IMMUNITY AND SUB-ACUTE RUMINAL ACIDOSIS

T. F. Gressley
Department of Animal and Food Sciences
University of Delaware

INTRODUCTION

Sub-acute ruminal acidosis (SARA) can occur as a consequence of feeding high energy rations to dairy cattle. During SARA, the rate of rumen short-chain fatty acid (SCFA) production exceeds SCFA absorption and results in an unhealthy depression of rumen pH. The severity of SARA is quantified based on the duration and magnitude of depression of rumen pH below a threshold (typically 5.6 or 5.8), and has been most well characterized in ruminally cannulated cows.

Consequences of SARA include depression and fluctuations in intake, reduced diet digestibility, reduced milk yield, reduced milk fat percent, gastrointestinal damage, liver abscesses, and lameness (Krause and Oetzel, 2006; Radostits et al., 2007; Plaizier et al., 2008). Though some of these effects can be remedied with management changes to resolve SARA, localized and systemic inflammation resulting from SARA can cause long term negative impacts on animal health and wellbeing. This review will discuss the impacts of SARA on the digestive tract, inflammation resulting from SARA, and steps that can be taken to reduce SARA.

IMPACT OF SARA ON THE RUMEN

During SARA, the increase in ruminally fermentable carbohydrates increases SCFA production and leads to shifts in the rumen microbiome. Several studies have used sequencing technologies to evaluate the changes in the rumen microbiome in response to SARA induction or high grain feeding. High grain feeding or SARA has been found to decrease in diversity of both the rumen fluid microbiome and the bacteria adhered to the rumen epithelium (Mao et al., 2013; Petri et al., 2013; Wetzels et al., 2017). When sequencing technologies have been used to characterize the rumen microbiome, at the phylum level, high grain diets tend to increase the relative abundance of *Firmicutes* and decrease the relative abundance of *Bacteroidetes* (Khafipour et al., 2009b; Mao et al., 2013), though effects are not always consistent across studies. The use of PCR to identify changes at the species level has demonstrated that high grain rations or a SARA challenge can result in a decrease in fiber fermenting bacteria including *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* and an increase in *Streptococcus bovis*, *Escherichia coli*, and *Megasphaera elsdenii* (Tajima et al., 2001; Khafipour et al., 2009b; Petri et al., 2013). Khafipour et al. (2009b) found that the increase in *E. coli* was positively correlated with the severity of SARA symptoms, leading them to conclude that increases in *E. coli* may be important to the etiology of SARA. In a follow up study, they found that the concentration of *E. coli* and *E. coli* virulence factors in rumen fluid was approximately 3 logs higher in cows given a grain based SARA challenge known to cause inflammation compared to alfalfa pellet induced SARA that does not trigger an inflammatory response

(Khafipour et al., 2011). Dysbiosis is a term used to describe an unhealthy shift in the bacterial community and has been associated with inflammatory diseases in humans and rodent models (Caesar et al., 2012; Vieira et al., 2013; Koboziev et al., 2014). A similar phenomenon may be occurring in the rumen and intestines as a result of SARA (Khafipour et al., 2016). Collectively, these data suggest that shifts in rumen bacterial communities in response to SARA are a key first step in the negative impacts of SARA on animal performance.

