OPPORTUNITIES TO IMPROVE GUT HEALTH IN RUMINANT PRODUCTION SYSTEMS

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INTRODUCTION

In the past decade there have been a large number of publications describing how the gastrointestinal tract (GIT) plays a pivotal role in health and disease (Spor et al., 2011). These findings coincide with the advancement of molecular based sequencing techniques that have allowed researchers to investigate the expression of genes in gut tissues and the microbial communities with great efficiency and specificity. This highly advanced branch of molecular research has recently been applied in livestock studies (Frank et al., 2011), leading to the term "gut-health" becoming a buzz-word in the animal nutrition industry. This is an interesting turn of events given that the industry has traditionally been rooted in highly quantitative research. In spite of the evolving interest in this field, the term "gut-health" remains loosely defined, even scientifically, thus careful consideration of what the gut-health promoting action of a particular nutrient or feeding strategy requires close consideration. In this review, the main principles of gut health will be defined, and a description of the key target areas for future advancement in ruminant production will be provided. Furthermore, we will examine what has been done thus far in the ruminant sector with respect to the development of nutritional additives that positively impact gut health.

DEFINING GUT HEALTH

The GIT is the largest organ in the body involved in digestion and nutrient absorption, and invests great effort into maintaining a fine balance between its highly dense resident gut microbiota and the gut-associated immune system. The absence of gut microbiota results in an underdeveloped gut-associated immune system and peripheral organs (e.g. the spleen; Guarner, 2006). Conversely, an altered gut microbiome is associated with chronic metabolic disorders (obesity), inflammatory bowel disease (IBD), allergies, and autoimmune conditions, including type1 diabetes (Sekirov et al., 2010). In addition to the gut microbiota, the GIT epithelial barrier plays a vital role in maintaining the health of the gut and the host. The epithelial barrier that physically separates microbiota in the gut lumen and the mucosal immune system contains nearly 70% of total leukocytes and 80% of total secreting cells of IgA (mucosal antibody) in the body (Vighi et al., 2008) and interacts with the gut microbiome to maintain intestinal homeostasis and gut health. Inasmuch, defining gut health should take all the involved components and their complex interactions into account.

MICROBIOTA AND GUT HEALTH

The mammalian GIT is considered to be sterile *in utero* and undergoes rapid colonization with an array of microbiota during and after birth. This process of colonization is influenced by maternal microbiota, and delivery mode during birthing (Fanaro et al., 2003), while diet, lifestyle and antibiotic treatments may also largely influence the microbial composition after birth (Fouhy et al., 2012; Rodriguez et al., 2015). The neonatal gut colonization is a crucial period for the developing gut and the naïve immune system (Hansen et al., 2012) and may have long-term health effects on the animal (Conroy et al., 2009). The "hygiene hypothesis" suggests that increased hygienic conditions in western countries has reduced infant exposure to microbes, resulting in higher incidences of atopic diseases (atopic eczema, allergic rhinitis and asthma) (Kalliomaki and Isolauri, 2002). The administration of probiotics or cultures of healthy gut microflora though has been shown to reduce the development of atopic eczema significantly (Kalliomaki and Isolauri, 2002). Therefore, both gut colonization and the composition of early microbiota are important factors for long-term gut health.

A recent study revealed that the establishment of host-specific gut microbiota is required for the development of the mucosal immune system (Chung et al., 2012). The development of mucosal T-lymphocytes in human-microbiota colonized mice was similar to that of germfree mice and the cell numbers were less than that of mouse-microbiota colonized mice (Chung et al., 2012). Further, the susceptibility to *Salmonella* infections was higher in human-microbiota colonized, compared to that of mouse-microbiota colonized mice (Chung et al., 2012). The same phenomenon has also been suggested in different livestock animals, such as swine (Mulder et al., 2011) and cattle (Oikonomou et al., 2013). For example, restricted exposure to microbiota during early life in piglets interferes with the development of gut epithelium, while promoting a greater immune activation (Mulder et al., 2011). Similarly, higher bacterial diversity and prevalence of *Faecalibacterium prausnitzii* during the first week of life have been shown to increase body weight gain and decrease diarrhea incidence in older calves (Oikonomou et al., 2013). These results highlight the importance of gut microbiota establishment and gut health in early life.

