-dwelling pH measurements, Duffield et al. (2004) determined that rumenocentesis is more sensitive **(Se)** for determining SARA (Se = 87%), than the oro-ruminal probe technique (Se = 50%), likely because of location sampled. Studies using ruminally fistulated cattle have demonstrated that the pH of the rumen differs based on the location sampled, with ruminal pH being lower in the central rumen than cranial dorsal or ventral rumen (Duffield et al., 2004). Tajaj et al. (2004) also reported pH to be lowest just below the rumen mat and increased toward the ventral rumen wall. Falk et al. (2016) recently evaluated a commercial wireless telemetry bolus (eBolus, eCow Ltd, Exeter, Devon, UK) and found reticular pH to be 0.24 pH units higher than ruminal pH on average. However, this evaluation was performed in dairy cows on a high roughage diet, and there is evidence that this discrepancy is not so great when high concentrate rations are fed (Seymour et al., 2016). Despite ongoing research, there is a need to define accurate diagnostics that help diagnose SARA as a measure to avoid negative outcomes for the health and productivity of beef and dairy cattle.

2.3 Rumenitis and Barrier Function of the Rumen and Gastrointestinal Tract

From the most apical to basal orientation, the ruminal epithelium is composed of the protective stratum corneum and stratum granulosum as well as metabolically active stratum spinosum, and stratum basale (Graham and Simmons, 2005). The multiple strata of the rumen epithelium allow this organ to dynamically respond to changes in luminal environment, and as ruminal pH declines and osmolality increases gross signs of physical damage to the ruminal epithelium can be seen. Pathological findings such as hyperemia, parakeratosis (thickening of the stratum corneum), erosions (loss of mucosal epithelium, not extending to the submucosal layer) and ulcerations (loss of mucosal epithelium extending to the submucosa) have been described (Steele et al., 2011).

Unquestionably, rumen ulcerations result in a clear loss of barrier function, however, it is important to note that cellular changes also occur at a histological level. In a case study, Steele et al. (2009) reported that reductions in pH to levels < 5.0 led to sloughing of the stratum corneum and damage to cellular junctions between the corneum and granulosum. Downregulation of major tight cell junction proteins claudin 1 and 4, and occludin, which are found in the stratum granulosum and spinosum has been reported subsequent to ruminal conditions of pH 5.3 in goats (Liu et al., 2013). It has been suggested that the down regulation of these proteins is an important precursor to the tissue remodeling, which takes place in the face of transition from high forage to high grain diets (Dionissopoulos et al., 2012). This transient increase in membrane permeability leads to the acidosis-liver abscess complex, whereby impaired rumen epithelial barrier function allows gram negative bacteria (primarily *Fusobacterium necrophorum*) to colonize and form micro abscesses in the ruminal epithelium. Bacterial emboli from these small abscesses shower the liver through portal circulation and form larger abscesses in the parenchyma of the liver (Tadepalli et al., 2009).

Laminitis is also a known sequelae of ruminal acidosis (Nocek, 1997; Nordlund, 2004; Stone, 2004.) and is most often seen at the end of the feeding period. The exact link between SARA and laminitis is not known, but carbohydrate overload is often implicated. In fact, laminitis has been successfully induced with an oligofructose challenge (Thoefner et al., 2004). The current theory suggests that laminitis is caused by altered hemodynamics of digital microvasculature due to circulating endotoxins and histamine, both of which have been found to be elevated with acidosis (Noeck, 1997). Histamine is a potent venous vasodilator and arterial constrictor, resulting

in increased microvascular blood pressure in the lamina causing seepage of serum, damage to the lamina, and release of laminar attachments to the distal phalange (P3). Intraruminal histamine concentration due to ruminal epithelial irritation and degranulation of mast cells (Randhawa et al., 1988) has been linked to ruminal acidosis. Histamine and endotoxin translocation across the epithelium damaged by rumenitis may result in a large (Aschenbach and Gäbel, 2002), albeit transient, load of vasoactive molecules into portal circulation and, if not cleared by the liver, at the hoof microvasculature.

Ruminal LPS has been shown to be elevated under conditions of SARA in cattle (Gozho et al., 2005; Khafipour et al., 2009). Lipopolysaccharide binding protein **(LBP)** can be measured in the serum as an indicator of translocated LPS (Bannermann et al., 2003) and has been shown in dairy cattle to be elevated for cattle with SARA (Dionissopoulos et al., 2012; Marchesini et al., 2013). It has been proposed that translocation of LPS occurs across the rumen epithelium (Nocek et al., 1997; Kleen et al., 2003) because of greater permeability of the forestomach tissues to large molecules (Penner et al., 2014). However, it has also been suggested that this translocation may occur in more distal regions of the gastrointestinal tract (Li et al., 2012). McCartney et al. (2016) found cecal LPS to be elevated well above ruminal LPS levels in cattle experiencing SARA. It should be recognized that the distal GIT may be a potential primary or secondary site for LPS translocation. The effect of SARA on hind gut acidosis and systemic inflammation and immune response is a further area of research interest (Gressley et al., 2011).

2.4 SARA and Inflammation

A systemic inflammatory response in cattle is accompanied by elevated concentrations of positive acute phase proteins haptoglobin (**Hp**) and serum amyloid A (**SAA**) (Eckersal and Bell, 2010; Paulina and Tadeusz, 2011). The function of Hp is to bind free hemoglobin **(Hb),** thereby reducing availability of Hb to bacteria. It also functions to reduce oxidative damage and up-regulate anti-inflammatory mediators (Ceciliani et al., 2012). Serum amyloid A is thought to have three possible functions including: 1) the binding of cholesterol and removing it from inflammatory sites; 2) opsonization of gram negative bacteria; and 3) modulation of the immune response by attracting monocytes and neutrophils as part of the innate immune system (Ceciliani et al., 2012). Understanding these functions helps to explain why both SAA and Hp are elevated in cattle experiencing acute or subacute ruminal acidosis (Gozho et al., 2005; Danscher et al., 2011;

Zebeli et al., 2012), although some studies suggest the response can be variable (Cannizzo et al., 2012, Khafipour et al., 2009). In most cases, acute phase proteins have been shown to be elevated within hours of the pro-inflammatory stimulus and remain elevated while the stimulus persists. However, following cessation of the pro-inflammatory stimulus, acute phase protein concentrations decline rapidly (Tothova et al*.,* 2013). The reported half-life of the Hp in cattle once it has bound to Hb is only 20 to 40 min before clearance by kupfer cells in the liver. In humans, the half-life of SAA is only 90 min (Ceciliani et al., 2012) and it seems logical that a similar half-life would occur for cattle. The short half-life of SAA and Hp may help explain variability in the APP response reported by Cannizzo et al. (2012) and Khafipour et al. (2009).

Another inherent problem with using APP as indicators is that they are not specific to ruminal acidosis. Both Hp and SAA are elevated in other conditions such as metritis, mastitis, laminitis (Tothova et al., 2013), and bovine respiratory disease (Iodate et al., 2015; Wolfger et al., 2015). Also, ruminal acidosis has the potential to induce an inflammatory response, and as such it is possible that ruminal acidosis may also compromise the ability of the bovine immune system to prevent other disease.

2.5 Clinical Signs of SARA

Ante-mortem diagnosis of SARA is difficult as the clinical signs are generally non-specific and transient. The most common clinical signs are described in the following sub-sections.

2.5.1 Variation in dry matter intake

Variable intake is a well-documented sign of SARA (Fulton et al.,1979; Plaizier et al., 2008; Danscher et al., 2015) and there are many proposed theories to explain this. The theories cover altered osmolality of the rumen contents affecting rumen motility and outflow (Owens et al., 1998); hepatic oxidation of propionate and subsequent satiety signals (Oba and Allen, 2003); reduced fiber digestibility leading to changes in mechanical stretch receptors in the rumen (Allen, 2000); and inflammatory signals (Ingvartsen and Anderson, 2000). In beef cattle, reduced ADG and G:F have been reported in response to SARA (Castillo-Lopez et al., 2014), which may be related to the aforementioned variable intake patterns, or in response to the inflammation and pathologic sequelae of SARA. Since these production measures are calculated on a pen level, they are not a reliable way to detect SARA in individual animals.

2.5.2 Depressed milk fat

In dairy cattle, SARA causes milk fat depression (Danscher et al., 2015; Bauman and Griinari, 2003; Peterson et al., 2003). Milk fat percentage is often used as an indicator of rumen health (Zebeli et al., 2010). In fact, milk fat percentages as low as 2.93% have been reported (Khafipour et al., 2009) when cattle are experiencing SARA. This is caused by partial inhibition of fatty acid biohydrogenation in the face of low ruminal pH. Specifically, low pH favors an isomerization step that converts linoleic acid from a cis-9 trans-11 isomer to trans-10, cis-12 and reduces subsequent biohydrogenation. The trans-10 cis-12 isomer is a potent inhibitor of lipid synthesis in the mammary gland (Peterson et al., 2003).

2.5.3 Hind gut acidosis

The appearance of diarrhea, foamy feces, and mucinous casts in feces have been reported as clinical signs of SARA (Radostits et al., 2007). This is likely due to a shift in fermentation of small particle carbohydrates to the hind gut and subsequent hind gut acidosis (Gressley et al., 2011). As discussed above, it has been proposed that the large intestine is the site for translocation of bacterial endotoxins that cause the inflammatory response reported with SARA (Li et al., 2012; Plaizier et al., 2012).

2.6 Ruminal Acidosis and Liver Abscesses

As discussed above, there are discrepancies in threshold values as well as measurement techniques that complicate calculations of prevalence. Animal factors must also be considered, as feeding behavior can influence DMI and subsequently ruminal pH. Logistically it is easier to determine the prevalence of SARA in dairy herds, and as such there have been many reports of prevalence (Enemark et al., 2009; Kleen and Cannizzo, 2012). In the beef and feedlot industry measuring prevalence poses more of a challenge. Group housing, animal handling constraints, and lack of real-time individual animal performance data prevent individual animal measurements from being routinely made. Because of these constraints, the majority of studies in beef cattle have been done in individually housed cattle, and therefore reported prevalence and risk for SARA in beef cattle may not accurately represent a large scale, group housed setting. Data regarding prevalence and severity of ruminal acidosis for feedlot cattle fed in a group setting is limited to

one study (Castillo-Lopez et al., 2014), and thus cannot be considered representative of all feedlots in western Canada in terms of management, or environment.

Traditionally the risk period for SARA in feedlot cattle has thought to be greatest during the diet transition period as there is a relatively rapid increase in the amount of concentrate in the diet with minimal reductions in DMI (Bevans et al., 2005). However, a recent surveillance study (Castillo-Lopez et al., 2014) reported increasing risk for SARA with increasing days on feed (DOF), and that the mean prevalence of SARA (pH < 5.5 for >180 min) increased from 0.7% during backgrounding to 37.8% during the final finishing phase. This suggests that the finishing phase may, in fact, be the greater risk period for SARA. In industry, this coincides with an increase in digestive related mortality that is observed late in the finishing period (Smith, 1998).

As liver abscesses are the most commonly described sequelae to ruminal acidosis (Nagaraja and Lechtenberg, 2007), the prevalence of liver abscesses at slaughter can potentially be used as an indicator of SARA in commercial feedlots. The most commonly used scoring system is the Elanco liver check, which has been described by Checkly et al. (2005). In brief, the scoring system consist of 4 categories: 0 (no abscesses); A- (1 or 2 small abscesses or abscess scars); A (2 to 4 well organized abscesses less than 2.5 cm in diameter); or A+ (1 or more large active abscesses with inflammation of surrounding liver tissue. More recently, the A and A+ categories have been combined, as it can be difficult to distinguish between the two categories. Examining livers at slaughter may provide insight into the ruminal environment; however, as abscesses are reported to heal within 50 to 70 d (Itabisashi et al., 1987), liver scoring systems at slaughter may limit information to the last 2 months of the finishing period. Alternately, the ruminal epithelium never fully recovers from ulcerative damage and scars known as “stars” develop and remain for life. As such, the examination of the ruminal epithelium for evidence of permanent “stellate scars” provides a better assessment of long term rumen health, although it is difficult to discern early life events from those occurring in the feedlot. In a large surveillance study of 19,229 cattle at slaughter, Rezac et al. (2014) reported that 31% of the cattle had liver pathology and 23% had rumen lesions. Of the cattle with rumen lesions, only 32% had liver abscesses, which may also suggest that not all cases of rumenitis result in liver abscesses, or that liver abscesses have healed before slaughter. This suggests that ruminal acidosis may be a larger problem than liver abscess data would indicate and that the use of post mortem monitoring has limitations for determining rumen health during the feeding period.

2.7 Mitigation of SARA

Mitigation of SARA occurs at the level of the individual animal, by adaptations in the animal themselves, and at the pen level, via management strategies implemented by the nutritionist or animal management team. Mitigation of SARA requires an in-depth understanding of the physiology and pathology described above.

2.7.1 Adaptations by the ruminant

In order to avoid ruminal acidosis, cattle reply on both physical and behavioral adaptations to regulate ruminal pH. Often these adaptations are triggered by the same changes in the luminal environment that induce SARA, and it is the animal's ability to adapt that prevents SARA from occurring.

The first and most well described adaptation is an increase in absorptive capacity of the ruminal epithelium. An increase in papillae surface area is well described following an increase in dietary grain during dietary adaptation (Estevam et al., 2016). An increase in surface area is known to increase the rate of functional SCFA absorption, and modulate rumen pH (Melo et al., 2013). Both the ruminal epithelial surface area (Gorka et al., 2009; Steele et al., 2011; Malhi et al., 2013) and epithelial blood flow (Storm et al., 2012) have been shown in dairy cattle to be increased with high luminal concentrations of butyrate. Likely butyrate is being used as an energy source to facilitate epithelial growth, which in turn facilitates absorption of other SCFA from the lumen. However, the same effect on epithelial blood flow is not apparent in beef cattle (Kristensen and Harmon, 2004), which could be explained by adaptations of dairy cattle to compensate for increased metabolic demands and negative energy balance associated with milk production.

The response of the rumen to elevations in SCFA is not limited to papillae growth, and changes have been reported at the cellular level. For example, an increase in expression of many important transport proteins and enzymes has also been shown to occur in response to butyrate supplementation or incubation (Laarman et al., 2013; Dengler et al., 2014). In dairy cattle, an increase in luminal SCFA during SARA challenge has been shown to alter the expression of extracellular matrix proteins, eliciting an adaptive remodeling process within 7 days (Dionissopolous et al., 2012).

At a cellular level, removal of SCFA from the rumen depends on either passive diffusion of SCFA in the protonated form (H-SCFA) or absorption of the dissociated form ($SCFA^- + H^+$) via the $SCFA^-/HCO_3^-$ transporters (Gäbel and Sehested. 1997; Gäbel et al., 2002; Aschenbach et al., 2009). The form in which most SCFA exist depends on the pH of the ruminal environment and when ruminal pH is > 4.8, the majority of SCFA will exist in the disassociated form. Thus, it could be expected that transporter mediated uptake may play a dominant role (Penner et al., 2009). In the face of high SCFA concentration, upregulation of transport proteins has been shown in goats (Yan et al., 2014), facilitating increased SCFA- removal; however, Schurmann et al. (2014) also reported an increase in passive absorption of SCFA and an increase in Na^+ flux across cellular membranes in Üssing chamber experiments after steers were adapted for 21 d to a moderate grain diet. This suggests that an increase in both passive and active removal of SCFA from the rumen occurs in response to a changing luminal environment.

Further support for a rapid adaptation response by the ruminal epithelium to changing luminal SCFA concentration is provided by the documented up-regulation of genes important for cholesterol biosynthesis in the first week of adaptation to a high concentrate diet (Steele et al., 2011). The provides an important protective mechanism, as it prevents accumulation of intracellular cholesterol, which can cause oxidative damage and altered cell membrane permeability. Dionissopolous et al. (2012) reported up-regulation of genes involved in protective function of the rumen and tight cell junction maintenance in response to ruminal butyrate infusion. In contrast, Wilson et al. (2012) did not report any improvement in ruminal barrier function of lambs fed supplemental butyrate. The discrepancy could be explained by species factors, differing butyrate dose, or length of adaptation period, 7 *versus* 14 d. Interestingly, in goats, tight cell junction proteins claudin-1 and 4 have shown to be upregulated in the colonic mucosa after exposure to a high concentrate diet (Tao et al., 2014). The same has been reported in monogastrics in response to elevated butyrate (Bordin et al., 2010), suggesting that the response to elevated SCFA concentration is not limited to the rumen epithelium and that protective mechanisms may be in place downstream to prevent hind gut acidosis and its sequelae.

Saliva production has traditionally thought to contribute to ruminal buffering, with concentrations of bicarbonate (126 mEql/L) and phosphate (26 mEq/L) and a pH of 8.6 (and Balch, 1961). Studies have shown that salivary secretion is positively correlated to DMI (Beauchemin et al., 1991; Maekawa et al., 2002) and is largely influenced by diet composition and chewing time;

with lower secretion for cattle fed high concentrate compared to high forage diets (Erdman, 1988; Beauchemin et al., 2008; Chibisa et al., 2016). However, extreme decreases in saliva secretion have been shown to concentrate the phosphate and bicarbonate in the saliva (Bailey and Balch, 1961), which may exist as a compensatory mechanism for decreased production. That said, the relationship between saliva production and the amount of time that ruminal pH is < 5.8 has been shown to be positive, indicating that saliva alone is not able to mitigate low ruminal pH (Penner and Beauchemin, 2010). Based on the information presented above, it is apparent that adaptive mechanisms exist that allow most animals to compensate for the increased production of SCFA. Perhaps not surprisingly, individual cattle susceptibility to ruminal acidosis has been reported (Brown et al., 2006; Penner et al., 2009), indicating that there is individual variation in these adaptations, and that likely there are multiple factors that have not yet been considered. With this in mind, management factors implemented by feedlot managers and nutritionists are often directed to the most susceptible of the population.

2.7.2 Management strategies for mitigation of SARA

Knowledge of the pathophysiology of ruminal acidosis has led to the development of many strategies used in the dairy and beef industry to help mitigate subacute acidosis caused by common feeding practices. These strategies can be grouped into three main categories: animal management; feed and feed delivery management; and microbial management. Each of these will be discussed in detail in the following section.

2.7.3 Animal management

Imposing a transient feed restriction followed by a grain challenge is a common technique used by researchers to induce ruminal acidosis (Bevans et al., 2005; Devries et al., 2009; Schwaiger et al., 2013). Subsequent re-feeding after feed restriction has been shown to decrease ruminal pH, with the lowest pH associated with the greatest level of restriction (Zhang et al., 2013). The reduction in pH induced after a period of low feed intake may be explained by a tendency of cattle to binge upon re-feeding. The marked increase in DMI may therefore cause a sudden increase in available carbohydrates to the rumen microbes and a reduction in pH. Interestingly, a marked reduction in ruminal pH following a short-term period of low feed intake can occur even with diets that would not normally induce ruminal acidosis (Albornoz et al., 2013; Zhang et al., 2013). Such

a situation may be typical in North American feedlots, where it is not uncommon for young calves to be transported 12 to 16 h to the feedlot, with an average rest time before processing these calves of only 24 h (Samuelson et al., 2016). It is therefore easy to imagine that these cattle would experience sufficient stress to cause a reduction in DMI during transport due to limited or no access to feed and upon arrival at the feedlot. Therefore, it is no surprise that an important factor for the health of newly received cattle is to encourage DMI. However, no studies have evaluated whether ruminal acidosis is common in newly received feedlot calves.