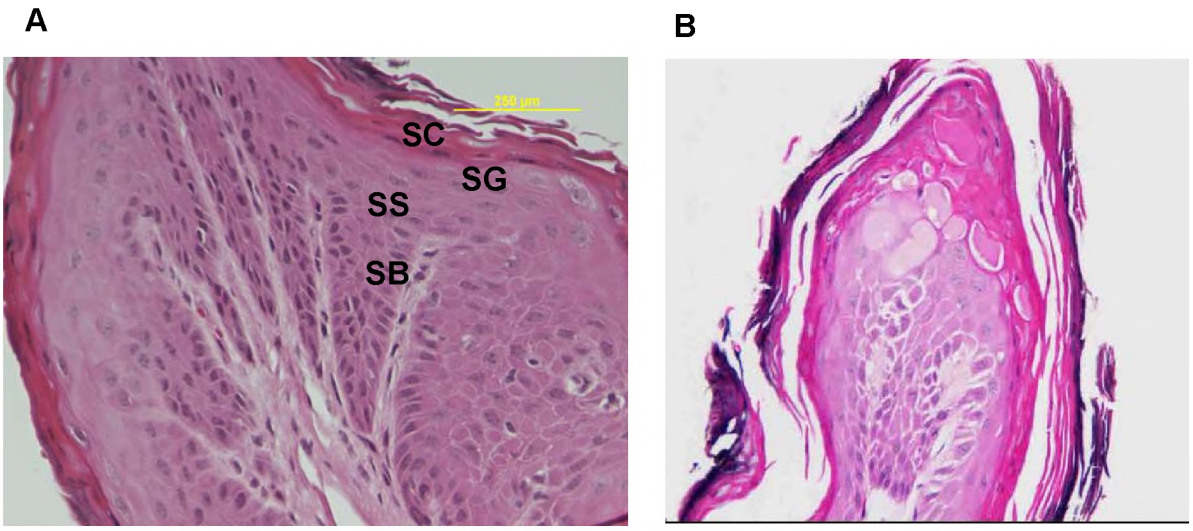
Concurrent with shifts in microbial populations, there is also an increase in rumen concentrations of potentially toxic and inflammatory compounds during SARA. The one that has received the most attention is lipopolysaccharide (**LPS**). Lipopolysaccharide is a component of gram negative bacterial cell walls, and is classified as an endotoxin because the presence of LPS within the body elicits an inflammatory response by mammalian cells. When animals are challenged with a SARA-inducing ration, the availability of fermentable carbohydrates initially results in logarithmic growth of bacteria, which is later followed by massive bacterial lysis in response to reduced availability of substrates, reduced rumen pH, and accumulation of fermentation end products (Zebeli and Metzler-Zebeli, 2012). Free LPS accumulates both during rapid growth and during bacterial lysis, resulting in increased rumen concentrations of LPS during SARA (Li et al., 2012). During an acute acidosis challenge in cows, rumen fluid collected following the challenge had increased endotoxin activity and became increasingly toxic when injected into mice (Nagaraja et al., 1978). These results led the authors to conclude that the effects of acidosis were mediated by systemic effects of rumen endotoxin. In addition, rumen concentrations of LPS were found to be negatively correlated with milk fat percentage and yield when cows were fed increasing levels of barley grain (Zebeli and Ametaj, 2009). Although rumen accumulation of LPS during SARA may be important for subsequent inflammatory responses, the immunoreactive properties of LPS differ among bacterial species, and Khafipour et al. (2009b) propose that the inflammatory response to SARA is due to *E. coli* LPS.

Other potentially harmful compounds produced during SARA include biogenic amines and ethanol (Ametaj et al., 2010). Ethanolamine is a biogenic amine that not only has potentially harmful effects on the host but has also been shown to enhance growth and virulence factor production by pathogenic bacteria (Saleem et al., 2012; Zebeli and Metzler-Zebeli, 2012). Histamine is another biogenic amine produced during SARA and its potential role during the inflammatory response to SARA will be discussed later in this review as it relates to laminitis.

The rumen epithelium serves as a selective barrier, allowing for absorption of SCFA while preventing entry and colonization by bacteria. Systemic effects of SARA are dependent upon a breach in this barrier. Structurally the rumen epithelium consists of four layers, the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Figure 1). In the healthy rumen, bacteria are loosely associated only with the stratum corneum. Tight junction proteins that regulate the permeability barrier are expressed most heavily in the stratum granulosum and to some extent in the stratum spinosum (Graham and Simmons, 2005). Connections among the stratum granulosum, stratum spinosum,

and stratum basale allow for the transport of SCFA from the rumen contents to the basal lamina (Graham and Simmons, 2005). The permeability barrier function of the rumen responds to changes in the animal or the rumen. For example, permeability is increased during oxidative stress, heat stress, and feed restriction (Mani et al., 2012; Zhang et al., 2013). Increased permeability may also be an adaptive response to higher grain diets to allow for increased uptake of SCFA (Zebeli and Metzler-Zebeli, 2012). Studies using isolated sections of rumen have also demonstrated increased permeability in response to acidification or hyperosmolality (Schweigel et al., 2005; Emmanuel et al., 2007).

Figure 1. A. Cross-section of a rumen papilla showing the stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and stratum basale (SB). **B.** Damaged papilla showing separation of stratum corneum.



CHANGES IN THE DIGESTIVE MUCOSA IN RESPONSE TO SARA

In addition to its role as a selective barrier, the rumen and intestinal epithelia helps direct immune function through its interactions with gut-associated lymphoid tissue (**GALT**). These microstructures are found throughout the digestive mucosa and consist of clusters of white blood cells and immune structures in close association with digestive epithelium. They range in size from large Peyer's patches to small isolated lymphoid follicles and consist of clusters of B and T lymphocytes interspersed with dendritic cells and phagocytes (Goto and Kiyono, 2012). Antigen presentation from M cells or dendritic cells causes B cells to be activated to IgA-secreting plasma cells. Secretory IgA then exits the columnar epithelial cells via transcytosis where it accumulates in the mucus to prevent attachment and translocation of target bacteria (Kamada et al., 2013). T cells in germinal centers within GALT are activated by receptor binding of microbial products and locally produced cytokines. In a healthy animal, tolerance of commensal gut microorganisms is facilitated by T cells with a regulatory phenotype that tend to suppress inflammatory responses by surrounding cells (Littman and Pamer, 2011).

