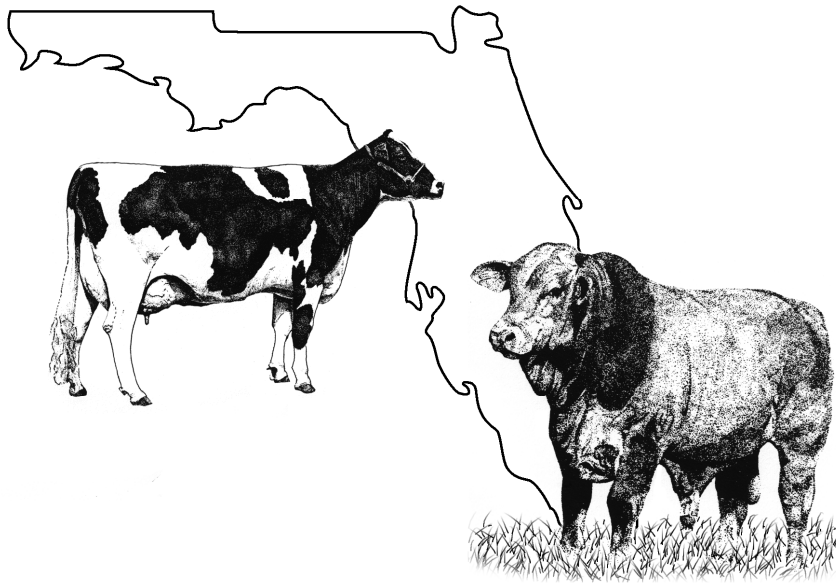
2025 Florida Ruminant Nutrition Symposium

36th Annual Meeting



February 24 - 26, 2025
Best Western Gateway Grand
Gainesville, Florida

PROCEEDINGS

UF UNIVERSITY of FLORIDA
IFAS
Department of Animal Sciences

2025

36th ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM

February 24 - 26, 2025
Best Western Gateway Grand Hotel
Gainesville, Florida

Department of Animal Sciences
University of Florida
Institute of Food and Agricultural Sciences
Gainesville, Florida 32611

Florida Ruminant Nutrition Symposium – February 24 to 26, 2025

Monday, February 24, 2025 – Mini-Symposium sponsored by Balchem Corporation *"Beef × Dairy Cattle: Advancing Beef Quality to the Next Level"*

2:00 PM **Dr. Clay Zimmerman**, Balchem Corporation. *Welcome and introductions*

2:05 PM **Dr. Tara Felix**, Penn State University *"How beef on dairy selection impacts beef and dairy production"*

2:50 PM **Dr. Brad Johnson**, Texas Tech University *"Beef x dairy cattle: advancing beef quality to the next level"*

3:35 PM **Dr. Albert De Vries**, University of Florida *"Beef-on-dairy: economic decisions making on the farm"*

4:20 PM **Dr. Tom Overton**, Cornell University *"Turbocharge your fresh cow diets"*

5:05 PM **Dr. Clay Zimmerman**, Balchem Corporation *"AminoShure-XL: redefining amino acid nutrition"*

5:30 PM ***Poolside Brazilian Barbeque***

Tuesday, February 25, 2025 - Pre-Conference *Sponsored* by Perdue Agribusiness *"Optimal Rumen Function to Drive Productivity and Animal Health*"

8:10 AM **Dr. Jonas de Souza**, Perdue Agribusiness. *Welcome and introductions*

8:20 AM **Dr. Jeff Firkins**, The Ohio State University "*Diet manipulation to improve nutrient digestibility and microbial protein synthesis*"

9:00 AM **Dr. Diwakar Vyas**, University of Florida *"Improving fiber utilization in dairy cows: technologies available to influence production outcomes"*

9:40 AM ***Refreshment Break***

10:10 AM **Dr. Alex Bach**, ICREA – Spain *"The role of buffers and alkalinizers to improve rumen function and animal performance"*

10:50 AM **Dr. Lance Baumgard**, Iowa State University *"Importance of gut health to drive animal performance and health"*

