Byproducts for Dairy Cows: Unlocking Their Value and Dealing with Their Limitations

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INTRODUCTION

Byproduct feeds have long been fed to ruminant animals. For example, an ancient Greek writer noted that in an attempt to supplement poor pastures, sheep on the Greek island of Ceos were fed fig leaves, olive leaves, and plant husks (Wilson, 2006). Today feed byproducts still originate from many human activities such as the production of food, fiber, beverages, and more recently bioenergy industries. Many byproducts are produced and available in large quantities and sold as commodities across the country and world; but it is also important to remember that byproducts produced are usually a secondary objective of some process (Crawshaw, 2004). Although they may contain a high concentration of nutrients and improve palatability of dairy rations; their existence, chemical composition, and nutrient availability may be affected by changes in the primary industry and production process. Nonetheless, the dairy industry has historically welcomed the availability of new byproducts and has also learned to adapt to changes in those commonly offered. Obviously, the type of byproducts vary by geographical location, but the objective of this work is to outline some major byproducts used by the dairy industry in the mid-south region of the U.S. and to outline their origin, chemical composition, and nutrient availability.

CORN MILLING BYPRODUCTS

Dry Milling

The dry milling industry produces the following feed products; distillers grains (**DG**), distillers grains and solubles (**DGS**), and distillers solubles (**DS**). Depending on the plant, and whether it is producing wet or dry feed, the proportion of DG and DS that are mixed together may vary. However, our current estimates are that wet distillers grains (**WDG**) + DS are approximately 65 % DG and 35 % DS (DM basis). Distillers grains (and DS) will hereby be referred to as either wet distillers grains (**WDDGS**) or dry distillers grains (**DDGS**) and our assumption is that both contain some solubles. The dry milling process is relatively simple. Specifically corn (or possibly some other starch sources) is ground, fermented, and the starch converted to ethanol and CO_2. Approximately 1/3 of the DM remains as the feed product following starch fermentation. As a result, all the nutrients are concentrated 3-fold because most grains contain approximately 2/3 starch. For example, if corn is 4 % oil, the WDDGS or DDGS will contain approximately 12 % oil; however more recently some of this oil is removed through centrifugation and the crude fat (**CF**) of these feeds may be as low as 6 %.

Feeding Distillers Grains to Dairy Cattle: How Much Can We Feed?

The American dairy industry consumes about 42 to 46 % (National Corn Growers Association, no date; Renewable Fuel Association, 2008) of the total DG produced in the U.S. Several studies have shown the effects of utilizing DG in dairy rations. It has generally been demonstrated to be an effective feed when incorporated into dairy feeding systems as it supports similar or higher milk yield than compared to control diets (Schingoethe et al., 2009). In feedlot diets inclusion of 20 % DDGS (DM) has resulted in greater economic returns (Buckner et al., 2008). It is likely that in dairy rations inclusion of DDGS results in a similar situation as it can replace proportions of highly priced feedstuffs, such as corn and soybean meal and even forages.

Even though DDGS have a valuable nutritional composition, dairy nutritionists tend to limit the inclusion of DDGS to 10 % of the dietary DM (Janicek et al., 2008; Schingoethe et al., 2009). Historically one reason for this is that the fat content was high, generally ranging between 10 and 12 % (Kleinschmit et al., 2006; Schingoethe et al., 2009). This may result in milk fat depression (**MFD**) due to the high content of polyunsaturated fatty acids (**PUFA**) present in DDGS, which has been observed experimentally. For example, Leonardi et al. (2005) reported a linear decrease in milk fat percentage as the inclusion of DDGS increased in the diet. This

reduction was only significantly different between 10 and 15 % DDGS when milk fat dropped from 3.33 to 3.24 %. Similarly, Hippen et al. (2010) reported that DDGS fed at 20 % of the diet resulted in a reduction in the concentration of fat in milk. These changes were slight and not very dramatic as diets with no DDGS averaged 3.21 % and 3.13 lb of milk fat; whereas diets with DDGS averaged 3.03 % and 2.82 lb. The reason for this reduction in milk fat is likely due to the high ruminal load of PUFA that may affect the extent of biohydrogenation and lead to accumulation of *trans* fatty acids that may ultimately cause MFD. The recent reductions in fat content of DDGS make the threat of MFD less likely (Ramirez-Ramirez et al., 2016)

When formulating a ration containing DDGS, nutritionists and producers must be careful to take into account not only the amount of neutral detergent fiber (**NDF**) in the diet but also the source of NDF. Ethanol byproducts have high content of fiber (from the bran fraction of the corn kernel); however it may not be effective fiber, meaning that it does not elicit high rates of ruminal motility, rumination activity, and saliva production. The end result of these factors is that ruminal pH may drop, leading to ruminal acidosis; which has the potential to exacerbate the negative effects of a high load of PUFA in the rumen. It is critical to fully understand the nutritional composition of DDGS, particularly as the fat content; nonetheless, it can also replace corn, which lowers the starch content of the diet and decreases the risk of developing low rumen pH (Ramirez Ramirez et al., 2015).

Nutrient Variation and Distillers Grains and Solubles

Investigations have demonstrated that there may be a high degree of variation in the nutrient content of co-products, such as DG, both within and across production plants (Knott et al., 2004; Spiehs et al., 2002). For example, Knott et al. (2004) demonstrated that the crude protein (**CP**) level in DG may range from 25 – 35 %, with variation also observed in fat (10-12 %), NDF (8-10 %) and phosphorus (0.8 – 1 %). These investigators note that one of the greatest sources of nutrient variation for DDG depends on the amount of solubles that were added to the grains. Along with the concentration of CP, the availability of these nutrients may also vary. Hence researchers are beginning to direct their attention towards creating practical methods for controlling this variation. Research from The Ohio State University (St-Pierre and Weiss, 2015) suggests that routine feed sampling is essential. Because it may be difficult and time consuming to sample and formulate rations based on lab results of individual loads, numerous load samples should be collected and analyzed over time. This will allow for estimation of the mean values and also the variation of these estimates. Consequently, it becomes possible to protect against underfeeding a nutrient, such as protein, by feeding an anticipated mean value of the feed.

Wet Milling

Compared to the dry milling process, the wet milling process is the more complex of the 2 because the corn kernel is partitioned into several components to facilitate high value marketing. For example, the oil is extracted and sold and the corn gluten meal, that contains a large amount of bypass protein, is commonly marketed to the dairy, poultry, or pet industries. Wet milling is a process that requires use of high quality (No. 2 or better) corn that results in numerous products that are produced for primarily human use. During this process, corn is *steeped* and the kernel components are separated into corn bran, starch, corn gluten meal (protein), germ, and soluble components. Wet corn gluten feed (**WCGF**) usually consists of corn bran and steep, with germ meal added if the plant possesses the capabilities. Wet CGF can vary depending on the plant capabilities. Steep liquor contains more energy than corn bran or germ meal as well as protein (Scott et al., 1997). Therefore, plants that apply more steep to corn bran or germ meal will produce wet CGF that is higher in CP and energy. Wet CGF contains 16 to 25 % CP, with a rumen undegradable protein (**RUP**) value of approximately 24 - 30 % CP (NRC, 2001). During wet milling, corn gluten meal is removed and marketed in higher value markets. Corn gluten meal *should not* be confused with CGF, as corn gluten meal contains approximately 60 - 65 % CP and a RUP value of approximately 64 - 75 % CP (NRC, 2001). Distinct differences exist for WCGF, even within companies, due to plant-to-plant variation.

A number of studies demonstrate the general concept that traditional forages may be partially replaced and byproducts may be included to maintain milk production. For example, VanBaale et al. (2001) observed that when fed diets containing 20 % WCGF, cows consumed more DM and produced more milk than those consuming diets higher in alfalfa hay, corn silage, and corn grain. Boddugari et al. (2001) demonstrated that a wet corn milling product, similar to WCGF, may be effective in diets for lactating dairy cows. When used to replace concentrate, the product could be included at 45 % of the ration DM and at over 60 % when used to replace

corn and forage. In a feeding trial these investigators also observed that, on average, cows consumed less feed but produced over 10 lb more milk when the WCGF replaced 50 % of the concentrate and 30 % of the forage of the control diet. These results suggest that the optimal inclusion level depends upon the feedstuffs being substituted for, as well as other ingredients contained in the ration.

Clearly the dairy cow is adaptable and can use non-traditional feedstuffs as sources of nutrients to make milk; however there clearly are limitations to her abilities. In a study designed to test the inclusion of corn gluten feed, Rezac et al. (2012) formulated diets in which both corn silage and alfalfa were completely removed from the ration and substituted with CGF and tallgrass prairie hay. On average, the complete removal of corn silage and alfalfa resulted in a reduction in the concentration of NE_L from 0.74 Mcal/lb to 0.72 Mcal/lb and resulted in a reduction of almost 5 lb of energy corrected milk (**ECM**). Certainly these results are not ideal; but the rations used in the study were dramatically different. For example, the concentration of starch was reduced from 21 to 13 % and forage NDF was reduced from 15 to 11 %. These treatments were designed to test strategies that could be used when the availabilities of traditional forages are poor and feeding conditions are not ideal. A more recent study evaluated the inclusion of WCGF at 20 or 30 % of the diet DM (Shepherd et al., 2014), both concentrations of inclusion maintained milk production and composition, but the authors suggested that the increase to 30 % requires careful consideration of effective fiber. Care should be taken to ensure that animals are consuming enough forage NDF to maintain healthy rumen conditions.

Effective Fiber Corn Milling Co-Products

Effective fiber is the portion of the diet that is believed to stimulate rumination, chewing activity, and saliva secretion; all of which is designed to help to maintain healthy rumen function and pH levels. Nutritionists are often concerned about rumen pH because, when pH levels fall below 6.0 fiber digestion may be impeded and milk fat levels may become depressed (Russell and Wilson, 1996). It is believed that rumen pH is a function of lactic acid and VFA production and is buffered by saliva (Maekawa et al., 2002). Because of this finding, it is a common practice to feed diets of longer particle size; therefore a greater amount of effective fiber, so that salvia production is stimulated. In support of this hypothesis, Krause et al. (2002) noted that the intake of particles > 19.0-mm was negatively correlated with the amount of time rumen pH was below 5.8. However, it is also known that diets should not be excessively long or coarse as they are more difficult to mix and may induce cattle to sort out ration ingredients (Kononoff et al., 2003). When co-products are used to substitute forage in the TMR, chewing activity is believed to be reduced due to the finer particle size. Nutritionists should not necessarily use this logic to infer that feeding co-products will result in lower rumen pH. In fact it is likely that diets may be balanced so that the inclusion of co-products will not influence rumen pH. When evaluating a dairy diet to determine a possible risk of subclinical acidosis, it is important to also consider levels of fiber and non-structural carbohydrates, along with their associated fermentability (Yang et al., 2001). Currently it is difficult to find robust feeding recommendations for effective fiber. Recently studies in which byproduct NDF replaced forage, concentrate, or both; have been conducted (Bradford and Mullins, 2012) and in some cases provide good examples for formulation but research on a field-ready, robust system to estimated effective fiber is still needed. Without this system it is wise to follow particle size recommendations previously established, which suggest that 3-8 % of the TMR should be retained on the top (19 mm) screen of the Penn State Particle Separator and 30 - 40 % should be retained on the second (8 mm) sieve (Heinrichs and Kononoff, 2002).

CANOLA MEAL

Canola is a trademarked name for rapeseed which contains < 2 % erucic acid in the oil and < 30 µmoles of alkenyl glucosinolates/g of oil-free DM. As a result canola meal contains less erucic acid and glucosinolates than conventional rapeseed meal (Bell, 1993). This is important because glucosinolates are bitter and negatively affect palatability and may even impair the uptake of iodine and interfere with the synthesis of thyroid hormones (Woyengo et al., 2016). In a summary of publication studies Huhtanen et al. (2011) reported that when fed to dairy cattle canola meal was at least as good as soybean meal and that some improved responses are due to increases in feed intake. It should however not be forgotten that feeding high concentration of canola meal may affect iodine status of the animals. Although feeding additional iodine to cattle has been shown to improve iodine status (Weiss et al., 2015), this practice is not common and additional research and recommendations must be made to fully understand the potential effects on humans consuming this milk.

OTHER NONFORAGE FIBER SOURCES

In a study designed and conducted at the William H. Miner Research Institute (Chazy, NY) to test the impact of feeding rations lower in both starch and forage, 4 treatments were formulated to contain decreasing proportions of forage (52, 47, 43 and 39 % of diet DM) by increasing the proportion of non-forage fiber sources (**NFFS**), namely wheat middlings (Farmer et al., 2014). Additionally, in an attempt to maintain energy and effective fiber in the rations, these investigators increased the proportion of rumen protected fat and wheat straw as the proportion of forage was reduced. In this study, DM intake increased with reducing forage but no differences were observed in milk production or composition. Interestingly, these ration strategies successfully maintained milk production over 94 lb/d and 3.6 % fat and 3.0 % protein. It should be noted while reducing forage in the ration, that this strategy involved careful attempts to maintain effective fiber. The reduction of forage did reduce the proportion of particles greater than 8.0 mm; however, no reductions were observed in rumination times, which suggests that effective fiber was still adequate.

In a similar study, Hall and Chase (2014) tested the impact of feeding varying proportions of chopped wheat straw and sugar beet pellets, which replaced a portion of both corn and alfalfa silage. Specifically forage was reduced from 61 % of the diet DM in the control to 40 % in the treatments containing variable mixes of straw and beet pulp pellets. The study included 48 cows in late lactation (average days in milk = 280 ± 79) and although the inclusion of the straw and beet pellets resulted in an increase in feed intake, the investigators successfully maintained fat and protein corrected milk yield. The partial replacement of forages with NFFS in close-up diets has also been evaluated at the William H. Miner Research Institute (Dann et al., 2007). In that study, oat hay was reduced from 30 to 15 % and beet pulp was increased to 15 % and fed to 64 cows from d -21 relative to expected calving date. Despite pronounced differences in ration particle size no differences were observed in periparturient intake or metabolism of production.

In vitro Laboratory Measures to Understand the Fermentability of Fiber

Today a number of assays are commercially available that attempt to measure the nutritional value of rumen feeds. For example investigators at Cornell University have developed an assay which attempts to estimate the RUP and intestinal digestibility of RUP (**dRUP**) in feed samples (Ross et al., 2013). Additionally, investigators at University of Wisconsin have developed an *in vitro* NDF fermentation assay to estimate total-tract digestibility (**TTNDFD**; Lopes et al., 2015a,b). Assays such as this hold great promise as the cost of routine testing feeds *in vivo* is prohibitive. These methods may be useful in screening feeds for differences between sources or manufacturing facilities. For example we have recently used the TTNDFD assay to test for differences in fiber digestion between DDGS originating from different corn-ethanol facilities (Dufour et al., 2017). In this study TTNDFD was observed to be 65.5 ± 1.59 % and differences between production sites were observed with differences > 10 %. It is difficult to identify driving factors responsible for observed differences in TTNDFD, but results support the notion that in addition to differences in chemical composition (Spiehs et al., 2002) differences in nutrient availability also exist between production facilities. The TTNDFD method represents an important and powerful tool to estimate *in vivo* fiber digestibility; but it should also be noted that the method does not account for selective retention of feed particles in the rumen (Huhtanen et al., 2007; Lopes et al., 2015b) which is affected both by particle fragility and particle size (Grant, 2010) and as a result it may be difficult to compare estimates of TTNDFD across feedstuffs.

CONCLUSIONS

The dairy cow is adaptable and can use byproduct feedstuffs as sources of nutrients to make a high quality food, namely milk. Although there are limitations in her ability to do so, extensive research has been conducted on the topic. This research on inclusion levels, chemical composition, and nutrient availability helps us understand how these byproducts can be included in a formulation. The dairy industry will continue to make extensive use of feed byproducts and the availability, type, and composition will likely change over time. To overcome these changes the practice of regular and consistent characterization of feed is important.

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Immune Dysfunction in Periparturient Dairy Cows: Insight into Pharmacologic and Dietary Immune Treatments

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INTRODUCTION

With a $40.5 billion gross domestic value for milk produced in the U.S. during 2013, the dairy industry was the third largest sector of the 2013 U.S. animal agriculture economic engine. The value of milk produced in 2013 represented 24 % of the total value of animal agriculture production; this figure had grown from $21-23 billion/y over a decade ago. The 2007 NAHMS Dairy Study reported that during 2006, 23.6 % of cows were culled from operations, 26.3 % and 23 % were removed for reproductive and udder health problems (USDA, 2007). In addition, 16.5 % of cow mortalities were due to mastitis. Clearly, the economic value of controlling mastitis pathogens is immense. Most economic analyses of the cost of mastitis cite a 10 % production loss as only one part of the overall cost of the disease. A majority (65 to 70 %) of losses are associated with decreased milk yield resulting in lower production efficiency; the remaining costs are attributed to treatment. In addition to these direct losses, mastitis causes significant problems in milk quality control; dairy manufacturing practices; quality and yield of cheese; nutritional quality of milk; antibiotic residue problems in milk, meat and the environment; and genetic losses due to premature culling. These additional costs are very significant and are not always included in economic analyses of mastitis costs.

Because of the need for a safe, economical, and stable supply of food, those of us serving the livestock health industry must be prepared to provide the best quality advice and care in managing our nation's dairy herd. For dairy producers, the critical factor in providing a low somatic cell count milk supply is keeping cows free from mastitis. Mastitis is anything causing inflammation of the mammary gland, and infectious mastitis is caused by a plethora of microbial agents (Watts, 1988). Nearly half of the nation's herd of dairy cows will experience at least 1 episode of mastitis during each lactation. Research has already resulted in genetic selection for cows with lower somatic cell counts by the incorporation of this trait into the A.I. sire summary ranking indices. This approach mainly serves to reduce the normal increase in mastitis incidence that occurs as milk production goes up. Coliforms and environmental streptococci are the most common etiologic agents isolated from clinically severe mastitis cases on well-managed dairy farms (Anderson et al., 1982; Hogan et al., 1989). Clinical trials and experimental studies have demonstrated repeatedly *no benefits* of antibiotic therapy in cattle with clinical or subclinical coliform mastitis (Erskine et al., 1991; Jones and Ward, 1990; Kirk and Barlett, 1984). Hence, the advent of the *Escherichia coli* J-5 and other endotoxin core mutant vaccines in veterinary medicine many years ago provided us a tool to reduce the incidence and severity of clinical coliform mastitis (Gonzalez et el., 1989; Hogan et al., 1992a,b, 1995). However, there remains an unmet veterinary medical need of new ways to prevent or treat mastitis caused by environmental pathogens. For several years, research at the USDA's National Animal Disease Center in Ames, IA undertook a 2-fold approach for improving the dairy cow's resistance to mastitis - immunomodulation and genetic selection for superior immune systems. In this paper, we will focus on:

- The evidence for immune suppression in periparturient dairy cows,
- How this sets the cow up for infectious diseases such as mastitis, metritis and retained placental membranes, and
- Some of the early research on immune modulation of the transition dairy cow and how that impacted resistance to mastitis.

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ROLE OF THE IMMUNE SYSTEM IN MASTITIS

Immunity against infectious diseases of cattle is mediated by diverse, yet interdependent, cellular and humoral mechanisms. Many environmental and genetic factors influence the ability of livestock to mount effective defense strategies against the various pathogens and normal flora that they are exposed to throughout their lifetime. Innate resistance to infectious diseases reflects the inherent physiological attributes of an animal that make it more or less susceptible to disease development by a particular pathogen. There are several cell lineages that comprise the immune system (e.g., B-cells, T-cells, neutrophils, eosinophils, basophils, macrophages, and mast cells). Each of these cell types has distinct responsibilities in providing host defense. Innate immunity represents the various immune components that are not intrinsically affected by prior contact with an infectious agent (Roitt, 1994). Lymphocytes provide the adaptive immune reactions that are antigen specific in nature and possess memory for future encounters with the same pathogen. In this paper we will present a novel approach of immune modulation of the innate immune system as a potential means to reduce antibiotic usage in veterinary medicine.

Our first understanding of cellular immunity is more than a century old and it actually involves research into the causes of bovine mastitis and the immune response. In his 1908 Nobel Lecture the Russian zoologist, Elie Metchnikoff, described disease as consisting "of a battle between a morbid agent, the external microorganism, and the mobile cells of the organism itself. A cure would represent the victory of the cells, and immunity would be the sign of an activity on their part sufficiently great to prevent an invasion of microorganisms (Metchnikoff, 1908)." Metchnikoff cited the work of a Swiss veterinary expert, Zschokke, who found that "plentiful phagocytosis of streptococci in the battle against infectious mastitis in cows, was a good sign. When phagocytosis was insignificant or not present, the cows were written off as no longer capable of producing good milk." This was later extended to include the idea that not only must the phagocytes engulf the microorganisms, but that these devouring cells must utterly destroy the microorganisms. In some cases, the streptococci of mastitis were found to "destroy the phagocytes after being engulfed by them thus liberating themselves to carry on their deadly work."

Today we have a far more detailed knowledge of the cow's immune response to pathogens in the mammary gland (and elsewhere). Neutrophils are one of the most important cell types of native defense mechanisms because they respond quickly (within minutes) and do not require previous exposure to a pathogen to effectively eradicate the microbe. A major function of neutrophils is the phagocytosis and destruction of microorganisms that invade the body. Phagocytosis is probably the most widely distributed defense reaction, occurring in virtually all phyla of the animal world.

NEUTROPHILS ARE CRITICAL AGAINST MASTITIS

Native defenses of cattle are continually challenged by exposure to pathogens (bacteria, fungi, and viruses) and many factors affect the outcome of this interaction. Establishment of an infection in any organ or tissue is dependent upon a delicate balance between defense mechanisms of the body and the abilities of pathogens to resist unfavorable survival conditions. The neutrophil is one of the most important cells of the innate defense mechanisms because it can act quickly (within minutes) in large numbers, and in most cases, does not require previous exposure to a pathogen to effectively eradicate the microbe. Studies have shown that it takes approximately 1-2 h for neutrophils to accumulate in response to *E. coli* infection in tissues (Persson et al., 1988, 1992, 1993; Persson and Sandgren, 1992). What this means is that microorganisms will have a 2-h head start on the host immune response and any further delay in the inflammatory response will result in significantly more pathogens for the host to deal with. Unfortunately, delays in inflammatory responses in stressed animals are well documented (Shuster et al., 1996; Hill et al., 1979; Hill, 1981), and some of the mechanisms responsible for delayed inflammation have been identified (Lee and Kehrli, 1998; Burton and Kehrli, 1995; Burton et al., 1995). The importance of the neutrophil in protecting virtually all body tissues (especially against bacteria) has been repeatedly demonstrated experimentally and in nature (Schalm et al., 1964a,b; Jain et al., 1968, 1978; Ackermann et al., 1993, 1996; Gilbert et al., 1993a). Early and rapid accumulation of sufficient numbers of neutrophils is paramount in the ability of the host to effect a cure of invading pathogens (Anderson, 1983). Neutrophils can also release cytokines that in turn result in additional recruitment signals for more neutrophils (Canning and Neill, 1989; Cicco et al.,

1990; Goh et al., 1989; Ohkawara et al., 1989). Circulating *neutrophils represent the major recruitable host defense against acute tissue infection*, such as mastitis (Hill, 1979, 1981; Jain, 1968; Schalm et al., 1976).

IMMUNOSUPPRESSION IN THE PATHOGENESIS OF MASTITIS

A literal definition of immunosuppression is diminished immune responsiveness. This simplistic definition impacts a highly diverse system that affords protection against disease. Periparturient immunosuppression research was initiated by the observation that most clinical mastitis occurs in dairy cows in early lactation and the view that most bovine mastitis is caused by opportunistic pathogens and; therefore, these cows must be immunosuppressed. What evidence supported the hypothesis of periparturient immunosuppression? Practical experience teaches us that opportunistic infections are associated with severe compromises of host defense mechanisms. Over the past couple decades, an overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils in periparturient cattle (Figure 1) and sows has been generated in research institutes around the world (Shuster et al., 1996; Lee and Kehrli, 1998; Burvenich et al., 1994, 2007; Cai et al., 1994; Detilleux et al., 1994, 1995a,b; Dosogne et al., 1998, 1999; Guidry et al., 1976; Harp et al., 1991; Heyneman and Burvenich, 1989; Hoeben et al., 1997, 2000a,b; Ishikawa and Shimizu, 1983; Ishikawa, 1987; Ishikawa et al., 1994; Kehrli and Goff, 1989; Kehrli et al., 1989a,b; Kelm et al., 1997; Kimura et al., 1999a,b, 2002a,b; Lippolis et al., 2006; Löfstedt et al., 1983; Mehrzad et al., 2001, 2002; Monfardini et al., 2002; Nagahata et al., 1988, 1992; Nonnecke et al., 2003; Pelan-Mattocks et al., 2000; Shafer-Weaver and Sordillo, 1997; Sordillo et al., 1991, 1992, 1995; Stabel et al., 1991; Van Werven et al., 1997; Vandeputte-Van Messom et al., 1993). Periparturient immune dysregulation impacts the occurrence of infectious diseases of virtually any organ system of livestock (e.g., gastrointestinal, respiratory, and reproductive tracts all have increased disease incidence in postpartum animals).

First of all, there is an extremely high incidence of clinical disease in postpartum cows with nearly 25 % of all clinical mastitis occurring during the first 2 wk after calving. Clinical mastitis caused by virtually all pathogens (but especially coliform bacteria and streptococci other than *Streptococcus agalactiae*) has a very high incidence in early lactation. Cows must first become infected and then develop clinical mastitis. The rates of new intramammary infections (**IMI**) caused by environmental pathogens are highest during the first and last 2 wk of a 60-d, nonlactating period of dairy cows (Hogan et al., 1989; Smith et al., 1985a,b; Oliver and Mitchell, 1983). The rate of new IMI during these periods of peak susceptibility is 2 to 12 X higher than any other time in the production cycle of the cow. Most coliform and environmental streptococcal infections, established in the nonlactating period and that are present at parturition, result in clinical mastitis soon afterward (Smith et al., 1985a; McDonald and Anderson, 1981). The proportion of all cases of clinical coliform mastitis that develop during the first 2, 4, and 8 wk of lactation has been reported to be 25, 45 and 60 %, respectively (Malinowski et al., 1983; Jackson and Bramley, 1983).