The increased beneficial bacteria in the gut may influence gut health via different mechanisms, such as the prevention of enteric pathogens colonization, increasing digestive capacity, lowering of pH, and improving mucosal immunity (Uyeno et al., 2015). For example, *Bifidobacterium* protects the host against enteropathogenic infections by competing for nutrients and space, and by producing acetate (Hsieh et al., 2015). Additionally, *Bifidobacterium* has also been shown to closely regulate the intestinal epithelial barrier via the modulation of intercellular tight junction proteins (TJs) (Ulluwishewa et al., 2011). Given the intricate nature of these interactions and outcomes, it seems incredibly important to understand the role of microbiota in gut health with respect to individual animal species if manipulations of gut microbiota are to be used to improve health and production of livestock.

BARRIER FUNCTION AND GUT HEALTH

An important factor for gut health is maintaining proper epithelial barrier function of the GIT, which is highly orchestrated by the presence of nutrients and microbes within the gut (Shen et al., 2011). The barrier function of the GIT in the ruminal and intestinal epithelium are managed by a combination of cell junctions, including anchoring junctions (desmosomes, hemidesmosomes and adherence junctions), gap junctions and tight junctions (Turner, 2009). Cell junctions are the most common range of transmembrane proteins that interact with actin cytoskeleton of cells to maintain cell-to-cell adhesion in the intestinal epithelium (Ulluwishewa et al., 2011).

Proper regulation of cell junctions is crucial for the maintenance of intestinal homeostasis (Ulluwishewa et al., 2011). The increased permeability of the epithelial barrier is a common indication of different gastrointestinal diseases, such as IBD (Edelblum and Turner, 2009). Crohn's disease and ulcerative colitis also display variations in the expression of TJs from the claudin family (Edelblum and Turner, 2009). Cell junction-mediated changes in the epithelial barrier permeability are regulated by the production of cytokines (Edelblum and Turner, 2009). Other than the mucosal cytokines, dysbiosis in gut microbiota has also been shown to alter TJs leading to an increase in intestinal permeability during IBD (Hold et al., 2014).

In ruminants, the feeding of calf starter has been suggested to decrease alter the expression of TJs at the mRNA level during weaning transition (Malmuthuge et al., 2013). Moreover, increasing the diet in rapidly fermentable carbohydrates has been shown to decrease the expression of TJs in the rumen epithelium (Steele et al., 2011) and increase the expression of inflammatory genes in the hindgut epithelium (Tao et al., 2014). However, how these observed changes in cell junctions may impact intestinal permeability and gut health is still unclear. Also, while the role of cell junctions in gut health has been studied extensively in humans, the corollary knowledge in livestock species remains quite limited.

OPPORTUNITIES FOR GUT HEALTH IN RUMINANT PRODUCTION

There are several key phases and challenges in dairy production that can impact both GIT function and economic profitability, including pre-weaning, weaning, and the transition to highly fermentable diets.

Pre-weaning

The pre-weaned calf is the most at risk of all cattle on the farm, with digestive disorders and diseases resulting in morbidity and mortality rates above 50% and 10%, respectively, while the majority of which come from scours (USDA, 2007). An increase in intestinal permeability and fecal scores have been observed in younger calves (2-week-old) compared to their 4-week-old counterparts (Marquez, 2014), suggesting higher prevalence of disruptions in gut barrier function in younger animals. Therefore, it is important to understand the role of the intestinal microbiota and epithelial barrier

function in the prevention of calf diarrhea to improve gut health and to decrease calf deaths.

Weaning

The time of weaning for calves can be classified as one of the most dramatic GIT transformations in nature. Dairy and beef calves can suffer from weaning stress associated with gastrointestinal ailments, such as parakeratosis (Bull et al., 1965) as well as sudden and dramatically increased gut permeability (Wood et al., 2005). Opportunities to improve rumen development and lower gut adaptations would be immensely beneficial in decreasing the stress associated with weaning in ruminants.

