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Effects and immune responses of probiotic treatment in ruminants

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Abstract

Gut microbial colonization and establishment are vital to ruminant health and production. This review article focuses on current knowledge and methods used to understand and manipulate the gut microbial community in ruminant animals, with a special focus on probiotics treatment. This review highlights the most promising of studies in this area, including gut microbial colonization and establishment, effect of gastrointestinal tract microbial community on host mucosal innate immune function, impact of feeding strategies on gut microbial community, current probiotic treatments in ruminants, methods to manipulate the gut microbiota and associated antimicrobial compounds, and models and cell lines used in understanding the host immune response to probiotic treatments. As a lot of work in this area is done in humans and mice, this review article also includes up-to-date knowledge from relevant studies in human and mouse models. This review is a useful resource for scientists working in the areas of ruminant nutrition and health, and to researchers investigating the microbial ecology and its relation to animal health.

Keywords

gut microbial colonization; probiotic treatment; immune response; ruminants

Gut microbial colonization and establishment

During fetal development, intestinal tissues proliferate while the rumen is underdeveloped at birth. The rumen proliferates from 30% to 70% gastrointestinal tract (GIT) capacity (Warner, 1956), while the intestinal tissues develop earlier to enable the digestion and absorption of nutrients from colostrum. The initial colonization of the ruminant GIT is suggested to derive from the environment and maternal feces, saliva, skin, and vaginal canal (Meale et al., 2016), while on-farm practices (*e.g.*, housing, contact with dam/ewe), diet, and development contribute to GIT microbial establishment. As the ruminant ages, diet and GIT development

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are key contributors to the changes in the GIT bacterial communities (Malmuthuge et al., 2014). Bacteria, archaea, and fungi are the first microbiota to colonize the GIT, whereas rumen ciliates are not present within the first week after birth. Rumen ciliates may colonize by mouth to mouth contact with a faunated adult. However, specific ruminal conditions are required for their establishment (Eadie and Hobson, 1962). Archaea and fibrolytic bacteria colonize the GIT of the young ruminant within 20 minutes after parturition (Guzman et al., 2015).

The bacteria belonging to the genera *Bifidobacterium*, *Faecalibacterium*, and *Lactobacillus* are suggested to be of great importance to the young ruminant as they aid in GIT development and have demonstrated their effectiveness as probiotics. Specifically, bacteria belonging to the genus *Faecalibacterium* produce butyrate, a volatile fatty acid (VFA) that promotes ruminal and intestinal development. *In vitro* experiments suggest that butyrate enhances the gut barrier function through increased expression of tight junction protein-related genes (Wang et al., 2012). The lactic acid-producing bacterial genera *Bifidobacterium* and *Lactobacillus* are typically more prevalent in the pre-weaned ruminant because of an abundance of oligosaccharides in milk. Rey *et al.* (Rey et al., 2014) reported the relative abundances of the bacterial genus *Bifidobacterium* in the rumen to be stable from birth to 83d in age. The bacterial genus *Lactobacillus* was undetectable in ruminal fluid from 2-day old and 15–83d old calves, while low relative abundances (1.2%) were present at 3–15d in age (Rey et al., 2014). Fecal relative abundances of the genera *Bifidobacterium*, *Faecalibacterium*, and *Lactobacillus* were shown to be greater in pre-weaned (1.21, 4.07, and 2.87%, respectively) than post-weaned (0.34, 0.94, and 0.20%, respectively) calves (Meale et al., 2016). Song et al. characterized the hindgut digesta and mucosa-associated microbiota from calves at birth, 7, 21, and 42 days of age. Newborn and 7d-old calves had greater relative abundances of mucosa-associated *Lactobacillus* and potential pathogenic bacteria *Escherichia-Shigella* and *Salmonella* than 21 and 42-d old calves. There was a positive correlation between the relative abundance of *Faecalibacterium* and molar proportions of butyrate, the both of which peaked at 21d of age (Song et al., 2018). McFarland et al. showed that fecal bacterial operational taxonomic units (OTU) related to the genera *Faecalibacterium* and *Lactobacillus* decreased or disappeared as calves aged (Dill-McFarland et al., 2017). As the ruminant reaches adulthood, the rumen contains greater relative abundances of the genus *Prevotella* and members of the bacterial family *Succinivibrionaceae* along with increased microbial diversity (Dill-McFarland et al., 2017).

Effects of GI tract microbial community on host mucosal innate immune function

GIT commensal microbiota are involved in the development of the intestinal architecture and immunomodulatory processes in a symbiotic and beneficial way. Immediately after birth, ruminants go through a process of colonization of foreign microorganisms. The archetypal molecules produced by the commensal microorganisms mediate the development of the host immune system. For example, *Bacteroides fragilis*, a gram-negative anaerobe, colonizes the mammalian lower GIT, with beneficial properties in ameliorating inflammatory symptoms. It was reported that the Zwitterionic polysaccharides produced by

B. fragilis played several immunomodulatory roles in directing the maturation of the developing immune system, including correcting systemic T cell deficiencies, adjusting T helper cell 1 (Th1)/T helper cell 2(Th2) imbalances and directing Lymphoid tissue biogenesis (Mazmanian et al., 2005). On the other hand, the host immune response helps the beneficial microbes to establish a robust colonization and spatial segregation between the host and the microbes. In a recent study by Donaldson and co-authors (Donaldson et al., 2018), the immunoglobulin A (IgA) antibody produced by the host in response to the *B. fragilis* capsule helps anchor the bacteria to the epithelial surface, providing a colonization advantage. In comparison, in mice lacking IgA, the bacterium was less successful at colonizing the intestinal surface and subsequently less efficient in maintaining long-term stability. RegIII γ is an antibacterial lectin expressed in intestinal epithelial cells. It promotes the spatial separation of commensal microbiota and host in the intestine, and maintains a lectin-mediated, symbiotic host-microbial relationship (Cash et al., 2006; Vaishnavi et al., 2011). Consistent with these findings, studies in germ-free animals revealed that significant immune system deficiency is associated with a lack of gut microbiota composition and metabolic activity of the intestinal microbiome (Okada et al., 1994; Bouskra et al., 2008).

Specific microbes having demonstrated roles in promoting inflammatory (Ivanov et al., 2008; Ivanov et al., 2009), or anti-inflammatory (Mazmanian et al., 2008; Round and Mazmanian, 2010) responses in the gut. As a prominent human commensal species, *B. fragilis*, mediates the conversion of CD4⁺ T cell into Foxp3(+) regulatory T cells, which suppress IL-17 production, and therefore, protect the host against inflammatory insults (Round and Mazmanian, 2010; Round et al., 2011). Furthermore, polysaccharide A produced by *B. fragilis*, is capable of protecting animals from intestinal inflammation through the suppression of IL-17 production (Mazmanian et al., 2008). Thus, when selecting beneficial probiotics with the aim to manipulate the GIT microbiome, the immunoregulatory properties are important factors to consider (Kechaou et al., 2013).

Effects of feeding regime on gut microbiota

The composition of the intestinal microbiota is dependent on diet, antibiotic use, host genetics and other environmental factors (De Filippo et al., 2010; Maslowski and Mackay, 2011). Alterations in the commensal bacterial populations have significant effects on fiber digestion and fermentation, vitamin synthesis, and regulation of inflammatory responses (Maslowski and Mackay, 2011). The microbiota enable the host to absorb nutrients from complex dietary carbohydrates that can't be digested by mammalian enzymes (Zebeli and Metzler-Zebeli, 2012). Specifically, microbial fermentation produces VFAs, which have effects on immunoregulation, metabolism and ruminal epithelium maturation (Baldwin and McLeod, 2000; Maslowski and Mackay, 2011; Laarman et al., 2012). Maintaining the ecological balance among the gut microbiota via nutrition is crucial for the health of the host (Zebeli and Metzler-Zebeli, 2012).

The quantity of carbohydrate in the feed has significant effects on the ruminal microbiome. The main digestible component in grain-based cattle diets is starch, which rapidly ferments in the rumen and generates a large amount of VFA (Klevenhusen et al., 2017). Feeding cattle concentrates rich in starches increases the risk of subacute ruminal acidosis, which is due to

the accumulation of VFA in excess of the rumen buffering capacity (Aschenbach et al., 2011). Recent studies have demonstrated that feeding highgrain diets modifies the rumen microbial population in favor of amylolytic and lactic acid-producing populations at the expense of fibrolytic microbiota (Fernando et al., 2010; Petri et al., 2013; McCann et al., 2016). These changes to the ruminal microbiome can lead to a reduction of fiber degradation that can result in decreased feed efficiency (Klevenhusen et al., 2017).