Due to logistics of behavioral monitoring in feedlots, few studies have investigated the effect of competition at the bunk for beef cattle. However, for dairy cattle, tendencies have been shown for competition to influence DMI and increase feeding rate (Proudfoot et al., 2009) and this likely occurs in beef cattle as well. In two separate studies, Schwartskopf-Genswein et al. (1999; 2002) found decreased bunk attendance rates for group housed cattle compared to individually housed cattle. Less frequent bunk visits likely resulted in large, infrequent meals, thus increasing the risk for SARA. Encouraging frequent bunk attendance by multiple feedings/day and ensuring bunk space meets recommendations outlines in the Alberta Feedlot Management Guide (Winchell, 2000) have likely been under emphasized as strategies for reducing risk of SARA.

Pen conditions and environmental stress have also been shown to influence feedlot performance (Mader, 2003). Particularly, the effect of heat stress on feeding behavior of feedlot cattle has been well documented (Hahn, 1995; Koknaroglu et al., 2008; Schwartzkopf-Genswein et al., 2002). A weak positive correlation has been found between mean ambient temperature and prevalence of ruminal acidosis (Castillo-Lopez et al., 2014), which may be due to altered meal frequency or size, although this effect was confounded by days on feed, DMI, and BW. The same study speculated whether mud depth influenced attendance at the feed bunk and subsequent ruminal pH. In a companion study, (Castillo-Lopez et al., unpublished) it was found that only minimum ruminal pH was lowest with greatest mud depth after melting, and despite muddy pen conditions, performance parameters were unaffected by mud depth. Overall, both the prevalence and severity of acidosis in this feedlot were very low. Nevertheless, the influence of temperature, pen conditions, and environmental factors on DMI in feedlot cattle must be considered when making management decisions to mitigate ruminal acidosis.

2.7.4 Feed and feed delivery management- step-up diets

The value of step up diets has been well known in the feedlot industry since the 1960's and this management tool has had anecdotal success at minimizing ruminal acidosis during the transition period. However, data supporting the use of step-up diets for mitigation of SARA in modern commercial settings is lacking. The typical step up diet is designed to incrementally increase the proportion of concentrate in the diet allowing for the rumen epithelium and the microbial population time to adapt to the changing feed substrate. A recent survey of feedlot nutritionists by Samuelson et al. (2016) found that the majority of respondents implement a 4 step, 6-d per step transition program with the initial diet consisting of 40.7% roughage. Therefore, the typical step up period lasts around 24 - 27 d, although transitions have been described in as few as 10 d (Hironaka et al., 1980).

The shift in the microbial population that is occurring during the transition from high-forage to high-grain diets can be described as a transition from cellulolytic bacteria to amylolytic bacteria. The shift occurs anywhere from 24 h to 3 d post transition, depending on severity of rumen conditions (Brown et al., 2006). Fernando et al. (2010) described changes in the microbial population in response to changing dietary substrate from 100% forage to 80% concentrate (% DM) over 4 weeks as follows: an increase in the phylum *Bacteriodes* and a reduction in *Fibrobacteres*, increase in lactate using *Megasphaera elsdenii,* and an initial increase in *Streptococcus bovis* that was managed by the end of the diet adaptation. It must be noted that identification of the diverse populations of microbes in the rumen has just begun and advances in gene sequencing technology will facilitate further investigation into the way these populations change within the ruminal environment and changes in the activity of the microbial populations.

In addition to the microbes, there is a greater concentration of SCFA in the rumen when the diet fermentability increases along with a shift in predominant SCFA from acetate to propionate and butyrate (Chibisa et al., 2016; Penner et al., 2009; Steele et al., 2012). As discussed in previous sections, this change in luminal environment leads to physical and physiological adaptation of the rumen epithelium that increase the absorptive capacity of the rumen epithelium. Dirksen et al. (1985) documented these changes occurring within 4 wk of diet change, although more recent work suggests that these adaptations occur earlier (Schurmann et al., 2014; Dieho et al., 2016). Despite the suggestion for a prolonged adaptation response, Schwaiger et al. (2013) reported no difference in severity of acidosis after acidosis challenge between cattle that had been

fed the finishing diet for a long (34 d) compared to a short (8 d) duration. The same study did find; however, that the cattle fed the long term high grain diet recovered more quickly than those only exposed to the high grain diet for 8 d. This may be because the short adapted cattle had less time to preform adaptive functions.

2.7.5 Feed and feed delivery management- diet composition

The inclusion of fiber in the diet is perhaps the simplest, but most overlooked method of preventing ruminal acidosis. Production economics of feeding a high grain diet are discussed above, and inclusion of forage in a feedlot finishing diet is largely considered production limiting, as forage inclusion in the diet results in decreased G:F (Yang and Beauchemin, 2009) and decreased DMI (Chibisa et al., 2016). However, inclusion of physically effective fiber (**peNDF**) in the diet is paramount for management and prevention of digestive disorders, by reducing diet fermentability, stabilizing the ruminal environment via stratification and stimulating chewing activity, resulting in greater ruminal buffering per unit of fermented feed by saliva (Beauchemin et al., 2008). Particle size of the forage contributes to peNDF, and cattle experiencing SARA will sort for large particle forages in an attempt to self-regulate ruminal pH (Hendriksen et al., 2015). However, this results in variable and inconsistent concentrate intake, and as such, increasing forage particle length does not provide the same rumen stabilization effect as increasing F:C (Yang and Beauchemin, 2009). As discussed previously, rate of saliva production is greater in cattle fed high forage diets than high concentrate diets (Chibesa et al., 2016), which is likely due to the slower consumption rate of the high peNDF feed (Beauchemin et al., 2008).

2.7.6. Feed and feed delivery management -feed additives

In an attempt to mimic the natural buffering effects of saliva, buffering compounds are often added to the feed. Limestone, sodium bicarbonate (**SB**) and magnesium oxide (**MgO**) are dietary additives marketed to increase ruminal pH by buffering H^+; however, the efficacy of dietary buffers is questionable. For example, SB was shown to increase ruminal pH in dairy cattle fed corn silage based diets (Hu and Murphy, 2005), but did not increase ruminal pH on pasture based diets (Clayton et al., 1999). The benefit is likely limited by logistics of incorporating an effective quantity of buffer into the ration. For example, inclusion of SB at 2% DM results in 2.5 mol of buffer, which is far exceeded by production of SCFA, produced at rates of 2.75 to 7.5 mol/kg DM

(Clayton et al., 1999). Furthermore, supplementing SB at 1% DM to sheep on a hay and concentrate diet prior to a feed restriction challenge prevented metabolic acidosis, but had no effect on ruminal pH (Laskoski et al., 2014). This again suggests that supplementing buffers at low levels is not enough to compensate for SCFA production in the rumen, but may be enough to improve buffering mechanisms systemically. Therefore, it has been suggested that the efficacy of dietary buffers is in fact due to other mechanism. The most common theory being that consumption of limestone, SB, or MgO leads to increased water consumption by cattle, thus decreasing retention time of starch particles and providing a "wash out" effect for rumen SCFA's (Russell and Chow, 1993). Interestingly, unlike sorting for particle size, cattle will not select for SB when posed with a SARA challenge (Keunen et al., 2003). Addition of SB has also shown to increase the proportion of acetate, thereby altering the acetate to propionate ratio, and often SB is supplemented in conjunction with antimicrobials, which helps to offset this shift.

Antimicrobials are probably the most common feed additive used in North American dairy and beef production. Of the in-feed antimicrobials, monensin sodium is likely the most commonly utilized, and virginiamycin (**VM**) less so, as use is extra label in Canada. They act to selectively inhibit gram positive microbes in the rumen, causing a shift in the microbial population. This results in greater propionate production and improved energy and protein metabolism from the diet (Bergen and Bates, 1983; Guo et al., 2010; Montano et al., 2015). Concurrently, DMI is reduced and feed efficiency is improved (Duffield et al., 2012). This effect on intake is likely the primary mechanism by which ionophores help modulate ruminal pH as increasing the monensin concentration reduced DMI without a corresponding change in pH (Wood et al., 2016). Treatment of cattle with VM has resulted in a reduction in L-lactic acid accumulation *in vitro* (Clayton et al., 1999). A tendency for a reduction of *Streptococcus bovis* in cattle fed virginiamycin has also been shown through real-time PCR (Guo et al., 2010). Despite the benefit of using antimicrobials in the diet, there is increasing societal pressures to reduce their use in beef production. As such, non-antibiotic alternatives to monensin and more prominently, VM, are being sought out.

One such product is an oral polyclonal antibody preparation (**PAP**) against some of the most pathogenic rumen microbes, *Streptococcus bovis*, *Fusobacterium necrophorum,* and numerous clostridial bacteria. Pacheco et al. (2012) compared performance and carcass characteristics from *Bos indicus* bulls fed monensin *versus* PAP and found the only difference to be a slightly lower dressing percentage with the latter. Interestingly, rumen lesion scores of bulls

fed polyclonal antibodies were less than those of bulls fed monensin. The same PAP has been investigated in acidosis challenge situation, where no significant difference in ruminal pH or number or concentration of *Streptococcus bovis* was found (Blanch et al., 2009), however numerical trends were observed. It is apparent that PAP has the ability to alter the rumen environment, and further studies are warranted to determine if this technology has potential in North American production systems. A second technology is the administration of direct fed microbials. One such product includes a strain of *Megasphaera elsdenii*. When dosed at the beginning of the diet adaptation, *Megasphaera elsdenii* has been shown to successfully reduce the prevalence of ruminal acidosis in cattle fed low roughage diets (Leeuw et al., 2009) or under short adaptation periods (Drouillard et al., 2012), and even reduce treatment for bovine respiratory disease in feedlots (Miller, 2013). Although none of the above studies found a performance benefit to dosing cattle with *Megasphaera elsdenii*, it is still recommended for improving health of cattle when low forage or rapid adaptation protocols are utilized in feedlots.

2.7.7 Feed and feed delivery management -bunk management

Bunk management is perhaps the most important mitigation strategy for ruminal acidosis, as it dictates timing of feeding and amount of feed provided to the cattle which may change on a daily or even hourly basis. Allocation of bunk space can also influence animal behavior through competition as discussed above. The most commonly utilized bunk management technique, restricted feeding or "slick bunk" management, where cattle are fed as close to *ad libitum* as possible without overfeeding is used to increase predictability of intake, growth performance and feed efficiency by limiting sorting and preventing over consumption (Pritchard and Bruns, 2003). Although it must be considered that this type of bunk management may lead to increased competition for feed, which may limit the DMI of individual cattle. This practice allows for consistent timing of feed delivery. However, without multiple feed deliveries per day, cattle could be without feed for extended periods during the day, which has been shown to decrease ADG (Erickson et al., 2003) compared to *ad libitum* feeding. Erickson et al. (2003) also demonstrated that slick bunk managed cattle experienced greater daily variations in ruminal pH, however the use of monensin in these diets decreased variation in pH and cattle were fed twice daily.

Feeding cattle to maximize performance and minimize digestive disease is a complex task, and a successful management program implements many if not all of the mitigation strategies

discussed above. Failure of any of these aspects can cause problems ranging from mildly decreased performance to catastrophic digestive disease and death of animals. As such it is important that mitigation of ruminal acidosis be first on the minds of producers and researchers alike. Changing feed sources and limit antimicrobial use in the future make this area an important one for continuing research.

2.8 Performance and Economics

As mentioned previously, the reason for feeding high energy diets to cattle in both the dairy and feedlot industries is increased production and improved production economics. In North America, the cost of feed is second only to the cost of feeder cattle (Canfax, 2013), and improvements in G:F can make a large difference to the bottom line of an operation. Cattle that reach finishing weight faster also spend less time in the feedlot resulting in a positive outcome for production economics. Due to the more energy dense nature of the feed substrate, efficiency of gain is usually improved for high concentrate diets (Koenig and Beauchemin, 2011; Leeuw et al., 2009). Unfortunately, digestion of readily fermentable carbohydrate comes with the inherent risk of ruminal acidosis. Moreover, the sequelae of ruminal acidosis can also have significant economic and welfare implications in both dairy and beef production systems.

Dry matter intake has been shown to be reduced (albeit transiently) during both acute and subacute acidosis (Brown et al., 2000; Fulton et al., 1979; Marchesini et al., 2013), and evidence of rumenitis at slaughter (rumen scars) has been associated with reduced ADG and dressing percentage (Rezac et al., 2014; Thompson et al., 2006). Moreover, a recent study indicated that the severity of ruminal acidosis was negatively associated with G:F and ADG (Castillo-Lopez et al., 2014). Similarly, severe (A+) liver abscesses are associated with reduction in ADG and a decrease in cattle performance, including DMI, G:F, dressing percentage, and HCW (Brink et al., 1990; Jorgensen et al., 2007). When decreased performance is considered along with discounts of $5.78 per liver (USDA, 2014) or more at slaughter, the economic impact can be substantial.

In both the dairy and feedlot sector, lameness often results in decreased production and weight loss (Enting et al., 1997; Tessitore et al., 2011) or at least reduced ADG (Castillo-Lopez et al., 2014). The responses are perhaps due to an increase in time spent lying (Solano et al., 2016) and decrease in time spent standing at the bunk. There is no treatment for laminitis, and often these cattle are sent for salvage slaughter resulting in a discounted price. Although there is not much

current literature describing laminitis in feedlot cattle, a survey of 3 Alberta commercial feedlots reported the prevalence of lameness in chronic pens to range from 32.8 to 52.8% (Tessitore et al., 2011). With both economic and welfare implications, the need for understanding and prevention of laminitis is very important for both the dairy and feedlot industries.

In the current social environment, there is a growing awareness and concern for animal welfare in both the dairy and beef industries and each of the sequelae discussed previously certainly have negative welfare implications. The relationship between ruminal acidosis and animal welfare and economic consequences associated with ruminal acidosis and its sequelae emphasize the need to develop strategies to mitigate ruminal acidosis.

2.9 Conclusions

Ruminal acidosis is a complex and multifactorial disease, and its occurrence depends on the rumen microbial population, animal factors, and the environment. Subacute ruminal acidosis is most often induced in the pursuit of improved performance in terms of dairy or beef production. The clinical signs are vague and easily missed, yet the consequences of the disease can have both economic and animal welfare implications. Fortunately, many strategies have been developed to mitigate risk for SARA, and most dairy and feedlot producers implement these strategies on a daily basis. Even so, data suggests that SARA is still prevalent in both dairy and feedlot production. With increasing feed costs and increasing consumer interest in production practices, it is important that research is continued in this area to improve our understanding of SARA, its risk factors, and develop new mitigation strategies.

3.0 DEFINING RISK FOR LOW RETICULO-RUMINAL PH DURING THE DIET TRANSITION PERIOD IN A COMMERCIAL FEEDLOT IN WESTERN CANADA

This chapter presents an experiment performed in partial fulfillment of a Beef Cattle Research Cluster Grant investigating the prevalence of sub-acute ruminal acidosis in commercial feedlots. The goal of this study was to focus on the diet transition period in terms of the risk and severity of ruminal acidosis.

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Author contributions: Wiese, Penner, and Campbell were responsible for experimental design. Hendrick was the feedlot veterinarian and contributed intellectually to study design and discussion. McKinnon, McAllister and Beauchemin were contributors to the original BCRC grant proposal and contributed intellectually to study design and discussion.

3.1 ABSTRACT

The objective of the current study was to measure reticulo-ruminal pH (**RRpH**) in cattle in a commercial feedlot setting to determine the incidence and extent of low RRpH for steers and heifers as they transition to a high-concentrate finishing diet. Reticulo-ruminal pH was measured in 16 ‘mixed breed’ steers (4 hd/pen with 4 pens) and 16 ‘mixed breed’ heifers (4 hd/pen with 4 pens) housed in commercial feedlot pens with 227 ±13 and 249 ± 6 hd/pen cohort steers and heifers, respectively for the diet transition period. Cattle were transitioned from a diet of 46.5% forage and 53.5% concentrate to a diet of 9.5% forage and 90.5% concentrate on a DM basis using a 40-d transition with 5 dietary steps with the diets containing 41.4, 44.8, 49.8, 52.5, 55.1, and 64.0% non-fibrous carbohydrate. In addition, wheat replaced barley as the grain source during the dietary transition. Reticulo-ruminal pH was measured using orally administered pH measurement

devices that were retrieved at slaughter. Data were analyzed using a mixed model including the fixed effects of sex, diet, and the 2-way interaction to evaluate the effect of diet and sex, and with the fixed effects of sex, diet, and day relative to each dietary change along with the 2- and 3-way interactions to evaluate temporal responses as a result of diet change. A repeated measures statement was included for the effect of day. Both the mean and minimum RRpH values decreased as the proportion of concentrate in the diet increased ($P < 0.001$). The area and duration that RRpH was < 5.6 increased with greater inclusion of concentrate in the diet ($P < 0.001$). The number of cattle experiencing low RRpH, defined as pH < 5.6 for > 180 min, increased with increasing concentrate, and by the end of the 40-d dietary transition, 83% of the cattle had experienced at least one bout of low RRpH with most experiencing between 1 and 3 bouts/diet. These data are interpreted to suggest that cattle are at high risk for experiencing low RRpH during the dietary transition but that the extent of low RRpH is mild. Moreover, the data suggest that the risk for low RRpH increases with increasing proportion of concentrate in the diet. The results also suggest that susceptibility to ruminal acidosis may differ between steers and heifers.

3.2 INTRODUCTION

In North America, finishing cattle are generally fed diets containing greater than 85% concentrate (Vasconcelos and Galyean, 2007) as an approach to increase energy intake and improve G:F. However, diets that are highly-fermentable increase the risk of reducing reticulo-ruminal pH as the rate of acid production may exceed acid removal from the rumen (Penner et al., 2009a). In addition to feeding highly-fermentable diets, abrupt changes in dietary concentrate intake, and abrupt changes in DMI (Bevans et al., 2004; Brown et al., 2006; Holtshausen et al., 2013) can also predispose cattle to low RRpH. Hence, the transition from a moderately fermentable diet to a highly fermentable finishing diet has been considered as a high-risk period for low RRpH (Brown et al., 2006). As such, management and feeding practices seek to minimize the impact of low RRpH by gradually increasing the dietary concentration inclusion rate while minimizing variation in DMI.

While the diet transition period has been considered a high-risk period, the majority of studies evaluating ruminal pH have been conducted using individually housed cattle (Bevans et al., 2005; Brown et al., 2006; Holtshausen et al., 2013) where lack of competition and social stimulation may result in feeding behavior that differs from cattle in a pen setting (Castillo-Lopez

et al., 2014). A recent study (Castillo-Lopez et al., 2014) evaluating ruminal pH in feedlot cattle reported that the greatest prevalence and severity for low ruminal pH occurred late in the finishing phase, with minimal risk during the transition period. This study was performed at a small scale research facility, and further work was deemed necessary to substantiate these claims in a large scale commercial feedlot setting. The hypothesis of this study was that both steers and heifers would experience increased duration that RRpH was < 5.6 as they transitioned to the finishing diet. The objective of the current study was to measure RRpH in cattle in a commercial feedlot setting to determine the prevalence and severity of low RRpH for steers and heifers as they transition to a high-concentrate finishing diet.