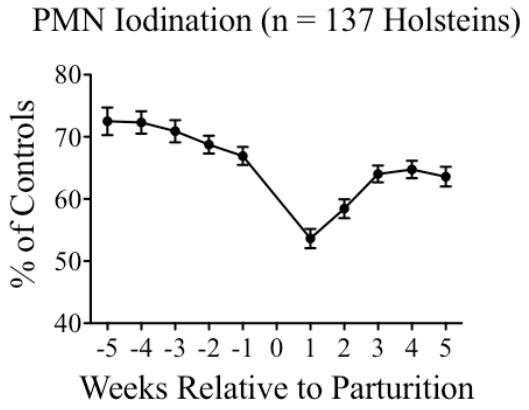

Figure 1. Neutrophil (PMN) iodination measures the myeloperoxidase-catalyzed halogenation of proteins, a phenomenon that takes place in phagolysosomes of neutrophils that have phagocytosed bacteria. *In vivo*, this halogenation disrupts the function of critical bacterial membrane proteins and results in the oxidative killing of the bacteria by the neutrophil. This bactericidal activity depends on a series of events to occur in the process of phagocytosis: successful opsonization and uptake of the bacteria by the β2-integrins into a phagosome, the generation of superoxide anion and its dismutation into hydrogen peroxide (H_2O_2), the fusion of the phagosome with a primary granule to produce a phagolysosome in which myeloperoxidase utilizes the H_2O_2 and cellular halides to halogenate the bacterial surface proteins. (Data from Detilluex, et al., 1995b.)

The second piece of evidence supporting the notion of immunosuppression in the pathogenesis of mastitis was that we are traditionally taught that opportunistic infections are associated with severe

compromises of host defense mechanisms. Most mastitis pathogens are considered opportunistic pathogens. These 2 points led to experiments evaluating how functional a cow's immune system is around calving time. Today the data tells us the immune system becomes progressively more compromised at the end of gestation, cows become more readily infected in the mammary gland, then as the immune system *bottoms out* the first week or two after calving, these subclinical infections begin to win the battle with the cow's immune system and clinical mastitis results.

WHAT CAUSES PERIPARTURIENT IMMUNOSUPPRESSION?

Many neuroendocrine changes develop in cows during the periparturient period. Periparturient hormone fluxes may adversely affect immune cell function. Surprisingly, there is no effect of estrogen on bovine neutrophil function either during the follicular phase of the estrous cycle in cows or after administration of high doses of estradiol to steers (Roth et al., 1982, 1983). However, supraphysiologic concentrations of estradiol have been reported to suppress neutrophil function (Bodel et al., 1972; Klebanoff, 1979). These high concentrations of estrogens may be germane to immunosuppression and the high new IMI rates prior to calving. Before calving, total plasma estrogen concentrations increase in the cow (at least 10 X greater than during estrus) (Comline et al., 1974). Moreover, during normal pregnancy, the progesterone binding capacity of human lymphocytes is increased (perhaps as a result of increasing estrogen levels) and the concentration of progesterone in serum during pregnancy combine as sufficient to reduce lymphocyte functions (Szekeres-Bartho et al., 1983, 1985). This raises the possibility that hormone sensitivities of immune cells during late gestation may be altered and result in functional changes in immune cells due to rising estrogen levels. Very high concentrations of both estrogens and progesterone are reached during the final days of gestation in cows (Comline et al., 1974). This may be germane to the onset of impaired lymphocyte function in the prepartum cow whose lymphocyte hormone binding capacity may be higher than that in barren cows.

Many of the hormonal and metabolic changes that prepare the mammary gland for lactation take place during the 3 wk preceding parturition. Lymphocyte and neutrophil function could be affected by prepartal increases in estrogen, prolactin, growth hormone, and/or insulin (Comline et al., 1974; Houdebine et al., 1985; Convey, 1974; Akers, 1985). During this critical period, the dairy cow's metabolism shifts from the demands of pregnancy to include those of lactation, with increased demands for energy and protein. Negative energy and protein balances that exist during early lactation may also contribute to impaired neutrophil function and, thus, account for a portion of the periparturient immunosuppression observed. The nutritional demands of lactation contribute to the duration of immune suppression (Kimura et al., 1999b; Nonnecke et al., 2003; Stabel et al., 2003) and postpartum neutrophil glycogen stores have been associated with postpartum uterine diseases (Galvão et al., 2010).

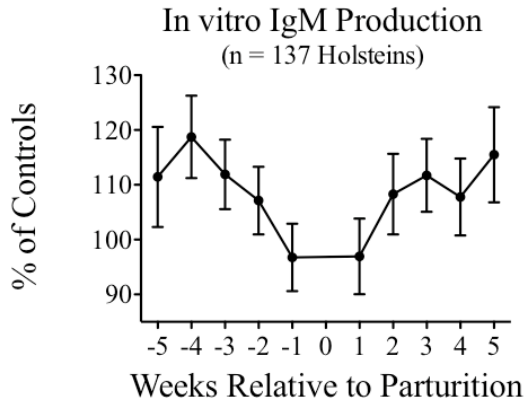

Figure 2. *In vitro* production of IgM by lymphocytes is reduced in the immediate week around calving time. (Data from Detilleux et al., 1995b.)

The specific physiological factors contributing to periparturient immunosuppression and increased incidence of clinical disease have not been fully elucidated. We do know, however, that there is a very broad-based suppression of immune function in cows the 1st wk or 2 after calving. Wide variation in leukocyte functional activities has been documented between dairy cows and between different production stages (e.g., around calving time) (Ishikawa, 1987, 1994; Nagahata et al., 1988, 1992; Guidry et al., 1976; Newbould, 1976; Manak, 1982; Gunnink, 1984a,b,c; Saad et al., 1989; Gilbert et al., 1993b). Most importantly, associations between neutrophil dysfunction and periparturient disorders in cows have been reported (Kelm et al., 1997; Kimura et al., 2002a; Cai et al., 1994). Periparturient immunosuppression is not limited to cattle. Investigations of immunosuppression and coliform mastitis in sows revealed depressed neutrophil function to be associated with the susceptibility to postpartum mastitis caused by *Escherichia coli*

(Löfstedt et al., 1983). Defects in lymphocyte function also contribute to immune suppression during the periparturient period (Figures 2 and 3). In addition to reduced antibody production, other impacted roles of lymphocytes in periparturient cows include reduced production of cytokines that activate and direct both innate and adaptive immunity (Detilleux et al., 1995; Ishikawa, 1987; Ishikawa et al., 1994; Manak, 1982; Wells et al., 1977; Kashiwazaki, 1984; Kashiwazaki et al., 1985).

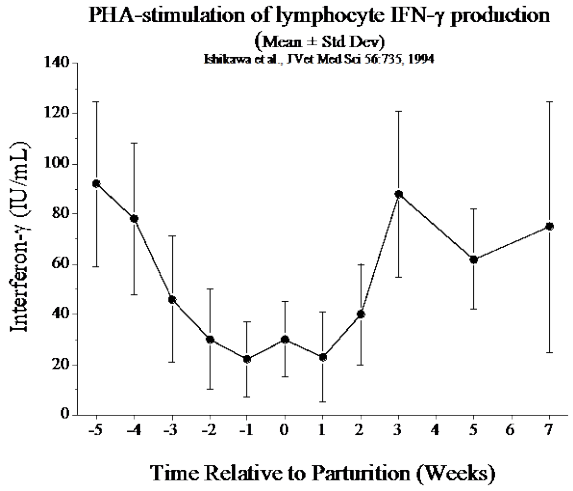

Figure 3. *In vitro* production of interferon- γ (INF- γ) by lymphocytes is reduced in the week around calving time. (Data from Ishikawa et al., 1994.)

Today it is well recognized that the bovine immune system is less capable of battling pathogens during the periparturient period. The periparturient cow has suppressed immune competence, manifest as reduced capacity for nearly all types of immune cells that have been studied. Interestingly, there may be a teleological reason for immunosuppression in the Th1 branch of the immune system that may be essential in preventing unwanted immune reactions against self and fetal antigens exposed to the mother's immune system as a result of normal tissue damage in the reproductive tract during parturition (Kehrli and Harp, 2001). However, an inadvertent and perhaps unintended consequence of this suppression of the Th1 branch of the immune system is that many of the cytokines normally produced by these cells are critical to fully activate neutrophils that are absolutely critical to the defense of the mammary gland. Without a fully functional cellular immune system, both adaptive and innate branches of the cellular immune system operate at diminished capacity for immune surveillance and pathogen clearance. This is the very circumstance that periparturient cows find themselves in and why it is so critical to manage transition cows to minimize their exposure to pathogens in the environment and to avoid metabolic disorders that might further stress their immune system.

The take-home message here is a multitude of factors of the immune system of a dairy cow become impaired as early as 2 - 3 wk before she actually gives birth (long before the elevation of endogenous cortisol which occurs from 36 h before to 36 h after calving). The cow's immune system then bottoms out and is seriously impaired for 1 - 2 wk after calving. This effect is known as periparturient immunosuppression. Regardless of its causation, periparturient immunosuppression makes the dairy cow highly susceptible to the establishment of new infections (particularly in the mammary gland) and the subsequent progression of these new subclinical infections into clinical disease (mastitis, metritis, and postpartum outbreaks of intestinal diseases such as salmonellosis, just to name a few).

WHAT ARE THE PROSPECTS FOR IMMUNOMODULATION TO PREVENT DISEASE?

Pharmacologic treatments that serve as immune modulators in cattle and other species have been under investigation for many years. Biotherapeutic immune modulators can be given to prevent or lessen disease symptoms caused by various pathogens (viral and bacterial). A general goal of such a biotherapeutic compound is to provide the desired effect on host immunity for a sufficient period of time to sustain immunity through a period of immune dysfunction the host is experiencing. In the past couple years 2 products have received approval by regulatory agencies that fall under this category but that work through very different innate immunity mechanisms.

According to the manufacturer, Zelnate™ (Bayer Healthcare LLC, Animal Health, Shawnee Mission, KS) was approved in 2015 as a USDA-Center for Veterinary Biologics approved immune modulator based on technology developed by Juvaris BioTherapeutics (Pleasanton, CA). As such, it represented a new class of drug for bovine respiratory disease (**BRD**) as an immune modulator; it is not an antibiotic nor a vaccine. Zelnate DNA Immunostimulant is a bacterial-produced plasmid DNA with a liposome carrier that stimulates the

innate immune system in cattle. Per the label claim, Zelnate is indicated for use as an aid in the treatment of BRD due to *Mannheimia haemolytica* in cattle 4 mo of age or older, when administered at the time of, or within 24 h after, a perceived stressful event. Although no peer-reviewed publications are available at this time, a summary of the technical studies conducted for regulatory approval is available: http://www.zelnate.com/static/documents/Zelnate-ChallengeStudy_Detailer.pdf.

In 2016, Imrestor™ (pegbovigrastim) (Elanco Animal Health, Indianapolis, IN) was approved by the Food and Drug Administration as the first and only immune restorative for periparturient dairy cows and heifers. Per the label claim usage, Imrestor reduces the incidence of clinical mastitis by 28 % in the first 30 d of lactation in dairy cows and heifers. Recent peer-reviewed studies describe the mechanism of action of pegbovigrastim and report an even greater reduction in clinical mastitis incidence in 4 studies conducted in the United States (Kimura et al., 2014; Hassfurther et al., 2015; Canning et al., 2017; McDougall et al., 2017).

Pegbovigrastim is a cytokine that is naturally part of a cow's immune system that works to turn on the innate immune response provided by neutrophils. Cytokines are a class of compounds that have been investigated for many years for potential biotherapeutic value. Administration of recombinant cytokines to modulate immunity in immunocompromised hosts is thought to prevent bacterial infections (Broxmeyer and Vadhan-Raj, 1989). In an effort to study methods to ameliorate the effects of periparturient immunosuppression, several scientists have evaluated various cytokines that are part of the cow's normal immune system (Sordillo et al., 1991b,1992; Zecconi et al., 1999, 2009; Sordillo and Babiuk, 1991; Campos et al., 1992; Sordillo and Peel, 1992). Granulocyte-colony stimulatory factor (**G-CSF**) is a cytokine that triggers the bone marrow to produce leukocytes – neutrophils in particular, which in turn, fight infectious disease. Human G-CSF has been successfully used for many years as an adjunct therapy for cancer patients undergoing chemotherapy. In a series of studies, G-CSF has been evaluated for its effects on bovine immunity and as a prophylactic against mastitis (Stabel et al., 1991; Kehrli et al., 1991a; Cullor et al., 1990a,b, 1992; Nickerson et al., 1989). Our research findings indicate no adverse effects and that it can reduce the incidence and severity of clinical coliform mastitis by 50 % during the 1st wk of lactation following experimental challenge (Kehrli, 1998). G-CSF has also been shown to be beneficial against *Staphylococcus aureus* and *Klebsiella pneumoniae* mastitis (Nickerson et al., 1989; Kehrli et al., 1991b). It is crucial to understand that immunomodulators work best in immunocompromised hosts; hence the periparturient period is an excellent time for such compounds to be given to cows as they will work to restore the immune system. Acceptable alternatives to the use of antibiotics in food animal practice need to be explored and the use of immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic approaches to prevent and combat infectious disease during periods of peak disease incidence. Research in the area of biotherapeutic immune modulation continues today (Kimura et al., 2014).

Dietary immune treatments are also an area of intense investigation. While not a major focus of this paper, considerable research has been done and managing optimal nutrition levels, with ingredients such as vitamin E and selenium, is well recognized to avoid immune impairment associated with nutrient deficiencies (Weiss et al., 1990, 1992, 1997; Hogan et al., 1990, 1992c, 1993, 1994; Smith et al., 1997). However, there is little evidence to support hyper-supplementation of nutrients such as these, as a means to enhance immune function.

Immunomodulatory feed ingredients have also received considerable research interest investigating possible beneficial effects on immunity and health in dairy cows. One such product, Omnigen-AF (Phibro Animal Health Corp., Teaneck, NJ), is perhaps the best studied product reported to enhance innate immunity parameters and increase milk production in dairy cows (Brandao et al., 2016; Leiva et al., 2017; Fabris et al., 2017; Wang et al., 2009; Ryman et al., 2013; Nace et al., 2014).

WHAT DOES THIS ALL MEAN FOR YOU?

Bovine mastitis is one of the most economically important diseases to the beef and dairy cattle industries. The pathogenesis is highly complex and involves many factors including various microbial etiologies, stress, management and environmental hygiene. Bovine mastitis has not been adequately controlled by vaccination or antibiotics. In many diseases, immunosuppression due to various stressors is responsible for increased susceptibility to bacterial colonization or growth. Over the past 50 y a considerable body of evidence of impaired neutrophil

and lymphocyte function in periparturient dairy cows has emerged that coincides with the high incidence of new intramammary infections 2 wk prepartum and clinical mastitis in early lactation. To overcome this immunosuppression, immunomodulatory agents have been and are being evaluated for their ability to prevent economic losses associated with periparturient diseases such as mastitis. Researchers have investigated immunomodulation as an approach to provide dairy farmers with a new tool to prevent infectious disease in their herds, although biotherapeutic products have not yet made it to the market place. The consequences of immune suppression are increases in infectious disease and premature loss from the herd, both of which add significantly to the cost of production and decrease the profitability of dairy farming. Simple solutions will not likely be found for something as complex as immune suppression; however, without additional significant research into this topic we can be assured that no progress will be made.

Production of milk from mastitis-free cows is quite simple, right? Keep your cows in clean, dry, and unstressful environments and feed them what they need, when they need it – far easier said than done! For years we have emphasized feeding cows optimal rations because the production and functional activities of leukocytes in combating microbial infection are complex and all involve expenditure of cellular energy, protein and other nutrients. The average cow has ~3500 neutrophils/µL of blood, this translates into ~1.4 x 10^{11} neutrophils in an 1800 lb Holstein cow. The circulating half-life of neutrophils is about 6 h, so the cow is replacing half of those cells every 6 h from bone marrow stores. Clearly, a significant component of the dietary energy and protein consumption for maintenance is spent on replenishment of immune cells. The negative energy and protein balance of dairy cows during the periparturient period and up to peak lactation undoubtedly influences immune function. We know that cows without the stress of lactation recover from periparturient immunosuppression within 1 wk after calving, whereas lactating cows remain immunosuppressed for 2 - 3 wk postpartum (Kimura et al., 1999a,b, 2002b). Today we have a new immune restorative to give transition cows. In combination with the best possible hygienic conditions and the best possible dietary management, we can further reduce the incidence of disease in early lactation and better enable cows to reach their full genetic potential.

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Immune Dysfunction in Periparturient Dairy Cows: Insight into Pharmacologic and Dietary Immune Treatments

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INTRODUCTION

With a $40.5 billion gross domestic value for milk produced in the U.S. during 2013, the dairy industry was the third largest sector of the 2013 U.S. animal agriculture economic engine. The value of milk produced in 2013 represented 24 % of the total value of animal agriculture production; this figure had grown from $21-23 billion/y over a decade ago. The 2007 NAHMS Dairy Study reported that during 2006, 23.6 % of cows were culled from operations, 26.3 % and 23 % were removed for reproductive and udder health problems (USDA, 2007). In addition, 16.5 % of cow mortalities were due to mastitis. Clearly, the economic value of controlling mastitis pathogens is immense. Most economic analyses of the cost of mastitis cite a 10 % production loss as only one part of the overall cost of the disease. A majority (65 to 70 %) of losses are associated with decreased milk yield resulting in lower production efficiency; the remaining costs are attributed to treatment. In addition to these direct losses, mastitis causes significant problems in milk quality control; dairy manufacturing practices; quality and yield of cheese; nutritional quality of milk; antibiotic residue problems in milk, meat and the environment; and genetic losses due to premature culling. These additional costs are very significant and are not always included in economic analyses of mastitis costs.

Because of the need for a safe, economical, and stable supply of food, those of us serving the livestock health industry must be prepared to provide the best quality advice and care in managing our nation's dairy herd. For dairy producers, the critical factor in providing a low somatic cell count milk supply is keeping cows free from mastitis. Mastitis is anything causing inflammation of the mammary gland, and infectious mastitis is caused by a plethora of microbial agents (Watts, 1988). Nearly half of the nation's herd of dairy cows will experience at least 1 episode of mastitis during each lactation. Research has already resulted in genetic selection for cows with lower somatic cell counts by the incorporation of this trait into the A.I. sire summary ranking indices. This approach mainly serves to reduce the normal increase in mastitis incidence that occurs as milk production goes up. Coliforms and environmental streptococci are the most common etiologic agents isolated from clinically severe mastitis cases on well-managed dairy farms (Anderson et al., 1982; Hogan et al., 1989). Clinical trials and experimental studies have demonstrated repeatedly *no benefits* of antibiotic therapy in cattle with clinical or subclinical coliform mastitis (Erskine et al., 1991; Jones and Ward, 1990; Kirk and Barlett, 1984). Hence, the advent of the *Escherichia coli* J-5 and other endotoxin core mutant vaccines in veterinary medicine many years ago provided us a tool to reduce the incidence and severity of clinical coliform mastitis (Gonzalez et el., 1989; Hogan et al., 1992a,b, 1995). However, there remains an unmet veterinary medical need of new ways to prevent or treat mastitis caused by environmental pathogens. For several years, research at the USDA's National Animal Disease Center in Ames, IA undertook a 2-fold approach for improving the dairy cow's resistance to mastitis - immunomodulation and genetic selection for superior immune systems. In this paper, we will focus on:

- The evidence for immune suppression in periparturient dairy cows,
- How this sets the cow up for infectious diseases such as mastitis, metritis and retained placental membranes, and
- Some of the early research on immune modulation of the transition dairy cow and how that impacted resistance to mastitis.

[1] No endorsements are herein implied. USDA is an equal opportunity provider and employer.

ROLE OF THE IMMUNE SYSTEM IN MASTITIS

Immunity against infectious diseases of cattle is mediated by diverse, yet interdependent, cellular and humoral mechanisms. Many environmental and genetic factors influence the ability of livestock to mount effective defense strategies against the various pathogens and normal flora that they are exposed to throughout their lifetime. Innate resistance to infectious diseases reflects the inherent physiological attributes of an animal that make it more or less susceptible to disease development by a particular pathogen. There are several cell lineages that comprise the immune system (e.g., B-cells, T-cells, neutrophils, eosinophils, basophils, macrophages, and mast cells). Each of these cell types has distinct responsibilities in providing host defense. Innate immunity represents the various immune components that are not intrinsically affected by prior contact with an infectious agent (Roitt, 1994). Lymphocytes provide the adaptive immune reactions that are antigen specific in nature and possess memory for future encounters with the same pathogen. In this paper we will present a novel approach of immune modulation of the innate immune system as a potential means to reduce antibiotic usage in veterinary medicine.

Our first understanding of cellular immunity is more than a century old and it actually involves research into the causes of bovine mastitis and the immune response. In his 1908 Nobel Lecture the Russian zoologist, Elie Metchnikoff, described disease as consisting "of a battle between a morbid agent, the external microorganism, and the mobile cells of the organism itself. A cure would represent the victory of the cells, and immunity would be the sign of an activity on their part sufficiently great to prevent an invasion of microorganisms (Metchnikoff, 1908)." Metchnikoff cited the work of a Swiss veterinary expert, Zschokke, who found that "plentiful phagocytosis of streptococci in the battle against infectious mastitis in cows, was a good sign. When phagocytosis was insignificant or not present, the cows were written off as no longer capable of producing good milk." This was later extended to include the idea that not only must the phagocytes engulf the microorganisms, but that these devouring cells must utterly destroy the microorganisms. In some cases, the streptococci of mastitis were found to "destroy the phagocytes after being engulfed by them thus liberating themselves to carry on their deadly work."

Today we have a far more detailed knowledge of the cow's immune response to pathogens in the mammary gland (and elsewhere). Neutrophils are one of the most important cell types of native defense mechanisms because they respond quickly (within minutes) and do not require previous exposure to a pathogen to effectively eradicate the microbe. A major function of neutrophils is the phagocytosis and destruction of microorganisms that invade the body. Phagocytosis is probably the most widely distributed defense reaction, occurring in virtually all phyla of the animal world.

NEUTROPHILS ARE CRITICAL AGAINST MASTITIS

Native defenses of cattle are continually challenged by exposure to pathogens (bacteria, fungi, and viruses) and many factors affect the outcome of this interaction. Establishment of an infection in any organ or tissue is dependent upon a delicate balance between defense mechanisms of the body and the abilities of pathogens to resist unfavorable survival conditions. The neutrophil is one of the most important cells of the innate defense mechanisms because it can act quickly (within minutes) in large numbers, and in most cases, does not require previous exposure to a pathogen to effectively eradicate the microbe. Studies have shown that it takes approximately 1-2 h for neutrophils to accumulate in response to *E. coli* infection in tissues (Persson et al., 1988, 1992, 1993; Persson and Sandgren, 1992). What this means is that microorganisms will have a 2-h head start on the host immune response and any further delay in the inflammatory response will result in significantly more pathogens for the host to deal with. Unfortunately, delays in inflammatory responses in stressed animals are well documented (Shuster et al., 1996; Hill et al., 1979; Hill, 1981), and some of the mechanisms responsible for delayed inflammation have been identified (Lee and Kehrli, 1998; Burton and Kehrli, 1995; Burton et al., 1995). The importance of the neutrophil in protecting virtually all body tissues (especially against bacteria) has been repeatedly demonstrated experimentally and in nature (Schalm et al., 1964a,b; Jain et al., 1968, 1978; Ackermann et al., 1993, 1996; Gilbert et al., 1993a). Early and rapid accumulation of sufficient numbers of neutrophils is paramount in the ability of the host to effect a cure of invading pathogens (Anderson, 1983). Neutrophils can also release cytokines that in turn result in additional recruitment signals for more neutrophils (Canning and Neill, 1989; Cicco et al.,

1990; Goh et al., 1989; Ohkawara et al., 1989). Circulating *neutrophils represent the major recruitable host defense against acute tissue infection*, such as mastitis (Hill, 1979, 1981; Jain, 1968; Schalm et al., 1976).

IMMUNOSUPPRESSION IN THE PATHOGENESIS OF MASTITIS

A literal definition of immunosuppression is diminished immune responsiveness. This simplistic definition impacts a highly diverse system that affords protection against disease. Periparturient immunosuppression research was initiated by the observation that most clinical mastitis occurs in dairy cows in early lactation and the view that most bovine mastitis is caused by opportunistic pathogens and; therefore, these cows must be immunosuppressed. What evidence supported the hypothesis of periparturient immunosuppression? Practical experience teaches us that opportunistic infections are associated with severe compromises of host defense mechanisms. Over the past couple decades, an overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils in periparturient cattle (Figure 1) and sows has been generated in research institutes around the world (Shuster et al., 1996; Lee and Kehrli, 1998; Burvenich et al., 1994, 2007; Cai et al., 1994; Detilleux et al., 1994, 1995a,b; Dosogne et al., 1998, 1999; Guidry et al., 1976; Harp et al., 1991; Heyneman and Burvenich, 1989; Hoeben et al., 1997, 2000a,b; Ishikawa and Shimizu, 1983; Ishikawa, 1987; Ishikawa et al., 1994; Kehrli and Goff, 1989; Kehrli et al., 1989a,b; Kelm et al., 1997; Kimura et al., 1999a,b, 2002a,b; Lippolis et al., 2006; Löfstedt et al., 1983; Mehrzad et al., 2001, 2002; Monfardini et al., 2002; Nagahata et al., 1988, 1992; Nonnecke et al., 2003; Pelan-Mattocks et al., 2000; Shafer-Weaver and Sordillo, 1997; Sordillo et al., 1991, 1992, 1995; Stabel et al., 1991; Van Werven et al., 1997; Vandeputte-Van Messom et al., 1993). Periparturient immune dysregulation impacts the occurrence of infectious diseases of virtually any organ system of livestock (e.g., gastrointestinal, respiratory, and reproductive tracts all have increased disease incidence in postpartum animals).

First of all, there is an extremely high incidence of clinical disease in postpartum cows with nearly 25 % of all clinical mastitis occurring during the first 2 wk after calving. Clinical mastitis caused by virtually all pathogens (but especially coliform bacteria and streptococci other than *Streptococcus agalactiae*) has a very high incidence in early lactation. Cows must first become infected and then develop clinical mastitis. The rates of new intramammary infections (**IMI**) caused by environmental pathogens are highest during the first and last 2 wk of a 60-d, nonlactating period of dairy cows (Hogan et al., 1989; Smith et al., 1985a,b; Oliver and Mitchell, 1983). The rate of new IMI during these periods of peak susceptibility is 2 to 12 X higher than any other time in the production cycle of the cow. Most coliform and environmental streptococcal infections, established in the nonlactating period and that are present at parturition, result in clinical mastitis soon afterward (Smith et al., 1985a; McDonald and Anderson, 1981). The proportion of all cases of clinical coliform mastitis that develop during the first 2, 4, and 8 wk of lactation has been reported to be 25, 45 and 60 %, respectively (Malinowski et al., 1983; Jackson and Bramley, 1983).

