Nutritional strategies to improve gastrointestinal health of dairy calves

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Abstract

Dairy calves are extremely susceptible to gastrointestinal disease during the pre-weaned period. The gastrointestinal immune system 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. Gastrointestinal health can be improved either by hastening the maturation of the intestinal immune system or by controlling infections in the intestines until the local immune system fully develops. Various nutritional strategies may function through 1 or both of these mechanisms and include yeast cell wall extracts, probiotics, other fermentation products, hyper-immunized egg protein, and spray-dried plasma proteins. In addition to various nutritional additives, the quantity and quality of milk solids that are fed to neonatal calves can influence not only the pre-weaning health, but have effects that persist later into life. Nutrition of neonatal calves is as important to calf health as any other management strategy.

Key words: calf, health, immune, nutrition

Résumé

Les veaux laitiers sont très susceptibles aux maladies gastro-intestinales dans la période précédant le sevrage. Le système immunitaire gastro-intestinal chez le veau part à zéro et se développe rapidement durant les premiers jours et les premières semaines de la vie. Les cellules du tractus gastro-intestinal représentent la première ligne de défense du système immunitaire. En attendant que les cellules ressemblent à celles des adultes, le veau est par conséquent plus à risque de développer des maladies gastro-intestinales. On peut améliorer la santé gastro-intestinale en accélérant la maturation du système immunitaire intestinal ou en contrôlant les infections dans l'intestin jusqu'à ce que le système immunitaire soit pleinement développé. Plusieurs stratégies d'alimentation font appel à l'un ou l'autre de ces deux mécanismes. Ces stratégies incluent des extraits de paroi cellulaire de levure, des probiotiques, d'autres produits de fermentation, des protéines d'œuf sur-immunisées et des protéines du plasma déshydratées par pulvérisation. En plus de ces additifs alimentaires variés, la quantité et la qualité des solides du lait qui sont donnés aux veaux néonataux peuvent influencer non seulement la santé avant sevrage mais aussi avoir des effets qui persistent plus tard dans la vie. L'alimentation des veaux néonataux est aussi importante pour la santé du veau que n'importe quelle autre stratégie de régie.

Introduction

Newborn calves are highly susceptible to disease from birth until weaning. The USDA NAHMS data varies from year-to-year, but approximately 1 out of every 10 calves alive within 24 hours of birth dies before weaning. Dairy producers consistently report about 60% of those deaths are attributed to gastrointestinal (GI) disease. Newborn calves are especially susceptible to GI diseases due to their undeveloped mucosal immunity at birth, and exposure to a wide variety of pathogens from either the environment or mismanaged colostrum.

The high mortality among calves is a significant economic loss for producers. Further, the time spent working with sick calves is expensive, but also influences the well-being of not only the calf, but also the employees. The NAHMS data also indicates that greater than 80% of calves with GI disease are treated with antimicrobials. There is pressure to decrease antimicrobial use in livestock industries, and nutrition is an attractive approach to improve GI health either through improved maturation of the GI immune system or preventing infections from causing disease while the GI immune system develops.

Some common nutrition supplement strategies in young calves are yeast cell wall extracts, such as β-glucans and mannanoligosaccharides (MOS), probiotic bacteria, IgY antibodies from chickens immunized against bovine enteric pathogens, plasma proteins, hydrolyzed yeast, and non-IgG extracts from colostrum. Further, the quantity and quality of milk solid nutrition influences not only enteric disease resistance during the neonatal period, but also may have long-term impacts on disease resistance later in life.

