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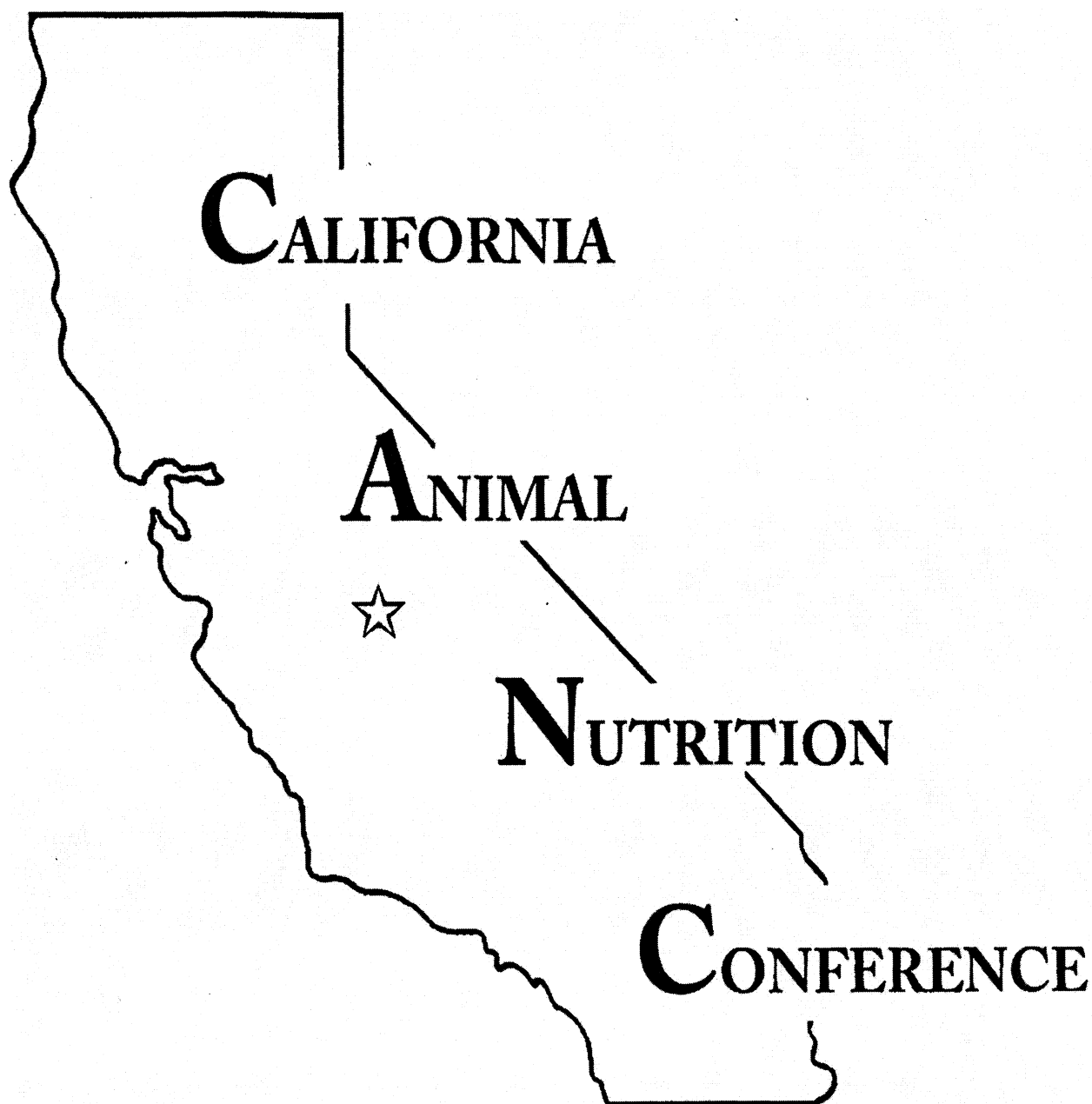
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California Animal Nutrition Conference – September 30th to October 1st, 2021(Sacramento)

Trace Minerals Sources: Where Have We Been, Where Are We Going?

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Introduction

Trace minerals (TM) have been recognized as important for almost 150 years. In 1874, it was reported that ashes from animal tissues were required for animal life (McCollum, 1956); however, it was not until the 1900s that individual trace minerals were recognized as essential using a mouse model. Many important TM discoveries and advances for cattle were made in the 1950s and 60s, including copper (Cu), zinc (Zn), and manganese (Mn) as essential nutrients for cattle. Early supplementation of TM started with oxides sources, and after World War II, manufacturing technologies advanced considerably, allowing for large-scale production of sulfate sources. Sulfate TM sources represented a significant nutritional advance for cattle at the time because they were more bioavailable than oxides (Sandoval et al., 1997). Organic sources of TM were first developed in the 1970s, and again, were a further advance over sulfate TM sources due to overall greater bioavailability than sulfates (Kincaid et al., 1986; Spears, 2003). Although organic TM have more bioavailability than sulfate TM, their high cost prevented feeding them as the sole TM source, leading to the use of inorganic/organic blends for commercial application. In the late 1990s, hydroxy TM (IntelliBond®) were developed and represented the next revolution in TM supplementation. Hydroxy TM have greater bioavailability than sulfates (Spears et al., 2004) and are more cost-effective than organic TM sources, making it possible to feed a 100% improved TM source to cattle.

Historical perspective

Copper occurs in plants in concentrations varying from 1 to 50 ppm on a dry matter basis (McDowell, 2017). This considerable variation is due to Cu soil concentration, soil physical properties, plant species, pH, plant maturity, fertilizers, etc. The essentiality of Cu was

discovered by Hart and Elvejhem in 1928 (McDowell, 2017), who first reported that it was required for growth and hemoglobin formation in rats. However, research with livestock animals dates from 1930 using cattle and sheep, and the first report showing Cu as an essential mineral for cattle used a Jersey heifer in Florida, which had a hemoglobin concentration of 5.9 g/100 mL, and after Cu, sulfate administration increased hemoglobin to 13.7 g/100 mL (McDowell, 2017).

Zinc was first recognized as essential for the fungus *Aspergillus niger* in 1869, but it was not until 91 years later that the essentiality of Zn was determined for cattle (McDowell, 2017). Zinc is the most abundant intracellular element, and it has been reported that the human genome encodes for more than 3,000 zinc-binding proteins, including enzymes and nuclear transcription factors (Sandstead, 2012). Due to its important role as a constituent of enzymes, Zn deficiency results in impaired growth, health, and performance.

Manganese deficiency was first described in swine in 1940 and was associated with enlarged joints and lameness. However, Mn deficiency in cattle was not reported until 1957 (McDowell, 2017). Soil Mn concentration can present a wide variation, ranging from less than 1 to 7,000 ppm. The skeleton accounts for about 25% of the total body Mn. It has been demonstrated that tissue concentration of Mn is not very responsive to alterations in intake (Miller, 1979), possibly due to the high regulation via homeostasis mechanisms.

Evolution of sources

Supplemental TM source knowledge is as important as determining animal requirements because some sources can be virtually unavailable to cattle (Spears, 2003). Copper is an essential TM; however, excess Cu can cause toxicity (Bradley, 1993). The essentiality and toxicity of Cu have guided research since the 1900s, helping determine both requirements and toxic levels. Cupric oxide (CuO) powder, a non-soluble compound with excessively strong chemical bonds, will not break down even under acidic conditions (Spears, 2003). The bioavailability of CuO has been evaluated by several authors and has consistently shown to be poorly absorbed in the gastrointestinal tract (Ledoux et al., 1991; Aoyagi and Baker, 1993; Baker, 1999). After 1990, CuO was no longer used on a large scale as a Cu supplement for livestock animals. However, not

all oxide sources are poorly used and absorbed, Zn oxide quality depends heavily on the manufacturing process and the quality of raw material used.

Furthermore, a challenge with Zn oxide sources is the large variability in quality and metal concentration found from different suppliers, resulting in less predictable bioavailability. Edwards and Baker (1999) evaluated the bioavailability of five Zn oxide sources in poultry (four feed grade sources and one analytical grade source) using total tibia Zn concentration. They reported relative bioavailability values ranging from 22 to 91%, using analytical grade Zn sulfate as the reference. The authors concluded that sources of Zn oxide have a large variability of metal content and bioavailability.

The replacement of oxides by sulfate sources of Cu, Zn, and Mn, represented an important advance in ruminant nutrition, providing animals with an economical and relatively more bioavailable mineral supply. Since World War II, sulfates have been used in animal nutrition and are commonly used as the standard to compare different TM sources (Shaeffer et al., 2017; VanValin et al., 2019; Jalali et al., 2020). Sulfate TM have weak ionic bonds that can be easily broken in an aqueous environment releasing free metal ions (Figure 1). Because Cu, Zn, and Mn are bound to their sulfate ligand via ionic bonds, the solubility of sulfate TM in the rumen is analogous to dissociation. Therefore, animals supplemented with sulfate sources of TM (STM) are highly prone to antagonist-induced deficiencies due to its high rumen solubility and antagonistic interactions. The antagonist interaction ultimately forms insoluble complexes that do not dissociate even after an acidic treatment, and these insoluble complexes are not available for absorption in the small intestine (Spears, 2003).

Inorganic - Sulfate	Organic	Inorganic - Hydroxy
A specific metal bound to a non-carbon containing ligand. Developed in the 1930's	A specific metal bound to a carbon/nitrogen-containing ligand. Developed in the 1970's	A specific metal bound via a coordinated covalent bond with a hydroxyl ligand. Developed in the 1990's
$CuSO_4$; $ZnSO_4$; $MnSO_4$	ZnAA; CuProteinate; ZnPolysaccharide	IntelliBond C[II] - $Cu_2(OH)_3Cl$ IntelliBond M - $Mn_2(OH)_3Cl$ IntelliBond Z - $Zn_5(OH)_8Cl_2 \cdot (H_2O)$

Figure 1: Chemical characteristics of sulfates, organic, and hydroxy trace minerals

Organic TM's (OTM) are defined as a mineral bound to carbon and/or nitrogen-containing ligands, such as amino acids, proteins, or polysaccharides. They were first developed in the 1970s to increase TM bioavailability. Overall, OTM have strong covalent bonds that protect the mineral from being released too early in the gastrointestinal tract, which may result in better bioavailability compared to sulfate sources (Osorio et al., 2012). Although OTM have more bioavailability than STM, the high cost of feeding OTM as the sole supplemental TM source meant that a combination with more economical sources, such as sulfate, was required. The most recent advancement in TM sources was the development of hydroxy TM (IntelliBond®) in the 1990s. Hydroxy TM (HTM) are a source of Cu, Zn, and Mn with low rumen solubility (Caldera et al., 2019) and higher bioavailability than sulfate sources (Spears et al., 2004; Shaeffer et al., 2017; VanValin et al., 2019). Hydroxy TM are considered to be an inorganic source because the metal is not bound to a carbon or nitrogen-containing ligand (Figure 1). The key novelty of the hydroxy TM are the strong covalent chemical bonds between the metal and the hydroxy ligand that are relatively insoluble in the rumen environment but are

broken down in the acidic conditions of the abomasum. Spears et al. (2004) reported that water solubility of hydroxy Cu was 0.6% after 24 hours, while Cu sulfate was 94.5%, and Cao et al. (2000) reported water solubility of Zn sulfate was 100%, while hydroxy Zn was 3% after 1 hour of constant mixing at 37°C. However, after treatment with HCl, the solubility of hydroxy TM ranged from 77 to 100% (Cao et al., 2000; Spears et al., 2004), demonstrating the ability to avoid interactions with dietary antagonists in the rumen and availability at the site of absorption in the small intestines. Caldera et al. (2019) evaluated rumen solubility of hydroxy sources of Zn, Cu, and Mn, using cannulated steers. The authors reported greater rumen solubility of Cu, Zn, and Mn sulfate compared to the HTM, in which Cu sulfate was more soluble than hydroxy Cu from 2 to 10 hours, Zn sulfate solubility was greater than hydroxy Zn from 2 to 20 hours, and Mn sulfate was more soluble than hydroxy Mn at 4 and 8 hours after bolus dosing. In the same study, Caldera et al. (2019) also evaluated the binding strength of these TM sources using a dialysis method. They concluded that HTM are less tightly bound to solid digesta than STM, indicating less interaction with dietary antagonists and greater availability for absorption in the small intestines.

Minerals are tightly regulated by homeostasis, which is one of the challenges with bioavailability studies. Therefore, one possible way to measure the bioavailability of minerals is to feed a diet below the animal's requirement creating a mineral-depleting condition, and then measure tissue accumulation or total body retention of the target mineral (Spears and Hansen, 2008). To further exacerbate the mineral depletion, antagonists such as molybdenum (Mo) and sulfur (S) can be added to the experimental diet. This approach was used by Spears et al. (2004) when comparing hydroxy Cu to Cu sulfate, using liver Cu accumulation as a measurement of bioavailability. The authors reported hydroxy Cu bioavailability as 196% compared to Cu sulfate (set as reference). In a similar study, VanValin et al. (2019) reported hydroxy Cu bioavailability as 112% compared to Cu sulfate. The bioavailability of hydroxy Zn was evaluated by Shaeffer et al. (2017) and was 204% compared to Zn sulfate (set as reference). Bioavailability numbers can vary due to several factors, such as animal TM status and physiological state; however, across these bioavailability studies, hydroxy sources of Cu and Zn resulted in consistently superior bioavailability compared to sulfate sources.

New frontiers on trace mineral nutrition

After years of research focusing on mineral deficiency and toxicity, the new frontier in mineral research focuses on the pre-absorptive effects of different TM sources. The negative impact of sulfate sources of Cu, Zn, and Mn on rumen fermentation has been determined mainly due to their high solubility and dissociation of free metal ions in the rumen. The most consistent negative effect reported is a decrease in neutral detergent fiber (NDF) digestibility when STM are supplemented as compared to HTM. By reducing NDF digestibility, sulfates also decrease total VFA concentration and alter individual VFA profiles (Guimaraes et al., 2019; 2020). Additionally, the source of TM has been shown to affect the fecal shedding of the bacterial *Treponema* genus, which is associated with bovine digital dermatitis (Faulkner et al., 2017b). This recent discovery is shedding some light on the mechanisms in which the source of Zn can affect hoof health. These new TM research frontiers are described in further detail below.

One recent advancement in TM nutrition for beef and dairy cattle was the determination of harmful effects of free metals ions of Cu, Zn, and Mn on ruminal fermentation, resulting in lower NDF digestibility when sulfate sources of these minerals were fed comparing to hydroxy (Faulkner et al., 2017b; Caldera et al., 2019; Miller et al., 2020). Ruminal microorganisms require TM for proper function and fermentation; however, it has been shown using in vitro techniques that high concentrations of these TM can be detrimental to bacterial growth. In fact, metals such as Cu and Zn have long been recognized as antimicrobial agents in the pharmaceutical industry (Lemire et al., 2013). Sala (1957) and Martinez and Church (1970) evaluated how individual trace minerals affect the ability of rumen microorganisms to digest cellulose and observed that even at low concentrations, they could negatively affect rumen microorganisms.

Mineral solubility can greatly affect the total concentration of TM available to rumen microorganisms because only soluble minerals are available for rumen microbial use. Therefore, Faulkner et al. (2017b) hypothesized that due to differences in rumen solubility, cows supplemented with hydroxy sources of Cu, Zn, and Mn would have greater fiber digestibility than cows supplemented with sulfates when at 10, 35, and 32 mg/kg DM of Cu, Zn, and Mn, respectively. Indeed, the apparent total tract NDF digestibility of cows fed HTM was 48.5% compared to 46.4% for cows fed STM ($P < 0.02$). However, the diet formulated using only corn

silage and alfalfa hay as forage sources resulted in a greater difference in NDF digestibility between the hydroxy and sulfate TM treatments than the diet formulated using by-products, suggesting a possible interaction between forage type and TM source.

Miller et al. (2020) evaluated the potential digestibility difference of BMR corn silage and conventional corn silage with different TM sources (HTM vs. STM). Interestingly, cows fed HTM had greater DM intake, greater kg of DM digested/d, and tended to have greater NDF digestibility ($P < 0.10$) than cows fed STM. Similarly, Hassan et al. (2011) observed that ewes fed Zn methionine had greater apparent digestibility of NDF and DM and greater total concentration of VFA's compared to ewes fed Zn sulfate, further indicating that TM source impacts nutrient digestibility.

A recent meta-analysis reported the effects of feeding hydroxy TM (IntelliBond®) on fiber digestibility across a wide range of diet types and composition (Ibraheem et al., 2021). Eleven comparisons were made using both dairy and beef animals. These studies were conducted under practical TM feeding levels, and on average, 12, 56, and 36 ppm of Cu, Zn, and Mn (respectively) were supplemented across all studies. Overall, the meta-analysis demonstrated that feeding HTM increased NDF digestibility by 1.7 percentage units compared to animals fed STM, regardless of diet type (Figure 2). When the method of NDF digestibility evaluation (in situ, marker, or total collection) was tested, the gold standard method of total fecal collection indicated feeding HTM increased NDF digestibility by 2.8 percentage units compared to animals fed STM (Figure 2). Meaning that a component as seemingly insignificant as TM source can have a significant impact on fiber digestibility.

Corn silage/grass hay in steers - High TM levels[1]   P=0.90
Corn silage/grass hay in steers - Low TM levels[1]   P=0.90   +0.02% P=0.97
Corn silage in lactating cows[2]   P=0.99
Corn silage in lactating cows[3]   P=0.79
Conventional corn silage in lactating cows[4]   P=0.10   +1.70% P=0.06
BMR corn silage in lactating cows[4]   P=0.10
By-product diet in lactating cows[5]   P=0.02
Corn/alfalfa silage in lactating cows[5]   P=0.02
Corn silage/steam-flaked corn in steers[6]   P=0.09   +2.80% P=0.01
Corn silage in steers[7]   P=0.04
Grass hay in steers[8]   P=0.04

■ In Situ Mean
■ Marker Mean
▨ Total Collection Mean

Bold indicates P-value ≤ 0.10

-10   -5   0   5   10

NDF Digestibility change: IntelliBond vs. sulfate trace minerals, % NDF
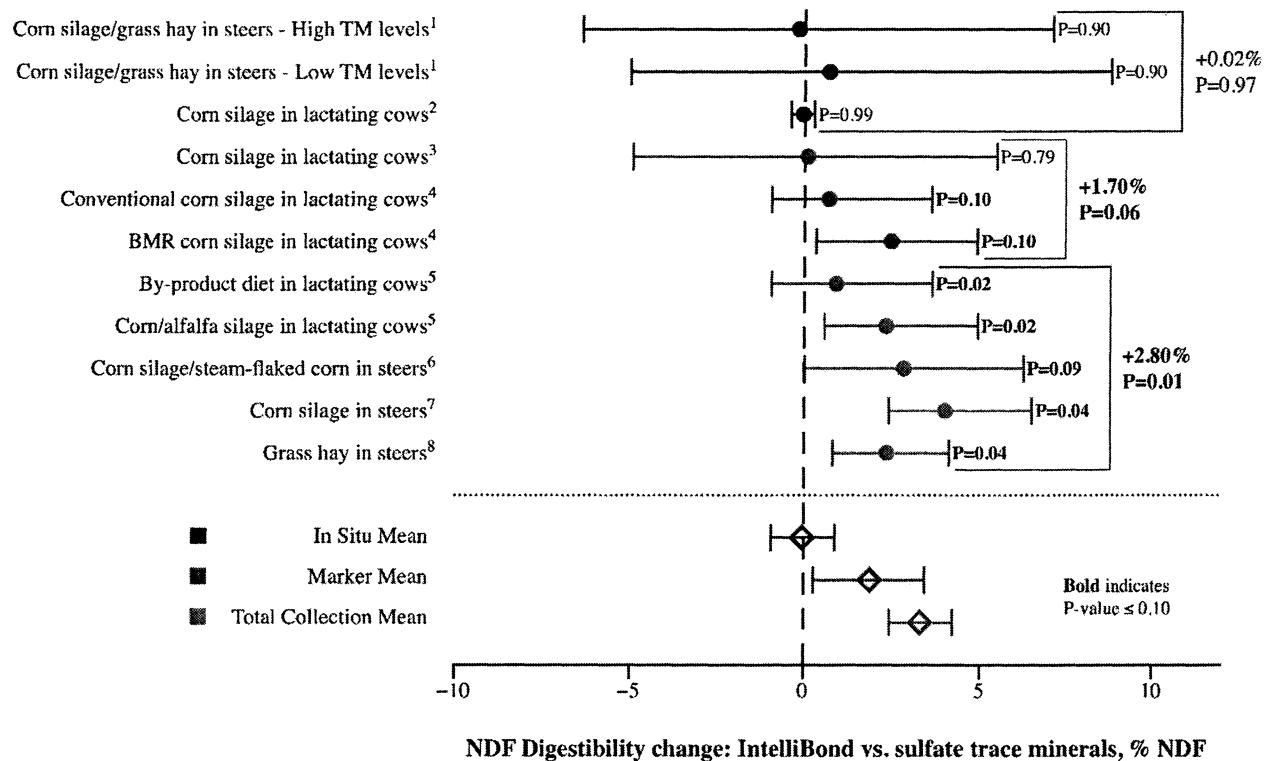
Figure 2: Forest plot from meta-analysis evaluating the change in neutral detergent fiber (NDF) digestibility when testing sulfate sources of copper, zinc, and manganese relative to IntelliBond C, Z, and M (Ibraheem et al., 2021).

