

Effects of Nutrition on the Immunity of Dairy Calves

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- Dairy calves are highly susceptible to enteric disease during the first few weeks of life as the gastrointestinal tract matures.
- Probiotics, prebiotics, and protein from either hyper-immunized eggs or plasma can improve enteric health during the first few weeks of life.
- Calves can digest, absorb, and utilize the additional protein and energy early in life when fed greater quantities of milk replacer.
- The risk for some enteric diseases are likely influenced by plane of nutrition from milk replacer, but it appears there might be a pathogen:host interaction.
- In contrast to early life, feeding greater quantities of milk replacer improves post-weaning health.

ABSTRACT

Dairy calves are extremely susceptible to gastrointestinal disease during the pre-weaned period. The risk for enteric disease decreases as the calf ages; therefore, it is important to break the pre-weaned period up into at least 2 distinct phases that likely need to be managed differently, early life (first couple weeks of life) and the remaining time the calf is fed milk or milk replacer. When a calf is born they have been exposed to very few if any microorganisms and some aspects of their gastrointestinal immune system are not fully developed. After birth, the calf is now in a microbial world and exposed to a greater quantity and diversity of

microorganisms. This adaptation is abrupt and dramatic and is a major stressor to a newborn calf. The gastrointestinal tract of the calf is naïve and develops rapidly during the first few days to weeks of life. The cells that make up the gastrointestinal tract are the first line of defense of the immune system; therefore, until the cells are more adult-like the calf may be at an increased risk for developing gastrointestinal diseases.

My laboratory recently tested the hypothesis that feeding greater quantities of milk solids during the first week of life would increase the percentage of dietary nutrients that were neither digested nor absorbed by the calf, which would increase the risk of scours. The data indicated that dairy calves during the first few weeks of life digest and absorb nutrients well, and when fed a greater plane of nutrition the additional nutrients were incorporated into tissue growth. However, the increased absorption of nutrients among calves fed greater quantities of milk replacer may increase the risk for enteric disease (Liang et al., unpublished).

A group of calves were challenged with an opportunistic enteric pathogen, *Citrobacter freundii*, at 10 d of life and the calves fed the greater plane of milk solids had greater rectal temperatures ($P = 0.021$) and numerically greater peak concentrations of plasma haptoglobin after the challenge (511 versus 266 ± 107.9 $\mu\text{g/mL}$; $P = 0.118$). The greater clinical response among the calves fed the greater plane of nutrition could be due to the numerically greater ideal mucosal height (921 versus 752 ± 59.1 μm ; $P = 0.059$). Our data also indicated that

calves fed greater planes of nutrition had increased fecal scores, but when the dry matter percentage was determined there were no differences. This suggests that fecal scores alone are inadequate as a measure of enteric health, especially when evaluating various planes of nutrition.

Others have reported that calves fed greater quantities of milk and challenged with *Cryptosporidium parvum* had reduced duration of scours and improved hydration (Ollivett et al., 2012). More data are needed to further investigate the mechanisms underlying this altered response to infectious diseases and understand how early life plane of nutrition influences gastrointestinal disease during that period. In addition, an interesting area of research is that the plane of nutrition of calves during the pre-weaned period improved future lactational performance.

Emerging data is suggesting that it may also improve the resistance to some diseases that persists past the pre-weaned period (Ballou et al., JDS *In Press*; Sharon and Ballou, unpublished). Calves that were previously fed a greater plane of nutrition from milk replacer had greater leukocyte responses after they were challenged orally with *Salmonella enterica* Serotype Typhimurium and subsequently had reduced measures of disease (Ballou et al., 2015). Similarly, another group of calves that were previously fed a greater plane of nutrition from milk replacer had reduced mortality and less clinical disease after they were challenged approximately a month after weaning with both bovine herpes virus-1 and *Mannheimia haemolytica* (Sharon and Ballou, unpublished).

More research is needed in this area before any conclusions should be made. In addition to plane of nutrition, the primary strategy to improve resistance to

gastrointestinal diseases during early life are focused on decreasing the interaction of potential pathogens with the cells of the calf's gastrointestinal tract. The uses of prebiotics, probiotics, hyper-immunized egg protein, and spray-dried plasma proteins were in many cases shown to decrease the incidence of gastrointestinal diseases and improve the growth of pre-weaned calves. In summary, nutrition influences leukocyte responses and disease resistance of calves in many ways, both directly by supplying specific nutrients and indirectly by potentially influencing the exposure to microorganisms. Again, I think it is important that we think about the pre-weaning period as 2 distinct phases that need to be managed differently, the first couple of weeks while the gastrointestinal tract is maturing, and the remainder of time the calf is fed fluid milk.