Gastrointestinal Development of the Neonatal Calf

A lot of the structures and functions of the GI immune system develop in utero; however, some aspects will only develop postnatal. There are many types of barriers that make up the GI immune system, and they include physical, chemical, immunological, and microbial barriers. Calves are born with vacuolated fetal-type enterocytes, which are important for the absorption of macromolecules from colostrum, but also increase permeability to microorganisms.[27,33] Vacuolated fetal-type enterocytes are replaced with adult-type enterocytes during the first week of life, which contributes to the GI barrier function.[19] The process of replacing vacuolated enterocytes with adult-type occurs proximal to distal intestines. Further, replacement of the immature vacuolated enterocytes with mature enterocytes decreases paracellular translocation by increasing tight junction proteins between the epithelial cells. The tight junction transcription factor, MAMDC4, increased during the first week of life.[27] Goblet cells in the GI mucosa are important producers of mucin, which is critical in the formation of the mucus layer over the apical membrane of the GI epithelium. The mucus layer forms a physical protective barrier, but also many immunological and chemical factors produced in the GI mucosa and submucosa are concentrated there, creating an effective chemical and immunologic barrier. Data from gnotobiotic mice suggest that formation of the mucus layer is dependent upon postnatal microbial colonization of the GI mucosa. These data indicate that the physical barriers of the GI immune system are compromised early in life, and likely contribute to increased risk for GI disease.

In addition to deficiencies in the physical barriers, many of the chemical and immunological factors that protect the GI mucosa are not fully developed at birth. The GI mucosa and submucosa are full of leukocytes and other cells that either produce host defense peptides that act like natural antimicrobials of the innate immune system or respond in an antigen-specific manner to produce IgA or become T-lymphocytes with helper, effector, or memory phenotypes. The host defense peptides are a group of small peptides that possess broad antimicrobial activity. The β-defensins are most studied class of host defense peptides in bovines.[18] The expression of human gastrointestinal host defense peptide synthesis appears to be influenced by postnatal microbial colonization of the gastrointestinal tract.[14] Very little is known regarding the development of gastrointestinal host defense peptides in calves during the preweaned period; however, another study reported that low levels of bronchoalveolar β-defensin-1 concentrations was associated with susceptibility to *Bordetella pertussis*-associated bronchopneumonia in neonatal pigs.[10] Further, they reported that administration of β-defensin-1 at the time of *Bordetella pertussis* infection prevented the development of bronchopneumonia. Nutritional modulation of host defense peptide production in a variety of animal species was reported and responded to a variety of nutritional supplementation strategies including: short chain fatty acids, vitamin D, and various probiotic bacteria.[31]

The subclass of immunoglobulin that is found predominately on mucosal surfaces is IgA. The primary T-lymphocyte populations were present in the peripheral blood of young calves at similar concentrations to adult cows.[15] In contrast, the B-lymphocyte populations developed over the first few months of life; therefore, it is suspected that active immunoglobin production is delayed until the B-lymphocyte populations develop. Salivary IgA concentrations were positively correlated with age from birth through adolescents in human subjects.[35] Therefore, these data further indicate that many of the components of the chemical and immunological barrier of the gastrointestinal tract develop during the preweaned period and likely contribute to the increased risk for GI disease in calves early in life.

Mucosal surfaces, including the GI tract, are colonized by a diverse group of microorganisms that play a synergistic role with the host. The majority of the trillions of microorganisms that live in the gastrointestinal tract are commensals and actually contribute to the microbial barrier of the gastrointestinal immune system. These microorganisms can influence immunity through various mechanisms, including: direct competition for substrates and space with potentially pathogenic microorganisms, secretion of antimicrobial factors, and/(or) modulation of other gastrointestinal mucosal immune responses. The calf develops *in utero* in a relatively sterile environment, and upon parturition and during the postnatal 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 (e.g. *Enterobacteriaceae*, *Streptococcus*, and *Staphylococcus)* dominating during the early postnatal period. There is a switch to where strict anaerobes (e.g. *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 GI tract is also compromised during early life and likely contributes to the greater incidence of enteric disease.

Colostrum

The importance of passive transfer of immunoglobulins (Ig) from colostrum to the calf is well established. In addition, colostrum and transition milk to a lesser degree have other factors that contribute to maturation of the immune system in a calf. These factors include essential nutrients such as carbohydrates, amino acids, and fats, but also non-nutritive and bioactive factors such as insulin-like growth factors (IGF) -1 and -2, growth hormone, IgG, IgA, IgM, and non-pathogenic bacteria crucial to establishing the symbiotic microbial ecology in the GI tract. Bioactive factors in colostrum have more local impacts in the small intestine than direct systemic effects.