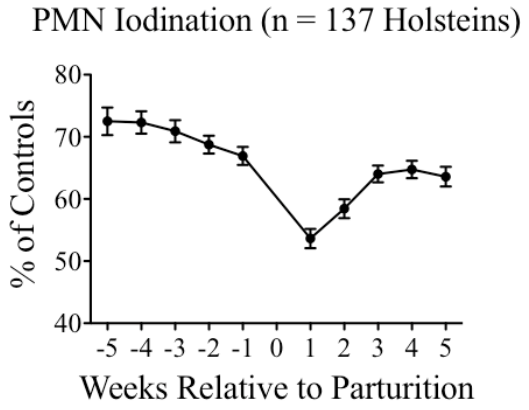

Figure 1. Neutrophil (PMN) iodination measures the myeloperoxidase-catalyzed halogenation of proteins, a phenomenon that takes place in phagolysosomes of neutrophils that have phagocytosed bacteria. *In vivo*, this halogenation disrupts the function of critical bacterial membrane proteins and results in the oxidative killing of the bacteria by the neutrophil. This bactericidal activity depends on a series of events to occur in the process of phagocytosis: successful opsonization and uptake of the bacteria by the β2-integrins into a phagosome, the generation of superoxide anion and its dismutation into hydrogen peroxide (H_2O_2), the fusion of the phagosome with a primary granule to produce a phagolysosome in which myeloperoxidase utilizes the H_2O_2 and cellular halides to halogenate the bacterial surface proteins. (Data from Detilluex, et al., 1995b.)

The second piece of evidence supporting the notion of immunosuppression in the pathogenesis of mastitis was that we are traditionally taught that opportunistic infections are associated with severe

compromises of host defense mechanisms. Most mastitis pathogens are considered opportunistic pathogens. These 2 points led to experiments evaluating how functional a cow's immune system is around calving time. Today the data tells us the immune system becomes progressively more compromised at the end of gestation, cows become more readily infected in the mammary gland, then as the immune system *bottoms out* the first week or two after calving, these subclinical infections begin to win the battle with the cow's immune system and clinical mastitis results.

WHAT CAUSES PERIPARTURIENT IMMUNOSUPPRESSION?

Many neuroendocrine changes develop in cows during the periparturient period. Periparturient hormone fluxes may adversely affect immune cell function. Surprisingly, there is no effect of estrogen on bovine neutrophil function either during the follicular phase of the estrous cycle in cows or after administration of high doses of estradiol to steers (Roth et al., 1982, 1983). However, supraphysiologic concentrations of estradiol have been reported to suppress neutrophil function (Bodel et al., 1972; Klebanoff, 1979). These high concentrations of estrogens may be germane to immunosuppression and the high new IMI rates prior to calving. Before calving, total plasma estrogen concentrations increase in the cow (at least 10 X greater than during estrus) (Comline et al., 1974). Moreover, during normal pregnancy, the progesterone binding capacity of human lymphocytes is increased (perhaps as a result of increasing estrogen levels) and the concentration of progesterone in serum during pregnancy combine as sufficient to reduce lymphocyte functions (Szekeres-Bartho et al., 1983, 1985). This raises the possibility that hormone sensitivities of immune cells during late gestation may be altered and result in functional changes in immune cells due to rising estrogen levels. Very high concentrations of both estrogens and progesterone are reached during the final days of gestation in cows (Comline et al., 1974). This may be germane to the onset of impaired lymphocyte function in the prepartum cow whose lymphocyte hormone binding capacity may be higher than that in barren cows.

Many of the hormonal and metabolic changes that prepare the mammary gland for lactation take place during the 3 wk preceding parturition. Lymphocyte and neutrophil function could be affected by prepartal increases in estrogen, prolactin, growth hormone, and/or insulin (Comline et al., 1974; Houdebine et al., 1985; Convey, 1974; Akers, 1985). During this critical period, the dairy cow's metabolism shifts from the demands of pregnancy to include those of lactation, with increased demands for energy and protein. Negative energy and protein balances that exist during early lactation may also contribute to impaired neutrophil function and, thus, account for a portion of the periparturient immunosuppression observed. The nutritional demands of lactation contribute to the duration of immune suppression (Kimura et al., 1999b; Nonnecke et al., 2003; Stabel et al., 2003) and postpartum neutrophil glycogen stores have been associated with postpartum uterine diseases (Galvão et al., 2010).

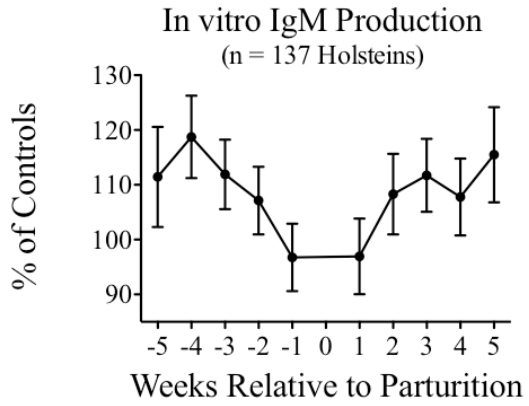

Figure 2. *In vitro* production of IgM by lymphocytes is reduced in the immediate week around calving time. (Data from Detilleux et al., 1995b.)

The specific physiological factors contributing to periparturient immunosuppression and increased incidence of clinical disease have not been fully elucidated. We do know, however, that there is a very broad-based suppression of immune function in cows the 1st wk or 2 after calving. Wide variation in leukocyte functional activities has been documented between dairy cows and between different production stages (e.g., around calving time) (Ishikawa, 1987, 1994; Nagahata et al., 1988, 1992; Guidry et al., 1976; Newbould, 1976; Manak, 1982; Gunnink, 1984a,b,c; Saad et al., 1989; Gilbert et al., 1993b). Most importantly, associations between neutrophil dysfunction and periparturient disorders in cows have been reported (Kelm et al., 1997; Kimura et al., 2002a; Cai et al., 1994). Periparturient immunosuppression is not limited to cattle. Investigations of immunosuppression and coliform mastitis in sows revealed depressed neutrophil function to be associated with the susceptibility to postpartum mastitis caused by *Escherichia coli*

(Löfstedt et al., 1983). Defects in lymphocyte function also contribute to immune suppression during the periparturient period (Figures 2 and 3). In addition to reduced antibody production, other impacted roles of lymphocytes in periparturient cows include reduced production of cytokines that activate and direct both innate and adaptive immunity (Detilleux et al., 1995; Ishikawa, 1987; Ishikawa et al., 1994; Manak, 1982; Wells et al., 1977; Kashiwazaki, 1984; Kashiwazaki et al., 1985).

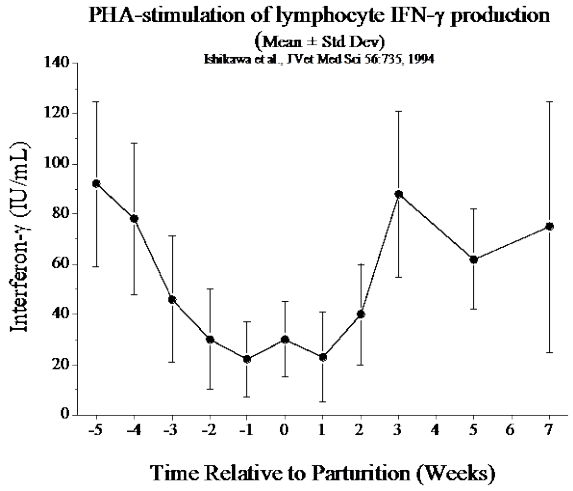

Figure 3. *In vitro* production of interferon- γ (INF- γ) by lymphocytes is reduced in the week around calving time. (Data from Ishikawa et al., 1994.)

Today it is well recognized that the bovine immune system is less capable of battling pathogens during the periparturient period. The periparturient cow has suppressed immune competence, manifest as reduced capacity for nearly all types of immune cells that have been studied. Interestingly, there may be a teleological reason for immunosuppression in the Th1 branch of the immune system that may be essential in preventing unwanted immune reactions against self and fetal antigens exposed to the mother's immune system as a result of normal tissue damage in the reproductive tract during parturition (Kehrli and Harp, 2001). However, an inadvertent and perhaps unintended consequence of this suppression of the Th1 branch of the immune system is that many of the cytokines normally produced by these cells are critical to fully activate neutrophils that are absolutely critical to the defense of the mammary gland. Without a fully functional cellular immune system, both adaptive and innate branches of the cellular immune system operate at diminished capacity for immune surveillance and pathogen clearance. This is the very circumstance that periparturient cows find themselves in and why it is so critical to manage transition cows to minimize their exposure to pathogens in the environment and to avoid metabolic disorders that might further stress their immune system.

The take-home message here is a multitude of factors of the immune system of a dairy cow become impaired as early as 2 - 3 wk before she actually gives birth (long before the elevation of endogenous cortisol which occurs from 36 h before to 36 h after calving). The cow's immune system then bottoms out and is seriously impaired for 1 - 2 wk after calving. This effect is known as periparturient immunosuppression. Regardless of its causation, periparturient immunosuppression makes the dairy cow highly susceptible to the establishment of new infections (particularly in the mammary gland) and the subsequent progression of these new subclinical infections into clinical disease (mastitis, metritis, and postpartum outbreaks of intestinal diseases such as salmonellosis, just to name a few).

WHAT ARE THE PROSPECTS FOR IMMUNOMODULATION TO PREVENT DISEASE?

Pharmacologic treatments that serve as immune modulators in cattle and other species have been under investigation for many years. Biotherapeutic immune modulators can be given to prevent or lessen disease symptoms caused by various pathogens (viral and bacterial). A general goal of such a biotherapeutic compound is to provide the desired effect on host immunity for a sufficient period of time to sustain immunity through a period of immune dysfunction the host is experiencing. In the past couple years 2 products have received approval by regulatory agencies that fall under this category but that work through very different innate immunity mechanisms.

According to the manufacturer, Zelnate™ (Bayer Healthcare LLC, Animal Health, Shawnee Mission, KS) was approved in 2015 as a USDA-Center for Veterinary Biologics approved immune modulator based on technology developed by Juvaris BioTherapeutics (Pleasanton, CA). As such, it represented a new class of drug for bovine respiratory disease (**BRD**) as an immune modulator; it is not an antibiotic nor a vaccine. Zelnate DNA Immunostimulant is a bacterial-produced plasmid DNA with a liposome carrier that stimulates the

innate immune system in cattle. Per the label claim, Zelnate is indicated for use as an aid in the treatment of BRD due to *Mannheimia haemolytica* in cattle 4 mo of age or older, when administered at the time of, or within 24 h after, a perceived stressful event. Although no peer-reviewed publications are available at this time, a summary of the technical studies conducted for regulatory approval is available: http://www.zelnate.com/static/documents/Zelnate-ChallengeStudy_Detailer.pdf.

In 2016, Imrestor™ (pegbovigrastim) (Elanco Animal Health, Indianapolis, IN) was approved by the Food and Drug Administration as the first and only immune restorative for periparturient dairy cows and heifers. Per the label claim usage, Imrestor reduces the incidence of clinical mastitis by 28 % in the first 30 d of lactation in dairy cows and heifers. Recent peer-reviewed studies describe the mechanism of action of pegbovigrastim and report an even greater reduction in clinical mastitis incidence in 4 studies conducted in the United States (Kimura et al., 2014; Hassfurther et al., 2015; Canning et al., 2017; McDougall et al., 2017).

Pegbovigrastim is a cytokine that is naturally part of a cow's immune system that works to turn on the innate immune response provided by neutrophils. Cytokines are a class of compounds that have been investigated for many years for potential biotherapeutic value. Administration of recombinant cytokines to modulate immunity in immunocompromised hosts is thought to prevent bacterial infections (Broxmeyer and Vadhan-Raj, 1989). In an effort to study methods to ameliorate the effects of periparturient immunosuppression, several scientists have evaluated various cytokines that are part of the cow's normal immune system (Sordillo et al., 1991b,1992; Zecconi et al., 1999, 2009; Sordillo and Babiuk, 1991; Campos et al., 1992; Sordillo and Peel, 1992). Granulocyte-colony stimulatory factor (**G-CSF**) is a cytokine that triggers the bone marrow to produce leukocytes – neutrophils in particular, which in turn, fight infectious disease. Human G-CSF has been successfully used for many years as an adjunct therapy for cancer patients undergoing chemotherapy. In a series of studies, G-CSF has been evaluated for its effects on bovine immunity and as a prophylactic against mastitis (Stabel et al., 1991; Kehrli et al., 1991a; Cullor et al., 1990a,b, 1992; Nickerson et al., 1989). Our research findings indicate no adverse effects and that it can reduce the incidence and severity of clinical coliform mastitis by 50 % during the 1st wk of lactation following experimental challenge (Kehrli, 1998). G-CSF has also been shown to be beneficial against *Staphylococcus aureus* and *Klebsiella pneumoniae* mastitis (Nickerson et al., 1989; Kehrli et al., 1991b). It is crucial to understand that immunomodulators work best in immunocompromised hosts; hence the periparturient period is an excellent time for such compounds to be given to cows as they will work to restore the immune system. Acceptable alternatives to the use of antibiotics in food animal practice need to be explored and the use of immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic approaches to prevent and combat infectious disease during periods of peak disease incidence. Research in the area of biotherapeutic immune modulation continues today (Kimura et al., 2014).

Dietary immune treatments are also an area of intense investigation. While not a major focus of this paper, considerable research has been done and managing optimal nutrition levels, with ingredients such as vitamin E and selenium, is well recognized to avoid immune impairment associated with nutrient deficiencies (Weiss et al., 1990, 1992, 1997; Hogan et al., 1990, 1992c, 1993, 1994; Smith et al., 1997). However, there is little evidence to support hyper-supplementation of nutrients such as these, as a means to enhance immune function.

Immunomodulatory feed ingredients have also received considerable research interest investigating possible beneficial effects on immunity and health in dairy cows. One such product, Omnigen-AF (Phibro Animal Health Corp., Teaneck, NJ), is perhaps the best studied product reported to enhance innate immunity parameters and increase milk production in dairy cows (Brandao et al., 2016; Leiva et al., 2017; Fabris et al., 2017; Wang et al., 2009; Ryman et al., 2013; Nace et al., 2014).

WHAT DOES THIS ALL MEAN FOR YOU?

Bovine mastitis is one of the most economically important diseases to the beef and dairy cattle industries. The pathogenesis is highly complex and involves many factors including various microbial etiologies, stress, management and environmental hygiene. Bovine mastitis has not been adequately controlled by vaccination or antibiotics. In many diseases, immunosuppression due to various stressors is responsible for increased susceptibility to bacterial colonization or growth. Over the past 50 y a considerable body of evidence of impaired neutrophil

and lymphocyte function in periparturient dairy cows has emerged that coincides with the high incidence of new intramammary infections 2 wk prepartum and clinical mastitis in early lactation. To overcome this immunosuppression, immunomodulatory agents have been and are being evaluated for their ability to prevent economic losses associated with periparturient diseases such as mastitis. Researchers have investigated immunomodulation as an approach to provide dairy farmers with a new tool to prevent infectious disease in their herds, although biotherapeutic products have not yet made it to the market place. The consequences of immune suppression are increases in infectious disease and premature loss from the herd, both of which add significantly to the cost of production and decrease the profitability of dairy farming. Simple solutions will not likely be found for something as complex as immune suppression; however, without additional significant research into this topic we can be assured that no progress will be made.

Production of milk from mastitis-free cows is quite simple, right? Keep your cows in clean, dry, and unstressful environments and feed them what they need, when they need it – far easier said than done! For years we have emphasized feeding cows optimal rations because the production and functional activities of leukocytes in combating microbial infection are complex and all involve expenditure of cellular energy, protein and other nutrients. The average cow has ~3500 neutrophils/µL of blood, this translates into ~1.4 x 10^{11} neutrophils in an 1800 lb Holstein cow. The circulating half-life of neutrophils is about 6 h, so the cow is replacing half of those cells every 6 h from bone marrow stores. Clearly, a significant component of the dietary energy and protein consumption for maintenance is spent on replenishment of immune cells. The negative energy and protein balance of dairy cows during the periparturient period and up to peak lactation undoubtedly influences immune function. We know that cows without the stress of lactation recover from periparturient immunosuppression within 1 wk after calving, whereas lactating cows remain immunosuppressed for 2 - 3 wk postpartum (Kimura et al., 1999a,b, 2002b). Today we have a new immune restorative to give transition cows. In combination with the best possible hygienic conditions and the best possible dietary management, we can further reduce the incidence of disease in early lactation and better enable cows to reach their full genetic potential.

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Ration Formulation Models: Biological Reality vs. Models

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ABSTRACT

Ration formulation programs are composed of basically 2 parts; the first is the model that represents nutrient requirements of the cow given her stage of life and level of production and the second is the algorithm that solves the ration to provide either the cheapest diet that meets the model (cow) requirements or maximizes milk income over feed costs. Early ration formulation programs used the simplex algorithm to solve the ration, which was based on maximizing or minimizing profit over cost based on linear model equations. The model, made up of linear, static nutrient relationships between milk production and nutrient inputs, was used to set nutrient requirements such as the tables in the Nutrient Requirements of Dairy Cattle (NRC, 1989 and earlier). At this level, the programs work, but are limited by the fact that life is not linear. As cattle eat more and produce more milk, the gain in milk production per unit of feed consumed gets smaller and smaller. As the focus changes from minimizing costs or maximizing profit to increasing efficiency, models and the algorithms used to solve them become more complicated. As the programs become more complicated, both the model and the algorithm influence the resulting ration solution. So to examine how well ration programs reflect reality or what nutrient inputs are really needed to formulate a diet, both the model and algorithm must be examined. The 2 main ration programs used today, **AMTS** (Agricultural Modeling & Training Systems, LLC) and **NDS** (Nutritional Dynamic Systems, RU.M.&N., Italy) both use the Cornell Net Carbohydrate and Protein System (**CNCPS;** Tylutki et al., 2008); but, because they use different solution algorithms and settings, will produce different rations.

INTRODUCTION

Models of dairy cow nutrient use are dependent on how nutrients are defined and how important those nutrients are to the nutritional physiology of the cow. Early ration formulation was based on nutrient definitions according to proximate analysis. However, proximate analysis had several problems including a non-homogenous category of nutrient, *Nitrogen Free Extract*, that had no relation to cow physiology and a lack of continuity between crude fiber (**CF**), and newer fiber analysis techniques, acid detergent fiber (**ADF**) and neutral detergent fiber (**NDF**). The basic idea of proximate analysis has stayed in nutrient analyses techniques through the use of total digestible nutrients (**TDN**) and more recently many analyses have been added to the basic framework of proximate analysis, and ADF, NDF through further development of CNCPS model. If all of the new analyses were needed to formulate a ration, laboratory costs would be extremely high. Therefore the goals of this paper are:

1) To use sensitivity analyses to determine the relative importance of a nutrient to the ration program,
2) To explore how changes in the nutrient affect the ration solution, and
3) To examine if ration program behavior would matter to the cow (reality).

The methods presented to evaluate the ration programs could be done by anyone and should be done before changing programs or upgrading to a new CNCPS model or algorithm.

Nutrient Descriptions

Chemical analyses of nutrients must be measurable with accuracy and precision, relevant to cow physiology, and must improve model predictions of production. Unfortunately none of the current systems meet all of these criteria. For instance, NDF was originally developed to quantify fiber from forages, but results for the same sample were not consistent. Due to the importance of feeds that contain both fiber and grain (i. e. corn silage), NDF was also used for high starch feeds. Because these feeds were nearly impossible to filter and complete the assay, the technique was modified to add amylase, noted as **aNDF**. But, since results were still not consistent (lack of precision), the ash content of NDF was removed (**aNDFom**). Then, because

whether a fiber was digestible in the rumen and therefore available to microbes for microbial growth would link NDF better with rumen physiology, digestible NDF (**dNDF as % NDF or NDFd as % DM**) was created. But these were determined chemically using an *in vitro* incubation system, which becomes more unlike rumen fermentation the longer it lasts. Consequently NDFd became defined according to length of incubation: **NDFd24** (24 h), **NDFd30** (30 h), etc. In recognition that some NDF is degraded more rapidly than others, NDF was also classified into undegradable NDF at 30 h (**uNDF30**), at 120 h (**uNDF120**) and at 240 h (**uNDF240**). These chemical analyses were used to define pool sizes in CNCPS for rapidly degrading NDF, slow degrading NDF, and unavailable NDF (lignin); respectively, to define how much NDF was potentially degradable (pdNDF = aNDFom - uNDF) and how much NDF was essentially not degradable at all in the rumen. While the development of these assays parallels how NDF has been observed to be degraded in the rumen, the nutrient NDF is not a substance that microbes degrade to produce specific products. Neutral detergent fiber is not unique and its components (cellulose, hemicellulose, lignin, and ash) are fermented through different pathways. Therefore NDF, while relevant to plant physiology, is not necessarily relevant to rumen physiology and so refining it further, according to rumen physiology, will not improve its representation of reality. It would be better to start with nutrient descriptions that were more homogeneous such as cellulose, hemicellulose, and lignin instead of trying to correct an already flawed nutrient description. This has been acknowledged by the developers of CNCPS and in a perfect world, the analyses to determine cellulose, hemicellulose, pectin, and lignin would already be developed and consistent with forage quality. Unfortunately this has not happened yet due to the focus on NDF.

METHODS

Evaluating the importance of a nutrient to the model and ration formulation

If a nutrient was measurable with accuracy and precision and a change in that nutrient supplied to the cow caused a change in cow health or production, the ration formulation program should reflect the importance of the nutrient. In modeling terms, the ration formulation should be sensitive to changes in the nutrient supplied by either changing the resulting ration or changing the requirements of other nutrients, or both. Essentially there are at least 2 questions that can be answered by this analyses:

1) "How important is it that I know that nutrient 's level in the feed (diet)?" or conversely "Should I spend the money for wet chemistry analyses?" and
2) "If I'm wrong about this nutrient's level in the feed, will it change the ingredient composition of the diet?"

The second question is impacted by both the nutrient requirement model and the algorithm used to solve the ration and may be different for different ration formulation programs. These analyses can be done by anyone and should be done before choosing which ration formulation program to use.

RESULTS AND DISCUSSION

The following are examples of these analyses using AMTS. Table 1 lists the baseline ration and ingredient constraints before any nutrients or nutrient variables were changed. The nutrient constraint column lists the nutrients that were constrained to get the ration solution. For each sensitivity analysis that compares changes in a model nutrient to ration changes, constraints were held constant and only the nutrient was changed.

Example 1.

Evaluate the importance of knowing physically effective (**pe**) factor in corn silage to meet the peNDF requirement for the ration. For a feed (corn silage), **peNDF = % NDF * pef** and pe factor is the percent of feed above the 1.1 or 4 mm screen of the Penn State Particle Sorter (**PSPS**). Physically effective NDF should be between 22 - 35 % according to constraints built into AMTS. Because corn silage is a major component of the baseline diet and pef is large for corn silage (82 %), pef was changed in 10 % increments to see the effect on ingredient content of the diet and peNDF. Figure 1 shows the impact of changing pef on peNDF. The dashed lines indicate the constraints for peNDF. For corn silage, there is not much impact on peNDF until pef is above 60 %. This makes sense because the amount of large fiber particles (above 4 mm) should be at least above 60 % of total corn silage. Figure 2 shows that as pef gets below 60 %, AMTS changes the ingredient composition of the TMR from corn silage to wheat silage. This also causes small changes in citrus, dried distillers grains (**DDG**), and corn to continue to balance the TMR. Above 60 % pef for an

Table 1. High milking cow ration solution (DMI 55 lb/d)

Ingredient	AMTS Ration (lb)	Min (lb)	Max (lb)	Nutrient Constraints
Corn silage	15	7	15	DMI
Wheat silage	0.3	0	6.5	ME
Corn	10	6	11	MP
Alfalfa	15	6	15	Rumen ammonia
Almond hulls	2	2	4.5	NFC
Dried distillers grains	3.5	2	3.5	peNDF
Wheat mill run	0	0	5	EE
Canola meal	0	0	6	Lys
Corn gluten	0.18	0	3.3	Met
Soybean meal	3.2	0	3.2	
Cottonseed	3.6	0	3.6	
Citrus pulp	0	0	1	
Molasses	0	0	0.65	

approximate 30 % change in pef, TMR peNDF changes by 5 %.

Reality Check

It is very difficult to get repeatable results with the PSPS. Results can commonly vary between 10 - 20 %. But, with only a 5 % change in peNDF of the TMR for a 30 % change in pef, getting good results from the PSPS is probably not an issue. However, it also implies that this number is not important for the ration (62 % pef is the same as 92 % pef) and could be excluded as a constraint and as a term in the program. In addition, particle size of the TMR can change greatly during mixing and feeding of the TMR due to mixing time, operating condition of the mixer wagon, and sorting of TMR by the cows (crowding, feeding frequency, etc.). Therefore including particle size as a constraint in a ration formulation program will not be a major contributor to impacting rumen function as it was originally intended.

Figure 1. Effect of changes in physically effective factor (pef) on peNDF. Constraints for peNDF is area between dashed lines.

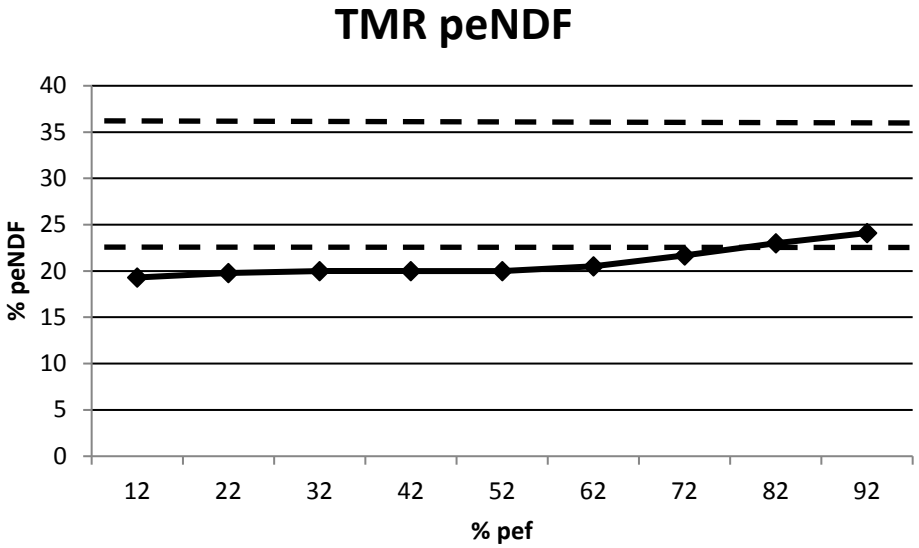
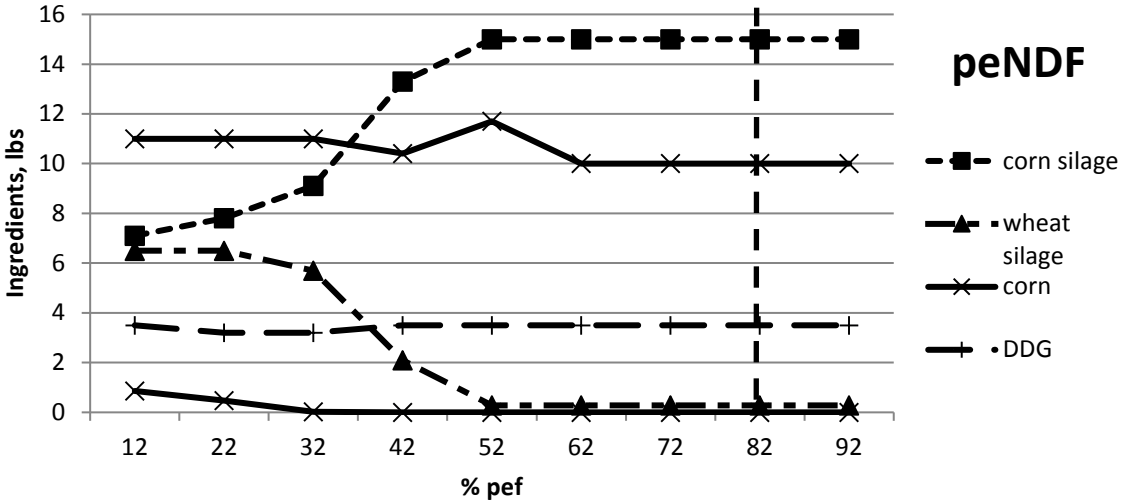


Figure 2. Changes in ingredient content of ration as pef is decreased. The pef for corn silage is depicted by the vertical dashed line.