11:30 AM ***Buffet Lunch***

Tuesday, February 25, 2025 – Symposium

1:10 PM **Dr. Saqib Mukhtar**, University of Florida "*IFAS/Animal Sciences update*"

1:20 PM **Dr. Robert Cousins**, University of Florida *"Mechanism of absorption, transport, and homeostasis of zinc in mammals"*

2:00 PM **Dr. David Fraser,** University of Sydney *"Mechanisms of Ca absorption in the gastrointestinal tract of ruminants"*

2:40 PM **Dr. Jerry Spears**, North Carolina State University *"Use of hydroxychloride trace minerals in diets of cattle"*

3:20 PM ***Refreshment Break***

3:50 PM **Dr. Jim Drackley**, University of Illinois *"Nutrient requirements of preweaning calves"*

4:30 PM **Dr. Javier Martín-Tereso**, Trouw Nutrition *"The untapped opportunity of early life nutrition"*

5:10 PM **Masroor Sagheer**, University of Florida *"Feeding rumen-protected choline during the peri-conceptional period programs postnatal phenotype of calves"*

5:30 PM ***Reception***

<u>Wednesday, Feb 26, 2025</u> – Symposium

8:10 AM **Dr. Mutian Niu**, ETH Zurich "*Exhaled breath approaches to assess rumen fermentations and metabolic changes in dairy cows*"

8:50 AM **Dr. John Goeser**, Rock River Lab *"New tools to assess and optimize forage quality and diet formulation"*

9:30 AM **Lais de Oliveira Lima**, University of Florida *"Life cycle assessment and net protein contribution of cow-calf operations in Florida"*

9:50 AM **Refreshment Break**

10:20 AM **Dr. Brad Johnson**, Texas Tech University *"Early life nutrition and muscle and adipose tissue deposition and feedlot performance"*

11:00 AM **Dr. Tara Felix**, Penn State University *"Beef on dairy: impacts on performance and carcass traits"*

11:40 AM Ruminant Nutrition Symposium Adjourns

2025 Symposium Speakers

Guests

Dr. Alex Bach, ICREA Spain
Dr. Lance Baumgard, Iowa State University
Dr. Jim Drackley, University of Illinois Urbana-Champaign
Dr. Tara Felix, Penn State University
Dr. Jeff Firkins, The Ohio State University
Dr. David Fraser, University of Sydney, Australia
Dr. John Goeser, Rock River Laboratories
Dr. Brad Johnson, Texas Tech University
Dr. Javier Martín-Tereso, Trouw Nutrition, the Netherlands
Dr. Mutian Niu, ETH Zurich, Switzerland
Dr. Tom Overton, Cornell University
Dr. Jerry Spears, North Carolina State University

University of Florida

Department of Animal Sciences

Dr. Robert Cousins
Dr. Albert De Vries
Lais de Oliveira Lima
Masroor Sagheer
Dr. Diwakar Vyas

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36th Annual Florida Ruminant Nutrition Symposium

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Diet Manipulation to Improve Nutrient Digestibility and Microbial Protein Synthesis

Jeffrey L. Firkins[1,a] *and Benjamin A. Wenner*[b]
[a]*The Ohio State University and* [b]*Feedworks USA, Ltd*

Introduction

Although we measure neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and acid detergent lignin (**ADL**), hemicellulose (NDF – ADF) is very diverse in chemical constituents, and cellulose (ADF – ADL) is very different in its crystallinity (Firkins et al., 2025). Besides different forages, differences in maturity and environmental factors also influence chemical components in fiber. For example, plant breeding to decrease lignin can result in plant adaptation that increases phenolic cross-linking to hemicelluloses. On a smaller scale, there are a diverse array of substrates and access by microbes to those substrates as they are dynamically shifting over time after colonization. The net result is a heterogeneous mix of substrates and microbes that increases variation among animals fed different diets and even animals fed the same diets.

