ADSA Foundation Graduate Student Literature Review: Developmental adaptations of immune function in calves and the influence of the intestinal microbiota in health and disease

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ABSTRACT

This graduate student literature review provides an examination of the ontological adaptations of the calf’s immune system and how the intestinal microbiota influences calf immune function in health and disease. Within dairy rearing systems, various nutritional and management factors have emerged as critical determinants of development influencing multiple physiological axes in the calf. Furthermore, we discuss how multiple pre- and post-natal maternal factors influence the trajectory of immune development in favor of establishing regulatory networks to successfully cope with the new environment, while providing early immune protection via immune passive transfer from colostrum. Additionally, our review provides insights into the current understanding of how the intestinal microbiota contributes to the development of the intestinal and systemic immune system in calves. Lastly, we address potential concerns related to the use of prophylactic antimicrobials and waste milk, specifically examining their adverse effects on intestinal health and metabolic function. By examining these factors, we aim to better understand the intricate relationship between current management practices and their long-term impact on animal health.

Keywords: Immune development, mucosal immunity, gut microbiome, antimicrobials

INTRODUCTION

Raising replacement heifers is a key aspect of the economic sustainability of dairy operations and represents almost 20% of total dairy production costs (Heinrichs, 1993). Calf management and nutrition during the preweaning period have been shown to affect not only health and growth rates, but also, impact age at first calving, milk production during first lactation, longevity, and culling rates (Aghakeshmiri et al., 2017; Abuelo et al., 2021). Many components of prevalent rearing systems such as colostrum management, early nutrition, and antimicrobial use have been shown to affect developmental programming influencing the trajectory of several physiological axes in the calf (Faber et al., 2005).

Despite the importance of proper nutrition and health management during the preweaning period, significant challenges remain around safely and effectively rearing heifer calves. The preweaning and weaning periods remain among the most challenging stages of a calf’s life, as reflected by the high morbidity and mortality rates (Urie et al., 2018). During the first weeks of life, calves have limited capacity to initiate immune responses as the immune system gradually matures over time. During this time, calves depend exclusively on the successful passive transfer of immunoglobulins (Ig) from colostrum to confer antibody-mediated protection against environmental pathogens. Failure of passive transfer can result in increased calf morbidity and mortality rates (Urie et al., 2018). During the first weeks of life, calves have limited capacity to initiate immune responses as the immune system gradually matures over time. During this time, calves depend exclusively on the successful passive transfer of immunoglobulins (Ig) from colostrum to confer antibody-mediated protection against environmental pathogens. Failure of passive transfer can result in increased calf morbidity and mortality rates (Urie et al., 2018). It is estimated that 5% to 6% of female dairy calves die during the preweaning period, and 33% suffer from at least one health disorder that requires treatment on farms in Canada and the United States (Urie et al., 2018). Moreover, disease incidence in male dairy calves is even higher, with mortality rates of 5–10% on veal farms across North America, and 25% to 87% of calves are treated at least once for disease (Scott et al., 2019).

In this review we will focus on the intrinsic characteristics of neonatal immunity, the profound impact that the intestinal microbiota has on shaping intestinal and immune development, and how different manage-
ment factors can impact calf health. Lastly, this review discusses potential avenues of future research based on our current understanding and knowledge gaps in the field of neonatal bovine immunology and microbiology.

**NEONATAL IMMUNITY**

At birth, calves are inherently susceptible to environmental pathogens, as reflected by the high incidence of mortality and morbidity during this phase (Urie et al., 2018). The synepitheliochorial placenta of the cow separates the maternal and fetal blood supplies, preventing in utero transmission of protective immunoglobulins (Ig). Therefore, the calf is born agammaglobulinemic and depends on passive transfer of immunoglobulins (Ig) from maternal colostrum to confer antibody-mediated protection against environmental pathogens (Windreyer et al., 2019). The process of ingestion of adequate amounts of IgG and other immune factors through colostrum is termed “successful transfer of passive immunity” and is the single most important management factor in determining the health and survival of neonatal calves (Godden et al., 2019). In addition to reducing the incidence of diseases during the preweaning period, adequate intake of colostrum is associated with long-term improvement in health and performance (Faber et al., 2005; Abuelo et al., 2021), including rates of gain after weaning, reduced age at first calving, improved first and second lactation milk production, and reduced tendency for culling during the first lactation. These benefits may be attributed to reduced incidences of disease, thereby sparing nutrients to stimulate growth during an important developmental period (Aghakeshmiri et al., 2017; Abuelo et al., 2021). Furthermore, colostrum has emerged as a critical factor shaping calf immune function during the early life, and its potential significance in programming immune development and function beyond the neonatal phase will be explored in the following section.