It is estimated that about 94.7% of total NDF digestion occurs in the rumen (Huhtanen et al., 2010). However, the meta-analysis performed by Ibraheem et al. (2021) used a dataset containing studies predominantly reporting total tract NDF digestibility, posing the question of whether the negative effect of STM on NDF digestibility is originated in the rumen or lower gut. To further investigate this question and the impact of STM and HTM on ruminal nutrient digestibility, five studies using a 48-hour in vitro fermentation system were performed. All trials aimed to compare the effect of different sources of Cu, Zn, and Mn on fermentation and microbial biomass production. Trials used equal concentrations of supplemental TM to compare sources. In four out of five studies, HTM increased apparent organic matter disappearance compared to STM, and HTM increased apparent microbial biomass production in all five studies compared to STM treatment (Figure 3). Overall, these data further indicate that HTM increases fiber digestibility by improving ruminal fermentation.
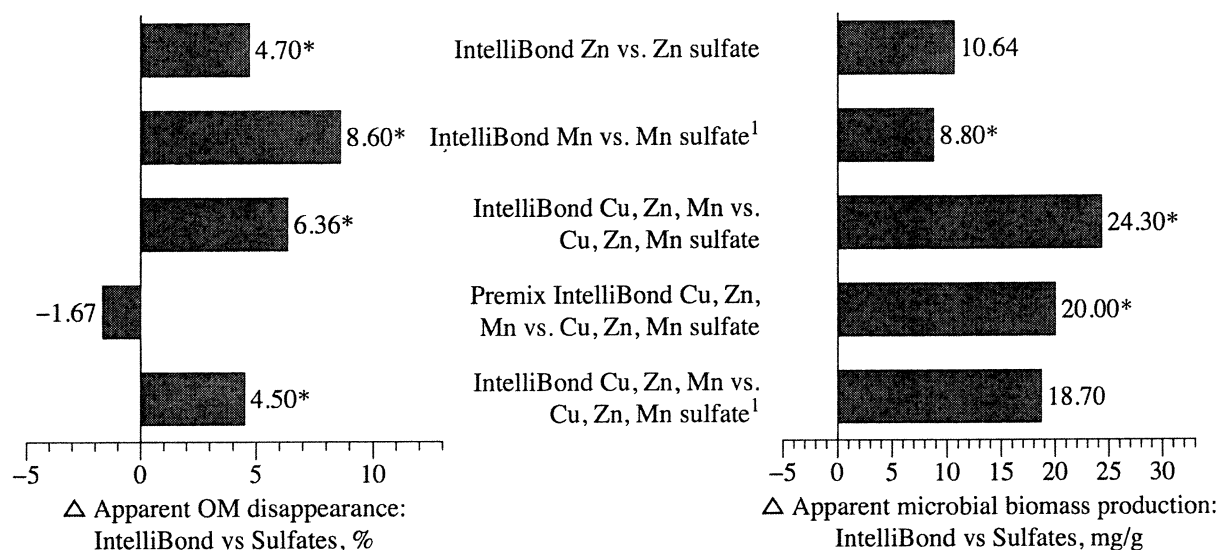
Figure 3: Change on apparent OM disappearance and apparent microbial biomass production when comparing IntelliBond and sulfate sources of supplemental Cu, Zn, and Mn using a 48-hours in vitro system (Fermentrics, Ontario, Canada). * indicate $P < 0.05$ original comparison. ([1]Brandao et al., 2021)

Sulfate TM have been shown to decrease total VFA concentration and alter individual VFA profiles. Therefore, three studies were carried out using cannulated steers, aiming to determine the effects of hydroxy Cu, Zn, and Mn compared with sulfate sources on total VFA concentration and individual VFA profile, using three different diets. The first diet was grass hay-based (Guimaraes et al., 2019), the second diet was corn silage-based (Guimaraes et al., 2020), and the third diet was a high concentrate feedlot diet (Guimaraes et al., 2021). Hydroxy TM supplementation increased total VFA concentration by 20% on the grass hay diet and 5% on the corn silage-based diet compared to the STM supplementation, with little to no changes on individual VFA profile (Figure 4). Furthermore, when the high concentrate diet was fed, there was a ten percentage unit increase in the molar proportion of propionate and a five percentage point decrease in the molar proportion of butyrate for steers fed hydroxy TM compared to sulfate TM (Figure 4). These results demonstrate that free metal ions released by sulfate sources of Cu, Zn, and Mn profoundly affect fermentation end-products, potentially reducing the energy

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available for the animal. These results also further strengthen our hypothesis that HTM improves NDF digestibility by improving ruminal fermentation.
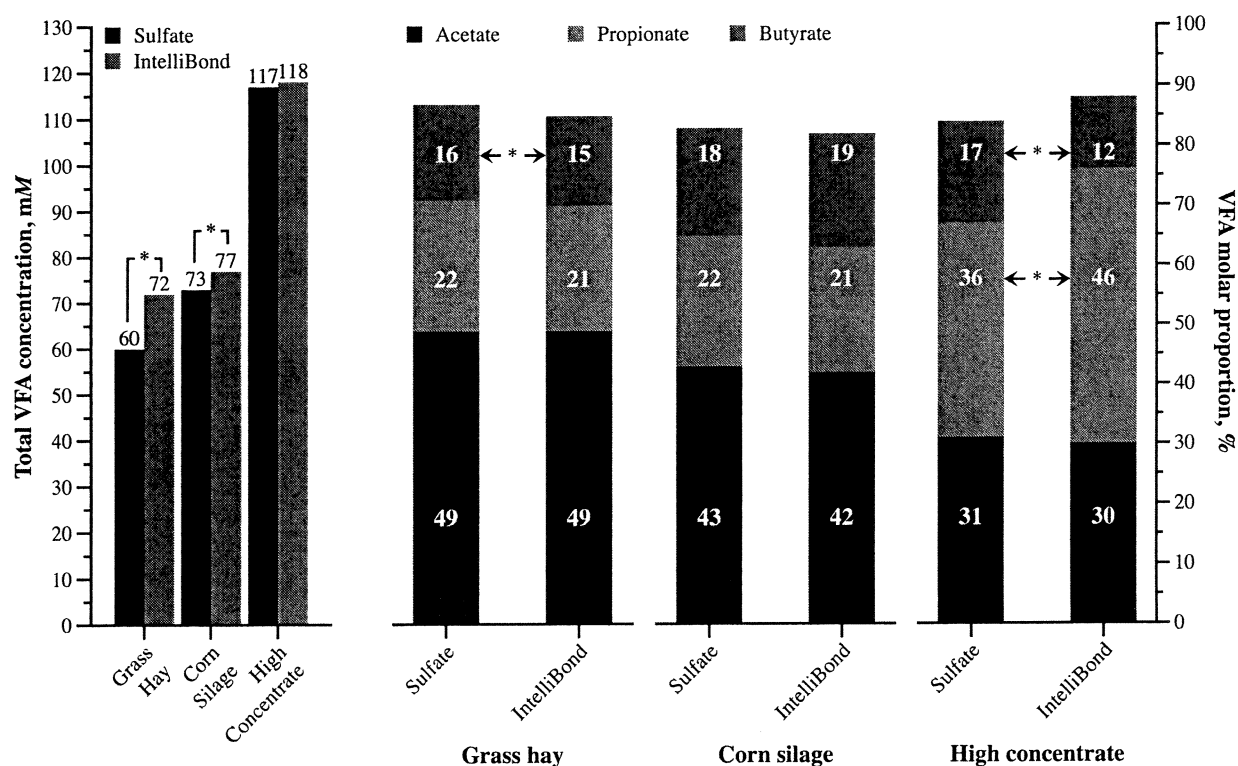

Figure 4: Changes in total volatile fatty acid (VFA) concentration and individual VFA profiles when three different diet types were fed to cannulated beef steers comparing sulfate sources of copper, zinc, and manganese to IntelliBond C, Z, and M. * = P < 0.05 (Guimaraes et al., 2019, 2020 and 2021).

Another recent discovery in TM research is related to bovine digital dermatitis and may shed some light on how different Zn sources may affect hoof health. The importance of adequate Zn status on skin and hoof health has been recognized for a long time (McDowell, 2017). However, the exact mode of action is not clear at this point. Digital dermatitis is a complex disease, and the *Treponema* genus of bacteria has been implicated as a causative factor. In a study evaluating the effect of Zn glycinate, Zn sulfate, or a combination of both using lactating dairy cows, it was hypothesized that the source of supplemental Zn would alter the relative abundance of fecal microorganisms implicated in digital dermatitis (Faulkner et al., 2017a). The authors reported a reduced relative abundance of the *Treponema* species in animals fed a combination of Cu and Mn sulfate with Zn glycinate when compared with sulfates alone, but the

relative abundance of *Treponema* species was similar when glycinate mineral sources and a combination of sulfates and glycinate sources were fed (Faulkner et al., 2017a). These results indicated that the Zn source could affect the fecal shedding of bacteria associated with digital dermatitis. In a similar study carried out at Iowa State University, lactating dairy cows were supplemented with 75 ppm of Zn from either hydroxy Zn (IntelliBond®) or Zn sulfate. The objective was to determine if the Zn source would affect the proportion of *Treponema* spp. in the feces of dairy cows (Wenner et al., 2021). A 67% reduction was observed in the relative fecal abundance of *Treponema* spp and when hydroxy Zn was fed compared to cows fed Zn sulfate (Figure 5), indicating that hydroxy Zn may help hoof health through better bioavailability and reduction in fecal shedding of bacteria implicated in digital dermatitis.

Cows fed IntelliBond Z had a 67% decrease in relative abundance of *Treponema* spp.

■ ZnSO₄ (n=11)   ▨ IntelliBond Z (n=12)
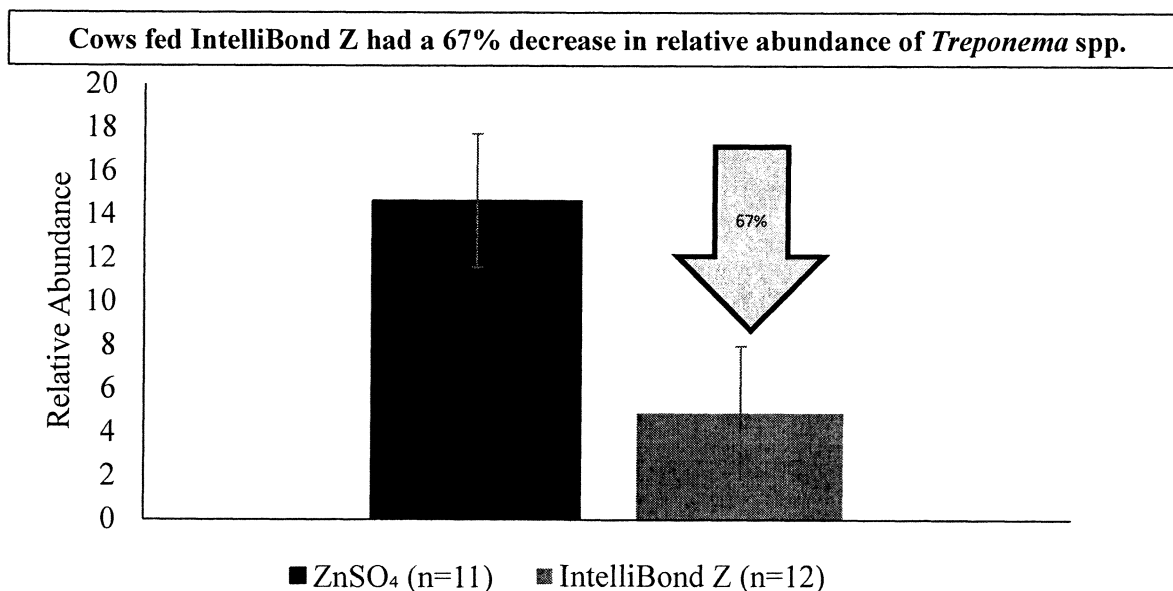
Figure 5: Relative abundance of *Treponema* spp. Of cows supplemented 75 ppm of zinc as either IntelliBond Zn or Zn sulfate (Wenner et al., 2021).

Summary

The main goal of supplementing trace minerals is to ensure that minerals will be available for absorption and that they will not negatively interact with other nutrients and dietary components. Sources of TM have evolved to meet the requirements and challenges of the

modern dairy cow. Modern TM nutrition should account for pre-absorptive and post-absorptive effects of different TM sources, providing animals with the best opportunity to optimize their productive performance.

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Mineral Nutrition of Dairy Cows:

Where Have We Been and Where Are We Going

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For more than 100 years, we have known that cows require certain minerals. Currently we know cows require at least 22 minerals, but at first only calcium, phosphorus and salt were deemed essential. Probably the factor that limited advances in mineral research most was the difficulty in measuring them. Available analytical methods were complicated, time consuming and not very sensitive and for many minerals, analytical methods were completely lacking. We now have instruments that can measure concentration of minerals in the parts per billion and parts per trillion ranges. Although our ability to measure minerals has vastly improved we still have limited knowledge on many aspects of mineral nutrition including : 1) molecular functions of minerals; 2) interactions among minerals and with other nutrients; 3) accurate estimates of absorption for most minerals; and 4) accurate and sensitive status indicators for most trace minerals.

The Last Century

Advances in mineral nutrition[1] have occurred at a slower pace than that for many other nutrients. The reasons for the slow pace were discussed by Weiss (2017), and include:

- Difficulty measuring minerals in feeds and other biological samples
- Lack of sensitive animal response measures
- Interactions of minerals with other minerals and other nutrients
- Experimental protocol requirements (long duration, large number of animals per treatment)

Forbes (1919) in the first paper published in the Journal of Dairy Science that was about minerals described the difficulties of mineral nutrition research and much of what he said is still true today.

" [Investigation is] difficult because the environment of the animal is composed of such a complication of influences that it is impossible to determine the proportionate contribution of each of them, especially of the obscure and intricate facts of mineral metabolism; and also because, on account of the supreme importance of mineral metabolism, the animal is so wonderfully protected by mineral reserves and other safety provisions that unfavorable effects of treatment are slow to

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[1] What is being written about minerals is also largely true for vitamins but this paper will be limited to minerals.

appear and are difficult to demonstrate in a clear-cut manner." (Forbes, 1919)

Requirements for Ca and P were included in the first NRC requirement book published (NRC, 1945) and were based largely on mineral balance studies. In those studies, output of Ca and P in feces, urine and milk were subtracted from Ca or P intake. If the value was negative cows were assumed to be below requirement. This approach has several problems including ignoring numerous functions of minerals, and is not used anymore to establish requirements. Calculated Ca and requirements for a 1400 lbs. cow producing 70 lbs. of milk based on NRC (1945)are about 84 and 63 g/day and using NRC (2001) requirements would be about 124 and 77 g/d. In other words, Ca requirements almost doubled but P requirements only increased about 20%. Before the 1960/s trace minerals were largely ignored probably because of the difficulty in measuring them. An exception was cobalt which started being studied in the 1930's. Researchers in Australia discovered that small amounts of dietary Co cured 'Coastal Disease'. Much later it was discovered that Co is needed by ruminants to make vitamin B-12. A paper on Co supplementation (Neal and Ahmann, 1937) contained a statement that has been a driving force in trace mineral nutrition:

"The specific symptoms that may be expected with a deficiency, or excess, of any element or compound are dependent on the ratio of that element or compound to all others in the ration." Neal and Ahmann (1937)

A major advance in mineral nutrition research was the development of flame spectroscopy. This is still the standard analytical instrument used to measure most minerals in dairy cattle nutrition. It is accurate and sensitive. The use of this instrument allowed us to easily measure the effects of mineral intake on concentrations of mineral in milk, plasma, and body tissues and some of these measures started being used to establish requirements. However, as more studies were conducted we determined that concentrations of minerals in plasma and milk are often very poor indicators of mineral status and are not good response measures for estimating requirements. Conversely liver Cu concentrations have become the gold standard for evaluating Cu status in cows. The value of hepatic concentrations of other minerals for evaluating status is not

well established at this time. The lack of sensitive biomarkers of mineral status is still a major block to the advancement of mineral research.

In 1989, the NRC (1989) published requirements for Ca and P for dairy cows (in g/d) and recommended dietary concentrations for 12 other minerals. Calcium and P requirements were determined factorially (requirements for maintenance, milk, growth, and reproduction were estimated and summed to determine total requirements). In some cases, the recommendations for the other minerals were based on published research on prevention of clinical disease (e.g., magnesium and grass tetany), for other minerals, the recommendations may have been based on production responses, and for some other minerals recommendations may have been observationally-based (e.g., cows fed diets with this concentration of manganese appear healthy and productive). To use the factorial approach an absorption coefficient (AC) for the mineral is needed. For example, in NRC (1989) Ca was assumed to be 45% available (i.e., AC = 0.45)and P was assigned an AC of 0.50.

NRC (2001)

The committee that developed the NRC (2001) took a different approach to mineral requirements than previous committees. The committee applied the factorial approach to almost all minerals (including trace minerals) and rather than using constant AC for all diets, the committee derived different AC for different dietary ingredients. Although some problems exist, this approach was a major advancement in mineral nutrition. With the exception of selenium, sulfur, iodine, and cobalt, requirements were given for absorbed, rather than total dietary mineral. The benefits of this approach include: 1) deficiencies can be prevented when poorly absorbed sources of minerals are fed (e.g., copper oxide); 2)safety factors can be reduced when highly available minerals sources are fed; and 3) supplemental mineral sources can be priced based on supply of available mineral rather than total mineral. Overall, this approach should increase the precision of mineral nutrition. The problems with this approach include: 1) difficulty in measuring AC for many minerals which means that in many cases, the AC is actually a constant rather than a variable; 2) difficulty in determining the maintenance requirement for many minerals; 3)failure to consider other functions of minerals (e.g., copper can enhance immune function

but we are unsure whether the maintenance requirement as currently measured includes this response).

Copper can be used as an example of how NRC (2001) improved mineral nutrition. A typical midwestern US diet without any supplemental Cu will contain about 7 mg/kg (ppm) Cu which is inadequate for a lactating cow. Adding about 100 mg of copper sulfate so that total diet Cu is 14 ppm will increase dietary Cu to about 14 ppm which is about 120% of absorbed requirement which is a good practical goal. If copper oxide was used to obtain a dietary Cu concentration of 14 ppm, the diet would only meet 87% of the absorbed Cu requirement.

An example of a problem with the NRC (2001) system is manganese. Based on NRC (2001), a typical diet with about 15 ppm Mn would provide 120% of the requirement and for most minerals 20% is an adequate safety factor. However, (Hansen, et al., 2006) reported that feeding pregnant beef heifers a diet with 16 ppm Mn resulted in clinical Mn deficiency in their newborn calves. The reason NRC (2001) grossly underestimated dietary (not absorbed) Mn requirement was because they overestimated the AC for Mn. This occurred because so little data on Mn absorption exists and the little data they could find had relatively high AC (approximately 0.0075). With the improvement in ability to electronically search the scientific literature, more data were found yielding an AC of about 0.004. Using this AC the diet would need to contain about 30 ppm to meet 120% of the Mn requirement. This illustrates the importance of obtaining accurate AC.

Where do we go from here?

In my opinion the three most important areas of mineral nutrition that need additional research are improved estimates for AC, identifying field applicable, sensitive measures of mineral status and determining whether the factorial approach is adequate for establishing mineral requirements.

Improving AC Estimates

The major limitation of the current NRC approach of using the factorial method to determine dietary requirements for minerals is the lack of good AC data. In NRC (2001) for most minerals, ingredients other than mineral supplements have the same AC.

Since diets are a blend of several feedstuff, for some minerals, using the same AC across feeds may not be a major issue for typical diets. However, many diets are not 'typical' and the current system does not adjust AC for known antagonists or enhancers of absorption that may be present in many diets. For some minerals such as P and Ca, variation in AC among feedstuffs may be great enough to affect diet formulation.

Specific research goals relative to AC:

1. *Development of commercially applicable methods to estimate AC for different feeds.* For example, in feeds P can be partitioned into inorganic P and organic P and each fraction has a different AC (0.84 for inorganic and 0.68 for organic) (Feng, et al., 2015). The assays to fraction P into organic and inorganic P are straightforward and commercial labs should be able to conduct the assay. The proportion of inorganic and organic P could then be used to estimate the AC for the feed. For example, the P in grass hay averages about 67% inorganic and 33% organic P which would give it an AC for total P of 0.79 (0.67*0.84 + 0.33*0.68). Soybean meal with 7% inorganic P and 93% organic P would have an AC for P of about 0.69. Currently I know of no other laboratory methods that can be used to estimate AC for other feeds.

2. *Incorporate more sources of variation in AC estimates.* Numerous dietary factors can affect absorption of minerals (both positive and negative). Some important 'real world' relationships include: the negative effect of high dietary (or water) sulfur (especially sulfate) on absorption of Cu, Mn, Se, and perhaps Zn, the additive negative effect of elevated sulfur in the presence of elevated molybdenum on Cu absorption, the negative effect of elevated dietary K on absorption of Mg, and the positive or negative effect of monensin on absorption of Mg. High iron may interfere with absorption of other metals such as Cu, Mn and Zn, and high Ca can reduce the absorption of Se. Some of these relationships have been quantified based on limited data. Increasing dietary K concentration linearly decreases absorption of Mg. However, the effect is dependent on the concentration of Mg in the diet. For typical midwestern US diets, the concentration of dietary Mg should increase

about 0.09 percentage units for every 1 percentage unit increase in dietary K above 1% of diet DM (Weiss, 2004)but this relationship is only valid when high quality magnesium oxide is the source of supplemental Mg. Based on data from only 1 experiment, feeding monensin in high K diets increased absorption of Mg by about 25% when magnesium oxide was fed but it decreased absorption by about 30% when magnesium sulfate was fed (Figure 1) (Tebbe, et al., 2018). More data quantifying relationships between minerals are needed so that ration software can include equations to adjust AC for diet composition.
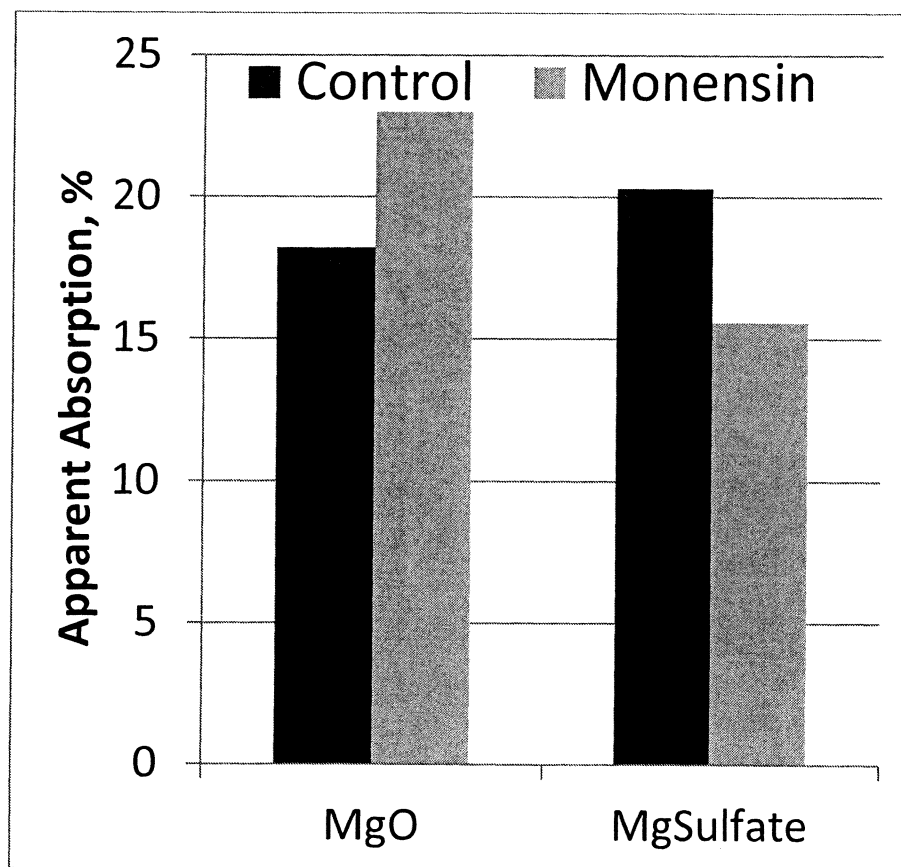

Figure 1. Apparent absorption of Mg by dairy cows fed either magnesium oxide (MgO) or Magnesium sulfate (MgSulfate) with or without monensin (approximately 350 mg/d). Diets contained 0.35% Mg and 2.1% potassium (Tebbe et al., 2018)

Identifying appropriate mineral status markers

For many minerals, health outcomes are used to determine their 'requirement'. For example, the NRC recommendation for Se is based largely on the amount of Se shown to reduce incidence of mastitis and retained placenta. For numerous reasons, health outcomes are not the best method to accurately determine requirements or recommendations. A better approach is to identify some biological marker that is highly correlated with health. The ideal marker should respond to changes in supply of absorbed mineral and should be field applicable (i.e., not highly invasive or expensive). An example of a reasonably good biomarker is concentration of Cu in liver. Although invasive, currently available biopsy tools make liver biopsies less stressful to cows. However, liver concentrations of many other trace minerals are not highly sensitive to changes in mineral supply. For most minerals (Ca, Mg, and Se are exceptions), blood or plasma concentrations are poor indicators of status. For some minerals, activities of specific metallo-enzymes (e.g., glutathione peroxidase and Se) are correlated with supply but for most minerals relationships between enzyme activity and supply of absorbed mineral are not strong. For other species, expression of certain genes are being used as status indicators and that approach needs to be evaluated for cattle. If (when?) good markers are identified, mineral research will become much more efficient.

Do minerals have 'non-factorial' effects?

The factorial approach accounts for minerals deposited in milk, body tissue, fetal tissue and the mineral that in inevitably lost in feces and urine (i.e., maintenance). However, we know that certain minerals can influence ruminal and intestinal bacteria and these effects do not require that the mineral be absorbed. For example, replacing supplemental zinc sulfate with organic Zn reduced the relative abundance of fecal *Treponema* (Faulkner, et al., 2017)Figure 2). Bacteria within that genus are associated with digital dermatitis. We do not know whether current requirements based on absorbed mineral are adequate to account for these effects. Perhaps we need to include a 'microbiome requirement'. Elevated intakes of sodium, potassium and to a lesser extent Mg can increase milk fat production. Production responses caused by increasing supply above requirements are not included in any factorial system. Ration

software could be modified to include basic factorial
requirements plus adjustment to account for these other
responses. These other responses would need to include economic
return.
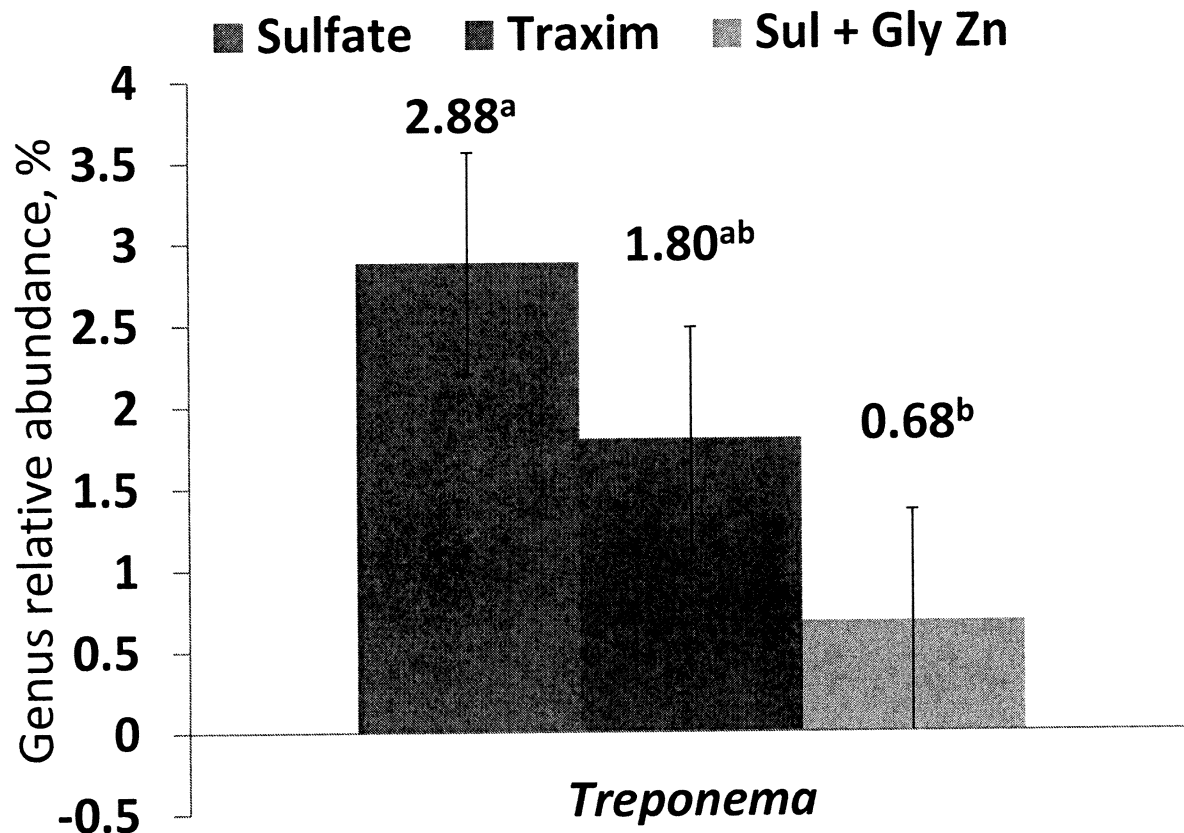
Figure 2. Relative abundance of fecal *Treponema* genus in cows
fed sulfate trace minerals (copper, manganese, and zinc),
organic trace minerals (Traxim; Pancosma, Geneva, Switzerland),
or sulfate copper and manganese with Traxim zinc (Faulkner et
at., 2016).

