

Effects of Housing and Nutrition on the Immunity of Dairy Calves

Michael A. Ballou

Department of Animal and Food Sciences, Texas Tech University, Goddard Building, Suite 108, MS 42123, Lubbock, Texas 79409
Email: michael.ballou@ttu.edu

■ Take Home Messages

- ▶ 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 egg 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 may be greater among calves fed greater quantities of milk replacer early in life.
- ▶ Feeding greater quantities of milk replacer improves post-weaning health.
- ▶ Group housed calves have increased calf starter intake and average daily gain compared to individually housed calves.
- ▶ Group housed calves have more active innate leukocyte responses, which could be attributed to greater microbial exposure. Therefore, if you are going to group house calves it is even more important to raise them in a sanitary environment.

■ 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 USDA National Animal Health and Monitoring System surveys (NAHMS, 1993, 2007) report that the national mortality rate of heifer calves from 48 hours 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 $\frac{1}{4}$ of all pre-weaned calves are therapeutically treated for scours, and the major causes of death from scours are either dehydration or

access of the pathogens to the blood causing septicemia. The high incidences of disease indicate we have much to learn about improving gastrointestinal disease resistance among pre-weaned calves. Colostrum management, including volume and the composition of fluid fed, the use of various additives such as prebiotics, probiotics, and proteins from hyper-immunized egg 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 (white blood cell) responses and disease resistance (Ballou, 2012; Ballou et al., *JDS In Press*; 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 Gastro-Intestinal 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 passive absorption 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 my discussion in this section 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 of the gastrointestinal tract and the tight junctions between those cells form a **physical barrier** that prevents luminal (intestinal) 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 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 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.

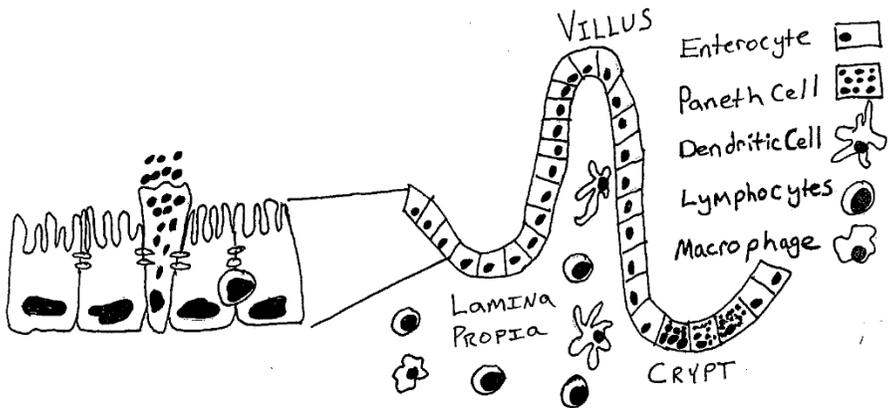


Figure 1. Schematic drawing of the small intestinal mucosa.

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

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. 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 postnatal 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 (cells that provide defense against microbes in the small intestine) 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. 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 weeks. 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 it is 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.

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 4L of colostrum within the first 6-12 hours 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. 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 egg 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. The common, commercially-available prebiotics are inulin, lactulose and the fructooligosaccharides (FOS) and mannanoligosaccharides (MOS). Probiotics is a vague term, but generally they 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 gastro-intestinal 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.

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; Heinrichs et al., 2003), whereas equally as many studies show no benefits to including either prebiotics or probiotics in milk (Morrill et al., 1995; Hill et al., 2008). 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 gastro-intestinal diseases in calves. The laying hens will produce immunoglobulins (IgY) and concentrate those proteins in their eggs; the IgY can recognize the pathogen, bind to it, and prevent its interaction with a calf’s gastro-intestinal tract. Inclusion of whole dried egg 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 the gastro-intestinal 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. Spray-dried plasma has been used for many years in the swine industry to improve pig 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; Hunt 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 weeks 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 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 two 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 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 gastro-intestinal 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 gastro-intestinal disease and subsequently death as well as investigate the mechanism(s) of action within the gastrointestinal immune system.

■ Plane Of Nutrition

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 may have improved future lactation performance (Soberon et al., 2012; Soberon and Van Amburgh, 2013). 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; Osorio et al., 2012; Hengst et al., 2012), 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 (20 vs 14.5 g/kg BW of milk solids per day) 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 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 (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 gastro-intestinal 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 up 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 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 (20 g/kg BW milk solids per day) had improved nitrogen retention during the first period (88.0 vs. 78.7 ± 1.20 ; $P=0.004$), but were not different from calves fed the reduced plane of nutrition (14.5 g/kg BW milk solids per day) 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. Apparent digestibility was likely underestimated among the calves fed the restricted milk replacer during the first period because an equal quantity of meconium feces was collected from the two treatment groups, and this would underestimate digestibility in the restricted-fed calves. 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.