Example 2.

How would the ration solution be changed if the starch content in corn was inaccurate?
Starch is a major component of non-fiber carbohydrate (**NFC**), which has a maximum limit of 40 % DM in the AMTS program. Corn was used to vary the amount of starch because it was the major contributor to starch in the diet. Changes in starch were counter balanced with changes in NDFom (Figures 3 and 4) and then sugar (Figures 5 and 6) to ensure the nutrient content of corn still summed to 100 %. Note that a decrease in corn starch content (about 10 %) replaced with NDFom caused a similar decrease in NFC (about 10 %) and large changes in the TMR, especially between corn and corn silage. But when starch was replaced with sugar, there was no change in NFC and very little change in the TMR. See Figure 6 where wheat silage is replaced with corn gluten (1:1 change by 0.13 lb).

Figure 3. Changes in NFC and TMR starch with replacing corn starch content with NDFom. Constraints for NDFom depicted by dashed line.

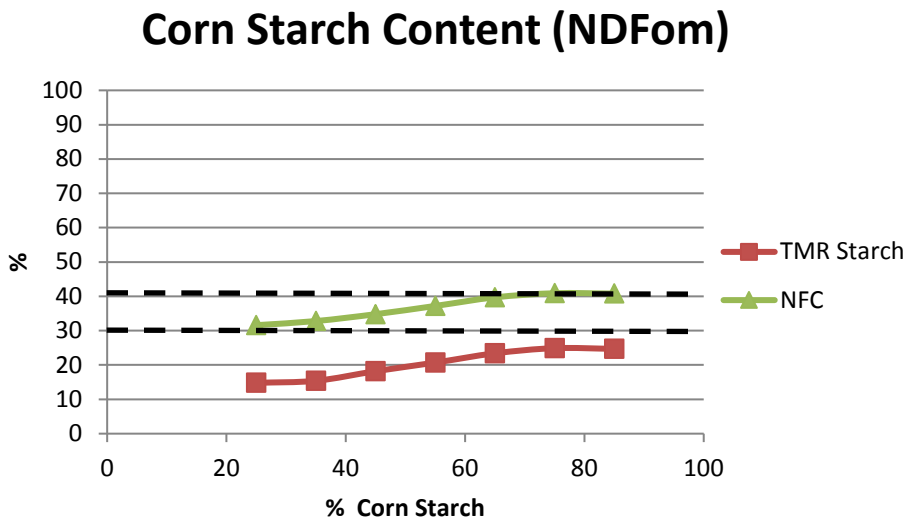
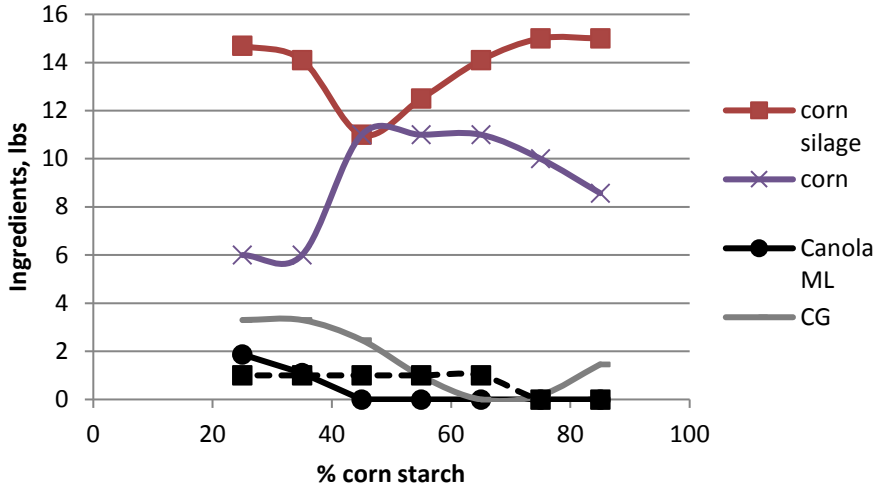


Figure 4. Changes in the ingredient composition in the TMR as a result of replacing starch percent in corn with NDFom.



Reality Check

Of all the macro nutrient analyses performed by laboratories, methods and results from starch analyses are the most variable. This analysis examines the impact on the ration solution if starch content in corn was wrong and either the *missing* nutrient percent ended up in NDFom or in sugars. If starch content is mis-identified as sugars, there is very little impact on the TMR ingredient composition; which also implies it may not be important to distinguish starch and sugars and sub-components of NFC. Knowledge of NFC may be enough. However, if starch content is mis-identified as NDFom, the impact to the TMR is much greater. Therefore it is important to know NFC and NDFom, but not-sub categories of nutrients within NFC.

Figure 5. Changes in NFC and TMR starch with replacing corn starch content with sugar. Constraints for sugar depicted by dashed line.

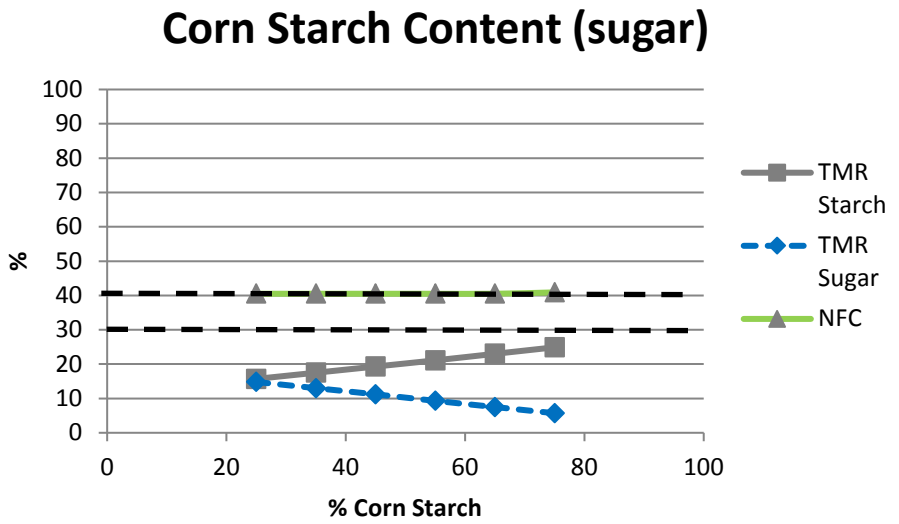
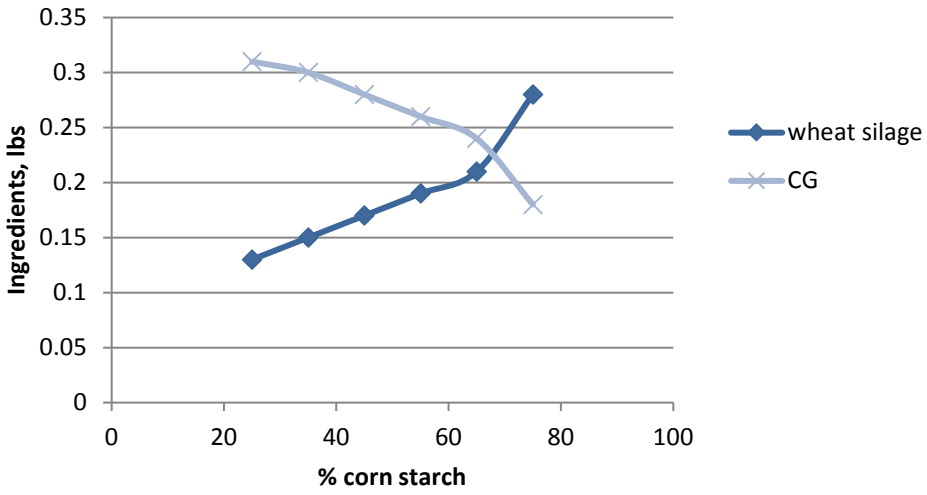


Figure 6. Changes in NFC and TMR starch with replacing corn starch content with sugar.



CONCLUSIONS

The Reality of Model Evaluation

Nutrient descriptions should be closely linked to how their nutrient inputs are described and measured. The CNCPS model has been very good at using well defined nutrient analyses to develop model concepts. However those nutrient definitions don't necessarily reflect differences in feed quality or changes in cow production. For the model, nutrient descriptions must adequately describe inputs for predicting cow physiology such as rumen function, ATP creation and use, and nitrogen and carbon for microbial growth. For the real cow, a change in a nutrient should result in a change in health or production. Unfortunately because cows are not usually managed or monitored individually, there is significant noise present in determining the impact of a nutrient in a real dairy herd. This makes model evaluation extremely difficult. For instance, glucose levels are extremely important in a transition dairy cow to prevent ketosis and the associated high economic costs of the disease. But until recently, subclinical ketosis, as defined by blood ketone (and glucose) levels, was largely ignored because cows generally did not show clinical signs and so the cost of the disease was thought to be inconsequential. However, once the associative effects of subclinical ketosis and their costs were estimated ($78/cow; Geishauser et al., 2001), prevention of subclinical ketosis (low blood glucose) through monitoring individual cows is becoming more common now. Using current nutrient descriptions, however, there is no way to predict glucose supply from a given diet with precision and accuracy. Even if we could predict glucose supply to the cow, there are many other health, stress, and management factors that would have a bigger impact than diet on glucose levels in cows at any one point in time. Therefore instead of trying to refine existing nutrients descriptions and analyses, it may be better to look to identifiers of feed quality that impact the production of the cow paying attention to methods of analysis that are precise and accurate.

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Recommendations for Vitamins and Trace Minerals for Dairy Cows

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SUMMARY

- The NRC (2001) requirements for most trace minerals and vitamins appear adequate, but modest safety factors (~1.2 to 1.5 X NRC) should be used to reduce risk.
- The trace minerals contained in basal ingredients, including forages, have some degree of availability and concentrations should not be set to 0.
- NRC (2001) requirements for Co and Mn are too low and concentrations need to be increased substantially.
- Be wary of long term overfeeding of Cu. Health issues may develop at dietary concentrations as low as 20 ppm when fed over long periods.
- Supplying vitamin E in excess of NRC (2001) requirement to peripartum cows provides health benefits.
- Supplying vitamin D in excess of NRC (2001) to cows with limited sun exposure may be needed to maintain adequate vitamin D status relative to general health.

INTRODUCTION

Providing adequate trace minerals and vitamins to dairy cows is essential for high production and good health; however feeding excess trace nutrients inflates feed costs and could be detrimental to production and cow health. Unfortunately quantifying the supply of available trace nutrients and their requirements is extremely difficult, which leads to a high degree of uncertainty relative to diet supplementation. This paper provides suggested strategies for formulating diets to provide adequate but not excessive amounts of vitamins and trace minerals under a variety of conditions. When this paper was written (Spring, 2017), the NRC was in the process of updating the *2001 Nutrient Requirements of Dairy Cows* publication. Since that publication, very little new information has been published on many vitamins and minerals; this paper will concentrate on those trace nutrients for which newer (published since 2000) information is available. These include cobalt (**Co**), chromium (**Cr**), copper (**Cu**), and manganese (**Mn**); vitamins D and E; and a few water soluble vitamins. The upcoming NRC may or may not reflect the opinions in this paper.

MINERAL SUPPLY

A major change that occurred in NRC (2001) was that requirements were calculated for absorbed mineral rather than total mineral. This was a major advance because we know minerals from some sources are more absorbable than minerals from other sources. However the use of absorbable mineral has limitations:

- Measuring absorption of many minerals is extremely difficult.
- Actual absorption data are limited; therefore most absorption coefficients (**AC**) are estimates.
- Absorption is affected by physiological state of the animal and by numerous dietary factors (many of which have not been quantified).
- For many of the trace minerals, the AC is extremely small and because it is in the denominator (i.e., dietary mineral required = absorbed requirement/AC) a small numerical change in the AC can have a huge effect on dietary requirement.

Concentrations of Minerals in Basal Ingredients

For most minerals of nutritional interest good analytical methods that can be conducted on a commercial scale at reasonable costs are available. Assuming the feed sample is representative, a standard feed analysis (using wet chemistry methods for minerals) should provide accurate concentration data for calcium (**Ca**), phosphorus (**P**), magnesium (**Mg**), potassium (**K**), sodium (**Na**), Cu, iron (**Fe**), Mn, and zinc (**Zn**). Labs can also routinely measure

sulfur (**S**) and chloride (**Cl**) but often these are separate tests. Most labs do not routinely measure Cr, Co, and selenium (**Se**) because the concentrations commonly found in feeds are lower than what commercial labs can reliably measure or because of contamination caused by routine sample processing, such as using a steel feed grinder (a major concern for Cr). Although we can get accurate total mineral concentrations data for basal ingredients, you must be careful when evaluating and using the data. Concentrations of minerals in feeds, even most macrominerals, are low. For example 1 ton of average corn silage (35 % dry matter (**DM**)) only contains about 2.5 g of Cu (to put this in perspective a penny weighs about 2.5 g).

Sampling error is a problem for most nutrients and when concentrations are low, sampling error is usually larger. From a survey we conducted on forages, sampling variation for trace minerals was greater than true variation. This means that mineral concentration data from a single sample should be viewed very suspiciously. The mineral concentration of soils is a major factor affecting the concentrations of most minerals in forages. Therefore averages of samples taken from a farm over time (up to a few years) or from a group of farms within a small geographic area (e.g., a few counties) should be a truer estimate of the actual mineral concentration of a forage than a single sample. In a normal distribution (the classic bell shaped curve) about half the samples have less than the mean or average concentration, about half the samples have more than the average, and about 95 % of the samples are within $\pm$ 2 standard deviation (SD) units of average. This means that if you know the average concentration and the SD you have a good description of the population. This information helps with risk assessment. If a feed has an average concentration of Mg of 0.4 % and SD of 0.01 % and the distribution is normal, about 95 % of the samples of that feed should have between 0.38 and 0.42 % Mg. With that information you should probably conclude it is not worth analyzing that feed for Mg, because even if your sample is 2 or 3 SD units from the mean it will have no effect on the diet or the animal. However when distributions are skewed, the average and the SD may not be good descriptors of the population. For many minerals, concentrations within feeds are not normally distributed (Figures 1 and 2). Often the distributions have long tails because concentrations cannot be less than 0 but can be extremely high for various reasons. Some samples have high concentrations of certain minerals because of soil contamination. The more skewed the data, the less valuable the average and SD become in describing the feed. The median is the

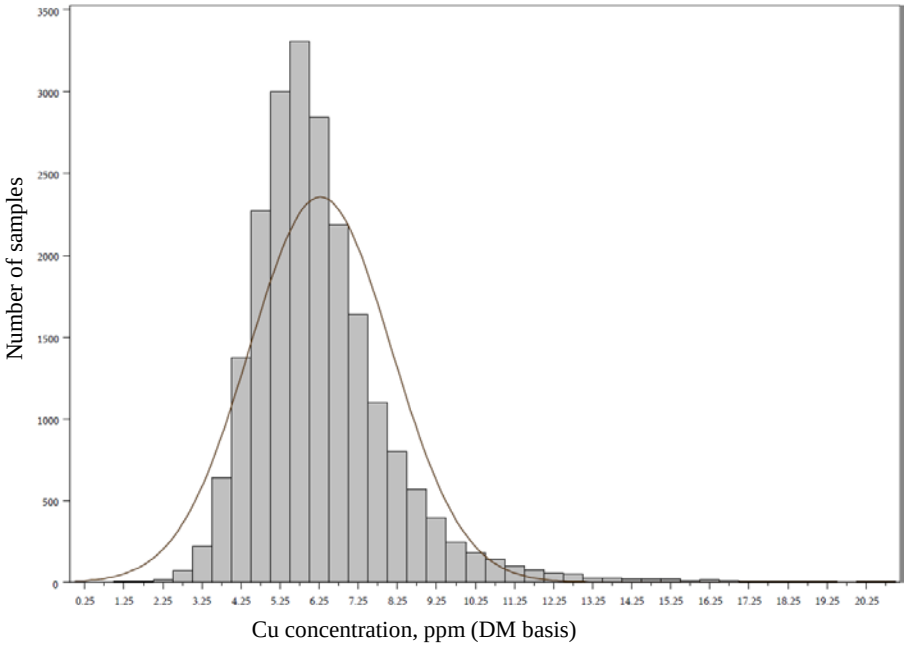

Figure 1. Distribution of Cu concentrations in corn silage grown throughout the U.S. The smooth line indicates a normal distribution while the bars indicate the actual distribution. (Knapp et al., 2015).

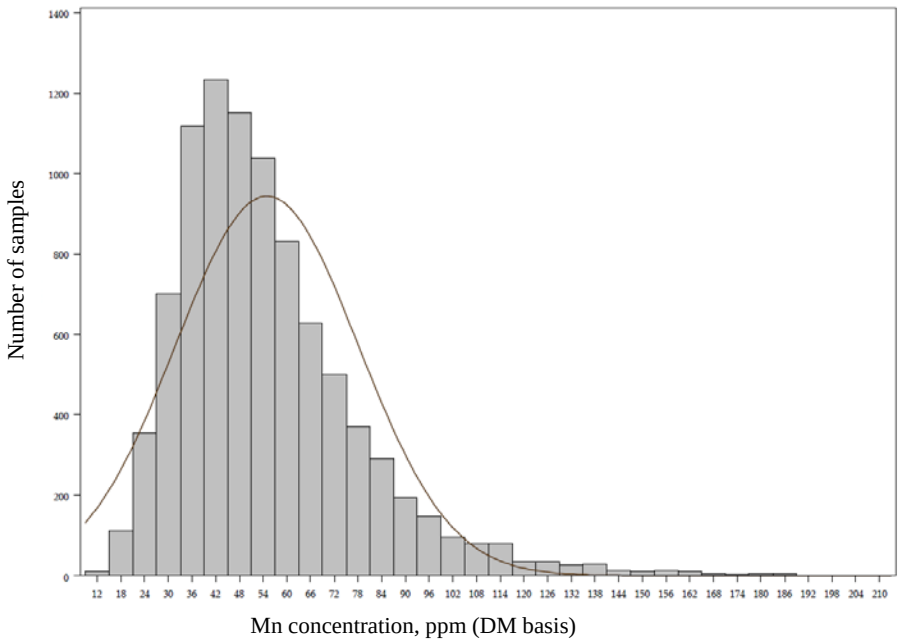

Figure 2. Distribution of Mn concentrations in mixed, mostly legume silage grown throughout the U.S. The smooth line indicates a normal distribution while the bars indicate the actual distribution (Knapp et al., 2015).

concentration where half of the samples have a lower mineral concentration and half of the samples have more mineral, and in a normal distribution the mean and the median are essentially equal. For concentrations of trace minerals and some macro minerals, the median is usually less than the average because their distributions are skewed. What this means is that for most situations, using the average trace mineral concentration (e.g., feed table data), overestimates the trace mineral concentration in the majority of samples. For skewed populations, the median is a better descriptor of the population than the mean; however simply replacing average concentration with median concentration does not fix all the problems associated with a skewed distribution.

As a distribution becomes more skewed, the risk that a specific feed will contain excess mineral increases. The Mn data shown in Figure 2 is a good example. That data has an average of 55 ppm and SD of 23. Assuming a normal distribution, one would expect about 2.5 % of the samples to have more than about 100 ppm (55 + 2 SD) and about 2.5 % of the samples to have less than about 9 ppm. However, no samples had less than 9 ppm and 5.2 % had more than 100 ppm. If your particular sample of mixed mostly legume silage was in the 5 out of every 100 samples with a very high Mn concentration, your diet would contain substantially more Mn than expected. Excess dietary Mn is rarely a problem for cows, but excess dietary Cu can be (discussed below). Corn silage in Figure 1 had a mean Cu concentration of 6 ppm with a SD of 1.8. With a normal distribution about 2.5 % of the samples should have more than about 10 ppm Cu. However, about 5 % of samples have more than 10 ppm Cu (i.e., twice the risk). If you formulate a diet assuming corn silage is 6 ppm Cu, but it really has 12 ppm, and corn silage comprises a significant portion of the diet, over the long term (months) excess dietary Cu could become a problem. The bottom line is that averages for trace mineral concentrations in forages (and perhaps other feeds) found in tables should be used with caution. Because of substantial sampling variation, data from a single sample should not be used. The best advice is to generate median values for trace minerals for forages grown within a limited geographical area.

Do Trace Minerals in Feeds Have Nutritional Value?

Essentially every feedstuff used in dairy diets contains some minerals. The question is, are those minerals biologically available to cows? Although survey data of nutritionists are lacking, based on personal experience it is not uncommon for nutritionists to set trace mineral concentrations in basal ingredients, or at least forages, at 0. This approach would be valid if the trace minerals in feedstuffs were not biologically available to cows.

Although substantial uncertainty exists regarding the absorption coefficients for most minerals in feeds, a portion of the trace minerals found in most (all?) feedstuffs is clearly available to cows. Tissues from wild ruminants such as deer (Wolfe et al., 2010) contain trace minerals indicating that absorption of basal minerals occur.

The NRC (2001) estimates that Cu, Mn, and Zn from basal ingredients are 4, 0.75 and 15 % absorbable. The AC assigned to basal ingredients are usually lower than AC for the sulfate form of minerals even though most of the trace minerals contained within plant cells would be in an organic form. The lower AC for trace minerals in basal ingredients may reflect an adjustment for soil contamination. Some trace minerals in basal feeds, especially forages, are in soil that is attached to the feed and those minerals are often in the oxide form (i.e., low availability). This suggests that feeds with substantially higher ash and trace mineral concentration than typical likely have AC that are lower than the NRC values for trace minerals. Concentrations of trace minerals substantially greater than median value should be discounted, but an exact discount cannot be calculated at this time; but those feeds would still contain some available mineral.

On average (and remember the issues with using averages), unsupplemented diets for lactating cows in the US based mostly on corn silage, alfalfa, corn grain, and soybean meal contain 7 to 9 ppm Cu, 25 to 35 ppm Mn, and 30 to 40 ppm Zn (specific farms may differ greatly from these ranges). For an average Holstein cow (75 lb of milk/d and 53 lb of dry matter intake (**DMI**)) using NRC requirements, basal ingredients supply about 80%, 235% (do not believe this), and 75 % of requirements for Cu, Mn, and Zn. Ignoring minerals supplied by basal ingredients can result in substantial over formulation for trace minerals.

EVALUATING TRACE MINERAL STATUS

The primary indicators of trace mineral status are often sick or poor producing animals. For both research purposes and practical diet formulation, more sensitive indicators or markers of mineral status are clearly needed. These would improve our ability to evaluate requirements, mineral sources, and diet adequacy. No biological measures are known which accurately reflect Zn, Mn, and Cr status in cattle. Plasma (or serum) Zn may be able to discern severe or clinical Zn deficiency, but too many other factors influence serum concentrations to make it a sensitive marker of Zn status (Kincaid, 2000). Cleft palate and other birth defects in calves (Hansen et al., 2006) are specific indicators of clinical Mn deficiency, but markers of marginal deficiencies have not been identified. New, enhanced analytical methods (mass spectroscopy) have greatly increased our ability to accurately measure plasma Mn and with additional research, plasma and liver Mn concentrations may have value as a status indicator.

Copper is stored in the liver and liver Cu concentrations are currently considered the gold standard for evaluating Cu status. Adult cattle liver Cu concentrations are deemed *adequate* between 120 – 400 mg/kg on a DM basis or approximately 30 – 110 mg/kg on a wet weight basis (McDowell, 1992). Over supplementation of Cu can result in Cu toxicity. Therefore, the range of adequate Cu status reflects both the minimum (110 or 30 mg/kg) and maximum (400 or 120 mg/kg) recommended concentrations of liver Cu on a DM or wet weight basis, respectively. The recommended range for liver Cu is the same for both Jerseys and Holsteins; however, livers from Jersey cows will usually have a greater concentration of Cu than those from Holsteins when fed similar diets. Liver Cu concentrations decrease when cattle are fed diets deficient in Cu and increase in a systematic manner as dietary Cu supply increases (Yost et al., 2002) which fits important criteria of a good marker of mineral status. Other Cu measures (e.g. enzyme activity, ceruloplasmin concentration) have been suggested as indicators of Cu status. However, liver Cu is mobilized during depletion to support cellular function and changes in enzyme activity or ceruloplasmin do not reflect status until the liver is depleted of the majority of its Cu stores.

Cobalt has no known nutritional function other than as a component of vitamin B_{12} so when we refer to Co status we really mean vitamin B_{12} status. Liver B_{12} concentrations reflect Co intake. Assumed adequate hepatic B_{12} concentrations are between 200-400 nmol/kg on a wet weight basis (Stangl et al., 2000). Similar to Cu, liver biopsies to determine B_{12} concentrations and subsequent Co status are invasive and not practical on a large scale (vitamin B_{12} is also difficult to measure). Dramatic increases in plasma concentrations of methylmalonic acid and homocysteine are able to indicate Co deficiency in cattle, but these metabolites are not sensitive enough to detect optimal Co status of cattle. (Stangl et al., 2000).

Selenium status of cattle can be evaluated by assaying Se concentrations in blood. Based on the

effects of Se supplementation on various biological responses, adequate serum (Weiss, 2005) and whole blood (Kommisrud et al., 2005) Se concentrations are around 0.06 µg/mL and 0.15 µg/mL, respectively. About 60 % of the Se in whole blood is in the erythrocytes, which have a half-life of almost 100 d in cattle. Therefore, whole blood Se is a more accurate long-term indicator of Se status compared to plasma or serum, which reflects short-term changes in Se intake. Whole blood glutathione peroxidase activity is often assayed to determine relative bioavailability of Se sources. However, glutathione peroxidase activity is somewhat dependent on the lab so adequacy must be evaluated compared with lab reference values. Selenium supplementation has been shown to increase Se concentrations in milk, but the relationship is highly dependent on Se source (Weiss, 2005). Concentrations also are usually lower than those found in plasma and can be difficult to measure accurately.