**Calf immune development**

In addition to conferring protection against extra-cellular pathogens, maternal immunoglobulins (Ig) present in colostrum also exerts an immunosuppressive action on calf naïve B cells in a process mediated by the interaction of the FC portion of colostrum Ig with the Fc gamma receptor II (FcγRII), also known as cluster of differentiation (CD) 32 (Figure 1). In cattle, CD32 is expressed by various immune cells, including B cells, monocytes, neutrophils, and dendritic cells. In the presence of maternal IgG antibodies bound to antigen, CD32 colligates the B cell receptor on naïve B cells with the antigen complex. This interaction activates the intracellular portion of CD32 which results in a reduction of intracellular calcium flux reducing antigen processing, ultimately limiting B cell activation and differentiation (Figure 1; Minskoff et al., 1998). In calves, CD32 is constitutively expressed by B lymphocytes, making them susceptible to suppression by maternal antibodies (Chattha et al., 2009). As the concentration of colostrum IgG wanes from calf’s circulation, the CD32 mediated inhibitory effect on B cell activation decreases overtime, allowing the calf to mount proper humoral immune responses.

At the end of gestation, the maturation of the fetal hypothalamic-pituitary-adrenal (HPA) axis leads to a substantial increase in fetal cortisol, triggering up-regulation of cyclooxygenase 2 and prostaglandin E2, enhanced estrogen concentrations and decreased progesterone production (Wood, 1999). This switch in fetal and placental hormones leads to increased myometrial contractions that aid in fetal expulsion (Challis 1971), with cortisol concentrations remaining elevated in calf circulation during the first week of life (Hoyer et al., 1990). This hormonal changes serves a dual role. It not only induces parturition, but collectively modifies cell function in CD4+ T cells during the first few weeks after birth to promote a Th2-biased response, effectively establishing an anti-inflammatory T cell phenotype in the neonatal calf (Harris and Barletta, 2001; Chase et al., 2008). Additionally, immune function during the first weeks of life is characterized by reduced phagocytic activity in neutrophils (Kampen et al., 2006), and reduced maturation and antigen presentation in dendritic cells (DC). Collectively, calf immune responses during the first weeks of life are compromised not only by their inherent naïve status, but also through a combination of pre and postnatal maternal factors that override calf immune function during this stage. This reorganization seems to prioritize immune protection through the transfer of passive immunity from colostrum, while allowing the calf’s own defense systems to gradually develop over time, effectively preventing aberrant immune responses against allergens from the new environment.

**Gamma Delta T Cells**

In addition to mediating B cell function, colostrum seems to promote the expansion of a group of non-traditional T cells, called Gamma delta T cells (γδ T cells), as colostrum-deprived calves have lower levels of γδ T cells in circulation (Figure 1; Krueger et al., 2016). In cattle, γδ T cells are a major lymphocyte subset, which at birth account for over 50% of all circulating lymphocytes, playing a dual role of conferring early protection against intracellular infections while preventing
exacerbated immune responses by promoting tolerance (Hoek et al., 2008; Guzman et al., 2014). These unconventional T cells possess a set of characteristics that set them apart from the traditional αβ T cells and are starting to emerge as a critical lymphocyte population in early life, endowed with both adaptive and innate-like characteristics, serving as a bridge between the 2 arms of the immune system (Guerra-Maupome et al., 2019).

Unlike naïve CD4⁺ T cells that can differentiate into several functional subsets based on signals from antigen-presenting cells (APCs), the phenotype of γδ T cells is pre-programmed during development in the thymus (Baldwin et al., 2021). In cattle, functional subsets of γδ T cells can be classified based on the expression of 2 different classes of the transmembrane glycoprotein receptor, workshop cluster 1 (WC1). The WC1 receptor serves as a costimulatory molecule, similar to CD4⁺ and CD8⁺ in traditional αβ T cells, and also serves as a pattern recognition receptor (Hsu et al., 2015). The WC1 receptor can be subdivided into WC1.1⁺ and WC1.2⁺. The WC1.1⁺ γδ T cell subset is implicated in the clearance of viral and bacterial infections, and upon activation, secretes interferon-gamma (IFN-γ) and interleukin 17 (IL-17; Megill et al., 2013), providing an early defense mechanism against intracellular infections during a period where effector functions of traditional αβ T cells are compromised.

