Effects of Nutrition on the Immunity of Dairy Calves

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TAKE HOME MESSAGES

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

ABSTRACT

Dairy calves are extremely susceptible to gastrointestinal disease during the pre-weaned period. The risk for enteric disease decreases as the calf ages; therefore, it is important to break the pre-weaned period up into at least 2 distinct phases that likely need to be managed differently, early life (first couple weeks of life) and the remaining time the calf is fed milk or milk replacer. When a calf is born they have been exposed to very few if any microorganisms and some aspects of their gastrointestinal immune system are not fully developed. After birth, the calf is now in a microbial world and exposed to a greater quantity and diversity of microorganisms. This adaptation is abrupt and dramatic and is a major stressor to a newborn calf. The gastrointestinal tract of the calf is naïve and develops rapidly during the first few days to weeks of life. The cells that make up the gastrointestinal tract are the first line of defense of the immune system; therefore, until the cells are more adult-like the calf may be at an increased risk for developing gastrointestinal diseases.

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

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

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

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

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

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

Keywords: Calf, Health, Immune, and Nutrition

INTRODUCTION

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

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

WHY ARE CALVES SO SUSCEPTIBLE TO GASTROINTESINTAL DISEASE?

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

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

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

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

Many of the components of the gastrointestinal immune system begin to develop as early as the first trimester of gestation; however, further maturation of many of these barriers occurs only after birth (Guilloteau et al., 2009). This process of rapid intestinal maturation is known as *gut closure* and contributes to the physical barrier. The enterocytes, the nutrient absorptive cells that make up the majority of cells in the intestinal epithelium, are considered fetal-type at birth because they are largely vacuolated and can absorb intact macronutrients through pinocytosis. These fetal-type enterocytes are quickly replaced by more adult-like enterocytes. This process occurs from the proximal to distal intestines and from the crypt to the villus tip; therefore, even though the majority of the gastrointestinal tract may have undergone *gut closure* in the day and a half after birth there likely persist vacuolated, fetal-type enterocytes toward the villus tip of the lower regions of the intestines for a longer period of time.

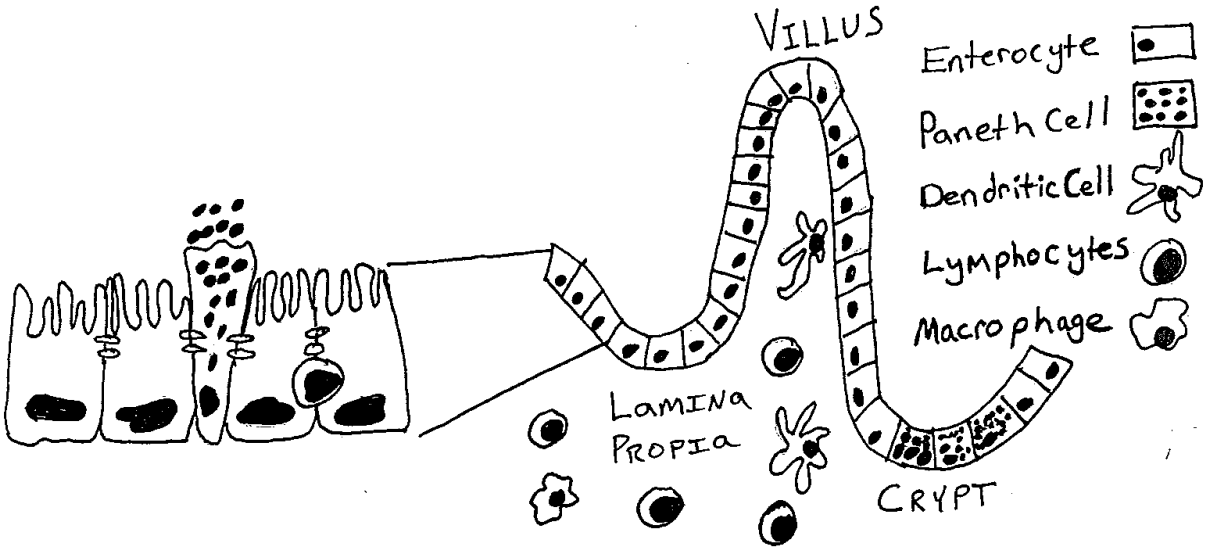

Figure 1. Schematic drawing of the small intestinal mucosa. The crypt-villus axis and common leukocytes found in the mucosa are shown on the right. The insert on the left is a magnification of the epithelial layer, depicting microvilli, tight junctions between epithelial cells, a goblet cell secreting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

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

The chemical and immunological barriers are also compromised during the early post-natal period. Paneth cells begin to develop during gestation; however, the number of Paneth cells and the antimicrobial secretions increase throughout life. Additionally, the adaptive arm of the immune system is naïve at birth and develops over the life of the animal as the calf is exposed and re-exposed to antigens. Therefore, sIgA concentrations and diversity are low and will remain low until the calf begins to develop it's own active immunity. Antibodies from colostrum are known to recirculate back to the mucosa of the intestines, and can offer some immediate protection from enteric pathogens; however, the half-life of many passively derived antibodies is 1 to 2 wk. Therefore, the gastrointestinal tract will become more susceptible to those specific microorganisms again until they develop their own active immunity against them. This is probably why many calves start developing localized enteric disease and scours during the 2nd or 3rd week of life. The fact is young animals will always be at an increased risk for infectious diseases until they develop their own active immunity. It's one of the benefits of getting older, the adaptive arm of the immune system becomes *wiser* because of what it has been exposed to and experienced.

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

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

calves is needed and should be a research priority.

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

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

Prebiotics, probiotics, and proteins from hyper-immunized eggs or spray-dried plasma all have shown some merit in improving the resistance to enteric disease. Prebiotics are dietary components that are not easily digested by the calf, but are used by bacteria in the lower intestines to improve their growth. Probiotics are a vague term, but generally are live microorganisms that provide *some* health benefit. At first glance this may seem bad, why would we want to improve the growth of bacteria in the lower intestines? As mentioned before, the intestinal tract is not sterile. Soon after birth, a wide range of bacterial species colonizes the gastrointestinal tract of calves. Most of these bacterial species do not pose any immediate threat to the survival of the calf and in the past were called *good bacteria* and, of which, many of the common probiotic species are routinely classified as, including: *Lactobacillus* species, *Bifidobacteria*, *Enterococcus faecium*, and *Bacillus* species. Remember that the microbial barrier of the intestinal tract soon after birth is colonized primarily by facultative anaerobes and subsequently becomes inhabited largely by strict anaerobes. Most of the probiotic microorganisms are strict anaerobes. Many of the probiotic species also have a direct bactericidal activity or compete with the more pathogenic microorganisms for limited resources. In addition, probiotics are themselves bacteria and they may *prime* the immune system of the calf by staying alert, as even the immune system recognizes the *good* bacteria as foreign. The common, commercially-available prebiotics available are the fructo-oligosaccharides (**FOS**), mannan-oligosaccharides (**MOS**), lactulose, and inulin.

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

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

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

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

In 2010, my lab evaluated the effects of supplementing a blend of prebiotics, probiotics, and hyper-immunized egg proteins to Holstein calves from immediately after birth through the first 3 wk of life (Ballou, 2011). Calves given the prophylactic treatment (n=45) were administered, directly into the milk, 5×10^9 colony forming units per day (from a combination of *Lactobacillus acidophilus, Bacillus subtilis, Bifidobacterium thermophilum, Enterococcus faecium*, and *Bifidobacterium longum*), 2 gm/d of a blend of MOS, FOS and charcoal, and 3.2 gm/d of dried egg protein from laying hens vaccinated against K99+ *Escherichia coli* antigen, *Salmonella typhimurium, Salmonella Dublin*, coronavirus, and rotavirus. Control calves (n=44) were not given any prebiotics, probiotics, or dried egg protein. All calves were fed 2 L of a 20 % protein/20 % fat, non-medicated milk replacer twice daily. Prior to each feeding fecal scores were determined by 2 independent trained observers. Briefly 1 = firm, well-formed; 2 = soft, pudding-like; 3 = runny, pancake batter; and 4 = liquid splatters, pulpy orange juice.

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

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

PLANE OF NUTRITION

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

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

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

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

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

Ongoing research in my laboratory is trying to understand the behavior and potential microbial exposure when calves are fed varying planes of nutrition and its influence on risk for enteric disease and immunological development. In fact, a few studies have shown that plane of nutrition during the pre-weaned period influences adaptive leukocyte responses. Pollock et al. (1994) reported that antigen-specific IgA and IgG_2 were reduced when calves were fed more milk. In agreement, Nonnecke et al. (2003) reported that less interferon-γ was secreted when peripheral blood mononuclear cells were stimulated with T-lymphocyte mitogens. However, not all data indicate that adaptive leukocyte responses are reduced when greater quantities of milk are fed; Foote et al. (2007) did not observe any difference in either the percentage of memory CD4+ or CD8+ T lymphocytes or antigen-induced interferon-γ secretion. All the leukocyte response data taken together suggest that calves fed lower planes of nutrition may have more active innate leukocyte responses driven by increased microbial exposure, which may explain the greater adaptive leukocyte responses. In a relatively sanitary environment this increased microbial exposure may improve adaptive immune development in the absence of clinical disease, but in a dirty environment it would likely increase the risk of enteric disease.

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

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

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

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

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

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

IMPLICATIONS

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

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

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

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Comparison of Sorghum Silage vs Corn Silage

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INTRODUCTION

Corn silage is the primary forage used in dairy rations across the United States, but many dairy producers also utilize sorghum silage as a part of their feeding program. There is renewed interest in sorghum silage as a primary forage because it requires less water to produce and is more drought tolerant than corn, which has become more important in many regions of the world where drought is common and water availability for irrigation is limited or restricted. However, sorghum silage has been considered lower quality forage compared with corn silage and has been used primarily in diets that require less energy than needed by high producing dairy cows. There is considerable variation in yield, fiber content and digestibility, and lodging potential of sorghum varieties commercially available for silage production. Research on the use of sorghum silage, especially grain sorghum, in dairy rations is more limited than that for corn silage; but indicates that improved varieties and genotypes of sorghum silage can support milk yield and component composition comparable with that of corn silage. While forage sorghum may not be a complete replacement of corn silage in all settings, it can be successfully used in rations fed to lactating dairy cows and offers an option for forage production.

CHARACTERISTICS OF SORGHUM SILAGE

Sorghum is a tropical summer annual with high yield potential when provided good fertility and moisture. Compared with corn, sorghum has proportionally more stem and less leaf and head/ear resulting in forage that has higher fiber concentrations (Contreas-Govea et al., 2010). Sorghum requires 40 to 53 % less water to produce a crop than corn (McCorkle et al., 2007), which is important in regions where water is limited or restricted. Miron et al. (2007) reported improved water efficiencies of 51 and 18 % for normal forage and brown midrib (**BMR**) forage sorghum silage compared with corn silage, respectively. The increase in water efficiency varies with the yield of the crop produced. The lower improvement observed in water efficiency for BMR forage sorghum reported by Miron et al. (2007) was due to the lower dry matter (**DM**) yield of that variety compared with normal forage sorghum and corn.

Several new genotypes of sorghum have been developed and made available for forage production that have improved forage quality and/or yield including BMR, high water soluble carbohydrate (**WSC**) or *sweet* varieties, photoperiod sensitive (**PS**) varieties, and brachytic dwarf varieties. The BMR varieties have lower lignin concentrations and greater neutral detergent fiber (**NDF**) digestibility. There are several naturally occurring genes that convey the BMR trait in sorghum. The two most common genes used in forage sorghum are bmr-6 and bmr-18. The DM yields of these varieties have been reported to be 10 % lower than conventional forage sorghum, but there is considerable variation among varieties. Forage sorghum naturally has a higher lodging potential than corn, especially when planted at high populations

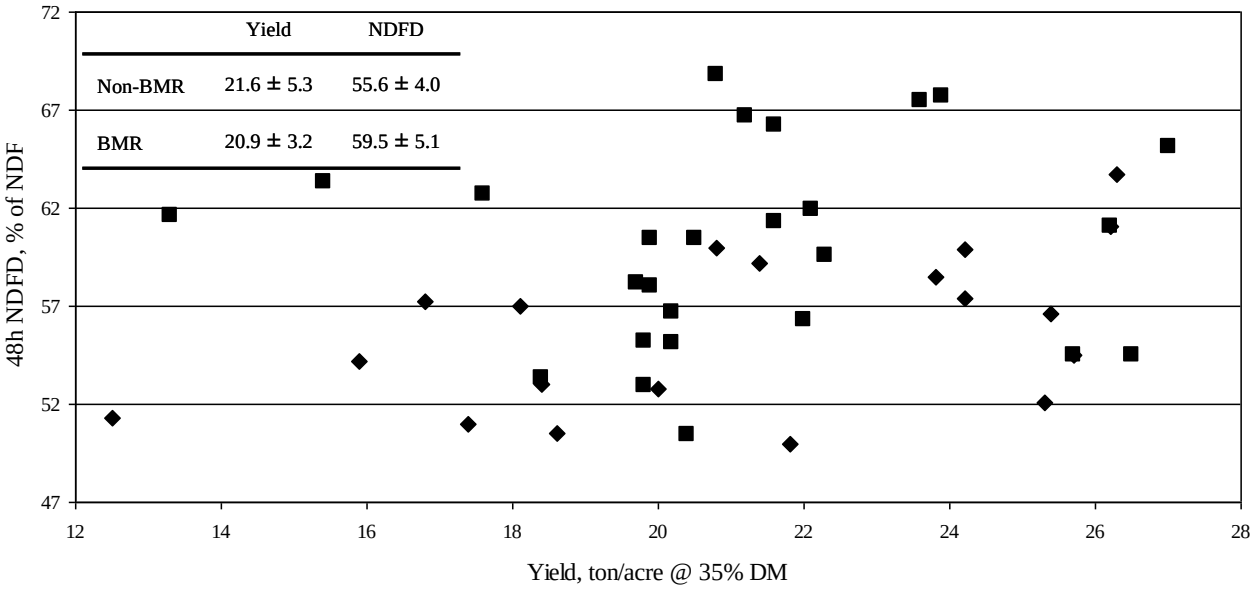

Figure 1. Relationship of yield and NDF digestibility of normal (♦) and BMR (■) forage sorghum varieties entered in the 2014 Texas Panhandle Sorghum Silage Trial (Bell et al., 2014).

and BMR varieties may have greater lodging potential because of the lower lignin concentrations. The WSC or sweet varieties contain more sugar which supports improved fermentation. The higher sugar content should provide more energy in support of milk synthesis or BW gain. Photoperiod sensitive varieties have delayed flowering which keeps the plant in a vegetative stage of maturity longer which should improve quality, but improvements in forage quality have not been consistently observed compared with normal forage sorghum. The PS varieties do have higher DM yield than normal sorghum varieties. Brachytic dwarf varieties have shorter internodes, greater leaf to stem ratio, and are considered to be more resistant to lodging. Many of the brachytic dwarf varieties also have the BMR gene and have become popular with dairy producers for forage production.

There is considerable variation in days to maturity, yield, plant height, lodging, and NDF digestibility among varieties. The extent of variation that exists is illustrated in Figure 1, which depicts the variation in DM yield and NDF digestibility, and Figure 2, which depicts the variation in DM yield and lodging of normal and BMR varieties entered in the 2014 Texas Panhandle Sorghum Silage Trial at Bushland (Bell et al., 2014). These figures illustrate the importance of reviewing variety test data to select varieties that have the combination of traits best suited for the forage quality and yield desired for specific feeding programs (i.e. high producing lactating cows versus dry cows or bred heifers).

Table 1. Chemical composition (mean ± standard deviation) of corn, normal forage sorghum, or BMR forage sorghum.

	Corn silage[1]	Forage Sorghum[1]		Forage Sorghum[2]		Grain sorghum[2]
Item	Normal	Normal	BMR	Normal	BMR	Normal
n =	8,640	1,498	132	26	34	8
DM, %	35.2 ± 4.9	32.8 ± 5.3	34.0 ± 6.5	32.3 ± 3.8	33.0 ± 3.0	32.6 ± 2.3
CP, %	8.1 ± 1.1	9.8 ± 2.4	10.6 ± 3.2	7.7 ± 1.0	7.9 ± 0.9	8.2 ± 0.3
ADF, %	25.3 ± 3.3	34.4 ± 4.59	34.3 ± 4.5			
NDF, %	40.9 ± 5.0	53.0 ± 6.8	54.2 ± 7.2	53.8 ± 9.2	49.4 ± 7.4	43.5 ± 2.4
NDFD, 30 h, %	56.5 ± 4.4	48.7 ± 7.0	54.0 ± 8.3			
NDFD, 48 h, %				54.6 ± 4.2	59.1 ± 5.0	58.8 ± 2.2
Lignin, %	3.2 ± 0.6	5.0 ± 0.9	4.6 ± 0.9	5.8 ± 1.1	4.8 ±0.8	4.7 ± 0.6
Sugar, %	1.3 ± 0.8	4.2 ± 2.3	5.3 ± 3.0			
Starch[3], %	32.1 ± 6.5	11.7 ± 8.0	10.3 ± 8.8	16.4 ± 9.6	20.0 ± 8.1	29.7 ± 3.2
Fat, %	3.2 ± 0.3	2.7 ± 0.4	2.9 ± 0.4	1.8 ± 0.5	2.1 ± 0.4	2.4 ± 0.2
Ash, %	4.1 ± 1.6	9.1 ± 3.5	8.9 ± 3.1			
Ca, %	0.25 ± 0.20	0.51 ± 0.35	0.44 ± 0.13			
P, %	0.23 ± 0.04	0.23 ± 0.06	0.26 ± 0.07			
Mg, %	0.16 ± 0.05	0.33 ± 0.11	0.32 ± 0.09			
K, %	1.14 ± 0.28	2.02 ± 0.76	2.23 ± 0.84			

[1]Analysis of silage samples submitted to Cumberland Valley Analytical Laboratory from January 1, 2013 through July 1, 2015.
[2]Analysis of unfermented samples from varieties entered in the 2014 Texas Panhandle Sorghum Silage Trial (Bell et al., 2014).
[3]Starch values reported for samples from Texas Panhandle Sorghum Silage Trial reflect concentrations at harvest.

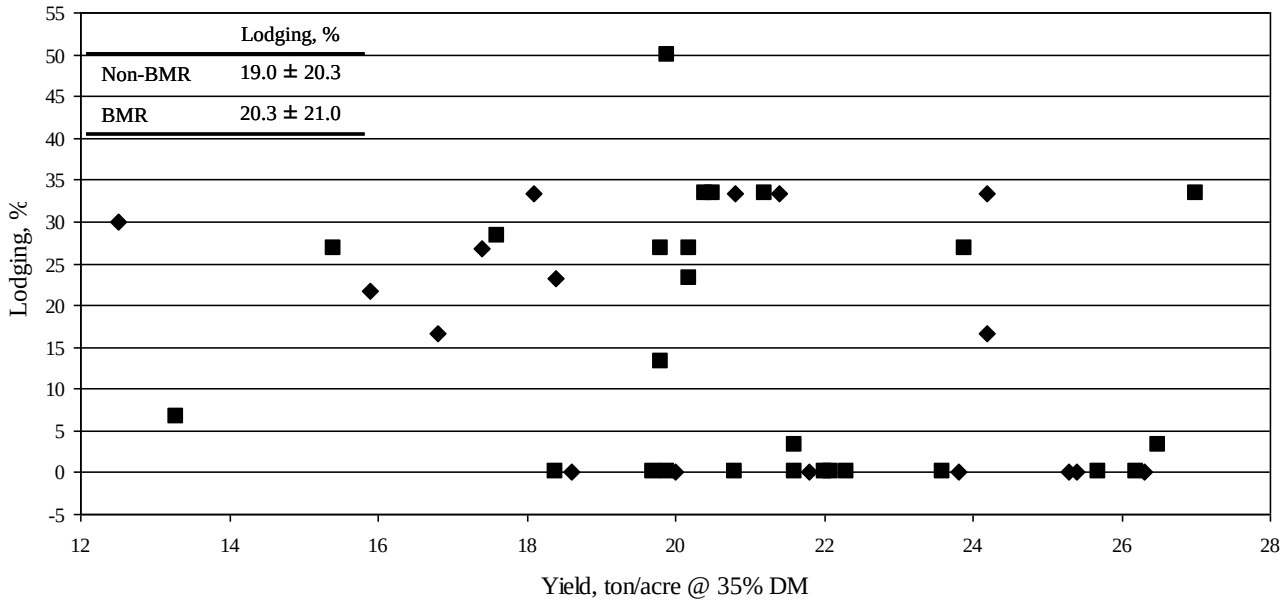

Figure 2. Relationship of yield and lodging of normal (♦) and BMR (■) forage sorghum varieties entered in the 2014 Texas Panhandle Sorghum Silage Trial (Bell et al., 2014).