Summary

Over the past several decades our knowledge about mineral
nutrition of dairy cows has increased immensely and we are now
at the fine-tuning point for many minerals. For typical cows in
typical conditions current mineral requirements (plus
appropriate safety factors) are usually adequate. However, under
atypical situations such as presence of antagonists in water or
diet current requirements may be inadequate. We need more

research on field applicable biomarkers. Good biomarkers may allow us to reduce degree of overfeeding without increasing the risk of deficiencies. Overfeeding minerals, in some cases, grossly overfeeding, is almost universally practiced. Modest safety factors should be applied when formulating diets but with good biomarkers we should be able to reduce (but not eliminate) safety factors. With increasing pressure to reduce manure excretion of minerals this will become even more important in the future. Lastly we need to ensure that the factorial system accounts for all the positive responses to increasing mineral supply. If this is not the case, we need to modify ration software to appropriately account for these responses.

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Methane, Cows, and Climate Change:
California Dairy's Path to Climate Neutrality

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INTRODUCTION

Climate change is a global issue that requires comprehensive and far-reaching solutions across all economic and demographic jurisdictions. The Paris Climate Agreement, adopted in 2015, sets out a global framework to address harmful climate impacts by limiting additional global warming to well below 2 degrees Celsius (°C) (1.5 °C goal). The accord recognizes regional differences and the need for specific actions across all jurisdictions, including developed economies providing leadership and assistance to developing nations in their climate mitigation efforts.

California continues to lead the United States and world in implementing measures to achieve emissions reductions of greenhouse gases (GHGs) that advance climate change. Toward this end, California has established ambitious goals for reducing GHG emissions (Senate Bill 32) by 40 percent by 2030 and 80 percent by 2050. Senate Bill 1383 (2016) also established specific goals for reducing short-lived climate pollutants (SLCPs), such as methane, by 40 percent from 2013 levels. Ultimately, California is working toward a goal of "net-zero" carbon emissions by 2045 (Executive Order B-55-18).

The U.S. dairy industry recently announced efforts to address climate change, boldly aiming for carbon neutral or better (net zero climate impact) by 2050 (Innovation Center for U.S. Dairy, 2020). As part of these important efforts, California's dairy farms are leading change and making significant progress in reducing the amount of GHG emissions released into the environment. Producing a glass of milk from a California dairy cow generates 45 percent less GHG emissions today than it did 50 years ago. This finding, recently published in the Journal of Dairy Science, comes from a life-cycle assessment of California dairy farms in 1964 and 2014, conducted by researchers at the University of California, Davis (Naranjo et al., 2020). Significant advancements in farming efficiency, feed crop yields, veterinary care, sustainable feed practices, and animal nutrition have helped reduce the environmental footprint of individual cows. Building on these gains, more can be done to lower the climate footprint of milk production in the coming decade.

California's dairy farmers are working closely with the California Department of Food and Agriculture (CDFA) and the California Air Resources Board (CARB) to further reduce dairy methane emissions. As the efforts continue, it is also important to improve our understanding of how methane and other GHGs contribute to climate impacts, as we seek to limit warming. Leading climate scientists are now recognizing that moderately reducing methane emissions can quickly stabilize the climate pollutant's powerful impact, and further reductions can actually offset the far more damaging impacts of carbon dioxide (CO_2), which accumulate in the atmosphere for hundreds of years.

California's Greenhouse Gas Emissions

California, the fifth largest economy in the world, is responsible for about 1 percent of all global GHG emissions. More than 80 percent of California's emissions come from the transportation (41 percent), industrial (23 percent) and electrical (16 percent) sectors. Even though California is the United States' largest agricultural producer—producing fruits, vegetables, nuts, livestock, and other commodities for much of the U.S. and world—the sector's GHG contribution is only 8 percent of the state's total. California's largest-in-the-nation dairy sector accounts for about half of the agricultural share, or 4 percent of the state's total GHG emissions. The U.S. dairy sector accounts for 2 percent of the nation's total GHG emissions.

While CO_2 is the primary GHG driving climate warming, methane (CH_4), nitrous oxide (N_2O), and refrigerants are also important GHGs in California. According to CARB, carbon dioxide accounts for about 83 percent of California's GHG inventory. In comparison, methane accounts for 9 percent, and N_2O accounts for about 3 percent. In addition to knowing how much of each gas is being emitted, understanding how each gas causes actual warming is most critical to fully understanding and addressing climate change. Recent work by leading climate scientists at the Oxford Martin School and Environmental Change Institute at Oxford University has shed light on important differences among these GHGs and their impact on climate change (Lynch, 2019).

Methane emissions are generated by a number of processes, both those resulting from human related activity (anthropogenic) and natural (biogenic). Fossil- fuel methane (more commonly known as "natural gas") results from the process of extracting coal or oil, or from leakage during the extraction, storage, or distribution of natural gas for homes and businesses. Fossil methane is largely converted to CO_2 when we burn natural gas in our homes, factories, buildings, and other businesses.

Biogenic methane emissions are created by wetlands, rice cultivation, and ruminant livestock, as well as the waste sector, when microbes digest organic matter in our landfills and sewage treatment plants. Animal agriculture activity (all livestock) in California represents the largest source of biogenic methane emissions, accounting for roughly 55 percent of all human-related methane emissions in the state. California is the largest dairy state, producing roughly 18.5 percent of the nation's milk (USDA, 2019). The dairy livestock sector accounts for about 45 percent of all methane emitted in the state (CARB, 2015), primarily from two sources. Roughly half (55 percent) of dairy methane emissions come from manure management (storage, handling, and utilization), and the remaining 45 percent comes from enteric emissions.

In ruminant animals, methane is produced during manure decomposition as well as during enteric fermentation, where microbes decompose and ferment plant materials in the first compartment of their stomach, known as the rumen. This methane is expelled by the animal through belching.

Fossil Methane vs. Biogenic Methane

Fossil methane impacts the climate differently than biogenic methane. Fossil methane, such as natural gas, is carbon that has been locked up in the ground for millions of years and is extracted and combusted in homes and businesses. The burning of fossil methane directly transfers carbon that was stored in the ground (geologic carbon) into the atmosphere as CO_2. That carbon continues to accumulate and persist in the environment, contributing to climate change for hundreds of years. Bottom line: Fossil methane increases the total amount of carbon in the atmosphere, which drives warming.

Biogenic methane from cows is part of a natural carbon cycle, where after about 12 years it is removed from the atmosphere. As part of photosynthesis, plants capture CO_2 from the atmosphere, absorbing the carbon and releasing oxygen. That carbon is converted into carbohydrates in the plant, which are then consumed by the cows, digested, and released from the cows as methane (CH_4). After about 12 years in the atmosphere, that methane is oxidized and converted into CO_2. These carbon molecules are the same molecules that were consumed by cows in the form of plants. As part of the biogenic carbon cycle, the carbon originally utilized by the plant is returned to the atmosphere, contributing no net gain of CO_2.

Global Warming Potential of California's Primary Greenhouse Gases

Each GHG captures and retains heat at a unique rate, known as its global warming potential or GWP (as shown in Table 1 as GWP 100). For example, CH_4 has 28 times the warming potential of CO_2 over a 100-year period. Understanding how emissions impact global climate; however, requires consideration of not just the potency, but also how long each type of GHG will last in the atmosphere (atmospheric lifetime).

This is particularly important for methane, as it is a SLCP, with emissions breaking down after about 12 years (Farlie 2019; Lynch, 2019). In contrast, a significant proportion of CO_2 emissions are expected to persist in the atmosphere for hundreds of years, or even longer (Farlie, 2019; Lynch, 2019). As a result, the treatment of all GHGs as CO_2 equivalent (CO2e) using GWP—and failure to consider the atmospheric removal of SLCPs—misrepresents the impact of methane on future warming (Frame et al., 2018; Cain, 2018). Recognizing this shortcoming, leading climate scientists expanded on GWP and developed GWP* (GWP-Star), which quantifies a GHG's actual warming potential, instead of just its CO_2 equivalence, by factoring in how much more or less methane is being emitted from a source over a period of time. GWP* appropriately builds on the conventional GWP approach employed in typical reporting of GHG emissions (Lynch, 2019). GWP* recognizes the rate and degradation of methane emissions, in addition to the total amount of CO_2 and other long-lived gases emitted (Lynch, 2019; Cain, 2018; Frame et al., 2018).

Climate Impact Potential/GWP* (GWP-Star)

Recognizing the important differences in how methane and carbon dioxide affect climate change is critical to quantifying their actual climate impacts. GWP* was developed to better and more completely account for the warming impacts of short- and long-lived gases and better link emissions to warming (Cain, 2018). GWP* is still based on GWP, but

recognizes how different gases such as methane affect warming (Cain, 2018).

Because CO2 emissions last in the atmosphere for so long, they can continue to impact warming for centuries to come. New emissions are added on top of those that were previously emitted, leading to increases in the total atmospheric stock or concentration of CO2. As a result, when additional CO2 is emitted, additional global warming occurs (Frame et al., 2018).

In contrast, methane emissions degrade in the atmosphere relatively quickly, after about 12 years, and do not act cumulatively over long periods of time. For a constant rate of methane emissions, one molecule in effect replaces a previously emitted molecule that has since broken down. This means that for a steady rate of methane release—as emitted by a constant number of dairy cows, for example—the amount of methane in the atmosphere (concentration) stays at the same level and does not increase. As a result, when a steady amount of methane is emitted for more than 12 years, no additional global warming occurs (Frame et al., 2018).

This improved understanding of how short-lived versus long-lived emissions affect climate differently is critical to addressing further global warming. Limiting climate change requires that we bring emissions of CO2 and other long-lived GHGs down to net-zero (Frame et al., 2018). For methane, however, it is possible to have steady ongoing emissions that do not result in additional warming (Frame et al., 2018).

This does not mean that methane can or should be ignored. Increasing methane emissions would result in significant warming. Because of its short-lived atmospheric lifetime, reducing methane emissions can lead to a drop in atmospheric concentration relatively quickly. So, reducing methane emission rates presents an important mitigation opportunity, which could reverse some of the warming the planet has already experienced (Lynch, 2019). Put simply, a reduction in methane emissions has climate cooling effects (Cain, 2018).

Climate-Neutral Dairy: Achievable in California's Near Future

Understanding how methane impacts global warming is critical to understanding the role of dairy production as a contributor to climate change. California's dairy sector is an excellent case in point. It is no longer growing and expanding production. The number of milk cows raised in the state reached a peak in 2008, around the same time that California passed its first climate policy (2006). Since then, the number of cows has declined by a little more than 7 percent (CDFA, 2017). Total milk production has also decreased in recent years. As a result, the amount of methane in the atmosphere contributed by California milk production is less today than in 2008, as more methane is being removed from the atmosphere each year through its natural breakdown process (biogenic methane cycle) than is created by fewer dairy cows.

California dairy farms are also taking important, voluntary steps to further reduce methane from farms by installing anaerobic digesters designed to capture methane. Other projects, such as compost pack barns

and solid separators, are designed to reduce methane production on farms. More than 213 dairy methane reduction projects have been incentivized with state funds to date (CDFA, 2019). These efforts alone are expected to achieve more than 2.2 million additional metric tons of GHG reduction each year, as the projects continue to be implemented (CDFA, 2019). Hundreds of additional dairy methane reduction projects are expected in future years.

As discussed earlier, enteric emissions (belching) from cows account for a significant share (45 percent) of total dairy methane emissions in California. Identifying solutions to reduce these emissions will also be necessary to meet state goals. While research into enteric emission mitigation is being conducted, and some feed additives show promise, commercially proven and cost-effective solutions are not yet available (Webinar on CARB's Analysis of Progress Toward Achieving Methane Emissions Target from Dairy and Livestock Sector, 2020).

Dairy farms also create other GHGs, such as CO_2 and nitrous oxide (N_2O), from the use of farm equipment for dairy management and the utilization of manure for growing crops. These emissions account for about 20 percent of all GHGs produced by the dairy production sector (Naranjo et al., 2020). Reducing or offsetting these emissions will also be necessary for the state's dairy production sector to achieve climate neutrality, or the point at which operations and resulting emissions are stable and no longer adding to global warming (no net global warming impact). California dairies are also reducing the amount of CO_2 they emit into the atmosphere through the adoption of solar energy and electrification of feed mixing and water pumping operations. Fossil fuel use per unit of milk produced has dropped by 58.5 percent from 1964 to 2014 (Naranjo et al., 2020). As dairy methane emissions are reduced further below current levels, then resulting cooling effects can offset some of the remaining CO_2 and other gases contributed by dairy production.

Conclusions

A continued focus on methane is necessary, as it is a powerful GHG and an important contributor to climate change. Under all scenarios, methane is significant, second only to carbon dioxide in terms of its overall contribution to global, human-driven climate change (Lynch, 2019). Over the last decade, global methane concentrations have increased (Lynch, 2019). Agriculture, including animal agriculture, is partially responsible for the increase, as dairy and meat production and consumption continue to expand globally, particularly in low- and middle-income countries. That notwithstanding, evidence is growing that shale gas production is a larger source of methane emissions than previously assumed (Howarth, 2019). Like every sector of the global economy, agriculture must do its part if we are to succeed in achieving the overarching goal of limiting global warming. Equally important, California acting alone cannot accomplish significant global dairy methane emission reductions.

Recognizing how methane impacts global climate is also critical to assessing whether the state and world are on track to meet the goals of the Paris Agreement and limit warming to well below 2°C. Comparing GHGs with each other using GWP* preserves the link between emissions and

warming or cooling of the atmosphere (Schleussner et al., 2019). It also provides an informative and better suited way to assess the relative merits of different options for reducing GHG emissions, especially in ambitious mitigation scenarios (Cain, 2019). More accurate expression of mitigation efforts in terms of their direct contribution to future warming also better informs burden-sharing and long-term policies and measures in pursuit of ambitious global temperature goals (Allen, 2018; Schleussner et al., 2019).

Reducing methane emissions and achieving climate neutrality is no small undertaking. California is among the most efficient producers of milk and dairy products, and its life-cycle carbon footprint (per gallon of milk produced) is among the lowest of any region in the world. Achieving these or similar levels of production efficiency (more milk with fewer cows) is a critical first step for other dairy regions to begin stabilizing methane emissions and work toward climate neutrality. The impact of such an accomplishment would have profound climate effects. Attaining California's level of production efficiency in all global dairy production regions could reduce total global GHG emissions by as much as 1.73 percent (E. Kebreab, calculations based on Naranjo et al., 2020 and FAO & GDP, 2018).

A full understanding of the potential climate impact of all greenhouse gases is also important in ensuring effective policies are developed to address methane and other flow pollutants in line with their effects. Dairy production primarily produces flow emissions (80 percent is methane) with smaller amounts of stock emissions, such as CO_2 and N_2O (Naranjo et al., 2020). Policy or consumption decisions that trade off and result in greater concentrations of CO_2 and N_2O, while reducing methane, may ultimately leave a warmer planet behind in the long term (Frame et al., 2018).

Adopting sustainable farming practices to vastly improve production efficiency is probably the single-most important step other dairy-producing countries can take to begin to stabilize regional and global methane emissions and begin to achieve climate neutrality. The United Nations Food and Agriculture Organization (FAO) estimates that improved management practices alone could reduce net global methane emissions by 30 percent (FAO, 2019). These efforts will be critical to reduce livestock methane emissions and present important opportunities for reaching global climate mitigation targets. Further reductions in methane emissions will lead to atmospheric concentrations falling relatively quickly, which could reduce some of the warming already experienced (Lynch, 2019).

Case Study: California Dairy Methane Reduction

Fully understanding the climate cooling potential of dairy methane reduction efforts in California is critical for state regulators and policymakers. California is seeking to reduce dairy methane emissions by roughly 7.2 million metric tons (MMT) per year by 2030 (40% reduction). What will this mean for California's overall emissions reduction goal of being "net zero" by 2045?

Achieving the state's goal of reducing dairy methane emissions by 7.2 MMTCO2e annually will provide about 20 MMT of annual reduction (cooling) equivalent each year from 2030 to 2045. These reductions will

be critical to mitigate continually accumulating CO2 emissions from other sectors of the economy, and the achievement of the state's "net zero" long-term goal. In the race to manage global warming, reducing methane can provide fast returns.

This analysis using GWP* shows the true value of the state's dairy methane reduction efforts and programs such as CDFA's Dairy Digester Research and Development Program (DDRDP) and Alternative Manure Management Program (AMMP), which are expected to incentivize more than half of the 7.2 MMT of methane reduction. This analysis also underscores the importance of continuing to fully fund these California Climate Investment Programs at a minimum of $85 million per year. (CARB Preliminary Analysis of Dairy Methane Reduction Progress, May 2020).

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Trace amounts of antibiotic is detrimental to the health of weaned pigs

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The objective of this experiment was to investigate the potential detrimental effects of trace amounts of antibiotic on weanling pigs' growth performance, systemic immunity, intestinal health, serum and intestinal metabolome, and intestinal microbiome using a disease challenge model. Thirty-four pigs (6.88 ± 1.03 kg body weight) were individually housed and randomly allotted to one of three treatments (9 to 13 pigs/ treatment). The three dietary treatments were control diet (CON), and 2 additional diets supplemented with 0.5 or 50 mg/kg of carbadox (TRA or REC), respectively. The experiment lasted 18 d [7 d before and 11 d after first inoculation (d 0)]. The *E. coli* F18 inoculum was orally provided to all pigs with the dose of D^{10} cfu/3 mL for 3 consecutive days. Pigs in REC had greater ($P < 0.05$) final body weight and lower $P (< 0.05)$ overall frequency of diarrhea, compared with pigs in other groups. Pigs in TRA had the lowest ($P < 0.05$) average daily gain and feed efficiency from d 0 to 5 post-inoculation (PI), highest ($P < 0.05$) percentage of β-hemolytic coliforms in feces on d 2 and 5 PI, compared with pigs in CON and REC. Pigs fed TRA had the greatest ($P < 0.05$) serum C-reactive protein on d 2 and d 5 PI and serum TNF-α on d 5 PI, compared with pigs in the other groups. Pigs in REC had increased ($P < 0.05$) mRNA expression of *ZO-1* and *OCDN*, and reduced ($P < 0.05$) *IL1B*, *IL6*, and *TNFA* in ileal mucosa on d 5 PI, compared with the CON. However, TRA up-regulated ($P < 0.05$) mRNA expression of *IL1B*, *IL6*, and *PTGS2* in ileal mucosa on d 11 PI, compared with REC. Differences in the metabolic profiles of serum and colon digesta, and microbial profiles of colon digesta were mostly found between pigs in TRA and REC. Supplementation of TRA differentiated serum and colon digesta metabolites associated with amino acids, carbohydrates, and purine metabolisms on d 5 PI, compared with pigs fed REC. Pigs in REC had the highest ($P < 0.05$) relative abundance of *Lactobacillaceae* and tended to increase ($P < 0.1$) the relative abundance of *Lachnospiraceae* in the colon digesta on d 5 PI. In conclusion, trace amounts of antibiotic have shown detrimental effects on growth performance and disease resistance of pigs challenged with *E. coli* F18, potentially by exacerbating systemic inflammation and altering metabolic and microbial profiles.

Key words: gut microbiome, host metabolome, immunity, pathogenic *E. coli*, post-weaning diarrhea, trace amounts of antibiotic, weaned pigs

Dairy Efficiency and the Impact of Different Diets

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Introduction

Food system sustainability is certainly a hot topic today. Between the burgeoning global middle class with its rising demand for animal-source foods and the current and future implications of climate change, many analysts are wringing their hands about the ways we produce and consume food around the globe.

In many analyses, the conclusion drawn is that animal-source foods are a luxury that the world simply cannot afford any longer as we approach a human population of 10 billion. This is a serious claim that the livestock sector has to engage with. Although individual behaviors in the short term are not likely impacted much by these assessments, longer-term policies and cultural shifts could completely change what agriculture looks like in the United States and globally.

What does efficiency really mean?

To dig into the details of food system analyses, we have to begin by understanding what the objective is, whether it is implicit or explicit. Some studies ask how we can feed the global human population with the least impact on the environment, whereas others ask how we can best meet all the nutritional needs of the peak human population on a fixed agricultural land base. Still other studies focus on limited fresh water supplies or net carbon emissions.

One thing that most analyses have in common is a desire to find the most efficient solution for feeding humanity. However, different analyses can come to very different conclusions because there is no single definition of efficiency. In fact, milk production, like production of essentially anything, requires a variety of resources – land, water, energy, etc. Efficiency can refer to output per unit of any of those inputs. Table 1 points to a number of key inputs or impacts of dairy production that have been used to describe dairy efficiency in different terms. It is very unlikely that any single dairy production system would be optimal for all of these inputs simultaneously. Therefore, the metric that is emphasized will result in an approach that optimized for that particular input, and not necessarily for others. The same is true for the overall food system.

Table 1. Literature evaluating U.S. dairy efficiency for different inputs.

Input resource	Estimated efficiency	Reference
Lactating dairy cows	23,777 lb milk / cow annually	USDA NASS (2021)
Feed dry matter	1.55 lb milk / lb DMI	
Human-edible feed DM	0.28 lb milk solids / lb human-edible DM	(Wilkinson and Lee, 2017)
Carbon	0.75 lb milk / lb CO_2-equivalent greenhouse gasses	(Capper and Cady, 2019)
Land	4,6245 lb milk / crop acre	(Capper and Cady, 2019)
Water	0.05 lb milk / gallon water	(Capper and Cady, 2019)
Surface and subsurface ("blue") water	0.026 lb milk / gallon available water	(Legesse et al., 2017)
Capital	1% annual return on equity	FINBIN (2021)
Human labor	667 lb milk / hour of labor	FINBIN (2021)
Total financial inputs (cost of production)	5.95 lb milk / $1 costs	FINBIN (2021)

The point of this is not that some metrics are more important than others. There are places in the world where land is clearly a constraint and land efficiency should be emphasized (e.g. India), whereas in other places, water may be a bigger constraint (perhaps California). In the end, a "real-world" food system has to balance efficiencies across all of these inputs, which is no simple task.

Traditionally, free-market supporters have considered the total cost of production to be as good a metric of total resource inputs as any. Assuming all inputs are priced at or near their true value, then the cost of the end product should reflect the sum of those inputs. Everyone involved in the U.S. dairy industry recognizes how brutely competitive it is, and it is frustrating and sad to see multi-generation farms exit the industry each year. The silver lining, however, is that this highly competitive environment has certainly

contributed to the massive gains in resource efficiency achieved by the industry over decades (Capper et al., 2009).

We should also note, however, that relying on cost of production to assess resource inputs is more or less rejected by those focused on the environment. One criticism is that at least some key inputs are *not* priced at their real value. For example, at least prior to the renewed surge in domestic oil and gas production, many have suggested that fossil fuel prices are heavily subsidized in the U.S., including through massive military and State Department spending to maintain a stable supply of oil and gas. Another example would be groundwater use, which in many states is tied to land ownership in systems established more than 100 years ago, and which do not limit use rates to sustainable levels, thereby decreasing the cost in the short term. Another hidden cost would be environmental impacts with costs that are borne in distant locations or in the future. A good example would be a mining operation that recovered and sold material in the early 1900's only to leave behind toxic waste that taxpayers in the 21st century are paying to clean up – the true cost of the enterprise was not borne by those purchasing the mined product 100 years ago.

Today, critics of the dairy industry would claim that long-term damage from greenhouse gas emissions, pollution of waterways with excess N and P, and even negative human health outcomes would all be hidden costs not reflected in the price of milk sold from farms. This is not to say that these criticisms are reasonable or accurate, but it points to the larger, complex way that industries are being put under the microscope today.

