POTENTIAL FOR FEED SUPPLEMENTS TO ENHANCE DAIRY COW HEALTH

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INTRODUCTION

The classic role of the dairy cattle nutritionist is to formulate rations that meet animal performance requirements. However, as the delicate interplay between animal nutrition and health continues to unfold, data are suggesting that dietary ingredients that do not meet specific nutrient requirements may still provide benefits to dairy cattle health. In ruminant nutrition, the best understood of these are certain minerals and vitamins that, when included either above current requirement levels or in more available forms, improve measures of animal health. In this example, the mineral or vitamin is absorbed and elicits a biological effect that boosts immune function. In addition to minerals and vitamins, specific supplemental fatty acids and amino acids can modify immune response and animal health. A more poorly understood area, but an area of active research, are those supplements that exert their effects entirely within the digestive tract. These include prebiotics and probiotics that modify microbial composition in the rumen and intestines and alter function of immune cells associated with the gut mucosa. Changes in function of these gut immune cells can cause downstream effects including an increase or decrease in gut mucosa integrity or activation or suppression of localized and systemic inflammation which in turn can affect whole animal health. This paper provides a brief discussion of some feed supplements that either improve or are suspected of improving dairy cattle health. The impact of nutrition on health is an area of active research and will likely yield additional nutritional tools to improve health of our dairy herds.

SYSTEMICALLY-ACTING FEED SUPPLEMENTS

Minerals and vitamins are the most well documented feed supplements that can enhance animal health. Although there are clearly benefits when animals are switched from a deficient state to a sufficient state, there is some evidence that supplementation of certain minerals and vitamins above current requirement levels may also provide benefits. In the case of trace mineral supplements, there is also evidence that chemical composition and consequent biological availability also impact health.

Lactating and transition dairy cows are under considerable oxidative stress. Intracellular and extracellular antioxidants function to neutralize free radicals and prevent damage to cells and their membranes. However, antioxidant systems cannot completely eliminate free radicals, and indicators of oxidative damage typically increase during periods of high oxidative stress including transition, heavy lactation, and heat stress. White blood cells are believed to be particularly susceptible to oxidative damage due to relatively high concentrations of unsaturated fatty acids on their membranes. Therefore, immune function may be compromised by oxidative stress more quickly than other biological systems.

Many of the trace minerals and vitamins that enhance cow health act at least in part through their function in antioxidant systems. Copper, manganese, and zinc all serve as cofactors for superoxide dismutase, an enzyme responsible for converting superoxide into oxygen and hydrogen peroxide. When heifers were marginally deficient in copper status, supplementation with copper sulfate improved clinical response to an intramammary *E. coli* challenge (Scaletti et al., 2003). In a subsequent study, supplementation using copper proteinate resulted in an improved response to the *E. coli* challenge compared to copper sulfate, suggesting a greater bioavailability of the organic proteinate than the inorganic sulfate (Scaletti and Harmon, 2012). In addition to zinc's function in superoxide dismutase, it is also a component of multiple enzymes and transcription factors and is important for a wide range of biological functions including the maintenance of dermal and hoof integrity. A study by Cope et al. (2009) found that lactating dairy cows fed diets below predicted requirements for zinc had increased somatic cell counts and milk amyloid A (an acute phase protein used as an indicator of systemic inflammation) compared to cows fed supplemental zinc. Since copper, manganese, and zinc all serve as cofactors in different forms of superoxide dismutase, multiple studies have evaluated the effect of changing level or bioavailability of all three of these on animal performance. We conducted a study using lactating cows that were supplemented with copper, manganese, and zinc in either inorganic sulfate or organic amino acid chelate forms (Nemec et al., 2012). Although we found no treatment effect on in vitro measures of neutrophil function, we did find an improved antibody response to vaccination against a foreign antigen for the cows fed the chelated forms compared to the sulfate forms. A recent study compared steers fed diets supplemented with amino acid complexed forms of copper, manganese, and zinc to steers fed diets with lower levels of those minerals (Gomez et al., 2014). There were also differences in cobalt and iodine supplementation between the two diets. That study found a benefit in hoof health for those steers fed the chelated minerals as evidenced by improved response to a digital dermatitis challenge (Gomez et al., 2014). Selenium also performs antioxidant functions primarily by acting as a cofactor for thioredoxin reductase enzymes that convert hydrogen peroxide to water. Some studies have found that marginal selenium status in dairy cattle is associated with increased milk somatic cell count and impaired white blood cell function (Hogan et al., 1993; Spears and Weiss, 2008). Dairy cattle selenium requirements are 0.3 mg/kg which is also the maximum supplementation level allowed (NRC, 2001). Though dietary selenium cannot be increased beyond this maximum, feeding selenium yeast instead of inorganic selenium provides a means to increase selenium absorption (Weiss and Hogan, 2005).