Transition to Rapidly Fermentable Diets

Dramatic shifts in rapidly fermentable carbohydrates are commonly associated with GIT ailments in ruminants. The most notable being ruminal acidosis, a disorder characterized by a depression of ruminal pH, which alters GIT microbiota and barrier function. Ruminal acidosis is estimated to affect a large proportion of lactating dairy and beef feedlot cattle, and as such, is of great interest to researchers seeking to develop feed additives to alleviate the detrimental impact of this digestive disorder.

FEEDING THE GUT

The history of animal nutrition has been largely based in quantitative analysis and assessment of ingredients and balancing for energy and protein. In addition to supplying a consistent and balanced source of nutrients, producers are now investing in ingredients that are fed for the sole purpose of improving health, a correlation that is not easily quantified in the form of milk or meat production. Within the livestock industry, the number of ingredients commonly used for gut health applications is far greater in monogastrics than in ruminants. Several bioactive ingredients exist with gut health applications, such as probiotics, prebiotics, metabolites, essential oils, and bioactive proteins and fats; however, the number of studies examining their effects and impact on ruminant health remains quite limited. To maintain the scope of this review, the most utilized bioactive ingredients in ruminant nutrition, such as probiotics, prebiotics and metabolites will be discussed. Other classes of ingredients, such as essential oils, bioactive fats and proteins have received limited attention in ruminants and thus will not be included in this review.

PROBIOTICS AND PREBIOTICS

A probiotic is defined as a live microorganism which when administered in adequate amounts confers a health benefit on the host. The expectation of a probiotic is to (1) promote the development of a healthy microbiota predominated by beneficial bacteria, (2) prevent enteric pathogen colonization, (3) enhance gut tissue maturation and integrity, and (4) improve mucosal immunity (de Lange et al., 2010). The manipulation of microbiota to improve gut health using direct fed microbials and

probiotics has been widely studied in human medicine and nutrition, as well as livestock nutrition. The commonly used probiotics in ruminant rations are live yeast (*Saccharomyces cerevisiae*), lactic acid producing bacteria (e.g., *Lactobacillus* and *Enterococcus* spp.) and fungi (e. g. *Aspergillus oryzae*). In ruminant production, probiotics were initially used in young ruminants to aid in the establishment of microflora for feed digestion and health. Further advancements in the field led to more focused research on fibre digestion and optimizing ruminal fermentation and health (McAllister et al., 2011). Most ruminant probiotic research is focused on dry matter intake and milk production, with limited attention given to the underlying mechanisms and overall health effects.

A recent effort has been made to reduce ruminal acidosis using direct fed microbials (Krehbiel et al., 2003). Ruminal acidosis is a common digestive disorder in the cattle industry, caused by the transition to highly fermentable diets designed to improve production (Nagaraja and Titgemeyer, 2007). In addition to the accumulation of short-chain fatty acids (SCFA) or lactate, the composition of rumen microbiota is also altered in cattle with ruminal acidosis (Nagaraja and Titgemeyer, 2007). Live yeast, such as *Saccharomyces cerevisiae*, has been shown to attenuate ruminal acidosis in cattle by altering the microbiota of the rumen (Chaucheyras-Durand and Durand, 2009; AlZahal et al., 2014). *Megasphaera elsdenii* has been successfully used to increase ruminal pH and decrease the production of lactate and has been recommended as a direct fed microbial to prevent high-grain diet induced acidosis (Krehbiel et al., 2003). This technology however is not currently available in the market. More research characterizing how probiotics can impact host-microbial interactions will provide more insight into how they can be formulated into rations to improve ruminal health.

The use of probiotics in calves has focused primarily on maintaining intestinal health in the first weeks of life or aiding in the development of the rumen during weaning. It has been well established that in certain environmental conditions, feeding lactic-acid bacteria during the pre-weaning phase is associated with improved weight gain (Frizzo et al., 2011). However, only a small number of samples evaluate health related metrics. For example, the administration of *Lactobacillus* and *Bifidobacterium* to newborn calves during the first week of life has been shown to increase weight gain, feed conversion ratios and health (Abe et al., 1995). Similarly, Timmerman et al., (2004) showed reduced diarrhea and improved health when calves were supplemented *Lactobacillus* in the milk. Probiotics have also been supplemented in the dry feed offered to calves to improve performance during the weaning period (Lesmeister et al., 2004; Yohe et al., 2015). Still, it remains unclear whether these benefits come from improved rumen growth or function, and thus they warrant further investigation.