Activities of lymphocytes and phagocytes within the gut of a healthy animal respond to the microbiome to elicit appropriate responses: tolerance and local immunosuppression in response to commensal organisms and inflammation and immune activation in response to a pathogenic challenge. Binding of bacterial components to pathogen recognition receptors such as toll like receptors (TLR) and NOD-like receptors is essential for homeostasis. Normal development of GALT structures is dependent upon functional pathogen recognition by these receptors. In addition to promoting proper GALT development, a symbiotic mix of commensal bacteria promotes mucus production and barrier function of the epithelium and inhibits colonization by competitive organisms (Kamada et al., 2013). The effects of the microbiome on GALT and mucosal functions are not only through direct interactions of microbial components with receptors but also through products of symbiotic microbes including short chain fatty acids (Brestoff and Artis, 2013). Dysbiosis can down-regulate these protective functions, stimulate mucosal inflammation, and potentiate colonization by pathogenic organisms.

Downstream inflammatory effects of SARA are dependent on a breach in the permeability barrier of the digestive epithelium. During SARA, some combination of increased osmolality, reduced pH, increased bacterial toxins such as LPS, and increased biogenic amines leads to disruption of the barrier function. A study using isolated rumen and colon tissue from steers demonstrated that LPS and decreased pH acted synergistically to reduce barrier function (Emmanuel et al., 2007). Once the epithelium has been breached, GALT cells may respond by triggering local inflammation and altering cytokine production; this in turn may further increase permeability, potentiate colonization by pathogenic organisms, enhance passage of bacteria and toxins across the epithelium, and increase the inflammatory response (Mani et al., 2012; Kurashima et al., 2013). When cows were switched from a 0% grain ration to a 65% grain ration, the rumen epithelium underwent dramatic changes including visible papillae lesions, decreased tight junctions, sloughing of the stratum corneum, and presence of bacteria in the stratum granulosum and stratum spinosum (Steele et al., 2011). Khafipour et al. (2011) found increased RNA levels of virulence and adhesion factors in *E. coli* isolated from rumen fluid during grain-induced SARA, indicating that SARA may increase the potential for pathogenic organisms to take advantage of a breach in epithelial integrity and colonize papillae.

Concurrent with local inflammation in the papillae are changes in epithelial cell cycle, adhesion protein expression, and SCFA absorption. Compared to cows fed high forage diets, high concentrate diets have resulted in dramatic differences in gene expression, including differences in genes for adhesion proteins and cell cycle regulation (Taniguchi et al., 2010; Steele et al., 2011; Ma et al., 2017). An in vitro study by Meissner et al. (2017) found that exposure of rumen tissue to reduced pH and increased SCFA disrupted epithelial barrier function and reduced expression of the tight junction proteins occludin, claudin-4, and claudin-7. Similarly, a study in goats revealed that a high grain diet decreased the epithelial integrity and the expression of claudin-4 and occludin in the omasum (Liu et al., 2014). Collectively, these studies demonstrate that SARA causes dramatic changes to the rumen epithelium including reduced barrier function. Injury to the rumen epithelium and changes to the cell cycle in response to SARA can result in parakeratosis or hyperkeratosis (Penner et al., 2011). Increased exposure of the more

basal epithelial layers to bacteria and toxins as a result of parakeratosis can further increase rumenitis and lead to the formation of microabscesses (Kleen et al., 2003).

Events that occur in the rumen during SARA are mirrored in the large intestine. An increase in intestinal carbohydrate fermentation typically occurs concurrent with SARA and leads to increased concentrations of SCFA and LPS, a reduction in pH, and damage to the intestinal mucosa (Bissell, 2002; Dijkstra et al., 2012; Li et al., 2012). Fecal indicators of SARA include diarrhea, frothy feces, increased particle size in feces, and presence of mucin casts in feces (Hall, 2002). Because the intestinal epithelium is composed of only a single layer of epithelial cells, it has been proposed that systemic inflammatory effects of SARA might be due to passage of bacteria or toxins through the intestinal mucosa (Oetzel, 2003). In fact, Khafipour et al. (2009a) found that the timing of the presence of LPS in the blood following a SARA challenge suggested LPS entered the circulation via the intestines instead of the rumen. Studies in goats found that compared to feeding high forage diet, a high concentrate ration resulted in evidence of colonic damage including epithelial injury, damaged tight junctions, increased markers of apoptosis, increased inflammatory cell infiltration, and greater gene expression of inflammatory mediators (Tao et al., 2014a; Tao et al., 2014b).