Vitamins E and C benefit dairy cattle health through their antioxidant activities. Vitamin E is a lipid soluble antioxidant that helps to stop free radical damage of lipids. Periparturient supplementation of vitamin E above NRC requirements can enhance neutrophil function and improve mammary gland health (Hogan et al., 1993; Spears and Weiss, 2008). Vitamin C is the primary water-soluble antioxidant and is important for regeneration of vitamin E. In addition, vitamin C serves a cofactor role in enzymes including several involved in collagen synthesis and, therefore, integrity of the skin. Weiss and Hogan (2007) supplemented periparturient cows with vitamin C and then subjected them to an intramammary lipopolysaccharide infusion a month following calving. Although supplementation did not affect measures of neutrophil function, it did reduce the elevation in somatic cell count following the intramammary challenge.

Vitamins and minerals without antioxidant activities can also benefit animal health. Vitamin A functions as a component of transcription factors that have a variety of downstream effects on growth, physiology, and health. Additional supplementation of vitamin A or precursors of vitamin A including β-carotene may have immunostimulatory activities and benefit mammary gland and uterine health (Spears and Weiss, 2008). β-carotene and other carotenoids also have antioxidant activities and may support immune health independent of their role as vitamin A precursors. Similar to vitamin C, B vitamins have not classically been supplemented to ruminant diets due to their synthesis by rumen microbes. There is some evidence that supplemental biotin may benefit hoof health in addition to a positive effect on milk production (Lean and Rabiee, 2011). Other B vitamins such as B12 or folic acid may potentially improve animal health as well. In addition to its actions on calcium homeostasis, vitamin D also directly moderates immune function. Nelson et al. (2010) found that bovine monocytes can both produce and respond to the active form of vitamin D, and another study by the same group found that cows were provided with some protection against an intramammary *S. uberis* challenge when a vitamin D precursor was administered in conjunction with the bacteria (Lippolis et al., 2011). Supplemental chromium may also benefit immune function due to its role in regulating insulin sensitivity. Both insulin sensitivity and immune function are suppressed in transition cows, and it is possible that supplementation with chromium to improve insulin sensitivity may have a positive effect on immune function. One study found that chromium supplementation to periparturient cows increased serum antibody response to a foreign antigen and in vitro lymphocyte proliferation (Burton et al., 1993), and a recent study suggested an improvement in uterine health following periparturient supplementation with chromium propionate (Yasui et al., 2014).

Minerals and vitamins are not the only supplements that can be absorbed and have beneficial effects on dairy cow health. Supplements commonly adopted in transition cow programs such as anionic salts and propylene glycol drenches have proven efficacy in improving transition cow health. The effect of supplemental dietary fatty acids on animal health is an area of active research and the interplay between lipid metabolism and health will be discussed by the next presenter at this conference. Additional supplementation of individual amino acids may also improve health as evidenced by improved measures of immune function in dairy cows supplemented with glutamine or methionine (Caroprese et al., 2013; Osorio et al., 2013). Supplementation of diets with mycotoxin binders can also enhance immune function. Although the binders themselves are not systemically active, they prevent the systemic effects of mycotoxins in the feed which tend to be immunosuppressive (Sharma, 1993; Mehrzad et al., 2011). If sufficient quantities of mycotoxins are present in the feed to elicit a negative immune response, then addition of mycotoxin binders can help to minimize these effects. Efficacy of an individual mycotoxin binder will depend on characteristics of the binder and the extent that it can neutralize the class of mycotoxin present.