In adult cattle, it has been shown that changes in dietary carbohydrates (inclusion of high quality hay containing higher sugar and metabolizable energy instead of concentrates with lower quality hay) shift the rumen epimural microbial population from predominantly *Firmicutes* to *Proteobacteria* with a higher abundance of *Campylobacter* (Petri et al., 2018). The effect of high-quality hay or high neutral detergent fiber feed versus starch-rich feed on the rumen microbial community diversity has varied among studies (Belanche et al., 2012; Petri et al., 2013; Klevenhusen et al., 2017; Petri et al., 2018). In a recent study, replacing fiber-rich hay with sugar-rich hay caused significant changes in the rumen bacterial communities at every taxonomic level; these alterations led to improvements in feed degradability and nutrient absorption (Klevenhusen et al., 2017). Changes to the ruminal microbiome, induced by feeding high quality hay, stimulated rumination, stabilized the ruminal pH, and improved total feed digestibility (Kleefisch et al., 2017). In beef cattle, the concentrate-based diet has been shown to cause an increase in the relative abundance of *Proteobacteria*; a high ratio of *Proteobacteria* to (*Firmicutes* + *Bacteroidetes*) was shown to be a good indicator of ruminal dysbiosis (Auffret et al., 2017). The changes to the rumen microbiota are therefore influenced by both diet and management practices (Shanks et al., 2011).

Early feeding regimes in calves have also been shown to have significant effects on the gut microbiota. In dairy calves, the gut microbiota is affected by colostrum administration in the first 2 to 6 hours of life. It has been shown that feeding heat-treated colostrum, compared to fresh colostrum or no colostrum, increases the relative abundance of *Bifidobacterium* and decreases the colonization of *Escherichia coli* in the small intestine (Malmuthuge, 2016). These results suggest that the impact of heat-treated colostrum is to enhance the establishment of beneficial microbiota and prevent pathogen colonization of the GIT (Malmuthuge, 2016). Following colostrum administration, calves on commercial dairies are fed whole milk or milk replacer with varying access to concentrates and forages. Pre-weaned dairy calves that were consuming calf starter, in addition to milk replacer, were shown to have increased ruminal fermentation capacity when compared to calves consuming milk replacer alone (Laarman et al., 2012). The increase in ruminal fermentation did not appear to cause a detrimental decrease in rumen pH in calves, which may indicate adaptation of the rumen epithelium to calf starter fermentation (Laarman and Oba, 2011). Additionally, the diet containing starter promoted greater diversity of bacterial taxa known to readily utilize fermentable carbohydrates (Dias et al., 2017), and the changes in the ruminal archaeal community were exclusively dependent on diet (Dias et al., 2017). Overall, diet and age were found to have simultaneous effects on the microbial commensal population (Dias et al., 2017). Access to concentrates in addition to milk and grass hay (compared to grass hay and milk alone) during the pre-weaning period, was found to alter the ruminal microbial population in lambs; some of these differences persisted 4 months after weaning while both

groups were receiving the same diet (Yanez-Ruiz et al., 2010). These studies indicate the potential feasibility of manipulating the microbial populations early in life using diets or dietary additives to promote health and productivity benefits in adults (Yanez-Ruiz et al., 2015).

Antibiotic administration may also have an effect on the GIT microbiota. Antibiotics are commonly used to treat bacterial infections in all animal species, but the effects of antimicrobial drugs on the microbial communities of the GIT are not well described (Oultram et al., 2015). In previous human studies, antibiotic treatment significantly decreased taxonomic richness, diversity and evenness of the fecal microbiota and increased the number of antibiotic resistance genes found (Jernberg et al., 2007; Dethlefsen et al., 2008). A recent study in humans, however, demonstrated that while fluoroquinolone and β -lactam antibiotics caused significantly decreased microbial diversity in fecal samples, there was an increase in the average microbial load (due to an increase in Gram-negative bacteria) in people treated with β -lactam antibiotics (Panda et al., 2014). In a recent study evaluating the effect of antibiotics on the fecal microbiota of dairy calves, calves treated with parenteral oxytetracycline had a reduced amount of *Lactobacillus* species in their fecal microbiota one week after treatment and calves treated with florfenicol had reduced species richness that persisted two weeks post-treatment (Oultram et al., 2015). A recent metagenomic study in feedlot steers demonstrated that administration of antibiotic feed additives (e.g. monensin, tylosin) reduced the abundance of Gram-positive bacteria; however, there was no correlation between the administration of antimicrobial feed additives and the presence of antibiotic resistance genes (Thomas et al., 2017). Overall, it is evident that parenteral and oral antibiotic administration modifies the GIT microbiota. Further studies will be required to investigate the impact of these changes on animal and human health as well as the development of antimicrobial resistance.

Current bacterial probiotics research in ruminants

There has been variable terminology used to describe microbial additives used as dietary interventions to affect rumen function and improve animal health and performance (Yoon and Stern, 1995). The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) definition of a probiotic was recently clarified in an expert consensus document: “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). The most frequently studied probiotics include species of *Saccharomyces* yeast (Newbold et al., 1996), the filamentous fungus *Aspergillus oryzae* (Mathieu et al., 1996; Jouany et al., 1998), *Bifidobacterium* and lactic acid bacteria (Chiquette et al., 2008; Weimer, 2015). Additional probiotics studied in ruminants have included *Bacillus*, *Enterococcus* and *Faecalibacterium* species (Uyeno et al., 2015). Probiotics have been associated with enhanced intestinal health through the stimulation of beneficial gut microbiota, improved mucosal immunity, and the prevention of enteric pathogens that typically colonize in the intestines of young ruminants (Uyeno et al., 2015). In addition to targeting the lower GIT, probiotics have been used to enhance ruminal fermentation. It was reported that the administration of the lactate-utilizing bacterium, *Megasphaera elsdenii*, increased ruminal butyrate production and feed intake in neonatal calves (Muya et al., 2015). However, treatment of dairy calves with *Lactobacillus plantarum*

and *Bacillus subtilis* were shown not to alter ruminal fermentation characteristics (Zhang et al., 2017). Current research continues to demonstrate the positive responses to early probiotic administration on animal performance and health, despite inconsistent effects on ruminal fermentation capacity (Table 1). Probiotics have the potential to enhance intestinal health by stimulating the development of a balanced microbiota with a predominance of beneficial bacteria, preventing the colonization of enteropathogens, increasing digestive capacity and improving mucosal immunity (Uyeno et al., 2015). Furthermore, demonstrated benefits of probiotic administration include increased body weight gain and reduced incidence of diarrhea (Timmerman et al., 2005; Goto et al., 2016).

Probiotic treatment based antimicrobial compounds

Several probiotic strains have been shown to produce a variety of antimicrobial compounds (Table 2) (e.g., VFA, hydrogen peroxide, nitric oxide, and bacteriocins) (Atassi and Servin, 2010; Chenoll et al., 2011). The production of potent antimicrobial compounds enables the probiotic bacteria to compete with native or pathogenic species (Eschenbach et al., 1989; Roos and Holm, 2002). The most commonly produced antimicrobial compounds are bacteriocins, which are produced by major lineages of Bacteria and, more recently, have been reported as universally produced by some members of the Archaea (Riley and Wertz, 2002b, a; Shand RF, 2008). Bacteriocins, antimicrobial peptides synthesized in the ribosome, are different from antibiotics in that they inhibit the growth of other closely related bacterial species or bacteria of the same strain, while antibiotics have a much broader killing spectrum that doesn't often affect closely related strains (Riley and Wertz, 2002b; Kralik et al., 2018). The bacteriocin family includes a diverse group of peptides, differing in size, microbial targets, immunity mechanisms, and modes of action and release.

Bacteriocin production has been considered an important trait in probiotic selection; however, there are few studies documenting the ability of bacteriocin production to influence the strain's ability to positively impact the health of the host (Corr et al., 2007). Bacteriocins may utilize a variety of mechanisms to reduce or prevent microbial infections within the GIT (Corr et al., 2009; Dobson et al., 2012). A potential function includes acting as colonizing peptides, allowing the probiotic strain to integrate with an already occupied niche (Hillman et al., 1987; Dawid et al., 2007; Gillor et al., 2009). Alternatively, bacteriocins may function by directly eliminating pathogens within the GIT via bacteriocidal activity (Corr et al., 2007; Le Blay et al., 2007). Recently, it was shown that lectin-like bacteriocins eliminate microbial rivals via contact-dependent disruption of outer membrane protein assembly machinery (Ghequire et al., 2018). Finally, bacteriocins may act as signaling peptides, inducing the host immune system or other bacteria via quorum sensing (Kleerebezem et al., 1997; Meijerink et al., 2010; van Hemert et al., 2010). The mechanisms of bacteriocin production and ecological function within the mammalian GIT are complex and will require further investigation.