One of the most abundant growth factors in colostrum is IGF-1, a member of the insulin family. IGF-1 increases proliferation of epithelial cells in small intestinal crypts of both piglets and calves.[36] Newborn calves supplemented with colostrum-extracted IGF-1 had greater villi height as well as proliferation of epithelial cells in the small intestine than control calves on d 5 and 8 of life; on the other hand, IGF-1 supplemented calves had decreased xylose absorption rate compared to control calves.[32] Growth-promoting hormones as well as IGF-1 target enterocytes and induce proliferation and differentiation to establish fully developed cells for digestion and absorption. Colostrum protects these bioactive proteins from digestion through a trypsin-inhibitor that can stop proteolytic digestion from occurring before the factors reach the small intestine.[12] The timing of supplementation of IGF-1 may be crucial in determining the impacts. A study conducted by our lab showed that supplementation of a colostrum extract product to high-risk Holstein bull calves did not have significant impacts on growth or health, but supplementation in that study started approximately 24 h after birth and continued in milk replacer for 3 d and was not given with colostrum.[8]

Immunoglobulin G (IgG) accounts for approximately 85 to 90% of the total Ig in the colostrum, and the other 10 to 15% is composed of IgA and IgM.[12] The IgG are absorbed into blood circulation within 24 h after calving and these immunoglobulins play a crucial role in humoral immunity for neonatal calves while the GI immune system develops. Further, the absorbed immunoglobulins play an important role in preventing infection while the GI immune system develops because of recirculation of IgG back into the lumen of the intestinal tract.[6]

Additionally, immunologically active leukocytes derived from colostrum including neutrophils, macrophages, T lymphocytes, and B lymphocytes are also absorbed from colostrum and may contribute to calf health. The B lymphocytes derived from maternal leukocytes secreted dimeric IgA in the newborn calves. Additionally, T lymphocytes secreted multiple cytokines to mediate innate immune responses.[16] However, the potential functions of neutrophils and macrophages in newborn calves remain uncertain.

Without proper colostrum quality or quantity, GI maturation can be delayed, which increases the time for exposure and susceptibility for infection and disease.[12] When a calf does not receive the quality or quantity of colostrum it needs and has a serum concentration of less than 10 mg/mL of IgG, then it is considered failure of passive transfer (FPT). Calves with FPT are at a greater risk than calves with proper passive transfer, and it is likely associated with reduced humoral immunity, but also delayed or impaired GI immune development. More research is needed to further understand the impacts of colostrum and feeding transition milk on GI immune development.

Nutritional Supplements

Prebiotics, probiotics, and proteins from hyper-immunized egg or spray-dried plasma were all reported to have 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 is a vague term, but generally refers to 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." They include many of the common probiotic species routinely classified, such as *Lactobacillus* species, *Bifidobacteria*, *Enterocooccus 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 fructooligosaccharides (FOS), mannanoligosaccharides (MOS), lactulose, and inulin.

In addition to providing substrates for growth for commensal bacteria, prebiotics may also have either immunomodulatory or binding characteristics that can improve enteric health in calves. β-glucans from fungal cell walls are a microbe-associated molecular pattern that can ligate the dectin-1 receptor on macrophages and neutrophils. β-glucans are subsequently endocytosed within macrophages, which activates the release of various cytokines and increases phagocytic activity. Another component that can be extracted from fungal cell walls is MOS. Purified MOS can competitively bind gram-negative bacteria that can potentially colonize the GI tract. Mammals do not have the enzymes to digest MOS, so the MOS-bacteria complex may exit the body without colonization or causing infection and disease. Many bacteria cause disease after attachment or internalization into enterocytes, and they do so using pili or fimbriae that are rich in lectins. Type-1 fimbriae, or pili, specifically bind mannose, and MOS has mannose receptors that can bind to those pathogens, thus not allowing the pathogen to bind with the GI mucosa.