3.3 MATERIALS AND METHODS

This experiment was conducted at a commercial feedlot (Coaldale, Alberta, Canada), during the spring (April to June) of 2015. Over the duration of the study, the average daily minimum and maximum ambient temperatures were 4.8ºC and 19.8ºC, respectively (Alberta Climate Information Services, Edmonton, AB, Canada). Average daily precipitation was 0.7 mm, with a total accumulated precipitation of 33.5 mm, and average daily humidity was 55.8%.

3.3.1 Animal management

Use of steers and heifers in this study was pre-approved by University of Saskatchewan Animal Care and Use Committee (protocol 20100021). A total of 1905, previously backgrounded, mixed breed yearling beef steers and mixed breed yearling heifers were available as the source population. Cattle were chosen for this study based on similarity in their previous nutritional exposure and target marketing date. Steers (n = 907) and heifers (n = 998) were separated by sex and housed in a total of 8 pens with an average of 227 ± 13 and 249 ± 6 hd/pen for steers and heifers, respectively. Pen dimension were 62.5 m × 68.5 m, and steers and heifers had (mean ± SD) 22.9 ± 0.8 and 25.4 ± 1.7 cm of linear bunk space. Within the source population, 16 steers (mean BW ± SD = 435.1 ± 32.8 kg) and 16 heifers (mean BW ± SD = 382.7 ± 49.4 kg) were randomly assigned as subjects for detailed monitoring resulting in 4 focal cattle/pen. The focal cattle were used for RRpH and lying behavior measurements.

At receiving, all cattle were treated with 500 μg/kg BW of a pour-on endectocide (Bimectin, Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) and an anabolic steroid

implant (Ralgro, Merck Animal Health, Madison, NJ). Cattle were vaccinated against clostridial diseases (Tasvax/Covexin 8, Merck Animal Health, Kirkland, QC, Canada), Bovine Rhinotracheitis Virus, Parainfluenza 3, Bovine Respiratory Syncytial Virus and Bovine Viral Diarrhea Virus (Pyramid 5FP, Boehringer Ingelheim, Burlington, ON, Canada). Depending on perceived risk category for disease, cattle were given no prophylactic antibiotic treatment (n = 16, 8H, 8S), an injection of tilmicosin (Micotil, Elanco, Guelph, ON) (n = 12, 8H, 4S), or an injection of oxytetracycline (Bio-Mycin, Boehringer Ingelheim, St. Joseph MO) (n = 4, 4S) upon arrival. The treatment protocol was consistent within pen.

3.3.2 Nutritional management and diet composition

Cattle were fed a diet containing dry-rolled barley grain, corn silage, alfalfa silage, and corn dried distillers grain (53.5:46.5 forage:concentrate, DM basis) for an average of 65.7 ±14.0 d (diet 1) prior to the start of the study. Subsequently, cattle were exposed to a 5-step, 40-d dietary transition program to adapt them to a high grain diet. The diet transition consisted of a gradual increase in the proportion of barley grain included in the diet at the expense of alfalfa silage and corn silage (Table 3.1). The dietary transition also included a switch from barley grain to wheat grain. On d 2 of diet 4, 3 barley straw bales weighing approximately 500 kg/bale with an unknown nutrient composition were added to all 8 pens to provide an additional source of effective fiber to combat digestive upset perceived by the feedlot staff. The straw bales were consumed within 2 d, representing approximately 2.9 ± 0.1 and 3.3 ± 0.2 kg/hd/d. The number of days that cattle were fed each diet, and the ingredient and chemical composition of the diets are provided in Table 3.1.

Table 3.1 Diet ingredient composition of a 5-step transition program used to adapt cattle from a high-forage diet to a high-concentrate finishing diet (diet 6) in a commercial feedlot. Cattle were previously adapted to diet 1 prior to the start of the study and were fed that diet for 65.7 ± 14.0 d.

	Diet[1]						
Variable	1	2	3	4	5	6H[3]	6S[4]
Days fed, no.	7	7	5	8	5	9	9
Ingredient, % DM							
Corn silage	30	30	31.5	31.5	23.5	9.5	9.5
Alfalfa silage	16.5	8.5	-	-	-	-	-
Barley grain	37.6	46.5	45	-	-	-	-
Wheat grain	-	-	8	55.32	66.22	78.9	78.9
DDGS	15	14	12	12	9	10	10
Limestone	0.8	0.9	1.1	1.1	1.2	1.5	1.5
Mineral and vitamin premix[2]	0.055	0.055	0.055	0.055	0.055	0.055	0.055
Chemical[5]							
DM %	67.6	68.2	70.1	72.8	73.6	81.6	81.6
CP	14.0	14.6	12.6	15.8	14.8	14.3	14.3
NDF	35.1	32.1	29.4	25.3	23.3	16.3	16.3
Starch	29.3	35	35.8	39	45.3	52.6	52.6
Ether extract	3.35	3.71	3.37	3.16	2.89	2.93	2.93
Ash	6.16	4.84	4.79	3.27	3.96	2.51	2.51
Non-fibrous carbohydrate[6]	41.39	44.75	49.84	52.47	55.05	63.96	63.96

[1]All diets contained tylosin (Tylan, Elanco, Division of Eli Lilly Canada Inc., Guelph, ON) to supply 11 ppm in the final diet, and monensin (Rumensin, Elanco) to achieve a final dietary concentration of 33 ppm.
[2]Mineral and vitamin premix (Gowans Feed Consulting, Wainwright, AB) contained 82,000 mg/kg Mn, 49,000 mg/kg Cu, 1,229.4 mg/kg Se, 490 mg/kg Co, 3300 mg/kg I, 245,000 mg/kg Zn, 9,000 KIU/kg vitamin A, 1,123 KIU/kg vitamin D, and 61,000 IU/kg vitamin E.
[3]Diet 6H was used for heifers and included melengesterol acetate (Zoetis Canada Inc. Kirkland QC) to supply 0.44 mg/hd/d.
[4]Diet 6S was used for steers only.
[5]Analyzed at Cumberland Valley Analytical Services Inc. (Hagerstown, MD).
[6]Non-fibrous carbohydrate was calculated as 100 - (ash + CP + NDF + ether extract)

Cattle were fed 3 to 4 times daily, with the first feeding occurring between 0730 h and 0800 h and the final feeding between 1200 h and 1400 h. On days of diet transition, the new diet was not offered until the second feeding of the day (approximately 1100 h). Diets were formulated to target an ADG of 1.8 kg/d and slick bunk management was used to ensure that no residual feed was remaining in the bunk at the end of each day. Diets were formulated using an Excel-based program using NRC (2000) equations and all diets included monensin (33 ppm) and tylosin phosphate (11 ppm). Feed samples were collected once weekly for the duration of the study and stored at -20°C until DM could be determined by drying the samples in a forced air oven at 55°C until achieving a constant weight. Samples were ground to pass through a 1-mm screen using a Christy Norris hammer mill (Christy and Norris, Christy Turner Ltd., Chelmsford, UK). Sub-samples of each feed ingredient were analyzed by Cumberland Valley Analytical Services (Hagerstown, WI) for DM, ash, CP, NDF, ADF, ether extract, starch, Ca and P using wet chemistry. Non-fibrous carbohydrate was calculated as 100 – (ash + CP + NDF + ether extract). A detailed description of the procedures used for wet chemistry has been described previously (Rosser et al., 2013).

3.3.3 Lying time

On the first day of diet transition, the 32 focal cattle were fitted with an accelerometer (HOBO® Pendant G Data Loggers, Onset Computer Corporation, Bourne, MA) to monitor standing and lying behavior. The accelerometers were fixed within small pouches using duct tape to attach the pouches to soft plastic leg bands (Multi-Loc® Leg Band, Bock's Identi Company, Mattoon, IL) and placed on the distal third metacarpal of the right front leg. The devices were programed to take measurements every 5 min for the duration of the diet transition period. At the end of the dietary transition, leg bands were removed and all but one device was recovered. However, two of the 31 recovered devices had lost data due to battery failures, leaving a total of 29 cattle with complete data. Data were downloaded using Onset Hoboware® Pro software version 3.3.2. (Onset Computer Corporation, Bourne, MA), which translated accelerometer readings into degrees of tilt in the x, y and z planes. As described by Ito et al. (2009), the x-axis was used to determine time spent lying. Due to the orientation of the accelerometers in the current study an x-axis < 120° was used to indicate lying. Daily pen averages were calculated for lying time.

3.3.4 Reticulo-ruminal pH

A pH measurement device (Penner et al., 2009b; Dascor Inc., Escondido, CA) was administered orally into the 32 focal cattle using a balling gun and the pH meter was set to record RRpH every 10 min for the duration of the dietary transition. Cattle were administered the pH measurement device at the same time as they were fit with the accelerometer. Each pH measurement device was 20.6 mm in diameter, 138 mm long, and weighed 245 g (Penner et al., 2009b). The pH measurement devices were retrieved from the reticulo-rumen at slaughter (described below) and data were transformed from mV values to pH using the regression data from the pre-measurement standardization. The pre-measurement standardization was conducted 1 d prior to administering the pH measurement device. No post-measurement standardization occurred as cattle were fed for another 100 d following the completion of this study.

To summarize the data, a daily start time of 1100 h was used as this time corresponded to the provision of the new diet during the transition period. Thus, pH data were assembled into days within each step of the dietary transition period. On d 1, both the previous (AM feeding) and new diets (PM feedings) were fed. Daily pen averages were calculated for mean, minimum, and maximum RRpH, and for the SD for mean RRpH (**SDpH**), duration of time RRpH < 5.6, area RRpH < 5.6 (magnitude $\times$ duration). Incidence and prevalence rates for low RRpH were were reported as bouts/diet, with a bout being defined to occur when RRpH was < 5.6 for ≥ 180 consecutive minutes (Gozho et al., 2005). Within diet, incidence for low RRpH was defined as the number of new cattle affected with a bout of low RRpH, whereas prevalence accounted for the total number of cattle experiencing a bout of low RRpH within each diet.

3.3.5 Body weight measurement

Individual BW for the 32 focal cattle were collected on a single day at the initiation of the study when accelerometers and pH measuring devices were administered and at the same time that accelerometers were removed (at the end of the diet transition period).

3.3.6 Slaughter

All cattle were slaughtered at a federally inspected processing plant (Cargill High River, High River, AB, Canada) at 141 days on feed. To collect pH measurement devices, the reticulum of each of the 32 focal cattle was opened by facility staff and devices were retrieved. If the device

was not found in the reticulum, the rumen was removed from the line and thoroughly searched. Two loggers were not recovered and one logger failed, leaving a total of 29 cattle with complete pH measurements. A total of 27 devices were retrieved from the reticulum, 3 from the rumen, and 2 were not recovered. After removal, the devices were rinsed with distilled water and stored in 0.3% KCL until data could be downloaded in the laboratory approximately 24 h later.

3.3.7 Statistical analysis

Data were analyzed using the MIXED procedure in SAS (version 9.2; SAS Inst. Inc., Cary, NC). The model included diet, sex, and the 2-way interaction as fixed effects. Pen was considered the experimental unit. An autoregressive correlation structure was used to account for the changes in diet over time, using averages for each diet, to evaluate the main effect of diet (Model 1). As the initial BW was greater for steers than heifers, a covariate adjustment was tested. The covariate adjustment was only significant for SDpH ($P = 0.001$) and hence was excluded from the model.

Model 2 evaluated the day effect during the first 5 d following each diet transition, and a compound symmetry correlation structure was used based on the best fit Akaike's and Bayesian Information Criterion. A duration of 5 d following each diet change was used as it represented the maximal number of days that were common among each diet, was similar to that reported by Samuelson et al. (2016) as the mean number of days for feeding step-up diets, and allowed for the comparison of response variables among diets. Thus, model 2 included diet, sex, day relative to the transition for each diet, and the 2- and 3-way interactions as fixed effects. Pen was included as a random effect. The start of test weight was initially included as covariate, but was removed as it did not account for a significant portion of the variation for any variables in this model.

For all analysis, least squared means were reported with the largest standard error of the mean (SEM) and when the *P*-value comparing more than 2 means was < 0.05, the Bonferonni post-hoc mean separation test was used. Statistical significance was declared when $P \leq 0.05$.

3.4 RESULTS

The average weight of the focal heifers (382.7± 32.8 kg) at the start of the study differed from steers (435.1 ± 49.4 kg; $P = 0.001$). Average BW at the end of the 40-d measurement period differed for heifers and steers with weights of 440.8 ± 51.7 and 511.7 ± 38.3 kg, respectively ($P < 0.001$).

3.4.1 The effect of diet and sex on reticulo-ruminal pH and lying time

There was an effect of diet on mean RRpH ($P < 0.001$) with mean RRpH being least while following the transition to diet 6, intermediate following the transition to diet 4 and 5, and greatest following the transition to diets 1, 2, or 3 (Table 3.2). Mean RRpH was also affected by sex with heifers having greater RRpH than steers when averaged over the diet transition period ($P = 0.040$). Minimum RRpH was also affected by diet ($P < 0.001$) and sex ($P < 0.01$) with values generally declining as cattle progressed during the diet transition and with heifers having greater minimum RRpH than steers. There was a diet × sex interaction ($P = 0.003$; Figure 3.1) for the SDpH with steers having greater SD than heifers during diet steps 3, 4, and 5, but lower SD than heifers when fed diet 6. In general, the SD for RRpH also increased as cattle transitioned from diet 1 to diet 5. Both the duration and area that RRpH was < 5.6 increased from diet 1 to diet 6, resulting in a diet effect for both variables with diet 6 having a greater duration and area following dietary change than all other diets ($P < 0.001$ Table 3.2). There was, however, no difference observed between steers and heifers for either the duration or area that RRpH was < 5.6 ($P = 0.11$ and 0.17, respectively).

There was a diet × sex interaction for lying time ($P < 0.001$; Figure 3.2). The time spent lying differed between steers and heifers when fed diet 1, 3, and 6 where heifers spent more time lying when fed diet 1 and less time lying than steers when fed diet 3. Lying time also differed by sex during diet 6, but in this case heifers spent more time lying than steers.

3.4.2 Evaluating the time-course for reticulo-ruminal pH, and lying time in response to dietary change

There was a diet × day interaction ($P < 0.001$; Table 3.3) for mean RRpH. Although there were differences among days within a diet, there was no repeatable pattern in the RRpH profile across diets (Figure 3.3). For example, the transition from diet 1 to diet 2 did not affect RRpH,

Table 3.2. The effect of diet, sex, and the diet × sex interaction for reticulo-ruminal pH, and lying time as cattle are transitioned from a high-forage to a high-concentrate diet in a commercial feedlot. Pen was considered the experimental unit with 4 pens/sex.

	Diet[1]							Sex			*P* values		
Variable	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	SEM[2]	Heifers	Steers	SEM[2]	Diet	Sex	Diet × Sex
Mean pH	6.45[a]	6.46[a]	6.42[a]	6.35[b]	6.31[b]	6.10[c]	0.025	6.38	6.33	0.0175	< 0.001	0.040	0.82
Min pH	6.05[a]	6.02[a]	5.99[a]	5.87[b]	5.79[c]	5.64[d]	0.030	5.93	5.86	0.02	< 0.001	0.010	0.36
SD pH	0.22[d]	0.26[c]	0.27[c]	0.33[b]	0.38[a]	0.33[b]	0.014	0.29	0.31	0.008	< 0.001	0.020	0.003
Duration pH < 5.6, min/d	10[c]	15[c]	36[bc]	48[bc]	71[b]	157[a]	16.3	44	68	10.2	< 0.001	0.106	0.32
Area < 5.6, pH × min/d	1.9[c]	2.4[bc]	6.4[bc]	6.7[bc]	8.8[b]	16.2[a]	2.61	5.7	8.4	1.42	< 0.001	0.17	0.32
Lying, min/d	781	805[b]	753[d]	830[a]	772[cd]	813[ab]	8.31	793	791	4.36	< 0.001	0.74	< 0.001

[abcd]Values with uncommon superscripts differ $P < 0.05$

[1]Diet forage to concentrate ratio (DM basis): diet 1 (46.5:53.5), diet 2 (38.5:61.5), diet 3 (31.5:68.5), diet 4 (31.5:68.5), diet 5 (23.5:76.5) diet 6 (9.5:90.5). In diets 1 and 2 the grain source was barley grain while wheat was included in diet 3 at 8% DM, and for diets 4, 5 and 6, wheat was the sole grain source. Cattle were previously adapted to diet 1 prior to the start of the study and were fed that diet for 65.7 ±14.0 d.

[2]Largest SEM reported.

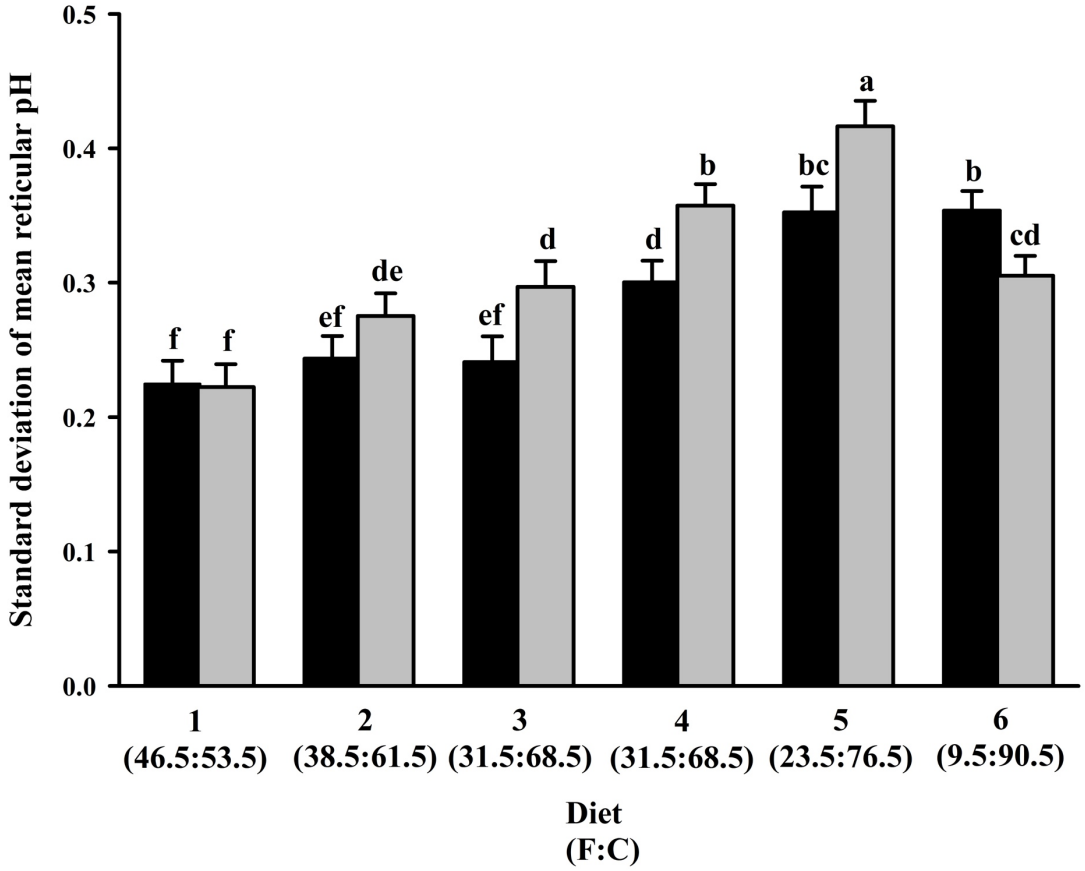

Figure 3.1. The interaction between sex and diet for the standard deviation of mean reticulo-ruminal pH (diet × sex interaction; P = 0.003). Both heifers (black bars) and steers (grey bars) were exposed to a 5-step (40-d) diet transition program. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and vertical bars with uncommon superscripts differ (P < 0.05). Pen was considered the experimental unit with 4 pens/sex.