Keywords: Calf, Health, Immune, and Nutrition

INTRODUCTION

It is well documented that dairy calves are extremely susceptible to enteric diseases and mortality during the first few weeks of life. The latest reports from the USDA's National Animal Health and Monitoring System (NAHMS, 1993; 1996; 2007) report that the national mortality rate of heifer calves from 48 hr of life to weaning is approximately 7.8 to 10.8 %. Producer perceived records indicate that scours account for 56.5 to 60.5 % of all pre-weaned deaths. Approximately ¼ of all pre-weaned calves are therapeutically treated for scours, and the major causes of death from scours are either dehydration or the pathogen gains access to the blood and causes septicemia. The high incidences of disease indicate we have much to learn about improving gastrointestinal disease resistance among pre-weaned calves.

Colostrum management, how much and the composition of fluid fed; the use of various additives such as prebiotics, probiotics, and proteins from hyper-immunized eggs or plasma proteins; and housing can all influence the health of pre-weaned dairy calves. In addition, there are a few data that indicate that early life nutrition can have long-term impacts on leukocyte responses and disease resistance (Ballou, 2012; Ballou et al., 2015; Sharon and Ballou, unpublished). There is a high incidence of respiratory disease among dairy calves which is the main contributor to the high death losses, 1.8 %, after weaning (NAHMS, 2007). This is an exciting area of research that needs to be addressed further.

WHY ARE CALVES SO SUSCEPTIBLE TO GASTROINTESTINAL DISEASE?

The calf is in a bit of a *catch-22* situation early in life because it requires the passive absorption of many macromolecules from colostrum and milk, but this also increases the risk of translocation of pathogenic microorganisms. The gastrointestinal tract of many neonates undergoes a rapid maturation after parturition, and the timing of this depends largely on the species of interest. There are large gaps in our knowledge regarding how the gastrointestinal tract of a calf changes early in life; however, using gastrointestinal morbidity/mortality risk as an indirect measurement, the maturation occurs quite rapidly over the first few weeks of life. There are many components to the gastrointestinal immune system (Figure 1). Most of the discussion that follows was derived from animal models other than the calf, but the general principles can still be applied to the calf.

The epithelial cells that make up the mucosal surface and the tight junctions between those cells form a *physical barrier* that prevents luminal contents from flowing

directly into systemic circulation. A breakdown in the tight junctions increases the likelihood of infectious disease because of increased bacterial translocation. Goblet cells are one of the types of epithelial cells found in the gastrointestinal tract, and they produce mucus that creates a layer that covers most of the intestinal epithelium. This mucus layer forms an additional physical barrier against potential enteric pathogens. Additionally, the mucus layer contains many antimicrobial factors that were secreted from immune cells in the intestinal mucosa. These antimicrobial factors include: defensins, lysozyme, and sIgA, and their function is to limit the interactions of live microorganisms with epithelial cells by creating a *chemical barrier*.

Many leukocytes are found in the mucosa of the gastrointestinal tract as well as large lymphoid aggregates are localized in the submucosa of the distal region of the small intestines. These leukocytes contribute to the *immunological barrier* of the gastrointestinal tract. The majority of leukocytes found in the gastrointestinal (sub)mucosa contribute to adaptive immune responses and create memory that will help to prevent subsequent infections. Macrophages are found in the mucosa and could be involved in the clearance of some microorganisms, but neutrophils are rarely found in the mucosa and are only present in a pathologic state. Trillions of commensal microorganisms live in the gastrointestinal tract and they have a symbiotic relationship with the calf. These commensal microorganisms are part of a *microbial barrier* that limits the colonization of the gastrointestinal epithelium with more potentially pathogenic microorganisms. These commensal microorganisms compete directly for substrates and space with the potentially pathogenic microorganisms and many of them produce antimicrobial factors

and stimulate mucus production that further restrict potential pathogens from infecting the calf. These barriers work together to create a competent **Immune System** of the gastrointestinal tract. A defect in any of these components can increase the risk for infectious disease.

Many of the components of the gastrointestinal immune system begin to develop as early as the first trimester of gestation; however, further maturation of many of these barriers occurs only after birth (Guilloteau et al., 2009). This process of rapid intestinal maturation is known as

gut closure and contributes to the physical barrier. The enterocytes, the nutrient absorptive cells that make up the majority of cells in the intestinal epithelium, are considered fetal-type at birth because they are largely vacuolated and can absorb intact macronutrients through pinocytosis. These fetal-type enterocytes are quickly replaced by more adult-like enterocytes. This process occurs from the proximal to distal intestines and from the crypt to the villus tip; therefore, even though the majority of the gastrointestinal tract may have undergone *gut closure* in the day and a half after birth there likely persist vacuolated, fetal-type enterocytes toward the villus tip of the lower regions of the intestines for a longer period of time.