On the other hand, the dairy industry can counter that milk is not the only value generated by dairy farms. Dairies provide a means to keep landscapes green (whether in pasture or forage crops) and provide jobs in areas where depopulation is a significant problem, among other secondary benefits.

Focusing on the efficiency of converting crops to dairy products

The discussion above points to the difficulty of engaging with all of the various challenges to the role of the dairy industry in future food systems at one time. However, we can tackle the more serious critiques and explore their validity. One such complaint is the assertion that feeding animals to produce animal-source foods is a waste of resources because it is inherently less efficient to use animals as an intermediate (with unavoidable inefficiencies) rather than just growing crops for direct human consumption (Cassidy et al., 2013).

It is certainly the case that animals can never convert 100% of consumed nutrients into meat, milk, or eggs. In fact, lactating dairy cattle typically convert only about 30-35% of the crude protein they consume into milk protein. In critiques of ruminant agriculture in particular, the relatively low feed efficiency of cattle compared to poultry or fish production is often pointed out as a reason to shy away from ruminant products.

How fair is this? Well, on a crude basis these figures are accurate. However, it is also true that a vegetarian living on a diet of 90% lettuce will have to consume far more food than someone consuming a typical American diet. Does that mean they are less efficient? Well, no – in fact, on a nutrient basis, the vegetarian would likely be forced to be far *more* efficient because their intake of calories and protein would be less than that of a typical person. Assessing inputs on the basis of feed mass is simply illogical, because (for example) the inputs to produce a pound of corn grain are much greater than that required to produce a pound of corn silage. The simple feed conversion assessment tilts the table against any forage-based system. This is especially egregious in diatribes against the beef industry, where the majority of animal days are spent on arid or highly erodible pasture ground where few other options for agricultural productivity exist. One cannot readily take a cow-calf pair off a few acres of rangeland in Nevada and start a vegetable farm.

The critical role of industrial co-products in the U.S. dairy industry

Another flaw in the typical assessments of feed use for cattle is the failure to consider the huge quantities of human-inedible products that we feed. Production of all sorts of goods - from t-shirts to energy drinks to almonds - generates high-fiber co-products that have little to no value to society outside of providing feed for ruminants. In addition to wasting nutrients that can contribute to the food system, if these industries were forced to landfill these products (e.g., cottonseed, corn gluten feed, almond hulls), they would substantially increase the amount of methane generated in landfills.

A national survey of 350 dairy farms estimated that the typical lactating dairy cow consumes a diet containing 19% byproducts on a dry matter basis (Tricarico, 2016). A separate analysis estimated that more than 137 million tons of U.S. byproducts are fed to animals each year (Knapp, 2015). If this amount of material was diverted to landfills, it would double the annual mass of material going to landfills, and because it is all organic matter, this would likely increase landfill methane production by 3-fold or more, negating the reduction of methane production that could be achieved by eliminating ruminant agriculture.

Is it possible to produce a meaningful quantity of milk with zero land use?

While I do not personally agree with all of its tenants, my collaborators and I were interested in engaging with the concept that livestock production should be focused on making use of "ecological leftovers" (Garnett, 2009). This proposal suggests that (1) arable land should primarily be designated to plant-based food for humans, (2) animals should be fed biomass unwanted by or unsuitable for humans, and (3) semi-natural grassland should be used for livestock, if grazing can be justified by reasons other than meat and milk production (e.g., biodiversity or soil conservation).

Furthermore, we wanted to tackle the question of whether dairy cattle can be net contributors of human-edible nutrients under the scenario where arable land (and/or irrigation water) is a primary constraint. Because most acres used for growing dairy forages could grow human food crops, we asked not just whether we could displace feeds that are directly used by both humans and livestock (e.g., corn grain), but also whether we could avoid using forages that displace human-edible crop ground (e.g., corn silage, alfalfa). We sought to face the "livestock are a net loss" argument without setting up a straw man to knock down.

Designing a lactation ration without any products grown specifically for the purpose of feeding animals was a challenge. We utilized byproducts of biofuel (spent algae waste), sweetener (corn gluten feed), and starch (wheat midds and straw) production. The most challenging aspect was to meet energy requirements with straw as the forage source, and also to balance for essential amino acid supply. We evaluated responses to the zero land use diet vs. a conventional Kansas diet in 12 mid-lactation cows.

How did cows respond? The very high-producing multiparous cows on the study dropped from 97 to 84 lb/d of energy-corrected milk yield, but the primiparous cows were able to maintain similar productivity, at 87 vs. 83 lb/d for conventional and zero land use diets (standard error: 4.5 lb/d). The take-away is that a diet like this was adequate to support more than 80 lb/d of milk production, at least over the course of a few weeks.

Figure 1. Description of diets used in experiment to investigate responses to a "zero land use" lactation diet (Takiya et al., 2019). Diet ingredients (e.g., corn grain) or components of the ingredients (e.g., the grain in corn silage) are denoted as human-edible by the arrows.

What is "suitable" for human consumption?

Choice Scenario

% of diet	Control diet	Zero land use diet	% of diet
27	Corn gluten feed	Corn gluten feed	32
22	Alfalfa	Hominy feed	27
21	Corn grain	Wheat straw	21
18	Corn silage	Algae	10
6	Soybean meal	Wheat middlings	4
4	Cottonseed	Molasses	3
2	Limestone	Limestone	2
		Blood meal	1

1.5 kg edible feed protein / day   0.06 kg edible feed protein / day

1.2 kg milk protein / cow / day   1.1 kg milk protein / cow / day

More central to our effort, though, was the exploration of the return to the human food system by cows fed these two diets. By accounting for ingredients – and components of ingredients – that could have been consumed by humans, we were able to determine whether the milk output provided more or less energy and protein than the nutrients invested in the cows.

As shown in Table 2, the conventional diet resulted in slightly less milk energy (82% recovery) and protein (95% recovery) than the human-edible inputs. Does this mean that it is wasteful to use dairy cows to produce milk? No, this is an overly simplistic interpretation. For one thing, the human-edible protein going into these cows almost certainly had a poorer digestibility than milk protein, and beyond that, the essential amino acid profile of milk protein is nearly ideal for human needs (Broderick, 2018). Furthermore, milk is ready for consumption after pasteurization, whereas the feed inputs would nearly always be consumed after further processing, likely with similar losses of energy and protein content.

Although energy and protein are the most critical macronutrient classes needed by people and animals, currently they are not the most limiting nutrients in the global food system (Ritchie et al., 2018). In fact, those nutrients with the narrowest gap between global supplies and human requirements include calcium, folate, and – in the absence of animal-source foods – amino acids like lysine. To dig into just one example of these potentially limiting nutrients, we also determined return on human-edible lysine investment. Unlike with total energy and protein, the conventional diet generated a clearly positive lysine return (155% recovery). This points to one huge advantage of ruminants – the ability to nurture ruminal microbes that can upgrade protein quality by creating essential amino acids from the most basic chemical building blocks (Virtanen, 1966).

Table 2. Return on human-edible nutrient investment in feeding lactating dairy cattle under two dietary scenarios (Takiya et al., 2019).

Input resource	Efficiency of dairy cattle fed a conventional diet	Efficiency of dairy cattle fed a "zero land use" diet
Human-edible protein	0.82 calories / human-edible calorie	7.7 calories / human-edible calorie
Human-edible metabolizable energy	0.95 lb milk protein / lb human-edible protein	20.4 lb milk protein / lb human-edible protein
Human-edible lysine	1.55 g milk lysine / g human-edible lysine	471 g milk lysine / g human-edible lysine

Although these results show that macronutrients can be generated at near breakeven recoveries and at least one micronutrient was a net positive return for the conventional diet, we also investigated the

extent to which these returns could be amplified by purposely designing a zero land use diet. In that treatment, because almost nothing was considered human-edible in the current food system, recoveries of energy, protein, and lysine shot up to 770%, 2,040%, and 47,100% of human-edible inputs. Obviously, in the scenario where society needs ruminants to exist "on the margins", we have the opportunity to produce dairy products with very large net contributions to human nutrient supplies.

What is the practical relevance of these findings? It's certainly not possible for all dairies to feed diets like this – for starters, the supplies of byproducts included in the zero land use diet are inadequate to feed the entire U.S. dairy herd today. Furthermore, this diet would likely fail in some other measures of sustainability, as milk yield dropped and return on other investments (e.g., infrastructure) would be diminished. Nevertheless, this experiment demonstrates the feasibility of a diet entirely built on food system leftovers, at a surprisingly high level of production. Ruminants are incredibly adaptable beasts!

Take-home points and implications for food system debates

One key point highlighted in these efforts is that we do not raise livestock primarily to produce dietary energy. Studies that point to the loss of energy through livestock (Cassidy et al., 2013), though nominally accurate, miss the point entirely. We used animal-source foods for high-quality protein and micronutrients that are hard to acquire from plants. Dismissing animal agriculture as wasteful due to caloric losses is as logical as suggesting that lettuce be abandoned as a crop due to its low human energy supply (just ~3 million Calories / acre vs. ~15 million Calories / acre for corn). We don't raise lettuce for energy, and we don't milk cows primarily for energy. The critical role of micronutrients from dairy products in the American diet has been highlighted recently (Liebe et al., 2020).

Secondly, food systems will adapt to changing scenarios, whether from economic pressures, lack of resource availability, or policies. Ruminants can make key contributions to the food system whether they are fed conventional rations, diets composed largely of leftovers, or primarily grass. Balancing what is best for mankind and the planet is quite tricky, but the solution is not to myopically pursue a single metric of efficiency that ignores the multiple inputs and costs to production of any foodstuff. There truly is no such thing as a free lunch!

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Feeding and managing for high milk components

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The shift to multiple component pricing in much of the U.S. dairy industry about 20 years ago has resulted in greatly increased focus on maximizing yields of milk fat and protein at the farm level. Farms that ship high amounts of components (> 6.5 lbs of fat and protein per cow per day) achieve that through both high milk yield and component percentages at or above breed averages. Figure 1 depicts milk fat and protein yield in 39 farms participating in the PRO-DAIRY Dairy Profit Monitor Program. Our work suggests that yield of milk components accounts for about 50% of the herd-to-herd variation in Income Over Feed Cost, a metric that is tied directly to both cash flow and to overall farm profitability.
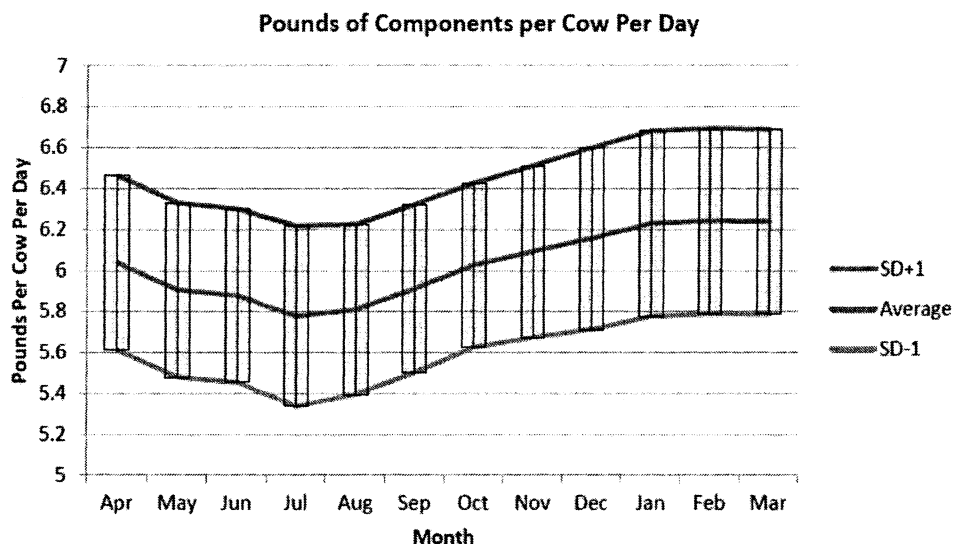

Pounds of Components per Cow Per Day

Figure 1. Pounds of milk fat and true protein shipped per cow per day by 39 farms participating in the PRO-DAIRY Dairy Profit Monitor program from April 2020 to March 2021 (Augello and Karszes). Accessed 7/14/21 at https://cals.cornell.edu/pro-dairy/publications/e-leader

Factors affecting milk fat

Our understanding of the factors affecting milk fat production in the cow has improved greatly over the past 20 years. Milk fat is made up of fatty acids; those from C4 to C14 are synthesized exclusively in the mammary gland from acetate and beta-hydroxybutyrate precursors derived from ruminal fermentation; those C18 or greater are exclusively derived either from dietary absorption of these fatty acids or mobilization from adipose tissue with uptake by the mammary gland; and the C16 fatty acids which come from both sources.

There are a number of non-nutritional factors that affect milk fat (e.g., genetics/breed, days in milk, a strong seasonal pattern (Salfer

et al., 2020), heat stress, and overstocking (Woolpert et al., 2016; 2017), but nutritional management (both ration formulation and also implementation) also has great influence on milk fat synthesis (Dewanckele et al., 2020). Among nutritional factors, ruminal fermentation of carbohydrates and balance between highly fermentable carbohydrates and buffering provided by fiber components of the ration, the amount and source of fatty acids in the diet (Jenkins and Harvatine, 2014), feed anti-quality factors such as mycotoxins and spoilage yeasts (Santos et al., 2014), and particle size of the TMR (McCarthy et al., 2018) can interact to decrease milk fat synthesis. For cows to recover from low milk fat, research suggests that 10 days to 2 weeks following a ration change or change in nutritional management should be sufficient (Rico and Harvatine, 2013). In addition to these factors that may decrease milk fat, research suggests that feed additives such as buffers (Iwaniuk and Erdman, 2015), yeasts and yeast cultures (Poppy et al., 2012), amino acids (Zanton et al., 2014), and added fat sources containing higher levels of palmitic acid may increase milk fat content and yield (Lock et al., 2013). Furthermore, increased acetate production (likely through higher NDF digestibility) may also have positive effects on milk fat percentage and yield (Urrutia and Harvatine, 2017).

Factors affecting milk protein and the efficiency of nitrogen use

Our understanding for the factors affecting the efficiency of protein (nitrogen) use in the cow and ability to improve the conversion of dietary protein to milk protein has also increased over the past 20 years. Improving amino acid supply and balance through the supplementation of rumen-protected sources of methionine (Zanton et al., 2014) and either rumen-protected lysine or high quality rumen-undegradable protein sources that are high in lysine can increase yields of milk and milk protein (Noftsger and St. Pierre, 2003). Dietary energy supply is also an important consideration (Lapierre et al., 2020) and higher energy supply through fermentable carbohydrate likely promotes greater milk protein synthesis. Newer versions of the CNCPS nutrition model predict amino acid requirements of the cow based upon energy supply (LaPierre et al., 2019), which is similar to how amino acid requirements have been expressed for pigs.

Newer analytical technologies are available and being implemented in many regions that are giving dairy producers and their nutritionists enhanced milk composition analysis including milk fatty acid profiles. In particular, de novo fatty acids are correlated strongly with both milk fat and milk true protein percentages and independently of milk yield (Barbano and Melilli, 2016). This information likely provides insight into opportunities to enhance percentages and yields of milk fat and true protein. Further research both at the commercial farm level and in research trials will improve our ability to interpret and make use of this enhanced information.

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MANIPULATING THE DAIRY CALF MICROBIOME TO IMPROVE DOWNSTREAM MILK PRODUCTION EFFICIENCY

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INTRODUCTION

Over the past 60 years, average milk yield for US dairy cattle has increased nearly four-fold, including a 16% increase over the last decade (Blayney 2002). Although it is difficult to directly compare efficiency over long time periods due to changes in feeding and production practices, dairy cows of today, by any measure were more efficient and environmentally benign than the dairy cows of 1944 (Capper et al. 2009). A variety of factors have driven this increase, including improved genetics; thrice-daily milking; the use of bovine somatotropin; photoperiod adjustment; and nutritional advances such as improved feeds and better ration balancing (VandeHaar and St-Pierre 2006, Connor, Hutchison et al. 2012). Continued advances that increase milk production are highly desirable, given that domestic consumption of dairy products is expected to increase over the next decade (Westcott 2010). Moreover, additional improvements in feed efficiency are considered essential to promote economically and environmentally sustainable dairy production in the future (Connor 2015), and there are a variety of routes on how this might be accomplished.

Experiments with groups of animals fed the same diet indicate that there are substantial differences in production efficiency among dairy cows (Arndt et al. 2015; Connor et al. 2012). While these differences are often ascribed to differences in animal genetics (Pryce et al. 2012; VandeHaar et al. 2016), there is accumulating evidence that differences in efficiency are associated with differences in the composition of the ruminal microbial community that is solely responsible for the conversion of feed into nutrients usable by the animal. A properly functioning rumen degrades plant matter and provides, through the action of its resident microbial consortium (Figure 1), a ready supply of volatile fatty acids (VFAs) usable by the host for maintenance, growth and product (viz., milk) formation (Van Soest 1994). The success of an individual host depends upon the ruminal microbiota's ability to ferment feed to VFAs and the host's capacity to utilize these nutrients. Because VFAs are microbially derived products, reduced VFA levels in ruminants have been linked to a reduced or deficient microbial community with poor feed conversion efficiency. For dairy cows, specific levels of certain VFAs are known to have a significant impact on milk fat production. In particular, increases in propionate are known to be negatively correlated with milk fat production (Miettinen and Huhtanen 1996), whereas acetate and butyrate are known to have positive effects (Dijkstra, Boer et al. 1993, Miettinen and Huhtanen 1996, Wang, Li et al. 2012).

A number of approaches to optimize the ruminal microbiome for the purposes of improving production have been proposed, from dietary changes to promote the fermentative abilities of acetate- and butyrate-producing bacteria, to wholesale dosing of these bacteria within the rumen (i.e. a probiotic) (Varel, Yen et al. 1995, Krause, Bunch et al. 2001, Moallem, Lehrer et al. 2009, Thrune, Bach et al. 2009, Saxena, Sehgal et al. 2010, Mohammed, Stevenson et al. 2012). In general, these ruminal manipulation strategies have not been overly successful, likely due in part to our inability to properly characterize the ruminal microbial community or fully understand how it interacts with the host. As a result, it is difficult to adequately determine the impact that approaches such as feed manipulation may have on the overall functioning of the ruminal

microbiota. This challenge is not specific to ruminants but is a difficulty in all areas of host-microbial symbioses. Fortunately, recent advances in next-generation sequencing have permitted the characterization of currently unculturable microbes, resulting in major leaps in our understanding of the impact of microbes on overall host health. Importantly, these approaches are being applied to the ruminal microbial community (Jami and Mizrahi 2012, Li, Wu et al. 2012, Jami, White et al. 2014), and studies that employ metagenomics and 16S rRNA sequencing (Hess, Sczyrba et al. 2011, Jami, Israel et al. 2013) have changed our understanding of the ruminal microbiome, specifically with respect to their functional capabilities. As a result, we now have the opportunity to gain an unprecedented picture of the rumen ecosystem and its interplay with the host, and recent work by our group and others are beginning to provide insights into the dynamics of the ruminal microbiome.

THE RUMINAL MICROBIOME PLAYS A DIRECT ROLE IN MILK PRODUCTION

Recent work from our group has demonstrated differences in the ruminal bacterial communities among 14 cannulated cows within discrete periods of the lactation cycle over two successive lactations, and that some of these differences were associated with differences in milk production efficiency (MPE, defined here as energy corrected milk produced per unit of dietary dry matter
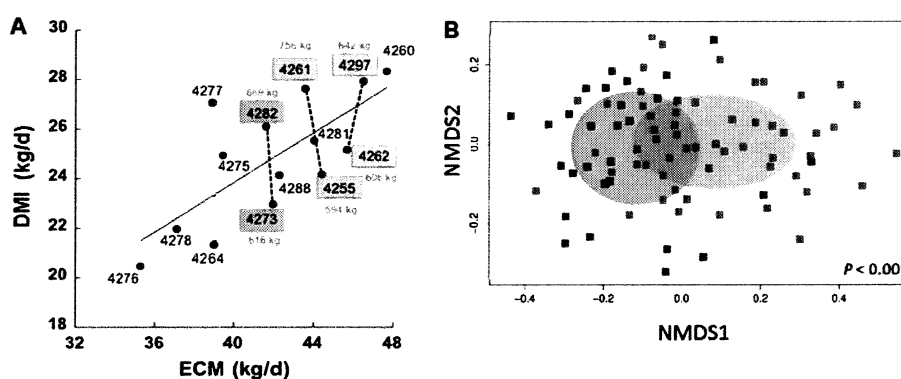

Figure 1. **Milk production efficiency in dairy cows is correlated to rumen microbiota composition.** A total of 14 cannulated cows were assessed as either high or low MPE (ECM/DMI) as shown in (A). Rumen microbiota analysis using Bray-Curtis dissimilarity, visualized as a PCoA, which plots similar microbiota closer to each other, shows with statistical significance a difference between the microbiotas of high MPE cows (green) and low MPE cows (red). Ellipses represent standard error.

intake [ECM/DMI]) (Fig. 1) (Jewell *et al.* 2015). A follow on study (Weimer *et al.* 2019) subjected three pairs of cows from our first study (Fig. 1a) with similar feed intake but different relative feed efficiencies to whole rumen contents swapping. We tracked both milk production efficiency, and rumen microbiota before and after the swap and found that we were able to alter milk production efficiency through wholesale alteration of the rumen microbiota (Fig. 2). Importantly, for all three of the low efficient animals that received rumen contents from the high-efficient pair, we found that they exhibited higher milk production efficiency for a period of one week after the swap, before returning to their lower pre-swap efficiency (Fig. 2a). Similarly, the high-efficient animals also experienced a shift and became low-efficient with the exception on one animal which became even more efficient after the swap. We also saw that the rumen microbial communities shifted across the course of the swap, where for each animal, the rumen microbiota eventually shifted back towards the original microbial community observed prior to the swap (Fig. 2b, c). This shift in both milk production efficiency and rumen microbiota suggests that the rumen microbiota is directly responsible for influencing milk production efficiency in dairy cows, and further indicates

that the host has a tremendous impact on modulating the composition of its rumen microbiota. Taken together, these data demonstrate that *wholesale changes to the rumen microbiota can alter milk production efficiency in dairy cows for a short period of time.*
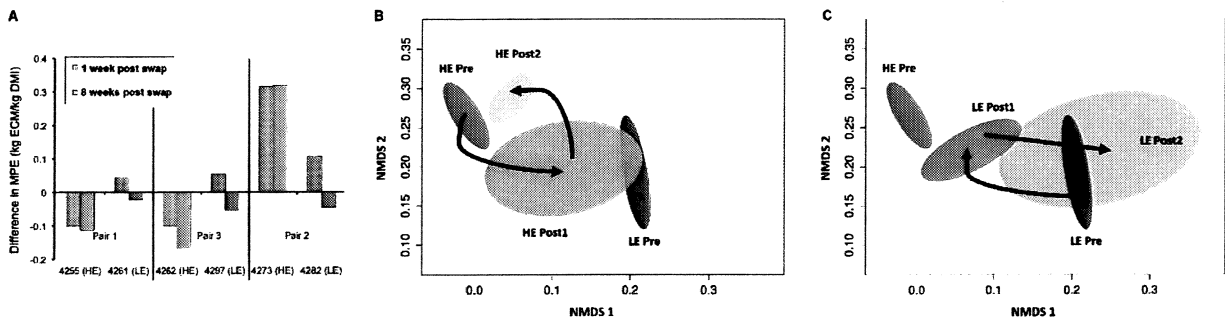

Figure 2. Whole rumen contents exchange between high and low MPE pairs of cows results in a temporary flip in efficiency accompanied by a shift in microbiota that eventually returns to the pre-exchange state. Tracking of MPE 1 week and 8 weeks after the swap revealed a temporal flip in efficiencies for 5 of 6 animals tested (A). Rumen microbiota analysis before and after the swaps show a gradual return to the pre-swap state that coincided with the MPE shift (B and C). Bray-Curtis dissimilarity plots (visualized using standard error ellipses) for Pair 1 shows the shift for both high efficient cow 4255 (B) and low efficient cow 4261 (C).