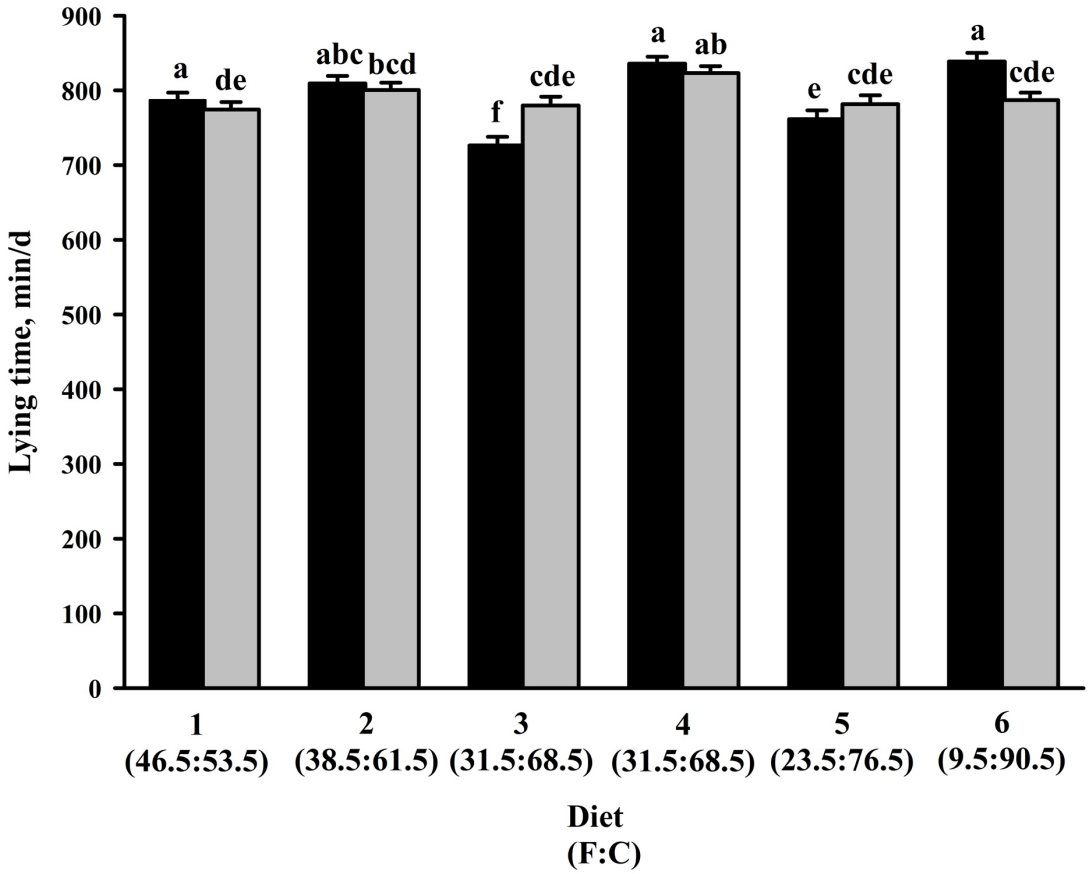

Figure 3.2. The interaction between sex and diet for lying time (diet × sex interaction; $P < 0.001$). Both heifers (black bars) and steers (grey bars) were exposed to a 5-step (40-d) diet transition program. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and vertical bars with uncommon superscripts differ ($P < 0.05$). Pen was considered the experimental unit with 4 pens/sex.

Table 3.3. The effect of day relative to diet change during the dietary transition period on reticulo-ruminal pH, and lying time for steers and heifers in a commercial feedlot. Pen was considered the experimental unit with 4 pens/sex.

	Day relative to the introduction of a new diet[1]						*P*-value			
Variable	1	2	3	4	5	SEM[2]	Day	Diet × day	Sex × day	Diet × day × sex
Mean pH	6.37^{a}	6.32^{b}	6.35^{a}	6.38^{a}	6.36^{a}	0.03	0.003	< 0.001	0.58	0.66
Minimum pH	5.86^{bc}	5.81^{c}	5.88^{ab}	5.91^{a}	5.88^{ab}	0.03	0.003	0.025	0.86	0.58
SD pH	0.33^{a}	0.33^{a}	0.30^{b}	0.32^{ab}	0.33^{a}	0.013	0.008	< 0.001	0.87	0.35
Duration pH < 5.6, min/d	62^{ab}	85^{a}	46^{bc}	27^{c}	56^{b}	10.8	<0.001	< 0.001	0.30	0.65
Area < 5.6, pH ×min/d	7.6^{b}	12.9^{a}	5.6^{b}	3.0^{b}	6.3^{b}	1.79	0.004	0.016	0.26	0.33
Lying, min/d	792^{ab}	785^{b}	797^{ab}	777^{b}	810^{a}	8.59	0.016	< 0.001	0.83	0.12

^{abc}Values with uncommon superscripts differ ($P < 0.05$).

[1]Diet forage to concentrate ratio (DM basis): diet 1 (46.5:53.5), diet 2 (38.5:61.5), diet 3(31.5:68.5), diet 4 (31.5:68.5), diet 5 (23.5:76.5) diet 6 (9.5:90.5). In diets 1 and 2 the grain source was barley grain while wheat was included in diet 3 at 8% DM, and for diets 4, 5 and 6, wheat was the sole grain source. Cattle were previously adapted to diet 1 prior to the start of the study and were fed that diet for 65.7 ±14.0 d.

[2]Largest SEM for the effect of day is reported.

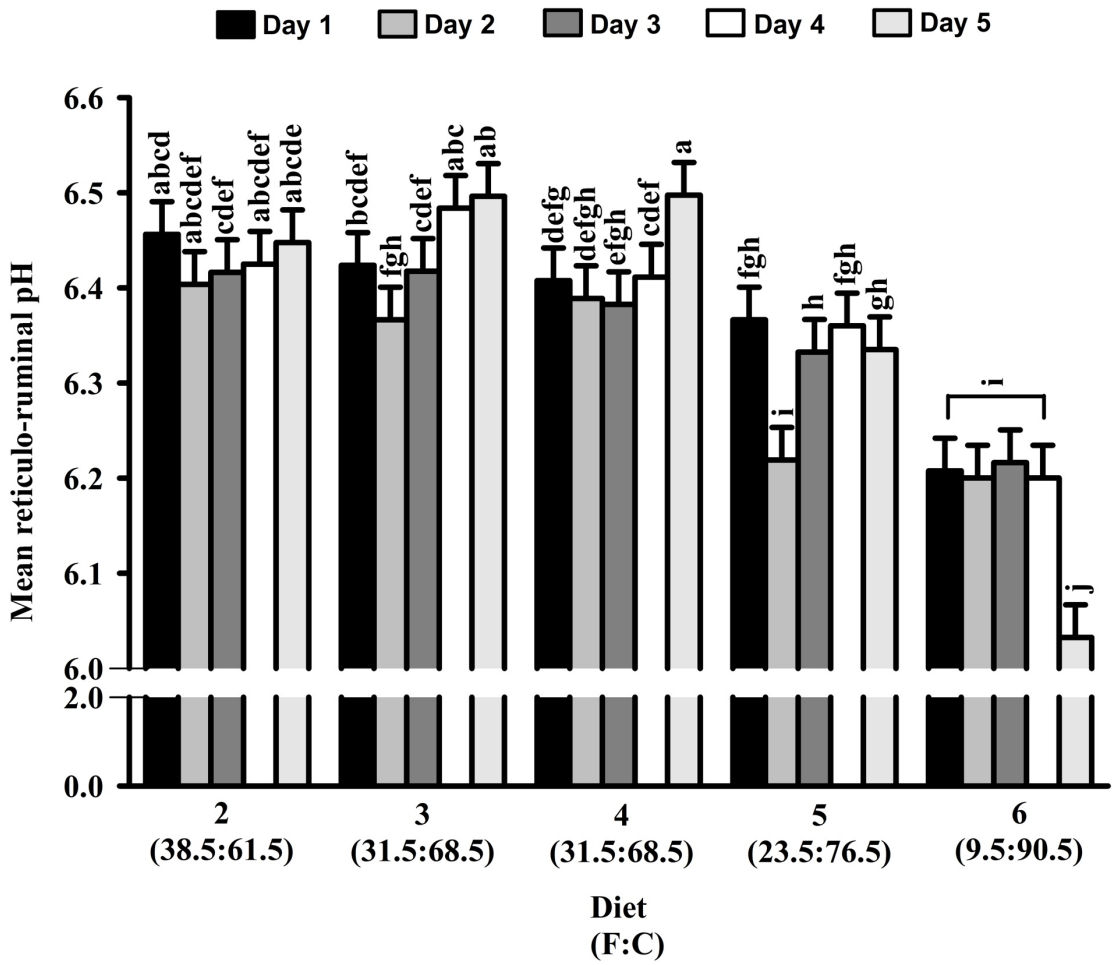

Figure 3.3. Average daily values for mean reticulo-ruminal pH (day × diet; $P < 0.001$) among days within a diet relative to the dietary transition on d 1 through 5 following each diet change in a 5-step diet transition program. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and bars with uncommon superscripts differ ($P < 0.05$). Pen was considered the experimental unit with 4 pens/sex.

whereas once cattle were fed diet 3, RRpH was lowest on d 2 relative to all other days. For diet 4, RRpH was greatest on d 5 with no differences occurring during d 1 to 4. When fed diet 5, mean RRpH decreased from d 1 to d 2 and then increased on d 3 with no differences observed between d 3, 4, or 5. In contrast, RRpH did not differ among cattle within the first 4 d on diet 6 but pH decreased on d 5.

There was also a diet × day interaction for minimum RRpH ($P = 0.025$; Table 3.3 and Figure 3.4). As was the case with mean RRpH, minimum RRpH did not follow a consistent pattern among days within a diet. For diet 2, there were no differences among the 5 days. However, when cattle were switched to diet 3, minimum RRpH was lower on d 2 than d 3, 4, and 5. The shift from a barley-based diet to a wheat-based diet (change from diet 3 to diet 4) initially decreased minimum RRpH but minimum RRpH increased over the 5-d period, however it should also be noted that cattle had access to straw bales during this dietary change. For diet 5, minimum RRpH was the least on the second day of feeding that diet, with no differences among days for diet 6.

A diet × day interaction was detected for SDpH ($P < 0.001$; Table 3.3 and Figure 3.5). The SDpH increased from the first d that cattle were fed diet 2 to d 2 with no differences thereafter. For diet 3, cattle had least SDpH on d 3 with values on d 1 and 2 being greater than d 4 and 5. No differences in SDpH were detected among days when transitioned to diet 4 but once transitioned to diet 5, SDpH increased and was greatest on d 5. Finally, SDpH was greatest on d 1 of diet 6 (but not different than the last d of diet 5) and decreased but stabilized for d 2, 3, 4, and 5 of diet 6.

The duration that RRpH was < 5.6 was affected by day ($P < 0.001$) and the diet × day interaction ($P < 0.001$; Table 3.3). For the transition to diet 2, there were no differences for duration among days, while duration was greater on d 2 and 3 than d 4 and 5 when cattle were transitioned to diet 3 (Figure 3.6). Changing from the barley-based diet to a wheat-based diet (transition to diet 4) resulted in greater SD d 2 of the diet 4 than d 3, 4, or 5. Cattle experienced greater duration of time < pH 5.6 on d 2 of diet 5 than d 1, 4, or 5 of the same diet. The transition to diet 6 resulted in greater duration below pH 5.6 on d 1 than day 3 and 4, with the duration being the longest on d 5. Overall, the duration was longer on d 5 of diet 6 than all other days across diets.

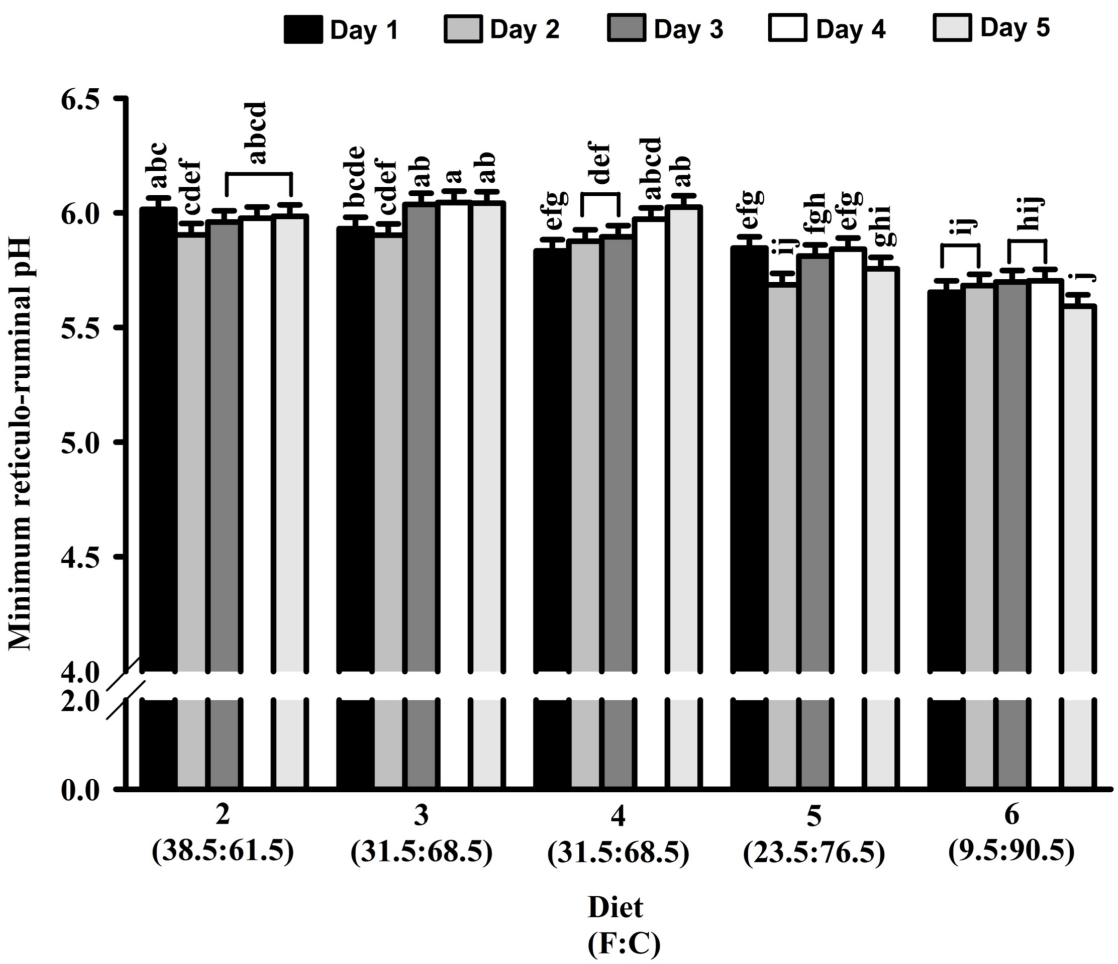

Figure 3.4. Average daily values for minimum reticulo-ruminal pH (day × diet $P = 0.025$) among days within a diet relative to the dietary transition on d 1 through 5 following each diet change in a 5-step diet transition program. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and bars with uncommon superscripts differ ($P < 0.05$). Pen was considered the experimental unit with 4 pens/sex.

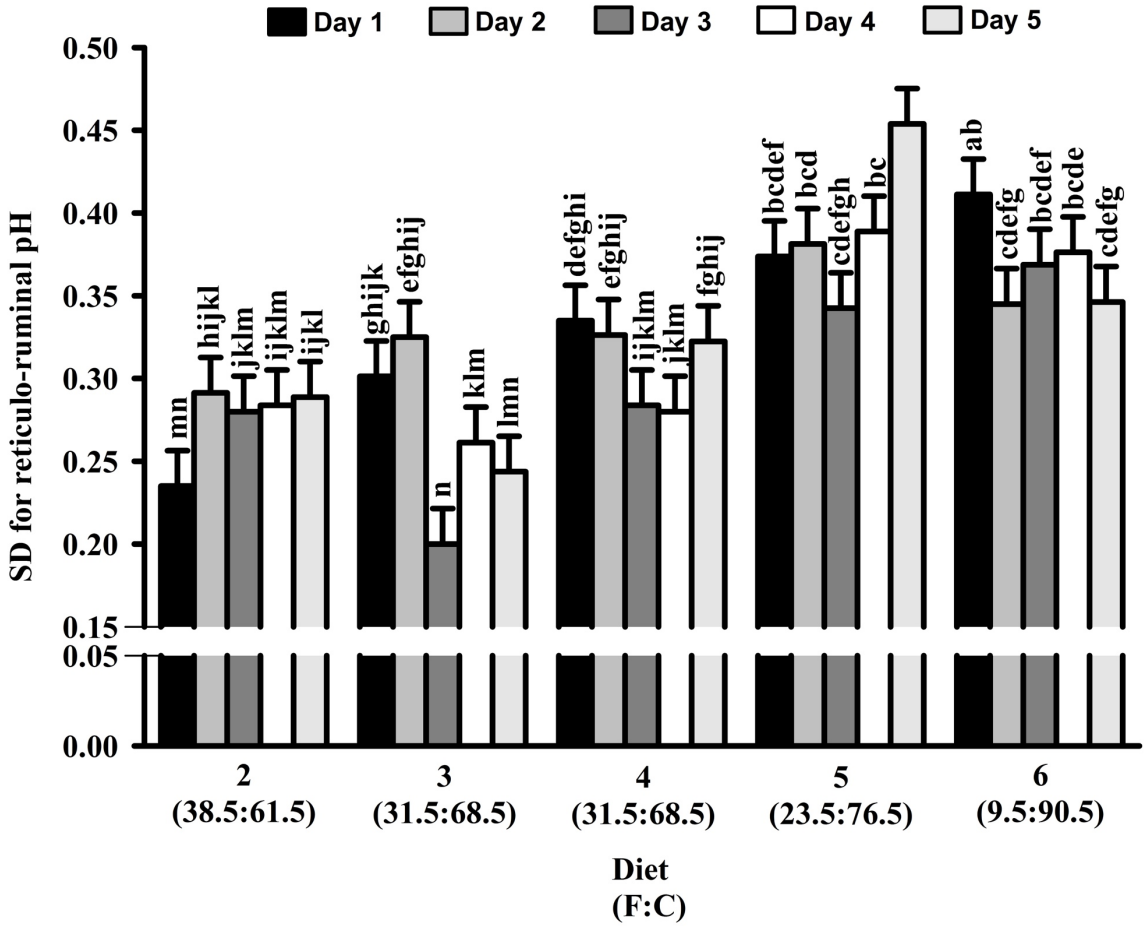

Figure 3.5. Average daily values for standard deviation from mean reticulo-ruminal pH (day × diet $P = 0.025$) among days within a diet relative to the dietary transition on d 1 through 5 following each diet change in a 5-step diet transition program. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and bars with uncommon superscripts differ ($P < 0.05$). Pen was considered the experimental unit with 4 pens/sex.