Isolated bacteriocins have potential applications in ruminants. Possible uses include as an alternative to antibiotic treatment, as a tool for reducing environmental contamination of zoonotic pathogens, and as a means to regulate ammonia production within the rumen. In the cow's rumen, *Streptococcus bovis* is a dominant bacterial species when the diet contains

large amounts of soluble starch (Wells et al., 1997; Zhang et al., 2015). Whitford et al. screened *S. bovis* cultures and found 20% of these strains inhibited other *Streptococci* (Whitford et al., 2001). Consistent with this finding, Mantovani *et al.* reported that 50% of *S. bovis* isolates from cattle fed hay- or grain-based diets inhibited the growth of *S. bovis* JB1 (Mantovani et al., 2001). The colicin-producer, *E. coli* strain Nissle 1917 (EcN), was found to produce microcin H47 and microcin M, both of which were shown to have antagonistic activity against enterobacteria (Patzner et al., 2003). Administering the EcN strain as a probiotic to dairy calves resulted in a significant reduction of neonatal diarrhea (von Buenau et al., 2005), which may have been due to antagonism of pathogenic enterobacteria. Compared to the control group, calves orally inoculated with a mixture of eight colicin E7-producing *E. coli* strains were reported to have significantly reduced shedding of *E. coli* O157:H7, a foodborne pathogen carried by at least 20% of the cattle. This observation suggested that the colicins may have the capability to inhibit or exclude *E. coli* O157:H7 in the GIT of cattle (Schamberger et al., 2004). *Butyrivibrio fibrisolvens* are ruminal microbes that are important for fiber digestion (Hungate, 1966), and many strains of *B. fibrisolvens* have been shown to produce bacteriocins that inhibit other *Butyrivibrio* species (Kalmokoff and Teather, 1997). Specifically, *B. fibrisolvens* JL5 was found to reduce the growth of obligate amino-acid fermenting bacteria, which may reduce ammonia production within the rumen (Rychlik and Russell, 2002).

Current understanding of host's immune response to probiotic treatment

The host mucosal immune system contributes to the maintenance of intestinal homeostasis and protective responses against pathogen infection (Yan and Polk, 2011). Probiotics play an intricate role in maintaining the delicate balance between necessary and excessive defense mechanisms. They can be used to stimulate or regulate epithelial and immune cells of the intestinal mucosa, which generates beneficial immunomodulatory effects. Specifically, the effects of probiotics on host intestinal mucosal defense system include: blocking pathogenic bacterial effects by producing bactericidal substances (Ocana and Elena Nader-Macias, 2004; EF et al., 2012), competing with pathogens and toxins for adherence to the intestinal epithelium (Perea Velez et al., 2007), and promoting intestinal epithelial cell survival (Yan et al., 2007; Yan et al., 2011).

The mechanisms of probiotic-induced immunomodulation mainly involve regulation of gene expression and signaling pathways in host cells. In human clinical studies, several probiotic genes/families involved in the regulation of host immune responses have been identified. Using isolated strains of a model probiotic organism, *Lactobacillus plantarum*, Meijerink et al (Meijerink et al., 2010) employed comparative genome hybridization and a random forest algorithm to successfully identify eight candidate genes in the *L. plantarum* genome that may modulate the cytokine response (IL-10 and IL-12) of dendritic cells to *L. plantarum*. Six of these genes (*lp_0422*, *lp_0423*, *lp_0424a*, *lp_0424*, *lp_0425* and *lp_0429*) were involved in bacteriocin secretion and production. The immunomodulatory effects of these genes were confirmed by comparing the cytokine production between targeted gene deletion mutants and wildtype strains. Additionally, probiotic strains have shown varied responses in different GIT locations in the host. In an *in vitro* GIT model, Weiss et al (Weiss and Jespersen, 2010) studied differentially expressed genes in *Lactobacillus acidophilus* during

passage through the GIT. They found that stress-response related genes, *GroEL*, *DnaK* and *ClpP*, were significantly up-regulated during gastric digestion, followed by a significant decrease in subsequent duodenal digestion. Genes encoding mucin-binding and fibronectin-binding showed significant up-regulation during incubation of duodenal contents and bile, indicating that probiotic strains led to different gene expression profiles in different locations of the GIT. Thus, it may be an ideal strategy to find a specific niche for each probiotic strain when designing probiotic-based feed treatment.

Probiotic-strain secreted compounds or proteins have been identified with beneficial effects to the host. p40, a *Lactobacillus rhamnosus* GG-derived soluble protein, was shown to prevent dextran sulfate sodium-induced intestinal injury and acute colitis (Yan et al., 2011). Intestinal epithelial apoptosis and the disruption of barrier function in a mouse model of colitis were decreased after treatment with p40. p40 treatment was also associated with reduced production of tumor necrosis factor (TNF- α), IL-6, keratinocyte chemoattractant, and interferon- γ production, suggesting p40's role in regulating innate immunity and the Th1 immune response (Yan et al., 2011). In the study of *Lactobacillus reuteri* RC-14, a human vaginal isolate (Reid et al., 1987), was found to be capable of inhibiting the staphylococcal quorum-sensing system *agr*. And Li et al (Li et al., 2011) found two compounds secreted by *L. reuteri* RC-14 that were able to inhibit the expression of toxic shock syndrome toxin-1. The beneficial effects of probiotic bacterial strain secreted compounds were manifested by targeted genome modification. The phosphoglycerol transferase gene mediates lipoteichoic acid (LTA) biosynthesis in *Lactobacillus acidophilus* NCFM (NCK56). Oral administration of LTA-deficient *L. acidophilus* NCFM significantly protected mice against experimentally induced colitis (Khan et al., 2012). Along with other studies on LTA (Grangette et al., 2005; Claes et al., 2010), it was proposed that LTA synthesized in *L. acidophilus* induces the inflammatory response, and its absence significantly attenuates intestinal inflammation.

Probiotics affect the host innate and adaptive immune responses through modulating the functions of dendritic cells, macrophages, and T and B lymphocytes (Kwon et al., 2010; Evrard et al., 2011). A mixture of probiotics, consisting of *B. bifidum*, *L. acidophilus*, *L. casei*, *L. reuteri* and *Streptococcus thermophilus*, stimulated regulatory dendritic cells that expressed high levels of IL-10, TGF- β and COX-2, and also induced both T-cell and B-cell hypo-responsiveness and downregulated Th1, Th2, and Th17 cytokines without inducing apoptosis (Kwon et al., 2010). In addition, the probiotic *L. rhamnosus* Lcr35 was shown to induce a dose-dependent increase in dendritic cell secretion of pro-Th1/Th17 cytokines (e.g., TNF, IL-1 β , IL-12p70, IL-12p40, and IL-23) (Evrard et al., 2011).

Models and cell lines used to study host response to probiotic treatment

The establishment of *in vitro* models is critical for the understanding of molecular and cellular interactions between probiotic and pathogenic microorganisms within the host. Bovine intestinal epithelial cells (IEC) have important roles in nutrient absorption and regulation of immune barriers. Primary cell cultures of bovine IEC are ideal models for investigating the beneficial effects of probiotic strains on the bovine host (Zhan et al., 2017). Using a novel clone cell method, Zhan et al. (Zhan et al., 2017) successfully cultured

primary bovine IEC collected from duodenal, jejunal, and ileal tissue of a healthy lactating Holstein cow. Cultured bovine IEC can be passed for about five generations, with cellular aggregates and clusters loosely adherent at day two of culture. More importantly, the cultured bovine IEC displayed positive reactions against IEC markers, cytokeratin 18 and E-cadherin. Another set of epithelial cell specific markers include cell to cell adhesion molecules (Hollande et al., 2003), ZO-1 and β -catenin, both of which were also expressed in bovine IEC (Miyazawa et al., 2010).

The immunology of bovine IEC has been evaluated by several groups. In the study to validate bovine IEC as a model system to study antiviral responses, Chiba et al (Chiba et al., 2012) confirmed toll-like receptor (TLR) 3 expression. Additionally, bovine IECs showed a significant response to stimulation with the TLR3 agonist poly(I:C). This involved a pronounced up-regulation of pro-inflammatory cytokines and type I interferons. These responses were consistent with various intestinal viral infections of cattle and other hosts (Rollo et al., 1999; Xu et al., 2009). By evaluating the expression of TLRs in bovine IEC in response to a challenge with heat-stable Enterotoxigenic *E. coli* (ETEC), Takanashi et al. (Takanashi et al., 2013) observed recognition of pathogen associated molecular patterns (PAMP) by IEC, demonstrated by production of cytokines and activation of MAPK and NF- κ B pathways. The authors also found that *Lactobacilli casei* OLL2768 had the capacity to attenuate the heat-stable ETEC PAMP induced pro-inflammatory response in bovine IEC (Takanashi et al., 2013). Findings from this study concluded that bovine IEC are suitable models for studying the molecular mechanisms underlying the protective activity of probiotics against pathogen-induced inflammation and for selecting lactic acid bacteria with immunoregulatory functions (Takanashi et al., 2013).