THE ESTABLISHMENT OF THE RUMINAL MICROBIOTA IN DAIRY CALVES IS HIGHLY VARIABLE

Given the previous results, we reasoned that making changes to the adult ruminal microbiota is challenging at best. Therefore, we sought to determine if influencing the establishment of the ruminal microbiota from birth through adulthood was feasible. To address this, we first tracked the gastrointestinal tract (GIT) microbiota of developing calves from birth through first lactation (Dill-McFarland, Breaker *et al.* 2017). The aims of this study were to determine how calves acquire their ruminal microbiota, and how much variability exists within this community as calves transition through weaning into adulthood. Our study shows that the calf GIT microbiota prior to weaning is highly variable and fluctuates significantly as the animals move into the weaning transition. After weaning, the GIT microbiota again shifts significantly and the proper establishment of the rumen microbiota occurs, likely facilitated by a transition to fibrous feed. Importantly, after the weaning transition, the heifer microbiota becomes adult-like and the adult rumen microbiota is maintained stably over time, as shown by the tight clustering of the rumen microbiotas from all animals in our study in years 1 and 2 (Fig. 3). These data corroborate our previous studies showing that the cow rumen microbiota is highly resistant to change, and that
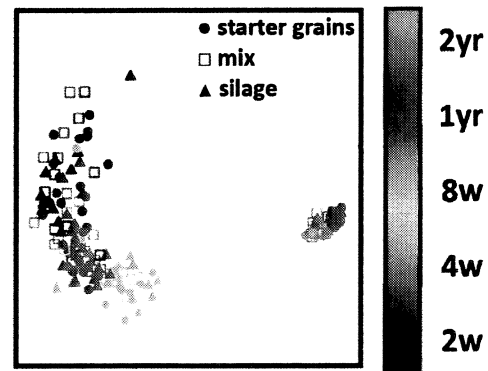
Figure 3. Tracking the GIT microbiota in developing calves from birth to adulthood on different diets. Regardless of diet, there is substantial fluctuation of the GIT microbiota from birth to weaning as shown (left side of the PCoA). Once animals reach adulthood, their microbiota form a tight cluster away from the calf GIT microbiota that persists into 2 years of age and lactation. Dots on the PCoA represent individuals at different times.

the host can modulate its composition back to stability even when significant alterations are made (Fig. 2b, c). However, what our data does suggest is that *there is a window of opportunity during the weaning transition, prior to the establishment of the adult-like rumen microbiota, where alterations could be made that would have a long-lasting effect and directly impact milk production efficiency in the adult cow.*

ALTERING THE ESTABLISHMENT OF THE CALF RUMINAL MICROBIOTA MAY LEAD TO DOWNSTREAM CHANGES IN MILK PRODUCTION EFFICIENCY

To capitalize on this potential window of opportunity during the weaning transition, we have embarked upon a third trial where we are attempting to alter the establishment of the ruminal microbiota in calves. To accomplish this, we dosed cohorts of calves on a weekly basis from birth until weaning with rumen contents obtained from either a high MPE or low MPE cow. Our rationale is that presentation of fully formed ruminal microbiota may overwhelm the stochastic nature of environmentally-acquire microbes, leading to the establishment of that adult microbiome in the rumen of the developing calves. The hypothesis is that the establishment of a ruminal microbiome from the high MPE donor would results in increased downstream MPE in recipient calves, relative to those calves receiving a ruminal microbiome from the low MPE donor. We performed this experiment and tracked the GIT microbiota of our cohorts across development and recently completed sampling of each cohort's first lactation cycle.

SUMMARY

As demand for improved milk production efficiency in dairy cows increases, novel approaches are required to enhance existing methods such as genetic selection and diet alteration. With advances in our ability to characterize entire microbial communities, there is opportunity to manipulate or alter the ruminal microbiome for improved digestion and energy efficiency. We have conducted a number of studies that establish a direct role in milk production efficiency by the ruminal microbiome, and showed that it is fairly challenging to alter the adult rumen microbiome. We then sought to determine if altering the ruminal microbiome during establishment in dairy calves is a feasible alternative. Our studies show that the acquisition and establishment of ruminal microbes in dairy calves is highly stochastic and likely acquired from the general farm environment. Moreover, stark changes in the ruminal microbiome occur during the weaning transition when calves are introduced to greater amounts of fiber. We have sought to capitalize on this window of opportunity by dosing calves with ruminal microbiomes from adult dairy cows in an effort to establish a known community in the rumens of developing calves. In this way, acquisition of an optimized ruminal microbiome may lead to improved downstream milk production efficiency. Taken together, we believe these efforts, coupled with other approaches, may substantially impact our ability to improve milk production efficiency in dairy cows.

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Update on Estimating Energy Supply and Energy Requirements for Dairy Cows

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Estimated energy balance is an essential output of ration formulation/evaluation software, but energy balance is calculated from estimated energy intake and estimated energy requirements, both of which are exceedingly difficult to estimate accurately. However, over the past few decades progress has been made that increase our ability to estimate both energy supply and requirements more accurately. The most common energy system used in the U.S. is the net energy-lactation (NEL) system which will be the system discussed in this paper.

Energy supply

In the NEL system, losses of energy (Figure 1) via feces, urine, methane and diet-induced thermogenesis (i.e., heat increment) are subtracted for dietary gross energy (GE) supply to yield NEL supply. Gross energy of a diet is mostly a function of the concentrations of ash (0 Mcal of energy), CP (average of 5.6 Mcal/kg), fat (9.4 Mcal/kg), carbohydrates (average of 4.2 Mcal/kg) and everything else (averages about 4 Mcal/kg). Fecal energy losses (averages about 33% of gross energy) are extremely variable among diets; heat increment (averages about 20% of gross energy) may also be variable among diets but measurement error can be high so we do not know if the variation among diets is actually caused by diet or measurement error. Indigestibility is the largest single loss and is the most difficult to estimate accurately. With the exception of GE, labs cannot measure the other forms of energy; rather they are estimated from feed and diet components using equations or mathematical models. Some models include various nutrient pools with varying rates of digestion and passage; whereas, other systems use simpler equations that do not implicitly include rates.

This discussion is limited to use of a summative equation that does not include pools or rates. About 35 years ago, we developed an equation to estimate TDN of feeds using commonly measured feed components (Conrad et al., 1984). The equation was substantially modified in 1992 (Weiss, et al., 1992), and in 2001 it was incorporated into the NRC (2001) but was altered to estimate digestible energy (DE) rather than TDN. After years of use, weaknesses have been identified and we modified the equation again in 2018 (Weiss and Tebbe, 2018). The major modifications included replacing nonfiber carbohydrate (NFC) with starch and residual organic matter (ROM) and updating digestibility of fatty acids.
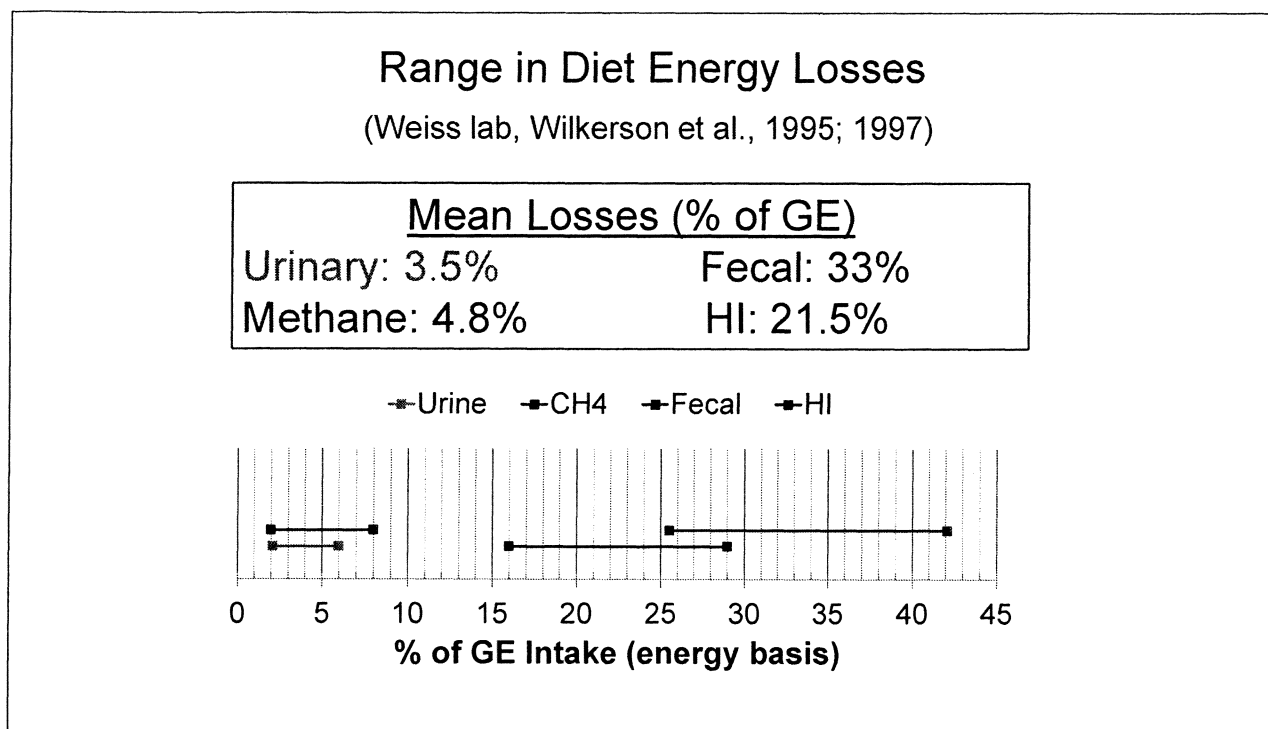
Figure 1. Average and ranges in energy losses by dairy cows. GE = gross energy; HI = heat increment

The current equation (Weiss and Tebbe, 2018) estimates the digestible energy (DE) provided by CP, NDF, fatty acids (FA), starch, and the unaccounted for residual organic matter (ROM). The truly digestible fraction of CP is estimated using acid detergent insoluble protein (ADICP). Alternatively, if you have accurate estimates of the rumen degradable and undegradable protein fractions(RDP and RUP) then digestible CP equals RDP (assumed to be 100% digestible) plus digestible RUP which is calculated using estimated RUP digestibility values (e.g., NRC, 2001) or could be estimated using certain lab assays. Digestibility of NDF is estimated using lignin or 48 h in vitro NDF digestibility (Lopes, et al., 2015). True digestibility of FA from basal ingredients is assumed to be 74%, digestibility of starch is feed specific (Table 1) and the true digestibility of ROM is set at 96% (Tebbe, et al., 2017). Metabolic fecal energy varies based on dietary concentration of ROM and estimated microbial protein but will average about 0.3 Mcal/kg of diet DM. The digestible fractions are then multiplied by their estimated heat of combustion (4.2 Mcal/kg for NDF and starch, 5.6 for CP, 9.4 for FA, 4.0 for ROM and 4.4 for metabolic fecal energy). The calculated DE is for cows consuming about 3.5% of their BW in dry matter (i.e., the average of the database used to generate the digestibility coefficients)

$$
\begin{aligned}
\text{DE (Mcal/kg)} = \ & (CP*e^{-0.012*ADICP} * 0.056) \\
& + (NDF*0.75*(NDF-Lignin)*(1-((Lig/NDF)^{0.667}))*0.042 \\
& + (0.74*FA*0.094) \\
& + (Starch*StDig*0.042) \\
& + (0.96*ROM*0.04) \\
& - 0.3
\end{aligned}
$$

Where nutrients are expressed as percentage of feed or diet DM except ADICP which is expressed as a percent of CP. StDig is a feed constant starch digestibility coefficient (Table 1). The NDF term in the equation can be replaced by NDF*(0.12+0.0061*IVNDFD48)*0.042 where IVNDFD48 is percent of NDF digested in vitro after 48 h. Equation from Lopes et al. (2015).

Table 1. In vivo starch digestibility measured in dairy cows consuming about 3.5% of BW. Sources: (Bal, et al., 1997, Ferraretto, et al., 2013, Firkins, et al., 2001).

Feed	Starch digestibility
Dry ground corn (adjust for particle size)[1]	90
High moisture corn (adjust for particle size)	96
Steam-flaked corn (adjust for flake density)	94
Sorghum, dry ground	83
Corn silage, mature	85
Corn silage, typical	89
Barley, rolled	92
Wheat, rolled	93
Other feeds (inadequate data, overall mean for diets is used)	91

[1] An example of adjusting starch digestibility based on particle size is shown in Figure 2.

The DE values calculated using the above equation is accurate for typical at moderate DM intakes. However, factors not included in the equation can affect digestibility and diet DE concentrations. These factors include:

- Increasing DMI can reduce DM digestibility mostly because of decreased NDF digestibility
- Increasing dietary starch concentration decreases NDF digestibility
- Increasing dietary cation anion difference (DCAD) increases NDF digestibility
- Increasing CP or RDP can increase NDF digestibility

Some of these effects have been quantified and can be added to the equation. For example, increasing dietary starch by 1 percentage unit, decreases NDF digestibility by about 0.5 percentage units (Ferraretto, et al., 2013). The equation above was generated from cows fed diets that averaged about 25% starch so deviation from 25% starch can be factored into the NDF digestibility term. Increasing DMI decreases NDF digestibility by about 1.1 percentage unit for every 1 percentage unit increase in DMI as a percentage of BW (de Souza, et al., 2018). Digestibility of starch may also decrease as DMI increases. The effects of DCAD and CP have not been quantified adequately so those factors cannot be included in the equations yet.
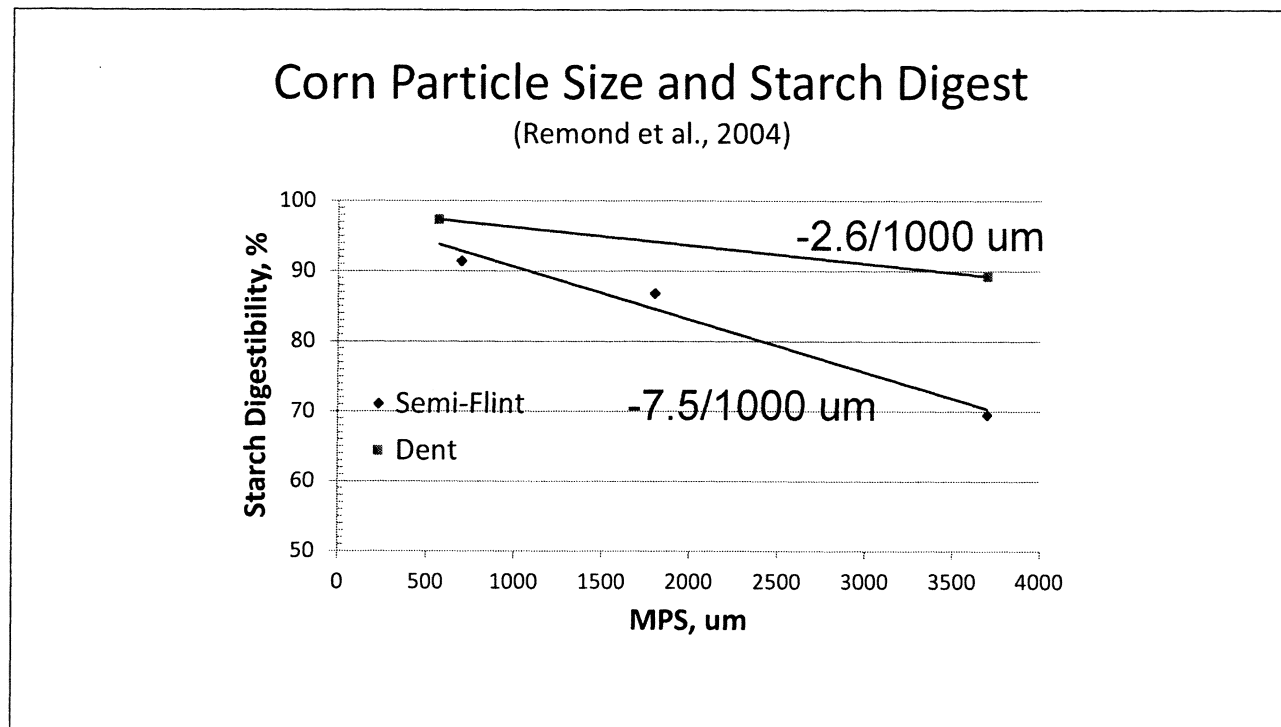
Figure 2. Effect of mean particle size (MPS) of dry corn grain (dent or semi-flint) on in vivo starch digestibility by dairy cows.

Once DE is calculated, methane production can be estimated using various equations such as the one developed by (Nielsen, et al., 2013):

Methane energy (Mcal/kg DMI) = 0.29*DMI - 0.35*FA + 0.041*digestible NDF

Where DMI is kg/d and FA and digestible NDF (as calculated above) are percentages of DM.

Urinary energy is more difficult to estimate accurately but reasonable estimates can be obtained by first estimating urinary nitrogen (UN) output (g/d) and then converting N output into energy output. Urinary N can be estimated using milk urea N (MUN) by equations similar to the one developed by (Kauffman and StPierre, 2001):

UN (g/d) = 0.026*BW * MUN

Where BW is kg and MUN is mg/dL

UN can be converted to Mcal/kg DMI by multiplying UN by 0.0146 and dividing by DMI (Morris, et al., 2021). Methane energy and urinary energy is subtracted from DE to yield metabolizable energy (ME). Calculating estimated methane and urinary energy should improve the accuracy of estimating ME when cows are fed high protein, high fiber, or high fat diets.

Little progress has been made on increasing the accuracy of converting ME to NEL and a constant efficiency still must be used. Using data from the

Beltsville energy lab, Moraes et al. (2015) determined the most accurate overall efficiency is 0.66. Therefore NEL (Mcal/kg) = ME*0.66

Energy Requirements

Current data suggest that the NRC (2001) NEL requirements for lactation and gestation are largely adequate. However, several studies have indicated that the equation for the maintenance requirement in NRC (2001) which has been in use since about 1982 likely underestimates the requirement for today's cows. Averaging across several studies, the current equation may underestimate maintenance requirement by about 25% (Figure 3). For a 650 kg cow, maintenance requirements would increase from about 10.3 Mcal NEL/d using the old system to about 12.9 Mcal of NEL/d using the revised estimate. For a cow producing 25 kg of milk/d this would represent about a 9% increase in total NEL requirements but for a cow producing 55 kg of milk/d it would represent a 6% increase in total NEL requirements. The total NEL requirement for a late gestation, dry cow would increase by about 20%.

Improvements in estimating energy supply and energy requirements will increase the accuracy of estimating energy balance of cows which should result in better diets and allow for better management of body condition.

NEL Maintenance Requirement

Historic: $0.08 \times BW^{0.75}$ (650 kg cow = 10.3 Mcal)

- Underestimates for modern dairy cow
  - Less body fat
  - Greater proportion of body as organs

Maint = $\sim 0.10 \times BW^{0.75}$ (650 kg cow = 12.9 Mcal)

Examples:

Ellis et al., (2006):   **0.085 to 0.095**
Moraes et al., (2015):   **0.088 to 0.124**
Agnew and Yan (2000): **0.118 to 0.160**

Figure 2. Suggested change in NEL requirement for maintenance based on several experiments.

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The Influence of Immune Activation on Transition Cow Health and Performance

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Introduction

Suboptimal milk yield limits the U.S. dairy industry's productive competitiveness, marginalizes efforts to reduce inputs into food production, and increases animal agriculture's carbon footprint. There are a variety of circumstances in a cow's life which activate the immune system and result in hindered productivity (i.e., metritis, mastitis, intestinal dysfunction). Although there are many etiological origins, a commonality among them is increased production of inflammatory biomarkers and markedly altered nutrient partitioning. We suggest periparturient immune activation contributes to hypocalcemia and ketosis in the transition period rather than these metabolites themselves being causative to future problems. We hypothesize that many post-calving undesirable phenotypes (reduced dry matter intake [DMI], hypocalcemia, elevated non-esterified fatty acids [NEFA], hyperketonemia) are a consequence of immune activation and not themselves causative of transition cow maladies. For a more detailed description of the areas covered herein, see our recent review (Horst et al., 2021).

Traditional Dogmas

Long-standing tenets describe a causal role of hypocalcemia, increased NEFA, and hyperketonemia in the incidence of transition diseases and disorders (Figure 1). Hypocalcemia has traditionally been considered a gateway disorder leading to ketosis, mastitis, metritis, displaced abomasum, impaired reproduction, and decreased milk yield (Curtis et al., 1983; Goff, 2008; Martinez et al., 2012; Chapinal et al., 2012; Riberio et al., 2013; Neves et al., 2018a,b). The proposed mechanisms by which hypocalcemia leads to these ailments include impaired skeletal muscle strength and gastrointestinal motility (Goff, 2008; Oetzel, 2013; Miltenburg et al., 2016), decreased insulin secretion (Martinez et al., 2012, 2014), and the development of immunosuppression (Kimura et al., 2006). Like hypocalcemia, increased NEFA and hyperketonemia are presumed causative to illnesses such as DA, retained placenta, metritis, reduced lactation performance, poor reproduction, and an overall increased culling risk (Cameron et al., 1998; LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010; Chapinal et al., 2011; Huzzey et al., 2011). Excessive NEFA mobilization and the affiliated increase in hepatic lipid uptake, triglyceride (TG) storage, and ketone body production has been traditionally believed to be the driving factor leading to ketosis and fatty liver (Grummer, 1993; Drackley, 1999). Additionally, elevated NEFA and ketones are thought to compromise immune function (Lacetera et al., 2004; Hammon et al., 2006; Scalia et al., 2006; Ster et al., 2012) and suppress feed intake (Allen et al., 2009). Thus, the magnitude of changes in NEFA, BHB and Ca have traditionally thought to be predictors of future performance and problems.
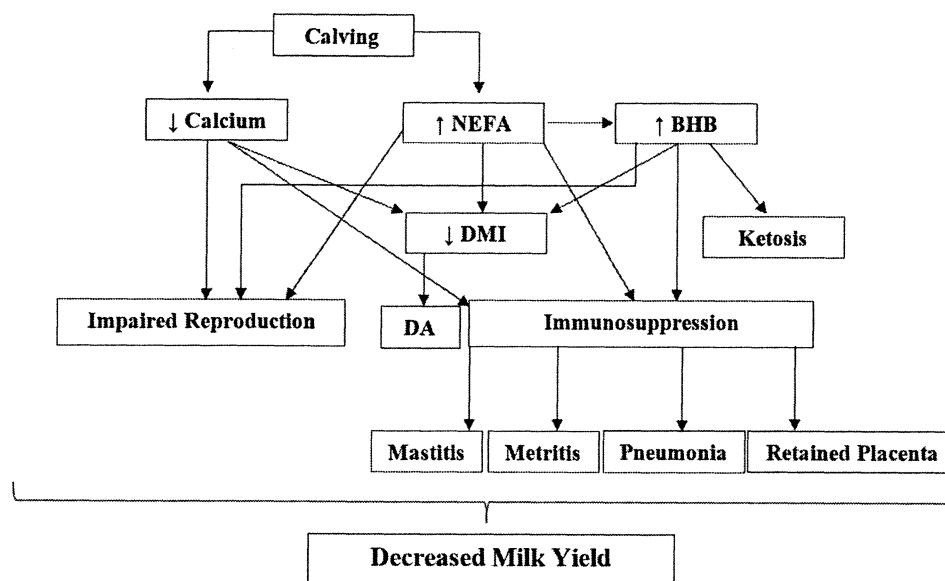
Figure 1. Traditional mechanisms by which hypocalcemia and increased NEFA and ketones are thought to cause poor transition cow health and performance

Inflammation in the Transition Period

Regardless of health status (Humblet et al., 2006), increased inflammatory biomarkers are observed in nearly all cows during the periparturient period (Ametaj et al., 2005; Humblet et al., 2006; Bionaz et al., 2007; Bertoni et al., 2008; Mullins et al., 2012). The magnitude and persistency of the inflammatory response seems to be predictive of transition cow performance (Bertoni et al., 2008; Bradford et al., 2015; Trevisi and Minuti, 2018). During the weeks surrounding calving, cows are exposed to a myriad of stressors which may permit endotoxin entry into systemic circulation and thereby initiate an inflammatory response (Khafipour et al., 2009; Kvidera et al., 2017c; Proudfoot et al., 2018; Barragan et al., 2018; Koch et al., 2019). The frequency and severity of these inflammation-inducing insults presumably determines the level of inflammation that follows (Bertoni et al., 2008; Trevisi and Minuti, 2018). Common origins of endotoxin entry include the uterus (metritis) and mammary gland (mastitis). Additionally, we believe the gastrointestinal tract may contribute as many of the characteristic responses (rumen acidosis, decreased feed intake, and psychological stress) occurring during the transition period can compromise gut barrier function (Horst et al., 2021).

Although an overt inflammatory response is present around calving, numerous reports have described a reduction in immune competence during this time (Kehrli et al., 1989; Goff and Horst, 1997; Lacetera et al., 2005). Traditionally, hypocalcemia and hyperketonemia have been primary factors considered responsible for periparturient immunosuppression (Goff and Horst, 1997; Kimura et al., 2006; LeBlanc, 2020), however, recent evidence suggests this is more complex than originally understood and that the systemic inflammatory milieu may be mediating the immune system to become "altered" and not necessarily "suppressed" around calving (Trevisi and Minuti, 2018; LeBlanc, 2020). Whether or not the "immune incompetence" frequently reported post-calving is

causative to future illnesses or is a consequence of prior immune stimulation needs further attention.