Circulating γδ T cells that constitutively express the coreceptor WC1.2⁺ as well as WC⁺ have been suggested...
to have an immunomodulatory function (Guerra-Mau-pone et al., 2019; Baldwin et al., 2021), based on the ability to produce anti-inflammatory cytokines such as IL-10, and TGF-β upon stimulation in vitro (Hoek et al., 2008; Guzman et al., 2014; Guerra-Mau-pone et al., 2019). Furthermore, in vivo depletion of WC1.2+ γδ T cells in cattle results in exacerbated humoral immune responses after a challenge with ovalbumin (Skeen et al., 2001; Guzman et al., 2014), and in mice depletion of γδ T cells after infection with Listeria monocytogenes induces septicemia (Skeen et al., 2001). In a previous study from our group, we showed that γδ T cells account for almost 60% of the lymphocyte pool in the blood of Holstein calves at birth which declines until weaning (Cangiano et al., In review). Furthermore, γδ T cells expressing the WC1.2+ receptor was the most abundant subset in circulation, accounting for 55% of all γδ T cells shortly after birth, declining in proportions and receptor expression after weaning.

Overall, developmental adaptations during early life coordinate immune function and development, promoting γδ T cell expansion possibly to provide early systemic protection (WC1.1+ subset) as well as to promote immune tolerance during a time when the calf’s immune system is being rapidly exposed to an increasing number of new antigens from the environment (WC1.2+ subset). Future work should focus on the development of tailored vaccination approaches aimed at stimulating γδ T cell proliferation as a preventive measure against neonatal diseases. As our understanding of neonatal immune function in cattle continues to mature, the prospect of harnessing γδ T cell for proactive immunization emerges as a compelling frontier in bovine health.

**INTESTINAL IMMUNE SYSTEM**

Of all the immune compartments, the intestinal immune system (IIS) is the largest in the body based on the number of immune cells, specialized anatomical structures, and routine microbial encounters (Chase and Kaushik, 2019). The intestinal host-microbial relationship is mutualistic in nature, however, the close association between an abundant microbial community and intestinal tissues is poised with health challenges. The IIS is physically separated from the lumen which is densely populated with microorganisms by a physical barrier that mediates its relationship with the external environment and has evolved several mechanisms to avoid unnecessary immune responses toward commensal microorganisms. One of the most important adaptations of the intestinal immune system is the development of mechanisms that reduce direct exposure of intestinal epithelial cells to microorganisms, largely reducing potential pathological outcomes (Hooper et al., 2012). Several immune mechanisms function together to stratify luminal microbes and minimize microbe-epithelial cell contact and prevent the induction of unnecessary systemic immune responses to maintain intestinal homeostasis. All the different components, cell types, and immune effectors that form the IIS work in concert to surveil and respond accordingly to microbial threats, maintaining a balance between tolerance and defense mechanisms.

A physical barrier separates the intestinal lumen from the internal environment and is made up of epithelial cells that form a tight monolayer (Vancamelbeke and Vermeire, 2017), structurally connected by a complex of tight junction proteins that regulate the intercellular passage of small molecules and ions (Meda et al., 2000). This epithelial barrier is covered by mucus secreted by specialized cells called goblet cells (Vancamelbeke and Vermeire, 2017), stratifying the intestinal contents to prevent the direct contact of luminal microbes with epithelial cells. Goblet cells produce a cross-linked proteoglycan gel called mucin that forms a dense inner layer, followed by a less cross-linked outer mucus layer. The outer layer is colonized by several microorganisms while the inner layer promotes a more sterile environment due to its high concentration of antimicrobial compounds and secretory IgA (sIgA), preventing most microorganisms from colonizing. Additionally, immune responses to microorganisms that manage to breach this first line of defense are largely confined to the intestine (Hooper et al., 2012). This compartmentalization avoids systemic ramifications of the local immune response and is a result of the programming of adaptive immune cells to travel back to the mucosa following activation and differentiation in Peyer’s patches or mesenteric lymph nodes (Maynard et al., 2012). The physical and chemical properties of the intestinal barrier are made up of numerous different cell types that develop from a common stem cell at the base of the intestinal crypts: enterocytes (absorptive), goblet cells (mucus-producing), Paneth cells (produce anti-microbial compounds), and microfold (M) cells (antigen processing and maintenance of intestinal tolerance). Beneath the epithelium lies the lamina propria, a loose connective tissue containing APCs, innate lymphoid cells, T cells and B cells (Chase et al., 2018; Gomez et al., 2019) and other immune mediators, such as complement, chemokines and cytokines (Chase and Kaushik, 2019).