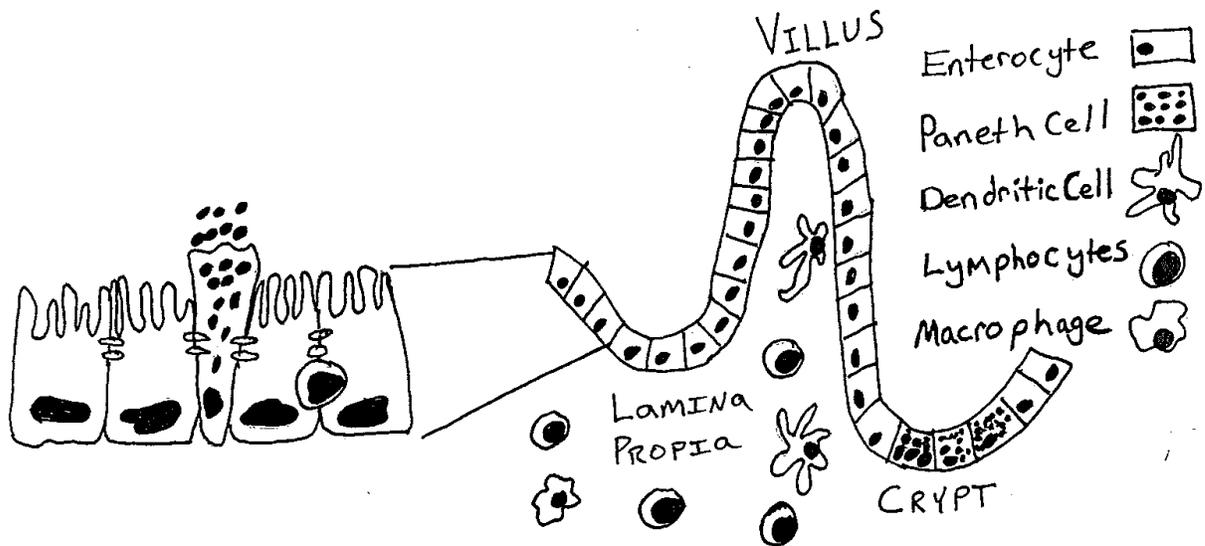


Figure 1. Schematic drawing of the small intestinal mucosa. The crypt-villus axis and common leukocytes found in the mucosa are shown on the right. The insert on the left is a magnification of the epithelial layer, depicting microvilli, tight junctions between epithelial cells, a goblet cell secreting mucus, and an intraepithelial lymphocyte.

In addition to transcellular absorption of macromolecules, the gastrointestinal epithelium may also be more prone to paracellular absorption because of reduced tight junctions between the enterocytes. The mucus layer that covers the intestinal epithelium is dynamic and cannot be studied with traditional histological methods; therefore, very little is known regarding the post-natal changes in the mucus layer. Goblet cells respond to microbial exposure by increasing mucus secretion; therefore, it is conceivable that the mucus layer develops further during the post-natal period. Intestinal motility and the movement of digesta through the gastrointestinal tract can also reduce colonization of potentially pathogenic microorganisms, so a reduced intestinal motility can also contribute to the high incidence of enteric disease. Therefore, the physical barrier of the intestines is compromised during the early post-natal period and likely contributes to the high incidence of enteric disease and bacterial translocation.

The chemical and immunological barriers are also compromised during the early post-natal period. Paneth cells begin to develop during gestation; however, the number of Paneth cells and the antimicrobial secretions increase throughout life. Additionally, the adaptive arm of the immune system is naïve at birth and develops over the life of the animal as the calf is exposed and re-exposed to antigens. Therefore, sIgA concentrations and diversity are low and will remain low until the calf begins to develop its own active immunity. Antibodies from colostrum are known to recirculate back to the mucosa of the intestines, and can offer some immediate protection from enteric pathogens; however, the half-life of many passively derived antibodies is 1 to 2 wk. Therefore, the gastrointestinal tract will become more susceptible to those specific microorganisms

again until they develop their own active immunity against them. This is probably why many calves start developing localized enteric disease and scours during the 2nd or 3rd week of life. The fact is young animals will always be at an increased risk for infectious diseases until they develop their own active immunity. It's one of the benefits of getting older, the adaptive arm of the immune system becomes *wiser* because of what it has been exposed to and experienced.

The calf *in utero* is developing in a relatively sterile environment and upon parturition and during the post-natal life they are exposed to a greater number and diversity of microorganisms. There is a progression in the microbial colonization of the gastrointestinal tract, with facultative anaerobes from the environment (ie: *Enterobacteriaceae*, *Streptococcus*, and *Staphylococcus*) dominating during the early post-natal period. There will be a switch to where strict anaerobes (ie: *Bifidobacterium*, *Bacteroides*, *Lactobacilli*, and *Clostridia*) will dominate and account for greater than 99 % of the bacteria in the intestines for the rest of the animal's life. Therefore, the microbial barrier of the gastrointestinal tract is also compromised during early life and likely contributes to the greater incidence of enteric disease.