The Importance of Glucose

To adequately recognize the connection between inflammation and transition period success, an appreciation for the importance of glucose is a prerequisite. Glucose is the precursor to lactose, the milk constituent primarily driving milk volume through osmoregulation (Neville, 1990). Approximately 72 g of glucose is required to synthesize 1 kg of milk (Kronfeld, 1982). A variety of metabolic adaptations take place in lactating mammals including increased liver glucose output and peripheral insulin resistance which allows for skeletal muscle to have increased reliance upon lipid-derived fuel (i.e., NEFA and BHBA) to spare glucose for milk synthesis and secretion by the mammary gland (Baumgard et al., 2017). The immune system is also heavily reliant on glucose when activated. The metabolism of inflammation (discussed below) has its own unique metabolic footprint to direct glucose toward the immune system. Consequently, when the onset of inflammation and lactation coincide, glucose becomes an extremely valuable and scarce resource.

Ketogenesis occurs when glucose is in short supply. This can come from a combination of factors including lack of substrate (i.e., reduced feed intake and ruminal fermentation) or high glucose utilization by other tissues (i.e., the immune system or mammary gland). When glucose demand is high, the TCA cycle intermediate oxaloacetate leaves the cycle to supply carbon for gluconeogenesis. Oxaloacetate is also the molecule that combines with acetyl CoA (the end-product of adipose-derived NEFA) to allow the TCA cycle to continue progressing. If the TCA cycle is limited in its progression due to lack of oxaloacetate, acetyl CoA enters into ketogenesis. The link between onset of lactation, immune system activation, and lack of glucose leading to ketogenesis may help to explain the metabolic footprint of a poorly transitioning dairy cow.

Metabolism of Inflammation

Inflammation has an energetic cost which redirects nutrients away from anabolic processes (see review by Johnson, 2012) and thus compromises productivity. Upon activation, most immune cells become obligate glucose utilizers via a metabolic shift from oxidative phosphorylation to aerobic glycolysis (not anaerobic glycolysis typically learned about in biochemistry classes), a process known as the Warburg effect (Figure 2).

This metabolic shift allows for rapid ATP production and synthesis of important intermediates which support proliferation and production of reactive oxygen species (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In an effort to facilitate glucose uptake, immune cells become more insulin sensitive and increase expression of GLUT3 and GLUT4 transporters (Maratou et al., 2007; O'Boyle et al., 2012), whereas peripheral tissues become insulin resistant (Poggi et al., 2007; Liang et al., 2013). Furthermore, metabolic adjustments including hyperglycemia or hypoglycemia (depending upon the stage and severity of infection), increased circulating insulin and glucagon, skeletal muscle catabolism and subsequent nitrogen loss, and hypertriglyceridemia occur (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005). Interestingly, despite hypertriglyceridemia, circulating BHB often decreases following LPS administration (Waldron et al., 2003a,b; Graugnard et al.,

2013; Kvidera et al., 2017a). The mechanism of LPS-induced decreases in BHB has not been fully elucidated but may be explained by increased ketone oxidation by peripheral tissues (Zarrin et al., 2014). Collectively, these metabolic alterations are presumably employed to ensure adequate glucose delivery to activated leukocytes.
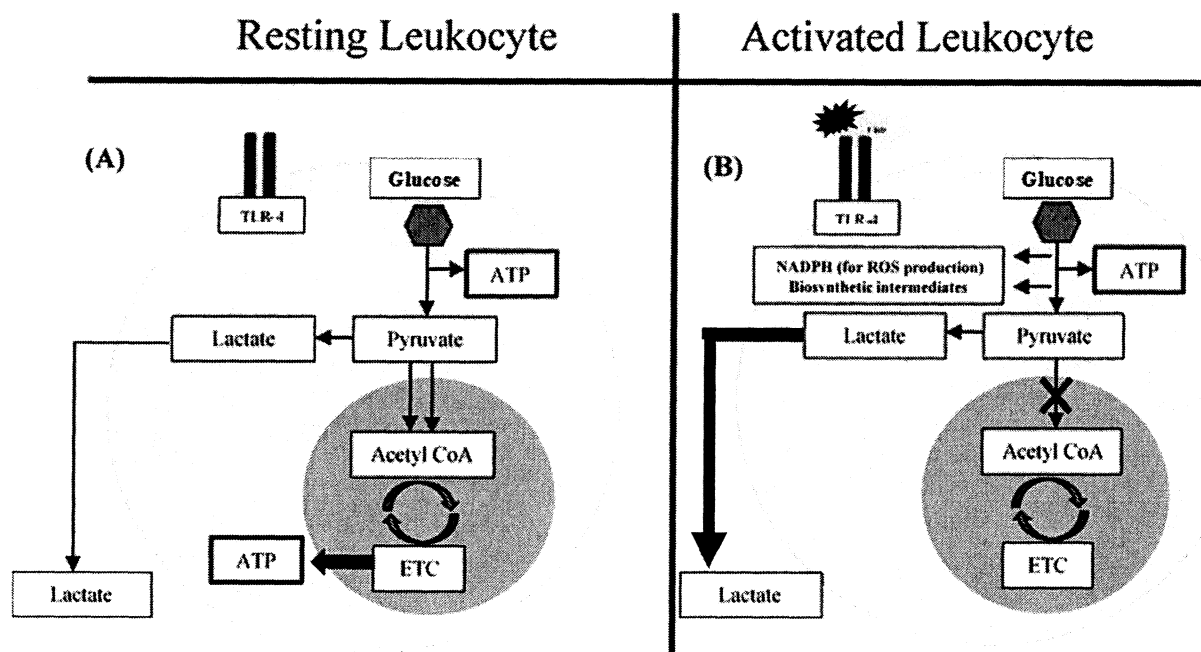

Figure 2. Metabolic pathway of a resting (A) vs. activated (B) leukocyte

Energetic Cost of Immune Activation

The energetic costs of immunoactivation are substantial, but the ubiquitous nature of the immune system makes quantifying the energetic demand difficult. Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by an intensely activated immune system during a 12-hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in mid- and late-lactation cows, growing steers and growing pigs were 0.64, 1.0, 0.94, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively; Kvidera et al., 2016, 2017a,b, Horst et al., 2018, 2019). A limitation to our model is the inability to account for liver's contribution to the circulating glucose pool (i.e., glycogenolysis and gluconeogenesis). However, both glycogenolytic and gluconeogenic rates have been shown to be increased during infection (Waldron et al., 2003b; McGuinness, 2005) and Waldron et al. (2006) demonstrated that ~87 g of glucose appeared in circulation from these processes. Furthermore, we have observed both increased circulating glucagon and cortisol (stimulators of hepatic glucose output) following LPS administration (Horst et al., 2019) suggesting we are underestimating the energetic cost of immunoactivation. The reprioritization of glucose trafficking during

immunoactivation has consequences as both are considerable glucose-demanding processes. Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool, etc.).

Inflammation and Metabolic Disorders

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptions to support glucose sparing for milk production. Early lactation dairy cows enter a normal physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and typically enter negative energy balance (NEB; Drackley, 1999; Baumgard et al., 2017). During NEB, cows mobilize NEFA in order to partition glucose for milk production in a homeorhetic strategy known as the "glucose sparing." However, increasing evidence suggests that chronic inflammation may be an additional energy drain that initiates the sequence of these disorders (Bertoni et al., 2008; Eckel and Ametaj, 2016) and this is supported by human, rodent, and ruminant literature which demonstrate effects of lipopolysaccharide (LPS) and inflammatory mediators on metabolism and hepatic lipid accumulation (Li et al., 2003; Bradford et al., 2009; Ilan et al., 2012; Ceccarelli et al., 2015). We and others have demonstrated that cows which develop ketosis and fatty liver postpartum have a unique inflammatory footprint both pre- and post-partum (Ohtsuka et al., 2001; Ametaj et al., 2005; Abuajamieh et al., 2016; Mezzetti et al., 2019; Figure 3). Because the activated immune system has an enormous appetite for glucose, it can exacerbate a glucose shortage by both increasing leukocyte glucose utilization and reducing gluconeogenic substrates by inhibiting appetite. Reduced DMI is a highly conserved response to immune activation across species (Brown and Bradford, 2021) which can further increase NEFA mobilization and hepatic ketogenesis (Figure 4).
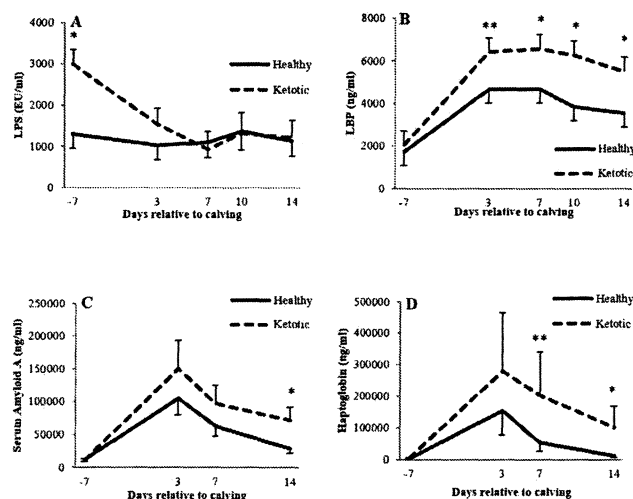

Figure 3. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows

Inflammation and Subclinical Hypocalcemia

Subclinical hypocalcemia remains a prevalent metabolic disorder afflicting ~25% of primiparous and ~50% of multiparous cows in the United States (Reinhardt et al., 2011). Although no overt symptoms accompany SCH, it has been loosely associated with poor gut motility, increased risk of DA, reduced production performance (i.e., milk yield and feed intake), increased susceptibility to infectious disease, impaired reproduction, and an overall higher culling risk (Seifi et al., 2011; Oetzel and Miller, 2012; Caixeta et al., 2017). Recent reports indicate that the severity of negative health outcomes observed in SCH cows appears dependent on the magnitude, persistency, and timing of SCH (Caixeta et al., 2017; McArt and Neves, 2020). For example, Caixeta et al. (2017) classified cases as either SCH or chronic SCH and observed more pronounced impairments on reproductive performance with chronic SCH. Similarly, McArt and Neves (2020) classified cows into 1 or 4 groups based on post-calving Ca concentrations: normocalcemia (>2.15 mmol/L at 1 and 2 DIM), transient SCH ($\leq$ 2.15 mmol/L at 1 DIM), persistent SCH ($\leq$ 2.15 mmol/L at 1 and 2 DIM), or delayed SCH (> 2.15 mmol/L at 1 DIM and $\leq$ 2.15 mmol/L at 2 DIM). Cows experiencing transient SCH produced more milk and were no more likely to experience a negative health event when compared to normocalcemic cows, whereas the opposite (i.e., higher health risk and hindered productivity) was observed in cows experiencing either persistent or delayed SCH. Clearly not all cases of SCH are equivalent; in fact, transient hypocalcemia appears to be correlated with improved "health" and productivity and this may explain why inconsistencies exist in the relationship between SCH and reduced productivity and health (Martinez et al., 2012; Jawor et al., 2012; Gidd et al., 2015). However, it remains unclear why despite successful implementation of mitigation strategies, SCH remains prevalent, why SCH is associated with a myriad of seemingly unrelated disorders, and what underlying factors may be explaining the different "types" of SCH.

Impressively, immune activation was originally hypothesized by early investigators to be involved with milk-fever (Thomas, 1889; Hibbs, 1950), but until recently (Eckel and Ametaj, 2016) it has rarely been considered a contributing factor to hypocalcemia. Independent of the transition period, we and others have repeatedly observed a marked and unexplainable decrease in circulating calcium following LPS administration in lactating cows (Griel et al., 1975; Waldron et al., 2003; Kvidera et al., 2017b; Horst et al., 2018, 2019; Al-Qaisi et al., 2020). Infection-induced hypocalcemia is a species conserved response occurring in humans (Cardenas-Rivero et al., 1989), calves (Tennant et al., 1973; Elsasser et al., 1996;), dogs (Holowaychuk et al., 2012), horses (Toribio et al., 2005), pigs (Carlstedt et al., 2000) and sheep (Naylor and Kronfeld, 1986). Additionally, hypocalcemia occurs in response to ruminal acidosis in dairy cows (Minuti et al., 2014). It is unlikely that cows (even those that are presumably "healthy") complete the transition period without experiencing at least one immune stimulating event and we are likely underestimating its contribution to postpartum hypocalcemia. In summary, it is probable that immune activation is at least partially explaining the incidence of SCH in the postpartum period (Figure 4). It is intriguing to suggest that cases of delayed, persistent, and chronic SCH recently described by Caixeta et al. (2017) and McArt and Neves (2020) may be related to the severity of the periparturient inflammatory response. This hypothesis may explain why these cases of SCH are associated with reduced "health",

as these represent direct consequences of immune activation rather than being related or caused by decreased Ca.

In addition to SCH, there are on-farm milk-fever situations that are biologically difficult to explain. For example, even while strictly adhering to a pre-calving calcium strategy, there remains a small percentage (~<1%) of cows that develop clinical hypocalcemia. Additionally, reasons for why a mid-lactation cow develops milk-fever are not obvious. Further, there appears to be an undecipherable seasonality component to clinical hypocalcemia in the southwest and western USA that coincides with the rainy season. Inarguably, there remain some aspects of Ca homeostasis that continue to evade discovery.

Conclusion

New evidence and thinking around inflammation is challenging the traditional dogmas surrounding hypocalcemia, elevated NEFA, and hyperketonemia as the causative factors in transition cow disease. We suggest, based upon the literature and on our supporting evidence, that activation of the immune system may be the causative role in transition cow failure rather than the metabolites themselves as inflammation markedly alters nutrient partitioning and these metabolites as a means of supporting the immune response (Figure 4). More research is still needed to understand the causes, mechanisms, and consequences of immune activation and how to prevent immune activation or support its efficacy to provide foundational information for developing strategies aimed at maintaining productivity.
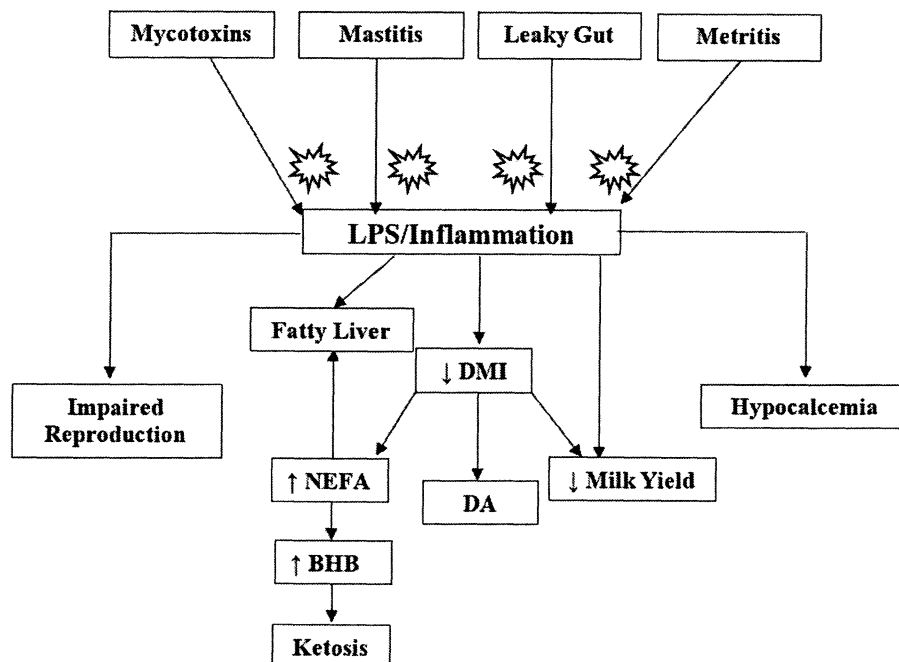

Figure 4. Potential downstream consequences of immune activation. In this model, decreased feed intake, hypocalcemia, excessive NEFA, hyperketonemia and hepatic lipidosis are not causative to poor transition cow performance and health, but rather a reflection of prior immune stimulation.

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Uncovering hidden or under recognized nutrition and economic opportunities on dairy farms

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Over the past 15 years, dairy farms have recognized sizable economic returns and losses. Learning from dairy farm margin summaries and projections reported by Mark Linzmeier, www.marginsmart.com, our industry has recognized dairy margins fluctuating between ($5.00) and $8.00 per hundredweight (cwt). Recently, the estimated net income before tax for an average 1,500 cow dairy in 2018, 2019 and 2020 has been ($1.34), $1.25 and $1.25 per cwt, respectively. In general, our US and California dairies are operating on thin margins at best throughout the past half decade. Average is not acceptable; thus, we are constantly seeking margin and efficiency opportunities. Forward thinking dairies are tracking feed conversion efficiency and other key performance indicators that are directly related to their balance sheet.

In my experience working with leading dairy consultants, allied industry experts, and business minded dairies, we're recognizing $0.25 or greater margin opportunities per cow per day in some unique spots on the farm. In some cases, $0.05 to $0.10 margin opportunities are captured by recognizing moisture or nutrient content differences relative to that expected. The economic opportunities can be attributed to both forages and commodity feeds. These unrecognized opportunities can appear in two different ways: an abrupt change or a subtle trend over time.

In other experiences, margin opportunities have been uncovered through unlocking more energy in each pound of feed consumed. This has been a focal point with many research efforts; however, a simple beginning point is to focus on starch and grain.

The aim with this article will be to review several economic opportunities rooted in unique nutrition trends that may exist for dairy farms within California and across the US. A secondary aim is to discuss and summarize a recent case study considering variation in almond hull rumen digestion.

Corn silage varies more than many recognize

As I've worked with dairies that have dug deeply into their nutrition program to find margin opportunities, the nutrition evaluations have taken numerous forms. It starts with forages. For example, in 2013 one dairy began sampling their forages every 2 weeks to recognize substantial starch content changes within their 13,000+ ton of corn silage piles. I distinctly remember the case, as the nutritionist at the dairy began adjusting the ground corn in the diet to balance against the week to week changes in silage starch content. In this case, corn silage was changing much more than we had previously conceived and I've witnessed this situation play out with other silages and farms.

We tend to think of corn silage as a consistent crop, which is true relative to hay. However, corn silage is a heterogeneous feed consisting of the ear and the stalk. The ear is filled with grain and starch, whereas the stalk and leaves are filled with fiber. Fiber digestibility is extremely important; however, fiber is always less digestible than starch and grain. For example, total tract NDF digestibility (TTNDFD, % of aNDF) averages roughly 42% for CA corn silage whereas total tract starch digestibility (TTSD) will average 92 to 95%. While fiber and starch contain a similar caloric value per pound, dairy cattle are only able to unlock roughly half the calories in fiber relative to starch. Hence, the nutrition value with silage begins with the ear to stover ratio. Environmental and agronomic management practices that improve grain yield will ultimately increase forage quality in general assuming harvest maturity and moisture are appropriate.

With the extreme drought experienced in the West recently, coupled with variable weather patterns year to year, the grain and starch content in silage may deviate substantially between fields relative to that we'd recognized a decade ago. In the past I've hypothesized that a well-made silage pit or pile effectively blends different fields; however, experience is now suggesting otherwise.

To visualize an exemplary starch content change with an aggressively sampling dairy, Figure 1 details the 4-sample average with corn silage starch content for a 7,000-ton pit over the duration of feed out. In this case, the dairy sampled the forage several times per week to characterize the nutrition changes. Based upon this experience and others like this, there

could be under-recognized corn silage starch content opportunities for many dairies throughout the US. Dairy cows may not

Feed libraries are a rough starting point

With grain prices at near historically high levels, feed costs are increasingly scrutinized. Many dairies and consultants are evaluating alternative protein or energy options. Often, we seek to identify the best cost option(s) and drive down feed costs while maintaining performance. Our comparative efforts with diet software or feed evaluation tools rely upon robust and accurate moisture and nutrient content values. Recent experience is suggesting that feed library values are only a rough starting point for nutrition evaluations.

For example, in Figure 2 the crude protein content (% DM) in canola meal is shown for several hundred samples analyzed by Rock River Laboratory, Inc. over the past two calendar years. Canola meal across the US has been averaging roughly 39 to 40 % of dry matter, and this is well above a feed library value at 38.7% of dry matter. In this instance, canola meal may be a value opportunity for the dairy industry, with one unit of crude protein equating to roughly 18 pounds of crude protein value per ton and an economic value of roughly $8 per ton. This value proposition is not limited to canola meal. In conjunction with forward thinking dairies more frequently testing their feeds, soybean meal and other costly commodities have been found to substantially deviate from feed library base values. These differences have often been due to subtle trend changes over time which cows didn't respond to, but the check book may have.

St-Pierre and Weiss (2017) have extensively evaluated dry matter and nutrient variation within and between farms. Their group observed substantial feed variations that should be accounted for over time. The authors recognized that variance can be partitioned between farms and within farms. While the science is sound, measuring and managing these economically impactful variances has proven challenging for dairies and nutritionists. Part of the challenge has been rooted in sampling error and limited replication in sampling. St-Pierre and Weiss (2017) commented that feed library nutrition values may be more realistic than a single sample due to sampling error. This concept is important to grasp. Single samples must be interpreted with caution. There is sampling error associated with

any single sample that complicates interpretation. However, as more samples are tested over time and more data available, we can observe statistically meaningful trends and cut through the sampling variation. With larger database insight and through novel intensive feed sampling programs, many have confirmed that feed library values can be improved upon in appropriately depicting the feed's changing moisture content or nutritive value over time.

Differentiate between abrupt and subtle trends over time

Both commercial experience and research studies with dairy cattle have clearly demonstrated cows respond to abrupt changes in moisture or nutrient content. For example, following a rainfall event the moisture content in exposed feeds increases. If left unadjusted, the cows' diet will become unbalanced and can contribute to digestive upset and performance losses. Alternatively, different hay lots are known to affect dairy performance if substantially different in nutritive value and fiber digestibility. In practice, abrupt changes in nutrient supply often elicit a positive or negative response on farm and then samples are taken, and adjustments are made. However, dairy herd responses to subtle trends and changes in nutrient or moisture content over time are far less studied and understood.

Meaningful feed trends can be buffered by cows

Two studies have documented that dairy cattle appear capable of buffering changes in protein and moisture changes over a short period of time (St-Pierre and Gerstner, 2005; McBeth et al., 2013). In these studies, substantial dietary changes were made to mimic nutrient or moisture changes. However, dairy cow performance did not change significantly, or as would be predicted by dairy nutrition models. In these studies, the dairy cows apparently exhibited some intrinsic ability to buffer protein or energy changes. More recently over the past two years, numerous commercial dairy experiences have been in line with the research observations described above.

Coming back to Figure 1 detailing corn silage starch content during a 9-month feed out period, the 4-sample moving average clearly detailed that starch content in the silage changed substantially on numerous occasions. During one 6-week period, starch content decreased from 37 to around 32% of dry matter, which represented roughly 1.6 pounds of corn grain equivalents for this diet feeding around 20 pounds silage dry matter per cow.

However, this dairy herd did not recall dairy herd performance relative to the measured starch trends. This single observation is intriguing and warrants further evaluation, however if assume the dairy herd was able to buffer this change in some capacity then this observation would be in agreement with those of the researchers described previously. Beyond dairy performance, in this case the change in starch content over time within the corn silage represented roughly $0.20 per cow per day in corn grain equivalents from mid-May to July. Often, we react to changes in performance or components to initiate adjustments. However, based on this field observation and others like this, there may be subtle economic opportunities that dairy cows do not present to us through performance.

Dry matter pounds fed match expectations

In the cases described above, nutrient concentration and supply were affected. One additional and more simple set of cases corresponding to moisture changes should be considered to help uncover hidden feed cost opportunities. Forage and wet feed moistures have been recognized to change from week to week. Most dairies recognize the need to monitor moisture content of their wet feeds several times per week. There are near-infrared reflectance spectroscopy (NIR) instruments that are now available that have greatly improved a farm's ability to check moisture content and make adjustments in a short period of time. However, in my experience very few have checked moisture content with dry and purchased feeds.

Often, feeders or managers use tag guarantees or feed library values within feed management software programs to build dairy recipes. Recent experience has shown there can be substantially less moisture than what tag guarantees list as a limit. In these cases, less moisture equates to more dry matter tons per truckload than what dairies or nutritionists recognize. Consider the following case study visualized in Figure 3, with two different soybean meal sources.

In this case, two different soybean meal sources guaranteed to be equivalent at roughly 47.5% crude protein (as-is) differed substantially in moisture content. Each of the soybean meals met or exceeded tag guarantee requirements, however one of the soybean meal sources was consistently 1.5 to 2.0 units lesser in moisture content. The ramifications of this difference equate to roughly 40 pounds per ton more dry soybean meal and roughly $8

per ton value at $400 per ton price point. Economic minded dairies can capitalize on cases like this and adjust for less moisture content in their purchased feeds.

Ensure purchased grain is being used by cows

Another hidden economic opportunity is nested within corn grain. In the West, we tend to feed less corn grain and starch due to the greater amount of high sugar or digestible fiber coming in with by-product feeds. However, at 18% starch concentration within a 55-pound dry matter intake, starch and corn grain equivalent in the diet represents roughly $1.65 per cow in feed costs at $6 per bushel corn. Hence, the economic ramifications associated with limited starch digestion can be sizable.

