Understanding the host-microbiome interactions in dairy calves: intervention opportunities to improve calf performance

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Abstract

The evidence of the role of gastrointestinal microbiota in gut health of neonatal dairy calves is accumulating and has revealed that early life microbiome plays important roles in calves' development, growth and health. However, the comprehensive understanding of the gut microbiome at composition and function levels in pre-weaned calves is limited, especially for the lower gut. This paper will include to date knowledge about the initial colonization of the microbiota in the lower gut of newborn calves and how they establish in the gut from birth to pre-weaning stages, and what are the key functions of the gut microbiome, and how they may affect the calf productivity in the long term. This mini review aims to provide some insights and opportunities for novel strategies to improve calf health through manipulation of their gut microbiome.

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Introduction:

Calves have an undeveloped gastrointestinal tract (GIT) when they are born. The maturation of the gut is modulated by many factors, including external ones such as nutrients, rearing management and environment and internal ones such as genetics, and the colonization and establishment of the microbiome. The mammalian gut microbiome consists of diverse groups of prokaryotic and eukaryotic microbes, which colonize the gut immediately after birth with different succession orders. Microbial colonization during early life is a dynamic process and the initial colonization is largely affected by the delivery methods, environment and material transmissions. Early life microbiota plays an important role in influencing host animal's development and growth. The commensal microbiota has many functions, including polysaccharide digestion, protecting the host against pathogens, stimulating host immune system development, detoxifying toxic metabolites (Tanaka and Nakayama, 2017), and impacting intestinal barrier development (Petersson et al., 2011), which can all affect intestinal barrier function, immune system development, metabolism, and health. To date, the comprehensive understanding of the gut microbiome and its functions are still limited.

Gut microbiome in pre-weaned dairy calves :

A recent study detected the labeled *Enterococcus faecium* admitted to the pregnant mice in the amniotic fluid and meconium of newborn mice after sterile cesarean section (C-section), suggesting that maternal microbiota could be transmitted to newborn mice in the utero (Jiménez et al., 2005). Although some evidences have suggested the microbial colonization starting *in utero*, it is still debatable. For neonatal calves, Mayer et al. (2012) reported that *Citrobacter*, *Lactococcus*, *Leuconostoc* and *Lactobacillus* are the first gut colonizers and changing patterns of several bacterial genera. *Citrobacter* spp., appeared in all calves after birth

and disappeared after 24 h, and *Clostridium* spp. is one of the dominant bacteria between 24 h and 48 h of life based on the microbial colonization process in feces of dairy calves from birth to 42 days of life. In addition, *E. coli* is the dominant genus from 24 h to day seven after birth, and its abundance starts to decrease from day 3 to day 7, whereas the population of *Bacteroides* spp. including *B. fragilis* and *B. vulgatus* began to increase at this time (Mayer et al., 2012). This study indicated that gut microbial colonization process of pre-weaned ruminant is similar to human, which was firstly colonized by facultative bacteria, following by obligating anaerobic. Additionally, Malmuthuge et al. (2014) described the intestinal microbiota composition of three-week old dairy calves in terms of using different sample types (Mucosa vs. Digesta). *Bacteroides*, *Prevotella*, *Lactobacillus*, *Sharpea*, *Faecalibacterium* and *Burkholderia* genera are predominant in mucosa-attached bacterial community, whereas, *Bacteroides*, *Prevotella*, *Lactobacillus*, *Clostridium*, *Sharpea* and *Faecalibacterium* are predominant genera in digesta-associated community (Malmuthuge et al., 2014). Moreover, *Bacteroides-Prevotella* and *Clostridium coccoides-Eubacterium rectale* groups have higher relative abundance during the first 12 weeks after birth based on sequence-specific rRNA cleavage analysis (Uyeno et al., 2010).