A prebiotic is a non-digestible feed ingredient that can be used to alter the composition or metabolism of the gut microbiota in a beneficial manner. In practice, prebiotics have been used almost exclusively to increase the proportion of *Bifidobacterium* and *Lactobacillus* in the gut (Gibson et al., 2004). Most of the work with prebiotics has been conducted in calves, leaving a paucity of information regarding mature cattle. For example, feeding fructooligosaccharides enhances the growth

performance of veal calves by decreasing feed conversion ratios and increasing carcass weight; however, the possible mechanisms behind these performance measures were not investigated (Grand et al., 2013). Recent galactooligosaccharide prebiotic supplementation research in newborn calves has shown increases in the abundance of *Lactobacillus* and *Bifidobacterium* (Marquez, 2014), underscoring the potential to improve gut health via increasing the establishment of beneficial bacteria. This same study also showed that intestinal permeability was not affected by prebiotics, which suggests that they may not influence gut health via modulating intestinal epithelial barrier, but only via promoting the colonization of beneficial microbiota. The effect of supplementing milk with the prebiotics inulin and lactulose on GIT immunology of pre-ruminant calves was recently evaluated and the mRNA expression of genes involved in inflammation were downregulated in the intestine (Masanetz et al., 2011). These studies showcase that both prebiotics and probiotics influence microbiota and the overall health of the host; however, the particular mechanisms and modes of action require more research in ruminants.

METABOLITES

The most studied metabolites with respect to ruminants are SCFA, which are the end-products of microbial fermentation in the rumen and hind gut. They are also commonly regarded as luminal growth factors and increasing their production alters GIT function in ruminant and non-ruminant models (Sakata and Yajima, 1978). Of the SCFA, butyrate has been reported to be the most potent stimulator of epithelial proliferation in colonic epithelial cells, and is the primary energy source of the ruminant GIT. The supplementation of butyrate is known to induce ruminal epithelial proliferation *in vivo* (Sakata and Tamate, 1978). Recent research in mature dairy cows showed that genes involved in differentiation and growth were activated in the rumen epithelium by ruminal butyrate infusions (Baldwin et al., 2012). Furthermore, butyrate supplementation in dairy cows during acidosis impacted the cytokine and host defense immune expression (Dionnisopoulous et al., 2013). Interestingly, young calves fed a milk replacer fortified with butyrate exhibited increased ruminal papillae length, width, and surface area (Gorka et al., 2011), suggesting cross-talk between the lower gut and rumen.

While ruminant studies have focused primarily on butyrate, research in monogastrics has identified a significantly larger catalogue of metabolites that can be used to improve GIT health. For example, precursors of butyrate have been studied as a mean of increasing butyrate supply (de Lange et al., 2010). In addition, osmoregulator metabolites, such as betaine have been shown to improve gut barrier functions (Eklund et al., 2005) and medium chain fatty acids have been shown to increase the proportion of beneficial bacteria within the GIT (de Lange et al., 2010). This body of knowledge in monogastric research provides a useful framework to explore and implement new nutritional technologies in ruminant production.

ADDITIONAL INGREDIENTS

Over the past decade an array of novel ingredients designed to improve gut health have entered the market. One class of ingredients that has received attention, as an alternative to antibiotics, is phytochemicals (also referred to as essential oils) derived from plant extracts. Phytochemicals are volatile plant components that are known for their antimicrobial activity and have been shown to have a positive impact on GIT microbial activity and community structure (de Lange et al., 2010). Recent research highlights how essential oils influence not only the microbiota but also neuroendocrine system function of the host (Furness et al., 2013). To date, the majority of studies have only evaluated the use of phytochemicals in relation to production parameters (growth and milk production) without evaluating the specific measurements of GIT function.