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 whereas equally as many studies show no benefits to including either prebiotics or probiotics in milk.[1,20] 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 of 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 *Lactobaccilus acidophilus* strains, behave similarly. 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 gastro-intestinal 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 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.[30]

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 weeks of life.[2] Calves given the prophylactic treatment (n=45) were administered directly into the milk 5 x 10^9 colony forming units per day (from a combination of *Lactobacillus acidophilus, Bacillus subtilis, Bifidobacterium thermophilum, Enterococcus faecium,* and *Bifidobacterium longum*), 2 grams per day of a blend of MOS, FOS and charcoal, and 3.2 grams per day 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 liters 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 according to Larson et al. (1977). 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 days 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 days 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 improve gastrointestinal health and could be an alternative to metaphylactic antibiotic use. Future research should determine the efficacy of that 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.

Milk Solid 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.[34] 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.[3,24] Many studies observed that the calves fed the greater plane of nutrition had more loose feces or greater fecal scores while others reported no differences in fecal scores.[2,3,5,24,25] 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.[17]

It was unknown whether the digestibilities of nutrients of 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 gastro-intestinal 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.[17] 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, averaging 88% across treatments for the first week of life. We separated the first week of life into 2 three-day periods and observed a tendency (P=0.058) for more of the intake energy to be captured as metabolizable energy during the second period (85.9 vs 91.2 ± 2.0; first and second 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 vs 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 vs 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 more greatly underestimate the calves fed the restricted quantity of milk replacer. 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 further investigated.

Over the past 10 years, 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.[2,3,17,25] The results indicate that plane of nutrition influences leukocyte responses of calves.[2,3,25] 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 and a greater neutrophil oxidative burst.[3,25] 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 gastrointestinal immune development 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. Antigen-specific IgA and IgG_2 were reduced when calves were fed more milk.[28] In agreement, less interferon-γ was secreted when peripheral blood mononuclear cells were stimulated with T-lymphocyte mitogens.[24] However, not all data indicate that adaptive leukocyte responses are reduced when greater quantities of milk are fed; some studies did not observe any difference in either the percentage of memory CD4+ or CD8+ T lymphocytes or antigen-induced interferon-γ secretion.[11] 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 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 vs 1.9 days.[29] 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 days of age.[26] 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 days 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 vs 266 ± 108 µg/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 vs 752 ± 59.1 µm; P = 0.059). Current data indicate that there likely is a pathogen:host interaction on the effects that plane of nutrition influences 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 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 be influence leukocyte responses and disease resistance among calves after they are weaned.[2,3,4] 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.[3] 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 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 x 10^7 colony-forming units of a *Salmonella enterica* serotype *Typhimurium*.[4] 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 days 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.

My lab recently 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 milk replacer (Sharon and Ballou, unpublished). Each calf was challenged intranasally with 1.5x10^8 plaque forming units of bovine herpesvirus-1 per nostril and 3 days 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 per plane of nutrition and bacteria dose combination; N=30). Calves were observed for 10 days 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 days 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 milk replacer nutrition can influence the health of dairy calves within 1 month 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 month 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. Colostrum is more than just passive transfer of immunoglobulins, and many bioactive substances in colostrum hasten maturation of the gastrointestinal immune system. Proper colostrum management is essential for initiating development of the gastrointestinal immune system. Supplementing prebiotics, probiotics, and proteins from hyper-immunized egg or spray-dried plasma were also shown to reduce the incidence of gastrointestinal disease. The mechanistic strategy is to improve gastrointestinal immune development and/or reduce the interaction of a potential pathogen with the gastrointestinal mucosa.

Increasing the plane of nutrition in the first few weeks of life 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 replacement solids among healthy calves. 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.

Conflicts of Interest

Michael Ballou is an equity owner in MB Nutritional Sciences, LLC. Neither Emily Davis nor Yu Liang have a conflict of interest.

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