The IIS possesses several mechanisms to recognize microbial-associated molecular patterns (MAMPs) through germline-encoded pathogen recognition receptors (PRR) expressed by intestinal epithelial cells and resident immune cells (Hooper and Macpherson, 2010). These include transmembrane receptors, such...
as toll like receptor (TLR) 2 and 4, and C-type lectin receptors, and intracellular PRRs such as TLR8 and TLR9, NOD Like receptors, and RIG like receptors. Sensing of commensal microbiota through the PRRs, such as TLRs, induces tissue repair and epithelial cell proliferation (Maynard et al., 2012), and in germ-free mice, these signals can be rescued by bacterial cell wall components, such as LPS or flagellin (Hill and Artis et al., 2010). The presence of microorganisms or microbial constituents in the vicinity of mucosal surfaces are recognized by APCs that upon activation stimulate the production of IL-23, which in turn, promote the expression of IL-22 by innate lymphoid cells. This cascade of events stimulates epithelial cell proliferation and the secretion and release of antimicrobial peptides, such as RegIIIγ, and α-defensins by intestinal epithelial cells and Paneth cells, respectively (Figure 2). Furthermore, APCs are continuously sampling antigens from the luminal environment and presenting them to naïve B and T cells on mesenteric lymph nodes. This leads to antigen-specific immune responses that promote the production of dimeric IgA, the most abundant Ig in the intestinal mucosa. This IgA gets transported across the epithelial cells by a polymeric immunoglobulin receptor which adds a secretory component to IgA, extending its half-life (Maynard et al., 2012; Chase and Kaushik, 2019). Additionally, slgA aids in antigen sampling from the intestinal lumen, improving antigen presentation by APCs to B and T cells in the Peyer’s patches (Mantis et al., 2002). Both antimicrobial peptides and slgA represent one of the main non-inflammatory defense mechanisms of the gut and help to shape the composition of the intestinal microbiota in the vicinity of epithelial cells (Pabst et al., 2016).

The intestinal microbial community exerts a profound influence on the intricate network of the IIS, regulating the delicate equilibrium between immune tolerance and effector function. Alterations that reduce microbial diversity, such as abrupt changes in diet and antimicrobial use, can have significant repercussions for overall health, especially during early life where it can have a long-term impact on the trajectory of immune development. In the next section, we explore the dynamic interactions between the host and the microbial community within the intestinal microbial ecosystem, highlighting the importance of the intestinal microbiome on gut health and immune function in both health and disease.

**ROLE OF THE INTESTINAL MICROBIOTA IN HEALTH AND DISEASE**

The advent of culture-independent techniques, such as next-generation sequencing to study the ecological complexity of the intestinal microbiota and its impact on host physiology, have revolutionized the field of microbiology and broadened the lens through which to explore the functional roles of the microbiota on host health and disease. Based on the broadscale implementation of sequencing of the 16S ribosomal RNA gene and whole genome sequencing techniques over the last 2 decades, it has become increasingly clear that the intestinal microbiota serves an important role in protecting health by shaping metabolic and immune function both locally and systemically (Michaudel et al., 2020). Recent estimates suggest that the human intestine is colonized by microbes that surpass the number of human cells by about 10-fold, and the collective number of microbial genes that make up the intestinal microbial metagenome is about 100 times larger than the human genome (Sender et al., 2016).

The intestinal microbiota has likely evolved under selective pressure to effectively degrade undigestible plant carbohydrates and polysaccharides, enhancing the host digestive efficiency (particularly in the case of ruminants), ensuring a stable and nutrient-rich ecological niche (Hooper et al., 2012). In addition to increasing the digestive capacity of the host, intestinal microbes provide important non-nutritive factors, such as vitamin K and B12 (Conly et al., 1994). Furthermore, intestinal microbial residents and their constituents have developed robust and interconnected networks that physically and metabolically restrict access to available resources in the gut to non-resident bacteria and pathogens which are not well-adapted to colonize the gut under normal ecological conditions (Maynard et al., 2012). Therefore, through competitive exclusion, the intestinal commensal microbiota prevents the invasion of opportunistic pathogens (Figure 2).