The average chemical composition of corn, normal and BMR forage sorghum, and grain sorghum samples submitted to a commercial lab from the Plains and Southeast and analysis from the Texas Panhandle Sorghum Silage Trial (2014) are presented in Table 1. The standard deviations reported for each nutrient in Table 1 provide an indication of the variation observed in each nutrient for each of the forages. The differences reflect differences among varieties, stage of maturity, and changes during storage. In general sorghum silages have higher concentrations of protein, NDF, lignin, sugar, and ash; but lower starch and fat compared with corn silage. The BMR silages have similar composition as the normal varieties except for less lignin concentrations and higher NDF digestibility, which is typical for BMR varieties. The recommended stage of maturity for harvesting sorghum is early to late dough to optimize fiber and starch digestibility. Harvesting earlier than late vegetative or early head stage of maturity will result in silage with very low DM (< 25 % DM), which will result in excess seepage and a higher potential for undesirable fermentation characterized by higher concentrations of acetic and butyric acids and ethanol. Harvesting later results in lower starch digestibility.

PRODUCTION RESPONSE

Performance of lactating dairy cows fed sorghum silage differs depending on type of forage sorghum fed. Nichols et al. (1998) did not observe any difference in dry matter intake (**DMI**), yield of milk or component composition of cows fed diets based on either tropical corn silage or normal forage sorghum. However, tropical corn has higher lignin and lower starch concentrations than normal corn silage. Grant et al. (1995) compared the performance of lactating cows fed diets containing 65 % forage provided by normal or BMR forage sorghum, second cutting alfalfa silage, or corn silage. The DMI was lowest for diets based on alfalfa silage and highest for BMR forage sorghum compared with normal forage sorghum and corn silage. Milk yield and percentage fat and protein were lower for cows fed the normal forage sorghum diet compared with the other forages. No differences were observed in milk yield or component composition of cows fed BMR forage sorghum compared with corn silage or alfalfa silage. Aydin et al. (1999) reported the results of 2 additional trials from the same laboratory. In the first trial dietary NDF content of diets with normal and BMR forage sorghum was higher than those based on corn or alfalfa silage (39.7, 40.3, 29.1, and 34.3 % of DM, respectively). The differences in dietary NDF content did not affect DMI, which averaged 23.4 kg/d. Yield of milk, fat, and protein was highest with corn silage, intermediate for BMR forage sorghum and alfalfa silage, and lowest for normal forage sorghum. In the second trial, diets were based on a blend of alfalfa silage (17.5 % of DM) and either normal forage sorghum, BMR forage sorghum, or corn silage (35.3 % of DM) and contained similar concentrations of NDF (32.3, 31.6, and 31.9 % of DM, respectively). In this trial milk yield was higher for BMR forage sorghum compared with normal forage sorghum, but was not different from corn silage. No differences were observed in yield or percentage of milk components.

Oliver et al. (2004) compared normal forage sorghum, BMR genotypes -6 and -18 with corn silage. Each of the diets contained 40 % of the dietary DM from one of the 3 forages plus an additional 10 % from alfalfa hay. Diets were balanced to provide similar

CP, NDF, and starch concentrations. No differences were observed in DMI among treatments, but milk yield and milk fat percentage and yield were lower for diets based on normal forage sorghum compared with BMR-6 and corn silage, but not different with BMR-18. Efficiency (4 % FCM/DMI) was lower with normal forage sorghum compared with the other treatments. Miron et al. (2007) reported the results of a trial comparing normal forage sorghum, BMR forage sorghum, and corn silage. No differences were observed in DMI, but milk yield was higher for corn silage and lowest for normal forage sorghum but not different from BMR forage sorghum. Milk fat percentage was lower with corn silage compared to both normal and BMR forage sorghum. Milk protein percentage was highest for corn silage, intermediate for BMR forage sorghum, and lowest for normal forage sorghum. Concentrations of MUN were higher for corn silage compared with normal and BMR forage sorghum.

Limited research has been conducted examining the effects of using forage sorghum in combination with other forages in diets fed to lactating dairy cows. Boyd et al. (2008) reported the results of a trial in which diets based on a blend of normal forage sorghum and ryegrass silage (50:50 or 75:25) and supplemented with either ground corn, hominy feed, or a 50:50 blend of corn and hominy were fed to mid-lactation Holstein cows. Diets contained similar CP, NDF, and energy concentrations although starch concentrations were slightly lower for the 50:50 compared with the 75:25 blend (20.7 and 24.6 % of DM, respectively). No differences were observed in DMI, milk yield, or concentrations of components; but yield of milk fat tended to be higher and ECM yield and efficiency were higher for the 75:25 compared with the 50:50 blend. The authors suggested that the slightly higher starch content of the 75:25 provided by the sorghum silage potentially supported improved ruminal fermentation resulting in the improvements in yield of milk fat and ECM.

Table 2. Chemical composition of two corn (CS) and forage sorghum silage (FS) crops harvested in the summer (S) or fall (F)[1].

	Year	CSS	CSF	FSS	FSF
DM, %	1	46.6 ± 5.1	29.6 ± 2.0	28.7 ± 1.7	29.7 ± 3.4
	2	33.2 ± 2.3	36.4 ± 2.6	24.6 ± 0.5	27.3 ± 1.5
CP, %	1	8.0 ± 0.5	8.5 ± 0.3	9.0 ± 0.6	9.5 ± 0.6
	2	8.1 ± 0.4	8.2 ± 0.5	9.5 ± 0.5	11.3 ± 0.3
NDF, %	1	39.0 ± 1.1	38.3 ± 1.7	54.2 ± 1.7	55.1 ± 2.0
	2	39.0 ± 2.0	39.0 ± 1.7	56.1 ± 2.0	51.5 ± 0.8
NDFD,%	1	47.1 ± 2.8	53.0 ± 1.7	45.8 ± 3.3	37.4 ± 2.8
	2	52.8 ± 1.9	52.1 ± 3.5	51.0 ± 1.2	52.7 ± 0.8
ADF, %	1	24.5 ± 1.2	24.0 ± 1.3	35.9 ± 1.2	36.0 ± 1.8
	2	25.2 ± 1.6	22.8 ± 1.1	37.0 ± 0.8	34.0 ± 0.9
Ash, %	1	3.20 ± 0.35	4.19 ± 0.48	5.03 ± 0.28	4.73 ± 0.43
	2	3.20 ± 0.35	3.11 ± 0.18	5.02 ± 0.19	5.79 ± 0.40

[1]Trials were conducted in 2012 (Year 1) and repeated in 2014 (Year 2).

Table 3. Performance of lactating cows fed diet based on corn (CS) or forage sorghum silage (FS) harvested in the summer (S) of fall (F)[1].

	Year	CSS	CSF	FSS	FSF	SE	*P*
DMI, kg/d	1	21.4	23.1	22.6	21.1	1.2	0.57
	2	25.0	22.5	23.4	23.2	1.0	0.30
Milk, kg/d	1	32.2	33.4	32.9	33.5	1.5	0.92
	2	35.6	34.5	33.8	35.7	1.1	0.56
Fat, %	1	3.20[a]	2.91[a]	3.42[b]	3.53[b]	0.14	0.02
	2	3.61[d]	3.26[c]	3.70[d]	3.67[d]	0.12	0.06
Protein, %	1	2.80	2.70	2.64	2.69	0.05	0.15
	2	2.55	2.62	2.57	2.63	0.03	0.13
Lactose, %	1	4.63[a]	4.88[b]	4.87[b]	4.82[b]	0.40	0.01
	2	4.68	4.67	4.74	4.72	0.02	0.14
SNF, %	1	8.28	8.33	8.21	8.26	0.07	0.65
	2	8.07	8.09	8.13	8.15	0.04	0.68
ECM, kg/d	1	30.8	30.4	31.9	33.1	1.4	0.64
	2	34.6	35.4	32.7	36.3	1.0	0.15
Efficiency	1	1.44	1.32	1.41	1.57	0.09	0.55
	2	1.37	1.48	1.46	1.48	0.04	0.26
MUN, mg/dl	1	10.6[a]	13.4[b]	14.9[b]	15.3[b]	0.8	0.002
	2	8.2a	8.8a	11.5b	11.4b	0.31	<0.0001

[a,b]Means with unlike superscripts in the same row differ ($P < 0.01$)
[c,d]Means with unlike superscripts in the same row differ ($P < 0.10$).
[1]Trials were conducted in 2012 (Year 1) and repeated in 2014 (Year 2).

In recent years, brachytic dwarf varieties with the BMR-6 gene have been adopted by producers because of their lower lodging potential and ability to produce similar DM yield as normal forage sorghum varieties. In semi-tropical areas, forage sorghum will produce a second crop without replanting, which would reduce production cost. For the last few years, the University of Georgia has included a measurement of regrowth as part of the variety test data. For 2010 the variety test plots were planted on April 16 and the first crop was harvested on July 28 with a second harvest on October 18. The average DM yield for varieties was 7.9 ton/acre for the first harvest and 6.4 ton/acre for the second harvest. We have completed 2 trials comparing the performance of lactating dairy cows fed silage harvested from spring and summer corn crop with forage sorghum silage harvested from a brachytic dwarf variety planted in the spring and allowed to ratoon after the first harvest. The chemical composition of the silages harvested in 2012 (Trial 1) and 2014 (Trial 2) are presented in Table 2. The composition of the first and second corn silages was similar except that the fall crop had lower concentrations of starch. The 2 forage sorghum silage crops were similar in composition and had higher concentrations of fiber and lower starch than corn silage. No differences were observed in DMI, milk yield, or component composition among the forages except that milk fat percentage was higher for both diets based on forage sorghum compared with corn silage (Table 3). Concentrations of MUN were lower for the first corn silage harvested in the summer compared with the other treatments. We repeated this trial in 2015 and the results are presented in Table 3. In agreement with the first trial, there were no differences in DMI or milk yield.

Table 4. Performance of lactating dairy cows fed diets based on corn (CS), whole plant grain sorghum (WPGS), or normal forage sorghum silage (FS)[1].

	CS	WPGS	FS	SE	*P*
DMI, kg/d	20.0	20.0	18.2	0.5	0.07
Milk, kg/d	25.4[a]	24.6[a,b]	23.6[b]	0.4	0.05
Fat, %	4.08	4.33	4.16	0.08	0.14
Fat, kg/d	1.03	1.06	0.98	0.02	0.09
Protein, %	3.36	3.28	3.31	0.07	0.31
Protein, kg/d	0.85[a]	0.81[a,b]	0.77[b]	0.02	0.05
4% FCM, kg/d	25.6	25.7	24.1	0.5	0.07
Efficiency	1.28	1.29	1.32	0.03	0.63
MUN, mg/dl	10.7[a]	11.9[a,b]	12.9[b]	0.02	0.05

[1]Colombini et al., 2012.
[a,b]Means in the same row with unlike superscripts differ ($P < 0.05$).

In contrast, milk fat percentage was lower for the second corn silage compared with the other forages and MUN concentrations were higher for both forage sorghum silages compared with the corn silages. No differences were observed in concentrations of milk protein, lactose, or SNF or efficiency of milk production.

Data on the feeding value of sweet sorghum are limited. Amer et al. (2012) reported lower milk yield and higher milk fat percentage for cows fed diets based on sweet forage sorghum plus corn silage compared with a control diet based on alfalfa and corn silage. Yield of enery-corrected milk (**ECM**) and efficiency of milk production was not different among diets suggesting that these varieties have potential for use in diets fed to high producing dairy cows. Additional data are needed to determine their full potential.

Limited research data are available on feeding grain sorghum silage to lactating dairy cows during the last 2 decades. In general grain sorghum has been considered to be higher quality when harvested before late dough stage of maturity than normal forage sorghum, partially because of the additional starch provided by the grain (Bolsen, 2004). No differences were observed in DMI, milk yield, or component composition of mid-lactation cows fed diets based on inoculated or un-inoculated corn silage compared with grain sorghum silage (Bolsen et al., 1989). Recently Colombini et al. (2012) reported the results of a trial comparing diets based on corn, whole plant grain sorghum, or normal forage sorghum silages (Table 4). The corn silage, whole plant grain sorghum, and normal forage sorghum provided 41.5, 36.7, and 28 % of the dietary DM, respectively, to maintain equal NDF concentrations. Starch was equalized using corn meal. No differences were observed in DMI or percentage milk fat and protein, but yield of milk and milk protein were lowest and MUN highest for normal forage sorghum compared with corn. Whole plant grain sorghum supported similar DMI, milk yield, and component composition as corn silage.

CONCLUSIONS

There is considerable variation in yield, lodging potential, and NDF digestibility of varieties currently available, so it is important that producers and their advisors study the available information to select varieties that can produce the yield and quality needed to support milk production. The available data indicate that BMR forage

sorghum or grain sorghum can support DMI, milk yield, and component composition comparable to that of corn silage; but diets based on regular forage sorghum will result in lower milk yield. Based on higher MUN concentrations observed when diets are based on sorghum, there is potential to improve dietary nitrogen utilization compared with corn silage.

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Mid-South Ruminant Nutrition Conference

"High Res Forage Testing"

August 20, 2015
Cliff Ocker
Director of Operations and Client Relations
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Characterizing Starch

Starch Concepts in the Ruminant

- We can do a reasonably good job of determining total starch in a feed material.
- We do not have a good means of characterizing of rumen degraded starch
- We do not have a good means of understanding passage rate of undigested starch
- As a result, we do not have a good understanding of partition of starch digestibility in rumen vs the hindgut.

Starch Concepts in the Ruminant

- Nutritionists would generally agree that we want to maximize starch digestion in the rumen up to the point where it significantly impacts the fiber digestibility.

Starch Feeds to Characterize

- Corn
- High Moisture Corn
- Barley, Wheat, Oats, Triticale
- Sorghum
- Milo
- Starch byproducts
- Corn Silage
- Sorghum silage
- Small grain silages
- Milo silage

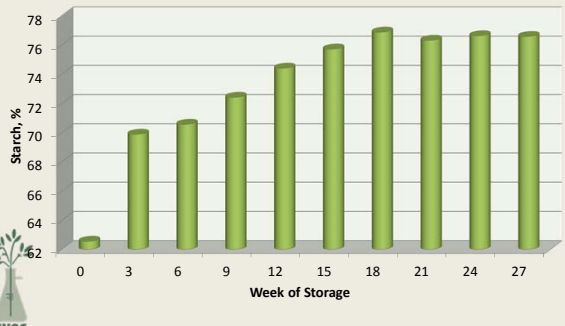
Relationship of Various Nutrients to Starch Digestibility in Corn Silage over Time in Storage

(CVAS, 2012 Crop Year, NE US Samples)

Storage Week	IVSD7	Total VFA	Lactic Acid	Soluble Protein	Ammonia
0	62.6	1.31	0.88	2.30	1.01
3	69.9	4.57	3.23	3.26	1.19
6	70.6	4.96	3.53	3.35	1.18
9	72.4	5.78	4.07	3.61	1.24
12	74.4	6.34	4.47	3.89	1.32
15	75.7	6.57	4.68	4.09	1.29
18	76.9	7.33	5.08	4.31	1.41
21	76.3	7.50	5.27	4.33	1.37
24	76.6	7.66	5.40	4.42	1.43
27	76.6	7.62	5.41	4.39	1.38

Impact of Storage Time on Starch Digestibility in Corn Silage

(CVAS, 2012 Crop Year, North-East US Samples)



Corn Silage Processing Score

- Measure of the % of starch in corn silage that passes through a 4.75mm screen
- Dried corn silage is shaken for 10 minutes on a Ro-Tap Sieve Shaker.
- Material not passing the 4.75 mm screen is collected and assayed for starch.
- Properly processed corn silage will have a processing score of greater than 60%, Optimum over 70%
- Poorly processed corn silage will lead to lower rumen starch degradation and lower total tract digestibility.

Rotap shaker showing 4.75mm screen and corn retained on the sieve

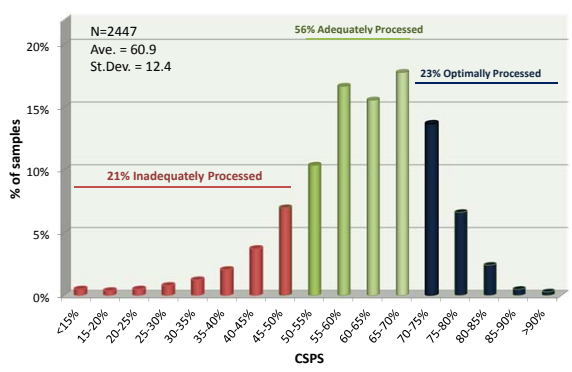
Industry Makes Advances in Corn Silage Processing

(CVAS Data, 2006 to 2014)

Crop Year	Number	Average	Percent Optimum	Percent Poor
2006	97	52.8	8.2	43.3
2007	272	52.3	9.2	37.9
2008	250	54.6	5.2	34.8
2009	244	51.1	6.1	48.0
2010	373	51.4	5.9	43.4
2011	726	55.5	12.3	33.1
2012	871	60.8	14.8	19.9
2013	2658	64.6	26.2	22.1
2014	4634	62.2	25.8	10.4

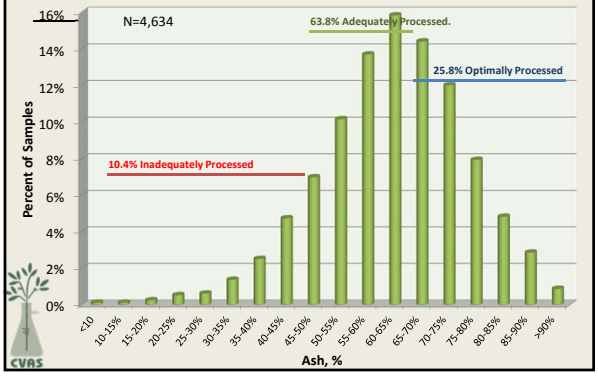
Distribution of Corn Silage Processing Scores

(CVAS, 2012 and 2013 Crop Years)



Distribution of Corn Silage Processing Scores

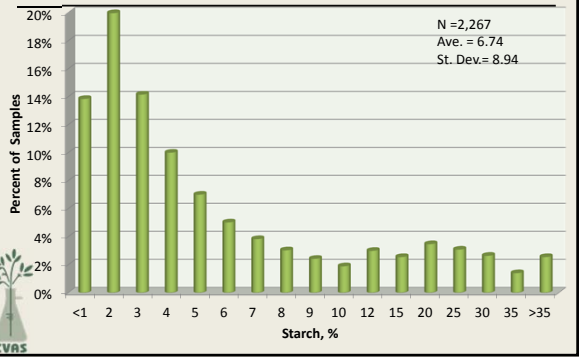
CVAS 2014



Apparent (whole tract) Digestibility

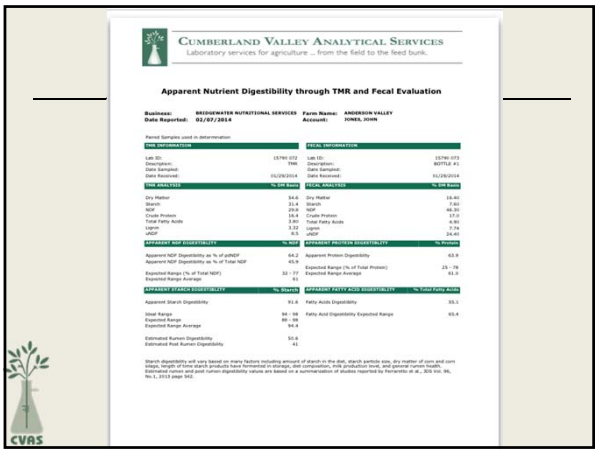
- There has been interest in evaluating fecal starch as an indicator of digestion efficiency.
- This approach has limited value because it does not account for beginning starch level or the concentration effect in the manure.
- One new approach is using indigestible NDF as a marker to relate the starting and ending starch levels.