RECOMMENDATIONS

Chromium

Chromium is a required nutrient; however, the NRC (2001) did not provide a quantitative recommendation. Furthermore, feeding diets with more than 0.5 ppm of supplemental Cr or from sources other than Cr propionate is not currently legal in the U.S. Cr is needed to transport glucose into cells that are sensitive to insulin. Because of analytical difficulties (e.g., normal grinding of feeds prior to chemical analysis can contaminate them with Cr) we do not have good data on Cr concentrations in feedstuffs. Some studies with cattle have shown that supplemental Cr (fed at 0.4 to 0.5 ppm of diet DM) reduced the insulin response to a glucose tolerance test (Sumner et al., 2007; Spears et al., 2012). Elevated insulin reduces glucose production by the liver and enhances glucose uptake by skeletal muscle and adipose tissue. These actions reduce the amount of glucose available to the mammary gland for lactose synthesis and this may be one mode of action for the increased milk yield often observed when Cr is supplemented. Most of the production studies evaluating Cr supplementation (studies used Cr propionate, Cr-Met, Cr-picolinate and Cr yeast) started supplementation a few weeks before calving and most ended by about 6 wk postpartum. Supplementation rates varied, but most were 6 to 10 mg/day (approximately 0.3 to 0.5 mg Cr/kg of diet DM). The median milk response from 30 treatments from 14 experiments was +4.1 lb/d (the SD among responses was 3.5 lb/d). About 75 % of the treatment comparisons yielded an increase in milk of more than 2 lb/d. Although a comprehensive meta-analysis is needed, based on this preliminary analysis of studies, increased milk yield of at least 2 lb/d is highly probable when approximately 0.5 ppm Cr is supplemented to early lactation cows. Whether this response would be observed throughout lactation is not known. The potential return on investment from milk can be calculated by using the value of milk and cost of feed plus the cost of the supplement and assuming a median response of about 4 lb/d of milk and an expected increase in DMI of about 2.8 lb. At this time, a milk response should only be assumed to occur up to about 42 DIM.

Cobalt

The current NRC requirement for Co is expressed on a concentration basis (i.e., 0.11 ppm in diet DM) rather than mg of absorbable Co/d basis. This was done because Co is mostly (perhaps only) required by ruminal bacteria and the amount they need is a function of how much energy (i.e., feed) is available to them. Although Co concentration data for feeds is very limited, the NRC requirement is for total Co and in many cases, basal ingredients would provide adequate Co. In studies conducted in WA, basal diets contained 0.2 to 0.4 ppm Co (Kincaid et al., 2003; Kincaid and Socha, 2007) but basal diets from WI contained 1 and 2 ppm Co (Akins et al., 2013). Data using growing beef animals (Stangl et al., 2000) found that liver B_{12} was maximal when diets contain 0.22 ppm Co (approximately twice as high as current recommendation). With dairy cows, liver B_{12} concentrations continued to increase as supplemental Co (from Co glucoheptonate) increased up to 3.6 ppm (Akins et al., 2013). In that study elevated liver B_{12} did not translate into any health or production benefits. Indicating that maximal liver B_{12} may not be necessary. Milk production responses to increased Co supplementation have been variable. One study reported a linear increase in milk yield in multiparious cows, but no effect in first lactation animals when supplemental Co increased from 0 to about 1 ppm. Older cows tend to have lower concentrations of B_{12} in their livers which could explain the parity effect. Based on current data, the NRC (2001) requirement does not result in maximal liver B_{12} concentrations in dairy cows. Across studies, when total dietary Co (basal plus supplemental) was about 1 to 1.3 ppm, maximum milk responses were observed. In some locations, basal ingredients may provide that much Co.

Copper

The NRC (2001) requirement for Cu is expressed on a mg of absorbable Cu/d basis and over a wide range of milk yields (40 to 150 lb), requirements range from about 7 to 15 mg of absorbed Cu /d under normal conditions. Because Cu is secreted in milk, as milk yield increases, the NRC requirement for Cu increases slightly. However, because DMI (and Cu intake) usually increases as milk yield increases, the dietary concentration of Cu needed to meet the requirement may not change as milk yield increases. Contrary to popular practice, diets for pens of high producing cows often do not need to contain higher concentrations of many trace minerals than diets for lower producing cows. Whereas fresh cow pens and dry cows, because of low DMI, often need to be fed diets with increased concentrations of trace minerals.

All trace minerals have antagonists that reduce absorption, but often these do not occur in real situations. All trace minerals are toxic, but for most of the minerals the intakes needed to produce toxicity are usually quite high. Copper, however, is unique among nutritionally important minerals in that it is toxic at relatively low intakes, which should dictate caution regarding over supplementation. On the other hand, Cu has numerous real world antagonists which mandate the need to over supplement in several situations. The NRC requirement assumes no antagonism (e.g., dietary S at 0.2 % of DM); however several situations commonly exists which result in reduced Cu absorption including:

- Excess intake of sulfur (provided by the diet and water),
- Excess intake of molybdenum (effect is much worse if excess S is also present),
- Excess intake of reduced iron (may reduce absorption and increase Cu requirement),
- Pasture consumption (probably related with intake of clay in soil), and
- Feeding clay-based 'binders'.

Most of these antagonisms have not been quantitatively modeled, and specific recommendations cannot be provided. When dietary sulfur equivalent (this includes S provided by the diet and the drinking water) is > 0.25 to 0.3 %, additional absorbable Cu should be fed. At higher concentrations of dietary equivalent S (0.4 to 0.5 %), cows may need to be fed 2 to 3 X NRC requirement when Cu sulfate is used. As an approximation, for an average lactating Holstein cow, for every 100 mg/L (ppm) of S in water add 0.04 percentage units to the S concentration in the diet to estimate dietary equivalent S. For example, if your diet has 0.26 % S and your water has 500 mg/L of S, dietary equivalent S = 0.26 + 5*0.04 = 0.46 %. Note that some labs report concentrations of sulfate, not S. If your lab reports sulfate, multiply that value by 0.333 to obtain concentration of S. In most situations dietary S will be < 0.25 % of the DM. Diets with high inclusion rates of distillers grains and diets that contain forages that have been fertilized heavily with ammonium sulfate can have high concentrations of S. Water S concentration is dependent on source. Water should be sampled and assayed on a regular basis (at least annually) to determine whether water is adding to the S load in the diet.

Although the presence of an antagonist justifies feeding additional absorbable Cu or using Cu sources that are more resistant to antagonism, no data are available indicating that the current NRC requirement is not adequate under normal conditions. Because of uncertainties associated with AC and the actual requirement, a **modest** safety factor should be used when formulating diets. Under normal situations, feeding 1.2 to 1.5 X NRC can be justified for risk management and it also should prevent excessive accumulation of Cu in tissues over the life of the cow. For an average lactating cow, NRC requirement for absorbed Cu is about 10 mg/day. Applying the 1.2 to 1.5 X safety factor, the diet should be formulated to provide between 12 and 15 mg of absorbed Cu/d. For an average Holstein cow fed a diet without any antagonists and using Cu sulfate as the source of supplemental Cu, the diet should be formulated to contain 12 to 15 ppm of **total** Cu (i.e., basal + supplemental). If using a Cu source that has higher availability than Cu sulfate, the safety factor would be the same; but because of a greater AC, the concentration of total Cu in the diet would be less because less supplemental Cu would be needed.

If antagonists are present, the NRC (2001) overestimates absorbed Cu supply and Cu supply will need to exceed NRC requirements. For an average Holstein cow fed a diet with substantial antagonists, total dietary Cu may need to be 20 ppm, or perhaps more, to provide 12 to 15 mg/d of absorbed Cu. Some specialty Cu supplements are less affected by antagonism (Spears, 2003) and under antagonistic conditions, those sources of Cu should be used.

Adequate absorbable Cu must be fed to maintain good health in dairy cows, however excess Cu is detrimental to cows. Acute Cu toxicity can occur but of a greater concern are the effects of long term overfeeding of Cu. When cows are overfed Cu, liver

Cu concentrations increase. If Cu is overfed for a short period of time (i.e., a few weeks) the change in liver Cu may be insignificant, but when Cu is overfed for many months, liver Cu concentrations can become dangerously elevated. Jerseys are at higher risk of Cu toxicity because they accumulate greater amounts of Cu in the liver than Holsteins (Du et al., 1996) however, toxicity can occur in Holsteins.

In non-lactating cows that were in good (or excess) Cu status and fed diets with approximately 20 ppm total Cu, liver Cu accumulated at an average rate of 0.8 mg/kg DM per day (Balemi et al., 2010). Although milk contains Cu, because of differences in DMI (and subsequent Cu intake), this accumulation of liver Cu is likely similar to a lactating cow fed a diet with 20 ppm Cu. Over a 305 d lactation, a cow fed a diet with ~20 ppm Cu (without antagonists) could accumulate ~250 mg/kg DM in the liver. Over 2 or 3 lactations, liver Cu concentrations would become extremely high. Classic toxicity is thought to occur when liver Cu concentrations are > 2000 mg/kg DM. Beef cattle are tolerant to extremely high liver Cu concentrations, and many of the studies used to establish the upper limit for liver Cu used beef cattle. However, beef cattle usually have short lifespans and may not be good models for dairy cows. Chronic copper poisoning is subclinical and can cause liver degeneration, which is evident based on elevated liver enzyme (AST and GGT) activities in plasma (Bidewell et al., 2012). Accumulating evidence suggests problems may start occurring at much lower concentrations of liver Cu (500 or 600 mg/kg DM). Activity of AST and GGT were significantly greater in heifers and bulls that had average liver Cu concentrations of 640 mg/kg DM compared with animals with average liver Cu of 175 mg/kg DM (Gummow, 1996). What was considered acceptable overfeeding of Cu (e.g., ~20 ppm supplemental Cu) may result in problems because of the duration of the overfeeding.

Manganese

The 2001 NRC greatly reduced the requirement for Mn compared with the earlier NRC. Based on NRC (2001) most lactating cows need between 2 and 3 mg/d of absorbable Mn, which based on typical DMI translates to 14 to 16 ppm of total Mn in the diet. However, the 2001 NRC probably greatly overestimated the AC for Mn. Seventy percent of the calves borne from beef heifers fed a diet with about 16 ppm Mn for the last 6 m of gestation displayed signs of classic Mn defiency (Hansen et al., 2006). Using Mn balance studies in lactating cows (Weiss and Socha, 2005; Faulkner, 2016), we estimated that lactating cows (average milk yield in the experiment = 84 lb/d) needed to consume about 580 mg of Mn to be in Mn balance. Based on the DMI in those experiments, that translated into a dietary concentration of ~30 ppm for total dietary Mn. As discussed above uncertainty exists and reasonable safety factors (i.e., 1.2 to 1.5 X) should be applied. For Mn, the starting point is 30 ppm and after the safety factor is applied, diets for lactating cows should have 36 to 45 ppm **total** Mn.

VITAMINS

Because of very limited data, the term requirement should not be used for vitamins. Rather we should use the term *Adequate Intake* or **AI**. This is the quantity of vitamin that has been shown to prevent health problems or result in statistically reduced prevalence or severity of disease. Some vitamins increase milk yields, but because effects on milk yields must be put into economic context (i.e., price of milk, price of feed, and cost of the vitamin) milk yield response should not be a major factor when setting AI. However this does not mean that supplementation rates that increase milk yield but do not affect health should not be used in situations where they are profitable. Data on concentrations of vitamins in basal ingredients is extremely limited or lacking entirely which adds to uncertainty. Concentrations of certain vitamins in feeds can be extremely variable (e.g., concentrations of tocopherol in hay crop forages can range from almost 0 to more than 150 ppm). Because supply of vitamins from basal ingredients will almost never be known, AI are usually based on supplemental vitamins. Adequate data are available to determine AI for biotin, niacin, and vitamins A, D, and E.

Vitamin A

NRC (2001) recommendations for vitamin A appear adequate for average cows (i.e., 110 IU of supplemental vitamin A/kg of BW). For a typical Holstein cow that equals about 70,000 IU/d. Milk contains about 0.3 mg of retinol/kg; therefore, high producing cows can secrete substantial amounts of A into milk. The average cow in the NRC (2001) database produced about 35 kg of milk/d (77 lb). For cows producing > 35 kg of milk, feeding an additional 1000 IU of vitamin A/d/kg of milk > 35 kg will replace what is secreted in milk (about 450 IU/lb of milk above 77 lb). For example for a Holstein cow producing 70 lb of milk/d, the adequate intake of vitamin A is 70,000 IU; but for a cow producing 100 lb of milk, the adequate intake would be 70,000 + [(100-77)*1000] = 93,000 IU/day. No data are

available showing NRC (2001) vitamin A recommendations for dry and prefresh cows are not adequate.

Vitamin D

Calcium homeostasis was long considered the primary function of vitamin D, but its effects on cells and animals go far beyond Ca including effects on immune function and health. The 2001 NRC requirement (30 IU of supplemental vitamin D/kg BW or about 20,000 IU/d for a Holstein cow) is adequate with respect to Ca; however it may not be adequate for optimal immune function. Using a plasma concentration of 30 ng of 25-hydroxyvitamin D/ml to indicate sufficiency, 45 or 50 IU/kg BW (about 30,000 IU/d) may be needed for lactating cows (Nelson et al., 2016). Cows that spend a few hours outside during summer months probably synthesize adequate vitamin D, but sun exposure in winter (in the US) probably lacks intensity for adequate synthesis rates.

Vitamin E

The 2001 NRC recommendations of 500 and 1000 IU/d of supplemental vitamin E for lactating and dry cows are adequate; however, sufficient data exists to justify increasing supplementation to 2000 IU/d during the last 14 to 21 d of gestation. This rate of supplementation has reduced early lactation mastitis and metritis.

Other Vitamins

Adequate consistent data exist to set the AI for supplemental biotin at about 20 mg/d. This inclusion rate often improves hoof health and milk production (Lean and Rabiee, 2011) . Niacin supplementation has been extensively researched, but the data are equivocal; about half the studies report a benefit and half report no effect. Supplementation at 12 g/d is more likely to elicit a production response (increased milk yield and milk component yields) in early lactation cows than the commonly used rate of 6 g/d. The majority of data do not support the use of niacin to reduce ketosis. Therefore, in most situations, the AI of supplemental niacin is likely 0. Supplemental rumen-protected choline usually increases milk yield in early lactation (Sales et al., 2010) and may help reduce fatty liver. The common supplementation rate is 12-15 g of actual choline/d but the choline must be rumen protected. Because the data on health is equivocal at this time, choline does not have an AI, but it may often be profitable because of its effect on milk yield. Supplemental B_{12} is not usually needed when diets contain adequate Co. Several experiments have been conducted with folic acid and many, but not all, showed a positive milk yield response. Questions remain regarding route of supplementation, rate of supplementation, and profitability; consequently at this time no AI can be established.

CONCLUSIONS

Adequate supply of trace minerals and vitamins improves the health and productivity of dairy cows; excess or inadequate trace nutrients can have the opposite effect. The 2001 NRC requirements for Cu, Zn, Se and vitamin A are adequate in most situations and only a modest safety factor should be applied for risk management. Because of regulations, no safety factor can be applied to Se. For Cu, numerous antagonists exist and in those cases, diets need to provide substantially more Cu than recommended by NRC or a high quality organic Cu should be fed. Although many situations dictate higher concentrations of dietary Cu, be aware of excessive Cu supplementation. Modest overfeeding of Cu for months or years can result in high liver Cu concentrations that may be negatively affecting cow health. Manganese requirement is likely much higher than 2001 NRC and Co requirement also likely needs to be increased. Cows benefit from greater amounts of supplemental vitamin E during the prefresh period and lactating cows without great sun exposure may benefit from additional vitamin D supplementation.

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Using Cow Monitoring for Nutritional Goals

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INTRODUCTION

Precision dairy monitoring is "the use of information and communication technologies for improved control of fine-scale animal and physical resource variability to optimize economic, social, and environmental dairy farm performance (Eastwood et al., 2012)." Precision dairy monitoring inherently lends itself to an interdisciplinary approach of different disciplines among informatics, biostatistics, ethology, economics, animal breeding, animal husbandry, animal nutrition, and process engineering (Spilke and Fahr, 2003). Precision technologies are successful in other industries. Originally, precision technologies started with confined swine and poultry and was named precision livestock farming (Frost, 2001). Though precision technologies originated in swine and poultry, they are successfully adaptable to many different species (Frost, 2001). However, cattle add a complexity to proper use of systems (Wathes et al., 2008).

Sensors fall into 2 categories that measure the response variable: attached or un-attached (Rutten et al., 2013). An attached sensor is one that is either on the cow, for example fitted to the cow's body with a strap, or is in the cow, as is the case with rumen sensing boluses. Un-attached sensors are ones that a cow can walk past, through, or over. Two specific forms of un-attached sensors sense a response variable in-line or on-line. An in-line sensor senses the response variable continuously, and sits in the milk line. On-line sensors take a sample automatically that is then analyzed by the sensor (Rutten et al., 2013). Technologies can be divided into 4 different processes that help alert the producer to a health event:

1) The technology that measures variables (e.g. activity),
2) The measured variable information is used in an algorithm that will provide information about the cow,
3) The information is used to provide advice in a decision support type model combined with economic information or other information devised from the producer and,
4) The decision about the health event is made by the producer, or is autonomously made by the technology itself.

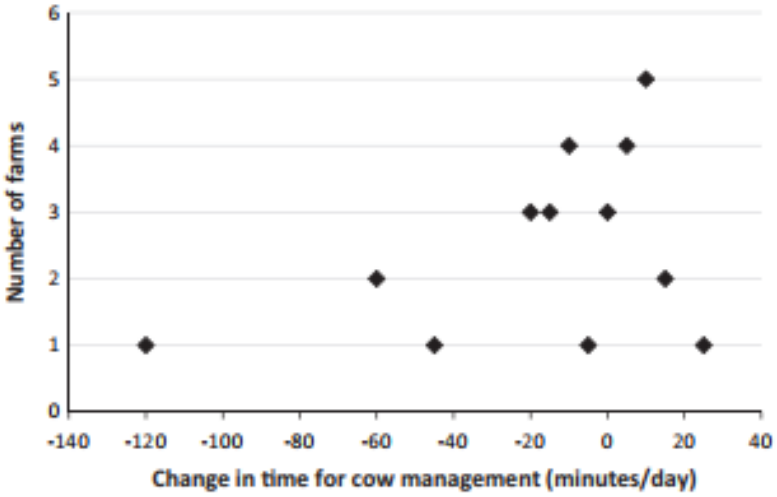
As the worldwide trend continues with smaller numbers of larger dairy farms (Bewley, 2010), producers have less time to monitor their herd; therefore, precision dairy monitoring technologies are able to help them monitor (Van Nuffel et al., 2015) and manage cattle individually (Wathes et al., 2008). Precision dairy monitoring technologies are becoming a reality as labor costs increase on farms (Rutten et al., 2013). Automatically measuring different behaviors saves producers' time and is less subjective (Bewley et al., 2010). However, a clear disconnect exists in the focus area of precision dairy monitoring technology research and on-farm application for the end user. Producers may not have established clear priorities yet for technology use and the market may be driving the research and application of precision dairy monitoring technologies (Wathes et al., 2008).

The dairy industry has gone through rapid changes in the last few years. Dairy producers have conventionally relied on labor; however, with technological advances more farms have adopted technology (Khanal et al., 2010). Khanal et al. (2010) found that larger farms adopted technology more than smaller farms, suggesting an economies of size benefit. As farms become larger, the amount of time spent with each cow diagnosing problems decreases. Utilizing a precision dairy monitoring technology could help producers move from reactive management to proactive management (Eastwood et al., 2012). Therefore, using precision dairy monitoring technologies can aid in early detection of health events (de Mol et al., 2013).

BENEFITS OF PRECISION DAIRY MONITORING TECHNOLOGIES

Benefits of precision dairy monitoring technologies are available both to the producer and the animal. Apparent benefits include increased efficiency, improved product quality, reduced economic costs, reduced opposing environmental impacts, and improved animal health and well-being (Bewley, 2010). Producer time budgets is also a

Figure 1. Farms using conventional milking systems decreased time spent on cow management on average by 10 min/d after investing in technologies[1].



[1]Figure was reproduced from Steeneveld, et al. (2015).

perceived benefit of adopting precision dairy monitoring technologies. Steeneveld et al. (2015) found that as producers adopted technologies, less time was spent on cow management (Figure 1). Though the researchers did not state exclusively, producers may have allocated more time toward important aspects of the farm, like business management. Automatic detection is an important piece of precision dairy monitoring technologies (Neethirajan, 2017). Early detection allows for more rapid recovery, reducing the spread of the disease, reducing the misuse of antibiotics, and reducing the related production, social, and economic consequences (Neethirajan, 2017).

Investment in Technologies

Although benefits of precision dairy monitoring technologies exist, adoption of these technologies is relatively slow and low compared to other industries (Bewley et al., 2010; Russell and Bewley, 2013). Adopting and applying a technology presents a significant investment for a producer; one which often has the challenge of choosing a single technology that will serve the producer for many years (Borchers and Bewley, 2015). More research is needed in investment economics and accuracy of technologies on farms because acquiring an unproductive technology could be detrimental to a producer; therefore, investments are made with caution (Borchers and Bewley, 2015). Borchers and Bewley (2015) designed a survey to assess considerations producers use to invest in a technology and to evaluate variables measured by technologies producers find most useful. Table 1 displays the standards that producers use when considering precision dairy monitoring technology adoption and their importance. Producers considered benefit-to-cost ratio as the most important criteria when investing in a technology, highlighting the need for more investment economic research. Table 2 displays the variables producers found most useful when using precision dairy monitoring technologies.

Before investing in a precision dairy monitoring technology, producer considerations and questions asked of the technology company may include:

1) What is the cost of the technology?
2) Are all technology parts under warranty?
3) How will the technology be used to manage the herd?
4) What is the customer service model of the company?
5) Is representation of the company available in the producer's area?
6) What is the sensitivity/specificity of the variable of interest?

Table 1. Standards producers use when considering precision dairy monitoring technology adoption and their importance[1].

Standard	Response, %				
	Unimportant	Of little importance	Moderately important	Somewhat important	Important
Benefit to cost ratio	0.9	0.0	3.7	31.5	63.9
Total investment cost	0.9	1.8	12.8	36.7	47.7
Simplicity and ease of use	0.9	0.9	10.1	47.4	40.4
Proven performance through independent research	1.9	0.0	7.5	53.3	37.4
Availability of local support	1.8	3.7	17.4	34.9	42.2
Compatibility with existing dairy practices and systems	0.9	4.6	11.9	46.8	35.8
Time involved using the technology	1.9	2.8	15.7	45.4	34.3

[1]Information for the table was reproduced from Borchers and Bewley (2015).

Validation and Usefulness of Technologies

Validation of technologies demonstrates that precision dairy monitoring technologies are viable for use in dairy cattle operations for management purposes. Third party groups validate many technological variables; however, not all are validated and the need for validation is strong. Many similar variables may have different results when measured on the same cow simultaneously. This may be due to the exact way the technology measures the variable, along with the algorithm the technology company has devised to output the measurement value. These differences may not mean that either technology variable is right or wrong, it may just mean that the measurement of the variable for each technology is different.

Validation of different precision dairy monitoring technologies that may help with meeting producers' nutritional goals is done by third party vendors. Canadian researchers compared the Hi-tag (SCR Engineers Ltd., Netanya, Israel) to observations made by 2 humans to validate measures generated. The researchers discovered that human observations and Hi-tag data were highly correlated, r = 0.96; $P < 0.001$, r = 0.92; $P < 0.001$, and r = 0.96; $P < 0.001$ in three trials (Schirmann et al., 2009). Similarly, Borchers et al. (2016) validated feeding and rumination behaviors in the CowManager® SensOor™ ear tag (Agis, Harmelen, the Netherlands), Smartbow® ear tag (Smartbow GmbH, Jutogasse, Austria), and Trackacow leg tag (ENGS, Rosh Pina, Israel). Where CowManager SensOor and Trackacow measured feeding behaviors; CowManager SensOor and Smartbow measured rumination behaviors. For feeding behaviors, CowManager SensOor and Trackacow both correlated well with visual observation at r = 0.88; $P < 0.01$ and r = 0.93; $P < 0.01$, respectively. For rumination behaviors, CowManager SensOor was less strongly correlated with visual observation than Smartbow at r = 0.69; $P < 0.01$ and r = 0.97; $P < 0.01$, respectively. Bikker et al. (2014) also found that the CowManager SensOor rumination time was also highly correlated to visual observation (r = 0.93; $P < 0.01$) and that eating time was less strongly correlated to visual observation (r = 0.86; $P < 0.01$). Kaniyamattam and De Vries (2014) found that an AfiLab real-time milk analyzer (Afimilk, Kibbutz Afikim, Israel) was not always in agreement with a Bentley 2000 analyzer (Bentley Instruments Inc., Chaska, MN); where fat, protein, and lactose correlations were 0.59, 0.67, and 0.46, respectively. Lohölter et al. (2013) validated a pH bolus (KB 3/04 bolus, Kahne Limited, New Zealand) and found that it was moderately correlated to manual pH measurements (r = 0.59; $P < 0.01$).

For producers to use technology for herd management purposes, the purchased technologies must perform at optimal levels. Researchers have removed data from cows or have removed cows entirely from data sets in research projects due to technologies not performing at optimal levels (de Mol et al., 2013; Borchers et al., 2016; Stone et al., 2017). In fact, de Mol et al. (2013) found that only 78 % of cow days in the model had viable measurements. The researchers found that the unreliability of the technology made it difficult to collect data consistently and stated that automated monitoring is only useful when technology systems are functioning at optimal performance levels.

Table 2. Variables producers find most useful when using precision dairy monitoring technologies[1].

Variable	Response, %				
	Not useful	Of little usefulness	Moderately useful	Somewhat useful	Useful
Mastitis	0.0	0.0	1.9	19.4	78.7
Standing estrus	0.0	0.9	2.8	16.5	79.8
Daily milk yield	0.0	09	6.4	11.9	80.7
Cow activity	1.8	1.8	5.5	16.5	74.3
Temperature	3.8	2.8	11.3	22.6	59.4
Feeding behavior	0.9	0.0	15.7	35.2	48.1
Milk components	0.9	4.6	13.8	27.5	53.2
Lameness	0.0	4.6	17.4	26.6	51.4
Rumination	3.8	3.8	18.9	28.3	45.3
Hoof health	0.9	3.7	19.4	39.8	36.1
Rumen activity	4.6	3.7	24.1	27.8	39.8
Lying and standing behavior	2.8	8.3	25.7	33.9	29.4
Rumen pH	5.5	11.0	26.6	29.4	27.5
Jaw movement and chewing activity	4.6	13.0	25.9	29.6	26.9
Respiration rate	7.5	13.2	29.2	32.1	17.9
Body weight	8.3	18.5	30.6	24.1	18.5
Body condition score	9.2	12.8	36.7	25.7	15.6
Heat rate	11.2	16.8	38.3	21.5	12.1
Animal position and location	19.3	23.9	31.2	13.8	11.9
Methane emissions	34.3	30.6	20.4	10.2	4.6

[1]Information for the table was reproduced from Borchers and Bewley (2015).