Therefore, from a systematic perspective, there are many holes in the gastrointestinal immune system defense during the early post-natal life. This greatly increases the relative risk for enteric disease. It is well known that what an animal is fed during the neonatal period will influence the development of the gastrointestinal immune system and enteric disease resistance. It should be noted that a lot more basic research on the development of the post-natal gastrointestinal immune system in

calves is needed and should be a research priority.

MATURATION OF THE GASTROINTESTINAL IMMUNE SYSTEM AND PREVENTING PATHOGEN-HOST INTERACTIONS

A common management strategy in the dairy industry is to feed approximately 4 L of colostrum within the first 6-12 hr of birth. Then calves are switched to either milk or milk replacer. It is well known that bioactive compounds in colostrum and transition milk directly influence the maturation of the gastrointestinal immune system. Our current colostrum management protocols are designed to ensure as many calves as possible get adequate passively derived immunoglobulins as possible. I don't want to down play the importance of passive transfer of immunoglobulins, because it is essential in preventing systemic and local enteric diseases while the gastrointestinal tract matures; however, current colostrum management programs completely ignore the role that colostrum and transition milk play in the maturation of the intestinal immune system. Enteric disease would likely be reduced if we fed calves to hasten the maturation of the gastrointestinal immune system. Most of our management decisions after feeding colostrum are aimed at reducing the interaction of potentially pathogenic microorganisms with the intestinal epithelial cells.

Prebiotics, probiotics, and proteins from hyper-immunized eggs or spray-dried plasma all have shown some merit in improving the resistance to enteric disease. Prebiotics are dietary components that are not easily digested by the calf, but are used by bacteria in the lower intestines to improve their growth. Probiotics are a vague term, but generally are live microorganisms that provide *some* health benefit. At first

glance this may seem bad, why would we want to improve the growth of bacteria in the lower intestines? As mentioned before, the intestinal tract is not sterile. Soon after birth, a wide range of bacterial species colonizes the gastrointestinal tract of calves. Most of these bacterial species do not pose any immediate threat to the survival of the calf and in the past were called *good bacteria* and, of which, many of the common probiotic species are routinely classified as, including: *Lactobacillus* species, *Bifidobacteria*, *Enterococcus faecium*, and *Bacillus* species. Remember that the microbial barrier of the intestinal tract soon after birth is colonized primarily by facultative anaerobes and subsequently becomes inhabited largely by strict anaerobes. Most of the probiotic microorganisms are strict anaerobes. Many of the probiotic species also have a direct bactericidal activity or compete with the more pathogenic microorganisms for limited resources. In addition, probiotics are themselves bacteria and they may *prime* the immune system of the calf by staying alert, as even the immune system recognizes the *good* bacteria as foreign. The common, commercially-available prebiotics available are the fructo-oligosaccharides (**FOS**), mannan-oligosaccharides (**MOS**), lactulose, and inulin.

Data on the influence of prebiotics and probiotics alone on the health of dairy calves is equivocal. There are data that show improvements in reducing scouring and improving growth (Abe et al., 1995); whereas equally as many studies show no benefits to including either prebiotics or probiotics in milk (Morrill et al., 1995). The lack of a clear effect in calves is likely due to many environmental factors. Research does however support that many prebiotics and probiotics are generally safe and do not have any adverse effects on calf health or performance. In fact, most regulatory

agencies around the world classify most prebiotics and probiotics as Generally Regarded As Safe (**GRAS**).

Lastly, it is important to note that not all probiotic species and further, not all strains of a specific species, ie: not all *Lactobacillus acidophilus* strains, behave similarly. Therefore, I would recommend only using probiotic species and strains that have been reported, through 3rd party research, to improve health and performance of calves. Additionally, viability/stability of the product should be confirmed as many of the probiotic species can become nonviable during processing and storage.

Another strategy to reduce the interaction of pathogenic microorganisms is to feed egg protein from laying hens that were vaccinated against the very microorganisms that cause gastrointestinal diseases in calves. The laying hens will produce immunoglobulins (**IgY**) and concentrate those proteins in their eggs, which can recognize the pathogen, bind to it, and prevent its interaction with a calf's gastrointestinal tract. Inclusion of whole dried egg from these hens decreased the morbidity due to various bacteria and viruses. In addition to the use of hyper-immunized egg protein, spray-dried plasma proteins can improve gastrointestinal health of calves. Spray-dried plasma is exactly like it sounds, plasma that is spray-dried to preserve the functional characteristics of the diverse group of proteins in plasma. The use of spray-dried plasma has been used for many years in the swine industry to improve the performance and health during the post-weaned period. The addition of spray-dried plasma proteins in milk replacer reduced enteric disease in calves (Quigley et al., 2002).