Distribution of Starch Values in Feces (CVAS 2012, Chemistry Methods)



Apparent (whole tract) Digestibility

- CVAS has developed NIR equations for 240 hour indigestible NDF in TMR and fecal material.
- Clients submit samples of TMR and associated fecal material to the laboratory.
- CVAS provides an analysis of the TMR and fecal material and a report of Apparent Digestibility for Starch, pdNDF, and Protein.
- This information can be used as a diagnostic tool to evaluate ration efficiency, evaluate additives and help make management decisions.



CUMBERLAND VALLEY ANALYTICAL SERVICES
Laboratory services for agriculture ... from the field to the feed bunk.

Apparent Nutrient Digestibility through TMR and Fecal Evaluation

Business: BRIDGEWATER NUTRITIONAL SERVICES **Farm Name:** ANDERSON VALLEY
Date Reported: 02/07/2014 **Account:** JONES, JOHN

Feed Samples used in determination

TMR INFORMATION	
Lab ID:	15791 072
Description:	TMR
Date Sampled:	
Date Received:	01/29/2014

TMR ANALYSIS	% DM Basis
Dry Matter	54.6
Starch	31.4
NDF	29.8
Crude Protein	16.4
Total Fatty Acids	3.80
Lignin	3.32
uNDF	8.5

APPARENT NDF DIGESTIBILITY	% NDF
Apparent NDF Digestibility as % of pdNDF	64.2
Apparent NDF Digestibility as % of Total NDF	45.9
Expected Range (% of Total NDF):	32 - 77
Expected Range Average:	61

APPARENT STARCH DIGESTIBILITY	% Starch
Apparent Starch Digestibility	91.6
Ideal Range:	84 - 98
Expected Range:	88 - 98
Expected Range Average	94.4
Estimated Rumen Digestibility	50.6
Estimated Post Rumen Digestibility	41

Starch digestibility will vary based on many factors including amount of starch in the diet, starch particle size, dry matter of corn and corn silage, how well starch products have fermented in storage, diet composition, milk production level, and general rumen health. Estimated rumen and post rumen digestibility values are based on a summarization of studies reported by Ferlantito et al., 2015 Vol. 96, No.1, 2013 page 562.

FECAL INFORMATION	
Lab ID:	15791 073
Description:	BOTTLE #1
Date Sampled:	
Date Received:	01/29/2014

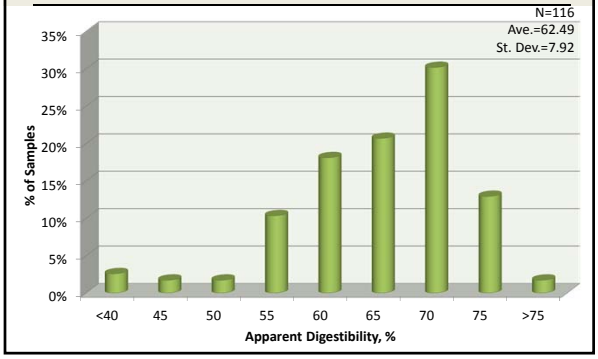
FECAL ANALYSIS	% DM Basis
Dry Matter	16.40
Starch	7.60
NDF	46.30
Crude Protein	17.0
Total Fatty Acids	4.90
Lignin	7.74
uNDF	24.40

APPARENT PROTEIN DIGESTIBILITY	% Protein
Apparent Protein Digestibility	63.9
Expected Range (% of Total Protein):	25 - 78
Expected Range Average	61.5

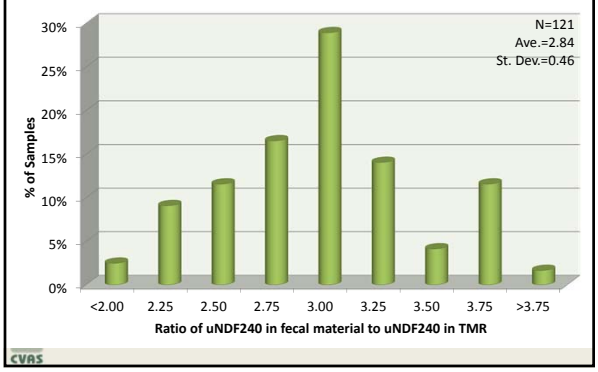
APPARENT FATTY ACID DIGESTIBILITY	% Total Fatty Acids
Fatty Acids Digestibility	55.1
Fatty Acid Digestibility Expected Range	65.4

CVAS

Distribution of Apparent Digestibility of TMR pdNDF Data

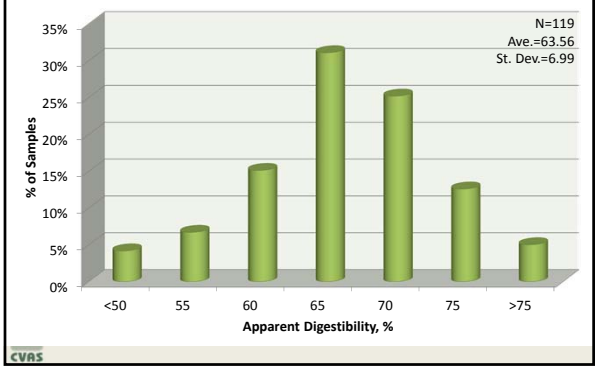


Distribution of Ratio of uNDF240 in Fecal Material to uNDF240 in TMR

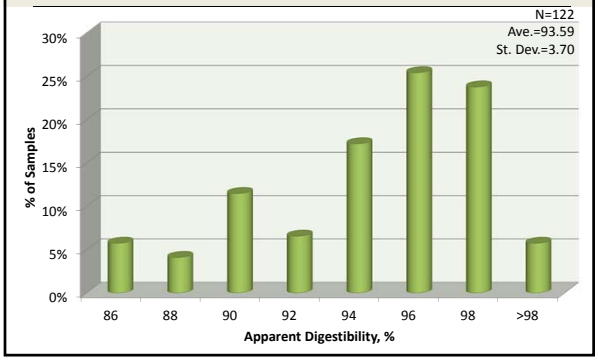


CVAS

Distribution of Apparent Digestibility of TMR Protein Data



Distribution of Apparent Digestibility of TMR Starch Data



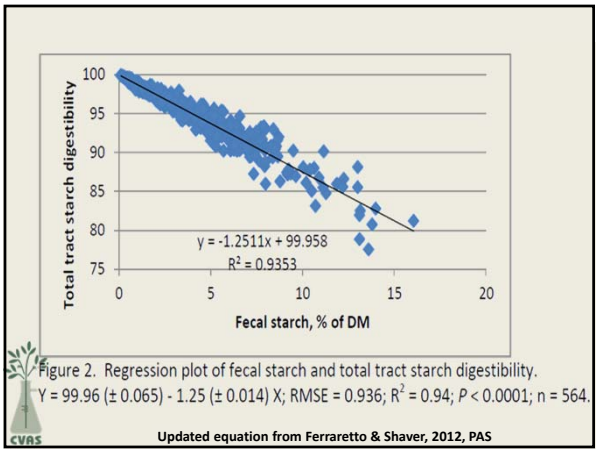


Figure 2. Regression plot of fecal starch and total tract starch digestibility.
Y = 99.96 (± 0.065) - 1.25 (± 0.014) X; RMSE = 0.936; R^2 = 0.94; *P* < 0.0001; n = 564.

Updated equation from Ferraretto & Shaver, 2012, PAS

In vitro and In situ

- In vitro methods are the most common used for starch digestibility evaluations in the U.S.
- The primary dairy laboratories in the U.S. have now all adopted this approach.
- At CVAS we maintain a 1800 flask incubation system and approximately 10 cannulated cows for In vitro and In situ work.
- CVAS provides significant In situ evaluations for protein, starch, and NDF.

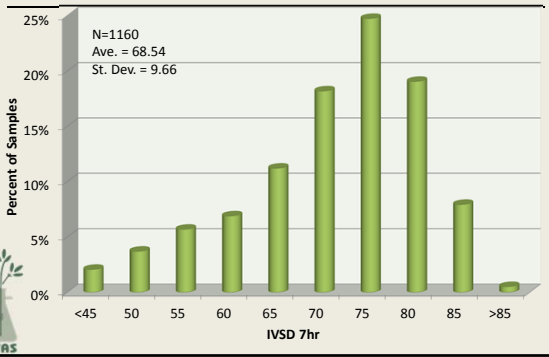
Comparison of 7hr in situ method with 7hr in vitro method for evaluating Starch Digestibility in Selected Samples (CVAS, 2013)

Feed Type	7hr in situ	7hr in vitro
Box Canyon Ground Corn (as is)	58.5	57.5
Box Canyon Ground Corn (ground	74.0	74.8
30# Flaked Corn GNE (as is)	44.5	40.8
30# Flaked Corn GNE (ground)	75.8	74.8
26# Flaked Corn GNE (as is)	53.9	46.7
26# Flaked Corn GNE (ground)	73.6	75.4
Ground Corn GNE (as is)	54.1	56.8
Ground Corn GNE (ground)	72.0	73.0

7-Hour In Vitro Starch Digestibility of Corn Samples (CVAS, 2010)

Feedstuff	No. of Samples	DM	7h IV Starch Digestibility	SD
Corn Grain	123	87.5	60.9	8.1
HM Corn	103	72.9	64.1	8.9
HM Ear Corn	20	58	73.9	8.5
Corn Silage	107	<28	80.1	7.5
Corn Silage	204	28 to 32	79.7	8.7
Corn Silage	224	32 to 36	77.5	9.5
Corn Silage	102	36 to 40	73.3	10.2

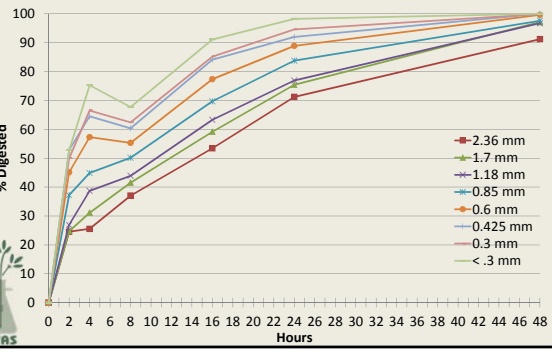
Distribution of IVSD 7HR in Corn Silage (CVAS, 2013)



Nutrient Characteristics of Sieved Fermented Corn Grain (CVAS, 2013)

Particle Size, MM	2.360	1.700	1.180	0.850	0.600	0.425	0.300	0.212
CP, %	9.3	8.5	8.5	8.6	7.9	6.6	6.4	5.8
ADF, %	6.8	6.9	6.1	4.2	3.2	2.3	2.3	2.6
NDF, %	14.3	13.9	12.1	8.6	5.9	4.0	2.6	2.8
Ash, %	4.24	4.19	2.45	1.88	1.76	1.56	1.21	0.95
Starch, %	66.4	67.4	69.6	75.4	78.7	81.6	83.7	84.9
Sugar, %	1.69	1.70	1.73	1.74	1.80	1.73	1.75	1.70
Fat, EE, %	3.78	3.96	3.89	3.49	2.77	2.66	2.48	2.49
SP%CP	11.5	8.73	7.98	6.71	6.13	2.35	3.35	1.25

Starch Digestion by Particle Size Over Time (CVAS, 2013)



Sampling Error & Technique

Weiss et al.

Studied over 448 samples, 8 farms, 14 days.

The variation attributed to sampling technique

Corn Silage	Hay Crop Silage
Dry Matter 25 to 55 %	5 to 30 %
NDF 15 to 65%	8 to 52 %
Starch 11 to 78 %	Protein 12 to 72%

Sampling Techniques

Bunker & Bag Silos – similar in sampling protocols.
Clean 5 gal bucket and clean surface.

Uprights – 2 to 3 gal of silage and proper subsampling

Hays and Baleage- a hay probe with sharp teeth.
Depending on the size of the crop – several probe samples are necessary.

Good samples are the foundation of good diet formulation.

NDFom

NDF (organic matter basis) or ash free

- What effects the ash level in forages?
- Why move to ash free?
- How does the lab make this adjustment?
- Does ash make that much difference?
- Does ash effect NDFD as well?

What effects ash level in forages?

- Rain splash of soil on a wilting crop
- Irrigation splash
- Flooding
- Incorporation of soil at harvest
- Incorporation of soil/mud while packing

Why move to ash free?

- To give credit where due…Dr. Charlie Sniffen had CPM built on ash free values
- Europeans has traditionally utilized an organic matter approach.
- Has not been perceived as a major issue and labs have not been volunteering to do this…
- Newer harvesting methods/equipment has increased soil contamination

How does the Lab make this adjustment?

- First we need to understand how an NDF is ran to understand the problem:
  - To extract NDF, a portion of the forage or feed material is boiled in a detergent solution that is buffered to a pH of 7.0, hence the term 'Neutral Detergent Fiber'
  - Some ash may be soluble in hot neutral detergent solution, but most will not.

How does the Lab make this adjustment?

- When the residue is collected on the glass fiber filter, the remaining insoluble ash is collected as well and appears as undigested fiber.
- For many samples this difference is small but can help explain some things for others.

To get to an 'ash free' basis, that filter and residue is placed into an ashing furnace at 600 degrees centigrade for two hours.

How does the Lab make this adjustment?

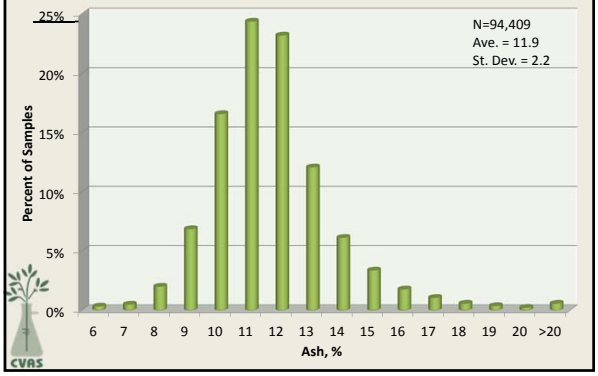
- After this treatment, all that is left is the glass fiber filter and the residual ash.
- This is weighed to determine ash content and by difference the Lab can determine the organic NDF that was present.
- See why the labs were not volunteering…? This can delay results by a day when done by chemistry.

Does ash make that much difference?

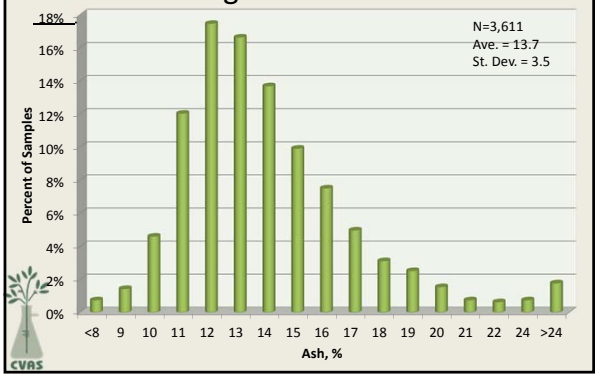
- Ash creates a challenge in the lab whether we are doing NIR or chemistry
- Fibers are inappropriately elevated creating a need for fibers to be reported 'ash free'

- Lets look at some data

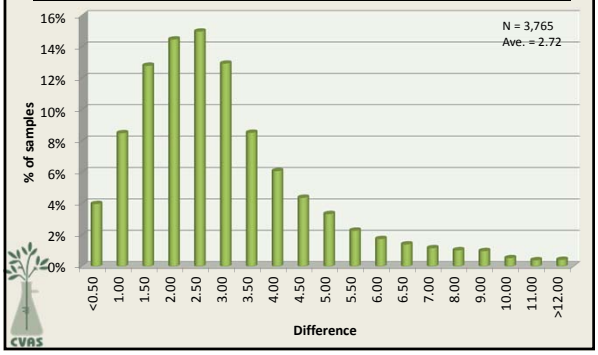
Ash in legume forages, CVAS 2013-2014



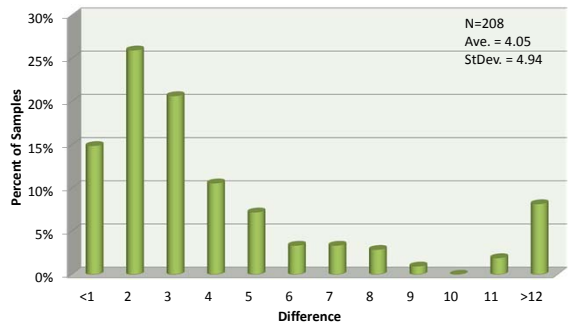
Distribution of Percent Ash in Legume Forages – West 2015



Distribution of Differences between NDF and NDFom in Haycrop Silage (CVAS, 2013)



Difference Between aNDF and aNDFom (organic matter basis) in Selected Sorghum and Sorghum/Sudan Samples
(CVAS, 2012 crop, chemistry)



aNDF - How does NIR see NDF?