Future directions

Probiotics have great potential in effective management of ruminant health. Multiple studies have shown that the microbial community in ruminants' GIT can be altered by various factors, including diet, probiotic treatments, age, and stress. Artificially introduced community changes may be achieved by well-defined probiotic treatments. And ideally, such GI microbial community changes may lead to increased production efficiency or strengthened host immunity. In depth, *in vivo* and *in vitro* studies of the dynamics and functional effects initiated by probiotic treatments can greatly enrich our understanding of when and how these treatments can benefit ruminants. The major areas of future studies include describing the structure and interactions of the gut microbiota, and functional relationships between the microbial community in the gut mucosa and the host cells. This would include comprehensive investigations that employ a "meta-omics" approach (*i.e.* metagenomic, metatranscriptomic, and metaproteomic) to investigate the dynamics between the GIT microbial community and host metabolism. Results from these studies can further the identification of a core set of well-defined microbial species for targeted health improvement and early development in ruminants.

The host mucosal innate immune function is largely affected by the GIT microbial community. The ruminal epithelium plays a major role in host mucosal innate immune function. Studies that specifically focus on the interaction between the mucosal microbial

community and the host ruminal epithelium will most likely facilitate the identification of key genes critical for host immune homeostasis. Ideally, with the application of targeted gene editing technology, one could engineer the genetic makeup of the microbiome to potentially enable optimal host health and productivity.

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References

- Aschenbach JR, Penner GB, Stumpff F, and Gabel G. 2011 Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *J Anim Sci* 89(4):1092–1107. doi: 10.2527/jas.2010-3301 [PubMed: 20952531]
- Atassi F, and Servin AL. 2010 Individual and co-operative roles of lactic acid and hydrogen peroxide in the killing activity of enteric strain *Lactobacillus johnsonii* NCC933 and vaginal strain *Lactobacillus gasseri* KS120.1 against enteric, uropathogenic and vaginosis-associated pathogens. *FEMS Microbiol Lett* 304(1):29–38. doi: 10.1111/j.1574-6968.2009.01887.x [PubMed: 20082639]
- Auffret MD, Dewhurst RJ, Duthie CA, Rooke JA, John Wallace R, Freeman TC, Stewart R, Watson M, and Roehe R. 2017 The rumen microbiome as a reservoir of antimicrobial resistance and pathogenicity genes is directly affected by diet in beef cattle. *Microbiome* 5(1):159. doi: 10.1186/s40168-017-0378-z [PubMed: 29228991]
- Balakrishnan VS, Schmid CH, Jaber BL, Natov SN, King AJ, and Pereira BJ. 2000 Interleukin-1 receptor antagonist synthesis by peripheral blood mononuclear cells: a novel predictor of morbidity among hemodialysis patients. *J Am Soc Nephrol* 11(11):2114–2121. [PubMed: 11053488]
- Baldwin R. L. t., and McLeod KR. 2000 Effects of diet forage:concentrate ratio and metabolizable energy intake on isolated rumen epithelial cell metabolism in vitro. *J Anim Sci* 78(3):771–783. [PubMed: 10764086]
- Belanche A, Doreau M, Edwards JE, Moorby JM, Pinloche E, and Newbold CJ. 2012 Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. *J Nutr* 142(9):1684–1692. doi: 10.3945/jn.112.159574 [PubMed: 22833657]
- Bouskra D, Brezillon C, Berard M, Werts C, Varona R, Boneca IG, and Eberl G. 2008 Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* 456(7221):507–510. doi: 10.1038/nature07450 [PubMed: 18987631]
- Cash HL, Whitham CV, Behrendt CL, and Hooper LV. 2006 Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 313(5790):1126–1130. doi: 10.1126/science.1127119 [PubMed: 16931762]
- Chenoll E, Casinos B, Bataller E, Astals P, Echevarria J, Iglesias JR, Balbarie P, Ramon D, and Genoves S. 2011 Novel probiotic *Bifidobacterium bifidum* CECT 7366 strain active against the pathogenic bacterium *Helicobacter pylori*. *Appl Environ Microbiol* 77(4):1335–1343. doi: 10.1128/AEM.01820-10 [PubMed: 21169430]
- Chiba E, Villena J, Hosoya S, Takanashi N, Shimazu T, Aso H, Tohno M, Suda Y, Kawai Y, Saito T, Miyazawa K, He F, and Kitazawa H. 2012 A newly established bovine intestinal epithelial cell line is effective for in vitro screening of potential antiviral immunobiotic microorganisms for cattle. *Res Vet Sci* 93(2):688–694. doi: 10.1016/j.rvsc.2011.10.002 [PubMed: 22047818]
- Chiquette J, Allison MJ, and Rasmussen MA. 2008 *Prevotella bryantii* 25A used as a probiotic in early-lactation dairy cows: Effect on ruminal fermentation characteristics, milk production, and milk composition. *Journal of Dairy Science* 91(9):3536–3543. doi: 10.3168/jds.2007-0849 [PubMed: 18765612]

- Claes JJ, Lebeer S, Shen C, Verhoeven TL, Dilissen E, De Hertogh G, Bullens DM, Ceuppens JL, Van Assche G, Vermeire S, Rutgeerts P, Vanderleyden J, and De Keersmaecker SC. 2010 Impact of lipoteichoic acid modification on the performance of the probiotic *Lactobacillus rhamnosus* GG in experimental colitis. *Clin Exp Immunol* 162(2):306–314. doi: 10.1111/j.1365-2249.2010.04228.x [PubMed: 20731672]
- Cocconier MH, Lievin V, Hemery E, and Servin AL. 1998 Antagonistic activity against *Helicobacter* infection in vitro and in vivo by the human *Lactobacillus acidophilus* strain LB. *Appl Environ Microbiol* 64(11):4573–4580. [PubMed: 9797324]
- Corr SC, Hill C, and Gahan CG. 2009 Chapter 1 Understanding the Mechanisms by Which Probiotics Inhibit Gastrointestinal Pathogens. *Advances in Food and Nutrition Research* 56:1–15. [PubMed: 19389605]
- Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, and Gahan CGM. 2007 Bacteriocin production as a mechanism for the antinfecitive activity of *Lactobacillus salivarius* UCC118. *P Natl Acad Sci USA* 104(18):7617–7621. doi: 10.1073/pnas.0700440104
- Dawid S, Roche AM, and Weiser JN. 2007 The blp bacteriocins of *Streptococcus pneumoniae* mediate intraspecies competition both in vitro and in vivo. *Infection and Immunity* 75(1):443–451. doi: 10.1128/iai.01775-05 [PubMed: 17074857]
- De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, and Lionetti P. 2010 Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* 107(33):14691–14696. doi: 10.1073/pnas.1005963107 [PubMed: 20679230]
- Dethlefsen L, Huse S, Sogin ML, and Relman DA. 2008 The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 6(11):e280. doi: 10.1371/journal.pbio.0060280 [PubMed: 19018661]
- Dias J, Marcondes MI, Noronha MF, Resende RT, Machado FS, Mantovani HC, Dill-McFarland KA, and Suen G. 2017 Effect of Pre-weaning Diet on the Rumen Archaeal, Bacterial, and Fungal Communities of Dairy Calves. *Front Microbiol* 8:1553. doi: 10.3389/fmicb.2017.01553 [PubMed: 28861065]
- Dill-McFarland KA, Breaker JD, and Suen G. 2017 Microbial succession in the gastrointestinal tract of dairy cows from 2 weeks to first lactation. *Sci Rep* 7:40864. doi: 10.1038/srep40864 [PubMed: 28098248]
- Dobson A, Cotter PD, Ross RP, and Hill C. 2012 Bacteriocin production: a probiotic trait? *Appl Environ Microbiol* 78(1):1–6. doi: 10.1128/AEM.05576-11 [PubMed: 22038602]
- Donaldson GP, Ladinsky MS, Yu KB, Sanders JG, Yoo BB, Chou WC, Conner ME, Earl AM, Knight R, Bjorkman PJ, and Mazmanian SK. 2018 Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* 360(6390):795–800. doi: 10.1126/science.aag0926 [PubMed: 29724905]
- Eadie JM, and Hobson PN. 1962 Effect of the presence or absence of rumen ciliate protozoa on the total rumen bacterial count in lambs. *Nature* 193:503–505. [PubMed: 13888936]
- EF OS, PM OC, Raftis EJ, PW OT, Stanton C, Cotter PD, Ross RP, and Hill C. 2012 Subspecies diversity in bacteriocin production by intestinal *Lactobacillus salivarius* strains. *Gut Microbes* 3(5):468–473. doi: 10.4161/gmic.21417 [PubMed: 22892690]
- Eschenbach DA, Davick PR, Williams BL, Klebanoff SJ, Young-Smith K, Critchlow CM, and Holmes KK. 1989 Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *J Clin Microbiol* 27(2):251–256. [PubMed: 2915019]
- Evrard B, Coudeyras S, Dosgilbert A, Charbonnel N, Alame J, Tridon A, and Forestier C. 2011 Dose-dependent immunomodulation of human dendritic cells by the probiotic *Lactobacillus rhamnosus* Lcr35. *PLoS One* 6(4):e18735. doi: 10.1371/journal.pone.0018735 [PubMed: 21533162]
- Fernando SC, Purvis HT 2nd, Najar FZ, Sukharnikov LO, Krehbiel CR, Nagaraja TG, Roe BA, and Desilva U. 2010 Rumen microbial population dynamics during adaptation to a high-grain diet. *Appl Environ Microbiol* 76(22):7482–7490. doi: 10.1128/AEM.00388-10 [PubMed: 20851965]
- Foditsch C, Pereira RV, Ganda EK, Gomez MS, Marques EC, Santin T, and Bicalho RC. 2015 Oral Administration of *Faecalibacterium prausnitzii* Decreased the Incidence of Severe Diarrhea and Related Mortality Rate and Increased Weight Gain in Preweaned Dairy Heifers. *PLoS One* 10(12):e0145485. doi: 10.1371/journal.pone.0145485 [PubMed: 26710101]