SYSTEMIC EFFECTS OF SUB-ACUTE RUMINAL ACIDOSIS

If bacteria or toxins enter the mucosa through a breach in the epithelium, they may trigger localized inflammation, enter the liver through the portal blood supply, or travel systemically through the lymphatics or blood. For example, both SARA and acute acidosis result in increased concentrations of endotoxin in both the hepatic portal vein and hepatic vein (Haubro Andersen et al., 1994; Chang et al., 2015). These results indicate that endotoxin generated in the digestive tract directly enters the liver where it can affect liver function and also exits the liver where it can trigger systemic effects. The presence of endotoxins in the liver, bloodstream, or lymphatic system can then trigger systemic inflammation (Eckel and Ametaj, 2016). For example, Chang et al. (2015) found that expression of inflammatory genes was up-regulated in the livers of cows fed a SARA inducing diet. Increased toxin flow to the liver can result in hepatocyte damage, and Bobe et al. (2004) noted that SARA can increase the likelihood of fatty liver which can further impair liver function. Further, SARA increases oxidative stress in the liver (Abaker et al., 2017), which can damage liver tissue and reduce the ability of the liver to detoxify gut-derived endotoxin. In addition, if live bacteria exit or bypass the liver, they can cause chronic inflammatory diseases in response to SARA such as pneumonia, endocarditis, pyelonephritis, and arthritis (Oetzel, 2007). Bacteria may also colonize the liver and form abscesses. *Fusobacterium necrophorum* is the primary agent isolated from liver abscesses in feedlot cattle, and the liver infection is secondary to infection of the rumen wall (Nagaraja and Chengappa, 1998). This normal inhabitant of the rumen increases in number in response to high grain diets and can opportunistically colonize a rumen wall that has been damaged by parakeratosis or rumenitis in response to SARA (Tadepalli et al., 2009). These are just a few examples of the negative effects of SARA on liver function, and it is likely that other bacterial products and toxins entering the liver as a result of SARA may also impair liver function.

One clear response of the liver to grain-induced SARA is production of acute phase proteins that can modify immune function and generate a systemic inflammatory response. Acute phase proteins include serum amyloid A, haptoglobin, LPS-binding protein, C-reactive protein, and α-1 acid glycoprotein. Their effects are multifaceted and include stimulating or suppressing an immune response, stimulating tissue repair, removing harmful compounds, isolating infectious agents, and preventing or modifying inflammation (Zebeli and Metzler-Zebeli, 2012; Eckel and Ametaj, 2016). In addition, endotoxins acting directly or indirectly through acute phase proteins can trigger release of inflammatory cytokines by the liver and tissues (Eckel and Ametaj, 2016). Plaizier et al. (2008) summarized results from multiple SARA challenge studies and proposed that LPS, inflammatory amines, or other products of bacteria that reach the liver stimulate release of acute phase proteins from the liver and generate a systemic inflammatory response. Thus, systemic inflammation does not appear to be dependent on bacterial compounds reaching the general circulation. In addition to their release by the liver, mRNA expression of acute phase proteins has also been detected in the gastric mucosa, indicating that the mucosa may contribute directly to this inflammatory response as well (Dilda et al., 2012).

Studies have also been aimed at evaluating why grain-based SARA challenges induce an increase in circulating acute phase proteins while alfalfa-based SARA challenges fail to do so. In a study using cows with ruminal and cecal cannulas, Li et al. (2012) found that although rumen concentrations of LPS increased in response to both types of challenges, cecal concentrations of LPS only increased in response to the grain-based challenge. They propose that translocation of LPS from the large intestine to the liver of grain-challenged animals might account for the increase in acute phase proteins. However, using challenge models that bypassed the rumen, we and others have been unable to generate similar increases in plasma acute phase proteins as found in response to high grain diets, perhaps due to the short-term nature of those challenges (Bissell, 2002; Mainardi et al., 2011). Khafipour et al. (2009b) found that of the microbiome shifts in response to SARA, rumen *E. coli* abundance, which increased only in response to grain-based SARA challenges, was most strongly associated with concentration of acute phase proteins in the blood. These results suggest that differences in bacterial products reaching the liver in response to dietary changes can differentially impact acute phase protein production. Those authors also suggested that increased LPS binding protein concentrations in the blood are a direct indicator of LPS translocation from the rumen to the liver (Khafipour et al., 2009a). As data on acute phase protein response to SARA continues to mount, it is becoming clear that direct passage of LPS or other bacterial products to the general circulation may not be necessary for the systemic inflammatory response to SARA. Instead, immune modulation at the level of the liver or even the gut mucosa seems to be sufficient to drive systemic inflammation.