The monogastric industry has a history of feeding bioactive proteins and fats to improve gut health. For example, plasma proteins and feed enzymes have been extensively used in monogastrics; however, their efficacy cannot be translated into ruminant production due to feed ingredient regulations and safety (de Lange et al., 2010). In addition, the use of essential fatty acids for improving gut immunity and health has been well characterized in monogastrics, but scarcely evaluated in ruminants (Garcia et al., 2015). There remain great opportunities to translate this knowledge to ruminant research, especially calves, which function in a similar physiological manner to monogastrics.

CONCLUSION

Gut health has become a popular topic in livestock agriculture, but the ruminant livestock are the least developed in the sense of scientific research and commercial application and provide the most opportunity for growth in the industry application. The recent influx of nutrition research investigating how ingredients can influence gut health represents a shift in the approach towards ruminant nutrition, which has been historically rooted in quantitative findings. In order to adequately and aptly discuss gut health, two major principles must be considered: microbiota and the barrier integrity of the GIT. There are several opportunities at different stages of life and different points in the production cycle (e.g. pre-weaning, weaning and transition to rapidly fermentable diets) to improve gut health in ruminants. The breadth of nutritional technologies now common in monogastric livestock species, but untested in ruminants, also offers valuable insight into potential developments and applications for the ruminant sector.

REFERENCES

Abe, F., N. Ishibash, and S. Shimamura. 1995. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. J. Dairy Sci. 73:2838-2846.

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.

Baldwin, R. L. VI, S. Wu, W. Li. C. Li, B. J. Bequette, and R. W. Li. 2012. Quantification of transcriptome responses of the rumen epithelium to butyrate infusion using RNA-seq technology. Gene Regul. Syst. Bio. 6:67-80.

Bull, L. S., L. J. Bush, J. D. Friend, B. Harris Jr., and E. W. Jones. Incidence of ruminal parakeratosis in calves fed different rations and its relation to volatile fatty acid absorption. J. Dairy Sci. 48:1459-1466.

Chaucheyras-Durand, F., and H. Durand. 2010. Probiotics in animal nutrition and health. Beneficial Microbes. 1:3-9.

Chung, H., S. J. Pamp, J. A. Hill, N. K. Surana, S. M. Edelman, E. B. Troy, N. C. Reading, E. J. Villablanca, S. Wang, J. R. Mora, Y. Umesaki, D. Mathis, C. Benoist, D. A. Relman, and D. L. Kasper. 2012. Gut immune maturation depends on colonization with a host-specific microbiota. Cell. 149:1578-1593.

Conroy, M. E., H. N. Shi, W. A. Walker. 2009. The long-term health effects of neonatal microbial flora. Curr. Opin. Allergy Clin. Immunol. 9:197-201.

de Lange, C. F. M., J. R. Pluske, J. Gong, and C. M. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science. 134: 124-134.

Dionissopoulos, L., A. H. Laarman, O. AlZahal, S. L. Greenwood, M. A. Steele, J. C. Plaizier, J. C. Matthews, and B. W. McBride. 2013. Butyrate-mediated genomic changes involved in the non-specific host defenses, matrix remodeling and the immune response in the rumen epithelium of cows afflicted with subacute ruminal acidosis. Am. J. Anim. Vet. Sci. 8: 8-27.

Edelblum, K. L. and J. R. Turner. 2009. The tight junction in inflammatory disease: Communication breakdown. Curr. Opin. Pharmacol. 9:715–720.

Eklund, M., E. Bauer, J. Wamatu, and R. Mosenthin. 2005. Potential nutritional and physiological function of betaine in livestock. Nut. Res. Rev. 18: 31-48.

Fanaro, S., R. Chierici, P. Guerrini, and V. Vigi. 2003. Intestinal microflora in early infancy: composition and development. Acta Paediatr. Suppl. 91:48-55.

Frank D. N. 2011. Promoting healthier humans through healthier livestock: Animal agriculture enters the metagenomics era. J. Anim. Sci. 89:835-844.

Frizzo, L. S., M. V. Zbrun, L. P. Soto, and M. L. Signorini. 2011. Effects of probiotics on growth performance in young calves: A meta-analysis of randomized controlled trials. Anim. Feed Sci. Tech. 169:147-156.

Fouhy, F., C. M. Guinane, S. Hussey, R. Wall, C. A. Ryan, E. M. Dempsey, B. Murphy, R. P. Ross, G. F. Fitzgerald, C. Stanton, and P. D. Cotter. 2012. High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob. Agents Chemother. 56:5811-5820.