- Fouladgar S, Shahraki ADF, Ghalamkari GR, Khani M, Ahmadi F, and Erickson PS. 2016 Performance of Holstein calves fed whole milk with or without kefir. *J Dairy Sci* 99(10):8081–8089. doi: 10.3168/jds.2016-10921 [PubMed: 27497895]
- Ghequire MGK, Swings T, Michiels J, Buchanan SK, and De Mot R. 2018 Hitting with a BAM: Selective Killing by Lectin-Like Bacteriocins. *MBio* 9(2)doi: 10.1128/mBio.02138-17
- Gillor O, Giladi I, and Riley MA. 2009 Persistence of colicinogenic *Escherichia coli* in the mouse gastrointestinal tract. *Bmc Microbiology* 9doi: Artn 165 10.1186/1471–2180-9–165
- Gillor O, Kirkup BC, and Riley MA. 2004 Colicins and microcins: the next generation antimicrobials. *Adv Appl Microbiol* 54:129–146. doi: 10.1016/S0065-2164(04)54005-4 [PubMed: 15251279]
- Goto H, Qadis AQ, Kim YH, Ikuta K, Ichijo T, and Sato S. 2016 Effects of a bacterial probiotic on ruminal pH and volatile fatty acids during subacute ruminal acidosis (SARA) in cattle. *J Vet Med Sci* 78(10):1595–1600. doi: 10.1292/jvms.16-0211 [PubMed: 27430197]
- Gotteland M, Andrews M, Toledo M, Munoz L, Caceres P, Anziani A, Wittig E, Speisky H, and Salazar G. 2008 Modulation of *Helicobacter pylori* colonization with cranberry juice and *Lactobacillus johnsonii* La1 in children. *Nutrition* 24(5):421–426. doi: 10.1016/j.nut.2008.01.007 [PubMed: 18343637]
- Granger M, van Reenen CA, and Dicks LM. 2008 Effect of gastro-intestinal conditions on the growth of *Enterococcus mundtii* ST4SA, and production of bacteriocin ST4SA recorded by real-time PCR. *Int J Food Microbiol* 123(3):277–280. doi: 10.1016/j.ijfoodmicro.2007.12.009 [PubMed: 18234384]
- Grangette C, Nutton S, Palumbo E, Morath S, Hermann C, Dewulf J, Pot B, Hartung T, Hols P, and Mercenier A. 2005 Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. *Proc Natl Acad Sci U S A* 102(29):10321–10326. doi: 10.1073/pnas.0504084102 [PubMed: 15985548]
- Guzman CE, Bereza-Malcolm LT, De Groef B, and Franks AE. 2015 Presence of Selected Methanogens, Fibrolytic Bacteria, and Proteobacteria in the Gastrointestinal Tract of Neonatal Dairy Calves from Birth to 72 Hours. *PLoS One* 10(7):e0133048. doi: 10.1371/journal.pone.0133048 [PubMed: 26186002]
- Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, and Sanders ME. 2014 Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 11(8):506–514. doi: 10.1038/nrgastro.2014.66 [PubMed: 24912386]
- Hillman JD, Dzuback AL, and Andrews SW. 1987 Colonization of the Human Oral Cavity by a *Streptococcus-Mutans* Mutant Producing Increased Bacteriocin. *Journal of Dental Research* 66(6):1092–1094. doi: 10.1177/00220345870660060101 [PubMed: 3476580]
- Hollande F, Lee DJ, Choquet A, Roche S, and Baldwin GS. 2003 Adherens junctions and tight junctions are regulated via different pathways by progastrin in epithelial cells. *J Cell Sci* 116(Pt 7):1187–1197. [PubMed: 12615962]
- Hummel A, Holzapfel WH, and Franz CM. 2007 Characterisation and transfer of antibiotic resistance genes from enterococci isolated from food. *Syst Appl Microbiol* 30(1):1–7. doi: 10.1016/j.syapm.2006.02.004 [PubMed: 16563685]
- Hungate RE 1966 *Rumen and Its Microbes* Academic Press, New York.
- Ishaq SL, Kim CJ, Reis D, and Wright AD. 2015 Fibrolytic Bacteria Isolated from the Rumen of North American Moose (*Alces alces*) and Their Use as a Probiotic in Neonatal Lambs. *PLoS One* 10(12):e0144804. doi: 10.1371/journal.pone.0144804 [PubMed: 26716685]
- Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, and Littman DR. 2009 Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139(3):485–498. doi: 10.1016/j.cell.2009.09.033 [PubMed: 19836068]
- Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, and Littman DR. 2008 Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 4(4):337–349. doi: 10.1016/j.chom.2008.09.009 [PubMed: 18854238]

- Jernberg C, Lofmark S, Edlund C, and Jansson JK. 2007 Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J* 1(1):56–66. doi: 10.1038/ismej.2007.3 [PubMed: 18043614]
- Jouany JP, Mathieu F, Senaud J, Bohatier J, Bertin G, and Mercier M. 1998 Effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on the digestion of nitrogen in the rumen of defaunated and refaunated sheep. *Animal Feed Science and Technology* 75(1):1–13. doi: 10.1016/S0377-8401(98)00194-1
- Kalmokoff ML, and Teather RM. 1997 Isolation and characterization of a bacteriocin (Butyrivibriocin AR10) from the ruminal anaerobe *Butyrivibrio fibrisolvens* AR10: evidence in support of the widespread occurrence of bacteriocin-like activity among ruminal isolates of *B. fibrisolvens*. *Appl Environ Microbiol* 63(2):394–402. [PubMed: 9023920]
- Kechaou N, Chain F, Gratadoux JJ, Blugeon S, Bertho N, Chevalier C, Le Goffic R, Courau S, Molimard P, Chatel JM, Langella P, and Bermudez-Humaran LG. 2013 Identification of one novel candidate probiotic *Lactobacillus plantarum* strain active against influenza virus infection in mice by a large-scale screening. *Appl Environ Microbiol* 79(5):1491–1499. doi: 10.1128/AEM.03075-12 [PubMed: 23263960]
- Khan MW, Zadeh M, Bere P, Gounaris E, Owen J, Klaenhammer T, and Mohamadzadeh M. 2012 Modulating intestinal immune responses by lipoteichoic acid-deficient *Lactobacillus acidophilus*. *Immunotherapy-Uk* 4(2):151–161. doi: 10.2217/Imt.11.163
- Kleefisch MT, Zebeli Q, Humer E, Kroger I, Ertl P, and Klevenhusen F. 2017 Effects of the replacement of concentrate and fibre-rich hay by high-quality hay on chewing, rumination and nutrient digestibility in non-lactating Holstein cows. *Arch Anim Nutr* 71(1):21–36. doi: 10.1080/1745039X.2016.1253227 [PubMed: 27858463]
- Kleerebezem M, Quadri LE, Kuipers OP, and de Vos WM. 1997 Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. *Mol Microbiol* 24(5):895–904. [PubMed: 9219998]
- Klevenhusen F, Petri RM, Kleefisch MT, Khiaosa-Ard R, Metzler-Zebeli BU, and Zebeli Q. 2017 Changes in fibre-adherent and fluid-associated microbial communities and fermentation profiles in the rumen of cattle fed diets differing in hay quality and concentrate amount. *FEMS Microbiol Ecol* 93(9)doi: 10.1093/femsec/fix100
- Kralik P, Babak V, and Dziedzinska R. 2018 The Impact of the Antimicrobial Compounds Produced by Lactic Acid Bacteria on the Growth Performance of *Mycobacterium avium* subsp. paratuberculosis. *Front Microbiol* 9:638. doi: 10.3389/fmicb.2018.00638 [PubMed: 29666620]
- Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, Nam JH, Rhee JH, Hwang KC, and Im SH. 2010 Generation of regulatory dendritic cells and CD4⁺ Foxp3⁺ T cells by probiotics administration suppresses immune disorders. *Proc Natl Acad Sci U S A* 107(5):2159–2164. doi: 10.1073/pnas.0904055107 [PubMed: 20080669]
- Laarman AH, and Oba M. 2011 Short communication: Effect of calf starter on rumen pH of Holstein dairy calves at weaning. *J Dairy Sci* 94(11):5661–5664. doi: 10.3168/jds.2011-4273 [PubMed: 22032389]
- Laarman AH, Ruiz-Sanchez AL, Sugino T, Guan LL, and Oba M. 2012 Effects of feeding a calf starter on molecular adaptations in the ruminal epithelium and liver of Holstein dairy calves. *J Dairy Sci* 95(5):2585–2594. doi: 10.3168/jds.2011-4788 [PubMed: 22541487]
- Le Blay G, Lacroix C, Zihler A, and Fliss I. 2007 In vitro inhibition activity of nisin A, nisin Z, pediocin PA-1 and antibiotics against common intestinal bacteria. *Lett Appl Microbiol* 45(3):252–257. doi: 10.1111/j.1472-765X.2007.02178.x [PubMed: 17718835]
- Li J, Wang W, Xu SX, Magarvey NA, and McCormick JK. 2011 *Lactobacillus reuteri*-produced cyclic dipeptides quench agr-mediated expression of toxic shock syndrome toxin-1 in staphylococci. *Proc Natl Acad Sci U S A* 108(8):3360–3365. doi: 10.1073/pnas.1017431108 [PubMed: 21282650]
- Marcondes MI, T. R. P., Chagas JCC, Filgueiras EA, Castro MMD, Costa GP, Sguizzato ALL and Sainz RD. 2016 Performance and health of Holstein calves fed different levels of milk fortified with symbiotic complex containing pre- and probiotics. *Trop Anim Health Prod* (DOI 10.1007/s11250-016-1127-1)
- Maldonado NC, Chiaraviglio J, Bru E, De Chazal L, Santos V, and Nader-Macias MEF. 2017 Effect of Milk Fermented with Lactic Acid Bacteria on Diarrheal Incidence, Growth Performance and