Laminitis and lameness are consequences of SARA, and it is likely that similar mechanisms to those driving systemic inflammatory responses to SARA also mediate hoof damage. In response to rumen acidosis, vasoactive substances including LPS and biogenic amines can be absorbed across the gut mucosa. Damage to the gut wall and

entry of bacterial products can drive formation of endogenous vasoactive products including cytokines and prostaglandins. The primary effect of these exogenous and endogenous compounds is dilation of arterioles and constriction of venules which at the level of the gut can enhance inflammation and increase entry of toxins (Shearer, 2011; Eckel and Ametaj, 2016). In the corium of the hoof, these vascular changes result in inflammation, hemorrhage, death of cells, activation of matrix metalloproteinases, and disruption of growth factor signaling (Shearer, 2011). Altered cell growth, cell damage, reduced oxygen and nutrient flow, and reduction of intercellular adhesion can cause sinkage of the pedal bone, damage to the corium, pain, and lesions (Nocek, 1997; Goff, 2006). Histamine that is absorbed from the gut or produced endogenously during inflammation has been proposed to play a key role in development of laminitis. In a study using bulls, Takahashi and Young (1981) demonstrated that grain overload and histamine injection to the digital artery acted synergistically to induce laminitis. As reviewed by Katz and Bailey (2012), equine laminitis resulting from starch overload occurs via a similar mechanism to that proposed in ruminants. A loss of barrier function in the gut allows for influx of bacterial products including LPS and amines into the portal circulation. The resulting inflammatory changes in liver and leukocytes, with or without systemic entry of toxins, is proposed to cause laminitis through vascular changes in the hoof, apoptosis, oxidative injury, and enzymatic degradation of the basement membrane (Katz and Bailey, 2012).

MANAGING COWS TO REDUCE THE IMPACT OF SARA

At the level of the rumen, causes of SARA can broadly be classified as management, environmental, and animal factors which reduce ruminal buffering capacity or increase ruminal SCFA accumulation. As reviewed by Stone (2004), buffering capacity can be increased by increasing dietary forage content and optimizing particle size to increase chewing and saliva flow, by addition of external buffers or alkalinizing agents to the ration, and by increasing the dietary cation anion difference of the ration. Risks of SARA can be reduced by following feeding recommendations including maintaining adequate particle size and physically effective fiber and avoiding excesses of fermentable carbohydrates (Stone, 2004). Buffering capacity can be reduced in response to heat stress or as a result of decreased chewing, for example due to feed sorting. Increasing dietary buffering during heat stress, practicing heat stress abatement, and preventing feed sorting can help to reduce the incidence of SARA. The rate of SCFA production and the risk for SARA can be increased in response to increased dietary proportion of grain, increased fermentability of grains or forages, increased feed intake, and management factors that lead to larger and less frequent meals. Fecal consistency should be regularly monitored, particularly following ration changes, for signs of SARA in the herd. Individual differences in SARA susceptibility may be related to feed intake variation, variation in saliva buffering, variation in SCFA uptake, and differences in endotoxin tolerance (Khafipour et al., 2009b).

Dietary supplements offer the potential to reduce the negative impacts of SARA, either by mitigating events in the digestive tract or by reducing subsequent inflammatory events. Inclusion of live yeast or yeast product can help to stabilize the rumen microbiome

to alleviate the negative effects of SARA (AlZahal et al., 2014). Other direct fed microbials including *Enterococcus faecium* or *Lactococcus lactis* may help to stabilize the rumen environment (Chiquette et al., 2015). Inclusion of feed supplements such as linseed oil or fish oil that contain high levels of omega-3 fatty acids may help to reduce the inflammatory response and tissue damage that can result from feeding high carbohydrate diets (Mani et al., 2012). Other dietary supplements such as biotin and zinc have the potential to strengthen epithelium to prevent tissue injury from SARA (Goff, 2006). A recent study also indicated that thiamine supplementation during a SARA challenge increased rumen pH, decreased rumen LPS, and reduced expression of inflammatory proteins in the rumen epithelium (Pan et al., 2017). The immunomodulatory agent OmniGen-AF (Phibro Animal Health, Teaneck, NJ) has been shown to reduce the systemic inflammatory response to LPS (Brandão et al., 2016), suggesting that it may help to alleviate SARA symptoms by reducing the systemic response to endotoxin derived from the digestive tract. Finally, as we continue to increase our understanding of pathologic bacteria that contribute to SARA-induced tissue damage, there may be potential to develop management strategies to reduce the competitive ability of those organisms. For example, Gill et al. (2000) found that vaccination against *Streptococcus bovis* reduced the severity of response to an acute acidosis challenge in sheep, and future development of vaccines against pathologic bacteria associated with SARA might be beneficial.