- Will see difference between aNDF by chemistry, aNDF by NIR, and aNDFom by chemistry
- Example: Legume, 15% ash
  - aNDF by chemistry 38.4%
  - aNDF by NIR 36.2%
  - aNDFom by chemistry 34.2%

Example of the Impact of Ash Contamination on NDF and NDF Digestibility Recovery

Sample	NDF	NDFom	NDFD30	NDFD30om
15081-068	54.6%	**48.3%**	56.3%	**65.9%**

Example of the Impact of Ash Contamination on NDF and NDF Digestibility Recovery

Sample	NDF	NDFom	NDFD30	NDFD30om
15081-68	54.6%	**48.3%**	56.3%	**65.9%**
15085-56	60.1%	**50.9%**	49.7%	**61.9%**

Labs traditionally have not run NDF on organic matter basis …

- Potential problems are generally not recognized
- Ash contamination is more of an issue today than 10 years ago
- Significantly more work / cost to lab, cost to client
- NIR calibrations generally do not exist for aNDFom (CVAS has developed these for forage equations)
- Not only NDF but NDF digestibility needs to be run on an ash-free basis
- Education / acceptance component

High Res Forage Testing

- NDF In vitro digestibility
  - Allows for proper ranking of forages and hybrids (plot study work)
  - Allows for more appropriate rate calculations, 6.5 Biology
  - Forages 30, 120, 240 Non Forages 12, 72, 120 time points
  - Properly labeling fast vs slow pools of NDFD
  - Great for troubleshooting herd performance

High Res Forage Testing

uNDF240

- Historically estimated as lignin * 2.4
- Based on early research by Van Soest
- 2.4 factor used within and across various feedstuffs
- Distinguished from "iNDF" which is a theoretical term
- U.S. Ration Models will be making the switch to 6.5 CNCPS
- More accurate rate predictions

Relationship Between uNDF as Lignin *2.4 and uNDF as uNDF240

	NDF	uNDF Lig2.4	uNDF240	Lignin Factor
Western Alfalfa	41.7	17.1	22.7	3.2
Legume	41.8	15.9	21.6	3.3
MM Legume	50.1.	16.5	24.3	3.5
Mixed	53.5	14.6	23.0	3.8
MM Grass	60.0	14.3	25.1	4.2
Grass	58.9	12.9	23.7	4.3
Corn Silage- Conv.	40.0	7.4	10.6	3.4
Corn Silage – BMR	40.4	6.2	8.0	3.1
Sorghum – Forage	59.6	9.8	18.0	4.4
Sorghum - Grain	48.5	10.5	9.7	2.3

NDF Characteristics of Byproduct Feeds (CVAS, 2014)

Feed Name	NDF	Dig NDF (% NDF)	uNDF (%NDF)	Kd (%/hr)	Lbs NDF/hr
Soy Hulls	69.9	96.3	3.7	10.6	0.72
Beet Pulp	46.4	84.2	15.8	15.4	0.60
Dry Distiller's Grains	35.3	88.8	11.2	6.9	0.22
Cotton Hulls	81.5	63.5	36.5	2.2	0.11
Almond Shells	61.2	19.9	80.1	4.1	0.05
Cotton Gin Trash	74.9	31.0	69.0	1.9	0.05
Rice Hulls	71.7	4.7	95.3	3.7	0.01

MSPE (Ross) uN Step 1: In vitro

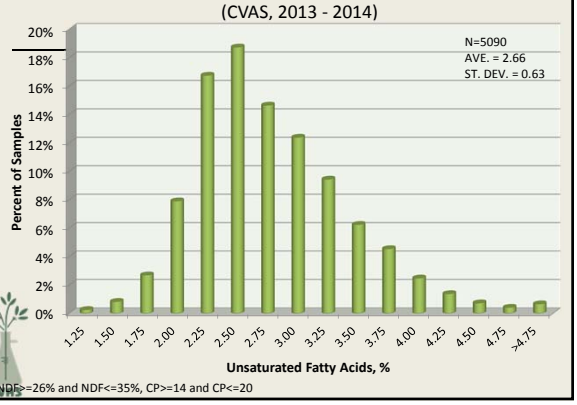
RUP is measured by incubating a sample in vitro with rumen fluid from high group lactating dairy cattle for 16 hours.

How do products compare?

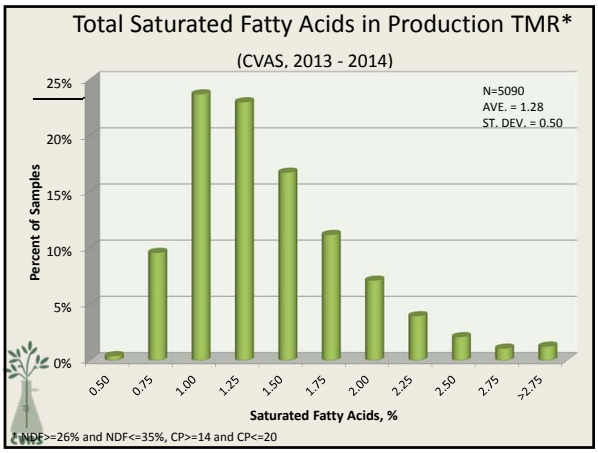
Source	SP, % CP	RUP at 16HR, % CP	RDP, % CP	Intest. Dig CP, % CP	Total Tract Digest. CP, % CP
Blood 1	58	40	60	37	97
Blood 2	9	91	9	74	82
Blood 2, Burnt	8	92	8	6	12
Soybean Meal	14	32	68	26	95
Canola	16	42	58	30	88
Gluten Meal	11	78	22	60	81
Commercial Soy 1	9	77	23	68	91
Commercial Soy 2	15	57	43	51	94
Commercial Blend 1	10	73	27	50	77
Commercial Blend 2	8	45	55	36	91

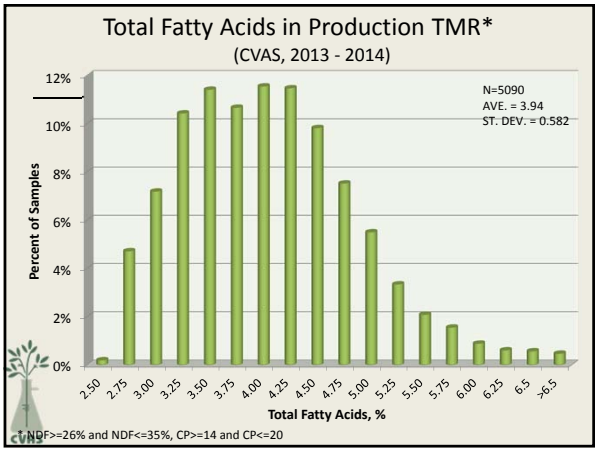
Unsaturated Fatty Acids, Production TMR*

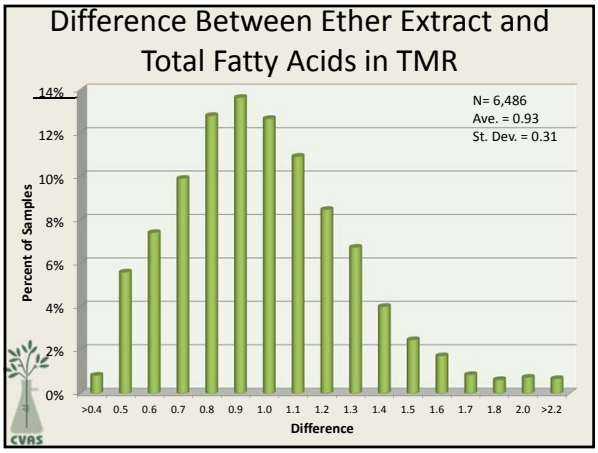
(CVAS, 2013 - 2014)



* NDF>=26% and NDF<=35%, CP>=14 and CP<=20


Total Saturated Fatty Acids in Production TMR*
(CVAS, 2013 - 2014)
N=5090
AVE. = 1.28
ST. DEV. = 0.50
Percent of Samples
25%
20%
15%
10%
5%
0%
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
>2.75
Saturated Fatty Acids, %
* NDF>=26% and NDF<=35%, CP>=14 and CP<=20


Total Fatty Acids in Production TMR*
(CVAS, 2013 - 2014)
N=5090
AVE. = 3.94
ST. DEV. = 0.582
Percent of Samples
12%
10%
8%
6%
4%
2%
0%
2.50
2.75
3.00
3.25
3.50
3.75
4.00
4.25
4.50
4.75
5.00
5.25
5.50
5.75
6.00
6.25
6.5
>6.5
Total Fatty Acids, %
* NDF>=26% and NDF<=35%, CP>=14 and CP<=20


Difference Between Ether Extract and Total Fatty Acids in TMR
N= 6,486
Ave. = 0.93
St. Dev. = 0.31
Percent of Samples
14%
12%
10%
8%
6%
4%
2%
0%
>0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
2.0
>2.2
Difference

Better Tools=Better Nutrition=Better Performance

- NDFom
- NDF Digestibility
- uNDFD 240
- Fermentation Evaluation
- Starch Characterization
- Apparent Nutrient Digestibility (TMR/Fecal)
- Multi Step Protein Evaluation
- Dry Methods/Sample Preparation
- CVAS Mobile App
- Database Summaries
- Report Validation

Conclusion

- Efficient utilization of starch in ruminant diets is dependent on being able to properly characterize starch across feedstuffs and processing methods.

CSPS

- A unified and animal relevant approach needs to be developed to accomplish this task.

Apparent Nutrient Digestibility

- NDF on an “ash free” or organic matter basis is a better way of characterizing true NDF in forages.

Mid-South Ruminant Nutrition Conference

“High Res Forage Testing”

Cliff Ocker
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The Food Safety Modernization Act: Regulatory Impact on Feed Manufacturing

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OVERVIEW

The Food Safety Modernization Act (**FSMA**) was signed into law on January 4, 2011 and provides the U.S. Food and Drug Administration (**FDA**) with sweeping new authorities and requirements. The law was a bi-partisan supported bill backed by the food and feed industries. It authorizes FDA to promulgate new rules for preventive controls, develop performance standards, create new administrative detention rules, provides authority for mandatory recall of adulterated products, and provides authority for hiring more than 4,000 new field staff among other provisions. It remains unclear whether Congress will provide sufficient funding to fully implement the law, but the FDA is proceeding with rulemaking to meet the court ordered deadlines that were established. The animal food final rule must be published by August 2015.

The centerpiece of the law is the hazard identification, written food safety plan, and preventive controls. These items are required of all feed, pet food, and ingredient facilities that process, pack, manufacture, or hold feed unless they are exempt as a *farm*, (facilities that feed their own animals on their own farms) or are classified as a very small business. The food safety plan must be available for FDA to review and copy. It encompasses several areas and requires recordkeeping for 2 yr. Basically, Congress requires FDA to do the following (quoted from the law):

"*The owner, operator, or agent in charge of a facility shall, in accordance with this section, evaluate the hazards that could affect food manufactured, processed, packed, or held by such facility, identify and implement preventive controls to significantly minimize or prevent the occurrence of such hazards and provide assurances that such food is not adulterated under section 402 or misbranded under section 403(w), monitor the performance of those controls, and maintain records of this monitoring as a matter of routine practice.*"

Regulations to implement this provision of the law were to be finalized by July 2012. FDA missed this deadline and was sued by food safety activists and is now under a court ordered mandate to finalize many of the FSMA regulations. This hazard analysis and preventive control regulation for animal food is due to be finalized by August 30, 2015.

IMPROVING FEED SAFETY

The intent of FSMA is to better protect human and animal health by helping to ensure the safety and security of the food and feed supply. FDA embraces preventing food safety problems as the foundation of a modern food safety system and recognizes the need for a global approach to food and feed safety. Thus, FSMA is designed to take a proactive approach by promoting continuous improvement through audits vs. compliance to regulatory requirements through inspections.

FDA states that ensuring the safety of animal food involves:

1) the safety of the food consumed by animals and
2) the safety of humans handling the food, particularly pet food.

The agency indicates the gaps in the current system to ensure the safety of animal feed include a lack of federal regulations for Current Good Manufacturing Practices (**CGMP**) to provide baseline requirements for non-medicated animal feed, pet food, raw materials, and ingredients. In addition, the agency feels that there is a lack of federal regulation relating to hazard analysis and preventive controls for all animal feed and ingredients. FSMA provides requirements for these areas.

Manufacturers of animal feed, pet food, raw materials, and ingredients will be responsible for ensuring the safety of their finished products. Each facility is responsible for identifying reasonably foreseeable hazards that may occur and determining the preventive controls necessary to minimize or eliminate the hazard. Manufacturers will establish CGMP to ensure the proper design, monitoring, and control of manufacturing processes are maintained. CGMP provide an environment where hazards may be controlled more effectively.

FSMA requires facilities to create an animal food safety plan, which includes hazard analysis and the development of preventive controls for reasonably foreseeable hazards. The food safety plan must include a supplier verification program, a recall plan, management of preventive controls, verification and validation activities for preventive controls, and a corrective action program. Records will be essential to demonstrate compliance.

The greatest risks for most feed manufacturing facilities come from outside of their facilities through raw materials and ingredients. Thus, an effective supplier verification program is critical to maintaining or improving the safety of animal food. Verification activities are required to ensure materials are obtained from approved suppliers and that reasonably foreseeable hazards are controlled.

While the FSMA requirements for animal food will not be final until August 30, 2015, facilities are developing programs and processes to ensure compliance with the new federal regulations. Based on the size of the facility, a business will have 1, 2, or 3 yr to comply with the requirements from the final rule on CGMP and hazard analysis and risk-based preventive controls for food for animals.

A facility that develops an effective quality and feed safety program to drive continuous improvement will reach compliance with the new FSMA requirements more efficiently and effectively. It is anticipated that facilities within the feed industry will seek third-party certifications to drive compliance with the new FSMA regulations and help gage their success with manufacturing safer animal food. Complete information on FSMA and its rules can be found at: www.fda.gov/fsma.

The American Feed Industry Association developed feed safety programs that mirror the FSMA approach, in that they require hazard analysis and development of preventive controls. The Safe Feed/Safe Food program can be utilized for feed and feeding ingredients. Separate programs for export to the European Union (**EU**), pet

food, and pet food ingredients also have been developed and are based on either the EU Hazard Analysis Critical Control Point (**HACCP**) approach or the global food safety initiative approach, which is also a HACCP program. More information about these programs can be found at www.safefeedsafefood.org.

Updates to the CNCPS v6.5 and a Perspective on the Future

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INTRODUCTION

The first complete version of the Cornell Net Carbohydrate and Protein System (CNCPS) was released in 1991, and was first published in 1992 and 1993 in a series of four papers (Fox et al., 1992; O'Connor et al.; 1993, Russell et al., 1992; Sniffen et al., 1992). The principal objective of CNCPS was to serve as a tool for both research development and feed formulation for cattle (Russell et al., 1992). In order to fulfill these goals, the CNCPS has been continuously under development by incorporating research outcomes into mathematical equations. As a consequence, several updated versions have been released over the last 20 yr (Fox et al., 2000; Fox et al., 2004; Tylutki et al., 2008). Moreover, several implementations of the program have been used by the industry to evaluate and formulate diets. Other updates to the model have included the refining of the feed library (Higgs et al., 2015) and an improvement in the equations to predict nitrogen excretion (Higgs et al., 2012). The latest version, CNCPSv6.5 (Van Amburgh et al., 2015), is used as a formulation and evaluation platform by AMTS.Cattle (Agricultural Modeling and Training Systems LLC; Cortland, NY), NDS (Ruminant Management & Nutrition; Reggio Emilia, Italy), DinaMilk (Fabermatica; Ostriano, Italy), and Dalex (Dalex Livestock Solutions; Los Angeles, CA).

More recently, development of the CNCPS has been focused on improving the prediction of amino acid (AA) requirements and supply for lactating dairy cattle. This has led to a number of changes within the model including updated AA profiles in the feed library, re-characterization of protein fractionation and pool assignments, and the adoption of a combined efficiency of utilization for essential amino acids (EAA) used for maintenance and lactation.

The objective of this paper is to provide a description of changes made to CNCPS in the last few years, which resulted in v6.5, and also to provide some discussion about the future of the model and how the current development group has incorporated more mechanistic and improved understanding of cattle biology, primarily in rumen and gut function, and how it alters our approach to formulating diets. The new version (v7.0) has been developed and evaluated on lactating cattle and the outcome will be discussed.

MODEL UPDATES

Protein Fractionation and Digestion Rates

The information provided by the CNCPS feed library, including estimations of digestion kinetics of protein fractions within each feed, are as important as any other component of the model structure. The CNCPS feed library includes more than 800 different feeds and was recently reviewed and updated using large datasets from commercial laboratories by Higgs et al. (2015). Updates to the feed library included a re-characterization of the non-protein nitrogen (NPN) fraction (PA) to ammonia (PA1) and the soluble true protein fraction (PB1) to soluble non-ammonia CP (PA2). A summary of the changing nomenclature in the equations used to calculate ruminal

degradation, outflow, and intestinal digestion are in Table 1.

Degradation rates of protein fractions were previously updated as described by Van Amburgh et al. (2007) which, along with re-assigning the soluble protein pools to flow with the liquid passage rate, represented a considerable improvement in the sensitivity of MP predictions. In this update, the PB2 pool (fiber bound protein) was linked to the CHOB3 pool (digestible NDF) and the PA1 pool was lowered to 200 %/hr from 10,000 %/hr. The more recent re-characterization of the PA1 pool from NPN to ammonia described by Higgs et al. (2015) shifted a considerable amount of protein from the PA1 to the PA2 pool. In the CNCPS, the PA1 pool does not contribute MP to the animal; whereas the PA2 pool can contribute up to 15 % of total AA flow to the small intestine (Reynal et al., 2007; Volden et al., 2002). Hence, this new configuration considerably increased the predicted MP supply. Van Amburgh et al. (2010) reported that MP predictions, prior to the most recent update, were in good agreement with observed milk. Therefore, the rates associated with PA2 and PB1 pools were re-calculated to ensure MP predictions were consistent with the previous predictions. The re-calculated rates are 10-40 %/hr and 3-20 %/hr for the PA2 and PB1 pool, respectively, and are consistent with literature reports (Lanzas et al., 2007b).

Table 1. Equations to compute pools, rumen degradation, and intestinal digestion for feed protein fractions.

Variables [1]	Description	Equations [2,3]
$PA1_j$	Ammonia	$ammonia_j \times (SolCP_j/100) \times (CP_j/100)$
$PA2_j$	Soluble non-ammonia CP	$SolCP_j \times CP_j/100 - PA1$
PC_j	Unavailable CP	$ADIP_j \times CP_j/100$
$PB2_j$	Slowly degradable CP	$(NDIP_j - ADIP_j) \times CP_j/100$
$PB1_J$	Moderately degradable CP	$CP_j - PA1_j - PA2_j - PB2_j - PC_j$
$RDPA1_j$	Ruminally degraded PA1	$DMI_j \times PA1_j$
$RDPA2_j$	Ruminally degraded PA2	$DMI_j \times PA2_j \times (kdPA2_j / (kdPA2_j + kp_j))$
$RDPB1_j$	Ruminally degraded PB1	$DMI_j \times PB1_j \times (kdPB1_j / (kdPB1_j + kp_j))$
$RDPB2_j$	Ruminally degraded PB2	$DMI_j \times PB2_j \times (kdPP2_j / (kdPB2_j + kp_j))$
$RDPEP_j$	Ruminally degraded peptides	$RDPA2_j + RDPB1_j + RDPB2_j$
$REPA2_j$	Ruminally escaped PA2	$DMI_j \times PA2_j \times (kp_j / (kdPA2_j + kp_j))$
$REPB1_j$	Ruminally escaped PB1	$DMI_j \times PB1_j \times (kp_j / (kdPB1_j + kp_j))$
$REPB2_j$	Ruminally escaped PB2	$DMI_j \times PB2_j \times (kp_j / (kdPB2_j + kp_j))$
$REPC_j$	Ruminally escaped PC	$DMI_j \times PC_j$
$DIGPA2_j$	Digestible PA2	$IntDigPA2_j \times REPA2_j$
$DIGPB1_j$	Digestible PB1	$IntDigPB1_j \times REPB1_j$
$DIGPB2_j$	Digestible PB2	$IntDigPB2_j \times REPB2_j$
$DIGFP_j$	Digestible feed protein	$DIGPA2_j + DIGPB1_j + DIGPB2_j$

[1] Subscript *j* means for the *j* th feed.

[2] SolCP: soluble crude protein ; CP: Crude protein; NDIP: neutral detergent insoluble protein ; ADIP: acid detergent insoluble protein ; NDF: neutral detergent fiber; ADF: acid detergent fiber; DMI: dry matter intake; IntDig: intestinal digestibility constants

[3] Kp is either liquid (kpl), forage (kpf), or concentrate (kpc).

Amino Acid Profiles

Comparison of feed AA profiles in the original CNCPS feed library with profiles of other databases used in the industry showed that there were inconsistencies among the data. Much of this can probably be attributed to the analytical methods used to generate data for the original AA CNCPS feed library (O'Connor et al., 1993). Methods used on some feeds were not adequate to correctly quantify sulfur AA and often represented only one sample. Thus, methionine concentrations of some feeds are lower than reality and the sample size used to populate the library may not best represent what is most commonly used in the industry. However, other feeds added after the original library developments, including many proprietary feeds, were analyzed using correct methodology which has led to inconsistencies throughout the library.