In 2010, my lab evaluated the effects of supplementing a blend of prebiotics,

probiotics, and hyper-immunized egg proteins to Holstein calves from immediately after birth through the first 3 wk of life (Ballou, 2011). Calves given the prophylactic treatment (n=45) were administered, directly into the milk, 5×10^9 colony forming units per day (from a combination of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium thermophilum*, *Enterococcus faecium*, and *Bifidobacterium longum*), 2 gm/d of a blend of MOS, FOS and charcoal, and 3.2 gm/d of dried egg protein from laying hens vaccinated against K99+ *Escherichia coli* antigen, *Salmonella typhimurium*, *Salmonella Dublin*, coronavirus, and rotavirus. Control calves (n=44) were not given any prebiotics, probiotics, or dried egg protein. All calves were fed 2 L of a 20 % protein/20 % fat, non-medicated milk replacer twice daily. Prior to each feeding fecal scores were determined by 2 independent trained observers. Briefly 1 = firm, well-formed; 2 = soft, pudding-like; 3 = runny, pancake batter; and 4 = liquid splatters, pulpy orange juice.

The prophylactic calves refused less milk ($P < 0.01$) during the first 4 d of life (57 vs 149 grams of milk powder). There were no differences in starter intake or average daily gain due to treatments. However, calves that received the prophylactic treatment had decreased incidence of scours ($P < 0.01$) during the first 21 d of life (25.0 vs 51.1 %). Scours were classified as a calf having consecutive fecal scores ≥ 3 . The intensity of disease in this study was low and only 1 out of 90 calves died during the experiment. These data support that a combination of prebiotics, probiotics, and hyper-immunized egg protein improves gastrointestinal health and could be an alternative to metaphylactic antibiotic use. Future research should determine the efficacy of prophylactic treatment in calves that are at a higher risk

of developing severe gastrointestinal disease, and subsequently death, as well as investigate the mechanism(s) of action within the gastrointestinal immune system.

PLANE OF NUTRITION

The interest in the plane of nutrition that calves are fed during the pre-weaned period has increased primarily because data indicate that calves fed a greater plane of nutrition have decreased age at first calving and they may have improved future lactation performance (Soberon et al., 2012). More large prospective studies in various commercial settings should confirm that calves fed greater planes of nutrition during the pre-weaned period have improved future lactation performance.

Most data on how plane of nutrition influences the health of calves during the first few weeks of life is limited to small, controlled experiments with fecal scores as the primary outcome variable (Nonnecke et al., 2003; Ballou, 2012). Many studies observed that the calves fed the greater plane of nutrition had more loose feces or greater fecal scores (Nonnecke et al., 2003; Bartlett et al., 2006; Ballou et al., 2015), while others reported no differences in fecal scores (Ballou, 2012; Obeidat et al., 2013). It is important to note, that no study has reported greater fecal scores among calves fed a lower plane of nutrition when compared to calves fed a greater plane of nutrition. It has been suggested that the greater fecal scores were not due to a higher incidence of infection or disease, but may be associated with the additional nutrients consumed. A couple of recent studies from my lab are confirming that calves fed greater quantities of milk solids early in life have greater fecal scores; however, when the dry matter percentage of the calves feces were determined there were no differences

between calves fed differing quantities of milk solids (Liang and Ballou, unpublished).

It was unknown whether the digestibilities of nutrients in calves fed varying planes of nutrition were different during the first week of life. Decreased nutrient digestibilities would likely increase the risk of enteric disease because the increased supply of nutrients to the lower gastrointestinal tract could provide a more favorable environment for pathogenic microorganisms to thrive. My lab recently tested the hypothesis that feeding a higher plane of nutrition during the first week of life would decrease the percentages of dietary nutrients that were digested and absorbed (Liang and Ballou, unpublished). Our justification for this hypothesis was that the reduced plane of nutrition during the first week of life would allow the gastrointestinal tract time to adapt to enteric nutrition, without overwhelming the system. However, after conducting a digestibility trial with Jersey calves during the first week of life we had to reject that hypothesis. In fact, there was no difference in the percentage of intake energy that was captured as metabolizable energy (**ME**), averaging 88 % across treatments for the first week of life. We separated the first week of life up into 2 three-day periods and observed a tendency ($P = 0.058$) for more of the intake energy to be captured as ME during the 2nd period (85.9 versus 91.2 ± 2.0 ; 1st and 2nd period, respectively); however, the first period was likely underestimated because residual meconium feces would decrease the apparent digestibility. There was a treatment x period interaction ($P = 0.038$) on the percentage of dietary nitrogen that was retained. The calves fed the greater plane of nutrition had improved nitrogen retention during the first period (88.0 versus 78.7 ± 1.20 ; $P = 0.004$), but was not different from calves fed the reduced plane of nutrition during the second

period (85.3 versus 85.0 ± 1.20 ; $P=0.904$). Most of the difference in nitrogen retention during the first period could be explained by differences in apparent nitrogen digestibility. It should be noted that apparent digestibility was likely more underestimated among the calves fed the restricted milk replacer during the first period because an equal quantity of meconium feces collected across the treatments during period 1 would underestimate the calves fed the restricted quantity of milk replacer more. The data from the digestibility study indicate that calves not only tolerate greater quantities of milk during the first week of life, but they incorporate those nutrients into lean tissue growth. The gastrointestinal immune system and implications to enteric health should be investigated further.