The intestinal microbiota establishes a relationship with the host that is mainly mutualistic in nature. This is achieved through bidirectional communication between the host and intestinal microbiota via a wide range of metabolites either from anaerobic fermentation of dietary components, such as short chain fatty acids (SCFAs), or from molecules produced by the host and modified by microorganisms, such as bile acids (Jia et al., 2018). Lastly, the host can recognize different microbial structural components through pathogen recognition receptors and respond accordingly. One of the main roles of the intestinal microbiota is the development of the immune system in neonates and the maintenance of gut health in adult animals. The maturation of the intestinal mucosa, Peyer’s patches, isolated lymphoid follicles (ILFs), and mesenteric lymph nodes relies on signals derived from sensing intestinal microbes during the colonization process. Germ-free mice do not develop lymphoid follicles and
are deficient in secretory IgA and mucus production. As a result, they can develop spontaneous necrotizing enterocolitis (Wopereis et al., 2014). Work from our group has shown that supplementation of a live yeast probiotic, *Saccharomyces cerevisiae boulardii* (SCB) to calves from birth to one week of age increased microbial diversity, stimulated secretory IgA (sIgA) production (Villot et al., 2020; Cangiano et al., 2023b), and reduced the incidence of severe diarrhea in pre-weaned calves, providing further evidence that a more mature microbial community can provide the necessary signals...
to stimulate immune development and increase resistance against opportunistic pathogenic bacteria.

During early life, the diet has a major impact on the community structure and diversity of the intestinal microbiota. Human studies comparing breastfed to formula fed infants have shown that breastfed infants have a greater proportion of *Bifidobacterium* and *Lactobacillus* (Fallani et al., 2010). In calves, it has been shown that delaying colostrum feeding or restricting colostrum, reduces microbial diversity and the proportion of *Bifidobacterium* and *Lactobacillus* (Malmuthuge et al., 2015). There are several mechanisms by which maternal milk and colostrum regulate the makeup of the intestinal microbiota in early life in favor of establishing regulatory networks to promote a mutualistic relationship (Maynard et al., 2012). Colostrum, and to a lower extent mature milk, contains a group of unconjugated glycans resistant to enzymatic digestion known as oligosaccharides (Fischer et al., 2018). These molecules act as prebiotics stimulating the development of key bacterial species, such as *Bifidobacterium*, *Lactobacillus* and *Bacteroides*, that possess enzymes that can ferment oligosaccharides into SCFAs and lactic acid (Fischer et al., 2018). In mice, colonization with *Bifidobacterium* spp. has been shown to promote SCFA production and to stimulate IL-10 production by immature DCs (Jeon et al., 2012). In addition, *Bifidobacterium*, and *Bacteroides* spp. can “forage” on mucin glycans and reside in the outer portion of the mucous layer (Martens et al., 2008). This group of bacteria produces lactate, decreasing pH in the vicinity of epithelial cells and competitively excluding other bacterial groups that cannot thrive in that environment. Colostrum also contains antimicrobial compounds, such as lactoferrin and lysozymes, that prevents most microorganisms from colonizing the inner mucus layer, reducing the microbial load in close proximity to epithelial cells (Laforest-Lapointe et al., 2017). Furthermore, lactoferrin supplementation have been shown to reduce neonatal mortality in preterm infants and preweaned calves (Manzoni et al., 2014; Habling et al., 2017).

Certain microbial groups can ferment undigestable plant carbohydrates and polysaccharides and produce SCFAs, secondary metabolites that play beneficial roles in maintaining the structural integrity and function of the IIS. Intestinal microbiota derived SCFAs act on goblet cells to stimulate mucin release and serves as a major energy source for the colonocytes, stimulating cell turnover and proliferation (Donohoe et al., 2012). Also, SCFAs act as signaling molecules, inhibiting histone deacetylases, and activating histone acetyltransferases (Liu et al., 2012). This induces IL-18 secretion, which in turn, stimulates mechanisms of cell repair on epithelial cells and mucin production in goblet cells. Additionally, SCFAs seem to promote class switching to IgA and enhance IgA secretion on B cells, further enhancing immune exclusion in a mechanism that involves rewiring of B cell energy metabolism (Kim et al., 2016). Lastly, SCFAs mediate intestinal immune function by inducing the expansion of a group of regulatory cells named T regulatory cells (Tregs). This is mediated first by inhibiting the maturation of DCs and second by promoting the production of transforming growth factor β (TGF-β) by epithelial cells and DCs (Atarashi et al., 2013). This promotes IL-10 secretion from DCs and promotes CD4+ T helper cell differentiation into Tregs. In ruminants, however, results from in vitro studies suggest that Tregs, although present in circulation, do not seem to promote immune tolerance, at least in vitro (Hoek et al., 2008). Several studies have suggested that in ruminants this function might be carried out, at least in part, by WC- and WC1.2+ subsets of γδ T cells (Hoek et al., 2008; Guzman et al., 2014).

