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Understanding the gut microbiome of dairy calves: Opportunities to improve early-life gut health[1]

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ABSTRACT

Early gut microbiota plays a vital role in the long-term health of the host. However, understanding of these microbiota is very limited in livestock species, especially in dairy calves. Neonatal calves are highly susceptible to enteric infections, one of the major causes of calf death, so approaches to improving gut health and overall calf health are needed. An increasing number of studies are exploring the microbial composition of the gut, the mucosal immune system, and early dietary interventions to improve the health of dairy calves, revealing possibilities for effectively reducing the susceptibility of calves to enteric infections while promoting growth. Still, comprehensive understanding of the effect of dietary interventions on gut microbiota—one of the key aspects of gut health—is lacking. Such knowledge may provide in-depth understanding of the mechanisms behind functional changes in response to dietary interventions. Understanding of host–microbial interactions with dietary interventions and the role of the gut microbiota during pathogenesis at the site of infection in early life is vital for designing effective tools and techniques to improve calf gut health.
Key words: microbiome, microbiota, early-life gut health, calves

INTRODUCTION

The role of the gut microbiome (the microbial taxonomic composition and their collective genomes) in the development and function of the gastrointestinal tract and in gut health across animal species has been widely recognized. The presence of gut microbiota is necessary for the development of the intestinal epithelium, the mucosal layer (Sharma et al., 1995), and lymphoid structures (Mebius, 2003), as well as for the differentiation of immune cell repertoire (Smith and Garrett, 2011). The use of germ-free rodent models has successfully explained the role of gut microbiota in modulating the anatomy and physiology of the mammalian gastrointestinal tract and intestinal-mucosa-associated immune system (Sommer and Bäckhed, 2013). A recent attempt to cross-colonize human gut microbiota in mice suggested that only host-specific microbiota could trigger the maturation of the mucosal immune system (Chung et al., 2012); it is therefore important to study host–microbial interactions that affect host health within a species.

Although the human gut microbiome has received the most attention due to its immense importance for human health, more and more studies are investigating the importance of gut microbiota in the health and production of livestock (Kogut and Arsenault, 2016). Cattle provide milk and meat to meet growing demands for animal proteins as the human population increases. They can convert low-quality dietary substrates that are unsuitable for human consumption into high-quality animal protein (meat and milk) through sustainable farming (Eisler et al., 2014). However, the cattle industry is facing many challenges—one of them being the high rates of preweaned calf mortality (USDA, 2010)—that affect replacement herds. Enteric infections in neonatal calves are one of the major causes of calf death (Uetake, 2013), despite preventive measures. Moreover, with the new regulations limiting the prophylactic use of antimicrobials (Smith, 2015), the need for alternative approaches to minimize diarrhea incidence in neonatal calves is urgent.

Methods to improve calf gut health during the preweaning period are necessary to minimize calves' susceptibility to enteric infections. Gut health is an ambiguous term used to describe multiple factors that contribute to maintaining disease-free status in the gastrointestinal tract (Bischoff, 2011). Manipulation of the gut microbiome, a key factor that influences gut health (Bischoff, 2011), is one option for improving calf gut health. However, a better understanding of

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host–microbial interactions in the preweaned calf gut is needed to design effective microbial manipulation tools and techniques. The aim of this review was to summarize available information on the gut microbiota and mucosal immune system of preweaned dairy calves and critically evaluate the microbial manipulation methods currently in use to improve calf gut health to identify knowledge gaps that need to be addressed to improve dairy calf health.

ASSESSMENT OF GUT MICROBIOTA: WHERE ARE WE?

More research into the gut microbiota of dairy calves has become available with the development of culture-independent molecular-based approaches. Among them, next-generation sequencing (**NGS**) has become affordable for many researchers as a way of including microbial analysis in their studies. However, not all studies provide clear definitions of terms, especially in animal nutrition research. For example, *microbiota* and *microbiome* are often used interchangeably, but *microbiota* is the composition of a microbial community, usually identified by targeted sequencing of 16S rRNA gene using amplicon sequencing, and *microbiome* is the total genetic information of a microbial community (microbiota), which can be identified using whole-genome sequencing of the metagenome.