Over the past 7 yr, my laboratory has conducted research to better understand how the plane of nutrition during the pre-weaned period influences leukocyte responses and resistance to infectious disease during the pre- and immediate post-weaned periods (Ballou, 2012; Obeidat et al., 2012; Ballou et al., 2015; Liang and Ballou, unpublished; Sharon and Ballou, unpublished). The results indicate that plane of nutrition influences leukocyte responses of calves (Ballou, 2012; Obeidat et al., 2013; Ballou et al., 2015). In 2 studies, we reported that when calves were fed a lower plane of nutrition their neutrophils were more active during the pre-weaned period, as evident by increased surface concentrations of the adhesion molecule L-selectin (Figure 1) and a greater neutrophil oxidative burst (Obeidat et al., 2013; Ballou et al., 2015). After weaning the elevated neutrophil responses were no longer apparent in either of those studies. The exact mechanisms for the more active neutrophils among the low plane of nutrition calves are not known; but could be due to increased microbial exposure because of increased non-nutritive suckling, altered

microbial ecology of the gastrointestinal tract, or reduced stress among the calves fed the low plane of nutrition. If the neutrophils are more active because of increased microbial exposure, calves fed a lower plane of nutrition could be at an increased risk for disease during the pre-weaned period if exposed to more virulent pathogens.

Ongoing research in my laboratory is trying to understand the behavior and potential microbial exposure when calves are fed varying planes of nutrition and its influence on risk for enteric disease and immunological development. In fact, a few studies have shown that plane of nutrition during the pre-weaned period influences adaptive leukocyte responses. Pollock et al. (1994) reported that antigen-specific IgA and IgG₂ were reduced when calves were fed more milk. In agreement, Nonnecke et al. (2003) reported that less interferon- γ was secreted when peripheral blood mononuclear cells were stimulated with T-lymphocyte mitogens. However, not all data indicate that adaptive leukocyte responses are reduced when greater quantities of milk are fed; Foote et al. (2007) did not observe any difference in either the percentage of memory CD4⁺ or CD8⁺ T lymphocytes or antigen-induced interferon- γ secretion. All the leukocyte response data taken together suggest that calves fed lower planes of nutrition may have more active innate leukocyte responses driven by increased microbial exposure, which may explain the greater adaptive leukocyte responses. In a relatively sanitary environment this increased microbial exposure may improve adaptive immune development in the absence of clinical disease, but in a dirty environment it would likely increase the risk of enteric disease.

How plane of nutrition influences resistance to enteric disease is even less clear than how the leukocyte responses are

affected. Quigley et al. (2006) reported that feeding a variable, greater plane of nutrition to high-risk Holstein bull calves, purchased from a sale barn and raised on bedding contaminated with coronavirus, increased the number of days calves had scours by 53 % and also increased the number of days calves received antibiotics, 3.1 versus 1.9 d. In contrast, a more recent study reported that calves fed a greater plane of nutrition had improved hydration and fecal scores improved faster when they were challenged with *Cryptosporidium parvum* at 3 d of age (Ollivett et al., 2012).

In a recent study from my lab, we orally challenged calves fed either a restricted plane or a greater plane of milk replacer at 10 d of age with an opportunistic pathogen, *Citrobacter freundii* (Liang and Ballou, unpublished). The calves fed the greater plane of nutrition had a greater clinical response to the challenge as evident by increased rectal temperatures ($P = 0.021$) and numerically greater peak plasma haptoglobin concentrations (511 versus $266 \pm 108 \mu\text{g/mL}$; $P = 0.118$). There also was a tendency for total mucosal height of the ileum to be increased among calves fed the greater plane of nutrition (921 versus $752 \pm 59.1 \mu\text{m}$; $P = 0.059$). The increased surface area of the lower gastrointestinal tract could partially explain the increased clinical response among the calves fed the greater planes of nutrition. Current data indicate that there likely is a pathogen: host interaction on the effects that plane of nutrition influence enteric disease resistance. Larger data sets with naturally occurring disease incidence and more experimentally controlled relevant disease challenges that are focused on the gastrointestinal immune system are needed before definitive conclusions can be made on the role that plane of nutrition plays on enteric health of calves during the first few weeks of life. However, current data do not support that

feeding greater planes of nutrition during the first few weeks of life are going to dramatically reduce enteric disease, so if you hear, “We have high incidences of disease and death in dairy calves because we restrict the quantity of milk they are fed” this is likely not true.