Fecal starch content has been clearly linked to total tract starch digestion (TTSD) in dairy cattle (Fredin et al., 2014). Dr. Jim Ferguson and Dr. Mike Hutjens have taught the industry to begin using this tool over a decade ago (Hutjens, personal communication). More recently, we have analyzed commercial fecal starch data and calculated population statistics to set new goals for today's dairy farms. As of the past 5 years, the 15th percentile for starch content in all dairy manure samples is roughly 0.5% of dry matter. This corresponds to 99% total tract starch digestion and is our new goal. In Figure 4, fecal starch results for 2 years' worth of commercial dairy samples from across the United States can be visualized. Recognize that the trendline moves over time, however the average result is nearly always at 2.5% fecal starch or greater. Assuming a 55 pound dry matter intake and 18% dietary starch as described above, 2.5% versus 0.5% fecal starch projects to roughly 0.4 pounds more undigested corn per cow and $15,000 per year economic opportunity for every 1000 cows. Projections such as this with dairy cattle can be determined using an open access web application available from Rock River Laboratory, Inc. The application can be accessed here: https://rockriverlab.shinyapps.io/FecalStarchCalc/

Almond hull sugar content and digestion: a case study

Almond hull nutritional value has been recognized to vary substantially (DePeters et al., 2020) while the inclusion rates within CA dairy diets continue to increase. In a recent collaborative effort with Dr. Mike Socha and Dr. Daryl Kleinschmit (Zinpro Corp., Eden Prairie, MN), we evaluated the sugar content, profile and in situ rumen digestion of 11 commercially sourced almond hull samples. The samples were provided by dairy

nutritionists from the Central Valley in CA in summer of 2020.
The almond hulls were all prime and of pollinator variety and
were cleaned of sticks and trash prior to further processing. The
entire sample was processed without subsampling. The samples were
initially mashed with a mortar and pestle to simulate dairy
cattle mastication and then subjected to macro in situ rumen
digestion for 0, 3, 7, and 16 h in 3 lactating dairy cattle
consuming a high forage diet. A subsample of the mashed sample
was further ground to pass a 1 mm screen for individual sugar
analysis by high performance liquid chromatography for sucrose
(Suc), glucose (Glu) and fructose (Fru). The preliminary results
are presented in population statistics Tables 1 and 2. The
coefficient of variation in sugar content (Suc+Glu+Fru) was
roughly 10% for the sample set. The in situ rumen sugar digestion
appears to be extensive, with a majority of sugar being digested
quickly (Table 2). However, the dry matter digestion was more
variable, with an approximately 10% coefficient of variation
evident at all rumen digestion time points from 3 to 16 hours.

These preliminary results of this sample set should be
interpreted with caution and further analysis is warranted.
However, in alignment with other prior results such as those
presented by DePeters et al (2020), these data suggest
substantial variation in the nutritional and rumen digestion
characteristics with prime almond hulls. While this case study
focused on sugar content and dry matter digestion, fiber content
should also be considered. Fiber can be an indicator of sticks
and trash. In figure 4, fiber content of 322 commercial samples
analyzed by Rock River Laboratory West are summarized for the
past 5 years. Fiber content appears to vary substantially, with
the 15th and 85th percentile database values separating by 20%
units of aNDF or more in recent years. These data may help guide
nutritionists and dairy farmers in decision making, by
highlighting meaningful variation.

Summary

This article leans heavily upon recent experience and case
studies. Admittedly, we have much yet to learn and research.
However, the dairy industry is evolving at a rapid pace due to
the demand for continuous efficiency gains. Whether discussing
emissions, environmental stewardship, feed conversion efficiency,
income over feed costs, or another efficiency metric, recognize
that each of these are highly correlated. Our collaborative goal
is to produce more with less, and improve the profitability of

dairy farms and agribusinesses. Within this article, I've discussed several different areas that may contribute to this collective aim.

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Figure 1: Example corn silage starch content, % DM, for a commercial dairy over a 9 month period.

Moving Averages Plots

Starch

4 Sample Rolling Average

Sample Date
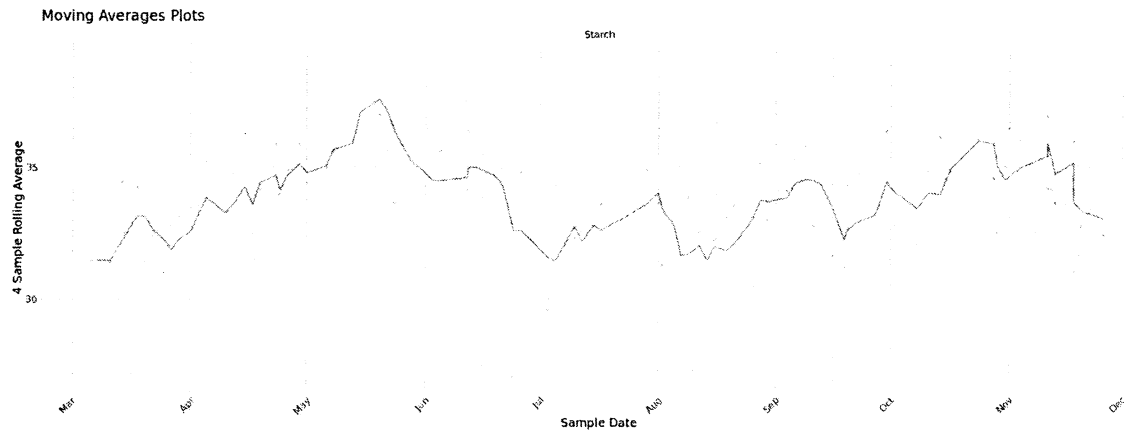
Figure 2: Canola Meal crude protein content, %DM, by calendar year for commercial feed samples analyzed by Rock River Laboratory, Inc. in 2020 and 2021. The vertical black lines within each distribution plot represent the 15th, 50th and 85th percentiles, respectively.

Year to Date Plot

CropYear

2021

2020

36   38   40   42   44

CrudeProtein
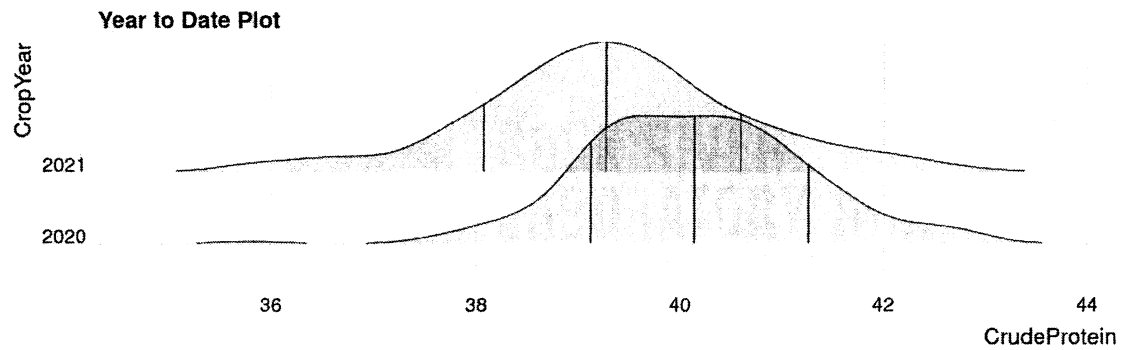
Figure 3: Total moisture content, %, for two different soybean meal sources over a 90 day period.

Moving Averages Plots

Moisture

Figure 4: Dairy cattle fecal starch content, %DM, for samples submitted to Rock River Laboratory, Inc. from 2019 through 2021. The flat red line equates to the 15th percentile and goal of 0.5% dry matter fecal starch content. The trendline represents the moving average over a 2-year time period.

DAIRY FECAL STARCH RESULTS

Figure 5: Almond hull fiber content, %DM, for 322 almond hull samples analyzed by Rock River Laboratory West from 2017 through 2021. The vertical black lines within each distribution plot represent the 15th, 50th and 85th percentiles, respectively.

Year to Date Plot

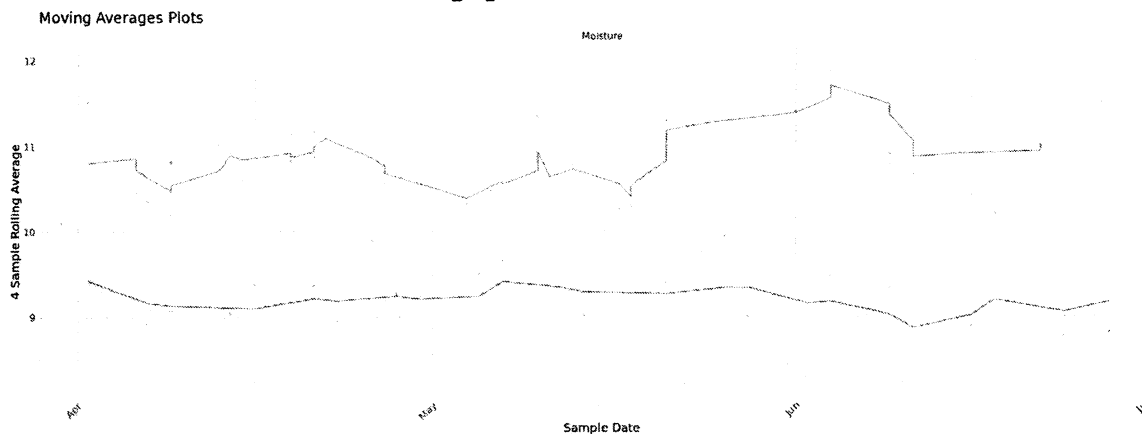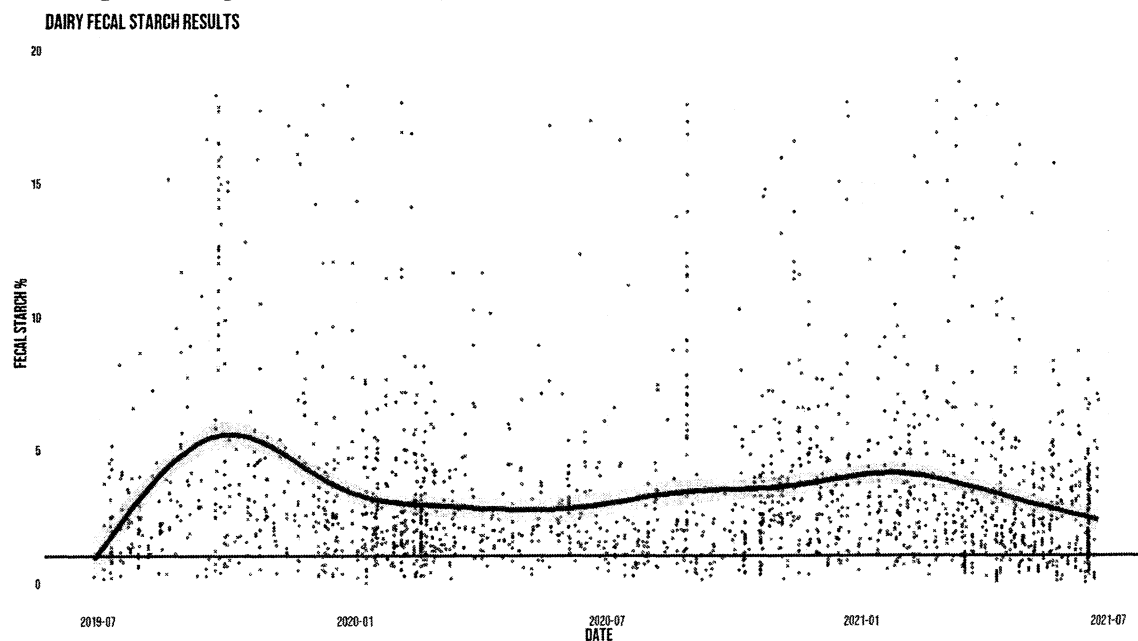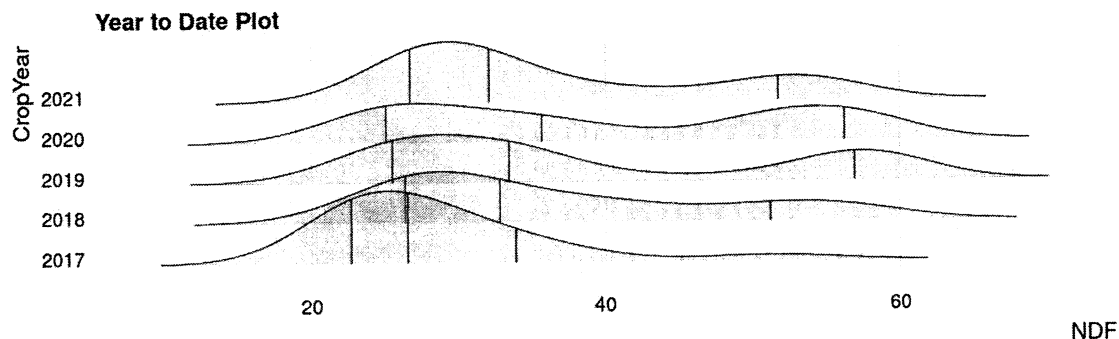Table 1: Almond hull sugar profile and content, %DM, for Prime Pollinator variety samples collected from CA in 2020.

Nutrient	Total N	Mean	Standard Deviation	15th Percentile	85th Percentile
Glucose, % DM	11	7.9	0.9	6.7	9.0
Fructose, % DM	11	11.9	1.1	10.0	13.0
Sucrose, % DM	11	4.4	1.3	3.1	6.4
Total sugar (Glu+Fru+Sucr)	11	24.2	2.7	20.6	27.4

Table 2: Almond hull in situ rumen dry matter and total sugar digestion, % of nutrient, for Prime Pollinator variety samples collected from CA in 2020. Total sugar includes Glucose, Fructose and Sucrose.

Rumen incubation period, h	In situ rumen digestion, % of nutrient	Total N	Mean	St. Dev	15th Perc.	85th Perc.
0	Dry matter digestion	11	23.2	3.2	18.5	26.7
0	Total sugar digestion	11	69.1	7.1	62.7	78.8
3	Dry matter digestion	11	45.3	4.5	39.6	50.4
3	Total sugar digestion	11	96.8	3.0	94.4	98.9
7	Dry matter digestion	11	49.0	4.1	44.3	54.2
7	Total sugar digestion	11	99.4	0.9	98.2	100.0
16	Dry matter digestion	11	58.2	6.0	53.8	67.6

| 16 | Total sugar digestion | 11 | 100.0 | 0.0 | 100.0 | 100.0 |

Fiber and starch digestibility in corn silage

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Introduction

Whole-plant corn silage (**WPCS**) is the predominant forage used in dairy cattle diets worldwide. On average, approximately 138 million tons of fresh corn forage was harvested in the United States in 2020. Besides providing energy for maintenance and lactation, coarser WPCS particles stimulate chewing and salivation, rumination, gut motility and health, regulate feed consumption, modulate feeding patterns and are the structural basis of the ruminal mat, which is crucial for ruminal digestion. Starch and fiber are the main sources of energy for dairy cows fed corn silage-based diets and therefore improvements in digestibility of these nutrients may increase milk production or reduce feed costs through enhanced feed efficiency. Greater digestibility of WPCS fiber is desired for productivity, profitability, and environmental reasons. The purpose of this paper is to review selected recent developments and strategies that may influence the nutritive value of WPCS with focus on digestibility of fiber and starch.

Stover fraction – fiber digestibility

Incomplete fiber digestion reduces the profitability of dairy production by limiting intake and hence, animal productivity. A 1%-unit increase in in vitro or in situ forage NDF digestibility (**NDFD**) is associated with 0.40 and 0.55 lb/d increases in DMI and 4% fat-corrected milk production, respectively (Oba and Allen, 1999). Lignin is the key obstacle to fiber digestion as it obstructs the enzyme access to the digestible fiber fractions, cellulose, and hemicellulose. In addition, rumen microorganisms cannot breakdown lignin. Due to its importance to animal performance, this association between lignin and other fibrous fractions (i.e. cellulose and hemicellulose) is considered in many diet formulation models. This undigested or indigestible NDF fraction is estimated using either lignin or quantified as the proportion of NDF remaining after in vitro or in situ ruminal incubations (i.e. 240 h uNDF). Thus, the reduction of lignin or undigested NDF fractions in forages improves fiber digestibility.

Genetic improvement

Improvements to fiber digestibility of forages are often accomplished by reducing lignin or undigested NDF concentrations (Grant and Ferraretto, 2018). Brown midrib mutant forages (e.g., corn and sorghum) consistently have lower lignin concentrations compared to conventional forages (Sattler et al., 2010) resulting in greater milk production when the BMR forages are fed. In this context, several studies have

reported greater DMI, passage rate, and rate of NDF digestion in cows fed BMR compared to conventional corn silage. In a meta-analysis of published studies, Ferraretto and Shaver (2015) reported increases in total tract NDFD (44.8 vs. 42.3% of intake), DMI (55.0 vs. 53.0 lb/d), yields of milk (85.5 vs. 82.2 lb/d) and protein (2.60 vs. 2.49 lb/d) for cows fed BMR diets instead of conventional corn silage diets. These benefits are associated with lower rumen gut fill as conventional forage-based diets may have lower rates of passage and digestion, causing physical constraints in the rumen that limit intake.

Genetic improvement resulted in BMR hybrids that are higher yielding than earlier hybrids. Nevertheless, it is important to account for lower yields of certain BMR hybrids than conventional hybrids when deciding on which hybrid to grow (Adesogan et al., 2019). Such lower yields may be outweighed by the improved animal performance from BMR hybrids, but the magnitude of the improvements may vary from farm to farm based on the prevailing conditions. Producers should consider establishing guidelines for using BMR hybrids such as feeding them to high-producing cows in early lactation while feeding less digestible conventional hybrids to cows in mid-to-late lactation. Such guidelines should be based on discussions with dairy nutritionists and crop consultants who are acquainted with the specific conditions of the dairy farm.

Chop height

A harvesting management option to reduce lignin concentration is chop height. With enhanced chop height more lignin is left with the portion that remains in the field, and thus, digestibility of the harvested material is greater (Ferraretto et al., 2018). However, DM yield is reduced as the row-crop head is raised. This is consistent among several studies conducted across the United States. However, decreased DM yields are offset by an increase in the milk per ton estimates at the higher chop height. Greater milk estimate is a response to the greater fiber digestibility and starch concentration of the harvested material. Also, increased quantities of high-chop silage could be included in the diet, rather than corn grain being added to the diet, providing an economic benefit to implementing increased chop heights. Recently, Paula et.al (2019) conducted a meta-analysis to evaluate the effects of chop height on nutrient composition and yield of WPCS. Yield of DM was reduced by 0.52 ton/acre for each 10-inches of increased chop height. However, for each 10-inches of increase in chop height there was an increase of 2.18, 2.08, and 2.02%-units in DM, starch, and in vitro NDF digestibility, respectively. A negative linear effect was observed for NDF, with a 2.48%-unit decrease per 10-inches of increase in chop height. Using these responses, we calculated the expected effect of increasing chop height from 6 to 26 inches, these results are reported in Table 1. Briefly, we used a baseline corn silage nutrient composition harvested at 6 inches and simulated what the response would be for 16 and 26 inches. Perhaps in the future these simulations could be used in team discussions among farmers, nutritionists, and crop

consultants to determine individual farm priorities for maximum yield versus higher quality prior to the establishment of new chop height guidelines.

Table 1. Predicted effects of chop height on whole-plant corn silage nutrient composition, digestibility, and yield.

Item	Normal chop height[1]	Simulation[2]	Simulation[2]
Cutting height, inches	6	16	26
NDF, % of DM	37.7	35.2	32.7
Starch, % of DM	37.5	39.6	41.6
ivNDFD[3], % of NDF	49.6	51.6	53.6
Yield, ton/acre	8.9	8.4	7.9

[1]Data from Ferraretto et al. (2017).
[2]Predicted using equations from Paula et al. (2019).
[3]Ruminal in vitro NDF digestibility at 30 h.

Plant population

Plant population could be used to increase yield per area without compromising nutritive value of WPCS. Thus, the combination of greater plant population with increased chop height could be of interest to maintain yield while increasing silage of WPCS. In a previous study (Ferraretto et al., 2017), we compared 2 chop heights (6 vs. 24 inches) and 4 plant populations (26,000, 32,000, 38,000 and 44,000 plants per acre). This experiment was conducted in Wisconsin. As aforementioned, increased chop height improved the nutritive value of whole-plant corn forage at the expense of yield. In contrast, plant population affected yield but not quality of whole-plant corn forage. No interactions were observed. However, effects of plant population may be affected by location and season. Diepersloot et al. (2021) aimed to evaluate the effect of plant population on yield, nutrient composition and ruminal in vitro NDF digestibility at 30 of whole-plant corn forage grown in Florida during the summer of 2016 and spring of 2017. Plant population effects on nutrient composition were inconsistent across seasons. In the spring, increasing plant population from 26,000 to 30,000 plants per hectare increase DM yield. However, DM yield of the summer crop was not affected by the same change in plant population.

Kernel fraction - starch digestibility

The energy value of WPCS contributed by starch is approximately 50%. An increase in starch digestion may lead to better nutrient utilization and decreased feed costs. However, starch digestibility of WPCS may be affected by several factors.

Kernel processing

It is well established that the use of kernel processors with 1 to 3 mm (0.04 to 0.12 inches) roll gap settings enhances kernel breakdown at harvest. Degree of kernel processing in WPCS, however, is impacted by other factors. Delayed WPCS harvest, for example, influence the breakdown of kernels. Kernel hardness (as evidence by the amount of vitreous endosperm proportion) increases with increased DM content of WPCS (Ferraretto et al., 2018) which in turn causes kernels in very dry WPCS to be less susceptible to breakage during kernel processing at harvest. Other factors, such as proper processor maintenance from wear, frequent quality-control monitoring of kernel breakage during harvest, and adequate TLOC and roll-gap settings for the chopper and processor used are also crucial for obtaining optimal kernel processing. It is always important to remember that optimal kernel processing requires constant monitoring of silage physical characteristics throughout harvesting.

Storage length

Keeping silage longer in the silo improves starch availability (Kung et al., 2018) and prolonged storage became an important practice for herds feeding WPCS, high-moisture corn and earlage. Table 2 exemplifies this effect and has a prediction of the potential implication to energy availability of WPCS.

Table 2. Time in the silo effect on starch digestibility of corn silage

Storage length, days	0	30-45	90-150	240-270
ivSD[2], % of starch	60.3	67.4	70.2	77.7
TDN, % of DM	68.0	70.5	71.5	74.1
NEL[3], Mcal/lb	0.78	0.81	0.82	0.86

[1]Corn silage – 35% DM, 7.1% CP, 36.3% NDF, 2% EE, 35.0% starch, 1.5% ash, and 52% NFC.
[2]Starch digestibility values based on the review article by Kung et al. (2018).
[3]Calculated according to NRC (2001) but separating starch and non-starch NFC digestibility with modified equations used by the Feed Grain Evaluation System (Hoffman et al., 2012).

Briefly, starch digestion in the rumen requires that starch granules be accessible for microbial degradation. Starch accessibility is increased when the intricate starch-protein matrices (i.e. zein proteins) surrounding starch granules are broken down. Hoffman et al. (2011)

observed a decrease in zein protein concentrations, as well as an increase in concentrations of soluble CP and ammonia-N, when high-moisture corn was ensiled for 240 d. These data suggested that proteases in the silo were responsible for degrading the zein protein matrix surrounding starch granules in corn kernels. This effect is caused primarily by bacterial proteases. Besides, the continuous decrease in pH and accumulation of acids as fermentation progresses favors the activity of kernel proteases. A spike in starch digestibility occurs during the initial fermentation. This initial spike is followed by a gradual but less pronounced increase over time.

Another benefit of ensiling and prolonged storage is the disruption of the starch-protein matrix during fermentation which may dissociate starch granules and thereby reduce kernel mean particle size. Table 3 summarizes the findings of fours studies evaluating the effect of ensiling and (or) prolonged storage on corn silage processing score (**CSPS**; % of starch passing through the 4.75 mm sieve).

Table 3. Storage length effect on corn silage processing score[1]

Storage length, days	0	30	120	240	*P* <
Ferraretto et al., 2015 trial 1	50.2	61.1	–	–	0.01
Ferraretto et al., 2015 trial 2	60.3	63.6	67.2	68.4	0.08
Agarussi et al., 2020	28.8	–	28.8	–	0.97
Saylor et al., 2020	62.4	59.7	64.8	67.7	0.01

[1]Corn silage processing score – % of starch passing through the 4.75 mm sieve.