- Microbiological and Blood Profiles of Newborn Dairy Calves. Probiotics Antimicrob Proteins doi: 10.1007/s12602-017-9308-4
- Malmuthuge N 2016 Role of Commensal Microbiota in Neonatal Calf Gut Development (Thesis). Education and Research Archives
- Malmuthuge N, Griebel PJ, and Guan le L. 2014 Taxonomic identification of commensal bacteria associated with the mucosa and digesta throughout the gastrointestinal tracts of preweaned calves. *Appl Environ Microbiol* 80(6):2021–2028. doi: 10.1128/AEM.03864-13 [PubMed: 24441166]
- Mantovani HC, Kam DK, Ha JK, and Russell JB. 2001 The antibacterial activity and sensitivity of *Streptococcus bovis* strains isolated from the rumen of cattle. *Fems Microbiology Ecology* 37(3): 223–229. doi: 10.1016/S01686496(01)00166-0
- Maslowski KM, and Mackay CR. 2011 Diet, gut microbiota and immune responses. *Nat Immunol* 12(1):5–9. doi: 10.1038/ni0111-5 [PubMed: 21169997]
- Mathieu F, Jouany JP, Senaud J, Bohatier J, Bertin G, and Mercier M. 1996 The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentations in the rumen of faunated and defaunated sheep; Protozoal and probiotic interactions. *Reprod Nutr Dev* 36(3):271–287. doi: 10.1051/rnd:19960305 [PubMed: 8766732]
- Mazmanian SK, Liu CH, Tzianabos AO, and Kasper DL. 2005 An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122(1):107–118. doi: 10.1016/j.cell.2005.05.007 [PubMed: 16009137]
- Mazmanian SK, Round JL, and Kasper DL. 2008 A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453(7195):620–625. doi: 10.1038/nature07008 [PubMed: 18509436]
- McCann JC, Luan S, Cardoso FC, Derakhshani H, Khafipour E, and Looor JJ. 2016 Induction of Subacute Ruminant Acidosis Affects the Ruminant Microbiome and Epithelium. *Front Microbiol* 7:701. doi: 10.3389/fmicb.2016.00701 [PubMed: 27242724]
- Meale SJ, Li S, Azevedo P, Derakhshani H, Plaizier JC, Khafipour E, and Steele MA. 2016 Development of Ruminant and Fecal Microbiomes Are Affected by Weaning But Not Weaning Strategy in Dairy Calves. *Front Microbiol* 7:582. doi: 10.3389/fmicb.2016.00582 [PubMed: 27199916]
- Meijerink M, van Hemert S, Taverne N, Wels M, de Vos P, Bron PA, Savelkoul HF, van Bilsen J, Kleerebezem M, and Wells JM. 2010 Identification of genetic loci in *Lactobacillus plantarum* that modulate the immune response of dendritic cells using comparative genome hybridization. *PLoS One* 5(5):e10632. doi: 10.1371/journal.pone.0010632 [PubMed: 20498715]
- Michetti P, Dorta G, Wiesel PH, Brassart D, Verdu E, Herranz M, Felley C, Porta N, Rouvet M, Blum AL, and Cortesy-Theulaz I. 1999 Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (johnsonii) La1 on *Helicobacter pylori* infection in humans. *Digestion* 60(3):203–209. doi: 10.1159/00007660 [PubMed: 10343133]
- Miyazawa K, Hondo T, Kanaya T, Tanaka S, Takakura I, Itani W, Rose MT, Kitazawa H, Yamaguchi T, and Aso H. 2010 Characterization of newly established bovine intestinal epithelial cell line. *Histochem Cell Biol* 133(1):125–134. doi: 10.1007/s00418-009-0648-3 [PubMed: 19830445]
- Mota-Meira M, Lacroix C, LaPointe G, and Lavoie MC. 1997 Purification and structure of mutacin B-Ny266: a new lantibiotic produced by *Streptococcus mutans*. *FEBS Lett* 410(2–3):275–279. [PubMed: 9237644]
- Mota-Meira M, LaPointe G, Lacroix C, and Lavoie MC. 2000 MICs of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against bacterial pathogens. *Antimicrob Agents Chemother* 44(1):24–29. [PubMed: 10602718]
- Muya MC, Nherera FV, Miller KA, Aperce CC, Moshidi PM, and Erasmus LJ. 2015 Effect of *Megasphaera elsdenii* NCIMB 41125 dosing on rumen development, volatile fatty acid production and blood beta-hydroxybutyrate in neonatal dairy calves. *J Anim Physiol Anim Nutr (Berl)* 99(5): 913–918. doi: 10.1111/jpn.12306 [PubMed: 25817063]
- Newbold CJ, Wallace RJ, and McIntosh FM. 1996 Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *Brit J Nutr* 76(2):249–261. doi: 10.1079/Bjn19960029 [PubMed: 8813899]
- Ocana VS, and Elena Nader-Macias M. 2004 Production of antimicrobial substances by lactic acid bacteria II: screening bacteriocin-producing strains with probiotic purposes and characterization of