Although the intestinal microbiota plays many beneficial functions for the host, certain factors, such as restriction of colostrum intake, abrupt diet changes, treatment or prophylactic antimicrobial use, feeding of waste milk and abrupt weaning can compromise intestinal microbiota, making the ecological environment more vulnerable to opportunistic pathogen invasions (Tamburini et al., 2016). In human and mice model studies, changes in the composition of the intestinal microbiota that result in the functional loss of important bacterial groups and decreased microbial diversity can create an opportunity for pathogenic infections and are associated with development of disease (Levy et al., 2017). This process, termed dysbiosis, can disrupt important immune regulatory networks that normally restrict intestinal inflammation and affect the function of the intestinal barrier.

**ANTIMICROBIAL USE IN CALF REARING AND ITS IMPLICATIONS ON IMMUNOMETABOLIC FUNCTION**

One of the most well documented contributors to intestinal microbial dysbiosis in both human and animal studies is the use of antimicrobials (Fallani et al., 2010). Due to the high incidence of mortality and morbidity during the preweaning period, antimicrobials have been traditionally used by the dairy industry as both therapeutic treatment and for disease prevention (Urie et al., 2018; Uyama et al., 2022). The practice of prophylactic antimicrobial administration to calves has been challenged in recent years due to the absence of consistent data on its efficacy in preventing disease, as well as concerns regarding antimicrobial resistance (Aghakeshmiri et al., 2017; Buss et al., 2021). The most common antimicrobials for prophylactic use for the prevention...
of enteric disease are neomycin and oxytetracycline, with 9% of dairy facilities still relying on this combination as their main antimicrobial additive (USDA, 2018). In addition, based on a recent survey (Uyama et al., 2022), 20% of farms in Canada feed calves with unsellable milk from cows treated for disease. This milk, commonly referred to as waste milk, can contain antimicrobial residues and a high number of microbial and somatic cells. In human neonates, regardless of the source of antibiotics, exposure during early life modifies the intestinal microbial community structure (Fallani et al., 2010; Woperies et al., 2014), and in preweaning calves is correlated with a reduction in the abundance of beneficial bacteria, such as Faecalibacterium, and an increased abundance of pathogenic E. Shigella and Enterococcus (Amin et al., 2021). However, to our knowledge, no studies have examined the impacts of waste milk on mucosal immunity.

The impact of antimicrobials on immune function

Alterations caused by environmental factors, such as antimicrobial use, can have lasting impacts on health and productivity (Aghakeshmiri et al., 2017). In humans, the use of antimicrobials during the neonatal period is correlated with the development of atopic allergies and asthma later in life (Arrieta et al., 2015). This process seems to be mediated by the disruption of TLR signaling between commensal microbiota and immune and epithelial cells in the intestine. This in turn leads to dysregulation of B cell antibody production and class switching, resulting in increased IgE and elevated levels of basophils in circulation (Hill et al., 2012). In addition, decreased abundance of Bifidobacterium in the intestine has been shown to disrupt immunological tolerance to commensal microbiota and food allergens due to reduced expansion of T regulatory subsets (Jeon et al., 2012). Moreover, a decline in microbial diversity and SCFAs diminishes the stimulatory signals exerted by the microbiota that normally promotes proper intestinal barrier function. This consequently leads to decreased epithelial cell proliferation, a thinning of the mucosal layer, and a decline in the production of secretory IgA (Tamburini et al., 2016). These factors collectively elevate the direct contact between epithelial cells and the intestinal environment, triggering immune responses that promote intestinal inflammation (Figure 3). In studies conducted in both humans and mice, administration of antibiotics leads to alterations in the composition of the intestinal microbiota resulting in the functional loss of important bacterial groups. This process disrupts important immune regulatory networks that normally restrict intestinal inflammation and affect the function of the intestinal barrier, ultimately leading to aberrant immune function in adulthood (Arrieta et al., 2017).