In contrast to health during the first few weeks of life, the plane of nutrition calves are fed during the pre-weaned period seems to influence leukocyte responses and disease resistance among calves after they are weaned (Ballou, 2012; Ballou et al., 2015; Sharon and Ballou, unpublished). Jersey bull calves that were fed a greater plane of fluid nutrition had improved neutrophil and whole blood *E. coli* killing capacities after they were weaned when compared to Jersey calves fed a more conventional, low plane of nutrition (Ballou, 2012). These effects were only observed among the Jersey calves in this study and not the Holstein calves. In a follow-up study, Jersey calves that were previously fed a greater plane of nutrition from milk replacer had a more rapid up-regulation of many leukocyte responses, including neutrophil oxidative burst and the secretion of the pro-inflammatory cytokine tumor necrosis factor- α , after they were challenged with an oral bolus of 1.5×10^7 colony-forming units of a *Salmonella enterica* serotype *Typhimurium* (Ballou et al., 2015). The increased activation of innate leukocyte responses among the calves previously fed the greater plane of nutrition reduced ($P = 0.041$) the increase in plasma haptoglobin and those calves also had greater concentrations of plasma zinc. The calves fed the greater plane of nutrition also had improved intake of calf starter beginning 3 d after the challenge ($P = 0.039$). These data indicate that the Jersey calves previously fed a greater plane of nutrition had improved disease resistance to an oral *Salmonella typhimurium* challenge approximately a month after weaning.

Recently, my lab completed a viral-bacterial respiratory challenge on calves a month after weaning that were previously fed either a restricted quantity or a greater plane of nutrition milk replacer (Sharon and Ballou, unpublished). Each calf was challenged intranasally with 1.5×10^8 plaque forming units of bovine herpes virus-1/ nostril and 3 d later were given either 10^6 , 10^7 , or 10^8 colony forming units of *Mannheimia haemolytica* intratracheal in 50 mL of sterile saline (n=5 / plane of nutrition and bacteria dose combination; N=30). Calves were observed for 10 d after the *Mannheimia haemolytica* challenge. The bovine herpes virus-1 challenge decreased calf starter intake by 21.2 % in both plane of nutrition treatments. The *Mannheimia haemolytica* challenge further decreased calf starter intake, but again was not different between planes of nutrition (7.6 %). All calves survived the entire observation period, but 2 calves were euthanized (were completely anorexic and did not respond to antimicrobial / anti-inflammatory treatments) 2 d after the end of the observation period and 2 calves died within a week of completing the observation period. All calves that died or were euthanized were previously fed the restricted plane of nutrition (1, 2, and 1 calves challenged with 10^6 , 10^7 , or 10^8 *Mannheimia haemolytica*, respectively). Necropsies of all 4 calves were consistent with severe pneumonia. Hematology and plasma data during both challenges indicated that calves previously fed the restricted quantity had a greater clinical response as evident by greater percentages of neutrophils in peripheral circulation ($P = 0.041$) and plasma haptoglobin concentrations ($P \leq 0.097$). Therefore, the calves previously fed the restricted quantities of milk replacer had a more severe response to the combined viral-bacterial respiratory challenge, and the

response was relatively independent of the *Mannheimia haemolytica* dose.

Therefore, the 3 studies from my lab are promising that early plane of nutrition from milk replacer can influence the health of dairy calves within 1 mo of weaning. Further, it appears that both enteric and respiratory health is improved with feeding greater planes of nutrition during the pre-weaned period. As was noted for enteric health during the pre-weaned period, larger data sets with naturally occurring disease and additional experimentally controlled challenges with leukocyte responses are needed before definitive conclusions can be drawn. Further, it is of interest whether or not the improved health observed within 1 mo of weaning would persist later into life and improve resistance to other diseases that are common during the life cycle of dairy cattle, including gastrointestinal, respiratory, metritis, and mastitis.

IMPLICATIONS

Dairy calves are extremely susceptible to disease in the first few weeks of life, which may be related to the naïve gastrointestinal immune system of calves. Increasing the plane of nutrition in the first week or 2 appears to increase fecal scores, although the dry matter percentages of the feces were not different. Additionally, the digestibility of nutrients during the first week of life is great and does not appear to be impaired by feeding a greater quantity of milk replacer solids. However, resistance to enteric disease during the first few weeks of life does appear to be influenced by plane of nutrition, but more data are needed before more definitive conclusions can be made. Some early data are suggesting that feeding a greater plane of nutrition during the pre-weaned period may improve leukocyte responses and disease resistance of calves that extends beyond the pre-weaned period;

but as with the effects of plane of nutrition on risk for enteric disease, more data are needed before we fully understand how early life plane of nutrition influences disease resistance later in life.