There are dynamic changes in the intestinal microbiota of ruminants during the pre-weaning period since the pre-ruminants are considered as monogastric before their rumen are developed. There is a conflict about the most predominant phylum in the ruminant gut based on previous publications (Table 1). *Firmicutes* is reported to be the most abundant phylum during the first seven weeks of life in feces of dairy calves (Oikonomou et al., 2013; Foditsch et al., 2015), while others suggest that *Bacteroidetes* is the most predominant phylum in fecal samples of pre-weaned calves (Uyeno et al., 2010; Klein-Jöbstl et al., 2014). Such difference may be due to different breed, calf management strategy, and the sampling method. Malmuthuge et al. (2014) indicated that *Firmicutes* (57.6%) was the predominant phylum in the digesta, while *Bacteroidete*s phylum dominates the mucosa-associated microbiota in the small intestine when studying the microbial community using lumen and tissue samples separately. However, most of studies on gut microbiota in dairy calves are based on fecal samples since sample collection process is not invasive (Uyeno et al., 2010; Oikonomou et al., 2013; Klein-Jöbstl et al., 2014). A few researches have used local intestinal tissue and content samples for microbial profile analysis (Malmuthuge et al., 2012, 2014, 2015). In dairy calves, the supplement of calf starter during weaning period does not affect small intestinal bacterial density and lactic acid bacterial populations, however, it tends to increase the number of bacterial phylotypes (Malmuthuge et al., 2013). Additionally, the effect of antimicrobial bacitracin methylene disalicylate (BMD) on dairy calf intestinal microbial community has been reported. The abundance of potential pathogenic *Escherichia*, *Enterococcus* and *Shigella* increased, while the abundance of beneficial *Roseburia*, *Faecalibacterium* and *Eubacterium* decreased (Xie et al., 2013). In the meantime, neonatal calves fed with milk replacer supplemented with prebiotics tend to have more Lactobacilli in their feces than calves fed with milk replacer and antibiotics (Heinrichs et al., 2009). Moreover, the host genetic effect on gut microbiota in dairy calf has been reported that the rectal microbiota of the calf is more similar with its twin sister compared to other calves who are raised in the same place (Mayer et al., 2012). Therefore, selecting the representative intestinal samples as well as taking the host genetics into account are need to assess the microbial profiles in the gut of neonatal and pre-weaned calves and how they can be affected by different farm management.

Functions of the gut microbiota

Lower gut microbiota plays an important role in microbial fermentation indigestible or unabsorbed of carbohydrates including the indigestible polysaccharides (resistant starch, non-starch polysaccharides), and some monosaccharides and disaccharides (oligosaccharide, lactose, fructose) (Chassard et al., 2010; Schwab and Gänzle, 2011; Wei et al., 2012; Ze et al., 2012). For example, *Bacteroides* is mainly responsible for resistant starch and xylan fermentation, and *Roseburia* utilize resistant starch, xylan and oligosaccharides. Additionally, *Ruminococcus* consume resistant starch and cellulose, and *Bifidobacterium* utilize oligosaccharide (Chassard and Lacroix, 2013). Short chain fatty acids (SCFAs) are the main microbial fermentation products, mainly including acetate, propionate and butyrate. Butyrate is the major energy source for colon epithelium cells (Rowe and Bayless, 1992) and is related to host immune function and inflammatory modulation by inhibiting NF-κB activation (Lührs et al., 2002) as well as affecting in gut integrity by regulating expression of genes encoding tight junction proteins (e.g., claudin-1 and Zonula Occludens-1) (Wang et al., 2012). Acetate serves as an energy source to be circulated from the blood to the peripheral tissues, such as the liver, where acetate participates lipogenesis and cholesterol synthesis (Bergman, 1990; Bäckhed et al., 2004). Propionate also is circulated into the liver, and is used for gluconeogenesis and cholesterol synthesis regulation (Venter et al., 1990; Reilly and Rombeau, 1993). These suggest that SCFAs are crucial to the host, not only that they are used as energy source, but also they are related to host metabolism and health.