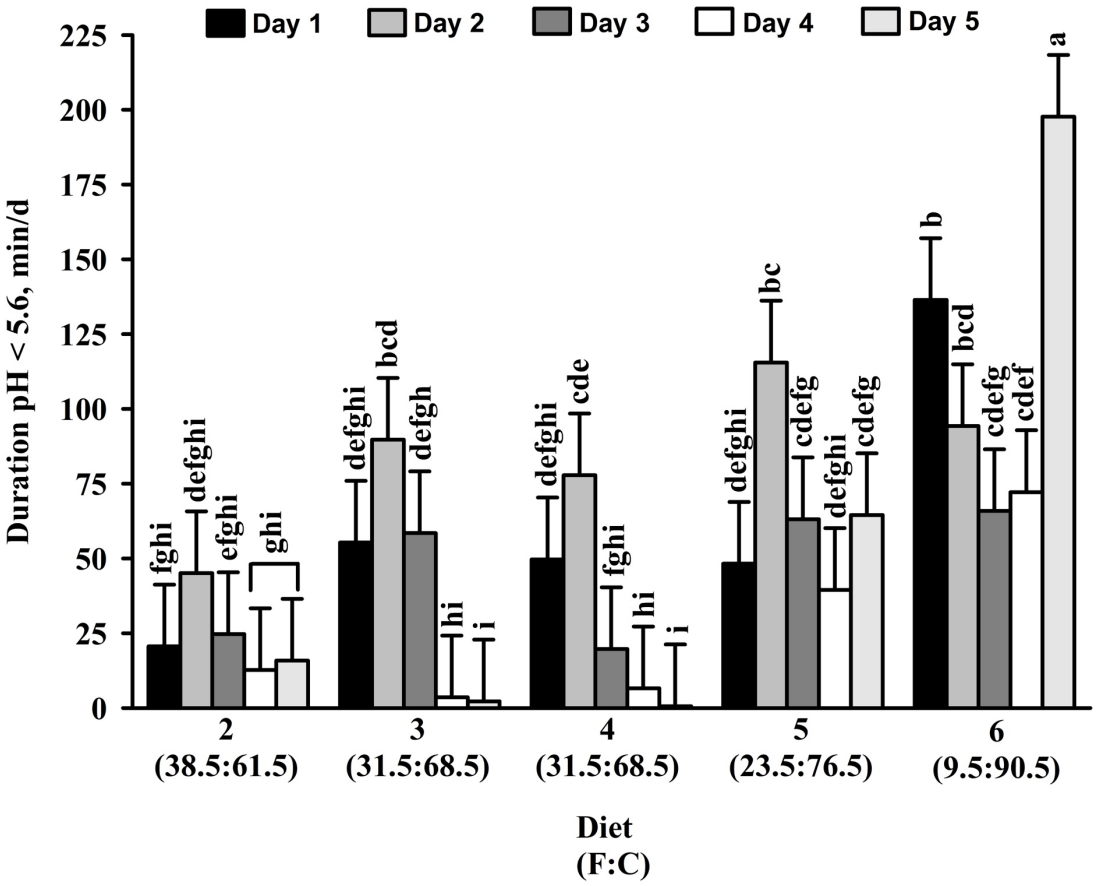

Figure 3.6. Average daily duration (min/d) that reticulo-ruminal pH was below 5.6 on d 1 through 5 of each diet change in a 5-step diet transition program (day × diet; $P < 0.001$). All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and bars with uncommon superscripts differ ($P < 0.05$). Pen was considered the experimental unit with 4 pens/sex.

Area that RRpH < 5.6 was affected by a diet × day interaction ($P = 0.016$; Table 3.3). In general, the area that RRpH was < 5.6 was greatest on d 2 of diets 3, 4, and 5, relative to d 4 and 5 of each respective diet (Figure 3.7). For diet 6, the area < pH 5.6 was greater on d 5 than d 2, 3, and 4.

There was also a diet × day interaction for lying time ($P < 0.001$; Table 3.3) with no distinct pattern among days within a diet (Figure 3.8). For the transition to diet 2, no differences were detected among days while for diet 3, lying time was least on d 4 relative to the other days within the diet and among diets. For diet 4, cattle spent less time lying on d 3 relative to all other days, but when transitioned to diet 5, less time was spent lying on d 1 with d 2 and 4 being intermediate, with cattle spending more time lying on and d 3 and 5. For diet 6, less time was spent lying on d 2 with no differences among days.

3.4.3 Prevalence of SARA

Over the entire diet transition period (40 d) 83% of the focal cattle in this study experienced at least 1 bout of low RRpH, defined by a case definition when RRpH was < 5.6 for > 180 min (Figure 3.9). Some cattle experienced a greater number of bouts than others with 16 cattle experiencing between 1 and 3 bouts, 6 experiencing 4 to 6 bouts, 1 experiencing 7 to 10 bouts, and 1 experiencing 13 bouts. Only 5 of the 29 cattle with complete pH data did not experience a single bout of reticulo-ruminal acidosis. Within each diet, cattle mostly experienced between 1 to 3 bouts of acidosis, except for diet 6, where 2 cattle experienced 7 to 10 bouts of acidosis. However, it should be noted that cattle were fed diet 6 for a longer duration, increasing the opportunity for sub-acute reticulo-ruminal acidosis to occur.

While not analyzed statistically, the average number of bouts per day within individual diets was calculated and found to increase from 0.43 bouts/d in diet 1 to 4.4 bouts/d in diet 6. Diet 4, however, had a lower daily prevalence than diet 3 (Figure 3.10).

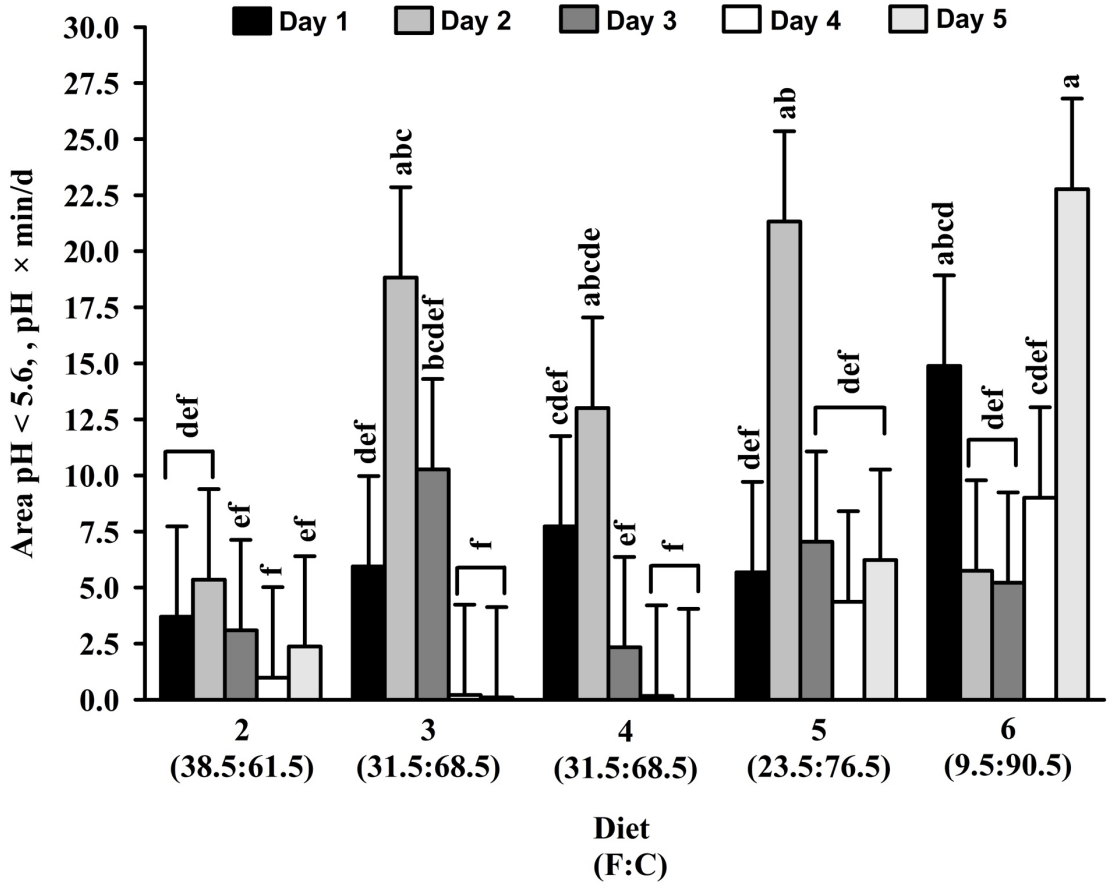

Figure 3.7. Average daily area (min × pH/d) that reticulo-ruminal pH was below 5.6 on d 1 through 5 of each diet change in a 5-step diet transition program (day × diet; *P* = 0.016). All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and bars with uncommon superscripts differ (*P* < 0.05). Pen was considered the experimental unit with 4 pens/sex.

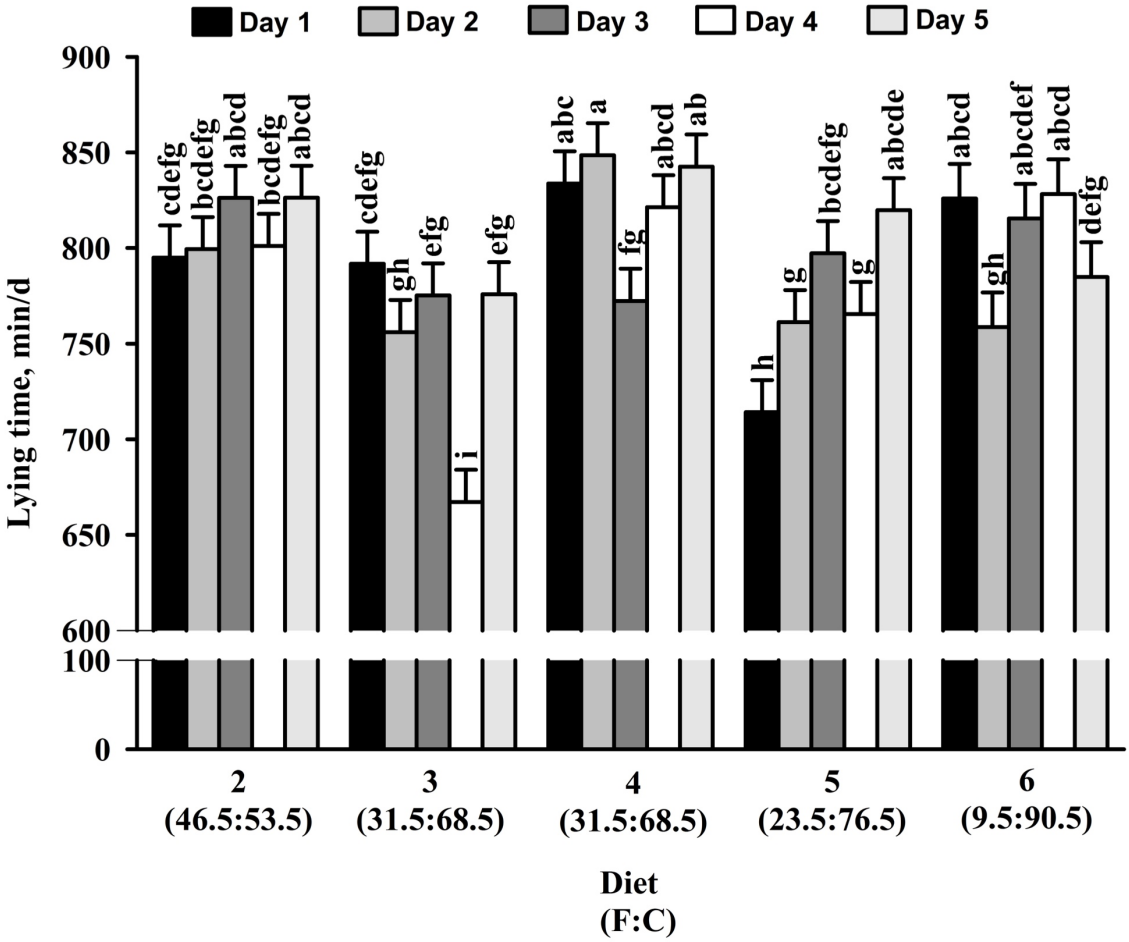

Figure 3.8. Average time spent lying (min/d) on d 1 through 5 following each diet change in a 5-step diet transition program (diet × sex interaction $P < 0.001$). All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and bars with uncommon superscripts differ ($P < 0.05$). Pen was considered the experimental unit with 4 pens/sex.

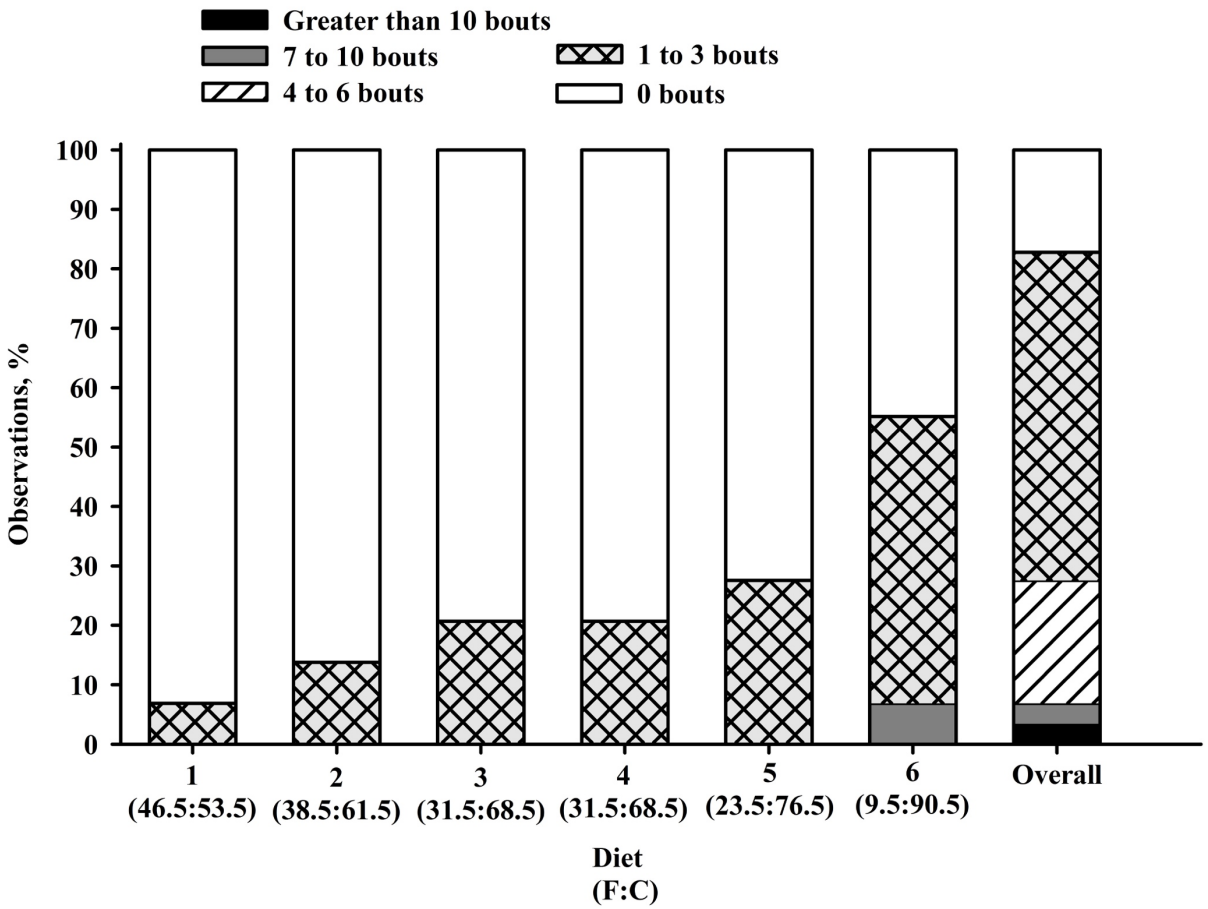

Figure 3.9. Proportion of cattle experiencing no bouts, 1 to 3, 4 to 6, 7 to 10, or greater than 10 bouts of reticulo-ruminal acidosis as affected by diet and cumulative throughout the dietary transition. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d and ruminal acidosis was defined to occur when ruminal pH was < 5.6 for > 180 min. Data from individual cattle (n = 29) were used to calculate proportions.

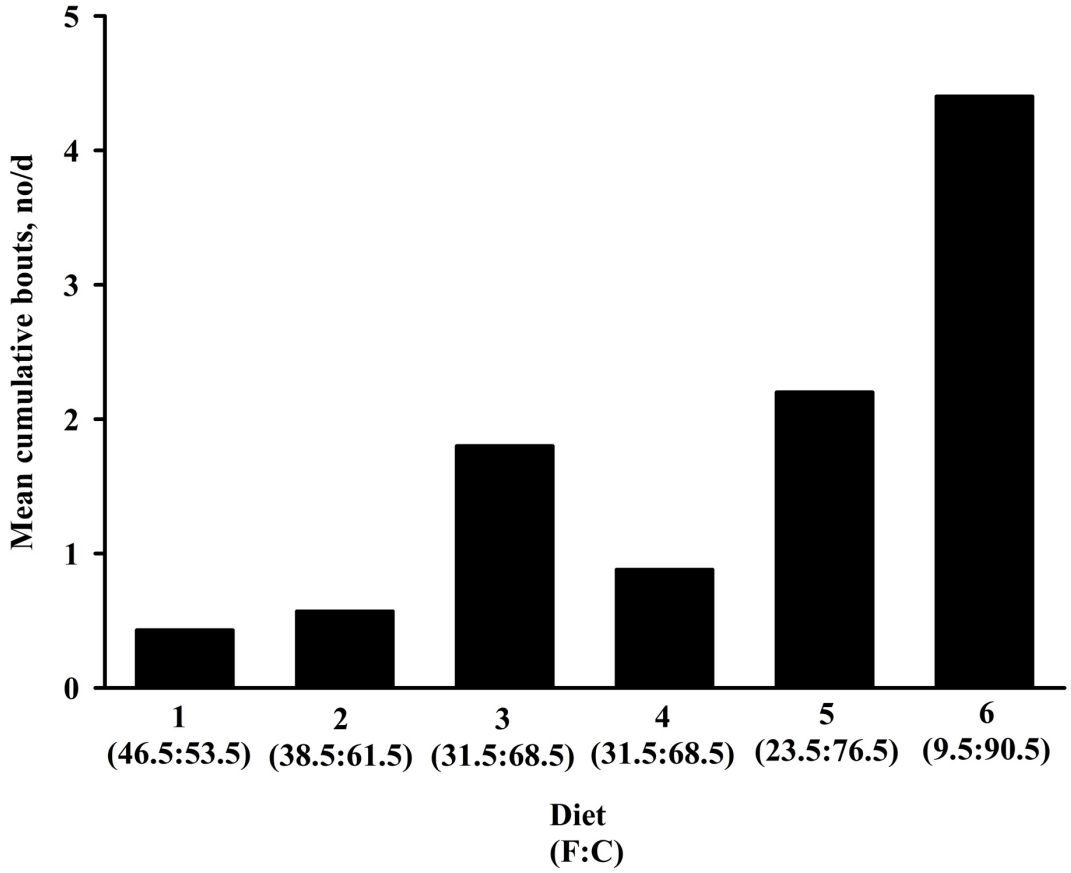

Figure 3.10. Number of daily bouts of reticulo-ruminal acidosis as cattle progressed through a 5-step diet transition program. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Vertical bars indicate the occurrence of bouts within diet. It should be noted that time spent on each diet differed, with diets 4 and 6 being 8 and 9 days long, respectively. Data from individual cattle (n = 29) were used to assess the number of bouts.