To improve the consistency and accuracy of AA profiles in the CNCPS feed library, profiles were updated using datasets provided by Evonik Industries AG (Hanau, Germany), Adisseo (Commentary, France), and taken from the NRC (2001). Data provided were mean values from analyses completed in the respective companies' laboratories or published in the NRC (2001). In all cases, AA analyses were completed on the whole feed and are expressed in the CNCPS on a percent CP basis (equivalent to NRC, 2001). This differs from previous versions of the CNCPS where AA were expressed as a percent of the buffer insoluble residue (O'Connor et al., 1993). Analyzing AA on the buffer insoluble residue is analytically challenging and much larger databases exist for analyses of whole feed samples. Amino acids in the soluble fraction also contribute up to 15 % of the AA flowing out of the rumen undegraded (Reynal et al., 2005) which are not present in the buffer insoluble residue. For these reasons the AA profiles were changed to being expressed on a whole feed basis.

To update the feed library, the most appropriate profile was assigned based on data availability and was used as received by the source without alteration. If profiles for specific feeds were not available in the datasets provided, current CNCPS values were retained. Proprietary feeds were not changed and were assumed to be analyzed using appropriate methods that provided adequate AA recoveries. Table 2 has examples of AA profiles from the old and new feed library.

Amino Acid Utilization

Another area of consideration has been the efficiency of AA utilization used by the CNCPS. Currently, AA requirements for maintenance and lactation are derived using two separate efficiencies of use as described by Fox et al. (2004). Lapierre et al. (2007) discussed the biological correctness of this assumption and suggested when considering the distribution of enzymes for AA catabolism and the dominate role the liver plays in modifying peripheral AA supply, using a combined efficiency of use makes more sense. Doepel et al. (2004) calculated a single efficiency of use for each essential AA using a meta-analysis of 40 published papers involving abomasal, duodenal, or intravenous infusions of casein or free AA (Table 3). In this version of the CNCPS, we adopted the efficiency that represented what was considered to be 100 % of MP supply from the work of Doepel et al. (2004) as described by Lapierre et al. (2007) and believe this to be a more representative efficiency that can be evaluated among variable ME allowable milk supply.

Table 2. Comparison of old and new amino acid profiles from selected feeds in the CNCPS feed library. Values from the old library are expressed as percent buffer insoluble residue. Values from the new library are expressed as percent CP from the whole feed.

Ingredient		Met	Lys	Arg	Thr	Leu	Ile	Val	His	Phe	Trp
Alfalfa hay, 17 CP 46 NDF 20 LNDF	Old	0.7	6.0	6.4	5.0	9.3	6.0	7.1	2.6	6.3	1.8
	New	**1.3**	**4.8**	**4.2**	**4.0**	**6.7**	**3.9**	**5.0**	**1.9**	**4.6**	**1.4**
Mixed hay, 13 CP 56 NDF 14 LNDF	Old	0.7	4.4	4.6	3.9	7.4	4.4	5.5	1.8	4.9	1.6
	New	**1.4**	**4.3**	**4.5**	**4.0**	**6.8**	**3.8**	**4.9**	**1.8**	**4.3**	**1.4**
Corn silage unprocessed, 35 DM 45 NDF coarse	Old	0.8	2.1	1.9	2.1	6.4	2.4	3.2	1.1	2.9	0.1
	New	**1.6**	**2.8**	**2.3**	**3.4**	**8.5**	**3.4**	**4.5**	**1.7**	**3.9**	**0.7**
Blood meal	Old	1.1	9.3	5.0	4.7	13.4	0.9	9.1	6.5	7.9	1.9
	New	**1.2**	**8.7**	**4.3**	**4.6**	**12.3**	**1.1**	**8.2**	**5.9**	**6.8**	**1.4**
Soybean meal, 47.5 % CP solvent	Old	1.3	6.5	7.7	4.8	8.7	4.0	4.4	2.7	5.2	1.4
	New	**1.3**	**6.1**	**7.3**	**3.9**	**7.6**	**4.5**	**4.7**	**2.6**	**5.1**	**1.3**
Canola meal, expelled	Old	1.4	6.7	6.8	4.9	8.0	4.9	6.4	4.0	4.7	1.2
	New	**2.1**	**5.7**	**6.1**	**4.4**	**7.0**	**4.2**	**5.3**	**2.6**	**4.0**	**1.5**
Corn distillers, light spirits	Old	1.2	2.1	4.2	3.1	9.1	2.8	5.2	1.8	4.2	1.6
	New	**2.0**	**2.8**	**4.3**	**3.7**	**11.7**	**3.7**	**4.9**	**2.7**	**4.9**	**0.8**
Corn gluten, feed dry	Old	2.1	1.2	3.2	2.9	16.2	4.3	5.0	2.5	6.5	0.4
	New	**1.6**	**3.1**	**4.6**	**3.6**	**8.5**	**3.0**	**4.7**	**2.9**	**3.5**	**0.5**

Table 3. Combined efficiencies of amino acid utilization for both maintenance and lactation (adapted from Doepel et al. (2004) and Lapierre et al. (2007)) based on values derived from the data set at 100 % of the metabolizable protein requirement.

	Amino Acid								
	Arg	His	Ile	Leu	Lys	Met	Phe	Thy	Val
Efficiency	0.58	0.76	0.67	0.61	0.69	0.66	0.57	0.66	0.66

EVALUATION

Evaluation Dataset Development

Three different data sets were developed from both the literature (references not provided here), and from farm data from regional nutritionists to evaluate lysine (Lys) and methionine (Met) requirements, supply, rumen N balance, and milk yield predictions.

The first dataset (AA set), was compiled from studies where Lys, Met, or both were increased either by intestinal infusion or by feeding in ruminally protected form. In total 19 studies were selected and concentrations of digestible Lys (8 studies forming 43 treatments) and Met (11 studies forming 50 treatments) in protein truly digested were calculated for control and treatment groups. A dose-response approach was used to define required Lys and Met concentrations in MP for maximal protein synthesis according to Rulquin et al. (1993). Reference values of 6.80 and 2.43 % were identified intermediate to the lowest and highest concentration values for Lys and Met in MP, respectively. Predicted concentrations of Lys in MP varied between 4.99 and 9.30 % of MP and for Met between 1.69 and 2.85 % of MP. Positive and negative values for production responses were calculated using the reference values for control and treatment groups. Responses of milk protein yield (g/day) and the predicted concentrations of Lys and Met (% of MP) were evaluated by regression procedures.

The second dataset (rumen set) was compiled from studies where post-ruminal N flows were assessed with the omasal sampling technique (Ahvenjärvi et al., 2000; Huhtanen et al., 1997; Reynal and Broderick, 2005). A recent meta-analyses (Broderick et al., 2010; Huhtanen et al.; 2010) on omasal sampling suggested that it is a reliable alternative to measuring nutrient flows via duodenal cannula. Moreover, the use of a triple marker system is more robust and reduces variation caused by the multiple and diverse markers used with post-ruminally cannulated animals. Therefore, to avoid inducing variation due to cannula position and the variety of markers used we included only studies with the omasal sampling technique. In total, 19 peer-review studies with 74 treatments were included.

The third data set (lactation set) was compiled from studies published in the Journal of Dairy Science between 2001 and 2012. Lactation trials were included for dairy cows in different stages of lactation (early, mid, and late). Studies with cross over design (Latin square, Box-Behnken, etc.) and with few experimental units ($n < 6$) were excluded from the data set. In total, 103 lactation studies were pre-selected, by which 55 with 200 treatments met the criteria for incorporation into the data set. The criteria for each study were:

a. description and chemical analysis of the ration fed for each treatment,
b. inclusion of each feed included into the ration,

c. information of actual dry matter intake (DMI), and
d. information on milk yield and milk composition for each treatment.

This dataset was enhanced by incorporating farm data from nutritionists in the Northeast U.S. that were willing to share their data. From the regional nutritionists 15 farms with 50 different diets were included.

A spreadsheet version of the CNCPS was used to conduct the model simulations for this study. Information on feed chemistry required by the CNCPS to run a simulation was used as reported by the study. When incomplete information was presented, values were predicted using the procedures described by Higgs et al. (2015). Animal information required to run a simulation in the CNCPS included a description of housing conditions, body weight (BW) and BW change for period studied, body condition score (BCS) and BCS change during the period studied, stage of lactation, and stage of pregnancy. If stage of pregnancy, BW, and BCS were not provided, CNCPS default values were used. When BW change was available, but BCS change was not, the final BCS (in CNCPS as the target BCS) was calculated from BW change assuming that empty body weight (EBW) changes, on average, 13.7 % for each unit of BCS change (Fox et al., 1999; and NRC, 2001). To calculate EBW from BW the following equations were used:

EBW = 0.851 * Shrunk BW (SBW), and
SBW = 0.96 * BW

Therefore, EBW = 0.81696 * BW

Statistical Analysis

Statistical analysis was conducted with JMP (SAS). To describe the relationships between increasing concentrations of Lys and Met in MP and protein yield responses, a broken line model with a plateau was used. According to the NRC (2001), this linear model was either equal to or superior to other models for describing protein content and protein yield responses to increasing amounts of both Lys and Met in MP. The model consisted of a linear regression line to a break point followed by a plateau:

$Y_{ij} = \beta_0 + \beta_1 X_{ij}$, when $X \leq C$

$Y_{ij} = \beta_0 + \beta_1 C$, when $X > C$

Where, Y_{ij} = the expected outcome for the dependent variable Y observed at repetition $_j$ of the continuous variable X in study $_i$, β_0 = the overall intercept across all studies, β_1 = the overall slope of Y on X across all studies, and C = the break point.

For the lactation and rumen datasets, a mixed effects model using the restricted maximum likelihood (REML) procedure was used to analyze the data as proposed by St-Pierre (2001):

$Y_{ij} = \beta_0 + \beta_1 X_{ij} + s_i + b_{1i} X_{ij} + \varepsilon_{ij}$,

Where, Y_{ij} = the expected outcome for the dependent variable Y observed at repetition $_j$ of the continuous variable X in study $_i$, β_0 = the overall intercept across all studies, s_i = the random effect of study $_i$, β_1 = the overall slope of Y on X across all studies, b_{1i} = the random effect of study $_i$ on the slope of Y on X, X_{ij} = the data associated with repetition$_j$ of the continuous variable X in study$_i$, and ε_{ij} = random variation.

To evaluate the performance of the model several statistics were calculated. The squared sample correlation coefficients reported were based on either the BLUP (R^2_{BLUP}) or model predictions using a mean

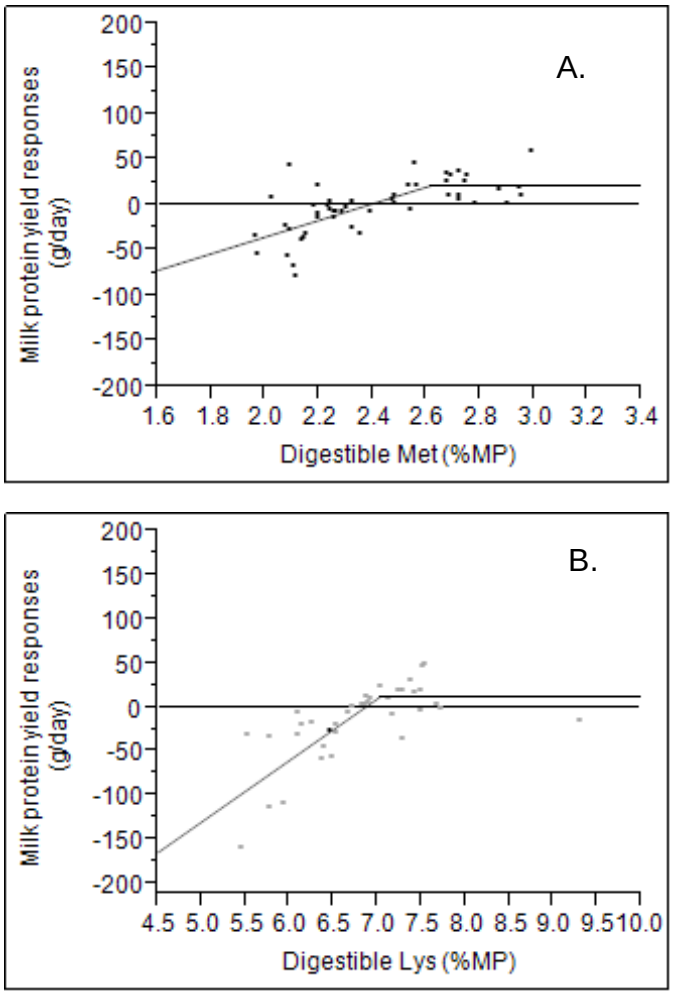


Figure 1. Milk protein yield responses as a function of digestible methionine (A) (Met; y = -219 + 92.65*Met and y = -219 + 92.65*2.60 for the linear and the plateau part of the model, respectively) and lysine (B) (Lys; y = -478 + 70.02*Lys and y = -478 + 70.02*7.00 for the linear and the plateau sections of the model, respectively).

study effect (R^2_{MP}). The Bayesian information criterion (BIC) was used as the statistical criterion to indicate the goodness of model fit, where lower values indicate a better fit. The residuals (predicted – observed) were visually examined for any patterns as well as for any potentially confounding factors. Additional model adequacy statistics were calculated to give further insight into the accuracy, precision, and sources of error in each model (Tedeschi, 2006). Mean square prediction errors (MSPE) were used to indicate accuracy. A decomposition of the MSPE was also performed to give an estimation of the error due to central tendency (mean bias), regression (systematic bias), and random variation. Concordance correlation coefficients (CCC) were used to simultaneously account for accuracy and precision. Concordance correlation coefficients can vary from 0 to 1, with a value of 1 indicating that no deviation from the Y = X line has occurred.

RESULTS AND DISCUSSION

Lys and Met Requirements

The plots of model predicted concentrations of Lys and Met (%MP) and the corresponding responses of milk protein yield are presented in Figure 1. The breakpoint estimates for Lys and Met for maximal milk protein yield were 7.00 and 2.60 % of MP, respectively. Similar break points were reported for NRC (2001) and the previous version of CNCPS. The CNCPSv6.1 estimated Lys breaking point at 6.93 % of MP and that of Met at 2.34 % of MP (Whitehouse et al., 2013). Current estimations require slightly higher Lys, and 11 % higher Met supply to optimize protein yield responses, which can be attributed to the updated AA profiles in the feed library.

Efficiency of AA Use

To evaluate the updated efficiency of AA use included in the CNCPS, the data set used to determine the optimum proportion of Met and Lys in MP was used to perform a regression of model predicted AA balance (g Met/d) against the concentration of Met in the diet (Met % MP). Using the new efficiencies (Table 3), the regression line intercepted the Y axis at approximately 2.6 % dietary Met relative to total MP (Figure 2), similar to the breakpoint derived in Figure 1 A. The studies used to perform this analysis were specifically designed to be both sufficient and limited in Met supply in order to observe a dose response. Hence, one would expect the model to predict both positive and negative Met balance. Using the old efficiencies of AA use, the regression line intercepts the Y axis at 2.0 %

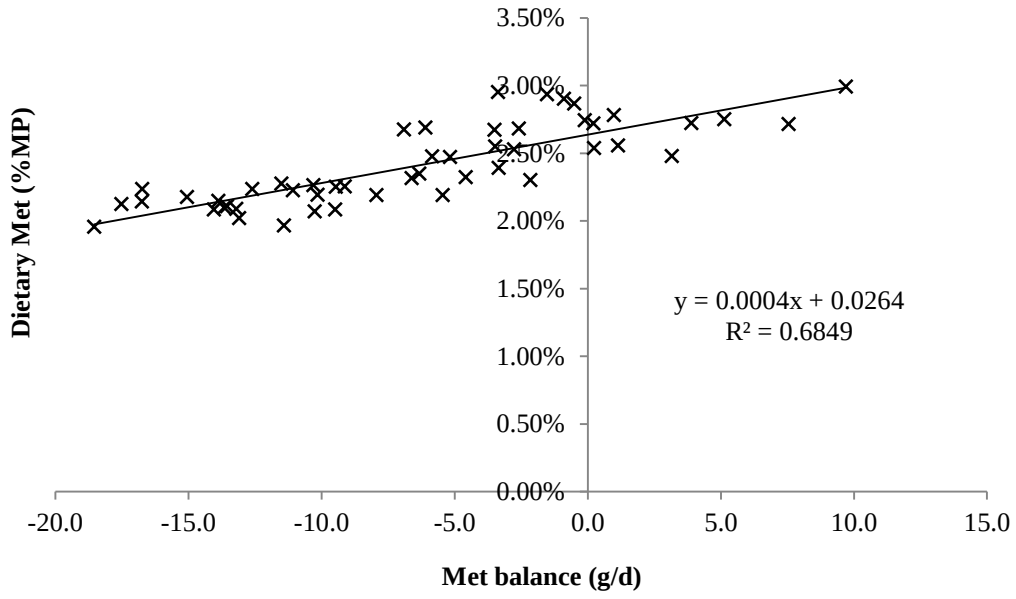


Figure 2. Model predicted Met balance (MP Met supply less requirement; g Met/d) versus dietary Met (% MP) with updated efficiencies of use of absorbed amino acids.

dietary Met (% MP) and no diets are predicted to have negative Met balance, contrary to expectations. Using the new efficiencies (Figure 2), there is a balance of both positive and negative Met balance among the data set. This suggests the new efficiencies of use allow the model to more adequately represent the true gram per day requirements of EAA.

Rumen Degradation

Updates to the digestion rates, passage rate assignments (Van Amburgh et al., 2010), and pool characterization (Higgs et al. 2015; and Lanzas et al., 2007a) have made MP predictions by the CNCPS more sensitive than previous versions of the model (Van Amburgh et al., 2010). The ability of the model to predict the various nitrogen fractions leaving the rumen was evaluated against omasal flow data. Studies in the compiled dataset reported measures of ruminal undegraded N (RUN), non-ammonia N (NAN) and bacterial N (BactN) flows. The dataset represented a wide range of diets and nutrient compositions (Table 4). The omasal flow of BactN and RUN ranged from 78 to 480 and from 7 to 326 g/d, respectively (Figure 3). The model predicted post-ruminal flows of NAN (R^2 = 0.97; RMSE = 24.57) and RUN (R^2 = 0.91; RMSE = 21.93) well, but with the current rates and pools size descriptions, underestimates BactN ($\beta1$ = 1.55) and overestimates RUN ($\beta1$ = 0.73). However, there is a uniform offset which provides a prediction of NAN that is robust with little bias (NAN; R^2 = 0.98; RMSE = 26.77; $\beta1$ = 1.17). The variance component analysis indicated that most of the variance is attributed to the study effect and not residuals, even though residual influence was higher for BactN (Table 6).

Milk Yield Prediction

Diets with a wide range of nutrients were included in the evaluation data set (Table 5). Previous evaluations of the CNCPS were conducted using specific experimental datasets of a few studies conducted at Cornell University (Fox et al., 2004; Tylutki et al., 2008). The first limiting nutrient (MP or ME) was regressed on the observed milk yield, and results demonstrated the capability of CNCPS to predict the first limiting nutrient. The current evaluation reinforced the ability of the latest version to accurately predict the most limiting nutrient: the first limiting nutrient (MP or ME) was predicted with an R^2 = 0.95 and a RMSE = 1.77. Further, the development of a large dataset provided the opportunity to evaluate the model over a wide range of production and dietary conditions.