The intestinal microbiota is important in shaping development of innate immune system in neonatal animals. Compared to germ free mice, gnotobiotic piglets colonized with *Lactobacillus* spp., *Colostridium, Roseburia Intestinalis* have been reported to have higher concentration of IgA and IgM in the serum compared to germ-free piglets (Laycock et al., 2012). suggesting that commensal microbiota stimulates the IgA and IgM's secretion in the gut. Gut microbiota is also associated with adaptive immune system homeostasis in neonatal animals, mainly related to T and B cells development. Previous studies also found that the introduction of *Bacteroides fragilis* to germ-free mice is related to Th1 response and regulation of the imbalance of TH1 and TH2 cells (Mazmanian et al., 2005), suggesting that specific bacteria are inducers of T cells. Similar to T cells, decreased number of plasma B cells was reported in the gut of germ-free animals (Crabbé et al., 1968). All the studies mentioned above imply the importance of commensal microbiota in the immune system development of the animals. However, such aspect has not been well studied in the ruminants.

Maintaining a healthy intestinal barrier is of significant importance to the host. The intestinal barrier has many defense mechanisms against pathogens, including mucus layer, epithelial integrity, and epithelial cell turnover (Kim et al., 2010). Mucus layer contains mucin, digestive enzymes and antimicrobial peptides which inhibit bacteria to penetrate into the inner layer (Ashida et al., 2011). Additionally, intestinal integrity is important to inhibit the translocation of pathogens to subepithelial layer. For example, strains of *Bifidobacterium* have been proven to affect gut integrity by strengthening tight junctions *in vitro* (Hsieh et al., 2015). The healthy intestinal barrier is of significant important to the neonates, and the breakdown of the barrier function predisposes the gut to the risks from intestinal pathogens and toxins (Bjarnason et al., 1994). Early life microbiota has been proven to be closely related to intestinal barrier

development. The immature gut barrier in preterm infants is closely related to necrotizing enterocolitis (NEC) (Grave et al., 2007). Through the mechanism for the NEC is still not clear, the administration of probiotic *Bifidobacterium infantis* protects the intestinal barrier by strengthening the tight junctions in neonatal mice model with NEC (Bergmann et al., 2013), indicating that probiotics may help to reduce NEC in premature infants by improving intestinal barrier function.

Commensal bacteria serve as a major luminal barrier to compete against the pathogens by the following mechanisms, competing for nutrients, producing metabolites (antibiotics such as bacteriocins), stimulating host immune defense, and accelerating gut motility to prevent pathogens colonization (Abt and Pamer, 2014). For example, *Bifidobacterium* inhibits Enterohemorrhagic *Escherichia coli* infection and Shiga toxin release by producing acetate in the murine gut (Fukuda et al., 2011). Meanwhile, commensal bacteria drive intestinal epithelial cells to secrete anti-microbial peptides, inhibiting pathogens expansion in mice (Cash et al., 2006). Moreover, commensal *Bifidobacteria breve* stimulates an immunoregulatory response by generating exopolysaccharide, which inhibits *Citrobacter Rodentium* expansion (inducing colonic crypt hyperplasia) in mice (Fanning et al., 2012). Overall, the above findings from mice and human suggest that commensal bacteria are crucial to prevent pathogens colonization in the gut, however, However, the mechanisms behind the how the microbiota contribute to host intestinal barrier function, immune system development, metabolism, and health are not well defined in dairy calves.