Over the past 7 years, my laboratory has conducted research to better understand how plane of nutrition during the pre-weaned period influences leukocyte responses and resistance to infectious disease during the pre- and immediate post-weaned periods. In our studies the lower plane of nutrition were fed 405 to 450 g of a 20/20 milk replacer per day, irrespective of whether they were Holstein or Jersey calves. Jersey calves fed the higher planes of nutrition were fed 680 to 735 g of a 28/25 milk replacer per day, whereas Holsteins were fed 1,050 to 1,180 g of a 28/20 milk replacer per day. The results indicate that plane of nutrition influences leukocyte responses of calves (Ballou, 2012; Obeidat et al., 2013; Ballou et al., In Press, JDS). 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 evidenced 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., In Press, JDS). 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 the 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 (3.1 vs. 1.9) calves received antibiotics. In contrast, a more recent study reported that calves fed a greater plane of nutrition had improved hydration, and fecal scores improved faster when the calves were challenged with *Cryptosporidium parvum* at 3 days of age (Ollivett et al., 2012). In a recent study, 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 evidenced by increased rectal temperatures and numerically greater peak plasma haptoglobin concentrations (511 vs. 266 ± 108 $\mu\text{g/mL}$). 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). 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 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. 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 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., In Press, JDS). The increased activation of innate leukocyte responses among the calves previously fed the greater plane of nutrition calves reduced 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. 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 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. Each calf was challenged intranasally with 1.5×10^8 plaque forming units of bovine herpes virus-1 per nostril and 3 days later was 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 and plasma haptoglobin concentrations. 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 gastro-intestinal and respiratory disease, metritis, and mastitis.

■ Calf Housing

The majority of dairy calves in the US are individually housed during the pre-weaned period. This management strategy was widely adopted for many reasons, including: individual assessment of health, individual therapeutic treatments are given more easily, reduced horizontal transmission of diseases, and prevention of non-nutritive suckling (especially when fed restricted quantities of milk solids). A recent study indicated that calves housed in groups of 3 had more active neutrophils in peripheral circulation than individually housed calves (Cobb et al., 2014). The more active neutrophils were already apparent 10 days after initiation of housing treatments, and persisted until weaning. All calves in this study were fed a high plane of nutrition (1,050 g of a 28/20 milk replacer per day), which helped reduce, but not eliminate non-nutritive suckling. We hypothesize that the increase in active neutrophils among the group-housed calves was due to increased microbial exposure due to calf-to-calf interactions. If the risk of microbial exposure is increased with group housing, then the risk for enteric disease during the first few weeks of life would also likely be increased while the gastrointestinal immune system is undergoing maturation. Therefore, it may benefit the health and wellbeing of calves to be individually housed for the first few weeks while the gastrointestinal immune system undergoes maturation and the risk for morbidity and mortality greatly reduces.

The benefits of group housing calves are in the social interactions among the calves. Increased calf starter intake is commonly reported among group-housed calves and was suggested that either social teaching or competition led to the increased starter intake. Others have reported greater calf starter intakes during the neonatal period (de Paula Vieira et al., 2010); however, in our study we only observed the difference after weaning, when calf starter intake increased greatly in both treatments (Cobb et al., 2014). Our study also showed that calves relied more on calf-to-calf interactions rather than calf-to-human interaction when they were group housed. In addition, when calves were randomly grouped into larger pens of 5 a month after weaning, the calves that were previously group-housed had improved average daily gain during the grower phase. If calves are fed restricted quantities of milk solids, individual housing is the preferred management because those calves will perform excessive non-nutritive suckling, significantly impairing the wellbeing of those calves. However, if calves are fed greater quantities of milk I think group housing could be introduced prior to weaning, but again I would continue to individually house calves for the first few weeks of life until the risk of morbidity and mortality is greatly reduced.

■ Conclusions

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 definitive conclusions can be made. Some early data suggest 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 egg or spray-dried plasma were all shown to reduce the incidence of gastro-intestinal disease. If you have a high early mortality I would recommend you look into using a research-backed product with prebiotics, probiotics, or proteins from hyper-immunized egg or spray-dried plasma.

There are pros and cons for both individual and group-housing situations of pre-weaned calves. If calves are fed restricted quantities of milk solids, they should be individually housed until after weaning because the negative effects of non-nutritive suckling among the calves will outweigh any of the positive effects. If calves are fed greater quantities of milk solids, group housing can be introduced before weaning. The major benefits are that they consume more calf starter, have greater average daily gain, and have less of a slump in performance when they are moved into larger groups after weaning. I would still suggest to individually house calves for the first couple weeks of life, as the risk for both disease and death are extremely great. Individually housing calves for the first few weeks of life will reduce additional environmental microbial exposure by reducing calf-to-calf interactions, improve the detection and monitoring of sick calves, and make individual therapeutic treatments easier to administer.

■ Acknowledgements

Many current and past graduate students and visiting scientists have helped collect most of the data presented in this paper. I would like to thank Clayton Cobb, Dr. Lindsey Hulbert, Yu Liang, Dr. Belal Obeidat, Tyler Harris, Matthew

Sellers, Dr. Amanda Pepper-Yowell, Devin Hansen, and Kate Sharon. I would also like to acknowledge that some of this work was conducted in collaboration with Dr. Jeff Carroll with the USDA-ARS Livestock Issues Research Unit located in Lubbock, TX. I appreciate our collaboration and would like to thank his lab group for all their hard work, especially Jeff Dailey.

■ References

- 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. 2012. Immune responses of Holstein and Jersey calves during the preweaning and immediate postweaned periods when fed varying planes of milk replacer. 95:7319-7330.
- 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. 2014. Plane of nutrition influences the performance, innate leukocyte responses, and the pathophysiological response to an oral *Salmonella typhimurium* challenge in Jersey calves. *In Press, J. Dairy Sci*
- 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.
- Cobb, C.J., B.S. Obeidat, M.D. Sellers, A.R. Pepper-Yowell, D.L. Hanson, and M.A. Ballou. 2014. Improved performance and heightened neutrophil responses during the neonatal and weaning periods among outdoor group-housed Holstein calves. *J. Dairy Sci.* 97:930-939.
- 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.
- Foot, 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, 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. 2007. Dairy 2007: Heifer calf health and management practices on U.S. dairy operations, 2007. 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. Bowman, 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.