CONCLUSIONS

Sub-acute ruminal acidosis impairs cow performance and health. Rumenitis is the initial insult of SARA and results in inflammatory and immune activation which reduces energy available to support production, allows for transfer of bacterial products across the gut epithelium, and can damage tissues including the liver and hoof. Sub-acute ruminal acidosis will likely continue to be a problem for the dairy industry as high energy diets are required to support high levels of milk production. Careful attention to nutritional management and development of new SARA mitigation strategies may help to reduce its impact in the future.

REFERENCES

Abaker, J. A., T. L. Xu, D. Jin, G. J. Chang, K. Zhang, and X. Z. Shen. 2017. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. J. Dairy Sci. 100:666-678.

AlZahal, O., L. Dionissopoulos, A. H. Laarman, N. Walker, and B. W. McBride. 2014. Active dry *Saccharomyces cerevisiae* can alleviate the effect of subacute ruminal acidosis in lactating dairy cows. J. Dairy Sci. 97:7751-7763.

Ametaj, B. N., Q. Zebeli, F. Saleem, N. Psychogios, M. J. Lewis, S. M. Dunn, J. G. Xia, and D. S. Wishart. 2010. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics 6:583-594.

Bissell, H. A. 2002. Post-ruminal starch infusion in dairy cattle: implications for inflammatory response and animal health. M.S. Thesis University of Florida.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87:3105-3124.

Brandão, A. P., R. F. Cooke, F. N. Corrá, M. B. Piccolo, R. Gennari, T. Leiva, and J. L. M. Vasconcelos. 2016. Physiologic, health, and production responses of dairy cows supplemented with an immunomodulatory feed ingredient during the transition period. J. Dairy Sci. 99:5562-5572.

Brestoff, J. R. and D. Artis. 2013. Commensal bacteria at the interface of host metabolism and the immune system. Nature Immunol. 14:676-684.

Caesar, R., C. S. Reigstad, H. K. Backhed, C. Reinhardt, M. Ketonen, G. O. Lunden, P. D. Cani, and F. Backhed. 2012. Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. Gut 61:1701-1707.

Chang, G. J., K. Zhang, T. L. Xu, D. Jin, J. F. Guo, S. Zhuang, and X. Z. Shen. 2015. Epigenetic mechanisms contribute to the expression of immune related genes in the livers of dairy cows fed a high concentrate diet. Plos One 10. ARTN e0123942 DOI:10.1371/journal.pone.0123942.

Chiquette, J., J. Lagrost, C. L. Girard, G. Talbot, S. Li, J. C. Plaizier, and I. K. Hindrichsen. 2015. Efficacy of the direct-fed microbial *Enterococcus faecium* alone or in combination with *Saccharomyces cerevisiae* or *Lactococcus lactis* during induced subacute ruminal acidosis. J. Dairy Sci. 98:190-203.

Dijkstra, J., J. L. Ellis, E. Kebreab, A. B. Strathe, S. Lopez, J. France, and A. Bannink. 2012. Ruminal pH regulation and nutritional consequences of low pH. Anim. Feed Sci. Tech. 172:22-33.

Dilda, F., L. F. Pisani, M. M. Rahman, S. Modina, I. Tessaro, P. Sartorelli, F. Ceciliani, and C. Lecchi. 2012. Distribution of acute phase proteins in the bovine forestomachs and abomasum. Vet. J. 192:101-105.

Eckel, E. F. and B. N. Ametaj. 2016. Invited review: Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. J. Dairy Sci. 99:5967-5990.

Emmanuel, D. G., K. L. Madsen, T. A. Churchill, S. M. Dunn, and B. N. Ametaj. 2007. Acidosis and lipopolysaccharide from *Escherichia coli* B:055 cause hyperpermeability of rumen and colon tissues. J. Dairy Sci. 90:5552-5557.

Gill, H. S., Q. Shu, and R. A. Leng. 2000. Immunization with *Streptococcus bovis* protects against lactic acidosis in sheep. Vaccine 18:2541-2548.

Goff, J. P. 2006. Major advances in our understanding of nutritional influences on bovine health. J. Dairy Sci. 89:1292-1301.

Goto, Y. and H. Kiyono. 2012. Epithelial barrier: an interface for the cross-communication between gut flora and immune system. Immunol Rev 245:147-163.

Graham, C. and N. L. Simmons. 2005. Functional organization of the bovine rumen epithelium. Am J Physiol-Reg I 288:R173-R181.

Hall, M. B. 2002. Rumen acidosis: carbohydrate feeding considerations. Pages 51-61 in In: Proc. 12th Int. Symp. on Lameness in Ruminants. Orlando, FL.