Previous work from our group, demonstrates that prophylactic administration of the antimicrobial neomycin for either 14 or 28 d increases intestinal permeability, as reflected by the increase in plasma recovery of the orally administered marker Chromium-EDTA (Cangiano et al., 2023a). This is likely mediated by the observed reduction in species richness and abundance of key bacterial groups related to intestinal health in early life such as Lactobacillus and Bifidobacterium reducing colonization resistance against pathogenic bacteria (Figure 3).

Although no studies have directly investigated the long-term impacts of antimicrobial use during the preweaning period in calves, epidemiological data suggests that antimicrobial treatment during this period is correlated with lower performance and increased incidence of disease later in life. Calves treated with antimicrobials during the preweaning period, but not after weaning, have lower conception rates, increased time to first calving, increased risk of leaving the herd earlier due to health complications, and reduced milk production during their first lactation (Aghakeshmiri et al., 2017; Abuelo et al., 2021). This suggests that in cattle, disruption of the intestinal microbiota by antimicrobial administration during early life might also impact immune development and health in previously unanticipated ways. In commercial herds, the productive lifespan of dairy cows continues to be much shorter than the natural life expectancy due to high culling rates despite large improvements in cow comfort and genetic selection (De Vries and Marcondes 2020). Therefore, it is important to understand how different management practices in early life impact immune development to determine if certain health and metabolic disorders in the adult cow stem from maladaptations during early development.

The impact of antimicrobials on metabolic function

In recent years, a growing body of evidence suggests that alterations to the intestinal microbiota, due to antimicrobial exposure in early life, is linked with increased adiposity and appears to be a risk factor in the development of several metabolic disorders in humans (Cox et al., 2014). In mice, reduced microbial diversity in early life can lead to dyslipidemia and increased fat deposition (Bäckhed et al., 2004), and in farm animals, antimicrobials are routinely used to improve efficiency of gain and fat deposition. One of the metabolic axes that seems to be disrupted by antibiotic use is the metabolism of bile acids (BA; Ipharraguerre et al., 2018). Besides their classical role of lipid emulsifiers, in recent...
years, bile acids have been shown to play an important role in the systemic regulation of several metabolic and inflammatory pathways and their function is affected by the composition of the intestinal microbiota. Bile acids serve as nutrient-sensing molecules alerting peripheral tissues of incoming nutrients during the postprandial state (Chiang, 2009). They are also involved in the regulation of lipid metabolism and inflammation via dedicated nuclear and cell surface receptors, constitutively expressed in the intestines, liver, peripheral adipose tissues, and certain immune cells (De Aguiar Vallim et al., 2013).

The concentration of BAs within each compartment of the enterohepatic system is tightly regulated by a complex of dedicated nuclear and membrane-bound receptors. In the liver, BAs interact with the nuclear receptor FXR, which reduces BA synthesis and downregulates hepatic lipogenesis by repressing the expression of sterol-responsive element binding protein 1c (Hirokane et al., 2004). The hepatic recovery of BAs from the portal vein is incomplete, and a small proportion of BA escape the enterohepatic system and enter systemic circulation. These BAs interact with TGR5 and FXR expressed in adipose tissue, skeletal muscle to activate transcriptional networks that control the expression of genes involved in lipogenesis, gluconeogenesis, and inflammation (Figure 4; Lefebvre et al., 2009; Ipharraguerre et al., 2018). Adipose tissue-resident macrophages also express FXR and TGR5 and BA-mediated activation, reduces inflammation and increase insulin sensitivity (McGavigan et al., 2017). Additionally, FXR activation in adipose tissue promotes cell proliferation by promoting peroxisome proliferator-activated receptor gamma activity (Abdelkarim et al., 2010). Lastly, FGF-19 promotes energy expenditure in

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**Figure 3.** Alterations caused by environmental factors, such as antimicrobial use, can negatively impact calf intestinal health and metabolic function. Decreased in microbial diversity and specifically in abundance of *Bifidobacterium* and *Lactobacillus* results in a reduction in short chain fatty acids (SCFAs) diminishing the stimulatory signals exerted by the microbiota on proper intestinal barrier function. This consequently leads to decreased epithelial cell proliferation, a thinning of the mucosal layer, and a decline in the production of secretory immunoglobulin A (IgA). These factors collectively elevate the direct contact between epithelial cells and the intestinal environment, triggering immune responses that promote intestinal inflammation. Additionally, the antimicrobial induced dysbiosis and reduction in *Bifidobacterium* and *Lactobacillus* alters the profile of bile acids (BA) and the products of microbial transformation of BA (2BA) leading to a dysregulation of BA signaling altering transcript abundance of genes related to metabolic function and inflammation in peripheral adipose tissue.
brown adipose tissue by increasing receptor affinity of TGR5 to BA (Watanabe et al., 2006).