Economics of Technologies

Investing in precision dairy monitoring technologies is usually a complicated chore. The standard net present value approach can be misleading and the costs and benefits of acquiring new technologies is often complex and requires interactions of many variables (Bewley et al., 2010). A real dearth of information exists for economics of investing in technologies, especially when using the technologies to detect health events. However, Steeneveld et al. (2015) researched the overall economics of investing in technologies. The researchers discovered that farms using automated milking systems had a total capital costs of €9.72 and €13.97/100 kg of milk before and after technology adoption, respectively. However, labor costs and variable costs did not change. For farms with conventional milking systems economic change did not occur for capital costs, labor costs, or variable costs after implementing a precision dairy monitoring technology. Farms with automated milking systems saw an increase in total revenue from €43.93 to €46.38/100 kg of milk before and after technology adoption, respectively. The authors speculated that the increased revenue may have been from the increase in milk production after technology implementation. Farms with conventional milking systems saw no change in revenues after technology adoption.

Available Precision Dairy Monitoring Technology Variables

The amount of technologies and variables being measured on the market is growing and in some sense, is saturated for a few measured variables. As precision dairy monitoring technologies grow, new variables and ways to monitor these variables have been fashioned (Borchers and Bewley, 2015). Variables currently measured include daily milk yield, milk components, step number, body temperature (at various places on or within the cow), milk conductivity, automatic estrous detection, daily body weight (Bewley, 2010), animal position/location, blood in milk content, activity (neck, head or total activity), jaw movements and chewing activity, lameness, progesterone, **LDH** (lactate dehydrogenase), **BHB** (beta-hydroxybutyrate), lying times, lying bouts, standing time, mastitis, milk flow, milk time, milk yield, rumen pH, somatic cell count, standing heat, vacuum in milk line, rumination time, feeding time, feeding bouts (Borchers and Bewley, 2013), and body

condition score. Other proposed measured variables include reticular contractions, heart rate, vaginal mucus electrical resistance, odor, glucose, acoustics, color (an indicator of cleanliness), infrared udder surface temperature, and respiration rates (Bewley, 2010).

Utilizing Precision Dairy Monitoring Technologies for Nutritional Goals

When trying to meet nutritional goals, rumination and feeding behavior monitors are at the forefront of one's mind; however, automated body condition scoring, in-line sensors monitoring milk components, and rumen pH boluses will also aid in meeting nutritional goals. Rumination and feeding behavior monitors historically have been used to monitor and detect health events instead of meeting producer's nutritional goals. This empirical data is missing, especially feed intake for individual cows, which can hinder herd management decision making (McParland and Berry, 2016). Bach et al. (2007) discerned differences in automatically recorded feeding behavior between lame and sound cows and Van Hertem et al. (2013) discerned differences in automatically recorded rumination behavior for lame cows on day of lameness diagnosis compared to sound cows. Gonzalez et al. (2008) discovered that cows diagnosed with ketosis had decreased feed intake, feeding time, and feeding rate automatically recorded by roughage intake control feeders.

Body condition score assesses body reserves on an animal and can be used as an indirect gauge of reproductive and health status of an animal. Body condition reflects energy balance in cows (Fischer et al., 2015). Bewley et al. (2008) used digital images to discern body condition scores accurately. However, researchers stated that future efforts should automate this system to predict body condition scores. An automated system does exist currently, but more research is warranted for on-farm application.

Dairy farms offer a unique environment in that 2 to 3 times daily, cows are milked, offering a biological sample that could be used to analyze in-line the physiological state of the cow. Therefore, the daily analysis of milk and milk constituents provides a way to conduct daily farm management and decision making (McParland and Berry, 2016). Infrared spectroscopy (the scattering of light) is used to quantify milk quality variables already; therefore the information already gathered can be applied directly to on-farm applications. McParland and Berry (2016) discovered that the accuracy of predicting energy intake, energy balance, and feed efficiency was 0.88, 0.78, and 0.63, respectively, using spectroscopy. The authors did state that further investigation is warranted for on-farm applicability.

Subacute ruminal acidosis is when the rumen pH is below 5.5 for 3 h/d (Stone, 2004; Blowey, 2015). Continuous sensing of rumen pH may help determine the state of subacute ruminal acidosis in cattle (Lohölter et al., 2013); however, sensor drift when measuring rumen pH is a real concern. Further investigation on sensor drift is warranted (Lohölter et al., 2013). When using rumen pH boluses, it is unclear if the bolus depicts clear value to the producer (Rutten et al., 2013). The same may also be true with other sensors, but just because automation of a variable can occur, it does not necessarily mean as an industry we know what to do with the data. As an example, if we automatically detect daily body condition scores on cows as they exit the parlor, how would the producer use that knowledge to better manage the herd. As an industry, more work needs to be conducted to evaluate how the vast amount of information on individual cows may help with farm management and decision making on-farm. With all sensors, there is a need for improvement, heightened detection, and better data performance (Rutten et al., 2013).

CONCLUSION

Precision dairy monitoring technology is still in the early stages of development. Therefore, when investing in a technology producers may want to evaluate the following aspects:

1) What is the cost of the technology?
2) Are all technology parts under warranty?
3) How will the technology be used to manage the herd?
4) What is the customer service model of the company?
5) Is representation of the company available in the producer's area?
6) What is the sensitivity/specificity of the variable of interest?

More research is needed to understand the economic consequences of investing in a technology, especially to target specific health or feeding events. There is still a lack of information surrounding how feeding behavior, rumination behavior, automated body condition score, and rumen pH boluses can help producers meet individual nutritional goals. However, even with the lack of information of how

precision dairy monitoring technologies may be applied to on-farm situations, the future looks bright.

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Vitamin Panel Supplementary Comments:

Aftermath: Global Vitamin Shortage: What did we learn in ruminants?

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Introduction

In late 2017, several factors resulted in an unprecedented drop in global vitamin supply and subsequent rise in prices. This was coupled with outages in some specialty markets, such as certain vitamin forms for liquid feeds. In the case of vitamin A (retinyl acetate) and vitamin D3, prices reached 3 to 10 times greater than previous typical levels and local availabilities were widely affected. These price increases and shortages resulted in many discussions and strategies at all levels of the ruminant feeds supply chain. Nutritionists and producers re-evaluated vitamin supplementation strategies, based on actual or perceived shortages and as an attempt to control input costs. Commonly asked questions included:

1. Why and how did this happen?
2. Are there alternatives to vitamins?
3. How low can we go, for how long, and which segments are most sensitive to vitamin levels?
4. If we reduce vitamin levels, what will happen?

Producers and nutritionists demonstrated notable creativity, resourcefulness, and strategic thinking, utilizing input from university researchers, re-interpretation of "old" data, and simply managing on the belief that green grass would grow quickly in early 2018.

What Happened?

Within weeks of one another, there were two major events that dramatically affected the vitamin supply and pricing (Figure 1). It is important to keep in mind that there are actually very few vitamin plants in the world and each plant only makes a handful of different vitamins.

- Beautiful China Policy: China is a major vitamin producer, and most industries have been affected by strict environmental quality policies. These policies came to a head in late 2017, resulting in total plant shutdowns of some major vitamins A and E producers in China, which affected global supply very quickly.
- BASF Declares Force Majeure: October 31, 2017 fire shuts down citral plant. Citral is an intermediate in both A and E manufacture, and BASF is a major player.

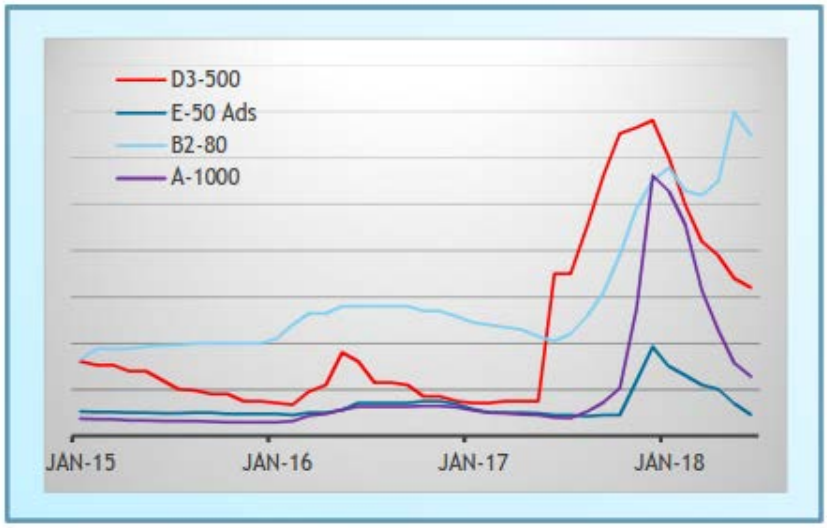

Figure 1 Source: Mark Engstrom

Alternative strategies employed

Additional antioxidants and vitamin sparing. Because vitamins are understood by all to be essential micronutrients, the vast majority of our customers and producers expected that there would be no suitable alternatives for vitamins. That said, many nutritionists and producers looked for ways to stretch or "spare" vitamin resources, especially those vitamins with antioxidant roles. Strategies included improved trace mineral nutrition, inclusion of additional antioxidants, or substituting pro-vitamins compounds, such as beta-carotene. In ruminants the best alternative to supplemental vitamins is the forage supply.

Fresh forages. Forages can supply up to 40,000 IU of vitamin A activity per kg of dry matter in fresh pasture; unfortunately, that drops to near-zero levels following harvest, storage, ensiling, or winter stockpiling. Sunshine converts plant 7-dehydrocholesterol to D3 in the skin of unconfined animals. Fresh plant material can contribute to vitamin E supply but falls far short of being a major contributor.

Injectable vitamins. Injectable micronutrients have become more widespread for a variety of reasons, including shortages and greater promotion by suppliers. A popular injectable trace mineral source provides Zn, Mn, Se, and Cu in a 1 ml/200 lb dose. A 6-ml injection for a beef or dairy cow would provide 360 mg Zn and 30 mg Se, for example, at a cost to the producer of $3 per head. Strategic use would be pre-fresh or at breeding time and, although the product provides no additional vitamins, producer perception is that antioxidant status and improved plane of nutrition are enough to prove beneficial. At $3 per head, MU-Se provides 30 mg Se and 408 IU of vitamin E in an adult cow dose and is in widespread in pre-fresh animals. Although the costs of the additional micronutrients are quite high when compared with in-feed vitamin E and Se,

the perceived and psychological benefits of injecting needed micronutrients at the appropriate time, outweigh the expense in many producers' minds, especially during the last 6 months.

Anecdotal experience and recommendations with reduced vitamin levels

Dairy

With such an extreme supply event, when looking at supplementation strategies anecdotally, the motivation of the consultant or feed company is not always clear. In some cases, clear lack of supply or the threat of running out may have been the prime motivation for cutting levels. In other cases, reducing vitamin levels was purely economic. Unfortunately, dairy economics could not have had poorer timing relative to the vitamin cost run-up. For example, some producers simply directed consultants to keep mineral (base mix) costs the same as before (the run-up). In such cases, vitamins had to be radically reduced in the mineral supplements to comply.

Vitamin nutritional strategies varied somewhat by region. In the Southwest US (marginal milk economics), several dairy consultants greatly reduced vitamin A supplementation, eliminated or radically lowered D3 depending on sun exposure and maintained E levels as high as possible through the close-up phase. Several consultants recommended no supplemental micronutrients in dry cows for 40 days before the closeup period, or depended upon hay supply for whatever vitamins the cow received. As perhaps reported from the depth of the vitamin supply crisis (and just afterward), the most common behavior reported by feed manufacturers and consultants may have been that supplementation levels were generally cut in half with productions classes and time coming into play here. We might argue that this was not particularly aggressive, considering the severity of the shortage and how that affected ration cost. Perhaps this is also the reason that there were few reports of health problems that could clearly be tied to vitamin deficiency.

In the Midwest, Dr. Bill Weiss, the leading university dairy vitamin expert related several strategies used by feed distributors:

1. "Greatly reduced" vitamin A supplementation from 2X NRC to 1X NRC levels: to 75,000 IU/hd/d for both lactating and dry cows. This would be near the bottom of an optimum vitamin nutrition (OVN) range.
2. No change in D3; usually fed within OVN optimum vitamin nutrition range
3. Vitamin E—strategic reductions in dry cows to 1000 IU/hd/d; 500 IU/hd/d for lactating cows. These correspond to the minimally acceptable OVN range.
4. Consultants and feed manufacturers were most reluctant to reduce E levels. This suggests the industry has a good understanding of key vitamin issues including research supporting health parameters and limited capacity for body storage.

Beef

Not surprisingly, there was probably more evidence possible vitamin problems in the field with beef cattle. For example, a veterinarian in Montana cow/calf country contacted DSM, asking about weak/dying calves and vitamin A deficiency. Liver samples were sent to the diagnostic laboratory, and assays showed essentially zero vitamin A (retinol): liver levels were < 1 ppm (normally 1.5 to 4.5 ppm for that age). Forage base was poor, and vitamin supplementation came from "unimpressive" molasses block intake—a perfect storm for calf deficiencies in an early calving season where colostrum is the first/best source of vitamins for newborns, followed by (nonexistent) green pasture.

The winter of 2017-2018 was exceptionally bitter in Alberta. Cow condition was very poor and calf losses were very high in some instances. In Western Canada, university extension personnel, in concert with DSM, provided vitamin education for beef cow/calf producers, and recommended strategically dividing their herds into less and more susceptible segments. A number of feed manufacturers did reduce vitamin levels in mineral supplements and other beef products. The Canadian Feed Inspection Agency relaxed requirements for levels of vitamin A and E in registered feeds until at least September of this year. Even with this latitude, most manufacturers apparently did not reduce these vitamins down to the permissible 50% reduction compared with their registered formula.

In the U.S., a few companies substantially reduced A and E levels in some product lines. Interestingly, economics of reducing vitamin levels did not appear to be effective in increasing sales, that is, when products were clearly marked as having sub-optimal levels of vitamins.

What do we do now?

As prices and supply return to levels before the fourth quarter of last year, most consultants and manufacturers in the ruminant segments have already begun to re-evaluate any temporary reductions in vitamin levels; vitamin A in particular. Were there widespread deficiencies? Clinical symptoms could include: poor calf health, higher abortion rates, higher retained placenta incidence, increased infectious disease—these are non-specific and tough to evaluate in a short-term market disruption, and ruminants have unique resources (forage supply and several month's liver storage of retinol) to manage short term deficiencies., except in beef calves born on poor-quality pasture. Our experience was that consultants/producers were most afraid of E deficiencies (changed these levels the least) and least afraid of reducing/removing biotin (not associated with a deficiency; seen as discretionary).

Effect of altering energy and amino acid nutrition on health and reproductive performance of dairy cows

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SUMMARY

- Nutritional strategies and feeding management during pre-calving and post-calving periods impact health, productivity and fertility of high-producing dairy cows.
- Formulating diets to meet requirements of the cows but avoid over-consumption of energy may improve outcomes of the transition period and lead to improved fertility.
- Management to improve cow comfort and ensure good intake of the ration is pivotal for success.
- Rumen-protected methionine (RPM) added to the diet of Holstein cows during the transition period and early lactation improves the survival rate of preimplantation embryos. Embryonic death has been shown to drop from 19 percent to 6 percent in multiparous cows fed RPM.
- Cows fed RPM have more lipid droplets inside the preimplantation embryo, which could be used as energy by the embryos.
- Impacts of the transition program should be evaluated in a holistic way that considers disease occurrence, productivity, and fertility.

Key words: rumen-protected methionine; energy balance; fertility; transition period

Effect of calf hutch type on calf performance and calf hutch temperature humidity index

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Housing type calves are raised in can have a major effect on the environmental stress the calves' experience. In the Western portion of the United States (Texas, New Mexico, Arizona and California), 92.9% of Dairies house pre-weaned heifers in individual hutches outside (NAHMS, 2011). Plastic hutches are commonly used due to the ease for the producers to clean and move hutches. However, research evaluating differences in calf performance and temperature humidity index differences between hutches is scarce. The objective of this study was to evaluate four different calf hutch designs to determine if differences existed in calf performance and hutch temperature humidity index.

This study was conducted on a commercial calf ranch from July 10, 2017 to October 12, 2017. Calves (n = 120) were placed in one of four types of calf hutches. Eleven calves were removed from the study due to death. Hutch A (Full Open Pro Hutch, Calf-Tel Hampel Corp., Germantown, WI; Figure 1; n = 31) had vents on the top of the rear wall. Hutch B (Pro II Hutch, Calf-Tel Hampel Corp., Germantown, WI; Figure 2, n = 27) had an adjustable rear ventilation door. Hutch C (Pro II Hutch with lower vents, Calf-Tel Hampel Corp., Germantown, WI; Figure 3, n = 25) was similar to Hutch B but had two added circular vents on the rear wall of the hutch. Hutch D (Pro Hutch, Calf-Tel Hampel Corp., Germantown, WI; Figure 4, n = 26) was the same design as Hutch A but was elevated 15.24 cm in the rear by a custom bar lift. Calves were weighed before being placed in the hutches and only calves from 32 to 42 kg were used for the study. Calves were also weighed exiting hutches to evaluate growth performance. Temperature and humidity data loggers (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger U23-001, Onset, Bourne, MA) were placed inside each type of hutch as well as one outside the hutches at calf level to record ambient temperature and relative humidity. Temperature humidity index (**THI**) was computed using the following formula (NOAA and Administration 1976): THI = temperature (^{0}F) - [0.55 – (0.55 × relative humidity/100)] × [temperature (^{0}F) – 58.8]. Statistical analysis was performed in SAS (version 9.4, SAS Institute Inc., Cary, NC). The MIXED procedure was used to evaluate fixed effects of hutch type, farm origin, and initial calf weight and their two-way interactions on weight gain. The GLM procedure in SAS was used to evaluate the fixed effect of hutch type on THI.

No significant differences ($P \geq 0.05$) existed for weight gain between farms. Farms 1, 2, and 3 had overall weight gains of 59.69 ± 1.39, 58.28 ± 2.79, and 58.01 ± 1.28, respectively. This result was not surprising as we did not expect to see weight gain differences between the farms calves were sourced. No significant differences ($P \geq 0.05$) also existed for weight gain between hutches. Hutch A, B, C, and D had overall weight

gains of 60.32 ± 1.76, 59.93 ± 1.93, 57.09 ± 1.95, and 57.31 ± 1.98, respectively. We did not expect to see differences in weight gain as the calves were placed in the hutches within 7 days of each other and had to meet a weight criteria. The calves were also under the same management routine with the only difference being the hutch the calves were housed in. When outside THI was above 77, the THI was significantly different ($P <$ 0.05) between hutches. Hutch A, B, C, and D had THI of 80.38 ± 0.06, 78.78 ± 0.09, 79.21 ± 0.06, and 78.33 ± 0.07, respectively. Hutch D had the least average THI, the custom lift that elevated the hutch may have provided extra air flow to keep the hutch cooler. In conclusion, evaluating different calf hutch types may help producers chose the best hutch for their operation. No differences were observed in calf performance between the different calf hutch types. However, differences were observed in THI between the hutches. More work is needed in the future to evaluate the same study in more typical summer conditions.

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Exploring a U. S. Without Livestock

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What would happen if U.S. farmers stopped producing animals for food? Many have called for a move in that direction to address increasing concerns about U.S. health, eating habits, and climate change. A recent study calculated the impact of eliminating animal agriculture from the U.S. (http://www.pnas.org/content/114/48/E10301). Shifting land usage from food animal production to food crop production increased the total U.S. food supply by 23 percent. But what would the food supply look like? The U.S. now imports 51% and 39% percent, respectively, of the fruits and vegetables that we eat in the U.S. Limitations to growing these crops include climate, suitable soils, and the availability of water. In the study, we assumed that if farmers could profitably grow more of the high value crops, they would already be doing so. In the study, the types of food crops planted to tillable land formerly used for livestock were kept in the same proportions that those crops represent in our current system. Accordingly, most of the additional food produced would include high-calorie crops like corn and soybeans. The 415 million acres of permanent pasture and rangeland would go out of food production.
A complete shift away from food animal production would present major challenges to meeting America's nutritional needs. With no meat, milk, eggs, fish, or cheese in our diets, the U.S. population would not receive enough of several different essential dietary nutrients from the unsupplemented foods that would be available. Eliminating food animals would increase deficiencies in calcium, vitamins A and B_{12} and some important fatty acids. The last are important as they may help to reduce cardiovascular disease and improve cognitive function and vision in infants. Animal food products are the only available, non-supplemental sources of some fatty acids and vitamin B_{12}. Different types of carefully balanced diets -- vegan, vegetarian, omnivore -- can meet a person's needs and keep them healthy, but this study examined balancing the needs of the entire nation with the foods we could produce from plants alone. There's a difference between what's possible when feeding one person versus feeding everyone in the U.S.

Eliminating food animals from U.S. production reduced greenhouse gas emissions from agriculture, but not by the full 49 percent of agricultural emissions that animals currently estimated to contribute. Greenhouse gas increases associated with producing additional food crops, including producing synthetic fertilizer to replace manure, counterbalanced these reductions. Total U.S. greenhouse gas emissions dropped by 2.6 percent without farmed animals.

A take-home message from the study was that we need to expand the way we think about food production to account for the complex consequences of changing any individual piece within the wider food system.

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Feed coproducts: “We’re one, but we’re not the same”

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Introduction

Ruminant production systems, including milk production, have become more efficient over time. When comparing dairying practices and resources needed in 1944 to 2007, Capper et al. (2009) reported that over this time dairy producers used 21% less animals, 23% less feedstuffs, 35% less water, and 10% less land to produce the same one billion kg of milk. Despite this increase in efficiency, increased pressure for land use and high commodity prices over the past decade have increased feed costs for dairy farmers, challenging them to consider less costly sources of protein and fiber ((Bradford and Mullins, 2012). In doing so, feed coproducts have played a major role in dairy nutrition but they also represent a means by which efficiency of human food production is improved. This is because when animals consume coproducts they are using a feed resource that from a human perspective would be considered a waste product (VandeHaar and St-Pierre, 2006). To illustrate this, Karlsson et al. (2018) recently conducted an experiment in which all feedstuffs which could be considered human-edible product were removed from the diet of lactating dairy cows and replaced with feed coproducts. They examined these formulations and their effects on what is known as “human-edible feed conversion efficiency.” This is an index which is determined by measuring human-edible material produced by a system minus the human-edible material used by the same system. In this study replacing human edible material (cereal grains and soybean meal) with human-inedible by-products (beet pulp, DDGS, and canola meal) resulted in a net increase in human food protein production without lowering milk production. Although often advantageous, the use of coproducts in dairy cattle diets may also be challenging because they vary in availability and in chemical composition. The objective of this work is to outline the nutritional value of common feed coproducts and to discuss how those can be effectively included in dairy rations.

Recent Studies on Coproducts

In general, soybean meal is the preferred protein supplement for dairy cattle. This is because it is widely available and high in CP content (Huhtanen et al., 2011). Solvent extracted soybean meal contains approximately 54 % CP and 10 % NDF (DM basis). The rumen undegradable protein (RUP) content is approximately 43 % and this bypass protein is highly digestible (93 %) (National Research Council (U.S.) and Subcommittee on Dairy Cattle Nutrition, 2001)(NRC, 2001). In comparison, the RUP content and intestinal digestibility of RUP (dRUP) of canola meal is lower (36, and 75%, respectively). In contrast, the RUP content and dRUP in DDGS (51, and 85%, respectively) is higher than either soybean meal or canola meal (NRC, 2001). In a recent study conducted at the University of Nebraska-Lincoln experimental diets in which canola meal or DDGS replaced soybean meal and corn were formulated. These diets were fed to lactating Jersey

cows in an energy metabolism facility in which total fecal and urine is collected. Body heat was also indirectly measured using indirect calorimeters. The milk performance of cows consuming diets containing coproducts was very similar; however, modest reductions in the digestibility of CP and NDF were observed in cows consuming canola meal. These effects resulted in a trend in the reduction of net energy balance (energy available for both milk and tissue) for cows consuming canola meal.

We have also recently completed a study comparing blood meal and hydrolyzed feather meal (HFM). In this study HFM was titrated into in the diet of lactating Jersey cows and in doing so blood meal was removed. As above, measures were taken in an energy metabolism facility in which total fecal and urine was collected and body heat was also indirectly measured using indirect calorimeters. In this study there were no difference in feed intake or fat correct milk when HFM was fed. The inclusion of HFM did reduce digestibility of CP and milk protein, but surprisingly, measured supply of energy and that available to tissue and milk increased. The increased supply of energy is likely a result of fat from HFM that supplies digestible energy.

Conclusions

The use of coproducts by the dairy industry is a practice that will continue. Such a practice increases the environmental sustainability of the industry because coproducts are resources that are unfit for human consumption. Feeding studies indicate that these feeds also supply valuable nutrients but subtle differences in availability of nutrients require good understanding of chemical composition and in some cases in vitro testing provides information that can be contribute to our need for knowledge on whole animal nutrient supply and utilization.

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Impacts of various milk replacer supplements on the health and performance of high-risk calves

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INTRODUCTION

High-risk calves have an increased risk for morbidity and mortality due to failure of passive transfer (FPT), high exposure to pathogens, and increased stressors in the first few days of life. Because of the immature anatomy of the gastrointestinal tract and naïve immune system calves are especially susceptible to environmental bacteria and viruses. The main cause of disease in neonatal calves is scours due to *Escherichia coli* or *Salmonella* infections, resulting in a 7.8% mortality rate for pre-weaned heifers on U.S. dairy farms with at least 56.5% of those deaths being attributable to gastrointestinal disease (NAHMS, 2007).

When high-risk calves are exposed to disease there are ways to decrease the risk of morbidity and mortality through supplementation of milk replacer additives. Probiotics and yeast cell wall fractions are increasing in supplementation strategies as studies begin to show advantageous impacts acting with similar health and performance results to antibiotics. Supplementing probiotics and mannanoligosaccharides (MOS) to calves has been shown to increase performance and health measures including increases in BW, ADG, and decreases in fecal scores (Heinrichs et al., 2003; Ghosh et al., 2012). Supplementing β-glucan (BG) was observed to increase pro-inflammatory cytokine production, increase leukocyte and lymphocyte activation, and moderate the impacts of inflammation during sepsis in multiple species (Novak et al., 2009; Eicher et al., 2010). Supplementation of these compounds has shown equivocal results, but each appears to work through separate mechanisms of action.