GUT-ACTING FEED SUPPLEMENTS

Interplay between the gut mucosa, the microbiome, and inflammatory disease. A field of intense research in human health is the relationship among diet composition, gut microbiota, intestinal mucosa, and whole-animal health. Although data on these relationships are just beginning to emerge in production animals, it is likely that some of the findings from monogastric species will drive future supplementation strategies.

The intestinal mucosa is made up of different classes of epithelial cells. The cells in the greatest numbers are the columnar epithelial cells responsible for the absorption of dietary nutrients. Tight junctions between columnar epithelial cells are crucial for forming the physical barrier between the tissue and digesta. Interspersed among the columnar epithelial cells are a variety of specialized cells that help to protect the tissue from bacteria and toxins within the gut lumen. Goblet cells produce mucus that adheres to the luminal side of epithelial cells and provides an additional physical barrier. The mucus also houses a variety of bactericidal and bacteriostatic agents that prevent colonization by microbes that penetrate the mucus barrier. Many of these antimicrobial agents originate from Paneth cells that produce different compounds in response to stimuli from gut contents or surrounding cells. These antimicrobial agents include peptide defensin molecules as well as antimicrobial enzymes. M cells are another specialized class of epithelial cell that sample particulates from the gut lumen and present them to underlying immune cells.

Gut associated lymphoid tissue (GALT) consists of structures of white blood cells that are found throughout the digestive tract in close association with the epithelial cells. These range in size from large Peyer's patches to small isolated lymphoid follicles and consist of clusters of B and T lymphocytes interspersed with dendritic cells and phagocytes (Goto and Kiyono, 2012). Antigen presentation from M cells or dendritic cells causes B cells to be activated to IgA-secreting plasma cells. Secretory IgA then exits the columnar epithelial cells via transcytosis where it accumulates in the mucus to prevent attachment and translocation of target bacteria (Kamada et al., 2013). T cells in germinal centers within the gut associated lymphoid tissue are activated by receptor binding of microbial products and locally produced cytokines. In a healthy animal, tolerance of commensal gut microorganisms is facilitated by T cells with a regulatory phenotype that tend to suppress inflammatory responses by surrounding cells (Littman and Pamer, 2011). In some disease states associated with chronic inflammation there is a shift in microbiome composition and intestinal T cells move more towards pro-inflammatory Th17 phenotypes (Littman and Pamer, 2011).

Activities of lymphocytes and phagocytes within the healthy GALT respond to the microbiome to elicit appropriate responses: tolerance and local immunosuppression in response to commensal organisms and inflammation and immune activation in response to a microbial threat. Binding of bacterial components to pathogen recognition receptors such as toll like receptors (TLR) and NOD-like receptors (NLR) is essential for homeostasis. Normal development of GALT structures is dependent upon functional pathogen recognition by these receptors. In addition to promoting proper GALT development, a symbiotic mix of commensal bacteria promotes mucus production and barrier function of the epithelium and inhibits colonization by competitive organisms (Kamada et al., 2013). The effects of the microbiome on GALT and mucosal functions are not only through direct interactions of microbial components with receptors but also through products of symbiotic microbes including short chain fatty acids (Brestoff and Artis, 2013). Dysbiosis, an unhealthy shift in microbiome composition, can down-regulate these protective functions, stimulate mucosal inflammation, and potentiate colonization by pathogenic organisms. In humans, some disease states including inflammatory bowel disease are associated with a complete shift in intestinal population structure (Koboziev et al., 2014).