Findings from the initial two studies highlighted that the known benefits of extended fermentation on starch digestibility are a combination of the breakdown of zein proteins with a reduction in kernel particle size. A study by Agarussi et al. (2020) evaluated the effect of ensiling on CSPS of poorly processed silage. Samples from eleven WPCS hybrids were arbitrarily processed to achieve CSPS of 30% and ensiled for 0 or 120 d. As expected, no effects of ensiling on CSPS were observed. Furthermore, a recent study from Cornell University with four hybrids grown at two locations for two consecutive years did not present a consistent effect on CSPS. These studies highlighted that ensiling and prolonged storage length may improve CSPS but will not replace adequate processing at harvest.

Moreover, Saylor et al. (2021) harvested whole corn plants, separated the ears from the forage portion and shelled kernels. Corn kernels were either left intact or broken manually using a hammer. Intact and broken

kernels were each reconstituted with the chopped forage portion of the corn plant simulating "whole-plant" corn forage and were ensiled for 0 or 30 d. Ensiling increased in vitro starch digestibility only when kernels were broken. Harvesting corn silage with at least 70-75% CSPS prior to ensiling is advised.

Implications

Using brown midrib hybrids and increasing chop height have been among the most consistent and adopted strategies to increase WPCS fiber digestion and milk production by dairy cows. However, these benefits come at the expense of reduced forage mass harvested per area. Furthermore, research supports the use of inventory planning so a newly harvested crop would be fed only after three to four months in storage. Although prolonged storage of WPCS would be a valid management practice, several factors should be taken into consideration when implementing this practice. Prolonged storage requires proper silo management during filling, packing, and covering to ensure beneficial fermentation patterns.

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TECHNICAL SYMPOSIUM SPEAKERS

Virginia Brandao, Ph.D. is a Dairy Technical Manager at Micronutrients. She received her Ph.D. degree from the University of Florida, and her research was focused on using the dual-flow continuous culture system to estimate nutrient flow, ruminal fermentation, and model fermentation end-products responses to dietary manipulations. Currently she provides technical services for the western geography and oversees research projects in collaboration with external research facilities.

Bill Weiss, Ph.D., Professor emeritus was a Professor and Extension Specialist of dairy cattle nutrition at The Ohio State University but after more than 33 years on faculty, he retired in early 2021. His main research areas were factors affecting digestibility by dairy cows, relationships between minerals and vitamins and health of dairy cows, and developing methods to incorporate cow and diet variability into ration formulations. Dr. Weiss has published more than 140 journal articles and 450 proceedings and extension articles. He has won several ADSA awards and was named a Fellow of the American Dairy Science Association in 2015. He is also a member of ARPAS and a Diplomat of the American College of Animal Nutrition. He was a member of the 2001 NRC Dairy Committee and is serving as co-chair on the 2020 NRC Dairy Committee.

Joseph Kerns, President of Partners for Production Agriculture, has thirty years' experience working with producers, suppliers, and mills to support agricultural operations. He leads a team of professionals with the express purpose of understanding the financial parameters around agricultural markets so that those that he works with can thrive and prosper.

Frank Mitloehner, Ph.D., is a Professor and Air Quality Specialist in Cooperative Extension in the Department of Animal Science at the University of California, Davis. He received his MS degree in Animal Science and Agricultural Engineering from the University of Leipzig, Germany, and his PhD degree in Animal Science from Texas Technical University. Dr. Mitloehner is an expert for agricultural air quality, livestock housing and husbandry. Overall, he conducts research that is directly relevant to understanding and mitigating of air emissions from livestock operations, as well as the implications of these emissions for the health and safety of farm workers and neighboring communities. Dr. Mitloehner has served as chairman of a global United Nations Food and Agriculture Organization (FAO) partnership project to benchmark the environmental footprint of livestock production. He served as workgroup member on the President's Council of Advisors on Science and Technology (PCAST) and as member on the National Academies of Science Institute of Medicine (IOM) committee on "A Framework for Assessing the Health, Environmental, and Social Effects of the Food System".

CANC SPEAKERS

Steven Ford – Keynote Speaker - Inspirational speaker Steven Ford, son of President Gerald R. Ford and Betty Ford, offers highly personal keynote presentations that focus on ethics, character, integrity and family.

Barry Bradford, Ph.D., grew up on a seedstock beef cattle operation in Iowa. He completed dual BS degrees at Iowa State University and a doctorate in animal nutrition at Michigan State University. Bradford then served on the faculty at Kansas State University from 2006 to 2019, and in 2020 he returned to Michigan State as the Clint Meadows Chair in Dairy Management. Contributions by Bradford's group have largely focused on dietary utilization of byproducts in lactation diets, the impacts of post-calving inflammation, and the roles of nutrients as signals. In his current role, Bradford works directly with dairy producers to utilize both research and herd monitoring data to improve decision-making on dairy farms.

Thomas R. Overton, Ph.D., is Professor of Dairy Management and Chair of the Department of Animal Science at Cornell University. Tom is recognized widely for his research and extension efforts relating to nutritional physiology of the transition dairy cow. He serves as Director of the statewide PRO-DAIRY extension program at Cornell. He teaches the dairy cattle nutrition course for undergraduates and co-teaches a similar course for veterinary students. He served as Associate Director, Agriculture and Food Systems, for Cornell Cooperative Extension from 2014 to 2019. In this college-level position, he worked to build additional regional agriculture specialist extension teams and strengthened several college-level extension programs through his leadership. Tom assumed the role of interim chair of the Department of Animal Science in July 2019 and was appointed chair in November 2020. Tom has a B.S. degree from Cornell University and M.S. and Ph.D. degrees from the University of Illinois. He has authored or co-authored more than 90 peer-reviewed scientific publications and numerous conference proceedings, extension publications, and popular press articles. He was awarded the Cargill Animal Nutrition Young Scientist Award by the American Dairy Science Association in 2006 and the ADSA Foundation Scholar Award in 2007. In 2013, he was named a Faculty Fellow of the David R. Atkinson Center for a Sustainable Future at Cornell University.

Garret Suen, Ph.D., is an Associate Professor of Bacteriology at the University of Wisconsin-Madison. He received a B.Sc. in Biological Sciences and both a B.Sc. and M.Sc. in Computer Sciences from the University of Calgary. He then went on to complete a Ph.D. in Biology at Syracuse University before conducting post-doctoral work in the Department of Bacteriology at the University of Wisconsin- Madison. He has been active in the field of rumen microbiology and his lab is focused on altering and manipulating the rumen microbial community in an effort to improve milk production efficiency in dairy cows. Dr. Suen works at the interface between microbiology and animal sciences and he has leveraged his expertise in microbial ecology to better understand how the rumen microbial ecosystem interfaces with the host to support important phenotypes such as milk production. More recently, Dr. Suen's interest has included other aspects of dairy microbiology, including antimicrobial resistance and persistence of pathogens.

Bill Weiss, Ph.D., Professor emeritus was a Professor and Extension Specialist of dairy cattle nutrition at The Ohio State University but after more than 33 years on faculty, he retired in early 2021. His main research areas were factors affecting digestibility by dairy cows, relationships between minerals and vitamins and health of dairy cows, and developing methods to incorporate cow and diet variability into ration formulations. Dr. Weiss has published more than 140 journal articles and 450 proceedings and extension articles. He has won several ADSA awards and was named a Fellow of the American Dairy Science Association in 2015. He is also a member of ARPAS and a Diplomat of the American College of Animal Nutrition. He was a member of the 2001 NRC Dairy Committee and is serving as co-chair on the 2020 NRC Dairy Committee.

Tyler Bramble began his career as a research associate at Texas Tech University focusing on feed and nutritional management of livestock. He has held various commercial roles where he has had the opportunity to focus on nutrition and management of dairy cattle throughout the US and various countries. He also is a coauthor on two patents, systems and methods for estimating feed efficiency and carbon footprint for milk and meat producing animals. Tyler joined Cainthus as Portfolio Growth Lead in July 2018.

Sara Kvidera, Ph.D., grew up on a small row crop and beef cow calf farm in Traer, Iowa. She attended Kansas State for her B.S. in Animal Science where she also ran track and worked in a research lab. She completed a Ph.D. in Nutritional Science studying under Lance Baumgard at Iowa State in 2017. Sara has studied and published in areas related to dairy cow metabolism, heat stress inflammation, and gut health. She has worked as a technical consultant in the industry since 2017 and joined Elanco Animal Health in April of 2021. Sara lives in Granger, Iowa.

John Goeser, MS, Ph.D., grew up with the dairy industry, following in his father's footsteps as a dairy nutritionist. Goeser holds several degrees from the University of Wisconsin – Madison, including: B.S. degrees in Animal & Dairy Science and Agronomy; M.S. degrees in Plant Breeding & Genetics and Animal & Dairy Science and Ph.D. in Animal & Dairy Science. He is Animal Nutrition Director at Rock River Laboratory, Adjunct Assistant Professor at University of Wisconsin – Madison, Animal & Dairy Science Department and Agribusiness Consultant: Cows Agree Consulting, LLC. Goeser has offered agribusiness & dairy nutrition and management expertise for over a decade. He has been overseeing animal nutrition, technical support and research with Rock River Laboratory since 2012. In 2014, Goeser joined the UW-Madison Animal & Dairy Science Department as an adjunct professor, and also began privately consulting for agricultural businesses. Goeser's focus is improving our understanding of ruminant nutrition seed genetics and forage management, and feed hygiene in relation to feed conversion efficiency, sustainability, and agribusiness profitability.

Luiz F. Ferraretto, Ph.D., is originally from Brazil where he earned his B.S. in Animal Science from São Paulo State University in 2008. Immediately after the completion of his B.S. Degree, Luiz joined University of Wisconsin-Madison for an internship (2009) followed by a M.S. (2011) and Ph.D. (2015) in dairy science with focus on applied dairy nutrition and forage quality. After the completion of his Ph.D., Luiz joined The William H. Miner Agricultural Research Institute as a Post-doctoral Research Associate. From 2016 to 2020, he worked as Assistant Professor of Livestock Nutrition at University of Florida. Currently, Luiz is an Assistant Professor and Ruminant Nutrition Extension Specialist in the Department of Animal and Dairy Sciences at University of Wisconsin-Madison and his research interests are applied dairy cattle nutrition and management with emphasis on starch and fiber utilization by dairy cows, corn silage and high-moisture corn quality and digestibility, the use of alternative by-products as feed ingredients, and supplementation of feed additives to lactating cows.

California Animal Nutrition Conference
2021 Steering Committee

Chairperson: Jennifer Heguy, M.S., P.A.S. – UCCE Farm Advisor
Jennifer Heguy is a native of California's San Joaquin Valley. She received her B.S. in Animal Science, with an emphasis in Livestock and Dairy, at the University of California, Davis. In 2006, she received her M.S. degree at UC Davis, focusing on dairy cattle nutrition. Jennifer currently serves as the University of California Dairy Farm Advisor in Merced, Stanislaus and San Joaquin Counties where milk is a major agricultural commodity. Jennifer's major program focus is improving silage and feeding management practices on California dairies.

Vice Chairperson: Zachery Meyer, was raised in Ixonia, Wisconsin. He grew up immersed in his family's business, Rock River Laboratory. Meyer spent many hours helping in various jobs around the laboratory, seeing first-hand the dedication and commitment his father and the late Twilah Kulow had to the business and their customers. Meyer gathered business experience at Clear Channel and GE Medical while working toward his degree from the University of Wisconsin-Milwaukee. In 2007, Meyer resumed his involvement in Rock River Laboratory, starting as a soil sampler, moving to outside sales and eventually taking on his current role of director of operations. Meyer still gathers inspiration from the Rock River Laboratory employees and mentors who cultivated his drive for customer satisfaction and service, while continuing to learn and deepen his understanding of animal nutrition and agronomy. When he isn't building relationships with customers or overseeing laboratory operations, Zac spends his time playing or watching sports and sharing in family time with his wife and two young daughters.

Ex Officio: David Ledgerwood, M.S., P.A.S. graduated in 2004 with a BS degree in Animal Science focusing on livestock and dairy cattle from the University of California, Davis. Upon graduating he worked in the university ruminant nutrition lab with Dr. Ed DePeters as a lab technician performing feed nutrient and milk analysis while assisting graduate students run various ruminant nutrition focused research trials. In 2007 he graduated with a MS degree in Animal Biology focusing on ruminant nutrition working with Dr. DePeters. After graduation in 2007 he worked as a lab and research program manager in the field of animal behavior/welfare on the UC Davis campus performing various research trials focused on improving cow comfort. He

accepted a job with the Veterinary Medicine Teaching and Research Center in Tulare as a research program manager for the clinical department performing various research projects covering cow behavior, calf health, and nutrition. In April of 2011 he was offered a position with Western Milling LLC working as a dairy nutritionist. In 2018 David joined Novita Nutrition as a Technical Service Manager. In the Spring of 2019 David began working with Chr-Hansen.

Committee Members:

Ruben Almada, B.A.Sc., Turlock Dairy & Refrigeration was born and raised in the Hilmar, California area. Growing up on a dairy steered him toward a life in the dairy world. He graduated from California Polytechnic State University – San Luis Obispo in 2006 with a Dairy Science Degree. Upon completing his degree Ruben joined Cargill Animal Nutrition in the fall of 2006 where he was a Dairy Management Consultant for 3 years. He then joined Kemin Animal Health and Nutrition in July of 2010 as Key Account Manager covering California and Arizona for the Dairy Segment. In April of 2021 Ruben joined Turlock Dairy & Refrigeration as a Farm Mananagment Support Specialist helping dairymen merging cows with automation, He is married to his wonderful wife, Jennifer, and they have two children, Kinley (8) and Jaxson (6).

Virginia Brandao, Ph.D., Micronutrients is a Dairy Technical Manager. She received her Ph.D. degree from the University of Florida, and her research was focused on using the dual-flow continuous culture system to estimate nutrient flow, ruminal fermentation, and model fermentation end-products responses to dietary manipulations. Currently she provides technical services for the western geography and oversees research projects in collaboration with external research facilities.

Jamie Jarrett, Ph.D., grew up in Bakersfield, California on a farm surrounded by pigs, chickens, horses, cattle and basically any animal she could convince her dad to let her have. That passion for animals lead her to Cal Poly, where she earned a degree in Animal Science with a pre-veterinary concentration. While there, she developed a deep interest in research through her senior project, which led her to graduate school at Oklahoma State where she worked in a lab that concentrated on low-nutrient excretion experiments and antibiotic alternatives. She completed her Ph.D. in 2011 at Virginia Polytechnic Institute and State University, where she completed her thesis: *"Effects of exogenous phytase and forage particle length on site and extent of phytate digestion in lactating cows."* Jamie joined Alpha Dairy Consulting in 2017 as a nutrition consultant.

Brian Rainey, M.S., MBA, P.A.S., Pine Creek Nutrition Service, Inc. Upon graduating from Kansas State University, Brian made a gradual progression west seeking career fulfillment in working hands on with livestock producers. Brian joined Pine Creek Nutrition Service, Inc. in May 2010 and brings a science, business and industry portfolio to the consulting staff. Brian received a Bachelor of Science degree in Animal Science in 2001 from Kanas State University, Manhattan, KS, a Masters of Science in Ruminant Nutrition in 2004 from Montana State University, Bozeman and a Masters of Business Administration, with Distinction, Phi Kappa Phi, May 2010 California State University, Fresno.

Kyle Thompson, Ph.D. received his B.S. degree in animal science from Fresno State (2006) and his master's and Ph.D. degrees in animal science from Oklahoma State (2011/2015). He joined the Fresno State staff in the fall of 2016 after taking classes and teaching at Oklahoma State from January 2007-June 2016 and serving as the graduate student assistant manager of the campus dairy cattle center. His research included dairy nutrition research trials and lactating cow probiotics. He also assisted in research for bovine respiratory disease, rumen temperature bolus, milk production by weigh-suckle-weigh and swine antimicrobial replacements. He also assisted in 4-H and FFA Field Day dairy judging competitions. While in Stillwater, OK, he owned and operated Wild Acre Farms and Exotics, which raised ewes, game birds, free range hens and other fowl/animals, and produced grasses and winter wheat for grazing and hay production. As a Fresno State student, he worked in the sheep unit three years, served as a campus farm tour guide, and dairy unit herdsman and feed/hospital technician. He also worked as an exotic animal nutrition intern (2009) and global nutrition fellow at the San Diego Zoo (2013).

Beau Williamson, B.S., Adisseo USA Inc., was born and raised in Clovis, California. Being the son of an animal science professor – Scott Williamson, Ph.D – he grew up around animal agriculture. His passion for animal agriculture grew through his involvement with 4-H and FFA. This led him to attend school at Purdue University, where he graduated with a degree in agricultural economics. Beau began his career in the dairy industry at Elanco Animal Health and he currently works for Adisseo, a global leader in animal nutrition. Beau works with nutritionists and dairymen to help them meet key nutritional needs of their animals. When he is not working he loves spending time with his family, being involved in his church, and enjoying the great outdoors.

Honorary Member:

Amanda Gipe McKeith, Ph.D. is an Assistant Professor in the Department of Animal Sciences & Agricultural Education at Fresno State. She serves as the graduate program coordinator and co-advisor for both the Meat Science Club and Young Cattlemen's Association. Her research focus areas are how nutrition affects meat quality, processed meat ingredients, and food safety in food products. Dr. Amanda Gipe McKeith is originally from Merced, CA where she grew up on a 700-acre farm/ranch. Her family raises purebred registered Shorthorn cattle and grows corn silage, alfalfa, oats, and pasture hay. Amanda grew up showing livestock with her sister, Amy, and parents, Alpha and Sherri. Her family still runs the operation that now includes her brother-in-law, Tim and nephew, TJ as well as her husband Russell McKeith. Amanda serves as President for the National Shorthorn Lassie Association, and Treasurer for the California Shorthorn Breeders Association. Amanda received her A.A. in General Agriculture from Merced Community College, B.S. in Animal Science and Food Science at Kansas State University, M.S. in Animal Science with an emphasis in Meat Science from Kansas State University, and Ph.D. in Animal Science with an emphasis in Meat Science from The Pennsylvania State University. Amanda judged on both the Meats and Livestock Judging teams at Kansas State University. She coached meats judging teams at Kansas State and Western Kentucky University and livestock judging teams at Penn State and Western Kentucky.

CALIFORNIA ANIMAL NUTRITION CONFERENCE HISTORY

YEAR	CHAIRPERSON	COMPANY AFFILIATION
2021	Jennifer Heguy, M.S., P.A.S.	University of California, Coop. Ext.
2020	NO CANC CONFERENCE	
2019	David Ledgerwood, M.S., P.A.S.	Chr-Hansen
2018	Jason Brixey, M.S., P.A.S.	Pine Creek Nutrition Service
2017	Dr. Phillip Jardon, DVM, MPVM	Elanco Animal Health
2016	Dr. Phillip Jardon, DVM, MPVM	Elanco Animal Health
2015	Mr. Ben Tarr	Adisseo USA Inc.
2014	Dr. Jeffrey M. DeFrain	Zinpro Performance Minerals
2013	Mr. Doug DeGroff	Diversified Dairy Solutions, LLC
2012	Mr. Eduardo Galo	Novus International, Inc.
2011	Dr. Michael A. DeGroot	DeGroot Dairy Consulting
2010	Dr. Jim Tully	Pine Creek Nutrition Service, Inc.
2009	Mr. Michael Braun	Phibro Animal Health
2008	Dr. Luis Rodriguez	Zinpro Corporation
2007	Dr. Marit Arana	A.L. Gilbert Company
2006	Mr. Dennis Ervin P.A.S.	Prince Agri Products, Inc.
2005	Dr. Lawson Spicer	Nutri Management Inc.
2004	Dr. Luis Solorzano	Purina Mills, Inc.
2003	Dr. Alfonso Mireles, Jr.	Foster Farms
2002	Mr. Edmund Vieira	Pine Creek Nutrition Service, Inc.
2001	Dr. Melinda Burrill	California State Polytechnic University - Pomona
2000	Mr. Dave Fischer	Foster Farms
1999	Dr. M. Steven Daugherty	California State Polytechnic University - SLO
1998	Dr. Doug Dildey	Alltech, Inc.
1997	Ms. Carla Price	Nutritionist
1996	Dr. H.John Kuhl, Jr.	Nest Egg Nutrition
1995	Mr. Dennis Ralston	M. Rinus Boer Co., Inc.
1994	Dr. Doug Dildey	Alltech, Inc.
1993	Dr. Mark Aseltine	Consulting Animal Nutritionist
1992	Dr. Carl Old	MacGowan-Smith Ltd.
1991	Mr. Nick Ohanesian	Ohanesian & Associates
1990	Mr. Rod Johnson	M. Rinus Boer Co., Inc.
1989	Mr. Timothy Riordan	Nutri-Systems, Inc.
1988	Dr. Russ W. Van Hellen	Great West Analytical
1987	Dr. John E. Trei	California State Polytechnic University, Pomona
1986	Dr. A.A. Jimenez	Ancon, Inc.
1985	Dr. Wm. A. Dudley-Cash	Foster Farms
1984	Dr. Joel Kemper	Penny-Newman Co.
1983	Dr. Alex J. Kutches	O.H. Kruse Grain & Milling Co.
1982	Dr. Howard Waterhouse	Bell Grain & Milling
1981	Mr. Don Ulrich	Diamond Shamrock Chemical Co.
1980	Mr. Tom Geary	PMS-West, Inc.
1979	Dr. Frank Parks	Kemlin Industries
1978	Mr. Fred Pfaff	Zacky Farms
1977	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.
1976	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.

YEAR	CHAIRPERSON	COMPANY AFFILIATION
1975	Dr. R.D. Hendershott	Nulaid Foods
1974	Dr. R.D. Hendershott	Nulaid Foods
1973	Dr. Leland Larsen	Nutri-Systems, Inc.
1972	Dr. Leland Larsen	Nutri-Systems, Inc.
1971	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.
1970	Mr. Fred Pfaff	Balfour Guthrie
1969	Mr. Fred Pfaff	Balfour Guthrie
1968	Mr. Fred Pfaff	Balfour Guthrie
1967*	Mr. Gary L. Frame	J.G. Boswell Co.
1966*	Mr. Gary L. Frame	J.G. Boswell Co.
1965*	Mr. Arne Jalonen	Topper Feed Mills
1964*	Mr. Arne Jalonen	Topper Feed Mills
1963*	Dr. W.P. Lehrer	Albers Milling Co.
1962*	Dr. H.J. Almquist	The Grange Co.
1961*	Dr. H.S. Wilgus	The Ray Ewing Co.
1960*	Mr. Bert Maxwell	Nulaid Foods
1959*	Mr. Bert Maxwell	Nulaid Foods
1958*	Mr. Robert Caldwell	Anderson Smith Milling Co.
1957*	Mr. Emery Johnson	P.C.A., Los Angeles
1956*	Mr. Emery Johnson	P.C.A., Los Angeles
1955*	Dr. H.J. Almquist	The Grange Co.
1954*	Dr. H.J. Almquist	The Grange Co.
1953*	Mr. Clifford Capps	California Milling Co.
1951*	Mr. Dolph Hill	Golden Eagle Milling Co.
1950*	Dr. H.J. Almquist	The Grange Co.
1949*	Dr. H.J. Almquist	The Grange Co.
1948*	Dr. H.J. Almquist	The Grange Co.

* California Animal Industry Conference

History of the
California Animal Nutrition Conference

The California Animal Nutrition Conference (CANC) originated in the 1940's as the California Animal Industry Conference, sponsored by the California Grain & Feed Association (CGFA). CGFA wanted to expand the continuing education program into a forum encompassing animal health, nutrition and management. The expectations were that communications between (nutritionists) industry, educational institutions and regulatory agencies would be improved. In 1972, CGFA discontinued sponsoring the Animal Industry Conference.

After the conference was discontinued, a small group of nutritionists began meeting annually in Fresno. Two or three invited speakers from industry or the universities presented information on nutrition, especially poultry.

In 1975 a set of organizational bylaws were developed by the steering committee. CANC was established and was provided support by CGFA. The CGFA Board of Directors appointed a chairperson annually and approved the steering committee. In 1978, Dr. Frank Parks, the Chairperson, requested that CANC be granted independent status and be established as a self-governing committee of CGFA. This request was granted.

For a few years, meetings were held in Fresno and Corona, California. For a couple of years starting in 1978, CANC published "Nutri-Facts", a "newsletter" consisting of articles in animal production.

In 1979, donations were requested from industry companies to help keep registration fees low. During the 1980's and through the 1990's the attendance at CANC continued to grow as the quality of the conference improved and the conference became known nationwide. In the 1990's a pre-symposium was added. The pre-symposium is sponsored by a company selected by the CANC Steering Committee. This process allows the selected company to showcase its research and products. In the year 2000, posters on research by students were included.

Attendance at the conference has grown from 50 in the 1970's to over 300 attendees. To encourage attendance, different activities have been tried such as keynote speakers, skiing expeditions and a very successful barbeque dinner put on by the Animal Science Department at Fresno State University.

The California Grain & Feed Association has supported and allowed CANC to work and grow. The premise of the CGFA and CANC relationship is to work together to educate the feed industry with information for problem solving and to disseminate valuable research information. CANC is not an industry, university, or government entity, but a committee collectively working together for the good of agriculture in California.