3.5 DISCUSSION

Low RRpH has been the primary variable to evaluate ruminal acidosis in both beef (Bevans et al., 2005; Castillo-Lopez et al., 2014) and dairy cattle (Penner et al., 2007). However, Khafipour et al. (2009) suggested that low RRpH itself may not be a sole indicator for ruminal acidosis as alfalfa-pellet induced ruminal acidosis failed to induce systemic inflammation despite reducing ruminal pH and increasing ruminal lipopolysaccharide concentration. Although there are concerns over whether ruminal pH can be used as a sole indicator for ruminal acidosis, one recent study has demonstrated a negative association between G:F and the duration that ruminal pH was < 5.5 (Castillo-Lopez et al., 2014). Furthermore, some data, albeit limited, has directly associated low ruminal pH with liver abscesses (Amachawadi and Nagaraja, 2016) and low ruminal pH is suggested to be involved in pathogenesis of laminitis (Nocek, 1997). Given the association between ruminal pH and negative outcomes on animal health and productivity, research is needed to evaluate risk for low RRpH for feedlot cattle.

As a strategy to minimize risk for low RRpH, dietary transition protocols have been designed to enable cattle to adapt from a high-forage to a high-concentrate diet in a way that minimizes the risk for low RRpH. Such strategies include the use of ionophores that have been demonstrated to reduce DMI (Ellis et al., 2012; Wood et al., 2016) and the variability in DMI (Duffield et al., 2012). In the present study, all diets included monensin and it is speculated that the use of monensin may have contributed to the mild extent of low RRpH observed in the present study. Nevertheless, due to individual variation in the ability of cattle to regulate RRpH, dietary recommendations are usually made at a pen level, and directed to enable the most susceptible cattle to adapt using a gradual adaptation program (Brown et al., 2000, 2006; Bevans et al., 2005). However, the risk for, and susceptibility of individually fed cattle to low RRpH during dietary transition protocols are likely not equal to that of group housed cattle (Castillo-Lopez et al., 2014) and may be different when bunk space is limited (Holtshausen et el., 2016). Thus, the risk for low RRpH may differ under commercial settings from that observed in controlled studies. However, currently there is only limited evidence suggesting the risk for low RRpH is lower for cattle housed in group settings than when compared to individually housed or fed cattle (Castillo-Lopez et al., 2014).

A particular challenge to evaluate the incidence and extent of low ruminal pH under commercial settings is the ability to measure ruminal pH at regular intervals for an extended period

of time. In the present study we used an orally administered pH measurement device (Penner et al., 2009b). This approach results in the pH measurement device measuring reticular pH rather than the pH in the ventral sac of the rumen, as is the case for most studies with ruminally fistulated cattle. In fact, 90% of the pH measurement devices were retrieved from the reticulum at the time of slaughter, which is consistent with other studies reporting that pH measurement devices were retrieved from the reticulum when orally dosed in calves (Schurmann et al., 2014). However, for the 3 pH measurement devices located in the rumen, it is not clear whether movement to the rumen occurred post-slaughter rather than during measurement as previous studies have reported that the pH measurement devices used in the present study do not migrate among regions (Castillo-Lopez et al., 2014). That said, given that 10% of the pH measurement devices were recovered from the rumen, we describe reticulo-ruminal pH in the current study. The latter is an important consideration given that measuring pH in a consistent location is important due to pH stratification among regions of the reticulo-rumen (Smith et al., 1956; Duffield et al., 2004).

There is some debate on the relationship between ruminal pH and reticular pH. Previous studies have used a ruminal pH threshold of 5.5 (Penner et al., 2007; Castillo-Lopez et al., 2014) as an indicator for sub-acute ruminal acidosis in beef cattle. A recent study by Falk et al. (2016) reported reticular pH to be 0.24 ± 0.08 pH units greater and to have less variation than ruminal pH suggesting that a threshold pH used for ruminal measurements may not be suitable when reticular measurements are used. However, cows in that study were fed forage-based diets and it is likely that the forage-to-concentrate ratio may affect this relationship. More recently, Seymour et al. (2016) evaluated the relationship between reticular pH and ruminal pH as cattle transitioned to a high-grain diet and found that reticular pH varied more than ruminal pH and was not consistently greater than ruminal pH. As such, in the current study, we used a threshold pH of ≤ 5.6 for ≥ 180 min. While this definition is the same as that used for dairy cattle (Gozho et al., 2005), it is 0.1 pH units greater than that previously used for beef cattle (Schwaiger et al., 2014; Castillo-Lopez et al., 2014) and could be considered as a threshold suitable for a conservative estimate for the prevalence of subacute reticulo-ruminal acidosis.

3.5.1 Severity, incidence, and prevalence of low Reticulo-ruminal pH during the diet transition

This study suggests cattle were at a high risk for low RRpH, even with the conservative dietary transition protocol that was implemented. In fact, we observed that 83% of the cattle experienced low RRpH at least once during the study. Moreover, the risk for low RRpH increased with increasing concentrate inclusion in the diet, confirming that the risk is greater when cattle are fed a high concentrate diet (Castillo-Lopez et al., 2014). While the overall incidence was high, the majority of cattle only experienced between 1 and 3 bouts and only 1 individual experienced 10 bouts of low RRpH over the entire study. The variability in the response among cattle highlights the variation in the susceptibility of individuals as previously reported (Bevans et al., 2005; Dohme et al., 2008). It is important to note that with increasing concentrate inclusion in the diet, there was an increase in the proportion of affected cattle, rather than an increase in number of bouts. Collectively, these findings are interpreted to suggest that cattle can adapt to highly fermentable diets but these diets increase risk for low RRpH. That said, it should be noted that the number of days cattle were fed each diet differed, although even when incidence was standardized for day, values were still greatest for diet 6.

Although prevalence and incidence rates were high, the severity of low RRpH that cattle experienced was mild. On average, cattle in the present study only spent 10, 15, 36, 48, 71, and 157 min/d below pH of 5.6 in response to transitioning through diets 1 to 6 respectively. Past studies have reported durations of 2.9 to over 10 h below 5.6 as cattle transitioned from a moderate to a high grain finishing diet (Bevans et al., 2005) when housed in tie-stalls or between 0.75 and 13 h/d below pH 5.5 when fed with limited bunk space in a group setting (Holtshausen et al., 2016), although it should be considered that these transition protocols were less conservative than in the current study. Previous work by Castillo-Lopez et al. (2014) using cattle group-housed and fed in a bunk line feeder reported mean durations that ruminal pH was below 5.5 to be 4.1 min/d when fed a backgrounding diet, 12.2 min/d during diet transition, and 79 and 195 min/d during finishing. While differing adaptation protocols, feed ingredients, and environment limit direct comparisons among studies, it appears that individual housing or limiting access to bunk space may increase the severity of reticulo-ruminal acidosis. In the current study bunk space was on average, 22.9 and 25.5 cm for steers and heifers respectively and for the study of Castillo-Lopez et al. (2014), available bunk space was excessive at 52 cm. While bunk space may play a role, it

should be noted that cattle were offered 3 straw bales/pen that were consumed within 2 d during the adaptation to diet 4. While short-term, we cannot dismiss that the provision of straw may have had helped to stabilize RRpH. Collectively, these findings further support the notion that current dietary management approaches (dietary transition and bunk management) and reactions employed by feedlot staff (in the case of perceived low RRpH) during dietary transition are effective at mitigating the severity of low RRpH (Castillo-Lopez et al., 2014). That said, it should be acknowledged that the dietary transition protocol used in the present study was quite gradual compared to typical transition protocols, which usually span 17 to 30 d (Vasconcelos and Galyean, 2007; Millen et al., 2009; Samuelson et al., 2016), and have been accomplished in as few as 10 d (Hironaka et al., 1968). Therefore, it is possible that the conservative practice used in the current study may have led to underestimation of the prevalence of low RRpH or reduced the severity of low RRpH relative to that which was expected to occur. Additionally, bunk management, stocking density, and even social and environmental conditions differ vastly between feeding operations, and these factors have all been shown to affect feed intake and feeding behavior of cattle (Erickson et al., 2003; Pritchard et al., 2003; Wilson et al., 2005). Given that RRpH is influenced by numerous factors, substantial variation in the prevalence and severity of low RRpH among feedlots can be expected.

There is no doubt that the adaptation protocol used in the present study was gradual based on the extended duration. Nevertheless, cattle in the present study received rolled wheat as a grain source as they transitioned from diet 3 to diet 4 (wheat was included at 55% of dietary DM in diet 4 and the transition from diet 3 to 4 did not include any change in the F:C outside of the straw provision). Wheat is generally thought to increase the risk of ruminal acidosis, but as He et al. (2015) observed, only tendencies for a reduction in mean ruminal pH could be detected when wheat replaced barley in finishing diets. Thus, despite the inclusion of wheat, the mean and minimum RRpH values in the current study appear to be greater than those reported by He et al. (2015) where individually housed steers had a mean and minimum ruminal pH of 5.61 and 4.88 when fed a comparable diet (60% wheat; DM basis). Even mean and minimum RRpH values of 6.1 and 5.6, occurring in diet 6 (75% wheat; DM basis) are above those reported by He et al. (2015). As discussed above, differences between the present study and those of He et al. (2015) may be related to measurement of pH in the reticulo-rumen *versus* ventral sac of the rumen. Other

factors such as frequency of feeding (Soto-Navarro et al., 2000) and processing index of the grain (Owens et al., 1998; Gimeno et al., 2015) may also have differed.

3.5.2 Variability in the daily reticulo-ruminal pH values during dietary transition

The SDpH has been used to describe the variability of cattle to cope with increased diet fermentability (Bevans et al., 2005). Accordingly, we used the SDpH to evaluate the ability of cattle to adapt to the high-grain diet. The SDpH generally increased with increasing dietary concentrate, but the nature of the increase differed between heifers and steers. It is not clear why SDpH would differ between steers and heifers although steers were heavier and had a greater DMI (Appendix Figure I). In addition, we observed that the SDpH response differed among days within a diet (Figure 3.5) without any repeatable pattern. Likewise, there were no distinct patterns in the response for duration or area that RRpH was < 5.6 across days within a diet although responses on d 2 of the diet change appeared to be the greatest in most cases. Castillo-Lopez et al. (2014) also reported that for group fed cattle there is no distinct pattern in the severity of low ruminal pH among days during a dietary transition protocol. While the generalized pattern is not completely clear, our data may suggest that the second day of feeding the new diet may be the greatest risk period for low RRpH, even when the new diet was introduced at the second of 3 to 4 daily feedings.

3.5.3 Influence of sex on subacute reticulo-ruminal acidosis

Mean and minimum RRpH were both lower for steers than for heifers. We attempted to use BW as a covariate in the present study, however, BW did not account for a significant proportion of the variation. Moreover, heifers in the current study experienced a gradual increase in the SDpH, reaching a plateau once transitioned to diet 5. In contrast, the SDpH for mean RRpH in steers increased more rapidly than heifers reaching a maximum SDpH in diet 5 before decreasing for diet 6. It is not clear why heifers and steers responded differently for RRpH and SDpH; however, detection of causal factors is not possible with the current study. Further research is needed to evaluate whether steers and heifers differ in their risk for low RRpH and to develop potential management strategies that mitigate risk.

3.5.4 Lying behavior during the dietary transition

As there were no direct measurements of eating behavior in the current study, we intended to use lying time as an indicator of time spent not eating and hypothesized that lying time would increase as the duration of time RRpH < 5.6 increased. Lying time has been investigated as a predictor of animal health in dairy cattle and was found to decrease transiently after an acidosis challenge (DeVries et al., 2009). In the current study, cattle spent less time lying (and consequently more time standing and potentially eating) during diet 3, but increased lying time during diet 4, corresponding to a decrease in RRpH. However, feedlot staff perceived digestive upset during the transition from diet 3 to 4, and added straw bales to each pen. Thus, the change in lying time may also be related to consumption of straw as there is a positive correlation between lying and ruminating time (Hicks et al., 1989). As there is limited information regarding activity budgets for feedlot cattle, we are unable to determine the biological relevance of the altered lying time. Our study does, however, indicate that feedlot cattle spend approximately 800 min/d lying during the dietary transition period. Given the use of lying time in welfare assessments in dairy cattle and the establishment of a feedlot welfare assessment tool in Canada (National Cattle Feeders Association, 2016), future studies evaluating normal activity budgets of feedlot cattle are needed.

3.7 CONCLUSIONS

Results of this study suggest that there is high risk but low severity of low RRpH during a gradual dietary transition phase. Our data further indicate that steers may have greater risk for low RRpH; however, we cannot fully discount that the greater BW observed for steers may have contributed to greater DMI and thus lower RRpH.

4.0 RUMINAL PH, SHORT-CHAIN FATTY ACID CONCENTRATIONS, AND ACUTE PHASE PROTEIN CONCENTRATIONS IN STEERS DURING FINISHING FOR STEERS WITH AND WITHOUT RUMINAL AND LIVER PATHOLOGY

Chapter 4 was completed as part of a larger study funded by the Beef Cattle Research Cluster Grant investigating the prevalence of sub-acute ruminal acidosis in commercial feedlots. As such the study was carried out prior to Chapter 3 chronologically. The goal of this study was to identify the threshold of ruminal pH that elicited a pathological response, and to determine if this pathology could be related to a systemic inflammatory response. It was positioned as the 4th chapter in in this thesis because it begins to address the question of risk versus severity posed in Chapter 3.

Author contributions: Study design was completed by Wiese and Penner. Campbell contributed to statistical analysis. The manuscript was originally drafted by Wiese, with suggested comments from Penner, Campbell and Hendrick.

4.1 ABSTRACT

The objective was to determine whether ruminal fermentation and acute phase protein concentrations differed between steers with (PATH) and without (NOPATH) rumen and liver pathology at slaughter. Ruminally cannulated steers (n = 28) that had been adapted to a diet containing 5% barley silage and 95% concentration. Ruminal pH was measured every 10 minutes and ruminal short-chain fatty acid and serum acute phase proteins were measured weekly. Steers with PATH spent more time with ruminal pH < 5.2 (P = 0.03). Serum amyloid A was greater (P = 0.02) in PATH than NOPATH. Steers presenting rumen and liver pathology had greater duration below pH 5.2 and serum amyloid A suggesting these acute phase proteins may be useful ante-mortem indictors of pathology.

4.2 INTRODUCTION

In North America, production economics for finishing cattle supports the feeding of diets with a substantial grain inclusion rate. Unfortunately, high-grain low-forage diets increase the risk for ruminal acidosis (Castillo-Lopez et al., 2014; Wiese et al., 2017) potentially resulting in negative implications on the health and performance of individual cattle (Brown et al., 2000; Castillo-Lopez et al., 2014). Even sub-acute ruminal acidosis (SARA) has been reported to induce an inflammatory response in dairy cattle (Zebeli et al., 2012), likely due to the translocation of bacteria and endotoxins across the ruminal epithelium (Kleen et al., 2003) or distal gastrointestinal tract (Li et al., 2012). The notion of ruminal translocation is supported by the fact that the strain of *Fusobacterium necrophorum* isolated from abscesses in the rumen epithelium is the same strain as that isolated from liver abscesses (Narayanan et al., 1997). Moreover, Rezac et al. (2014) reported a positive association between liver abscesses and rumen lesions when assessed after slaughter. While the causative factors for liver abscesses and association between rumen and liver lesions have been assessed, few studies have characterized ruminal fermentation in relation to formation of rumenitis and liver abscesses. Therefore, the objective of this study was to determine whether ruminal fermentation and circulating acute phase protein concentrations differed between steers with no rumen or liver pathology and those with rumen and liver pathology at slaughter.

4.3 MATERIALS AND METHODS

4.3.1 Management of steers

This study was carried out at the University of Saskatchewan Beef Cattle Research and Teaching Facility (Saskatoon, SK, Canada) as part of a larger study (Castillo-Lopez et al., 2014). The current study took place during the final 5 weeks of the finishing period as pathology occurring during this time period would not be expected to have healed by slaughter. Details for cattle and feeding management have been described by Castillo-Lopez et al. (2014). In brief, 28 ruminally cannulated (Angus × Hereford) steers were used in this study. Cannulated steers weighed approximately 477 ± 39 kg at the onset of the present experiment, and the non-cannulated cohorts weighed 587 ± 33.8 kg. In each of the pens (n = 8), there were a total of 35 steers including 3 or 4 cannulated steers. Prior to the start of this study, steers were exposed to a 4-step diet transition program to allow adaptation to the finishing diet coupled with 67 days on the finishing diet. The finishing diet consisted of (DM basis): barley silage (5%); rolled barley grain (81.2%); canola meal

(4.9%); a barley-based mineral and vitamin supplement (8%); and limestone (0.9%). All diets were formulated to supply 33 mg/kg monensin (Elanco Animal Health, Indianapolis, IN) and 11 mg/kg tylosin (Elanco Animal Health, Indianapolis, IN). Steers were fed twice daily at 0900 and 1600 h, with the afternoon feeding adjusted to achieve 5% refusals (as fed basis). Dry matter intake was calculated for each pen and was, on average, 11.6 kg/d/steer for the duration of the study. Cannulated steer ADG for the study period was 1.5 ± 0.49 kg/d, compared to non-cannulated cohort steers who gained an average of 1.4 ± 0.6 kg.

4.3.2 Ruminal pH measurement

Ruminal pH was measured using indwelling pH measurement devices according to the protocol described by Penner et al. (2009) and Castillo-Lopez et al. (2014). The pH devices were set to record pH every 10 min for the duration of the study. Once every 2 wk, the data were downloaded and the pH devices were re-standardized in buffers of pH 4.0 and 7.0 at 38.5 ºC and placed back into the ventral sac of the rumen. Transformation of the millivolt data to pH values has been described by Castillo-Lopez et al. (2014).

4.3.3 Ruminal fluid and blood sampling

Ruminal digesta and blood samples were collected weekly between 0700 h and 0800 h, for the 5 wk preceding slaughter. Ruminal digesta samples were collected from the anterior and posterior ventral sac (250 mL/region), composited, and strained through 2 layers of cheese-cloth. Ten millilitres of the resulting ruminal fluid was transferred into a 15-mL tube containing 2 mL of metaphosphoric acid (25% wt/v) and stored at -20°C until further analysis. Samples were analyzed by gas chromatography (Hewlett-Packard 5890; Hewlett-Packard, Santa Clara, CA) for determination of short-chain fatty acid (**SCFA**) concentration following protocol described by Khorasani et al. (1996).

Blood was collected via jugular venipuncture at the same time as ruminal fluid sampling. Blood was collected into two 10-mL evacuated tubes containing either Na-heparin (158 IU) or a silica act clot activator (BD, Franklin Lakes, NJ) for plasma and serum, respectively. Plasma samples were immediately placed on ice and serum samples were stored at room temperature. All blood samples were transported to the lab and centrifuged within 4 h of collection and were stored in 5-mL aliquots at 20°C until analysis. Serum haptoglobin (**Hp**) concentration was determined

using a commercial sandwich ELISA (GenWay Biotech Inc. San Diego, CA). Samples were diluted (50-time dilution). Intra- and inter-assay CV's were 3.3% and 11% respectively. Serum amyoid A (**SAA**) was measured using a commercial multispecies ELISA (Tridelta Development Ltd, Kildare, Ireland) described by McDonald et al. (1991). Optimal dilution was determined to be 1:500, and the intra- and inter-assay CV were 8.3%. and 9.2% respectively.