Work in rodent models is beginning to demonstrate that chronic inflammatory diseases including metabolic syndrome, diabetes, and atherosclerosis are associated with shifts in intestinal microbiota (Caesar et al., 2012; Vieira et al., 2013). Although cause and effect are unclear, in certain instances transfer of healthy microflora to a sick individual can restore health and transfer of microbiota from a sick donor to a healthy recipient can cause disease. For example, mice lacking TLR5, a receptor that recognizes bacterial flagellin, developed metabolic syndrome and altered intestinal microbiome compared to wild-type mice (Vijay-Kumar et al., 2010). When their gut contents were transferred to healthy germ-free wild-type mice, the recipient mice developed characteristics of metabolic syndrome as well, indicating that the microbiome is important in both maintaining health and contributing to disease states. Such findings could be of relevance in the cow also, particularly in the transition period when cows experience some symptoms of metabolic syndrome including decreased insulin sensitivity, increased glucose tolerance, and increased mobilization of fatty acids.

Data in dairy cows. The interaction between changes in gut microbiome and dairy cattle health is most well documented as it relates to rumen acidosis and sub-acute rumen acidosis (SARA). A switch to a high grain diet or induction of SARA induces dramatic changes in the rumen fluid microbiome and in population structure of bacteria adhered to the rumen epithelium (Khafipour et al., 2009b; Chen et al., 2011). Sub-acute rumen acidosis-inducing diets also increase flow of fermentable carbohydrates to the intestines. This results in shifts in the intestinal microbiome as demonstrated by changes in fecal bacterial composition (Mao et al., 2012). These shifts in gastrointestinal bacterial communities in response to SARA are believed to be a key first step in the negative impacts of SARA on animal health and performance. In addition to the microbiome shift, acidosis also increases concentration of toxic and inflammatory compounds in the digesta (Ametaj et al., 2010; Li et al., 2012; Saleem et al., 2012) concurrent with a decrease in barrier function of rumen epithelium (Steele et al., 2011). Because the intestinal epithelium is composed of only a single layer of epithelial cells, systemic entry of bacteria or toxins in response to SARA may be more likely to occur in the intestinal mucosa than in the rumen. In fact, Khafipour et al. (2009a) found the timing of the presence of lipopolysaccharide in the blood following a SARA challenge suggested entry through the intestines instead of the rumen. Shifts in the microbiome, accumulation of toxins in the digesta, and mucosal damage in response to SARA likely shift nearby GALT structures from an anti-inflammatory to a pro-inflammatory state, which, based on rodent models, may result in further damage to the mucosa and systemic inflammation. This may be a contributing factor to the increase in circulating acute phase proteins observed in response to grain-based SARA challenges (Plaizier et al., 2008). Systemic inflammation resulting from SARA likely contributes to the negative impacts of SARA on animal health. Therefore, supplements currently used to maintain rumen pH including sodium bicarbonate, sodium sesquicarbonate, magnesium oxide, and yeast products could help to improve animal health by decreasing microbiome shift and reducing gut inflammation. Other supplements that can alter the rumen microbiome and potentially impact animal health include ionophores and essential oils.

Feeding of prebiotics and probiotics to monogastric animals can shift the intestinal microbiome, alter activities of GALT, and improve animal health. Although determining those effects in ruminants is a bit more complicated due to pregastric fermentation, it is likely that health benefits to feeding some prebiotics and probiotics would benefit dairy cattle health as well. We have shown that postruminal administration of oligofructose or pectin, both researched for their potential role as prebiotics in monogastrics, alter intestinal microbiome as reflected indirectly by shifts in fecal pH and volatile fatty acid composition (Gressley and Armentano, 2005; Mainardi et al., 2011). In mice, oligofructose fed as a prebiotic at 10% of the diet had positive effects on animal health as measured by decreases in gut and systemic inflammation and decreases in gut permeability (Cani et al., 2009). Although prebiotic use in dairy animals is currently limited mainly to milk replacer, there may be potential for postruminal or even ruminal supplementation of prebiotics to improve health of mature dairy cattle.