Table 4. Input variables used for the rumen sub-model evaluation dataset.

	Mean	SD	Min	Max
Diet Composition (% DM)				
CP	16.1	2.55	9.9	20.7
RUP	5.9	1.33	2.9	9.2
RDP	10.2	1.81	6.2	14.5
NDF	34.6	9.02	22.7	59.5
Starch	23.8	11.66	44.1	1.1
Fat	4	0.84	2.6	6.2
Omasal flows (g/d)				
Non ammonia nitrogen (NAN)	481	176.8	87	778
Bacterial nitrogen (BactN)	316	123.8	78	480
Rumen undegraded nitrogen (RUN)	164	65.1	7	326

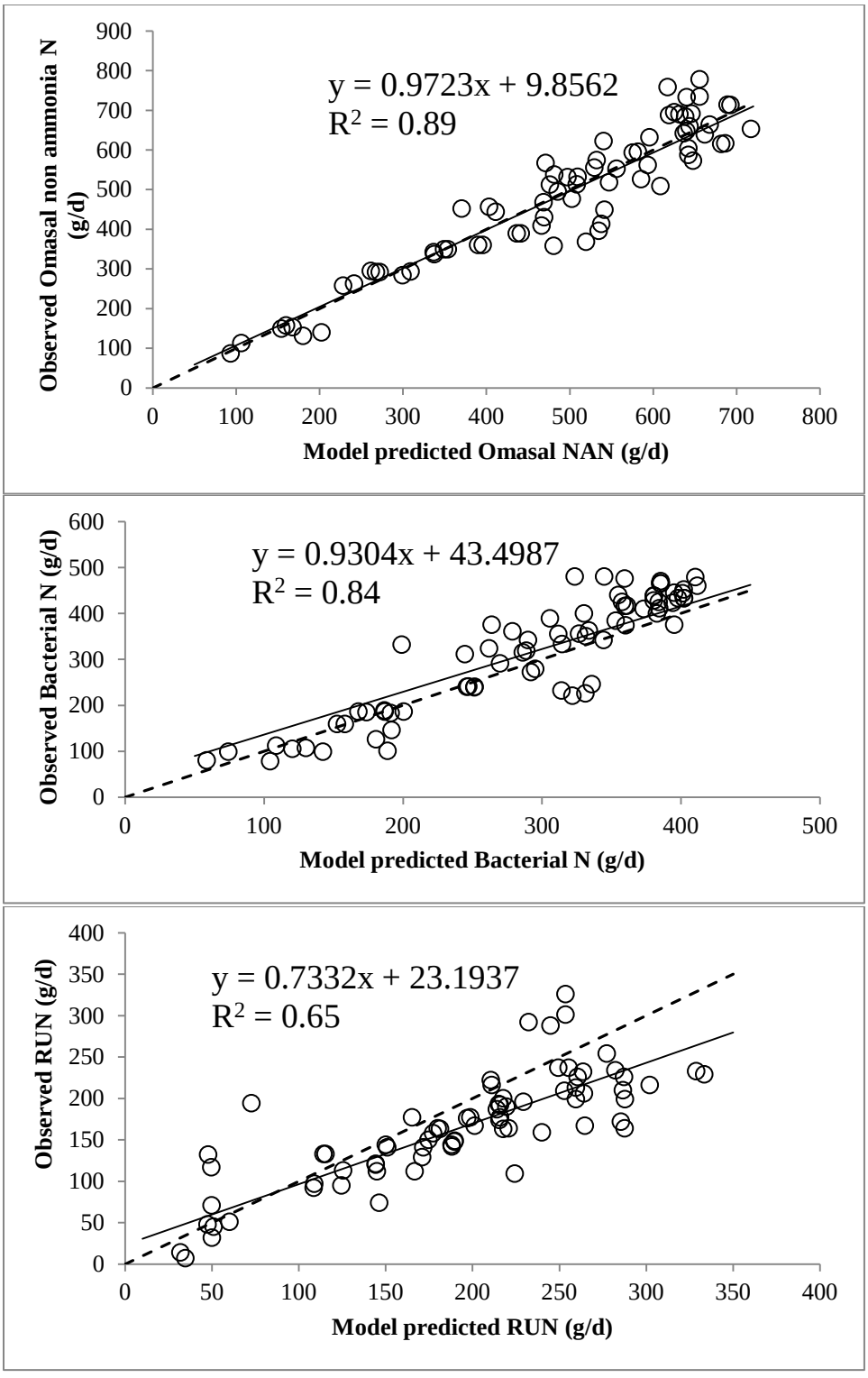


Figure 3. Observed versus model predicted values of: (A) non-ammonia nitrogen (NAN), (B) bacterial nitrogen (BactN) and (C) rumen undegradable nitrogen (RUN), assessed with a mixed effects model.

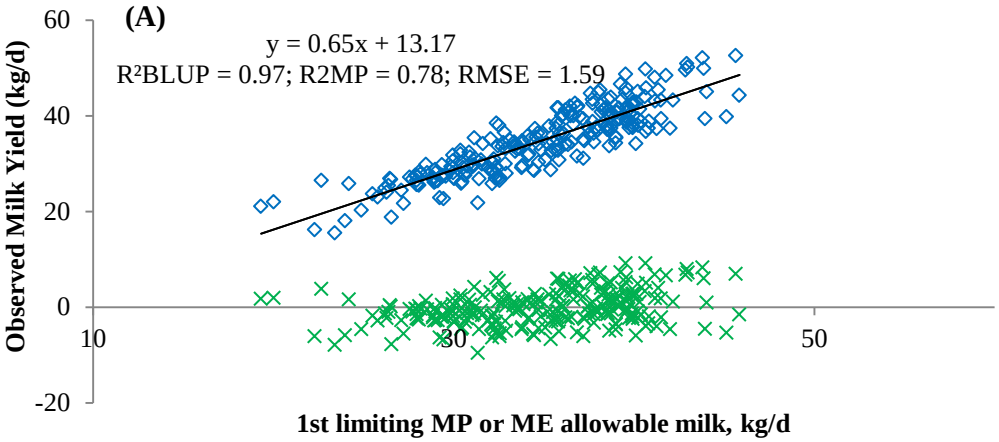
Results of the evaluation of ME and MP allowable milk yield are presented in Figure 4 and Table 6. Both MP and ME allowable milk were predicted reasonably well with an overall R^2 of 0.76 and a RMSE of 1.59 kg. In this evaluation, MP allowable milk was predicted with greater accuracy than ME allowable milk (R^2 = 0.82 and RMSE = 1.12 kg; R^2 = 0.76 and RMSE = 1.96 kg, respectively). An early attempt to evaluate CNCPSv6.0 when MP was the first limiting nutrient resulted in low precision (R^2 = 0.29; Van Amburgh et al., 2007). Since then, several updates to the model have been made (Higgs et al., 2012b; Van Amburgh et al., 2010; Van Amburgh et al., 2007) and among them, the updates to the protein fractionation and degradation rates have resulted in improved predictions and sensitivity of the model.

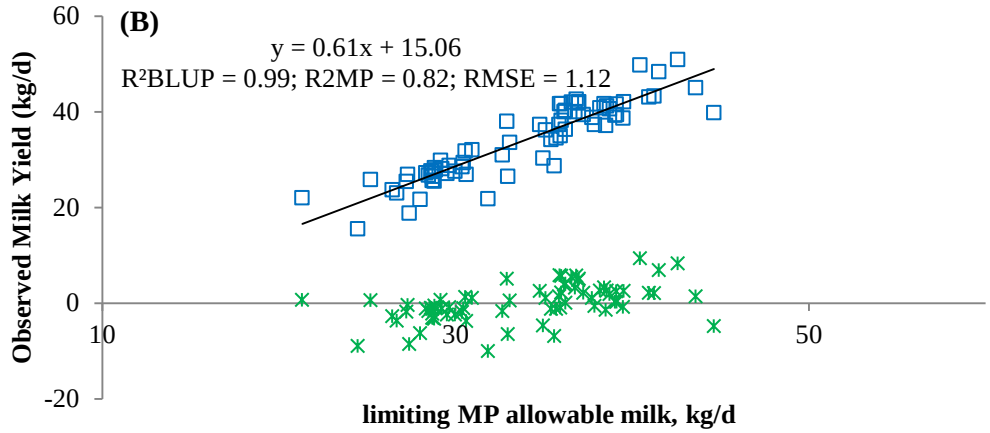
Within the data sets evaluated, it is more difficult to evaluate energy balance because typically information on BCS change and BW change are not reported. Also, BW change, depending on stage of lactation, is not a good indicator of energy balance due to changes in rumen fill and DMI; body water vs body fat changes; and physiological state (e.g. pregnancy related BW changes). Thus, the ability to describe ME allowable milk or ME balance among published data sets is more difficult and that outcome is reflected in the partitioning of error in the MSPE (Table 6), where the majority of the error is random and due to study and not systematic within the model.

Table 5. Cattle and production characteristics for the lactation evaluation dataset.

	Mean	SD	Min	Max
Diet Composition (%DM)				
CP	16.9	2.35	9.4	29.5
RUP	7.2	1.55	3.3	16.7
RDP	9.7	1.38	6.08	14.6
NDF	33.8	5.4	25.3	52.7
Starch	23.1	7.2	2.1	37.8
Fat	4.8	1.3	2.0	13.1
Animal Inputs				
Initial body weight, kg	623	44.4	525	737
Final body weight, kg	632	46.1	532	748
Initial BCS, 1-5 scale	2.92	0.374	1.1	3.6
Final BCS, 1-5 scale	2.96	0.384	1.2	4.4
DMI, kg	22.3	2.73	13.5	29.1
Production inputs				
Milk Yield, kg/d	34.6	7.14	15.5	52.6
ECM[1], kg/d	32.3	6.18	14.9	47.15
Milk protein, %	3.02	0.194	2.51	3.61
Milk fat, %	3.67	0.479	2.06	5.06

[1]ECM: energy corrected milk (Tyrrell and Reid, 1965)





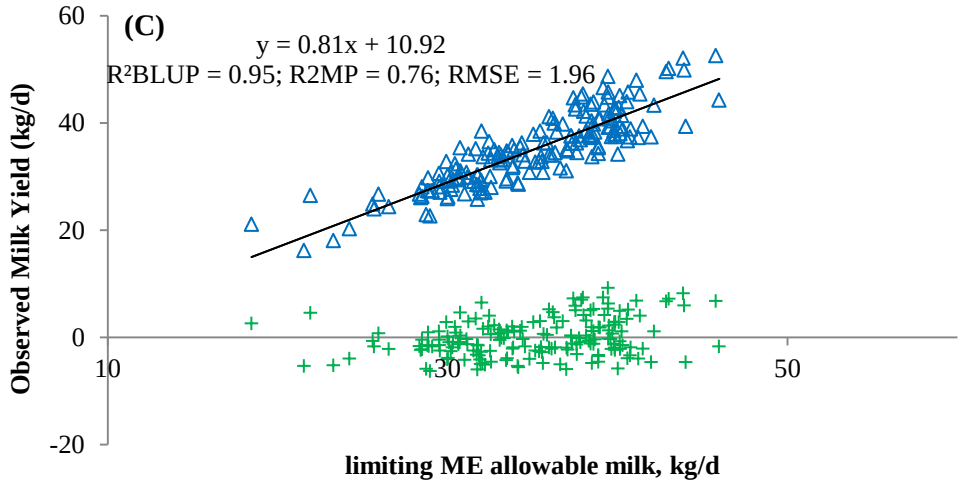


Figure 4. Observed versus model predicted values of: (A) first limiting MP or ME (◊;) and residuals (×), (B) MP limiting (□) and residuals (∗) and (C) ME limiting (∆) and residuals (+), assessed with a mixed effects model.

Table 6. Model adequacy statistics for the prediction of the first limiting nutrient (metabolizable protein or/and metabolizable energy; MP and ME, respectively) and of post ruminal flow of non-ammonia nitrogen (NAN), bacterial nitrogen (BactN) and rumen undegradable nitrogen (RUN).

				Variance Component[3]					MSPE partitioned[6] (%)		
	n	RMSE[1]	BIC[2]	Study	Slope	Residual	CCC[4]	MSPE[5]	U^M	U^S	U^R
Lactation											
MP or ME	250	1.56	1192	77.7	0.5	21.8	0.83	12.8	0.05	21.75	78.20
ME	177	1.77	870	67.0	0.6	32.4	0.84	11.8	0.55	16.33	83.12
MP	73	1.12	360	91.5	0.4	8.1	0.83	14.2	0.45	26.91	72.64
Post-ruminal flow (g/d)											
NAN	74	24.97	767	84.6	NS	15.4	0.68	14011	83.35	3.52	13.13
BactN	74	24.55	743	86.1	NS	13.9	0.31	17762	91.08	6.16	2.76
RUN	74	21.73	726	66.9	NS	33.1	0.71	1141.6	24.41	7.68	67.91

[1] Root mean square error
[2] Bayesian information criterion
[3] Percentage of variance related to the effect of study and random variation
[4] Concordance correlation coefficient.
[5] Mean square prediction error.
[6] U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, U^R = percentage of error due to random variation ($U^M + U^S + U^R = 100$).

MOVING THE CNCPS TO A DYNAMIC PLATFORM

Work is ongoing in the modeling group at Cornell to move the CNCPS to a more dynamic framework in order to more effectively capture the interactions of nutrient digestion, intake, and microbial growth. The majority of the work was conducted by Ryan Higgs as part of his Ph.D. where v6.5 was reprogrammed into Vensim (Ventana Systems, Harvard, MA), a visual, dynamic programming software and other components like protozoa, endogenous protein flow and recycling, and urea recycling were added to improve true protein supply predictions. Figure 5 is a schematic representation of a portion of the nitrogen transactions within the model. The nitrogen pools remain identical to v6.5 and modeling in this manner allows for more precise estimations of digestion, and also estimations of pool sizes in the rumen when the model reaches dynamic equilibrium.

After the model was reprogrammed in v7.0, it was important to evaluate the predictions on lactating cattle. As part of the modeling exercise the requirements for AA were re-derived using a similar approach to Doepel et al. (2004) and Lapierre et al. (2007). However, rather than expressing AA supply relative to MP supply, the AA requirements were expressed relative to ME (Figure 6, Figure 7, Table 7). The data in figure 6 were used to determine the grams of digested Met necessary to meet the expected Met requirement (g/g). That calculation was accomplished by estimating the use of Met at the point on the curve where the rate of change away from productive use was greatest. At that calculated intercept, we assumed the efficiency of use would be the greatest under the conditions described, which included the integration of ME. The *efficiency* of Met use was then used to recalculate Met requirement on both a gram per Mcal of ME basis (Figure 7A) or on an MP basis (Figure 7B). Again, the optimum amount of Met per Mcal of ME was identified by mathematically determining when the rate of change away from productive use was greatest and the AA were then described on a gram per Mcal of ME basis, just like a monogastric animal. This process was conducted for all EAA and resulted in the *optimum* values in Table 7.

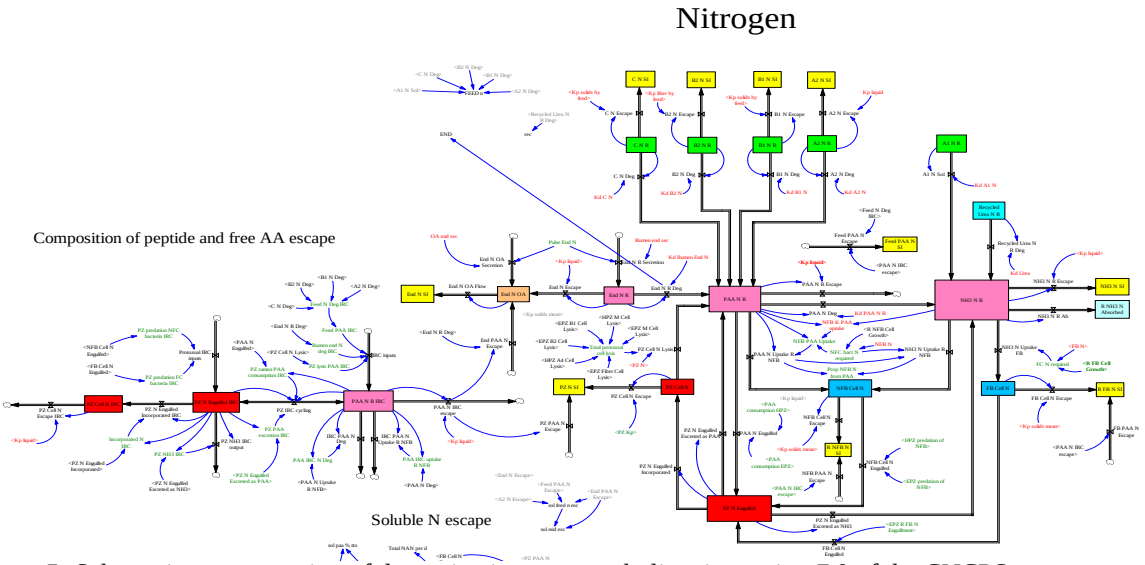


Figure 5. Schematic representation of dynamic nitrogen metabolism in version 7.0 of the CNCPS.

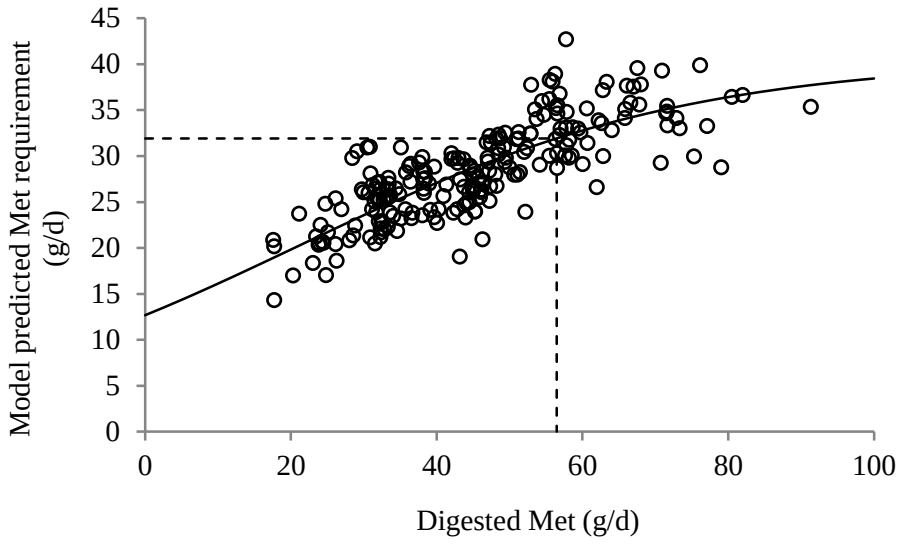


Figure 6. Logistic fit of model predicted Met requirement and Met supply. The dashed line represents the optimum ratio of Met requirement and Met supply.

To evaluate the model, 64 high producing dairy cows (100 ± 31 DIM) were randomly assigned to one of 4 treatments:

1) Base - limited in Met, MP, and rumen N,
2) Base+M - adequate in Met, but limited MP and rumen N,
3) Base+MU - adequate in Met and rumen N, but limited MP, and
4) Positive - adequate in MP and rumen N, while balanced for all EAA on a g/Mcal ME basis.

The chemical composition and ingredients used in each diet are in Table 8. Model predicted (CNCPS v7.0) dietary MP balance was −231, −310, −142, and 33 g/d for the Base, Base+M, Base+MU, and Positive treatments, respectively.