- a *Lactobacillus bacteriocin*. *Methods Mol Biol* 268:347–353. doi: 10.1385/1-59259-766-1:347 [PubMed: 15156045]
- Ocana VS, Pesce AA Ruiz Holgado De, and Nader-Macias ME. 1999 Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain. *Appl Environ Microbiol* 65(12):5631–5635. [PubMed: 10584033]
- Okada Y, Setoyama H, Matsumoto S, Imaoka A, Nanno M, Kawaguchi M, and Umesaki Y. 1994 Effects of Fecal Microorganisms Acid Their Chloroform-Resistant Variants Derived from Mice, Rats, and Humans on Immunological and Physiological-Characteristics of the Intestines of Ex-Germ-Free Mice. *Infection and Immunity* 62(12):5442–5446. [PubMed: 7960124]
- Oultram J, Phipps E, Teixeira AG, Foditsch C, Bicalho ML, Machado VS, Bicalho RC, and Oikonomou G. 2015 Effects of antibiotics (oxytetracycline, florfenicol or tulathromycin) on neonatal calves' faecal microbial diversity. *Vet Rec* 177(23):598. doi: 10.1136/vr.103320
- Panda S, El khader I, Casellas F, Lopez Vivancos J, Garcia Cors M, Santiago A, Cuenca S, Guarner F, and Manichanh C. 2014 Short-term effect of antibiotics on human gut microbiota. *PLoS One* 9(4):e95476. doi: 10.1371/journal.pone.0095476 [PubMed: 24748167]
- Patzter SI, Baquero MR, Bravo D, Moreno F, and Hantke K. 2003 The colicin G, H and X determinants encode microcins M and H47, which might utilize the catecholate siderophore receptors FepA, Cir, Fiu and IroN. *Microbiology* 149(Pt 9):2557–2570. doi: 10.1099/mic.0.26396-0 [PubMed: 12949180]
- Perea Velez M, Hermans K, Verhoeven TL, Lebeer SE, Vanderleyden J, and De Keersmaecker SC. 2007 Identification and characterization of starter lactic acid bacteria and probiotics from Columbian dairy products. *J Appl Microbiol* 103(3):666–674. doi: 10.1111/j.1365-2672.2007.03294.x [PubMed: 17714400]
- Petri RM, Kleefisch MT, Metzler-Zebeli BU, Zebeli Q, and Klevenhusen F. 2018 Changes in the Rumen Epithelial Microbiota of Cattle and Host Gene Expression in Response to Alterations in Dietary Carbohydrate Composition. *Appl Environ Microbiol* 84(12)doi: 10.1128/AEM.00384-18
- Petri RM, Schwaiger T, Penner GB, Beauchemin KA, Forster RJ, McKinnon JJ, and McAllister TA. 2013 Changes in the rumen epimural bacterial diversity of beef cattle as affected by diet and induced ruminal acidosis. *Appl Environ Microbiol* 79(12):3744–3755. doi: 10.1128/AEM.03983-12 [PubMed: 23584771]
- Rea MC, Dobson A, O'Sullivan O, Crispie F, Fouhy F, Cotter PD, Shanahan F, Kiely B, Hill C, and Ross RP. 2011 Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. *Proc Natl Acad Sci U S A* 108 Suppl 1:4639–4644. doi: 10.1073/pnas.1001224107 [PubMed: 20616009]
- Reid G, Cook RL, and Bruce AW. 1987 Examination of strains of lactobacilli for properties that may influence bacterial interference in the urinary tract. *J Urol* 138(2):330–335. [PubMed: 3599250]
- Rey M, Enjalbert F, Combes S, Cauquil L, Bouchez O, and Monteils V. 2014 Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. *J Appl Microbiol* 116(2): 245–257. doi: 10.1111/jam.12405 [PubMed: 24279326]
- Riley MA, and Wertz JE. 2002a Bacteriocin diversity: ecological and evolutionary perspectives. *Biochimie* 84(5–6):357–364. [PubMed: 12423779]
- Riley MA, and Wertz JE. 2002b Bacteriocins: evolution, ecology, and application. *Annu Rev Microbiol* 56:117–137. doi: 10.1146/annurev.micro.56.012302.161024 [PubMed: 12142491]
- Rodriguez-Palacios A, Staempfli HR, and Weese JS. 2017 High Doses of Halotolerant Gut-Indigenous *Lactobacillus plantarum* Reduce Cultivable Lactobacilli in Newborn Calves without Increasing Its Species Abundance. *Int J Microbiol* 2017:2439025. doi: 10.1155/2017/2439025 [PubMed: 28596790]
- Rollo EE, Kumar KP, Reich NC, Cohen J, Angel J, Greenberg HB, Sheth R, Anderson J, Oh B, Hempson SJ, Mackow ER, and Shaw RD. 1999 The epithelial cell response to rotavirus infection. *J Immunol* 163(8):4442–4452. [PubMed: 10510386]
- Roos K, and Holm S. 2002 The Use of Probiotics in Head and Neck Infections. *Curr Infect Dis Rep* 4(3):211–216. [PubMed: 12015913]

- Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, and Mazmanian SK. 2011 The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 332(6032): 974–977. doi: 10.1126/science.1206095 [PubMed: 21512004]
- Round JL, and Mazmanian SK. 2010 Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A* 107(27):12204–12209. doi: 10.1073/pnas.0909122107 [PubMed: 20566854]
- Rychlik JL, and Russell JB. 2002 Bacteriocin-like activity of *Butyrivibrio fibrisolvens* JL5 and its effect on other ruminal bacteria and ammonia production. *Appl Environ Microbiol* 68(3):1040–1046. [PubMed: 11872448]
- Saleem AM, Zanouny AI, and Singer AM. 2017 Growth performance, nutrients digestibility, and blood metabolites of lambs fed diets supplemented with probiotics during pre- and post-weaning period. *Asian-Australas J Anim Sci* 30(4):523–530. doi: 10.5713/ajas.16.0691 [PubMed: 28002935]
- Schamberger GP, Phillips RL, Jacobs JL, and Diez-Gonzalez F. 2004 Reduction of *Escherichia coli* O157 : H7 populations in cattle by addition of colicin E7-producing *E-coli* to feed. *Appl Environ Microb* 70(10):6053–6060. doi: 10.1128/Aem.70.10.6053-6060.2004
- Shand RF, L. K. 2008 Archaeal antimicrobials: an undiscovered country. In: Blum P, editor. *Archaea: new models for prokaryotic biology* Caister Academic; Norfolk:233–242.
- Shanks OC, Kelty CA, Archibeque S, Jenkins M, Newton RJ, McLellan SL, Huse SM, and Sogin ML. 2011 Community structures of fecal bacteria in cattle from different animal feeding operations. *Appl Environ Microbiol* 77(9):2992–3001. doi: 10.1128/AEM.02988-10 [PubMed: 21378055]
- Sharma AN, Kumar S, and Tyagi AK. 2018 Effects of mannan-oligosaccharides and *Lactobacillus acidophilus* supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves. *J Anim Physiol Anim Nutr (Berl)* 102(3):679–689. doi: 10.1111/jpn.12878 [PubMed: 29493022]
- Shin MS, Han SK, Ryu JS, Kim KS, and Lee WK. 2008 Isolation and partial characterization of a bacteriocin produced by *Pediococcus pentosaceus* K23–2 isolated from Kimchi. *J Appl Microbiol* 105(2):331–339. doi: 10.1111/j.13652672.2008.03770.x [PubMed: 18540969]
- Takanashi N, Tomosada Y, Villena J, Murata K, Takahashi T, Chiba E, Tohno M, Shimazu T, Aso H, Suda Y, Ikegami S, Itoh H, Kawai Y, Saito T, Alvarez S, and Kitazawa H. 2013 Advanced application of bovine intestinal epithelial cell line for evaluating regulatory effect of *Lactobacilli* against heat-killed enterotoxigenic *Escherichia coli*-mediated inflammation. *BMC Microbiol* 13:54. doi: 10.1186/1471-2180-13-54 [PubMed: 23497067]
- Thomas M, Webb M, Ghimire S, Blair A, Olson K, Fenske GJ, Fonder AT, Christopher-Hennings J, Brake D, and Scaria J. 2017 Metagenomic characterization of the effect of feed additives on the gut microbiome and antibiotic resistome of feedlot cattle. *Sci Rep* 7(1):12257. doi: 10.1038/s41598-017-12481-6 [PubMed: 28947833]
- Timmerman HM, Mulder L, Everts H, van Espen DC, van der Wal E, Klaassen G, Rouwers SM, Hartemink R, Rombouts FM, and Beynen AC. 2005 Health and growth of veal calves fed milk replacers with or without probiotics. *J Dairy Sci* 88(6):2154–2165. doi: 10.3168/jds.S0022-0302(05)72891-5 [PubMed: 15905445]
- Uyeno Y, Shigemori S, and Shimosato T. 2015 Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes Environ* 30(2):126–132. doi: 10.1264/jsme2.ME14176 [PubMed: 26004794]
- Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, Ley R, Wakeland EK, and Hooper LV. 2011 The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science* 334(6053):255–258. doi: 10.1126/science.1209791 [PubMed: 21998396]
- van Hemert S, Meijerink M, Molenaar D, Bron PA, de Vos P, Kleerebezem M, Wells JM, and Marco ML. 2010 Identification of *Lactobacillus plantarum* genes modulating the cytokine response of human peripheral blood mononuclear cells. *BMC Microbiol* 10:293. doi: 10.1186/1471-2180-10-293 [PubMed: 21080958]
- von Buenau R, Jaekel L, Schubotz E, Schwarz S, Stroff T, and Krueger M. 2005 *Escherichia coli* strain Nissle 1917: Significant reduction of neonatal calf diarrhea. *Journal of Dairy Science* 88(1):317–323. doi: DOI 10.3168/jds.S0022-0302(05)72690-4 [PubMed: 15591395]