At the end of finishing, all steers were transported to a federally inspected abattoir (Plains Processors, Carman, MB, Canada), where they were slaughtered in 3 groups over a period of 4 d. For each steer, assessment of liver abscesses was conducted by a single trained person using the Elanco system: 0 (no abscesses); A- (1 or 2 small abscesses or abscess scars); A (2 to 4 well organized abscesses less than 2.5 cm in diameter); or A+ (1 or more large active abscesses with inflammation of surrounding liver tissue) as described by Brink et al. (1990). Rumen scoring was modified, combining the A- and A scores to fit with industry convention. Additionally, the rumen of each steer was opened along the lateral pillar of the ventral sac to allow for washing and visualization of the entire ventral and caudo-ventral blind sac. Rumen pathology was determined by an objective third party trained professional that was blinded to the liver abscess score. The rubric used to score rumen lesions is described as follows: 0 = healthy rumen with large papillae and normal coloration; 1 = hyperemia/discolouration, clumped papillae; 2 = erosion or regeneration from previous erosion; 3 = evident ulceration or appearance of stellate scars.

4.3.4 Data and Statistical Analysis

Ruminal pH data were summarized by week, and the mean pH, minimum pH, and average daily time spent below the pH thresholds of 5.8, 5.5, and 5.2 were calculated. Preliminary statistics indicated that week was not significant and thus, weekly values were subsequently averaged across the 5-wk measurement period. Weekly measurements of ruminal acetate, propionate, and butyrate concentrations, as well as blood SAA and Hp were also averaged over all 5 wk. A case definition was created for pathological lesions associated with SARA: steers with liver scores of A, or A+, or with rumen scores of 2 or 3, were defined as having pathology (**PATH**), whereas steers with rumen scores of 0 or 1 and liver scores of 0 were defined as having no pathology (**NOPATH**). Descriptive and inferential statistics were carried out on all data (Stata version 13.1, College Station, TX, USA). Normality was determined by Shapiro-Wilk normality test. Variables with normally distributed data were subject to the two-sample t-test, and non-normal data were analyzed

using a non-parametric approach (Wilcoxon Rank Sum test). Normally distributed data included SAA, acetate, mean ruminal pH and duration that pH was < 5.8. All other data were non-normally distributed. Significance was declared when $P < 0.05$ and tendencies are discussed when $0.05 < P < 0.1$.

4.4 RESULTS AND DISCUSSION

As reported by the National Beef Quality Audit 2010-11 (Beef Cattle Research Council. 2012), the prevalence of liver abscesses in Canadian fed cattle is 30%, and North American incidence has been reported to range from 12 to 32% (Nagaraja and Lechtenberg. 2007). In the current study, the prevalence of liver abscesses in the cannulated steers was 46% with 25% scoring A+ and 21% scoring A. The prevalence of liver abscesses in non-cannulated cohorts was 24%; 14.7% A+ and 9.8% A. The difference in liver scores could be explained by a slower line speed, and the ability to palpate the livers at the small-scale abattoir where the cannulated steers were slaughtered. However, the lighter initial body weight and increased ADG of the cannulated steers could also be contributing factors. A detailed description of pathology scores for the 28 cannulated steers is described in Table 4.1, but broadly; 8 steers were classified as NOPATH, consisting of 5 steers with no pathology and 3 steers with only mild rumen lesions. The PATH group (n = 20) consisted of 7 steers with only rumen pathology, 2 steers with only liver pathology, and 11 steers with both rumen and liver pathology.

Although ruminal pH is used as a primary diagnostic for ruminal acidosis (Bevans et al., 2005; Castillo-Lopez et al., 2014), minimum and mean ruminal pH were not different between PATH and NOPATH steers ($P > 0.10$; Table 4.2) suggesting that minimum and mean pH are not sensitive predictors indicating risk for ruminal pathology or liver abscesses. However, when the duration below 5.2 was assessed, it was observed that PATH steers spent on average 62 min/d with ruminal pH < 5.2, compared to only 8.3 min/d for NOPATH steers ($P = 0.03$; Figure 4.1). There was no difference between PATH and NOPATH for the duration that ruminal pH was < 5.5 ($P = 0.36$), although numerically, only the PATH steers experienced rumen pH < 5.5 for > 180 min, which is the case definition for SARA described in the study by Castillo-Lopez et al. (2014). In addition, steers with PATH tended to spend more time with ruminal pH < 5.8 than NOPATH ($P = 0.05$). Collectively, this data suggests that the duration that ruminal pH is < 5.2 may be a reasonable indicator for the risk for ruminal and liver lesions. Unfortunately, the study

Table 4.1. Distribution of rumen and liver pathology found in 28 cannulated steers at slaughter

	Rumen Pathology[1]				
Liver Score[2]	0	1	2	3	Total steers
0	5	3	4	3	15
A	1	2	1	2	6
A+	1	3	2	1	7
Total steers	7	8	7	6	28

[1]Rumen lesions are scored as follows: 0 = healthy rumen with large papillae and normal colouration; 1 = hyperemia/discolouration, clumped papillae; 2 = erosion or regeneration from previous erosion; 3 = evident ulceration or appearance of stellate scars.
[2] Livers were scored as follows: 0 (no abscesses); A (abscess scars or 1 to 4 well organized abscesses less than 2.5 cm in diameter); or A+ (1 or more large active abscesses with inflammation of surrounding liver tissue).

Table 4.2. Mean ± SEM for ruminal pH, short chain fatty acid concentrations, and acute phase protein concentrations of steers with and without pathology at slaughter.

	Case definition[1]		
	PATH (n = 20)	NOPATH (n = 8)	*P* - value
Ruminal pH			
Minimum	5.38 ± 0.05	5.40 ± 0.14	0.89
Mean	5.99 ± 0.05	6.12 ± 0.06	0.12
Duration pH < 5.8, min/d	547.4 ± 47.4	365.4 ± 71.7	0.05
Duration pH < 5.5, min/d	236.6 ± 44.6	118.4 ± 28.0	0.36
Duration pH < 5.2, min/d	62.0 ± 26.7	8.31 ± 5.81	0.03
Ruminal SCFA[2], m*M*			
Acetate	50.8 ± 0.52	49.4 ± 0.57	0.08
Propionate	34.4 ± 1.13	37.0 ± 0.64	0.22
Butyrate	9.59 ± 0.69	8.70 ± 0.59	0.64
Serum[3], μg/mL			
SAA	45.12 ± 3.47	31.04 ± 3.05	0.02
Hp	4.91 ± 0.78	2.28 ± 0.18	0.08

[1]Rumen and liver pathology were used to create a case definition. Steers with rumen scores of 0 or 1 and liver scores of 0 were categorized as NOPATH. Steers with liver scores of A-, A, or A+, or with rumen scores of 2 or 3, were categorized as PATH. Data are reported as means ± SEM.

[2]Short chain fatty acid concentrations for individual steers were measured from ruminal fluid samples taken weekly and averaged over the final 5 weeks of finishing.

[3]Haptoglobin (Hb) and Serum Amyloid A (SAA) concentrations were measured weekly for each steer. Results were then averaged over the final 5 weeks of finishing.

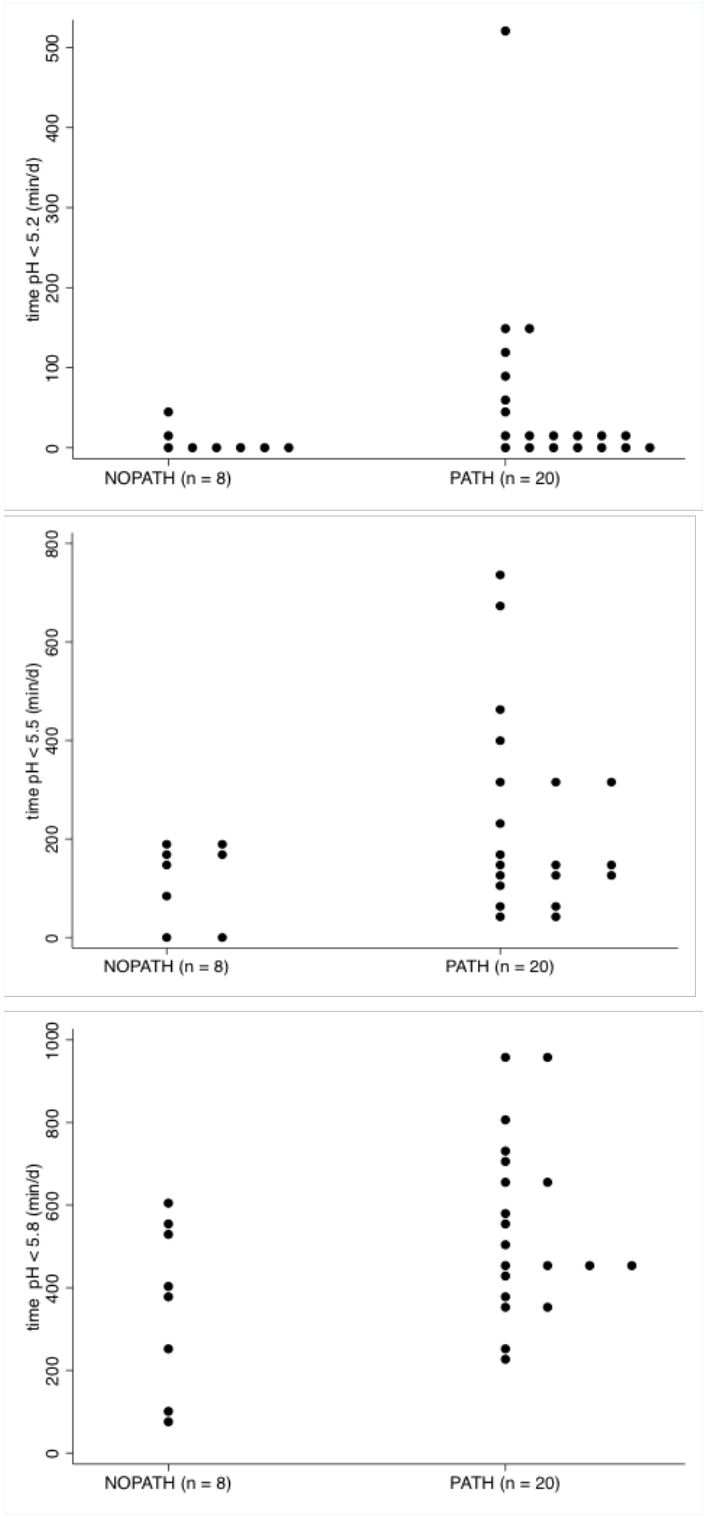

Figure 4.1. Dot plots of the time (min/d) spent below rumen pH 5.2 (top panel), 5.5 (middle panel) and 5.8 (bottom panel) by steers with (n = 20) and without (n = 8) rumen and liver pathology at slaughter. Measurements were collected daily during the final 5 weeks of finishing and averages of these values were used. The finishing diet was composed of 5:95 F:C (%DM).

was limited by its small sample size, and further work with larger numbers of cattle is needed to confirm these conclusions.

In addition to greater duration of a ruminal pH < 5.2, PATH steers tended to have greater concentration of acetate than for NOPATH steers ($P = 0.08$; Table 4.2); however, propionate and butyrate were not affected. Chen et al. (2012) also reported that steers susceptible to ruminal acidosis had greater acetate than resistant steers; although, they found that propionate and butyrate were also affected. While all cattle received the same diet, it was not possible to measure individual DMI in the present study and DMI may also have affected fermentation patterns (Duffield et al., 2012). It is also possible that inherent differences in the density and composition of the ruminal microbiota may alter the risk for ruminal acidosis and its pathologic sequelae (Chen et al., 2012). Evaluating these variables were out of scope of the present study but future research is needed to further determine causative factors that increase the risk for ruminal and liver pathology.

Although they are not specific to pathology of the liver and rumen lesions, acute phase proteins SAA and Hp were measured in this study as indicators of systemic inflammation. Steers with PATH tended ($P = 0.08$) to have greater concentrations of Hp and had greater SAA compared to NOPATH ($P = 0.02$; Table 4.2). The SAA concentration of PATH was in agreement with values reported for acute inflammation by Horadagoda et al. (1999) but less than that reported by Gozho et al. (2006) in steers after a SARA induction challenge, suggesting a chronic nature of the rumenitis-liver abscess disease complex. Moreover, liver abscesses have been shown to heal within 70 d (Itabisashi et al., 1987), and acute phase proteins have been shown to be elevated within hours of an inflammatory insult and decline rapidly after the stimulus is removed (Tothova et al. 2013). While the blood was collected infrequently (once/wk) in the present study, the data were averaged over a 5-wk duration to represent a chronic condition rather than to indicate acute changes. Thus, it is likely that the pathology identified at slaughter was the cause of the inflammatory response detected. Interestingly, the SAA concentrations of NOPATH were well above the 1.30 ± 0.44 μg/mL reported in healthy dairy cattle (Eckersall et al., 2006), but for both PATH and NOPATH steers, SAA concentrations were consistent with values reported by Gozho et al. (2006) during diet transition.

In addition to having significantly greater concentrations or a tendency for greater concentrations of Hp and SAA, PATH steers had greater variation (SEM) for both Hp and SAA than NOPATH steers (Figure 4.2). It is apparent that acute phase protein concentrations are highly

variable, as Huzzey et al. (2009) reported Hp concentrations of < 100 μg/mL to be within an acceptable level in healthy dairy cattle. In the current study, although there was only a tendency for a difference between groups ($P = 0.08$), both PATH and NOPATH steers were below the range suggested by Huzzy et al. (2009), with Hp concentrations of 4.91 μg/mL and 2.28 μg/mL, respectively. If the reported threshold for healthy cattle is indeed correct, the lower Hp response compared to SAA described in the current study could be due to the functional roles of each APP. The role of SAA is largely unknown, but opsonisation of bacteria, and scavenging of cholesterol are thought to be two functions (Ceciliani et al., 2012). It is possible that these roles of SAA are more suited to the pathophysiology of the rumenitis-liver abscess complex in a finishing steer that is off-feed, than are the hemoglobin binding and anti-inflammatory properties of Hp.

4.5 CONCLUSIONS

Overall, results of the current study suggest that the duration of time that ruminal pH is less than 5.2 and a chronic systemic acute phase protein response are greater for steers with a case definition including ruminal and liver lesions. Steers with rumen and liver pathology, on average, were only exposed to ruminal pH < 5.2 for only 1 h/d. To the authors' knowledge, this is the first study to suggest a ruminal pH threshold for predicting gross pathology of the rumen and liver.

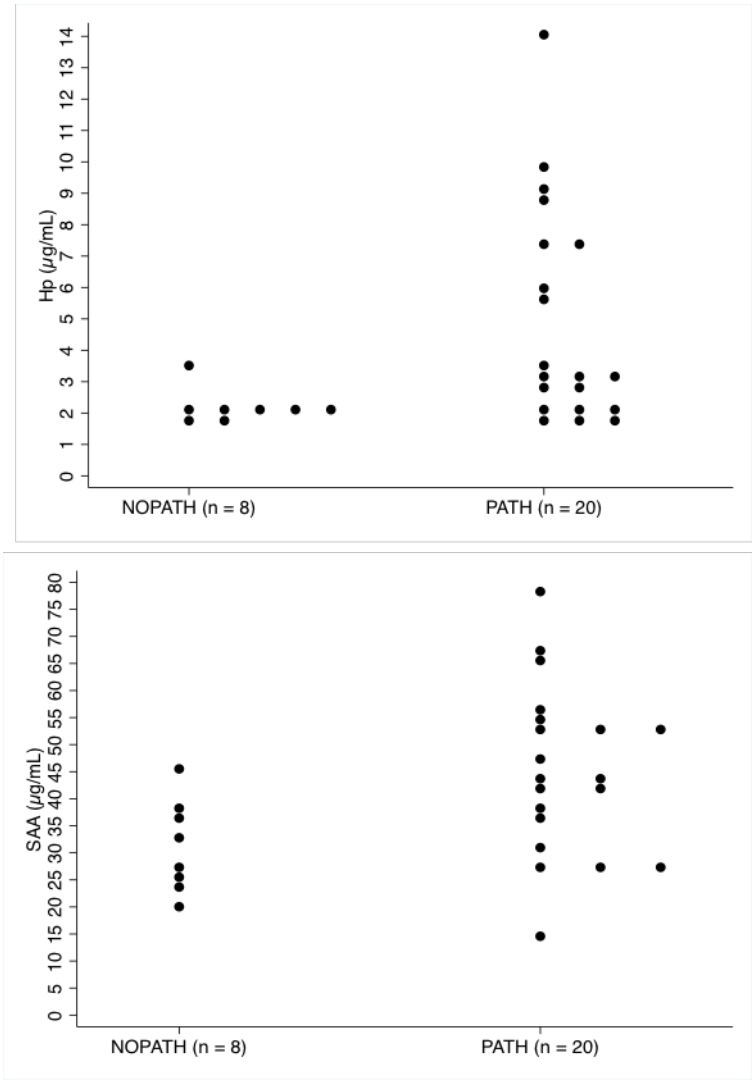

Figure 4.2. Dot plots of serum haptoglobin (top panel) and serum amyloid a (bottom panel) concentrations of steers with (n = 20) and without (n = 8) rumen and liver pathology at slaughter. Samples were collected weekly during the final 5 weeks of finishing and averages of these values were used.

5.0 GENERAL DISCUSSION

Ruminal acidosis has long been known to be an issue in the dairy industry, where the prevalence and effects of subacute ruminal acidosis have been well described (Kleen and Cannizzo, 2012). In contrast, the feedlot sector of the beef industry had to extrapolate from studies where cattle were housed and fed individually to define the risk for and consequences of ruminal acidosis in feedlot settings. It has been suggested that because of different social and environmental factors, individual animal data is perhaps not truly representative of commercial production systems, and only recently has work been conducted to determine the prevalence of ruminal acidosis in commercial settings (Castillo-Lopez et al., 2014). The aim of the experiments completed for this thesis was to determine the prevalence of reticulo- ruminal acidosis during the diet transition period in a commercial feedlot (Chapter 3), and to identify the ruminal fermentation patterns and serum acute phase responses that are associated with the known consequences of ruminal acidosis detected at slaughter (Chapter 4).

It is apparent when examining the literature, that there is no consensus amongst researchers for a definition of ruminal acidosis. As technology has improved, our ability to measure ruminal pH has evolved. In research, point source measurements from a cannulated animal or via rumenocentesis have now been replaced by indwelling continuous measurement devices (Penner et al., 2009a). These new measurement techniques have provided much insight to the ruminal environment, and have led to more refined definitions for ruminal acidosis, especially the subacute form. As such, many of the current definitions take into consideration both severity of pH depression and time, either described as time below a threshold, or area (a function of severity and time). However, a lack of consensus amongst researchers results in many different thresholds being used. For example, Castillo-Lopez et al. (2014) describes ruminal acidosis using a threshold of pH 5.5, Gozho et al. (2005), a threshold of 5.6, and Dohme et al. (2008) a threshold of 5.8. One of the intentions of this thesis was to advance the search for the most appropriate and clinically relevant definition of SARA. In chapter 3, a pH threshold of 5.6 was chosen in an effort not to underestimate the prevalence of SARA, as orally dosed pH measurement devices are often recovered from the reticulum, which has a lower pH than the rumen in cattle fed high grain diets (Seymour et al., 2016). Therefore using a lower pH threshold in this study would have risked underreporting of the prevalence of SARA in commercial feedlots.