Milk yield was not significantly different among the treatments, despite CP levels in the 13.5-13.6 % range. However, as the grams of AA per Mcal of ME approached the optimum, energy corrected milk yield increased (Table 9). The predictions of grams of AA increased in two ways, first by meeting the N requirements of the rumen and in the Positive control, by adding ingredients to meet the AA requirements. The Base+MU treatment was designed to ensure adequate ruminal N availability and this treatment was considered not necessary during the formulation of treatment diets; however, due to the significant shift in the protein content of the corn silage (from 9 % to 7 %), the treatment became quite useful to help us evaluate the ability of the model to predict rumen ammonia levels and microbial yield, which in turn impacted the grams of AA supplied from the rumen. The model predicted the depression in microbial yield due to the low rumen N status (Table 10) and this prediction coincided with plasma urea N concentrations below 6 mg/dL. A review of most studies where data are available would indicate that after the PUN

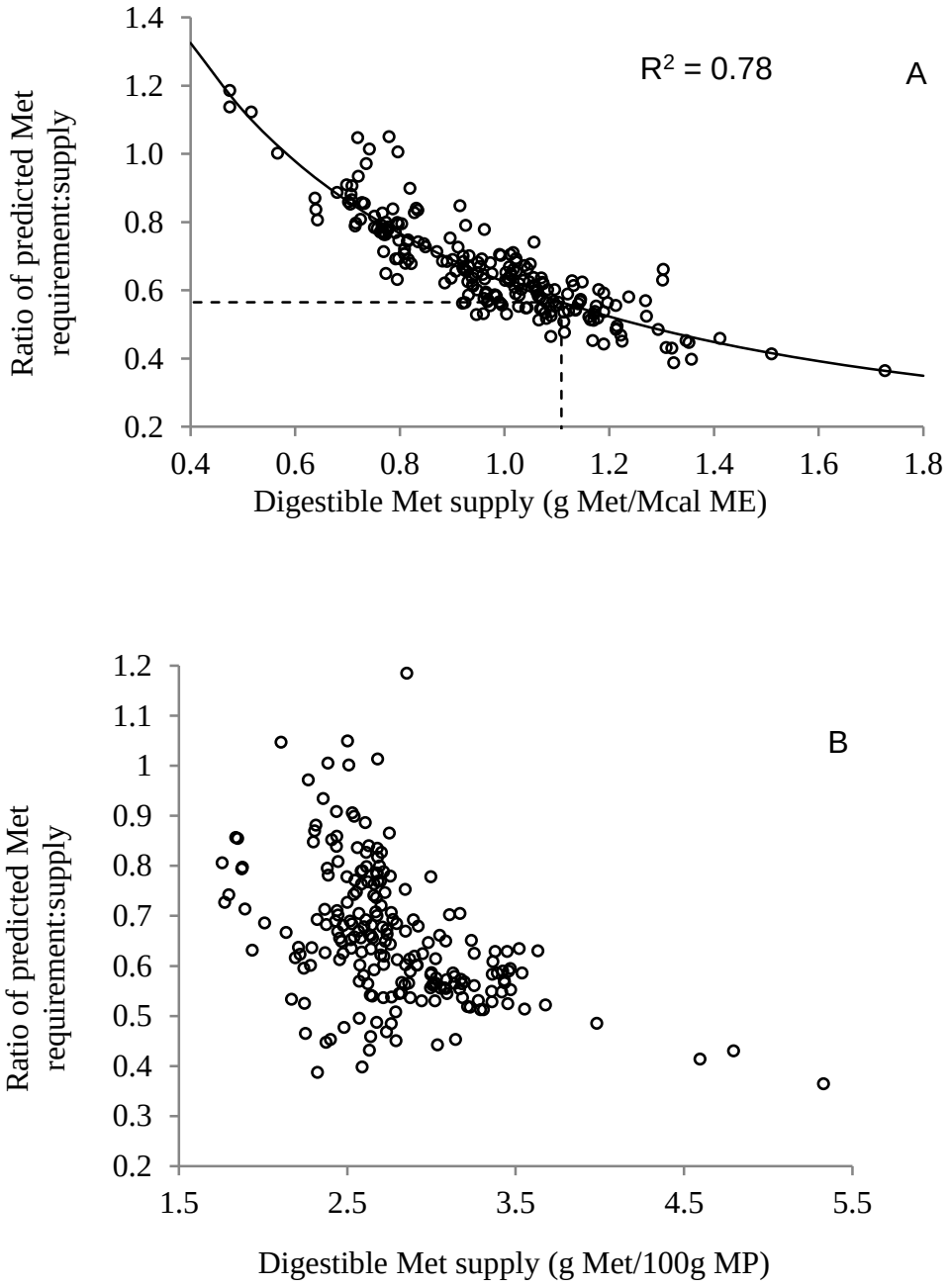


Figure 7. Relationship between model predicted Met requirement: supply and Met supply relative to ME (A) or MP (B). The dashed line in (A) represents the Met supply at the optimum ratio of model predicted Met requirement and supply. No significant relationship was determined in (B).

concentrations drop below 6 mg/dL, the blood pool of urea and urea production are not high enough to recycle adequate urea N to the gastrointestinal tract; thus the rumen goes into negative N balance and NDF digestibility is decreased. This data and the latest version of the model all coincide with these observations (Table 10). It is important to note that as the PUN decreased, there was a discrepancy between the MUN

Table 7. The predicted AA supply for each treatment compared with the calculated optimum supply (g digested AA/Mcal ME).

AA	Optimum	Base[1]	Base+M	Base+MU	Positive
Arg	2.04	1.85	1.86	1.96	2.15
His	0.91	1.01	1.01	1.05	1.19
Ile	2.16	1.83	1.83	1.94	2.00
Leu	3.42	3.64	3.65	3.81	4.15
Lys	3.03	2.83	2.82	2.98	3.09
Met	1.14	0.93	1.13	1.17	1.25
Phe	2.15	2.12	2.12	2.22	2.42
Thr	2.14	2.16	2.16	2.27	2.43
Trp	0.59	0.60	0.60	0.63	0.69
Val	2.48	2.33	2.33	2.45	2.62
Lys:Met	2.66	3.04	2.51	2.54	2.47

[1]Base = balanced for ME (assuming 45 kg ECM), but limited in MP and rumen N;
Base+M = balanced for ME and MP Met but limited in MP and rumen N;
Base+MU = balanced for ME, MP Met, with adequate rumen N, but limited in MP;
Positive = balanced for ME, MP, all EAA and adequate rumen N.

Table 8. Ingredient and nutrient profile of the Base diet, Base plus Met, Base plus Met and urea and Positive control diets.

Ingredient, % DM	Base	Base+M	Base+MU	Positive
Corn Silage	46.98	46.49	46.75	46.13
Grass Hay	8.53	8.53	8.42	8.46
Corn grain ground fine	15.73	15.84	15.66	15.12
Corn gluten feed	8.69	8.75	8.66	7.07
Soybean meal	6.21	6.25	6.18	7.89
Soyhulls	2.07	2.08	2.06	2.10
SoyPLUS	2.07	2.08	2.06	4.11
Molasses Dried	2.07	2.08	2.06	1.20
NutraCor	1.90	1.92	1.90	1.64
Urea	0.08	0.08	0.52	0.12
AjiPro-L	0.10	0.10	0.09	0.00
Smartamine M	0.00	0.08	0.08	0.09
Blood meal	1.66	1.67	1.65	2.18
Minerals and vitamins	3.92	4.05	3.91	3.88
Chemical components				
CP	13.5	13.6	14.6	15.6
SP, % CP	38.8	38.6	38.8	37.8
Starch	31.9	31.9	31.5	30.9
NDF	29.7	29.6	29.3	29.3
Ash	7.3	7.4	7.3	7.3
EE	4.7	4.7	4.6	4.4

Table 9. Dry matter intake, energy corrected milk (ECM) yield, milk yield, and milk components.

Item, kg/d	Base	Base+M	Base+MU	Positive	*P*-Value
Dry matter intake	24.1	24.5	24.8	24.7	0.717
ECM yield	38.5[a]	39.3[a]	40.0[a]	41.8[b]	0.005
Milk yield	40.0	40.6	40.7	41.8	0.288
True protein yield	1.13[a]	1.18[ab]	1.18[ab]	1.22[b]	0.009
Fat yield	1.30[a]	1.28[a]	1.34[ab]	1.41[b]	0.047
Lactose yield	1.93	1.94	1.95	2.00	0.344
Milk composition					
True protein, %	2.88[a]	2.93[ab]	2.96[b]	2.98[b]	0.009
Fat, %	3.31	3.20	3.34	3.51	0.078
Lactose, %	4.84	4.85	4.85	4.86	0.799

and the PUN concentrations, and we see this as PUN concentrations are measured below about 8 mg/dL. This suggests to us that below a certain range, most mid-infrared units are not calibrated properly and are insensitive to some of the changes in N metabolism that might be useful for diet formulation and diagnostics.

SUMMARY

Nutritional models can be evolutionary. The CNCPSv6.5 is the latest evolution in the CNCPS path and the final update for this version. Among the analytical improvements, error corrections, and new research implemented within the CNCPS framework, model accuracy has been improved. These changes allow the nutrition professional to reduce dietary CP levels while maintaining or improving production and profitability. More importantly, the feed descriptions for AA in the feed library are now current and in a form that allows any user to make updates and additions with contemporary AA analyses methods. This step provides the next opportunity to continue to develop the model to better predict the supply and requirements of AA for lactating and growing cattle. Further, the application of a combined efficiency of use of MP AA appears to provide a more consistent approach between AA supply and requirements that should improve the ability of the model to predict limiting AA and provide more sensitivity in determining a dietary approach to overcome the limitation. Finally, the model is being reprogrammed to incorporate more dynamic approaches to modeling and data analyses. Protozoal growth and yield, endogenous protein supply and digestibility, recycled urea N, and intestinal digestibility provided new insights into AA supply and were incorporated into the new model. Further, new estimates of AA requirements were developed on an energy basis, similar to monogastric animals and evaluated in lactating dairy cattle and with this approach and capability, dairy cattle were able to produce ~40 kg of milk on diets containing ~13.5 % CP and responded positively to improved AA balance on an ECM and ME basis.

Table 10. Nitrogen intake, milk and plasma urea N, N use efficiency, neutral detergent fiber digestibility, and bacterial growth depression due to predicted rumen ammonia N.

	Base	Base+M	Base+MU	Positive	*P*-Value
N intake, mg/dl	521.6[a]	532.1[a]	581.9[b]	615.1[c]	< 0.001
MUN, mg/dl	6.9[a]	7.3[a]	9.1[b]	10.4[c]	< 0.001
PUN, mg/dl	5.9[a]	5.7[a]	8.5[b]	8.7[b]	< 0.001
N use efficiency	0.37[a]	0.38[a]	0.35[b]	0.34[b]	< 0.001
NDF digestion %	40.8[ab]	40.5[b]	42.9[a]	42.9[a]	0.008
pd NDF digestion %	56.7[ab]	55.2[b]	59.0[a]	59.2[a]	0.011
Bacterial growth depression, %	16 %	17 %	4 %	2 %	

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Landscape of Formulation Platforms

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INTRODUCTION

Optimal diet formulation is critical for the profitability of dairy farms. Feed is usually the largest expense (50 to 75 %) and milk sales (fat, protein, and other solids) represent a significant portion of the revenue (80 to 95 % on most dairy farms). Maximization of income over feed costs (**IOFC**) as well as return on assets for a given dairy farm should be influential in ration formulation. Nutrition models are becoming more complex, as our understanding of the conversion of nutrients into milk and growth continue to evolve. Research has provided a plethora of knowledge of qualitative relationships (e.g. altered rumen biohydrogenation and production of conjugated linoleic acids); however, quantitative modeling of the complex biology of the cow is lagging behind in certain areas.

Application of nutrition models is useful to provide a baseline accounting system, directional guidance, and to improve our understanding of biology; however, it is imperative that one have an understanding of what nutrition formulation models can predict accurately and what they cannot predict well at all. Many nutrition models have continued to evolve and improve our ability to detect the most limiting nutrient(s), to predict apparent total digestible nutrients (**TDN**), to manipulate productive efficiency, and to predict excretion of important environmental emission compounds (VanAmburgh et al., 2015). However, nutrition models in general struggle to describe the interactions of nutrient concentrations (i.e. associative effects), diet effects on dry matter intake (**DMI**), and partitioning of nutrients for milk components and growth (Allen, 2011). Human intelligence and intervention is still a major factor in formulating economically optimal diets for dairy cattle.

If economics, environmental outputs, variables of the individual farm operation, and cow health were ignored, formulation of nutritional diets would be much simpler, as the primary goal would be maximum milk production. Unfortunately, the overarching ration parameters that nutritionists typically target are often not independent and some even are negatively correlated (feed efficiency vs. profit maximization). Software formulation strategies that increase predicted energy concentration of the diet typically do result in model-predicted higher energy allowable milk; however, changing the diet energy concentration often has negative effect(s) on factors such as neutral detergent fiber digestibility (**NDFD**), DMI, and rumen pH. Factors such as DMI, rumen pH, or predicted amino acid (**AA**) supply are, in fact, not considered quantitatively when using software optimizers to formulate diets. Because of this, it is prudent to design an array of formulation restrictions based on pragmatic, experience-based guidelines that take into account the intangibles of cow health and fermentation.

In general, ruminant formulation models will be underpinned on a nutritional requirement system. Users input milk yield, body weight (**BW**), days in milk (**DIM**), BW loss/gain, and environment characteristics. Dry matter intake is predicted based on BW, milk yield, and

DIM. Actual observed DMI is usually inputted during routine diet formulation; however, one must consider that any ration change may alter subsequent DMI. Most models (NRC, 2001 and CNCPSv6.5) predict dietary energy supply quite well, if the inputs and outputs are well-described (VanAmburgh et al., 2015); however, substantial departure of predicted vs actual energy supply can occur, largely due to the variation of diet NDFD (Weiss, 2010). Prediction of protein supply is quite varied across nutrition software platforms, as models differ in predicted microbial protein yield (empirical vs. mechanistic), efficiency of utilization of metabolizable protein (**MP**), rumen degradable protein (**RDP**) and rumen undegradable protein (**RUP**) fractions, and AA requirements (Schwab et al., 2014).

ENERGY PREDICTIONS BY NUTRITION MODELS

The NRC (2001) model and its derivations predict energy using the net energy system. Actual DMI and diet digestibility affect the conversion of dietary gross energy (**GE**) to digestible energy (**DE**). Higher DMI (i.e. intake over maintenance) and increased dietary TDN concentrations result in reduced conversion of diet GE to diet DE (NRC, 2001). What this means is that the calculated DE of a diet is always equal to or less (most cases) than the weighted average DE of the individual ingredients. For calculation of dietary metabolizable energy (**ME**), dietary DE and ether extract concentrations are considered. Usage of ME for maintenance, milk energy, and BW maintenance are fixed efficiencies, regardless of diet characteristics (except for fat concentration) in the NRC (2001). CPM-Dairy and CNCPSv6.5 estimate ME supply of diets by modeling of the apparent TDN (or DE) and by utilizing a fixed efficiency value for predicting energy utilized for milk production, growth, etc.

Dietary energy originates from primarily five fractions (NDF, starch, protein, fats, and other) and approximately 60 % of DE in a diet originates from starch and neutral detergent fiber (**NDF**) in a typical lactating cow diet (Weiss, 2010). Starch total tract digestibility is usually high and has been described as ranging from 92.6 to 93.9 % mean digestibility for major starch sources such as barley, corn, and wheat (Ferraretto et al., 2013). The apparent digestibility of starch usually does not vary substantially from diet to diet. Across 237 observations of total tract starch digestibility, the coefficient of variation for starch digestibility was 3.8 % (Weiss, 2010). However, the range of rumen degradable starch is quite variable, 54.1 to 78.9 % rumen digestibility across barley, corn, and wheat (Ferratetto et al., 2013). Within corn grain, rumen degradable starch can vary substantially depending on particle size, storage process, and endosperm characteristics. We know that this variation in site of starch digestion will affect DMI, microbial protein yield, and milk, fat, and protein yields; therefore, effective modeling of the site of starch digestion should be beneficial for field nutritionists. The NRC (2001) model and its derivations do not predict site of nutrient digestion. The CNCPSv6.5 model and its derivations do offer nutritionists some insight into fermentable starch concentrations of diets.

In contrast to the low observed variance associated with starch digestibility, variation in total tract NDFD is substantial. The coefficient of variation was 23.7 % for NDF diet digestibility across 237 observations (Weiss, 2010). Digestibility of NDF is usually model-predicted via a combination of lignin and *in vitro* NDF measurements; however, the relationship between lignin and *in vitro* digestible NDF has been shown to be quite variable. In the animal, the digestibility of NDF can be greatly affected

by DMI and other nutrient concentrations as well. The NRC (2001) model estimates NDFD using the "lignified surface area equation" or suggests that users can utilize 48-hr NDFD measurements (NRC, 2001). The CPM-Dairy model calculates the pool of potentially digestible NDF (**pdNDF**) using the following equation:

lignin * 2.4 = pool of pdNDF

CNCPSv6.5 determines the pool of pdNDF using the *in vitro* measurement of NDFD at 240 hr or the previously described equation (VanAmburgh et al., 2015). For some non-forage feeds, the measured NDFD at 120 hr and 240 hr appears to be significantly different than the previous lignin based equations utilized by the CPM-Dairy and NRC, 2001 models (Zontini et al., 2015). In some studies, the lack of a strong relationship between lignin and NDFD has also been demonstrated in forages and this has correlated with observed cow responses (Cotanch et al., 2014).

In vitro measurements for starch and NDFD of individual feed ingredients have value for understanding and ranking ingredients; however, one must consider that NDF and starch digestibility are not independent of dietary factors (e.g. DMI, starch concentration, rumen protein balance, and particle size). Predicted rumen fermentable starch concentration (as well as other carbohydrate fractions) should, at the minimum, provide directional inference when making diet formulation changes. However, it should be recognized that modeling fermentable starch is highly complex. The inability to predict the passage rate of individual feeds, represents one key limitation as the passage rate of some feeds, e.g. dry fine ground corn vs. high moisture corn, varies significantly (Ying and Allen, 2005).

Increases in digestibility of NDF usually cause increases in DMI which can somewhat depress overall diet digestibility. *In vitro* NDFD will typically be less than *in vivo* values, because of associative effects (Weiss, 2010). Highly fermentable diets (i.e. high starch content) will depress NDFD (Ferraretto et al., 2013). Replacing forage NDF with byproduct NDF increases the theoretical digestible NDF concentration of diets; however, the negative associative effects of increased passage rate and/or possible reductions in rumen pH may wipe out potential benefits of higher NDFD. For example, diets with similar model predicted energy concentrations (0.73 and 0.72 NE_L, Mcal/lb), but differing in forage NDF concentrations (22 % vs. 16.8 % DM) and analyzed 30 hr *in vitro* NDFD (59.8 vs. 62.7), resulted in the cows fed the lower forage NDF diet increasing DMI by 2.2 lb and producing numerically more milk (1.8 lbs; Weiss, 2012). However, cows fed the lower forage NDF diet had reduced milk fat concentrations and lower energy corrected milk (**ECM**) yield. The estimated dietary energy concentrations were 0.68 NE_L, Mcal/lb with the low forage NDF diet and 0.76 NE_L, Mcal/lb with the high forage NDF diet when accounting for DMI, body weight change, and ECM yield; which are very different estimates than the NRC, 2001 model had predicted. During routine diet formulation, consideration for the dietary effects on DMI and associative effects on rumen digestibility should be considered as the quantitative modeling of this effect is limited to nonexistent.