The objectives of this study were to determine the impacts of supplementing a blend of probiotics containing *Lactobacillus casei*, *Enterococcus faecium*, and *Saccharomyces cerevisiae*, β-glucan, a heat stable blend of *Bacillus subtilis* probiotic with mannanoligosaccharides, and IGF-1 molecules fractionated from colostrum on the performance and health of high-risk Holstein calves and to determine if any treatment had carry-over effects into the immediately post-weaned period.

MATERIALS AND METHODS

This study was a completely randomized design consisting of two 84-day periods with a total of 100 Holstein bull calves. Treatments were completely randomized and included a negative control group (CON), Mushroom β-glucan; ImmuOligo, Irvine, CA (BG) supplemented at 5mL/day, Immu-PRIME; Sterling Technologies, Brookings, SD (ImmPr) given at 0.75 grams per feeding for only the first 3 days of life, probiotic; PROVIDA Calf; MB Nutritional Sciences LLC, Lubbock, TX (PROVIDA) treatment of $2x10^9$ CFU/d of *Lactobacillus casei*, *Enterococcus faecium*, and *Saccharomyces cerevisiae*, and mannanoligosaccharide; CEREVIDA Calf MOS; MB Nutritional Sciences LLC, Lubbock, TX (MOS+Bs) treatment with $4x10^9$ CFU/d *Bacillus subtilis* and 3g/d of MOS. Upon arrival calves were weighed, randomly assigned a treatment and

administered a bolus of a full day's treatment, with control calves receiving a sham bolus. Peripheral blood was drawn immediately to assess passive immune status via total serum protein (TSP) analysis on a refractometer. All calves were enrolled in the study within 24 h of birth. Calves were housed outdoors in individual calf hutches (2.13 x 1.09 m Agri-Plastics, Cortland, NY). Calves were fed 700 g of milk replacer containing 22% CP and 20% fat (Milk Specialties, Eden Prairie, MN) and texturized calf starter *ad libitum* at 22% CP (Purina Ampli Calf, Nestle Purina, St. Louis, MO). All calves were vaccinated on d 28 with Inforce 3 (Zoetis Inc, Parsippany-Troy Hills, NJ) and Bovi-shield (Zoetis Inc, Parsippany-Troy Hills, NJ). Calves were stepwise weaned starting d 53 only being fed milk in the morning until d 56 when they were moved into randomized group pens after the morning milk feeding. Treatments were ceased at weaning and calves were co-mingled in groups of 10 to 12 calves per pen with treatments equally represented within each pen. Measurements of BW were taken on d 0, 7, 14, 21, 28, 35, 42, 49, 56, 70, and 84. Rectal temperatures were assessed on d 1, 4, 8, 11, 15, 18, and 22. Peripheral blood samples were taken on d 1, 3, 7, 14, 21, 42, 56, and 84. Blood was analyzed within 2 hours for a complete blood cell count on an IDEXX Procyte analyzer.

Statistical Analysis

All continuous, repeatedly measured data were analyzed as a repeated measure using the Mixed Procedure in SAS (SAS 9.4, Cary, NC). The model included fixed effects of treatment, time, and treatment x time. Initial BW and TSP were tested as covariates in the model and were retained in the final model if they were significant. Initial BW was a significant covariate for ADG and TSP for calf starter intake. Period was included as a random effect, and the subject of the repeated statement was calf nested within treatment. All appropriate covariance structures for unequal spacing and variance structures were analyzed and the most appropriate model was selected based on the lowest Bayesian Information Criterion. Differences of $P \leq 0.05$ were considered significant and a tendency was reported when $0.05 < P \leq 0.10$. Significant treatment x time interactions were further evaluated by sliced treatment differences at each sample time using a Duncan adjustment to control for the familywise error. All pairwise comparisons at each significant time were determined. Before analysis all data were trimmed using the Windsor method.

RESULTS AND DISCUSSION

This study investigated the impacts of four different nutritional supplement strategies on the health and performance of high-risk Holstein bull calves during both the pre-weaned period as well as carry over effects in the immediate post-weaned, comingled period. The data from the current study suggest that BG, MOS+Bs, and PROVIDA probiotics influenced the performance and some measures of health; however, the mechanisms of action appear to be different.

Body weight, TSP, starter intake, ADG, body measures, and blood metabolites are reported in Table 1. The TSP data was reported as average TSP per treatment and the percent of calves in each treatment with FPT (<5.2g/dL). There was a treatment difference for pre-weaned starter intakes from day 0 to day 28 (P=0.016). The BG supplemented calves consumed the most starter in the first month of life and CON calves consumed the least. Starter intakes did not differ for PROVIDA calves from any treatment except they were less than the BG calves from d 0 to d 28. The starter intake from d 29 through d 56 reflects the total pre-weaned starter intake as most of the pre-

weaned starter intake was consumed during this period. Total and post-weaned ADG did not differ between groups but a tendency for difference was detected for pre-weaned ADG (P=0.081; Table 1). The ADG in the pre-weaned period was greatest for the BG and PROVIDA calves and the CON calves gained the least. The ADG among the PROVIDA and BG calves during the pre-weaned period was a 3.92 and 2.80 kg improvement in pre-weaned BW gain, respectively. This is in an agreement with data from multiple studies that supplemented probiotic bacteria to young calves and observed increased ADG as well as BW (Abu-Tarboush et al., 1995; Mokhber et al., 2007; and Jatkauskas et al., 2010). The increase in ADG with probiotic supplementation may be due to a few mechanistic actions. Supplementation of 12 *Lactobacillus* strains decreased colonization of the GIT with 3 strains of *E. coli* and up to 5 strains of *Salmonella*. This is likely because some of the *Lactobacillus* species can adhere strongly to the small intestinal mucosa and epithelium and is suggested there is production of substances to decrease pathogenic growth (Jin et al., 2014). A study completed by Ewaschuk et al. (2012) recorded no difference in feed intake, ADG, or BW in BG supplemented pigs. However, in agreement with the current study, Dritz et al. (1995) supplemented a purified BG product to pigs and observed an increase in ADG as well as BW. The effect of feeding MOS to calves is equivocal with some studies reporting increased ADG, BW, and decreased fecal scores (Ghosh et al., 2012 and Berge et al., 2016) with other studies reporting no differences in ADG or final BW (Hill et al., 2008; Nargeskhani et al., 2010). In agreement with the pre-weaned calf starter intake data in this study, Heinrichs et al. (2003) reported that a MOS supplement increased starter intake at a younger age than a control group; however, there were no differences in calf performance at the end of the study. Similarly, Terre et al. (2007) observed no change in final BW but did report an increased pre-weaned starter intake for MOS supplemented calves.

The health of calves likely impacts the performance outcomes of many nutritional supplements. As reported by Gilliland et al. (1980), when calves were fed a probiotic *Lactobacillus* strain there was no difference observed between treatments due to the general good health of all the calves in the study. Similar findings in a *Bacillus sp.* based probiotic study where there were no differences in either calf starter intakes or ADG between a control and a probiotic supplemented group of healthy, unstressed calves (Riddell et al., 2008). The calves in the current study were high-risk bull calves from a commercial calf ranch, and despite intensive management of the calves there was still an overall 17% mortality in the study.

Calf health was evaluated daily over the course of the study. Average fecal scores were greatest during the 2nd week of life and were lowest among BG calves. However, there were no differences in the dry matter (DM) content of fecal samples among treatments throughout the entire study. Rectal temperatures were taken during the first 3 weeks of life, and contrary to the increased rectal temperatures in the MOS+Bs group, Kara et al. (2015) observed a decrease in temperature of dairy calves supplemented with MOS. The increase in rectal temperature on day 4 and 8 in the ImmPr calves may be related to the increased serum haptoglobin concentrations seen within the first week of life for ImmPr calves as well.

The greater calf starter intakes for PROVIDA calves during the second month of life coincided with a decrease in fecal scores. Fecal scores, fecal dry matter, rectal temperature, and hematology measures are all reported in Table 2. There was a treatment

x time interaction among fecal scores (P<0.0001). Fecal score differences are indicated in Figure 2 at week 2 (P=0.036), week 5 (P=0.017), week 6 (P=0.001), week 7 (P=0.001), and week 8 (P=0.002). During weeks 5, 6, and 7, the PROVIDA supplemented calves had the lowest fecal scores. Probiotics have been shown to decrease fecal scores during both the pre-weaned and post-weaned periods (Mokhber et al., 2007; Meale et al., 2017). Fecal scores during week 8 were greatest for CON calves, which corresponded with the reduced starter intake among CON calves. There was a treatment x time interaction among rectal temperatures (P=0.049). Rectal temperature differences are indicated in Figure 3 and occur at d 4 (P=0.010) and d 8 (P=0.006). Table 2 also contains blood metabolite data. There was a treatment x time interaction for serum glucose concentration (P=0.049). The treatment x time differences in serum glucose are indicated in Figure 1. The CON had decreased serum glucose concentrations on d 3 (P=0.007) and d 7 (P=0.107) when compared to all other treatment groups. Both CON and ImmPr calves had increased serum glucose concentrations at d 56 and d 84. The exact mechanisms leading to the greater glucose concentrations among these calves at these later time points is unclear; however, these 2 treatments had numerically lower calf starter intake when compared to the other 3 treatments. Therefore, the differences in serum glucose concentrations could be associated with nutrient availability or anatomical site of digestion. Serum haptoglobin was assessed as a measure of systemic inflammation and analyzed as AUC as well as concentration over time. Serum haptoglobin concentrations had a treatment x time interaction (P=0.010). Figure 4 shows the treatment interactions at d 7 (P=0.036), d 14 (P=0.033), d 42 (P=0.019), and d 56 (P=0.006). There was a tendency for a treatment interaction when haptoglobin was assessed using an area under the curve (AUC) approach (P=0.075), whereas BG, MOS+Bs, and PROVIDA all had reduced haptoglobin concentrations when compared to the CON and the ImmPr was not different than any other treatment. Haptoglobin AUC was greatest for CON calves and lowest for BG, MOS+Bs, and PROVIDA calves. Further, on d 7 the ImmPr and CON calves had the greatest serum haptoglobin concentrations, suggesting there may have been a greater exposure or lack of microbial control in the GIT of those calves in the first week of life. Some of the effect in the ImmPr calves may be associated with the greater proportion of FPT calves in that group at enrollment. Exposure and inability to combat bacterial or viral infection of the GIT could increase haptoglobin levels as a general marker inflammation. In agreement with the current study, Sandvik et al. (2007) that observed BG modulated inflammation in rats undergoing LPS-induced endotoxemia. Therefore, BG may reduce the intensity of the systemic inflammatory response through some mechanism. A study with pigs supplemented with BG reported a consistent decreased haptoglobin concentrations in the BG supplemented pigs compared to a control group from d 7 through d 28 (Dritz et al. 1995). Additionally, Liang et al. (2017) reported decreased serum haptoglobin concentrations among high-risk Jersey calves after an oral *Salmonella typhimurium* challenge if they were supplemented with the same blend of probiotic bacteria used in the current study. In contrast to the current study, Terre et al. (2007) observed no difference in serum haptoglobin concentrations between MOS supplemented calves and a negative control group.

The MOS+Bs calves had the lowest total leukocyte count, neutrophil count and percent, and the lowest lymphocyte count at all time points. Hematology data are reported in Table 3. Total leukocyte count did not have a treatment x time interaction

(P=0.360); however, there was a treatment difference (P<0.002). There was no treatment x time interaction for polymorphonuclear neutrophil (PMN) counts (P=0.224), but there was a treatment difference (P=0.003). Additionally, there was no treatment x time interaction for PMN percentage (P=0.290), but there was a treatment difference (P=0.005). Lymphocyte counts also differed with a treatment x time interaction (P=0.001). The differences in lymphocyte counts are illustrated in Figure 4 at d 3 (P=0.071), d 7 (P=0.043), d 14 (P=0.065), d 21 (P=0.054), d 42 (P=0.061), d 56 (P=0.003), and d 84 (P=0.010). There was no treatment x time interaction in the ratio of PMN to lymphocytes (P=0.189); however, the PMN:lymphocyte had a strong tendency for a treatment difference (P=0.051), whereas the BG treatment had the greatest PMN:lymphocyte. Neutrophil counts decrease over time in the first 6 to 8 weeks of life in dairy calves and may reflect maturation of the GIT immune system and/or exposure to enteric pathogens.

The GIT of young calves is colonized by a wide variety of bacteria that represent diverse phyla, and this colonization is dynamic during the first few months of life. A primary mode of action of MOS is to bind mainly gram-negative bacteria in the small intestine, which is assumed to decrease pathogenic exposure by those bacteria that may be in the environment. However, some of the beneficial microbes beginning to colonize the small intestine of neonatal calves are gram-negative. Many gram-negative bacteria express Type 1 Fimbriae, a type of adherence filament. Type 1 Fimbriae are mannose-specific filaments that induce agglutination and are expressed on many types of gram-negative bacteria, both pathogenic and commensal (Rendon et al., 2007; Lasaro et al., 2009). The high affinity of MOS for Type 1 Fimbriae of gram-negative bacteria may also be binding some of the beneficial gram-negative GIT microbes in addition to potentially pathogenic ones. This may be contributing to the reduced leukocyte counts in the MOS group. More research is needed to understand how MOS maybe affecting the microbial ecology of the GIT of calves early in life.

In contrast to the MOS+Bs treatment, the PROVIDA supplemented calves maintained the greatest lymphocyte counts throughout the entire study. The probiotic bacteria colonizing the GIT may be stimulating lymphocyte production similar to findings by Bai et al. (2004) on in vitro stimulation of intestinal epithelium by probiotics. In contrast, Fleige et al. (2009) observed a decrease in the lymphocyte population of calves fed probiotics. The ImmPr supplemented calves also had elevated lymphocyte counts on d 21 when compared to the MOS+Bs treatment. Blum et al. (2008) reported that supplementing IGF-1 to neonatal calves may stimulate lymphocyte development. The increased lymphocyte count in peripheral circulation among the ImmPr calves was demonstrated on d 21; therefore, the impacts of ImmPr on lymphocyte development are not understood.

The BG calves had increased neutrophil counts and neutrophil to lymphocyte ratios in peripheral circulation. Whether the BG stimulated granulopoiesis or reduced the marginating pool of neutrophils in circulation is not completely known. However, the latter appears plausible because the BG supplemented calves had the lowest L-selectin expression on the surface of peripheral blood neutrophils. Reduced L-selectin adhesion protein can increase the number of neutrophils measured in circulation because less neutrophils are loosely adhered to the vascular endothelium. Neutrophil L-selectin expression, phagocytosis and oxidative burst capacity data are reported in Table 3. There

was no treatment x time interaction for any data in this table ($P \geq 0.371$). The mean fluorescence intensity for neutrophil phagocytosis had a tendency for a treatment difference ($P=0.087$). Additionally, the neutrophil oxidative burst capacity had a treatment difference ($P=0.011$). The surface expression of L-selectin on neutrophils had a treatment difference ($P<0.0001$) and no treatment x time difference ($P=0.371$). The BG calves had the lowest L-selectin expression on neutrophils, the lowest numerical phagocytosis and oxidative burst capacity percentage, and the lowest phagocytosis and oxidative burst intensities. The exact reason for the attenuated neutrophil responses is unknown. Supplementing BG to lambs increased neutrophil phagocytosis and oxidative burst capacities when compared to a control (Wojcik, R., 2007). Further, supplementing rats with BG increased neutrophil functionality (Stier et al., 2014), and the potential killing activity as well as respiratory burst activity of phagocytes (Malaczewska et al., 2010). Harris et al. (2017) supplemented calves with a yeast cell wall extract that contained both MOS and BG and reported an increase in L-selectin expression in supplemented calves. Supplementing the PROVIDA probiotics also led to a similar low neutrophil oxidative burst MFI as the BG treatment. These data are in contrast to Indart et al. (2012) that observed an increase in neutrophil functionality when supplemented with probiotics. The CON and ImmPr calves had the greatest neutrophil oxidative burst capacity, implying the need for greater leukocyte function to fight off bacterial or viral infections.

CONCLUSION

Supplementing high-risk Holstein calves with either BG, MOS+Bs, or PROVIDA increases measures of performance and health, albeit by different mechanisms. Supplementing BG to high-risk calves likely decreases neutrophil oxidative burst and L-selectin expression, potentially moderates some systemic inflammation, and simultaneously stimulates starter intake in the first months of life. Feeding PROVIDA to high-risk calves increases starter intake and body weight gain, moderates a systemic inflammation response and stimulates lymphocytes. Supplementing MOS+Bs to high-risk calves decreases the lymphocyte and neutrophil populations while decreasing neutrophil functionality. However, MOS+Bs was shown to increase L-selectin expression on neutrophils, implying some stimulation may be occurring.

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Table 1. The effect of treatments on performance measurements in high-risk Holstein dairy calves

	Treatments[1,2]					Largest	Fixed Effects		
	CON	ImmPr	BG	MOS+Bs	PROVIDA	SEM	Trt	Time	Trt*Time
Item								*P* ≤	
Total serum protein, TSP[3]	5.50	5.24	5.47	5.31	5.28	1.22	…	…	…
Failure of passive transfer, %	25.0	50.0	35.0	45.0	40.0	…	…	…	…
Initial body weight, kg	41.7	39.1	41.7	39.2	40.1	1.238	0.242	…	…
Weaned body weight, kg	66.6	66.0	68.4	66.4	69.0	3.645	0.664	…	…
Final body weight, kg	91.6	90.2	91.5	89.8	94.2	5.239	0.825	…	…
Preweaned starter intake, kg	13.95	14.79	17.10	17.42	18.50	4.060	0.413	…	…
0 d to 28 d	0.99[a]	1.49[bc]	1.96[c]	1.75[bc]	1.36[ab]	0.274	0.016	…	…
29 d to 56 d	12.30	12.82	14.41	15.71	16.40	3.738	0.319	…	…
Average daily gain, kg/d	0.59	0.62	0.62	0.61	0.65	0.038	0.422	…	…
Preweaned average daily gain, kg/d	0.44[a]	0.47[ab]	0.49[b]	0.48[ab]	0.51[b]	0.033	0.081	…	…
Postweaned average daily gain, kg/d	0.89	0.89	0.89	0.85	0.92	0.048	0.879	…	…
Glucose, pg/mL	103.2	105.2	104.2	103.4	104.5	1.663	0.603	<0.0001	0.049
Urea Nitrogen, pg/mL	11.73	11.08	11.45	12.34	11.84	0.664	0.048	<0.0001	0.023

[1]Treatments included a control group which were fed a base diet of milk replacer and calf starter; ImmPr which were fed 1.5 g/d ImmPr first 3 d only; BG which were fed 1 g/d β-Glucan; MOS+Bs which were fed 3 g/d Mannanoligosaccharides + 4 x 10^9 CFU/d *Bacillus subtilis*; PRO which were fed a blend of 2 x 10^9 CFU/d *Lactobacillus casei* and *Enterococcus faecium* + 2 x 10^9 CFU/d *Saccharomyces cerevisae*.

[2]Differing superscripts within a row indicate a difference between means (P < 0.05).

[3]The Largest SEM for total serum protein was reported in this table as the largest standard deviation because TSP was calculated only as an average per treatment.

Table 2. The effect of treatments on health measurements in high-risk Holstein dairy calves

	Treatments[1,2]					Largest	Fixed Effects		
	CON	ImmPr	BG	MOS+Bs	PROVIDA	SEM	Trt	Time	Trt*Time
Item							*P* ≤		
Fecal Score, average by week	2.24	2.27	2.17	2.21	2.12	0.033	0.004	<0.0001	<0.0001
Fecal Dry Matter, %	21.2	21.0	22.4	19.7	19.7	1.169	0.375	<0.0001	0.916
Rectal Temperature, °C	38.6	38.7	38.6	38.6	38.6	0.03	0.006	<0.0001	0.049
Haptoglobin, μg/mL	247	246	207	228	234	17.472	0.075	<0.0001	0.010
Haptoglobin, μg/mL x 10^3 AUC	23.7[a]	20.8[ab]	19.0[b]	19.2[b]	19.2[b]	1.593	0.075	…	…
Hemoglobin, g/dL	10.6[a]	10.42[ab]	9.97[bc]	9.5[c]	10.64[a]	0.178	<0.0001	<0.0001	0.373
Red Blood Cell Count (M/μL)	8.78[a]	8.72[a]	8.38[b]	7.95[c]	8.78[a]	0.127	<0.0001	<0.0001	0.544
Total Leukocyte Count (10^6/μL)	9.94[a]	10.45[a]	10.59[a]	8.87[b]	10.6[a]	0.343	0.002	<0.0001	0.360
Neutrophils, 10^6/mL	4.45[a]	4.64[a]	4.97[a]	3.75[b]	4.69[a]	0.221	0.003	<0.0001	0.224
Neutrophil, %	44.7[ac]	44.0[ac]	46.0[ac]	41.8[b]	43.9[a]	0.797	0.005	<0.0001	0.290
Lymphocyte, 10^6/mL	4.47	4.68	4.63	4.13	4.79	0.116	0.001	<0.0001	0.003
Lymphocyte, %	46.2	46.94	44.86	47.91	46.61	0.959	0.224	<0.0001	0.246
Neutrophil:Lymphocyte	0.86[ab]	0.84[a]	0.94[b]	0.8[a]	0.83[a]	0.036	0.051	<0.0001	0.189

Table 3. Effects of treatment on hematology measurements in high-risk Holstein dairy calves

	Treatments					Largest	Fixed Effects		
	CON	ImmPr	BG	MOS+Bs	PROVIDA	SEM	Trt	Time	Trt*Time
Item							*P* ≤		
Neutrophil L-selectin, MFI x 10^3	78.9[a]	76.7[a]	69.2[b]	83.7[c]	76.1[a]	4.771	<0.0001	<0.0001	0.371
Neutrophil phagocytosis & oxidative burst, %	45.1	44.9	42.7	43.6	44.2	3.559	0.485	<0.0001	0.996
Neutrophil phagocytosis, MFI x 10^3	9.5[ab]	9.7[ab]	9.1[b]	10.6[a]	10.2[a]	1.530	0.087	<0.0001	0.996
Neutrophil phagocytosis CV, MFI	178.1	174.1	177.6	183.5	187.1	12.108	0.198	<0.0001	0.809
Neutrophil oxidative burst, MFI x 10^3	72.1[a]	71.5[a]	65.6[b]	67.8[ab]	65.5[b]	2.630	0.011	<0.0001	0.782
Neutrophil oxidative burst CV, MFI	109.2	108.7	107.2	109.5	109.5	1.945	0.611	<0.0001	0.749

[1]Treatments included a control group which were fed a base diet of milk replacer and calf starter; ImmPr which were fed 1.5 g/d ImmPr first 3 d only; BG which were fed 1 g/d β-Glucan; MOS+Bs which were fed 3 g/d Mannanoligosaccharides + 4 x 10^9 CFU/d *Bacillus subtilis*; PRO which were fed a blend of 2 x 10^9 CFU/d *Lactobacillus casei* and *Enterococcus faecium* + 2 x 10^9 CFU/d *Saccharomyces cerevisae*.
[2]Differing superscripts within a row indicate a difference between means (P < 0.05).

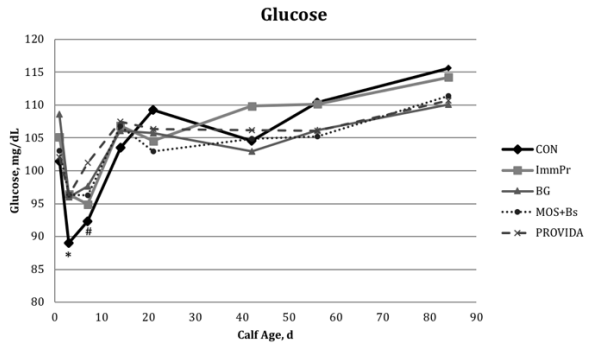

Figure 1. Serum glucose concentrations were measured on d 0, 3, 7, 14, 21, 42, 56, and 84. There was a treatment x time interaction (P=0.049). The CON treatment had lower glucose concentrations than all other treatments on d 3 ($P \leq 0.006$) and was reduced on d 7 when compared to PROVIDA ($P=0.009$). Largest SEM per time point are expressed as mg/dL and include 3.727, 2.271, 3.051, 2.571, 2.739, 3.131, 3.149, and 2.933 for d 1, 3, 7, 14, 21, 42, 56, and 84, respectively. An * indicates a treatment difference of ($P \leq 0.05$) and a # indicates a tendency for a treatment difference ($0.05 < P \leq 0.10$) when treatments were sliced by time.

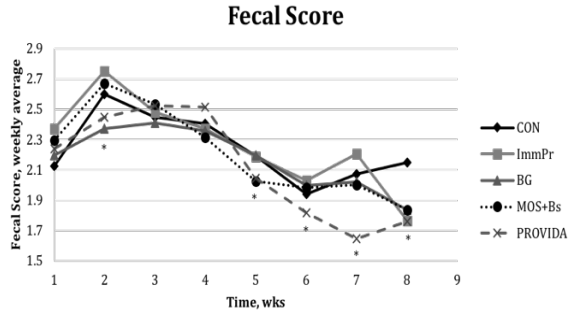

Figure 2. The fecal scores were averaged by week per treatment for weeks 1, 2, 3, 4, 5, 6, 7, and 8. There was a treatment x time interaction ($P<0.0001$). The BG calves had lower fecal scores during week 2 ($P \leq 0.087$). The PROVIDA calves had decreased fecal scores when compared to other treatments during weeks 5, 6, 7, and 8 ($P \leq 0.027$). Largest SEM per time point are expressed as fecal score weekly average and include 0.094, 0.104, 0.131, 0.108, 0.052, 0.046, 0.112, and 0.094 for d 0, 3, 7, 14, 21, 42, 56, and 84, respectively. An * indicates a treatment difference of ($P \leq 0.05$) when treatments were sliced by time.

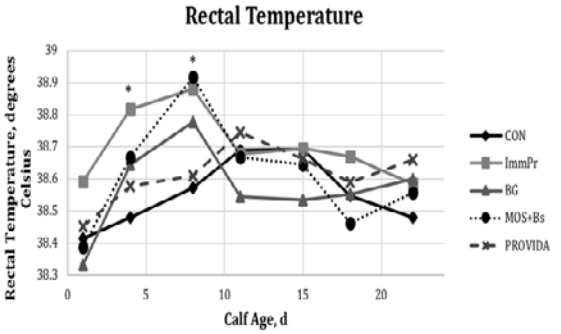

Figure 3. Rectal temperature was measured on d 1, 4, 8, 11, 15, 18, and 22. There was a treatment x time interaction ($P \leq 0.049$). The CON treatment had decreased rectal temperatures when compared to other treatments; CON vs. BG ($P \leq 0.091$) on d 4 and 8; CON vs. ImmPr ($P \leq 0.021$) on d 4 and d 8; and CON vs. MOS+Bs ($P \leq 0.064$) on d 4 and d 8. The ImmPr treatment had increased rectal temperatures when compared to other treatments; ImmPr vs. CON ($P \leq 0.021$) on d 4 and d 8; ImmPr vs. BG ($P=0.085$) on d 4; and ImmPr vs. PROVIDA ($P \leq 0.012$) on d 4 and d 8. Largest SEM per time point are expressed as degrees Celsius and include 0.096, 0.076, 0.080, 0.094, 0.072, 0.076, and 0.066 for d 1, 4, 8, 11, 15, 18, and 22, respectively. An * indicates a treatment difference of ($P \leq 0.05$) when treatments were sliced by time.