I believe that this thesis highlights the importance of differentiating between prevalence and severity of SARA. Based on our studies, prevalence and severity of pH depression should be considered independently when describing SARA. For example, although a large number of cattle (83%) experienced low RRpH during diet transition, most experienced very few bouts, and mean RRpH remained > 6.0 (Chapter 3). This suggests that despite a high prevalence of SARA in the group, most individual cattle were able to regulate RRpH and did not suffer severe SARA during the diet transition period. It should be noted that previously backgrounded yearling steers were used in this study, and it is expected that the risk for SARA in this class of cattle would be less than for freshly weaned calves. In addition, compared to industry standards (Samuelson et al., 2016) a relatively conservative diet transition protocol was utilized by this commercial feedlot; however, the inclusion of wheat in the diet provided enough potential for depressions in RRpH that we considered this to be valuable data, representative of commercial situations. Due to the nature of liver abscesses healing within 70 d of developing (Itabisashi et al., 1999) and the duration between the end of the measurement period and slaughter, we were not able to utilize liver abscess data to support any relationship between RRpH and pathology in chapter 3. However, based on results presented in Chapter 4, it seems that severity is an important factor to consider, as the duration of time that rumen pH was < 5.2 was associated with rumen and liver pathology at slaughter. Further work must be done to determine the robustness of this threshold (< 5.2) for predicting physiological response beyond pathology, but it certainly provides a promising place to start.

The rumenitis-liver abscess complex is the most common pathologic sequelae of ruminal acidosis seen at slaughter, and although rumens are not often examined, liver pathology is often used as an indicator of rumen health during the finishing period. Until this point, it was not known what ruminal pH pattern led to the development of gross pathology, or if this pathology was associated with a detectable acute phase protein response. Despite capturing a representative overall picture, the weekly sampling frequency was not enough to allow us to develop a sensitive prediction of the relationship between APP and pathology, and this, as well as a relatively small sample size, were limitations of the study.

The intent was to measure lipopolysaccharide binding protein (**LBP**) in weekly blood samples, however, due to an inability to estimate constant value with serial dilution we were unable to validate either of the two commercial enzyme-linked immunoabsorbance assay kits (Hycult

Biotech Inc., Plymouth Meeting, PA. USA; Cell Sciences, Inc., Norwood, MA.USA). As a result, this portion of the study was dropped and we cannot guarantee that the APP response observed in chapter 4 was in fact due to translocation of LPS across the rumen or gastrointestinal tract. Further validation work is therefore needed to determine if APP concentrations hold any promise as predictors of SARA and/or pathology. Nevertheless, this study (Chapter 4) was an important step in determining a biologically relevant threshold for defining ruminal acidosis. Yet, if the goal is to define ruminal acidosis in a way that is meaningful to the beef industry, it is important that we define a pH threshold that is both biologically and economically relevant.

Highly fermentable diets promote performance economics, but also increase the risk for low RRpH. As such, determining the pH threshold where performance and welfare of feedlot cattle are negatively affected will allow for timely intervention while preserving animal performance. Inclusion of fiber in finishing diets is one mitigation strategy suggested to mitigate acidosis. However, despite higher forage diets, SARA is still prevalent in the dairy industry (Kleen and Cannizzo, 2012). It has been suggested that inclusions of 30 to 33% peNDF would be required to successfully mitigate SARA in dairy herds (Zebeli et al., 2008); likely the same would be required in feedlot rations. A recent survey reports the proportion of forage included in typical beef finishing diets to range between 6 and 12% (DM basis; Samuelson et al., 2016). Fiber inclusion at levels suggested above would certainly be costly, as it would limit production efficiency, increasing the time feedlot cattle spend on feed, increasing the environmental resources required (deVries et al., 2015), and increasing methane production (Hales et al., 2014).

Production economics dictate the use of energy dense, high grain diets in feedlot cattle, despite increasing the risk for acidosis. Results from Chapter 3 suggest that conservative protocols to transition from high forage to high concentrate diets can be used to minimize the severity of ruminal acidosis, although the risk for SARA remains high. This again highlights the importance of finding a physiologically relevant threshold, because in beef cattle, SARA has been associated with reductions in ADG and G:F (Castillo-Lopez et al., 2014), and severe liver abscesses have been related to reduced performance (Brink et al*.,* 1990; Rust et al*.,* 1980, and reduced carcass quality (Rust et al., 1990; Brown and Lawrence, 2010), which all translate to lost profit for the feedlot. In addition, the cost of condemned livers can reach up to $4.00 USD/liver ($0.29/cwt live; USDA, Aug 2016) resulting in losses for the packing plants as well. Fortunately, the addition of antibiotics such as rumensin and tylosin to most feedlot diets help to mitigate SARA and prevent

liver abscesses. However, if these feed additives were to be eliminated, it could be expected that the prevalence of liver abscesses may increase and feedlot operators may face larger economic losses.

While we cannot disregard the potential negative welfare and economic implications of ruminal acidosis, it must be considered that risk and performance are likely related in a positive fashion. For example, the greater risk for steers than heifers in Chapter 3 is most likely explained by greater DMI (Appendix Figure I). In fact, previous work suggests that cattle with the greatest ADG are more likely to have mild liver abscesses (Wiese et al., 2015), likely also driven by DMI.

Considering individual animal susceptibility, and the difficulty in making individual animal measurements in feedlot settings, basing management decisions on the most susceptible cattle may in fact be production limiting for the majority of cattle, and therefore a poor economic decision for feedlots. It may therefore be most cost effective to define and manage ruminal acidosis on a per-pen basis. As such, it will be important to determine a threshold of SARA, where enhanced pen performance is balanced with individual animal health and welfare.

6.0 OVERALL CONCLUSIONS

Novel results presented in this thesis suggest that in a commercial feedlot situation, the diet transition period is not high risk when a conservative step up protocol is used. Results also suggest that prevalence and severity should be considered independently and that ruminal pH < 5.2 may prove to be a promising threshold for a biologically relevant definition of SARA. Future work is needed to identify and mitigate ruminal acidosis in at-risk groups, while maintaining performance at the pen level.

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8.0 APPENDIX

8.1 Dry Matter Intake During the Diet Transition

Data presented in this appendix were collected alongside data from chapter 3. It was omitted from that chapter based on the questionable relevance of pen based DMI correlating to individual animal pH data. Despite this, I believe that the overall trends are valuable and as such have been included them within an appendix.

8.2 RESULTS

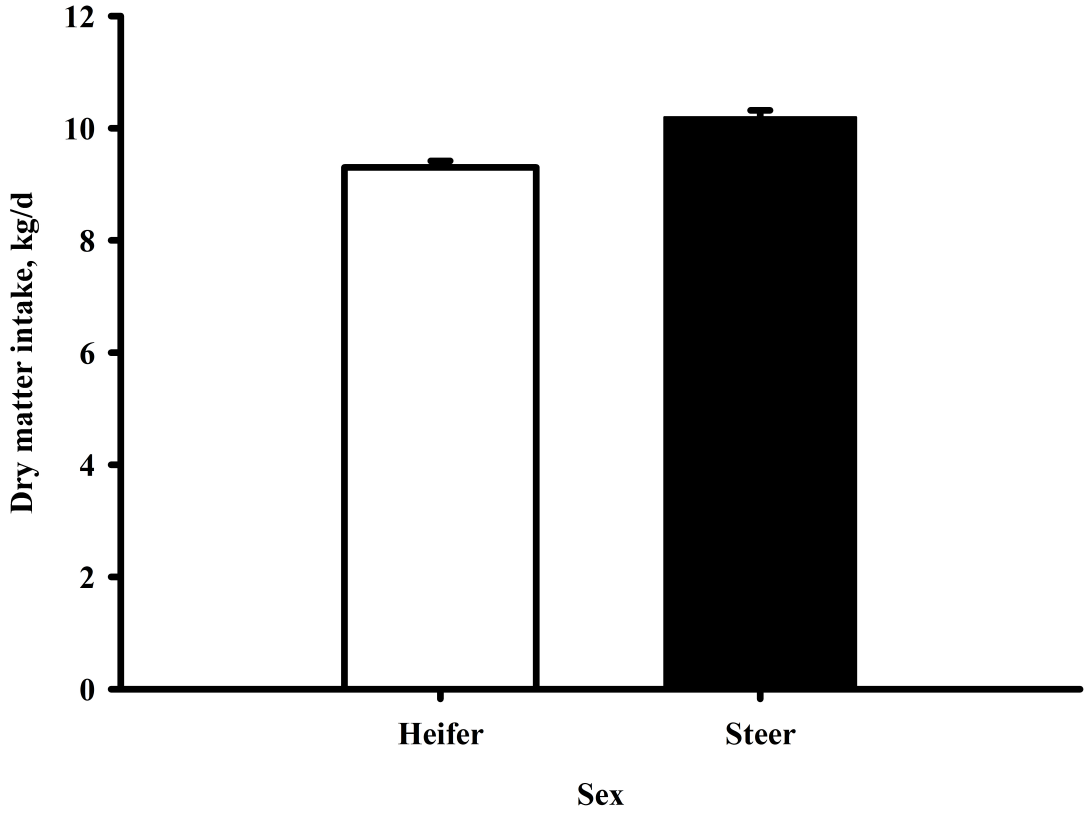
8.2.1 Effect of diet and sex on DMI during dietary transition

Dry matter intake was not affected by a diet × sex interaction ($P = 0.13$, data not shown), but DMI was greater for steers than heifers averaging 10.2 vs. 9.3 kg/d, respectively ($P < 0.001$; Appendix Figure I). Dry matter intake was also affected by diet ($P < 0.001$) with an increase in DMI from diet 1 to diet 3, reaching a peak of 10.2 kg/d, before declining to 9.4 kg/d for diet 6 (Appendix Figure II).

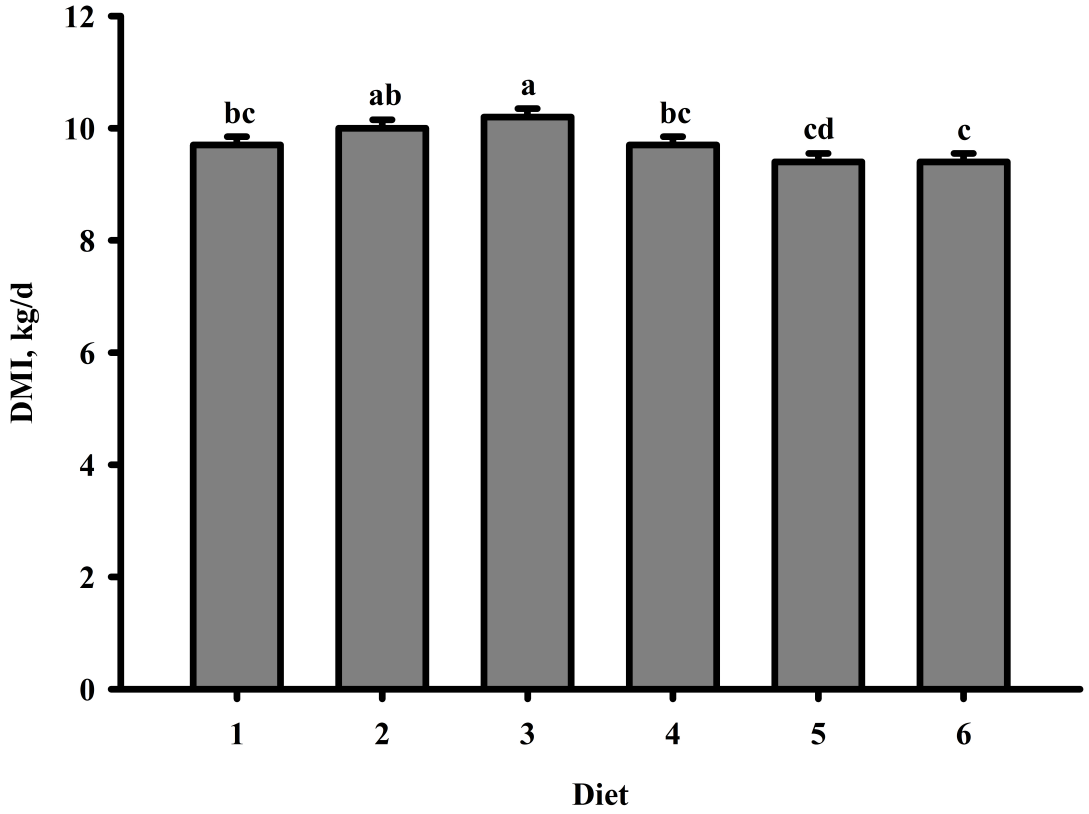
There was also a diet × day interaction ($P < 0.001$) for DMI, which occurred because DMI did not differ in the 5-d following dietary change when cattle were transitioned to diets 2 or 3 but a reduction in DMI was observed when cattle were fed diet 4 on d 5 (Appendix Figure III). The reduced intake persisted during diet 5 and 6 with the lowest intake occurring on d 3 and 4 relative to d 1 on diet 5 and on d 1, 2, and 3 relative to d 5 on diet 6.

8.3 DISCUSSION

Across all of the diets, the DMI of steers was greater than heifers. Greater intake for steers than heifers is common and most readily explained by the positive relationship between BW and DMI (NRC, 2000). When both heifers and steers were considered, variation in daily DMI occurred between and within diets. Past studies have also reported marked variability in DMI among days during the dietary transition period (Bevans et al., 2005; Brown et al., 2006; Holsthausen et al., 2016). It should be noted that in the present study, a reduction in DMI also occurred for diets 4, 5, and 6 that coincided with the replacement of barley for wheat. Wheat inclusion has been previously reported to reduce intake, potentially due to the increased energy



Appendix Figure I. Dry matter intake of steers and heifers over a 5-step (6 diet) transition period in a commercial feedlot. Values with uncommon superscripts differ (*P*<0.001; SEM=0.1173*).* The Diet forage to concentrate ratio (DM basis): diet 1 (46.5:53.5), diet 2 (38.5:61.5), diet 3 (31.5:68.5), diet 4 (31.5:68.5), diet 5 (23.5:76.5), and diet 6 (9.5:90.5). In diets 1 and 2 the grain source was barley grain while wheat was included in diet 3 at 8% DM, and for diets 4, 5 and 6, wheat was the sole grain source. Cattle were previously adapted to diet 1 prior to the start of the study and were fed that diet for 65.7 ± 14.0 d.

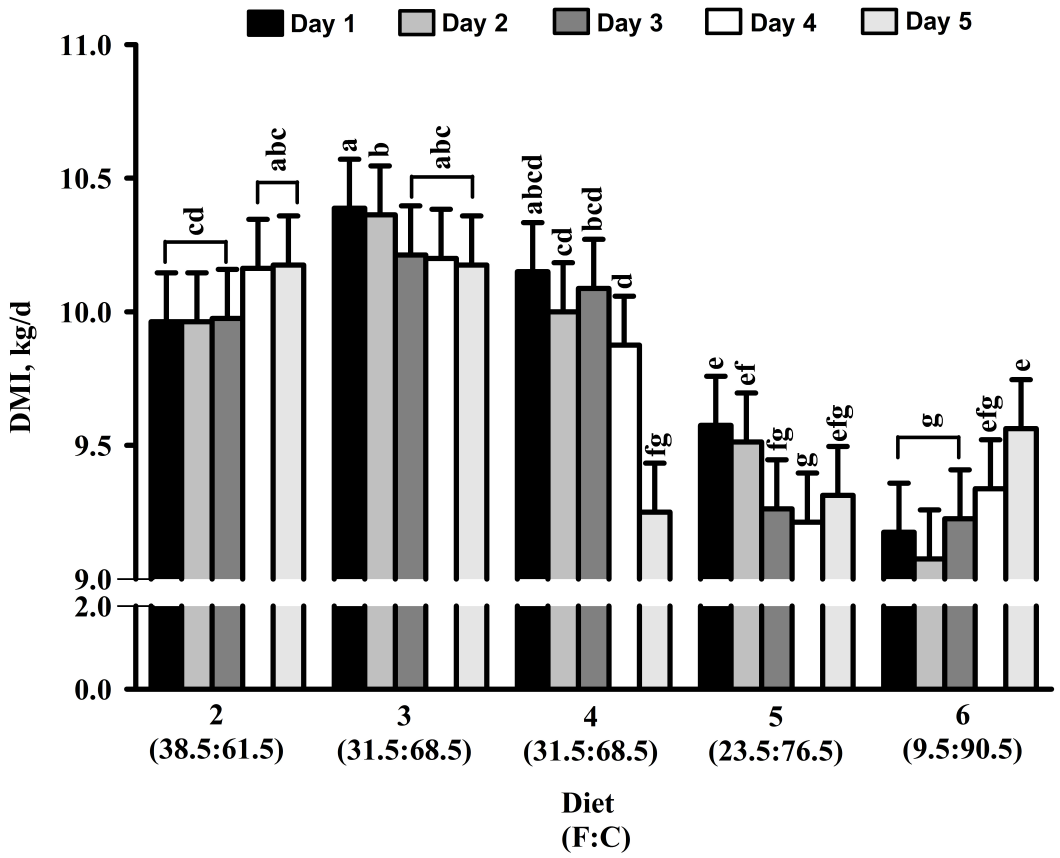


Appendix Figure II. Average Dry matter intake by diet of steers and heifers during the diet transition period. Values with uncommon superscripts differ (*P*<0.05; SEM=0.15*).* The Diet forage to concentrate ratio (DM basis): diet 1 (46.5:53.5), diet 2 (38.5:61.5), diet 3 (31.5:68.5), diet 4 (31.5:68.5), diet 5 (23.5:76.5) diet 6 (9.5:90.5). In diets 1 and 2 the grain source was barley grain while wheat was included in diet 3 at 8% DM, and for diets 4, 5 and 6, wheat was the sole grain source. Cattle were previously adapted to diet 1 prior to the start of the study and were fed that diet for 65.7 ± 14.0 d.

content of the diet (Owens et al., 1998; Zinn et al., 2008; He et al., 2015). However, we also observed that reticulo-ruminal pH decreased with increasing concentrate inclusion and several studies have reported a reduction in DMI in response to ruminal acidosis (Brown et al., 2006; Castillo-Lopez et al., 2014; Holtshausen et al., 2016). However, in the current study, DMI and reticulo-rumen pH values were not correlated ($R^2 < 0.08$; data not shown), likely because DMI was measured on a pen basis whereas reticulo-ruminal pH was the average of 4 focal cattle in each pen.

8.4 CONCLUSION

Despite the known relationship between DMI and ruminal pH, we were unable to use the DMI intake data in the current study (Chapter 3) to draw any conclusions. However, data supports that steers consume more than heifers, and as such might be at greater risk for SARA. This data also suggests that there is no predictable pattern to DMI in the days following a diet transition, when measured at a pen level.



Appedix Figure III. Average daily values for DMI (day × diet; $P < 0.001$) among days within a diet relative to the dietary transition on d 1 through 5 following each diet change in a 5-step diet transition program. All cattle were transitioned from a diet with a forage:concentrate ratio of 46.5:53.5 (diet 1; cattle were fed this diet prior to the start of the study for 65.7 ±14.0 d) to a diet with a forage:concentrate ratio of 9.5:90.5 (diet 6; DM basis) using 5 dietary steps over 40 d. Error bars indicate the SEM for the interaction and bars with uncommon superscripts differ ($P < 0.05$). Pen was considered the experimental unit with 4 pens/sex.