Yeast products have received a fair amount of study and have potential to be used as health promoting supplements in dairy cattle. Most studies have evaluated the effect of *Saccharomyces cerevisiae*, but there has been some work with other organisms including *S. boulardii* and *Aspergillus oryzae*. Studies indicate that feeding yeast supplements alters the rumen microbiome and can increase both fiber digestion and rumen pH (Chaucheyras-Durand and Durand, 2010; Pinloche et al., 2013). Yeast seem to function primarily as a prebiotic because similar changes in digestion occur whether yeast are alive or dead, although there can be a slight benefit to the live probiotic form (Oeztuerk et al., 2005; Oeztuerk, 2009). Work in monogastrics indicates that in addition to altering the intestinal microbiome yeast supplementation can benefit host health through interaction of yeast carbohydrate moieties (primarily mannan oligosaccharides β-glucans) with the gut mucosa. It is believed that these moieties can bind to pathogen receptors and, in so doing, alter functionality of the mucosa to increase barrier function and pathogen resistance (Munyaka et al., 2012). In piglets, feeding of live *S. boulardii* reduced translocation of orally gavaged *Escherichia coli* across the gut mucosa and into the mesenteric lymph nodes (Lessard et al., 2009). In non-challenged broilers, feeding a commercial yeast derived product resulted in decreased mRNA levels of inflammatory cytokines in the ileum and cecum (Munyaka et al., 2012). In another broiler study, feeding of live *S. boulardii* increased goblet cell density in the jejunum, tight junction mRNA levels in the jejunum and ileum, and secretory IgA concentration in the jejunum (Rajput et al., 2013). Feeding of live yeast or yeast products to dairy cattle may have a similar ability to enhance animal health by increasing mucosal barrier function and altering GALT activity. Inclusion of *S. cerevisiae* into grain was shown to improve health of young calves (Magalhaes et al., 2008). In a study using Holstein steers, we found that abomasal infusion of *S. boulardii* was able to reduce fecal volatile fatty acid and pH changes following an oligofructose challenge (Gressley et al., unpublished). The most convincing evidence that yeast may have a health benefit in older dairy cattle comes from data related to a commercially available product, OmniGen-AF (Prince Agri Products, Inc.), that includes yeast among other proprietary ingredients. Cows fed this product have improvements in neutrophil function as evidenced by changes in expression of genes including L-selectin and increased in vitro response of neutrophils to *E. coli* (Wang et al., 2009; Ryman et al., 2013). Anecdotal evidence also suggests that OmniGen-AF may be beneficial in preventing hemorrhagic bowel syndrome.

In calves, bacterial probiotics such as lactic acid producing bacteria may help to reduce intestinal growth of pathogenic microorganisms, but there has been little work to evaluate potential health benefits of probiotic organisms in older dairy animals (Chaucheyras-Durand and Durand, 2010). As reviewed by Ashraf and Shah (2014), *Lactobacillus* spp. and *Bifidobacterium* spp. fed as probiotics to rodents and humans have a range of experimentally measured immunostimulating effects including enhanced white blood cell function, increased antibody production, and improved responses to pathogen challenges. In addition, commercial products for humans with active *Bifidobacterium animalis*, *Lactobacillus* spp., *Bacillus clausii*, *S. boulardii*, *Clostridium butyricum*, or *E. coli* have clinically proven efficacy in treating a range of gastrointestinal disorders through their abilities to alter the gut microbiome, mucosal function, or GALT activity (Vieira et al., 2013). There have been some studies that have found immunomodulating effects of probiotics when fed to mature ruminants. A study in feedlot steers fed high concentrate diets found that feeding strains of *Propionibacterium* or *Enterococcus faecium* altered circulating concentrations of acute phase proteins (Emmanuel et al., 2007). Another study that fed *Bacillus subtilis natto* to lactating dairy cows suggested that a sufficient quantity of the fed organisms reached the intestines intact to affect intestinal fermentation as evidenced by a change in fecal ammonia and a shift in fecal microbiome (Song et al., 2014). Probiotics fed either intact or in ruminally protected forms to dairy cattle may have similar benefits to intestinal and overall health as observed in monogastrics.