In addition to plane of nutrition, the uses of prebiotics, probiotics, and proteins from hyper-immunized eggs or spray-dried plasma were all shown to reduce the incidence of gastrointestinal disease. If your calves have a high early mortality I would recommend you look into using a research-backed product with prebiotics, probiotics, or proteins from hyper-immunized eggs or spray-dried plasma.

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LITERATURE CITED

- Abe, F., N. Ishibashi, and S. Shimamura. 1995. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. *J. Dairy Sci.* 78:2838-2848.
- Ballou, M.A. 2011. Case Study: Effects of a blend of prebiotics, probiotics, and hyperimmune dried egg protein on the performance, health, and innate immune responses of Holstein calves. *Prof. Anim. Sci.* 27:262-268.
- Ballou, M.A., D.L. Hanson, C.J. Cobb, B.S. Obeidat, T.J. Earleywine, J.A. Carroll, M.D. Sellers, and A.R. Pepper-Yowell. 2015. Plane of nutrition influences the performance, innate leukocyte responses, and the pathophysiological response to an oral *Salmonella typhimurium* challenge in Jersey calves. *J. Dairy Sci.* 98:1972-1982.
- Bartlett, K. S., F. K. McKeith, M.J. VandeHaar, G.E. Dahl, and J.K. Drackley. 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. *J. Anim. Sci.* 84:1454-1467.
- De Paula Vieira, A., M.A. von Keyserlingk, and D.M. Weary. 2010. Effects of pair versus single housing on performance and behavior of dairy calves before and after weaning from milk. *J. Dairy Sci.* 93:3079-3085.
- Foote, M. R., B. J. Nonnecke, D. C. Beitz, and W. R. Waters. 2007. High growth rate fails to enhance adaptive immune responses of neonatal calves and is associated with reduced lymphocyte viability. *J. Dairy Sci.* 90:404-417.
- Guilloteau, P., R. Zabielski, and J.W. Blum. 2009. Gastrointestinal tract digestion in the young ruminant: ontogenesis, adaptations, consequences and manipulations. *J. Physiol. Pharmacol.* 60:37-46.
- Morrill, J. L., J. M. Morrill, and A. M. Feyerherm. 1995. Plasma proteins and a probiotic as ingredients in milk replacer. *J. Dairy Sci.* 78:902-907.
- National Animal Health Monitoring System. 1993. Dairy heifer morbidity, mortality, and health management focusing on preweaned heifers. Ft. Collins, CO: USDA:APHIS:VS.
- National Animal Health Monitoring System. 1996. Part 1: Reference of 1996 Dairy Management Practices. Ft. Collins, CO: USDA:APHIS:VS.
- National Animal Health Monitoring System. 2007. Dairy 2007: Heifer calf health and management practices on U.S. dairy operations. Ft. Collins, CO:USDA:APHIS:VS.
- Nonnecke, B. J., M.R. Foote, J.M. Smith, B.A. Pesch, and M.E. Van Amburgh. 2003. Composition and

functional capacity of blood mononuclear leukocyte populations from neonatal calves on standard and intensified milk replacer diets. *J. Dairy Sci.* 86:3592-3604.

Obeidat, B.S., C.J. Cobb, M.D. Sellers, A.R. Pepper-Yowell, T.J. Earleywine, and M.A. Ballou. 2013. Plane of nutrition during the preweaning period but not the grower phase influences the neutrophil activity of Holstein calves. *J. Dairy Sci.* 96:7155-7166.

Ollivett, T.L., D.V. Nydam, T.C. Linden, D.D. Bowmann, and M.E. Van Amburgh. 2012. Effect of nutritional plane on health and performance in dairy calves after experimental infection with *Cryptosporidium parvum*. *J. Am. Vet. Med. Assoc.* 241:1514-1520.

Pollock, J. M., T. G. Rowan, J. B. Dixon, and S. D. Carter. 1994. Level of nutrition and age at weaning: Effects on humoral immunity in young calves. *Br. J. Nutr.* 71:239-248.

Quigley, J.D., III, C.J. Kost, and T.A. Wolfe. 2002. Effects of spray-dried animal plasma in milk replacers or additives containing serum and oligosaccharides on growth and health of calves. *J. Dairy Sci.* 85:413-421.

Quigley, J.D., T. A. Wolfe, and T. H. Elsasser. 2006. Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites. *J. Dairy Sci.* 89:207-216.

Soberon, F., E. Raffrenato, R.W. Everett, and M.E. Van Amburgh. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. *J. Dairy Sci.* 95:783-793.