- Wang HB, Wang PY, Wang X, Wan YL, and Liu YC. 2012 Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig Dis Sci* 57(12):3126–3135. doi: 10.1007/s10620-012-2259-4 [PubMed: 22684624]
- Warner AC 1956 Criteria for establishing the validity of in vitro studies with rumen micro-organisms in so-called artificial rumen systems. *J Gen Microbiol* 14(3):733–748. doi: 10.1099/00221287-14-3-733 [PubMed: 13346034]
- Weimer PJ 2015 Redundancy, resilience, and host specificity of the ruminal microbiota: implications for engineering improved ruminal fermentations. *Frontiers in Microbiology* 6doi: UNSP 296 10.3389/fmicb.2015.00296
- Weiss G, and Jespersen L. 2010 Transcriptional analysis of genes associated with stress and adhesion in *Lactobacillus acidophilus* NCFM during the passage through an in vitro gastrointestinal tract model. *J Mol Microbiol Biotechnol* 18(4):206–214. doi: 10.1159/000316421 [PubMed: 20559014]
- Wells JE, Krause DO, Callaway TR, and Russell JB. 1997 A bacteriocin-mediated antagonism by ruminal lactobacilli against *Streptococcus bovis*. *Fems Microbiology Ecology* 22(3):237–243. doi: Doi 10.1016/S0168-6496(96)00095-5
- Whitford MF, McPherson MA, Forster RJ, and Teather RM. 2001 Identification of bacteriocin-like inhibitors from rumen *Streptococcus* spp. and isolation and characterization of bovicin 255. *Appl Environ Microbiol* 67(2):569–574. doi: 10.1128/AEM.67.2.569-574.2001 [PubMed: 11157218]
- Xu J, Yang Y, Wang C, and Jiang B. 2009 Rotavirus and coxsackievirus infection activated different profiles of toll-like receptors and chemokines in intestinal epithelial cells. *Inflamm Res* 58(9): 585–592. doi: 10.1007/s00011-009-0022-x [PubMed: 19296205]
- Yan F, Cao H, Cover TL, Washington MK, Shi Y, Liu L, Chaturvedi R, Peek RM Jr., Wilson KT, and Polk DB. 2011 Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGFR-dependent mechanism. *J Clin Invest* 121(6): 2242–2253. doi: 10.1172/JCI44031 [PubMed: 21606592]
- Yan F, Cao H, Cover TL, Whitehead R, Washington MK, and Polk DB. 2007 Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* 132(2):562–575. doi: 10.1053/j.gastro.2006.11.022 [PubMed: 17258729]
- Yan F, and Polk DB. 2011 Probiotics and immune health. *Curr Opin Gastroenterol* 27(6):496–501. doi: 10.1097/MOG.0b013e32834baa4d [PubMed: 21897224]
- Yanez-Ruiz DR, Abecia L, and Newbold CJ. 2015 Manipulating rumen microbiome and fermentation through interventions during early life: a review. *Front Microbiol* 6:1133. doi: 10.3389/fmicb.2015.01133 [PubMed: 26528276]
- Yanez-Ruiz DR, Macias B, Pinloche E, and Newbold CJ. 2010 The persistence of bacterial and methanogenic archaeal communities residing in the rumen of young lambs. *FEMS Microbiol Ecol* 72(2):272–278. doi: 10.1111/j.1574-6941.2010.00852.x [PubMed: 20236326]
- Yoon IK, and Stern MD. 1995 Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants. *Asian-Australasian Journal of Animal Sciences* 8:533–555.
- Zebeli Q, and Metzler-Zebeli BU. 2012 Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. *Res Vet Sci* 93(3):1099–1108. doi: 10.1016/j.rvsc.2012.02.004 [PubMed: 22370295]
- Zhan K, Lin M, Liu MM, Sui YN, and Zhao GQ. 2017 Establishment of primary bovine intestinal epithelial cell culture and clone method. *In Vitro Cell Dev Biol Anim* 53(1):54–57. doi: 10.1007/s11626-016-0082-5 [PubMed: 27561890]
- Zhang R, Dong X, Zhou M, Tu Y, Zhang N, Deng K, and Diao Q. 2017 Oral administration of *Lactobacillus plantarum* and *Bacillus subtilis* on rumen fermentation and the bacterial community in calves. *Anim Sci J* 88(5):755–762. doi: 10.1111/asj.12691 [PubMed: 27628956]
- Zhang XF, Zhang HB, Wang ZS, Zhang XM, Zou HW, Tan C, and Peng QH. 2015 Effects of dietary carbohydrate composition on rumen fermentation characteristics and microbial population in vitro. *Ital J Anim Sci* 14(3)doi: ARTN 3366 10.4081/ijas.2015.3366

Table 1.

Probiotic Treatment in ruminants

Probiotic	Host	Component affected by probiotic administration			Main Conclusions	Reference
		Fecal consistency	Feed efficiency	Body weight (BW) gain		
<i>Lactobacillus acidophilus</i>	buffalo calves	+	+	+	Improved feed efficiency, final BW gain, dry matter intake, and average daily gain	(Sharma et al., 2018)
Mixture of <i>Lactobacillus</i> species	dairy calves	+	n/a	+	The overall health index, including fecal consistency, was greater in treated calves. Calf morbidity and mortality tended to be lower in treated animals.	(Maldonado et al., 2017)
<i>Lactobacillus plantarum</i>	dairy calves	ND	n/a	-	High doses of the probiotic decreased BW gain and abundance of other fecal lactobacilli.	(Rodriguez-Palacios et al., 2017)
<i>Pediococcus acidilactici</i> and <i>Pediococcus pentosaceus</i>	lambs	n/a	ND	ND	No differences in pre- or postweaning average daily gains, milk intakes, or feed conversion ratios between treatments.	(Saleem et al., 2017)
Mixture of <i>Bacillus subtilis</i> , <i>Enterococcus faecium</i> , <i>Lactobacillus acidophilus</i> , and <i>Saccharomyces cerevisiae</i>	dairy calves	ND	ND	ND	No difference in BW gain, dry matter intake or feed efficiency of calves during pre- or post-weaning periods. Fecal scores tended to be improved in treated animals.	(M. I. Marcondes, 2016)
Kefir (<i>Acetobacter</i> , <i>Lactobacillus</i> spp., <i>Lactococcus</i> spp., and yeasts)	dairy calves	+	ND	ND	Dry matter intake, BW gain, and gain to feed ratios did not differ between treatments. Fecal scores were improved and days with diarrhea during the first 2 weeks of life were reduced in kefir-fed calves.	(Fouladgar et al., 2016)
<i>Bacillus</i> , <i>Paenibacillus</i> , and <i>Staphylococcus</i> isolates from moose	lambs	n/a	+	ND	BW gain and wool quality did not differ between treatments. Feed intake was decreased in animals provided the probiotic mixture.	(Ishaq et al., 2015)
<i>Megasphaera elsdenii</i>	dairy calves	n/a	n/a	ND	Greater dry matter intake, ruminal papillae width, density and butyrate, and no difference in average daily gain	(Muya et al., 2015)
<i>Faecalibacterium prausnitzii</i>	dairy calves	ND	n/a	+	Decreased mortality rate associated with severe diarrhea	(Foditsch et al., 2015)

*
ND: not determined

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Table 2.

Bacteriocins and their functions.

Producing strain	Bacteriocins	Function	Reference
<i>Bacillus thuringiensis</i> DPC 6431	Thuricin CD	Active against <i>Clostridium difficile</i>	(Rea et al., 2011)
<i>Enterococcus faecium</i>	lactococcin G, plantaricin A, enterocin X	Active against other lactic acid bacteria	(Hummel et al., 2007)
<i>Enterococcus mundtii</i> ST4SA	Enterocin	Inhibit <i>Enterococcus faecalis</i> , <i>S.aureus</i> & <i>Paeruginosa</i> , <i>K.pneumonia</i>	(Granger et al., 2008)
<i>Pediococci</i>	pediocins	Active against <i>Listeria monocytogenes</i>	(Shin et al., 2008)
<i>Escherichia</i>	colicins, microcins	Prevent <i>Salmonella</i> contamination in poultry industry	(Gillor et al., 2004)
<i>Lactobacillus salivarius</i> UCC118	Abp118	Active against <i>Listeria monocytogenes</i>	(Corr et al., 2007)
<i>Lactobacillus johnsonii</i> strain LA1	UN	Inhibit against <i>Helicobacter pylori</i> in	(Michetti et al., 1999; Gotteland et al., 2008)
<i>L.acidophilus</i> strain LB	UN	Inhibit <i>H. pylori</i> , <i>H. felis</i>	(Coconnier et al., 1998)
<i>L. salivarius</i> strain CRL 1328	BLIS	Inhibit <i>Enterococcus spp</i> , <i>Neisseria gonorrhoeae</i>	(Ocana et al., 1999)
<i>Streptococcus mutans</i>	Mutacin B-Ny266	Active <i>Staphylococci</i> , <i>Streptococci</i> , <i>Neisseria</i>	(Mota-Meira et al., 1997; Mota-Meira et al., 2000)
<i>Streptococcus salivarius</i> K12	Salivaricin A & B	Inhibit <i>S. pyogenes</i> , <i>S. sobrinus</i> , <i>S. mutans</i>	(Balakrishnan et al., 2000)