CONCLUSIONS

A variety of feed supplements have the potential to improve health of dairy cattle. Increasing supplementation levels or bioavailability of certain trace minerals and vitamins can improve various measures of immune function and health. Other supplements including probiotics and prebiotics can improve health by stimulating shifts in the ruminal or intestinal microbiome that increase mucosal barrier integrity and alter GALT activity to boost systemic immunity. As reviewed above, a range of nutritional tools exist that can be used to improve health of dairy cows. Transition cows are more likely to benefit from immunostimulatory supplements due to depressed immune function and increased disease susceptibility. However, effects of feed supplements on animal health tend to be subtle and individual changes are unlikely to have dramatic effects on disease measures. Also, individual animal and herd responses to a feed supplement will vary depending upon management factors and environmental stressors. However, as our understanding of efficacy and mechanism of action of various supplements continues to grow so will the potential for the nutritionist to balance rations for health as well as performance.

REFERENCES

Ametaj, B. N., Q. Zebeli, F. Saleem, N. Psychogios, M. J. Lewis, S. M. Dunn, J. G. Xia, and D. S. Wishart. 2010. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics 6:583-594.

Ashraf, R. and N. P. Shah. 2014. Immune system stimulation by probiotic microorganisms. Crit. Rev. Food Sci. Nutr. 54:938-956.

Brestoff, J. R. and D. Artis. 2013. Commensal bacteria at the interface of host metabolism and the immune system. Nature Immunol. 14:676-684.

Burton, J. L., B. A. Mallard, and D. N. Mowat. 1993. Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. J. Anim. Sci. 71:1532-1539.

Caesar, R., C. S. Reigstad, H. K. Backhed, C. Reinhardt, M. Ketonen, G. O. Lunden, P. D. Cani, and F. Backhed. 2012. Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. Gut 61:1701-1707.

Cani, P. D., S. Possemiers, T. Van de Wiele, Y. Guiot, A. Everard, O. Rottier, L. Geurts, D. Naslain, A. Neyrinck, D. M. Lambert, G. G. Muccioli, and N. M. Delzenne. 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58:1091-1103.

Caroprese, M., M. Albenzio, R. Marino, A. Santillo, and A. Sevi. 2013. Dietary glutamine enhances immune responses of dairy cows under high ambient temperature. J. Dairy Sci. 96:3002-3011.

Chaucheyras-Durand, F. and H. Durand. 2010. Probiotics in animal nutrition and health. Benef. Microbes 1:3-9.

Chen, Y. H., G. B. Penner, M. J. Li, M. Oba, and L. L. Guan. 2011. Changes in bacterial diversity associated with epithelial tissue in the beef cow rumen during the transition to a high-grain diet. Appl. Environ. Microb. 77:5770-5781.

Cope, C. M., A. M. Mackenzie, D. Wilde, and L. A. Sinclair. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. J. Dairy Sci. 92:2128-2135.

Emmanuel, D. G. V., A. Jafari, K. A. Beauchemin, J. A. Z. Leedle, and B. N. Ametaj. 2007. Feeding a combination of lactate-utilizing and lactate-producing bacteria modulates acute phase response in feedlot steers. Can. J. Anim. Sci. 87:251-257.

Gomez, A., N. Bernardoni, J. Rieman, A. Dusick, R. Hartshorn, D. H. Read, M. T. Socha, N. B. Cook, and D. Dopfer. 2014. A randomized trial to evaluate the effect of a trace mineral premix on the incidence of active digital dermatitis lesions in cattle. J. Dairy Sci. In press. DOI: http://dx.doi.org/10.3168/jds.2013-7879

Goto, Y. and H. Kiyono. 2012. Epithelial barrier: an interface for the cross-communication between gut flora and immune system. Immunol. Rev. 245:147-163.

Gressley, T. F. and L. E. Armentano. 2005. Effect of abomasal pectin infusion on digestion and nitrogen balance in lactating dairy cows. J. Dairy Sci. 88:4028-4044.

Hogan, J. S., W. P. Weiss, and K. L. Smith. 1993. Role of vitamin-E and selenium in host-defense against mastitis. J. Dairy Sci. 76:2795-2803.