Associative effects on rumen fermentation result from the interaction of all diet characteristics and feed intake. Linear optimization is much easier if NE_L is assigned to individual feed ingredients; however, this approach may lead to a predicted dietary NE_L concentration that ignores associative effects. Nutrition

software models that assign NE_L and/or MP concentrations to individual feed ingredients may over predict NE_L and MP diet concentrations and subsequently, milk yield. Nutritionists should be aware of whether their ration software estimates energy and MP based on values for individual feeds or if it is computed from the total diet. Lab reported energy values for feed ingredient are irrelevant in NRC (2001), CPM-Dairy, and CNCPSv6.5 based nutrition models, as these platforms do compute energy from the total diet.

The largest losses of energy occur during transformation of GE to DE and ME to NE. Research related to residual feed intake (**RFI**) has shown that heat increment (conversion of ME to NE) possibly contributes 37 % to the variation of observed RFI in the beef population (Herd et al., 2004). We have also known that the theoretical conversion efficiencies for carbohydrate to body fat, lipid to body fat, protein to body fat, and protein to body protein are different: 0.80, 0.96, 0.66, and 0.86 (Blaxter, 1989). Application of a mechanistic model (Baldwin, 1980) by simulating varying dietary acetate, propionate, lipid, and protein inputs yields very different efficiencies for milk production or growth. In addition, changes in AA supply or efficiency of MP efficiency usage likely are closely associated with changes in ME utilization for milk yield (VanAmburgh et al., 2015), which potentially represents an opportunity for more mechanistic modeling of the conversion of ME to NE. Overall, this suggests that efficiency might be improved through dietary manipulation if we better understood predicted metabolic end-products.

Individual feed ingredients can vary substantially in NDFD and starch fermentability and these factors will affect DMI, rumen health, partitioning of nutrients, and digestibility of the total diet. Modeling of *in vitro* digestibility measurements for feed ingredients is useful; however, one must recognize that the digestibility of a particular nutrient is not an independent variable in the cow and that digestibility in the cow of dietary nutrients (e.g. NDF) may be significantly different than *in vitro* measurements would suggest (positive or negative). More mechanistic models are needed to help us better understand and represent digestion to optimally formulate diets.

PROTEIN PREDICTIONS BY NUTRITION MODELS

Most nutrition models (NRC, 2001; CPM-Dairy; and CNCPSv6.5) predict MP supply and estimate MP allowable milk. Metabolizable protein is the summation of absorbed microbial protein, digestible RUP, and endogenous protein. The assumed efficiency of MP utilization for protein synthesis is 67 % for CNCPSv6.5 and NRC (2001) and 65 % efficiency for CPM-Dairy. NRC (2001) and its derivations predict microbial protein from model calculated diet TDN intake. Rumen degradable protein and RUP are predicted by fractionating protein into 3 pools (fractions A, B, and C) and rumen degradation rates are estimated using *in situ* data. Amino acid requirements were not established in the NRC (2001), therefore, are not explicitly provided in NRC (2001) based ration software programs. The CPM-Dairy and CNCPSv6.5 based models differ from the NRC (2001) with a more mechanistic prediction of microbial protein production, prediction of AA requirements, protein fractions, consideration of urea recycling, and several other factors (VanAmburgh et al., 2015). For more complete review of protein predictions by NRC and CNCPSv6.5 based models, please see the following papers:

Schwab et al., 2014; VanAmburgh et al., 2015.

Important considerations for evaluating commercial software programs are that estimation of MP is calculated from the diet, not individual feed ingredients. If MP is estimated on individual feeds vs estimated from the total diet, the associative effects of DMI, RDP, or ammonia concentrations, and carbohydrate digestibility are not considered. Least cost optimization for supply of MP does not consider the benefit of feeding a variety of protein feed ingredients and/or balancing for limiting AA versus a diet formulated with only corn protein. The benefits of providing an improved dietary AA profile have been well documented (Schwab et al., 2014); however, nutrition models do not consider the effect of diet on efficiency of MP utilized for milk protein synthesis, as an example. Improved quantitative modeling of carbohydrate metabolism, as discussed earlier in the paper on CNCPSv6.5, may provide a platform for improving our ability to optimize microbial protein yield, for troubleshooting diets with perceived protein supply issues, and for formulating lower CP diets to improve N efficiency.

NUTRITION MODEL FEED LIBRARIES

Accurate characterization of feed ingredients is critical for successful diet formulation in terms of meeting animal requirements, accuracy of model predictions, and economic selection of ingredients. Most nutritionists analyze forages and some concentrates on a routine basis for individual farms and do not rely on stock library values. This is highly recommended, as the individual farm has been shown to be a significant source of variation for forages and some concentrates (St-Pierre and Weiss, 2015). Chemical analyses for feed ingredients continue to evolve, in part driven by the increased mechanization of the CNCPS model. The major nutrient concentration inputs for the NRC (2001) model are DM, CP, NDF, lignin, fat, ash, minerals, and to a lesser extent, ADICP and NDICP. For the more mechanistic models, the major additional inputs are soluble CP; ammonia; NDFD at the following time points, 30 hr, 120 hr, and 240 hr; undigestible NDF; sugar; starch; starch 7 hr digestibility; total fatty acids; and volatile fatty acids (**VFA**; lactic, acetic, and butyric). The CNCPSv6.5 model calculates the rate of degradation of NDF (multiple time points of digestion) and starch (single time point of digestion).

While NDF and starch digestibility *in vitro* measurements are important for describing feed ingredients, inter-assay variation, lab-to-lab variation, and sampling variation will limit the accuracy of these absolute values for appropriate characterization in a mechanistic model. Sampling has been shown to contribute anywhere from 9.2 to 80.6 % of the variance for nutrient concentrations in feed ingredients (St-Pierre and Weiss, 2015). The use of data from a single sample should be avoided in ration formulation (particularly for populations that pose sampling representation issues, i.e. large corn silage bunker). Several commercial nutrition platforms possess a function to allow averaging (simple or weighted average) of analyses for individual feed ingredients. From a user standpoint, the ability to electronically import sample analyses and the ability to automatically average samples within the software are 2 software functions that users may want to consider when selecting a ration software platform.

As noted above, lab-to-lab variation needs to be considered and selection of a single lab for an individual farm is

recommended to remove this source of variance. Important nutrients such as NDF might be assayed slightly different from lab-to-lab and it is suggested that nutritionists pay attention to the assay being used by a given lab (Hall and Mertens, 2012). On average, 30 hr NDFD inter-assay variation was shown to be +/- 5 percentage units (95% confidence interval) and +/- 6.5 percentage units across labs for forages (Hall and Mertens, 2012). The repeatability of *in vitro* NDFD assay for ranking ingredients has been shown to be quite good. CPM-Dairy and CNCPSv6.5 utilize *in vitro* measurements as absolute values for prediction of digestibility of NDF and starch; therefore, users should pay attention to lab assay variance associated with these measurements. For example, determination of rumen starch degradation is complex, i.e. particle size, grain type, and fermentation (e.g. HMSC) (Ferraretto et al., 2013) and assay repeatability of *in vitro* 7 hr starch measurements may be suspect. Starch degradation (rate and passage) should be assessed across a range of starchy based ingredients. Caution is suggested when using *in vitro* results (especially from single samples) as absolute values in mechanistic models. If a nutritionist is utilizing multiple labs and a single sampling technique, the noise (variance unassociated with real ingredient change in starch degradation concentration) is likely quite high and, therefore, should be avoided within an individual farm. Nutritionists should always use their own experience and knowledge of feed ingredients (i.e. particle size, floury vs. vitreous endosperm) as part of a feedback loop for more accurately describing starch degradation rate in mechanistic models. In addition, special attention should be paid to base library values for feed ingredients, as those values might be outdated or significantly different than commercial lab reported values. For example, corn silage (35 % DM, 41 % NDF, processed, medium) in the CPM-Dairy and CNCPSv6.5 feed libraries is described with a starch degradation rate of 32 % hr, which infers an 89.4 % starch 7-hr digestibility value. This value for starch degradation may be outdated as genetics for the corn endosperm may have changed substantially in recent years. For example, the reported average 7-hr starch degradability of corn silage submitted from US-based accounts was 77.8 % and the standard deviation was 5.7 % (n=16,479) for the time period of January 1, 2015 to June 30, 2015 (Cumberland Valley Analytical Services, Hagerstown, MD, www.foragelab.com). When *in vitro* measurements are not conducted on major dietary ingredients, users should consider if they want to adjust library values for starch degradation rates in mechanistic models. For example, if a nutritionist is formulating diets with consideration of fermentable starch concentrations then concentrations across diets might look different, if the starch degradability of corn silage is measured on some farms and library values used on other farms. The degradation rate of starch (corn grain) has been shown to be the most sensitive input for prediction of MP milk within the CNCPSv6.5 model (Higgs et al., 2015). A change of 1 standard deviation increase in the degradation rate of starch in corn grain increased model MP allowable milk by 4.1 pounds (Higgs et al., 2015).

Model feed libraries are often utilized for concentrates such as corn grain, soybean meal, whole cottonseed, etc. and variation of most concentrates from farm-to-farm has been shown to be limited (St-Pierre and Weiss, 2015). However, differences appear to exist across nutrition model platforms and, in the case of the NRC (2001) database, the nutrient concentrations of some feeds may have changed over time (Yoder et al., 2014). Nutrient concentrations of common

Table 1. Various feed ingredients nutrient concentrations and calculated MP concentration from several formulation platforms and a summarized database.

Item[1]	CPMv3.0	CNCPSv6.5	NRC, 2001	Yoder et al., 2014[2]
Citrus Pulp, dry				
DM, %	88.6	88.6	85.8	87.0
CP, % DM	7.0	7.3	6.9	7.0
NDF, % DM	23.9	23.9	24.2	22.3
EE, % DM	3.1	2.9	4.9	2.8
MP, % DM	13.0	11.3	8.1	
Cost ($/lb of MP)	$0.93	$1.07	$1.55	
Soybean Meal, 48 %				
DM, %	90.0	90.0	89.5	88.3
CP, % DM	55.0	51.5	53.8	52.9
NDF, % DM	10.0	10.0	9.8	8.7
EE, % DM	2.8	2.8	1.1	1.6
MP, % DM	24.9	26.5	26.9	
Cost($/lb of MP)	$0.78	$0.73	$0.73	
Blood Meal[3]				
DM, %	90.0	90.0	90.2	89.9
CP, % DM	93.0	95.0	95.5	99.4
NDF, % DM	37.8	-	-	6.0
EE, % DM	2.0	1.5	1.2	0.7
MP, % DM	68.7	50.5	65.2	
Cost ($/lb of MP)	$1.23	$1.68	$1.30	
DDGS - Ethanol				
DM, %	88.8	88.8	90.2	89.6
CP, % DM	30.3	30.3	29.7	29.5
NDF, % DM	32.2	33.6	38.8	33.0
EE, % DM	14.5	14.5	10.0	12.6
MP, % DM	17.0	18.6	18.5	
Cost ($/lb of MP)	$0.58	$0.53	$0.52	

[1]Feed ingredient nutrient concentration values were obtained from the libraries of the respective nutritional models, CPM-Dairy v3.0, NDS Professional v3.8.10.01, and NRC (2001).
[2]Ingredient nutrient concentrations were obtained from summaries provided by Yoder et al., 2014
[3]Blood meal was listed with the following descriptions within each model; blood meal (CPM-Dairy v3.0, blood meal average (NDS Professional v3.8.10.01), and ring dried blood meal (NRC, 2001)
[4]Metabolizable protein was estimated using a standardized and balanced diet (52 % forage), cow description inputs, and intake at 54 lb of DMI across CMP-Dairy v3.0, NDS Professional v3.8.10.01, and NRC (2001)
[5]Prices of the ingredients were same across nutrition models and the prices were the following; citrus pulp - $215/ton, soybean meal 48 % - $350/ton, blood meal - $1525/ton, and distillers ethanol - $175/ton.

feed ingredients vary across ration software platforms, as well as predictions of model calculated nutrient (ME and MP) concentrations for some ingredients (**Table 1**). We often hear in the field that an individual model *prefers* certain feed ingredients. Feedstuffs such as blood meal may have significant differences across models with its calculated MP concentration (% of DM) varying 18.2 percentage units across CPM-Dairy, CNCPSv6.5, and NRC (2001). A feed high in sugar and soluble fiber content, such as citrus pulp, is predicted to provide significantly more MP in CPM-Dairy vs. an empirically based model such as NRC (2001). With the updates in the partitioning of N supply (ruminally and post-ruminally) of feed ingredients within CNCPSv6.5, some feed ingredients may have more or less predicted MP contributions compared to earlier versions of CNCPS (e.g. CPM-Dairy; Table 1).

DIET FORMULATION AND OPTIMIZATION

Formulation and optimization of diets usually involves adjusting the nutrient concentration of a diet (e.g. ME allowable milk) and the designation of optimal inclusion of individual feed ingredients to meet a specified supply of nutrients. A change in dietary energy concentration often leads to a change in DMI or the impact of an associative effect on digestibility (Conrad et al., 1964). For example, formulating for a higher energy concentration often leads to reduced DMI which means energy intake will be less than expected. Increasing dietary NDFD (e.g. BMR corn silage or byproduct NDF) will increase the predicted energy concentration of the diet by the model, but the observed response is potentially increased DMI and reduced observed dietary energy concentration. The inability of current models to predict changes in DMI from a diet is a limitation that must be considered during formulation and optimization. While a particular optimized diet solution might predict increased IOFC, if DMI changes from the resulting diet solution, then the improved IOFC model prediction may not occur and, in some cases, might be negatively affected from the dietary change.

While optimization of IOFC by a software model represents a tool for improving profitability on dairy farms, we must recognize the limitations of computer optimization. Optimizers evaluate feed ingredients in terms of nutrient concentrations (considered static) and costs. However, feed ingredient nutrient concentrations are not constant, but variable, and the level of variation in nutrient concentrations is substantial across some feeds (e.g. CP concentration of distillers grains vs. soybean meal). The associated economic costs of nutrient variation within feed ingredients is not considered by current model optimizers. Statistical algorithms for assessing the costs of variation of feeds during least cost formulation have been proposed and discussed (St-Pierre and Harvey, 1986). Least-cost solutions might lead to an increased likelihood of diets formulated that have greater negative associative effects (e.g. preference for high unsaturated fatty acid concentrated feed ingredients, which may increase risk of milk fat depression) as most nutrition models don't quantitatively model well-documented associative effects. Although the effects of associative effects are widely understood, nutritionists often only consider nutrient guidelines and not the quantitative relationships of associative effects within most commercially available ration software. Nutritional models cannot predict responses in milk component concentrations

from dietary changes and this limitation should be considered when formulating diets for increased milk yield, as most producers are compensated for milk component yield, not fluid milk. In summary, optimization has value for selection of feed ingredients to deliver a predetermined supply of nutrient(s); however, the limitations of optimizing for increased milk yield and/or IOFC should be considered, as the optimization algorithm does not consider that changes in DMI or the partitioning of nutrients that are likely to occur with a changed dietary nutrient concentration.

COMMERICAL NUTRITION MODELS

Most field nutrition models in the US that are available to the public are based on the NRC (2001) (or NRC, 1989), CPM-Dairy, or CNCPSv6.5 model framework. For this paper, only a few platforms, i.e. AMTS, NDS, CPM-Dairy, and Formulate2 software will be discussed. In the US, the CNCPSv6.5 model platform is licensed and marketed by the following companies; Agricultural Modeling and Training Systems (AMTS, https://agmodelsystems.com), Nutritional Dynamic System (NDS, www.rumen.it), and Dalex Livestock Solutions (www.dalex.com) to the author's knowledge. Trial versions of AMTS and NDS are both available for download from the respective websites. The latest CNCPS released feed library is contained within both nutritional software platforms. The CNCPS library contains the majority of commercial products utilized in dairy rations today. The NDS software also contains another feed library, RUMEN, which contains feed ingredients not provided in the CNCPS feed library and commercial feed products. AMTS and NDS platforms both contain nonlinear optimizers that allow optimization on dietary concentrations of a number of diet calculated nonlinear nutrients (i.e. MP-lysine supply). Other features pertaining to AMTS and NDS can be found on their respective websites. In general however, functions for managing pricing, electronic importing of feed analyses, creating mix composites, user nutrients, and an array of report formats exist in both of these software platforms.

CPM-Dairy v3.0 continues to be utilized by a number of field nutritionists from the author's observations. The CPM Dairy v3.0 software is available for download; however, the development of the model by Cornell University, The University of Pennsylvania, and the Miner Institute has officially ended. Based upon a recent review, the CPM Dairy v3.0 was evaluated and its ability to predict milk production from ME and MP supply at the farm level given animal inputs, appropriate feed characterization, and feed intake was concluded to be accurate by the authors (Tedeschi et al., 2008). The University of Pennsylvania has recently released an updated version of CPM v3.0 titled ***UPenn Dairy Ration Analyzer*** and the major updates are related to the liquid passage rate, efficiency of MP utilization, and NDF digestion parameters. Information related to the software and a demo version for download is available at: cahpwww.vet.upenn.edu/doku.php/software:dra:start.

Formulate2 is a commercial software platform that fully implements the NRC (2001) and contains an optimizer that accounts for the nonlinear equations present in the NRC (2001) model. Formulate2 is marketed and supported by Central Valley Nutritional Associates LLC (www.formulate2.com). A new Formulate2 version is currently under development and key updates involve moving to a new development platform (Delphi XE6) and improving user functionality. The current version of Formulate2 for download to demo

has been suspended in anticipation of the release of the new version. Formulate2 contains a robust nonlinear optimizer, a range of reports, and several user functionality options.

Other major commercial formulate software available are Spartan Dairy Ration Evaluator/Balancer version 3.0 (http://spartandairy.msu.edu/spartandairy/home), NittanyCow Dairy Ration Evaluator (http://www.nittanycow.com/App_content/home.aspx), and AminoCow (http://www.nittanydairynutrition.com/App_Content/aminocow.aspx). Several other nutrition software platforms do exist, but are not listed in this paper.

A number of factors appear to determine selection of ration formulation software by practicing nutritionists. These include computer software functionality, underlying biology of the model, robustness of the feed library, optimization functionality, linear or nonlinear estimation of calculated nutrients (e.g. ME allowable milk), cost of software, training and technical support, user functionality (e.g. user generated report(s) format, electronic import of feed analyses, database structure (i.e. diets, farms, feeds, prices, etc.)), and previous formulation software experience (mechanistic vs. empirical based). From a model structure standpoint, estimation of calculated nonlinear nutrients on the diet vs. on individual feeds, mechanistic vs. empirical modeling of apparent TDN (or conversion of GE to DE) and microbial protein yield, and the accuracy of model predictions should be key factors for selection of ration software.

SUMMARY

Diet formulation for lactating dairy cows is complex with many interacting factors to consider. Quantitative modeling continues to evolve with incorporation of new research that potentially may improve ration software models. In general, nutrition models account for the transformation of nutrients into NE with good accuracy when provided good descriptions of intake, BW, environment, milk production, and BW change. The advent of more mechanistic based models and the development of *in vitro* assays provide tools to better characterize and determine the economic value of feed ingredients. Ration models that quantitatively model major sources of variation, e.g. NDFD and site of starch digestibility, may have the potential to better predict on farm performance, be more useful for troubleshooting, and improve decision making related to ingredient selection. Mechanistic models may also improve the accuracy and sensitivity of predicting N supply to the cow.

Nutrition models are useful tools for addressing the complex issue of optimal diet formulation. However, one must recognize what nutrition models predict well and also the limitations of nutrition models. Nutritionists should probably keep in mind the instructive comment of Box (1979) that "All models are wrong, but some are useful." It is important to appreciate that current nutrition models do not predict the effect of diet on the following variables; DMI (limited consideration for associative effects), conversion of ME to NE (except fat), and partitioning of nutrients (e.g. milk components). The need for human intelligence is still immensely necessary for optimal ration formulation.

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