Haubro Andersen, P., M. Hesselholt, and N. Jarlov. 1994. Endotoxin and arachidonic-acid metabolites in portal, hepatic and arterial blood of cattle with acute ruminal acidosis. Acta Vet Scand 35:223-234.

Kamada, N., S. U. Seo, G. Y. Chen, and G. Nunez. 2013. Role of the gut microbiota in immunity and inflammatory disease. Nat. Rev. Immunol. 13:321-335.
Katz, L. M. and S. R. Bailey. 2012. A review of recent advances and current hypotheses on the pathogenesis of acute laminitis. Equine Vet. J. 44:752-761.
Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009a. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060-1070.
Khafipour, E., S. Li, J. C. Plaizier, and D. O. Krause. 2009b. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. Appl. Environ. Microbiol. 75:7115-7124.
Khafipour, E., S. Li, H. M. Tun, H. Derakhshani, S. Moossavi, and J. C. Plaizier. 2016. Effects of grain feeding on microbiota in the digestive tract of cattle. Anim. Frontiers 6:13-19.
Khafipour, E., J. C. Plaizier, P. C. Aikman, and D. O. Krause. 2011. Population structure of rumen *Escherichia coli* associated with subacute ruminal acidosis (SARA) in dairy cattle. J. Dairy Sci. 94:351-360.
Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. T. M. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): a review. J Vet Med A 50:406-414.
Koboziev, I., C. R. Webb, K. L. Furr, and M. B. Grisham. 2014. Role of the enteric microbiota in intestinal homeostasis and inflammation. Free Radical Bio. Med. 68:122-133.
Krause, K. M. and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim Feed Sci Tech 126:215-236.
Kurashima, Y., Y. Goto, and H. Kiyono. 2013. Mucosal innate immune cells regulate both gut homeostasis and intestinal inflammation. Eur. J. Immunol. 43:3108-3115.
Li, S., E. Khafipour, D. O. Krause, A. Kroeker, J. C. Rodriguez-Lecompte, G. N. Gozho, and J. C. Plaizier. 2012. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. J. Dairy Sci. 95:294-303.
Littman, D. R. and E. G. Pamer. 2011. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 10:311-323.
Liu, J. H., T. T. Xu, W. Y. Zhu, and S. Y. Mao. 2014. A high-grain diet alters the omasal epithelial structure and expression of tight junction proteins in a goat model. Vet. J. 201:95-100.
Ma, L., M. Zhao, L. S. Zhao, J. C. Xu, J. J. Loor, and D. P. Bu. 2017. Effects of dietary neutral detergent fiber and starch ratio on rumen epithelial cell morphological structure and gene expression in dairy cows. J. Dairy Sci. 100:3705-3712.
Mainardi, S. R., B. A. Hengst, S. J. Nebzydoski, L. M. Nemec, and T. F. Gressley. 2011. Effects of abomasal oligofructose on blood and feces of Holstein steers. J. Anim. Sci. 89:2510-2517.
Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. 2012. GROWTH AND DEVELOPMENT SYMPOSIUM: Endotoxin, inflammation, and intestinal function in livestock. J. Anim. Sci. 90:1452-1465.
Mao, S. Y., R. Y. Zhang, D. S. Wang, and W. Y. Zhu. 2013. Impact of subacute ruminal acidosis (SARA) adaptation on rumen microbiota in dairy cattle using pyrosequencing. Anaerobe 24:12-19.

Meissner, S., F. Hagen, C. Deiner, D. Gunzel, G. Greco, Z. M. Shen, and J. R. Aschenbach. 2017. Key role of short-chain fatty acids in epithelial barrier failure during ruminal acidosis. J. Dairy Sci. 100:6662-6675.

Nagaraja, T. G., E. E. Bartley, L. R. Fina, and H. D. Anthony. 1978. Relationship of rumen gram-negative bacteria and free endotoxin to lactic-acidosis in cattle. J. Anim. Sci. 47:1329-1337.

Nagaraja, T. G. and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci. 76:287-298.

Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80:1005-1028.

Oetzel, G. R. 2003. Subacute ruminal acidosis in dairy cattle. Adv. Dairy Technol. 15:307-317.

Oetzel, G. R. 2007. Subacute ruminal acidosis in dairy herds: Physiology, pathophysiology, milk fat responses, and nutritional management. Pages 89-119 in Proc. AABP 40th Annual Conference, Vancouver, BC, Canada.

Pan, X. H., L. Yang, Y. Beckers, F. G. Xue, Z. W. Tang, L. S. Jiang, and B. H. Xiong. 2017. Thiamine supplementation facilitates thiamine transporter expression in the rumen epithelium and attenuates high-grain-induced inflammation in low-yielding dairy cows. J. Dairy Sci. 100:5329-5342.