Kamada, N., S. U. Seo, G. Y. Chen, and G. Nunez. 2013. Role of the gut microbiota in immunity and inflammatory disease. Nat. Rev. Immunol. 13:321-335.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009a. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060-1070.

Khafipour, E., S. Li, J. C. Plaizier, and D. O. Krause. 2009b. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. Appl. Environ. Microbiol. 75:7115-7124.

Koboziev, I., C. R. Webb, K. L. Furr, and M. B. Grisham. 2014. Role of the enteric microbiota in intestinal homeostasis and inflammation. Free Radical Bio. Med. 68:122-133.

Lean, I. J. and A. R. Rabiee. 2011. Effect of feeding biotin on milk production and hoof health in lactating dairy cows: A quantitative assessment. J. Dairy Sci. 94:1465-1476.

Lessard, M., M. Dupuis, N. Gagnon, E. Nadeau, J. J. Matte, J. Goulet, and J. M. Fairbrother. 2009. Administration of *Pediococcus acidilactici* or *Saccharomyces cerevisiae boulardii* modulates development of porcine mucosal immunity and reduces intestinal bacterial translocation after *Escherichia coli* challenge. J. Anim. Sci. 87:922-934.

Li, S., E. Khafipour, D. O. Krause, A. Kroeker, J. C. Rodriguez-Lecompte, G. N. Gozho, and J. C. Plaizier. 2012. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. J. Dairy Sci. 95:294-303.

Lippolis, J. D., T. A. Reinhardt, R. A. Sacco, B. J. Nonnecke, and C. D. Nelson. 2011. Treatment of an intramammary bacterial infection with 25-hydroxyvitamin D-3. PLoS One 6(10). DOI 10.1371/journal.pone.0025479

Littman, D. R. and E. G. Pamer. 2011. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 10:311-323.

Magalhaes, V. J., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. Santos. 2008. Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J. Dairy Sci. 91:1497-1509.

Mainardi, S. R., B. A. Hengst, S. J. Nebzydoski, L. M. Nemec, and T. F. Gressley. 2011. Effects of abomasal oligofructose on blood and feces of Holstein steers. J. Anim. Sci. 89:2510-2517.

Mao, S. Y., R. Y. Zhang, D. S. Wang, and W. Y. Zhu. 2012. The diversity of the fecal bacterial community and its relationship with the concentration of volatile fatty acids in the feces during subacute rumen acidosis in dairy cows. BMC Vet. Res. 8.

Mehrzad, J., G. Klein, J. Kamphues, P. Wolf, N. Grabowski, and H. J. Schuberth. 2011. In vitro effects of very low levels of aflatoxin B-1 on free radicals production and bactericidal activity of bovine blood neutrophils. Vet. Immunol. Immunopathol. 141:16-25.

Munyaka, P. M., H. Echeverry, A. Yitbarek, G. Camelo-Jaimes, S. Sharif, W. Guenter, J. D. House, and J. C. Rodrigez-Lecompte. 2012. Local and systemic innate immunity in broiler chickens supplemented with yeast-derived carbohydrates. Poultry Sci. 91:2164-2172.

Nelson, C. D., T. A. Reinhardt, T. C. Thacker, D. C. Beitz, and J. D. Lippolis. 2010. Modulation of the bovine innate immune response by production of 1 alpha,25-dihydroxyvitamin D-3 in bovine monocytes. J. Dairy Sci. 93:1041-1049.

Nemec, L. M., J. D. Richards, C. A. Atwell, D. E. Diaz, G. I. Zanton, and T. F. Gressley. 2012. Immune responses in lactating Holstein cows supplemented with Cu, Mn, and Zn as sulfates or methionine hydroxy analogue chelates. J. Dairy Sci. 95:4568-4577.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. ed. Natl. Acad. Sci., Washington, DC.

Oeztuerk, H. 2009. Effects of live and autoclaved yeast cultures on ruminal fermentation in vitro. J. Anim. Feed Sci. 18:142-150.

Oeztuerk, H., B. Schroeder, M. Beyerbach, and G. Breves. 2005. Influence of living and autoclaved yeasts of S*accharomyces boulardii* on in vitro ruminal microbial metabolism. J. Dairy Sci. 88:2594-2600.

Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. J. Loor. 2013. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J. Dairy Sci. 96:6248-6263.

Pinloche, E., N. McEwan, J. P. Marden, C. Bayourthe, E. Auclair, and C. J. Newbold. 2013. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS One 8(7). DOI 10.1371/journal.pone.0067824

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet. J. 176:21-31.

Rajput, I. R., L. Y. Li, X. Xin, B. B. Wu, Z. L. Juan, Z. W. Cui, D. Y. Yu, and W. F. Li. 2013. Effect of *Saccharomyces boulardii* and *Bacillus subtilis B10* on intestinal ultrastructure modulation and mucosal immunity development mechanism in broiler chickens. Poultry Sci. 92:956-965.

Ryman, V. E., S. C. Nickerson, F. M. Kautz, D. J. Hurley, L. O. Ely, Y. Q. Wang, and N. E. Forsberg. 2013. Effect of dietary supplementation on the antimicrobial activity of blood leukocytes isolated from Holstein heifers. Res Vet Sci 95:969-974.

Saleem, F., B. N. Ametaj, S. Bouatra, R. Mandal, Q. Zebeli, S. M. Dunn, and D. S. Wishart. 2012. A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. J. Dairy Sci. 95:6606-6623.

Scaletti, R. W. and R. J. Harmon. 2012. Effect of dietary copper source on response to coliform mastitis in dairy cows. J. Dairy Sci. 95:654-662.

Scaletti, R. W., D. S. Trammell, B. A. Smith, and R. J. Harmon. 2003. Role of dietary copper in enhancing resistance to *Escherichia coli* mastitis. J. Dairy Sci. 86:1240-1249.

Sharma, R. P. 1993. Immunotoxicity of mycotoxins. J. Dairy Sci. 76:892-897.

Song, D. J., H. Y. Kang, J. Q. Wang, H. Peng, and D. P. Bu. 2014. Effect of feeding *Bacillus subtilis natto* on hindgut fermentation and microbiota of Holstein dairy cows. Asian Austral. J. Anim. Sci. 27:495-502.

Spears, J. W. and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76.

Steele, M. A., J. Croom, M. Kahler, O. AlZahal, S. E. Hook, K. Plaizier, and B. W. McBride. 2011. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. Am. J. Physiol.-Reg. I. 300:R1515-R1523.

Vieira, A. T., M. M. Teixeira, and F. S. Martins. 2013. The role of probiotics and prebiotics in inducing gut immunity. Front. Immunol. 4:445.

Vijay-Kumar, M., J. D. Aitken, F. A. Carvalho, T. C. Cullender, S. Mwangi, S. Srinivasan, S. V. Sitaraman, R. Knight, R. E. Ley, and A. T. Gewirtz. 2010. Metabolic syndrome and altered gut microbiota in mice lacking toll-like receptor 5. Science 328:228-231.

Wang, Y. Q., S. B. Puntenney, J. L. Burton, and N. E. Forsberg. 2009. Use of gene profiling to evaluate the effects of a feed additive on immune function in periparturient dairy cattle. J. Anim. Physiol. An. Nut. 93:66-75.

Weiss, W. P. and J. S. Hogan. 2005. Effect of selenium source on selenium status, neutrophil function, and response to intramammary endotoxin challenge of dairy cows. J. Dairy Sci. 88:4366-4374.

Weiss, W. P. and J. S. Hogan. 2007. Effects of dietary vitamin C on neutrophil function and responses to intramammary infusion of lipopolysaccharide in periparturient dairy cows. J. Dairy Sci. 90:731-739.

Yasui, T., J. A. McArt, C. M. Ryan, R. O. Gilbert, D. V. Nydam, F. Valdez, K. E. Griswold, and T. R. Overton. 2014. Effects of chromium propionate supplementation during the periparturient period and early lactation on metabolism, performance, and cytological endometritis in dairy cows. J. Dairy Sci. In press. DOI: http://dx.doi.org/10.3168/jds.2013-7796