Furness, J. B., L. R. Rivera, H-J. Cho, D. M. Bravo, and B. Callaghan. 2013. The gut as a sensory organ. Nat. Rev. Gastroentro. doi:10.1038/nrgastro.2013.180.

Garcia, M. J. H. Shin, A. Schlaefli, L. F. Greco, F. P. Maunsell, W. W. Thatcher, J. E. P Santos, and C. R. Stables. 2015. Increasing intake of essential fatty acids from milk replacer benefits performance, immune responses, and health of preweaned Holstein calves. J. Dairy Sci. 98:458-477.

Gibson, G. R., H. M. Probert, J. Van Loo, R. A. Rastall, and M. B. Roberfroid. 2004. Dietary modulation of the human colonic microbiota: updating the concepts of

prebiotics. Nut. Res. Rev. 17: 259-275.

Guarner F. 2006. Enteric flora in health and diseases. Digestion. 73:5-12.

Grand, E., F. Respondek, C. Martineau, J. Detilleux, and G. Bertrand. 2013. Effects of short-chain fructooligosacchrides on growth performances of pre-ruminant veal calves. J. Dairy Sci. 96:1094-1101.

Gorka, P., Z. M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, and R. Zabielski. 2011. Is rumen development in newborn calves affected by different liquid feeds and small intestine development? J Dairy Sci. 94:3002-3013.

Hansen, C. H. F., D. S. Nielsen, M. Kverka, Z. Zakostelska, K. Klimesova, T. Hudcovic, H. Tlaskalova-Hogenova, and A. K. Hansen. 2012. Patterns of early gut colonization shape future immune responses of the host. PLoS One. 7:e34043. doi:10.1371/journal.pone.0034043.

Hold, G. L., M. Smith, C. Grange, E. R. Watt, E. M. El-Omar, and I. Mukhopadhya. 2014. Role of the gut microbiota in inflammatory bowel disease pathogenesis: What have we learnt in the past 10 years? World J. Gastroenterol. 20:1192-1210.

Hsieh, C. Y., T. Osaka, E. Moriyama, Y. Date, J. Kikuchi, and S. Tsuneda. 2015. Strengthening of the intestinal epithelial tight junction by *Bifidobacterium bifidum*. Physiol. Rep. 3:e12327. doi: 10.14814/phy2.12327

Kalliomaki, M., and E. Isolauri. 2002. Pandemic of atopic diseases--a lack of microbial exposure in early infancy? Curr. Drug Targets Infect. Disord. 2:193-199.

Krehbiel, C. R., S. R. Rust, G. Zhang, and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81:E120-E132.

Lesmeister, K. E., A. J. Heinrichs, and M. T. Gabler. 2004. Effects of supplemental yeast (*Saccharomyces cerevisiae)* culture on rumen development, growth characteristics and blood parameters in neonatal dairy calves. 87:1832-1839.

Malmuthuge, N., M. Li, L. A. Goonewardene, M. Oba, and L. L. Guan. 2013. Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. J. Dairy Sci. 96:189-200.

Malmuthuge, N., Y. Chen, G. Liang, L. A. Goonewardane, and L. L. Guan. 2015. Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. J. Dairy Sci. In Press. doi: 10.3168/jds.2015-9607.

Marquez, C. J. 2014. Calf intestinal health: assessment and dietary interventions for its improvement. PhD Thesis. University of Illinois. Urbana-Champaign, USA.

Masanetz, S., W. PreiBinger, H. H. D. Meyer and M. W. Pfaffl. 2011. Effects of the prebiotics inulin and lactulose on intestinal immunology and hematology or preruminant calves. Animal 5:1099-1106.

McAllister, T. A., K. A. Beauchemin, A. Y. Alazzeh, J. Baah, R. M. Teather, and K. Standford. 2011. Review: The use of direct fed microbial to mitigate pathogens and enhance production in cattle. Can. J. Anim. Sci. 91:193-211.