Penner, G. B., M. A. Steele, J. R. Aschenbach, and B. W. McBride. 2011. RUMINANT NUTRITION SYMPOSIUM: Molecular adaptation of ruminal epithelia to highly fermentable diets. J. Anim. Sci. 89:1108-1119.

Petri, R. M., T. Schwaiger, G. B. Penner, K. A. Beauchemin, R. J. Forster, J. J. McKinnon, and T. A. McAllister. 2013. Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well as during and after an acidotic challenge. Plos One 8. ARTN e83424 DOI: 10.1371/journal.pone.0083424.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet. J. 176:21-31.

Radostits, O. M., C. C. Gay, K. W. Hinchcliff, and P. D. Constable. 2007. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10 ed. Elsevier, Philadelphia, PA.

Saleem, F., B. N. Ametaj, S. Bouatra, R. Mandal, Q. Zebeli, S. M. Dunn, and D. S. Wishart. 2012. A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. J. Dairy Sci. 95:6606-6623.

Schweigel, M., M. Freyer, S. Leclercq, B. Etschmann, U. Lodemann, A. Bottcher, and H. Martens. 2005. Luminal hyperosmolarity decreases Na transport and impairs barrier function of sheep rumen epithelium. J. Comp. Physiol. B 175:575-591.

Shearer, J. K. 2011. Rumen acidosis, metalloproteinases, peripartum hormones and lameness. Pages 207-215 in Proceedings of the 47th Eastern Nutrition Conference. Montreal, Quebec, Canada.

Steele, M. A., J. Croom, M. Kahler, O. AlZahal, S. E. Hook, K. Plaizier, and B. W. McBride. 2011. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. Am J Physiol-Reg I 300:R1515-R1523.

Stone, W. C. 2004. Nutritional apporaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. J. Dairy Sci. 87(E. Suppl.):E13-E26.

Tadepalli, S., S. K. Narayanan, G. C. Stewart, M. M. Chengappa, and T. G. Nagaraja. 2009. Fusobacterium necrophorum: A ruminal bacterium that invades liver to cause abscesses in cattle. Anaerobe 15:36-43.

Tajima, K., R. I. Aminov, T. Nagamine, H. Matsui, M. Nakamura, and Y. Benno. 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl. Environ. Microb. 67:2766-2774.

Takahashi, K. and B. A. Young. 1981. Effects of grain overfeeding and histamine injection on physiological-responses related to acute bovine laminitis. Jap. J. Vet. Sci. 43:375-&.

Taniguchi, M., G. B. Penner, K. A. Beauchemin, M. Oba, and L. L. Guan. 2010. Comparative analysis of gene expression profiles in ruminal tissue from Holstein dairy cows fed high or low concentrate diets. Comp. Biochem. Phys. D 5:274-279.

Tao, S. Y., Y. Q. Duanmu, H. B. Dong, Y. D. Ni, J. Chen, X. Z. Shen, and R. Q. Zhao. 2014a. High concentrate diet induced mucosal injuries by enhancing epithelial apoptosis and inflammatory response in the hindgut of goats. Plos One 9. ARTN e111596 DOI: 10.1371/journal.pone.0111596.

Tao, S. Y., Y. Q. Duanmu, H. B. Dong, J. Tian, Y. D. Ni, and R. Q. Zhao. 2014b. A high-concentrate diet induced colonic epithelial barrier disruption is associated with the activating of cell apoptosis in lactating goats. BMC Vet. Res. 10. Artn 235 DOI: 10.1186/S12917-014-0235-2.

Vieira, A. T., M. M. Teixeira, and F. S. Martins. 2013. The role of probiotics and prebiotics in inducing gut immunity. Front. Immunol. 4:445. DOI: 10.3389/fimmu.2013.00445.

Wetzels, S. U., E. Mann, P. Pourazad, M. Qumar, B. Pinior, B. U. Metzler-Zebeli, M. Wagner, S. Schmitz-Esser, and Q. Zebeli. 2017. Epimural bacterial community structure in the rumen of Holstein cows with different responses to a long-term subacute ruminal acidosis diet challenge. J. Dairy Sci. 100:1829-1844.

Zebeli, Q. and B. N. Ametaj. 2009. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. J. Dairy Sci. 92:3800-3809.

Zebeli, Q. and B. U. Metzler-Zebeli. 2012. Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. Res Vet Sci 93:1099-1108.

Zhang, S., R. I. Albornoz, J. R. Aschenbach, D. R. Barreda, and G. B. Penner. 2013. Short-term feed restriction impairs the absorptive function of the reticulo-rumen and total tract barrier function in beef cattle. J. Anim. Sci. 91:1685-1695.