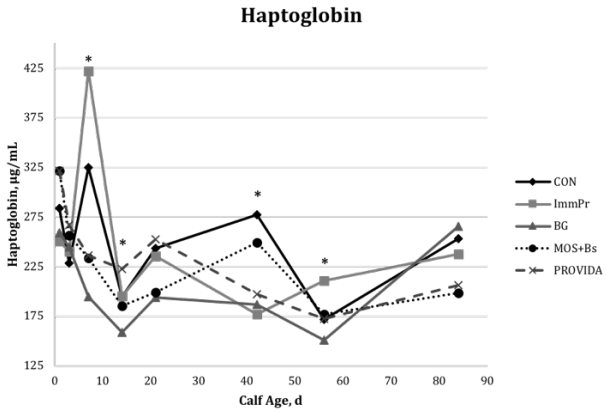

Figure 4. Serum haptoglobin concentration was measured on d 0, 3, 7, 14, 21, 42, 56, and 84. There was a treatment x time interaction ($P \leq 0.010$). On d 7 ImmPr had greater haptoglobin concentrations than BG, MOS+Bs, and PROVIDA ($P \leq 0.015$). On d 14, BG had decreased haptoglobin when compared to Con, ImmPr, and PROVIDA ($P \leq 0.068$). The CON treatment had greater concentrations on d 42 than ImmPr, BG, and PROVIDA ($P \leq 0.016$). On d 56 ImmPr had the greatest haptoglobin concentrations ($P \leq 0.035$). Largest SEM per time point are expressed as µg/mL and include 31.202, 24.013, 61.286, 19.575, 29.605, 31.639, 16.917, and 45.241 for d 1, 3, 7, 14, 21, 42, 56, and 84, respectively. An * indicates a treatment difference of ($P \leq 0.05$) when treatments were sliced by time.

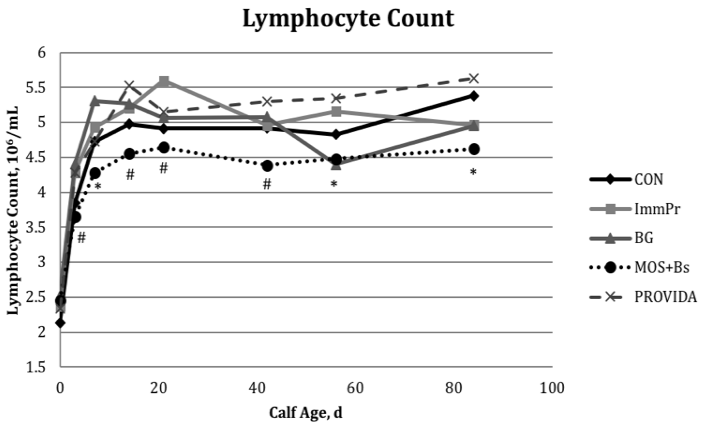

Figure 5. Lymphocyte count was measured on d 0, 3, 7, 14, 21, 42, 56, and 84. There was a treatment x time interaction ($P \leq 0.003$). The MOS+Bs treatment had decreased lymphocytes when compared to other treatments; MOS+Bs vs. CON ($P \leq 0.079$) on d 42, and 84; MOS+Bs vs. ImmPr ($P \leq 0.068$) on d 3, 7, 14, 21, 42, and 56; MOS+Bs vs. BG ($P \leq 0.05$) on d 3, 7, 14, and 42; MOS+Bs vs. PROVIDA ($P \leq 0.042$) on d 3, 14, 42, 56, and 84. Largest SEM per time point are expressed as 10^6/mL and include 0.183, 0.229, 0.237, 0.263, 0.244, 0.230, 0.214, and 0.231 for d 0, 3, 7, 14, 21, 42, 56, and 84, respectively. An * indicates a treatment difference of ($P \leq 0.05$) and a # indicates a tendency for a treatment difference ($0.05 < P \leq 0.10$) when treatments were sliced by time.

Livestock Vitamin Nutrition in Perspective

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Vitamins were identified as a group of unique and nutritionally essential organic compounds in the early 20th century. Prior to that time vitamin deficiency diseases were categorized along with infectious diseases with unknown causes. In a few cases such as vitamin C deficiency (scurvy) the presence or absence of certain foods in the diet (citrus fruits) were associated with the development or alleviation of deficiency symptoms. Development of the germ theory of disease helped pave the way for discovery of the vitamin deficiencies as the cause of non-infectious disease symptoms. Gradually, the persistence and ingenuity of researchers across several fields led to the identification of specific chemical compounds that were given the name vitamin based on their ability to alleviate specific deficiency symptoms and restore health in birds, rodents and other species, including humans.

While vitamins had been chemically identified it was not until the middle of the 20th century that large scale chemical synthesis and production of individual vitamins was achieved resulting in commercially available vitamin supplements. The synthesis and production of Vitamin A in the 1940's and 1950's was a classic example. Later, vitamin manufacturers studied factors affecting vitamin stability and developed specific product forms for individual vitamins that extended their shelf life and improved mixing and handling properties.

Prior to the advent of commercial vitamin sources, natural sources were used to prevent vitamin deficiency and improve livestock health and performance. Supplements such as wheat germ oil (vitamin E), liver extract (multiple vitamins), cod liver oil (fat soluble vitamins), alfalfa and other carotenes (vitamin A) and rose hips (ascorbic acid) were examples. Challenges with natural sources of vitamin activity include relatively low and variable vitamin concentrations coupled with poor stability.

As commercial vitamin supplements became more plentiful their cost came down. This trend continued into the early 2000's when additional global supplies became available. As a result, vitamin supplementation lost some prominence on the list of concerns for professional nutritionists. However, shortages have occurred when manufacturers have exited the market or experience production problems bringing vitamins supplementation levels and their justification back under scrutiny. These shortages can cause serious supply issues and cost increases for livestock producers. There are however, some upsides to the increased scrutiny of vitamin supplementation levels and rationales. Nutritionists are benefited by refocusing on this important class of micronutrients and their value to animal health and performance. Vitamin supplementation should be reviewed in the light of recent research to ensure that optimal levels

are being provided in diets. Optimal levels are those which provide the greatest overall health, performance and economic return from commercial livestock.

Stability and shelf life of vitamins are a valid concern. Vitamins are organic compounds with inherently unstable structures. Vitamins A, E, C, K and folic acid are the least stable, although it is not a straight forward relationship due to interactions of heat, light, free metals and pH in premixes and other media in which vitamin formulations are prepared and stored.

In addition, advanced product forms such as crossed linked beadlets (vitamin A,D), acetate esters (vitamin A, E), thiamin mononitrate and phosphorylated ascorbic acid are significantly more stable than less sophisticated product forms. A consultant would be wise to enquire as to the source(s) of vitamins used to prepare vitamin premixes and base mixes.

Straight vitamin product forms in the original, sealed container are stable for 12 months or longer as designated by the manufacturer. Vitamins in a well formulated premix without trace minerals or choline, with adequate levels of an appropriate carrier stored under cool, dry conditions are generally stable (>90%) for 3-6 months.

However, vitamins are commonly combined with trace minerals, choline and other nutrients in premixes of varying concentration and formulation. In these situations, the vitamins decay more rapidly in proportion to the relative “stress” of premix conditions. For example, a highly concentrated premix with copper sulfate is much more stressful on vitamins than one where copper is supplied by less reactive forms such as organically chelated or complexed sources or tribasic copper chloride. Oxidative reactions are especially damaging to the vitamins. Coelho (5) created a set of reference tables for vitamin stability and provided the following summary table as an example of net vitamin stability under a given set of conditions and storage times. The following Table 13 was prepared by Coelho (1).

Table 13. Vitamin stability in ruminant premixes and feeds

	1	2	3	4
	Vitamin Premix (TABLE 8)	Pelleting Temperature (TABLE 11)	Feed Storage Time (TABLE 10)	Total Vitamin Retention %
	2 Months	96°C	2 Weeks	1x2x3
A Beadlet	90	88	98	78
D_3 Beadlet	91	91	99	82
E Acetate 50%	92	91	99	83
Thiamine Mono	77	82	99	63
Riboflavin	91	84	99	76
B_{12}	96	95	100	90
Calcium Pantothenate	87	84	99	72
Biotin	89	84	99	74
Niacin	90	86	99	77

Synthetic antioxidants have been added to basic vitamin product forms such as cross-linked beadlets for many years to improve stability. Addition of antioxidants can help improve the stability of premixes containing both vitamins and trace minerals and/or choline and stored under conditions of elevated temperature and humidity. On-farm bulk storage of vitamin-mineral mixes in open bays would be a very good example of stressful storage conditions.

Stability of fat-soluble vitamins (A, D, E, K) are also affected by the presence or absence of unsaturated fats in the diet and the relative stability of the fat. Unsaturated fats are prone to oxidative rancidity. Oxidation is a progressive chain reaction that reduces the energy value of and leads to destruction of fat soluble vitamins in the diet and in the G.I. tract. Supplemental antioxidants can be used to stabilize unsaturated fats and prevent energy and vitamin losses.

During the recent period of vitamin shortages and price increases some nutrition consulting groups and feed companies have begun adding supplemental antioxidant blends to premixes and bagged feeds to help preserve vitamin activity during storage and feed out. Given the storage times and conditions of these products this is a prudent consideration.

From a practical standpoint the following guidelines may be used for premix formulation:

1. Use vitamin sources of known origin from a quality manufacturer with a strong quality assurance program. Such suppliers will have data available on vitamin product forms and their stability.

2. Follow manufacturer recommendations for premix formulation including the use of proper carrier materials to disperse the vitamins and bulk ingredients to meet bulk density and flow targets.

3. Use of a separate vitamin premix will improve storage stability compared to a vitamin-trace mineral combination. If vitamins and trace minerals must be combined then use of less reactive trace mineral sources (mainly copper, iron) and recommended levels of inert ingredients will aid stability.

4. Store vitamin premixes under the most favorable conditions available in the feed mill or storage facility avoiding extremes of temperature and humidity, keeping premixes in re-sealable containers and using up premixes within 4-6 months of receipt.

5. Consider addition of a high-quality antioxidant blend to higher cost, lower use rate premixes to improve shelf life and buffer against seasonal variations in temperature, humidity and use rates.

In addition, when a vitamin or vitamin-trace mineral premix is used in the formulation of a base mix or concentrate, resist the temptation to over-concentrate the final product, especially when liquids and reactive ingredients such as magnesium or calcium oxides, sulfates or chlorides, commercial fat sources and urea or ammonium salts are included in the formula. The use of 30-40% non-mineral, non-reactive ingredients such ground corn, wheat midds, distiller's grains, rice hulls etc. will maintain dispersion and separation of reactive ingredients, improve handling characteristics, reduce the likelihood of chemical reactions and help protect the vitamins from oxidative damage. The economic benefits of proper premix formulation with adequate levels of an appropriate carrier far exceeds any "savings" from using zero carrier ("filler") premixes or base mixes.

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New Concepts in Sugars and Starches for Dairy Cattle

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We continue to learn about factors that affect how diets function in dairy cattle. In the case of nonfiber carbohydrates such as starch and the water-soluble carbohydrates, we are also finding out what other factors outside of these carbohydrates affect their use. A few specific items that go beyond the basics:

- The more starch that's fed, the more rapidly it ferments, with the rate of starch in high moisture corn increasing more than with dry ground corn. At the same time, the measured yield of microbial nitrogen from organic matter truly fermented in the rumen was greater for the more slowly fermenting dry ground corn (Oba and Allen, 2003). This may have to do with how microbes handle rapidly available carbohydrates (see note on glycogen).
- Ruminally degradable protein, but not high moisture corn or dry ground corn, increased lactic acid detected in the rumen (Hall, 2013).

Water-soluble carbohydrates (WSC) are typically what we are talking about when we talk about "sugars". The WSC include monosaccharides such as glucose and fructose (yes, these are real sugars), disaccharides like sucrose and lactose (real sugars, too), as well as galacto-oligosaccharides such as stachyose and raffinose (not sugars), fructans (in cool season grasses, not sugars), and any other carbohydrates soluble in water. With the exception of lactose (milk sugar) which ferments more slowly, all of the WSC appear to be rapidly available to the rumen microbes. Key things to know about WSC use by microbes:

- If there is more WSC than RDP available, or the WSC is very rapidly available, microbes may store the WSC internally as glycogen, a carbohydrate much like starch. Overfeeding RDP is not a good solution to this. Production of glycogen slows down rumen fermentation and may help to maintain a higher rumen pH.
- Glycogen production requires energy – 1 ATP per glucose added to the chain (Stouthamer, 1973). This will reduce the amount of energy available to make microbes.
- In vitro, providing peptides (RDP) vs. urea gave a much higher yield of microbial nitrogen, and reduced production of glycogen (Hall, 2017).
- The more rapidly a WSC ferments, the more microbial protein is produced, even with substantial glycogen production (a dilution of maintenance?).

Among the WSC as compared to starch, feeding sugars tends to increase or maintain milk fat production. We are not sure exactly why this occurs, but it may be due to increased biohydrogenation of fatty acids in the rumen by microbes that utilize sugars (McKain et al., 2010). This could reduce the amount of bioactive fatty acids that have been associated with milkfat depression. Another basis could be the greater amount of butyrate produced from fermentation of sugars than from other carbohydrates (Strobel and Russell, 1986).

Butyrate makes up 30% of the fatty acids in the *sn*-3 position on milk triglycerides (Jensen, 2002), and provides 50% of the starting carbons in de novo synthesis of fatty acids in milk (Palmquist et al., 1969).

Another thing to consider with starch vs. WSC: the polysaccharide (starch) contains more carbohydrate than the mono- or disaccharides do. To put carbohydrates on an equal monosaccharide or free sugar, dry matter basis, the weight of water must be added hydrolyze the bond between sugars to release free sugars. For glucose, a monosaccharide, the free sugar value of 1 lb of glucose = 1 lb of free sugars. For disaccharides such as sucrose which have a single bond between the sugars, 1 lb of sucrose = 1.05 lb of free sugars. For a polysaccharide such as starch which has many bonds, 1 lb starch = 1.11 lb of free sugars. So, although we treat the carbohydrates as though equal weights have equal value, that is not really the case. However, it's their fermentation and digestion characteristics that will decide the amounts of products produced that ultimately matter to the cow.

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Re-evaluating dogmas of metabolic health in transition cows

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Introduction: There are a variety of situations in a farm animal's life when nutrient utilization is prioritized towards agriculturally unproductive purposes. Two well-known examples that markedly reduce production efficiency are heat stress and ketosis. Decreased feed intake, experienced during both situations, is unable to fully explain the decreased productivity. Additionally, both ketosis and heat stress are characterized by negative energy balance, body weight loss, inflammation, and hepatic steatosis. While the metabolism of ketosis and heat stress have been thoroughly studied for the last 40 years, the initial insult in the cascade of events ultimately reducing productivity in both heat-stressed and ketotic cows has not been identified. To that end, our data strongly implicates intestinally derived endotoxin as the etiological culprit in each case.

Ketosis: The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support milk production. Unfortunately, a disproportionate amount of herd culling occurs before cows reach 60 days in milk (NAHMS, 2008). Ketosis is arbitrarily defined as an excess of circulating ketone bodies and is characterized by decreases in feed intake, milk production, and increased risk of developing other transition period diseases (Chapinal et al., 2012). Epidemiological data indicate about 20% of transitioning dairy cows clinically experience ketosis (BHBA > 3.0 mM; Gillund et al., 2001) while the incidence of subclinical ketosis (>1.2 mM BHBA) is thought to be much higher (> 40%; McArt et al., 2012). Ketosis is a costly disorder (estimated at ~$300 per case; McArt et al., 2015) and thus it represents a major hurdle to farm profitability. Traditionally, ketosis is thought to result from excessive adipose tissue mobilization (Baird, 1982; Grummer, 1993; Drackley, 1999) which in turn contributes to fatty liver (hepatic steatosis) and excessive ketone body synthesis (Grummer, 1993).

Heat Stress: Heat stress negatively impacts a variety of production parameters and is a significant financial burden to animal agriculture. Heat-stress affects productivity indirectly by reducing feed intake; however, direct mechanisms also contribute as we have shown reduced feed intake only explains approximately 35-50% of the decreased milk yield during heat stress (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Direct mechanisms contributing to heat stress milk yield losses involve an altered endocrine profile, including reciprocal changes in circulating anabolic and catabolic hormones (Collier et al., 2006; Bernabucci et al., 2010; Baumgard and Rhoads, 2012). Such changes are characterized by increased circulating insulin concentration, lack of adipose tissue lipid mobilization, and reduced adipocyte responsiveness to lipolytic stimuli. Hepatic and skeletal muscle cellular bioenergetics also exhibit clear differences in carbohydrate production and use, respectively, due to heat stress. Thus, the heat stress response markedly alters post-absorptive carbohydrate, lipid, and protein metabolism through coordinated changes in fuel supply and utilization across tissues in a manner distinct from commonly recognizable changes that occur in animals on a reduced plane of nutrition (Baumgard and Rhoads, 2013).

Endotoxin: The Common Denominator?: Endotoxin, otherwise referred to as lipopolysaccharide (LPS) is a glycolipid embedded in the outer membrane of Gram-negative bacteria and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 2007). LPS-induced inflammation redirects nutrients away from anabolic processes that support milk and muscle synthesis (see review by Johnson, 1997, 1998) and thus compromises productivity and efficiency. Initial mechanisms responsible for altered nutrient partitioning during heat stress may be mediated by inflammation resulting from effects of heat stress on gastrointestinal health and function (Baumgard and Rhoads, 2013). As a result, heat stress increases the infiltration of luminal LPS into the portal and systemic blood (Hall et al., 2001; Pearce et al., 2013b). Furthermore, endotoxemia is common among heat stroke patients (Leon, 2007) and it is thought to play a central role in heat stroke pathophysiology, as survival increases when intestinal bacterial load is reduced (Bynum et al., 1979) or when plasma LPS is neutralized (Gathiram et al., 1987). Likewise, increased inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). We have demonstrated increased inflammation prior to ketosis diagnosis and in cows with no overt infection in the uterus or mammary gland (Abuajamieh et al., 2015). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus, mammary gland, and the gastrointestinal tract (Mani et al., 2012). We have demonstrated decreased milk synthesis during a pharmaceutical-induced model of leaky gut (Stoakes et al., 2014) and have also shown a simple 60% feed restriction alters gut morphology and increases circulating LPS (Stoakes et al., 2015a). Furthermore, experimentally-induced endotoxemia in dairy cattle has been linked to several metabolic and endocrine disturbances including decreased circulating glucose, abortion, leukopenia, disruption of ruminal metabolism, and altered calcium homeostasis (Griel et al., 1975; Giri et al., 1990; Waldron et al., 2003; Jing et al., 2014). Our data and the literature suggest the aforementioned pathological conditions in both heat stress and ketosis are likely mediated by LPS-induced inflammation and the subsequent changes in nutrient partitioning caused by immune system activation.

Energetic Cost of Immune System Activation: Upon immune system activation, immune cells switch their metabolism from oxidative phosphorylation to aerobic glycolysis, causing them to become obligate glucose utilizers in a phenomenon known as the Warburg Effect (Vander Hiden et al., 2009). Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by the immune system during a 12 hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in lactating cows, growing steers and growing pigs is 0.64, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively; Stoakes et al., 2015b,c,d). Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool, etc.).

Conclusion: Ketosis and heat stress are two of the most economically important pathologies which severely jeopardize the competitiveness of animal agriculture. Heat stress and ketosis affect herds of all sizes and almost every dairy region of the globe. We suggest, based upon the literature and on our supporting evidence, that LPS is the common etiological origin of both metabolic disorders. Collectively, we hypothesize that leaky gut and the resulting LPS markedly alters nutrient partitioning and is a causative agent in metabolic disruption during heat stress and ketosis. Identifying dietary approaches that can ameliorate gut barrier dysfunction is paramount in developing seasonal mitigating strategies.

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Utilizing Distillers Grains in growing heifer diets and effects of source on ruminal and intestinal digestibility

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Executive Summary: (Key words: Distillers Grains, Dairy Heifer, Rumen Degradability)

Over the years as the ethanol industry has developed the processing of distillers grains has also changed. There is a relative abundance of research conducted on feeding distillers grains to beef cattle and lactating cows, but less is available on feeding it to growing dairy heifers. Research (Anderson et al., 2009 and Anderson et al., 2015d) demonstrated that distillers wet grains with solubles (**DWGS)** can be fed as a large proportion of dairy heifer diets, but consequently dietary fat content increased. Other recent research (Anderson et al., 2015 a, b and c) determine if increased dietary fat from distillers dried grains with solubles (**DDGS**) affected growth, metabolism, and long-term performance of heifers. Thirty-three Holstein heifers (133 ± 18 d old) were used in a 24-wk RCBD feeding trial. Heifers were fed one of the following treatment diets: 1) a control diet containing corn and soybean products (**CON**), 2) a low-fat diet containing reduced-fat DDGS and corn (**LFDG**) and 3) a high-fat diet containing traditional high-fat DDGS (**HFDG)**. All had 40% grass hay, 25% corn silage, and 35% concentrate mix (DM basis). Diets were balanced to be isonitrogenous and isocaloric, but not isolipidic. The HFDG contained 4.8% fat (DM basis) compared to 2.8% in CON and LFDG, which had more starch. Body weights (BW), frame measurements, and blood samples for metabolite and hormone analyses were taken throughout the trial. Post-trial data were collected on reproduction and lactation. Body growth was similar among treatments. Total tract digestion of DM was similar among treatments, but CP and fiber digestion were greater in heifers fed HFDG compared to CON and LFDG. Most metabolites and metabolic hormones analyzed were similar among treatments. Cholesterol increased in heifers fed HFDG compared to CON and LFDG. Progesterone analysis indicated heifers fed HFDG were pubertal at lower BW and age compared to LFDG or CON. Fat from DDGS can be fed in replacement of starch from corn to growing pre-pubertal dairy heifers and maintain growth performance, nutrient utilization, and subsequent reproductive and lactation performance.

In follow-up, two studies were conducted to evaluate the effects of limit-feeding heifers DDGS with varying forage to concentrate ratios. First, a 16-wk feeding trial (Manthey et al.., 2016, 2017a and 2017b) was conducted using 48 Holstein heifers to evaluate effects of dietary treatment on dry matter intake (DMI), average daily gain (ADG), growth performance, rumen fermentation, and nutrient digestibility. Treatments were 1) 30% DDGS, with the diet fed at 2.65% of body weight (BW) (**30DG**), 2) 40% DDGS, with the diet fed at 2.50 % of BW (**40DG**), and 3) 50% DDGS, with the diet fed at 2.35% of BW (**50DG**). The remainder of the diet consisted of grass hay and 1.5% mineral mix. Heifers were individually limit-fed using Calan gates. There were no differences in growth parameters; however, gain: feed and nutrient digestibility increased

with increasing amounts of DDGS. There was a quadratic response of plasma urea nitrogen and a quadratic tendency for cholesterol. After heifers completed the feeding trial, data were collected to assess post trial reproductive and lactation performance, which were comparable among treatments. A second study (Manthey and Anderson, 2018) was conducted to determine the effects of feeding a corn and soybean product based concentrate mix or distillers dried grains with solubles (**DDGS**) concentrate mix with ad libitum grass hay to dairy heifers. A 16-wk feeding trial was conducted using 24 heifers to evaluate the effect of diet on DMI, growth performance, rumen fermentation, metabolic profile, and nutrient digestibility. Treatments were 1) corn and soybean product concentrate mix (**CON**), and 2) DDGS based concentrate mix (**DDG**). Both concentrate mixes were limit-fed at 0.8% of BW and grass hay was offered ad libitum. Dry matter intake and growth parameters did not differ between treatments. Rumen fermentation was shifted, but metabolic profile was maintained for heifers fed DDG. Results from these studies indicate that the fat and protein in DDGS can be used as a replacement for the starch in corn in limit-fed heifer diets with varying forage to concentrate ratios to maintain growth performance, nutrient digestibility, and metabolic profile without detrimental effects to long-term performance.

In all of our recent research with feeding heifers DDGS average daily gains have been between 0.95 – 1.0 kg/d and greater than anticipated according to formulations by the Dairy NRC, 2001. It is speculated that the NRC overestimates the energy requirements of heifers and underestimates the digestibility and energy content of DDGS. As the ethanol industry has evolved processing methods have continued to improve and change which means investigation into the quality, variation, and digestibility of dried distillers grains with solubles (DDGS) is warranted. Two methods, in vitro and in situ (Krogstad et al., 2018a and b), were used to evaluate the digestibility of 6 different DDGS samples (DG1, DG2…). The DDGS differed in source (ethanol plant) and fat content. It was hypothesized that fat content of the DDGS may impact rumen degradability and fiber utilization. The in situ experiment used 3 ruminally cannulated primiparous Holstein cows to evaluate the ruminal dry matter, fiber, and protein digestibility of the DDGS. The various DDGS samples were incubated in the cows for 0, 2, 4, 8, 16, 24, 48, 72, and 120 h. Dry matter and fiber degradation using two different bag types (Dacron vs. F57) were also compared. The in situ showed that the magnitude of the digestion was affected by bag type used. Total digestible protein (TDP) was also different across treatments, DG4 and DG5 being greater than the remaining DDGS. Rumen degradable NDF was also influenced by bag type with DG4 having the greatest RDNDF. Additionally, a 24 h rumen in vitro study was conducted with the same DDGS. It was found that although DM degradation did vary among the DDGS, fiber degradability varied less and was not affected as much by fat content as hypothesized. The VFA profile and gas production differences were observed among treatments, with DDGS favoring production of propionate over acetate. Differences in DDGS digestibility and utilization appear to be more dependent on other processing factors rather than fat content.

Overall, distillers grains performs well in heifers diets and is a very flexible feedstuff that can be used in a variety of inclusion rates and with different feeding strategies, provided overall nutrient requirements are met. Digestibility of DDGS is affected by source and methods of testing. Digestibility fractions vary compared to

previous research (Kleinschmit et al., 2007, and Cao et al., 2009) which warrants further investigation and demonstrates the quality of DDGS is evolving with the ethanol industry.

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