Effect of early life microbial shift on lifelong host health

Microbiota dysbiosis during early life has been reported to affect long-term performance on their mammalian host. In humans, the disturbance of the early life microbial colonization leads to a variety of disease in adults such as food allergies, atopic dermatitis and asthma (Stiemsma and Turvey, 2017). One case study that investigated the relationship between shifts in intestinal microbiota and the development of atopic dermatitis in Sweden and Estonia children showed that the children with atopic dermatitis have lower *Enterococcus* at one month old, lower *Bifidobacterium* at one year old, and higher *Clostridium* at three months of age when compared to healthy ones (Björkstén et al., 2001). In piglet, the diversity and composition of postnatal microbiota at day seven is suggested to be an indicator of post-weaning (day 35 after birth) diarrhea. Healthy piglets usually have a higher abundance of *Prevotellaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Lactobacillaceae* compared to those with diarrhea at day seven after birth (Dou et al., 2016). In addition, the higher relative abundance of *Faecalibacterium. prausnitzii* is reported to be related with higher weight gain and lower diarrhea rate in dairy calves at three weeks of life (Oikonomou et al., 2013). Therefore, the shifts in early life microbiota is important which can affect the long-term performance in human and animals, and those studies support that there is an opportunity in manipulating of early life intestinal microbiota to promote long-term animal health.

Future direction to intervene early life microbiota

Comparing to human, the research on gut microbial colonization in ruminants is scarce, preventing the effective and long last manipulation approaches. With the development of next generation sequencing method, we are getting more knowledge about the microbiota composition and functions, as well as the importance of early life microbiota on host life-long

health. One of the immediate implications is to discover effective technologies to intervene calf gut microbiome for their resilience to calf scours. Calf scours is one of the major causes of neonatal deaths and a major contributor of the therapeutic and preventative use of antimicrobials in cattle industry. In 2017, USDA reported that digestive problems in pre-weaned calves account for 7.7-11.6% in the US dairies and the calves are often treated with therapeutic (85.7% of dairy farms) or prophylactic (37.6% of dairy farms) antimicrobials [USDA. 2017 Death loss in US cattle and calves due to predator and nonpredator causes, 2015. USDA–APHIS–VS–CEAH. Fort Collins, CO.]. Enteric infection accounts for about 50% of the total death of neonatal dairy calves, which is usually caused by enteric pathogens infection (Cho and Yoon, 2014), suggesting maintaining and improving gut health during early life is essential for preventing infection and reducing mortality and morbidity of neonatal calves. With the federal regulations to control in-feed antimicrobials use in livestock and growing consumer awareness of antimicrobial resistance, the industry must adopt sustainable alternatives to increase host resilience to early life enteric infections in calves. Prebiotics and probiotics are at the forefront of the potential alternatives of antimicrobials to improve resilience to enteric infections in other livestock species, yet knowledge is limited on its efficacy as early life alternatives in cattle. Therefore, it is necessary to develop and apply practical methods to alter the gut microbiome during early life.

It has been reported that feeding heat treated (at 60 ℃, 60 min) colostrum enhanced the abundance of small intestinal beneficial bacteria (*Bifidobacterium*) and reduced the abundance of potential pathogenic bacteria (*E. coli*) colonization in calves compared to fresh colostrum (Malmuthuge et al., 2014), suggesting that feeding heat treated colostrum shape the bacterial composition towards a “good” direction. Meanwhile, delayed colostrum feeding to 12 h after birth has been shown to reduce the proportion of mucosa-attached *Bifidobacterium* spp., *Lactobacillus* spp., and ileum mucosa-attached *E. coli* in the colon of 2 day old calves, when compared to fed colostrum within one hour after birth (Fischer et al., 2018), implying that delaying colostrum feeding after birth may postpone bacterial colonization in the gut. Moreover, previous study found significant compositional differences at genus level when compared the effect of milk supplemented with antibiotics on fecal microbial profile with milk only, suggesting that antibiotics residues in the milk disrupt the fecal microbiota (Van Vleck Pereira et al., 2016). All the findings above indicate that early life microbial profile can be manipulated through nutritional management strategies, which may affect the long-term health of the dairy calves. Therefore, more research on the influence of nutritional management on early life microbiota are needed to determine the best approaches to alter the gut microbiome for the improved lifelong productivity.

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