Several microorganisms can modify the composition of the BA pool in the intestine through deconjugation and dihydroxylation enzymatic processes that reduce their microbicidal effects and modify their agonistic functions, thereby establishing a bidirectional communication with the host (Lefebvre et al., 2009). Secondary BA, the product of bacterial modification of primary BA, leads to changes in their agonistic function on target receptors in the intestine, liver, and peripheral tissues. The profile and concentration of these BAs largely depend on the composition of the intestinal microbiota, suggesting a direct link between the intestinal microbiota and metabolic function. The loss in functional diversity of the intestinal microbiota caused by antimicrobials can alter the biosynthesis of BA, leading to changes in their “instructive functions” on metabolism in peripheral tissues (Lefebvre et al., 2009; Ipharraguerre et al., 2018).

Several studies have shown that prophylactic use of antimicrobials induce changes in the abundance of several bacterial groups that express genes for deconjugation (bile salt hydrolases; BSH) and dehydroxylation of primary BA (BA-inducible genes; BAI), ultimately resulting in a dysregulation of BA metabolism by altering its profile and tissue distribution. This results in changes in transcript abundance of genes related to metabolic function and inflammation in peripheral adipose tissue and liver in rodents (Swann et al., 2011), piglets (Ipharraguerre et al., 2018), and calves (Cangiano et al. 2023a). We recently demonstrated that prophylactic administration of the anti-infective neomycin to Holstein calves leads to aberrant changes in BA concentration and profile in different compartments of the enterohepatic system. The dysregulation of BA metabolism by neomycin seems to be mediated by a reduction in the relative abundance of key bacterial groups such as Lactobacillus and Bifidobacterium, leading to alterations in transcript abundance of genes related to lipid metabolism and immune activation in adipose tissue and liver (Cangiano et al., 2023a; Figure 3). This suggests that antimicrobial administration during early life may impact calf development and health in previously unanticipated ways.

**CONCLUSIONS**

In summary, several components of dairy rearing systems have emerged as critical determinants of development across multiple physiological axes in the calf. Pre- and post-natal maternal factors seem to influence the trajectory of immune development in favor of establishing regulatory networks to successfully cope with the new environment, while providing early immune protection via colostrum. Moreover, microbial colonization plays a crucial role in promoting intestinal and immune development. This intricate process can be negatively impacted by several management factors such as poor colostrum management, prophylactic antimicrobials and waste milk, ultimately affecting the composition of the initial gut microbiome, with prolonged consequences for host health. However, the existing knowledge on the long-term effects of antimicrobial use is primarily based on correlational data obtained from epidemiological studies. Consequently, there are significant gaps in our understanding of how these practices affect immune and metabolic function in cattle long-term. Therefore, future studies should examine how management factors (i.e., prophylactic antibiotics, waste milk, colostrum management) in early life affect the developmental trajectory of immune and metabolic functions long-term to understand if certain metabolic and inflammatory disorders in the adult cow stem from maladaptations during early development.

**REFERENCES**


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**Figure 4.** Bile acid enterohepatic cycle and effects on systemic metabolism. Besides their classical role as lipid emulsifiers, bile acids (BAs) act as pleiotropic signaling molecules, serving as nutrient-sensing molecules by alerting peripheral tissues of incoming nutrients during the postprandial state. BAs are involved in the regulation of lipid metabolism and inflammation via dedicated nuclear and cell surface receptors, constitutively expressed in the intestines, liver, and peripheral adipose tissues. FXR = Farnesoid x receptor. BA = Primary bile acids. 2BA = Secondary bile acids. TGR5: Takeda G protein-coupled receptor 5. FGF19 = Fibroblast growth factor 19. SREBP1 = Sterol regulatory element-binding protein 1. CYP7A1: Cytochrome P450 Family 7 Subfamily A Member 1. LDL-R: Low-density lipoprotein receptor. Ostα/Ostβ = heterodimeric organic solute transporter α and β. PPAR-γ = Peroxisome proliferator-activated receptor gamma. Created with BioRender.com.