Mulder, I. E., B. Schmidt, M. Lewis, M. Delday, C. R. Stokes, M. Bailey, R. I. Aminov, B. P. Gill, J. R. Pluske, C-D. Mayer, and D. Kelly. 2011. Restricting microbial exposure in early life negates the immune benefits associated with gut colonization in environments of high microbial diversity. PLoS One. 6:e28279. doi: 10.1371/journal.pone.0028279.

Nagaraja, T.G., and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. J Dairy Sci. 90:E17-38.

Oikonomou, G., A. G. Teixeira, C. Foditsch, M. L. Bichalho, V. S. Machado, and R. C. Bicalho. 2013. Fecal microbial diversity in pre-weaned dairy calves as described by pyrosequencing of metagenomic 16S rDNA. Associations of *Faecalibacterium* species with health and growth. PLoS One. 8:e63157. doi: 10.1371/journal.pone.0063157.

Rodriguez, J.M., K. Murphy, C. Stanton, R. P. Ross, O. I. Kober, N. Juge, E. Avershina, K. Rudi, A. Narbad, M. C. Jenmalm, J. R. Marchesi, and M. C. Collado. 2015. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb. Ecol. Health Dis. 26:26050. http://dx.doi.org/10.3402/mehd.v26.26050.

Sakata T and H. Tamate. 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. J. Dairy Sci. 61:1109-1113.

Sasajima, N., T. Ogasawara, N. Takemura, R. Fujiwara, J. Watanabe, and K. Sonoyama. 2010. Role of intestinal *Bifidobacterium pseudolongum* in dietary fructo-oligosaccharide inhibition of 2,4-dinitrofluorobenzene-induced contact hypersensitivity in mice. Br. J. Nutr. 103:539-548.

Sekirov, I., S. L. Russell, L. Caetano, M. Antunes, and B. B. Finlay. 2010. Gut microbiota in health and disease. Physiol. Rev. 90:859–904.

Shen, L, C. R. Weber, D. R. Raleigh, D. Yu, and J. R. Turner. 2011. Tight junction pore and leak pathways: A dynamic duo. Annu. Rev. of Physiol. 73:283-309.

Spor, A., O. Koren, and R. Ley. 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. Nat. Rev. Microbiol. 9:279-290.

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

Tao, S., Y. Duanmu, H. Dong, Y. Ni, J. Chen, X. Shen, and R. Zhao. 2014. High concentrate diet Induced mucosal injuries by enhancing epithelial apoptosis and inflammatory response in the hindgut of goats. PLOS One. 9:e111596. doi: 10.1371/journal.pone.0111596

Timmerman, H. M., L. Mulder, H. Everts, D. C. van Espen, E. van der Wal, G. Klaassen, S. M. G. Rouwers, R. Hartemink, F. M. Rombouts, and A. C. Beynen. Health and growth of veal calves fed milk replacers with or without probiotics. J. Dairy Sci. 88:2154-2165.

Turner, J. R. 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9:799-809.

Ulluwishewa, D., R. C. Anderson, W. C. McNabb, P. J. Moughan, J. M. Wells, N. C. Roy. 2011. Regulation of tight junction permeability by intestinal bacteria and dietary components. J. Nutr. 141:769-776.

USDA, 2007. Part 1: Reference of dairy cattle health and management practices in the United States, NAHMS Dairy. pp. 1–128.

Uyeno, Y., S. Shigemori, and T. Shimosato. 2015. Effect of probiotics/prebiotics on cattle health and productivity. Microbes. Environ. 30:126-132.

Vighi, G., F. Marcucci, L. Sensi, G. DiCara, and F. Farati. 2008. Allergy and the

gastrointestinal system. Clin. Exp. Immunol. 153:3-6.

Wood, K. M., S. Palmer, M. A. Steele, J. A. Metcalf and G. B. Penner. 2015. The influence of age and weaning on permeability of the gastrointestinal tract in Holstein bull calves. J. Dairy Sci. In Press. doi: 10.3168/jds.2015-9393.

Yohe, T. T., K. M. O'Diam, and K. M. Daniels. 2015. Growth, ruminal measurements, and health characteristics of Holstein bull calves fed an *Apergillus oryzae* fermentation extract. J. Dairy Sci. 98: 6163-6175.