In the past, a targeted or desired region of the 16S rRNA gene was sequenced by using Sanger sequencing, after cloning of PCR products (Chakravorty et al., 2007; Tringe and Hugenholtz, 2008). With the development of NGS approaches that use bridge amplification (Illumina sequencing) or emulsion amplification (454 sequencing, SOLiD) of a single DNA strand, cloning of PCR products is no longer needed (Shendure and Ji, 2008). As well, NGS approaches generate high-throughput data by sequencing a large number of pooled samples after adding identifiers or barcodes, and they drastically decrease the cost of sequencing (Tringe and Hugenholtz, 2008). Of the NGS approaches, gut microbiologists prefer Roche 454 pyrosequencing and Illumina sequencing (Arrieta et al., 2014), which are associated with well-developed bioinformatics pipelines for community composition analysis such as quantitative insight into microbial ecology (QIIME) and mothur (Plummer et al., 2015).

In addition to targeted 16SrRNA amplicon-based sequencing, NGS can be used to sequence the genomic DNA and messenger RNA of a microbial community to study the metagenome and the metatranscriptome of a gut microbiome, respectively. They allow study of the functions of microbial communities while generating taxonomic composition data. Metagenomics-based approaches can be used to explore microbial gene composition and functional abundance; metatranscriptomics-based approaches can be used to investigate the active microbiome by measuring levels of gene expression (Hugenholtz and Tyson, 2008). The advantage of studying the metatranscriptome over the metagenome is that it allows for exploration of the active microbial community. Amplicon sequencing has been used widely to study the gut microbiota of preweaned calves as a way of identifying their composition (presence or absence of particular taxa) and how they can be affected by age, diet, or growth (Oikonomou et al., 2013; Klein-Jöbstl et al., 2014; Malmuthuge et al., 2014; Foditsch et al., 2015). To date, metagenome sequencing to explore the function of gut microbiota in preweaned calves is rare (Malmuthuge, 2016), and no attempts have been made to study the active microbial community of dairy calves using a metatranscriptome-based approach.

PREWEANED CALF GUT MICROBIOME: WHAT DO WE KNOW?

The calf gut microbiota has been studied mainly using fecal samples (Uyeno et al., 2010; Mayer et al., 2012; Oikonomou et al., 2013; Klein-Jöbstl et al., 2014; Tomassini, 2015), but a few studies have used local intestinal samples (Malmuthuge et al., 2012, 2014). These studies have reported the presence of a simple, less diverse bacterial community at birth that increases in complexity and diversity with growth, as a result of age and dietary changes (Uyeno et al., 2010; Mayer et al., 2012; Oikonomou et al., 2013; Klein-Jöbstl et al., 2014; Tomassini, 2015; Dill-McFarland et al., 2017). The first colonizers identified from calf meconium samples are *Citrobacter*, *Lactococcus*, *Leuconostoc*, and *Lactobacillus*, and meconium microbial composition is very similar to the fecal microbiota at 6 and 12 h after birth (Mayer et al., 2012). However, the similarity between fecal microbial composition and meconium samples decreases drastically after 24 h of life because of increased diversity (Mayer et al., 2012), indicating the establishment of a complex microbiome very early in life. Individual variations observed among calves decrease with calf age (Klein-Jöbstl et al., 2014), suggesting the establishment of a more similar microbial community in older calves. Bacterial groups detected in the fecal samples of preweaned calves revealed increasing or decreasing patterns in their relative abundance with calf growth. For example, the abundance of bacterial genera *Bifidobacterium*, *Lactobacillus*, *Fecalibacterium*, and *Enterococcus* decreases with growth (Uyeno et al., 2010; Oikonomou et al., 2013; Klein-Jöbstl et