Soluble fiber can be measured separately but is not recovered by the NDF assay and often is not measured in the field. Starch is now much more accurately measured than a couple of decades ago and is routinely measured, but starch availability in the rumen is difficult to assess and can interact with expectations for NDF degradability (**NDFD**) in the rumen. Sugars also can be measured separate from starch. However, if you include oligosaccharides as sugars, they vary among sources and, in contrast with starch, can increase NDFD. Certain fats also seem to interact with NDFD, but analysis should be verified by fatty acid (**FA**) analysis. In fact, NASEM (2021) explained why FA should be analyzed and ether extract basically buried in the same graveyard as crude fiber. In short, these interactions are not well understood and therefore not well modeled in our software. Hence, thinking about the specific substrates and not just their chemical measurements should help troubleshoot rations.

When modeling microbial protein production, our databases have gaps. For example, there are some studies on how FA affects microbial protein production, but there are few studies on soluble fiber and sugars. When assessing roles of forages and grains, there tend to be few details that can be used to assess meta-data (from combined studies) other than by discrete classification. Alfalfa has been studied compared with orchardgrass to show how fragility affects NDFD and dry matter intake (**DMI**). However, any such measurements of fragility are not consistently distributed across a variety of forages or different sources within a forage type. Undegraded NDF is widely assessed in the field, but this assay is not routinely measured in peer-reviewed research and, even then, often is only compared to in vitro assessments of NDFD.

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Similarly, we know how grain processing or vitreousness affects ruminal starch digestibility, but particle size is not routinely reported for grain and is not on a dry matter (**DM**) basis for forage (leaves and stems vary in DM). Again, this variation is not well described in models, so users need to be aware of mechanisms to lessen that variation.

Microbial protein production is a challenge to measure and relate to the factors that field nutritionists are evaluating on farms using predicted microbial protein supply. Even though such models will always be limited because there are not enough source data, we do know factors that will influence actual microbial protein from individual studies. These factors will be discussed, and some potential opportunities will be assessed in the paper and in the presentation. Throughout this discussion, we must remember that microbial protein is generally of a better amino acid (**AA**) profile than the rumen-degraded protein (**RDP**) from which it was derived and still far cheaper than metabolizable AA in more expensive rumen-undegraded protein (**RUP**). Hence, microbial protein should be optimized before using high quality RUP sources.

Why Emphasize Ruminal Fiber Degradation for Microbial Protein Synthesis?

Apart from its role to support milk fat production, NDFD should also be optimized to provide the base of degraded carbohydrates to synthesize AA and the energy from fermentation to convert those AA into microbial protein. Prediction of ruminal NDFD is problematic on its own but also for its role in predicting microbial protein supply. Firkins et al. (2025) emphasized both physiological and predictive limitations preventing optimization of NDFD. We must remember that prediction equations are empirical in derivation and thus represent an “average” expected response rather than being case-specific, so we must understand the physiological mechanisms and limitations to be on the upside of the mean when using those predictions.

One of the main physiological limitations is the negative associative effects of starch degradation on NDFD. Using treatment means (Ferraretto et al., 2013) and within-cow comparisons (de Souza et al., 2018), each 1% unit increase in starch decreases total tract NDF digestibility about 0.5 to 0.6% unit. Of course, starch is about twice the digestibility as NDF, so each 1% increase in starch is actually only ~ 0.75% net gain (subtracting the depressed NDFD). The carbohydrate chapter in NASEM (2021) describes how starch and fiber should be included in diets. Although we need enough starch to optimize microbial protein, increasing starch beyond this sliding optimum decreases efficiency of microbial protein synthesis (Firkins et al., 2007; Hackmann and Firkins, 2015b). Microbes compete for substrate, but surface area probably limits their access to that substrate. Increasing relative abundance through microbial growth increases likelihood to colonize newly ingested feed. However, degraded carbohydrate can be uncoupled from microbial protein synthesis yet still be beneficial to microbes if they are competitively accessing substrate and wasting the energy derived from fermentation. As an example, butyrivibrios can gain up to 50% more ATP per glucose than traditionally thought (Hackmann and Firkins, 2015a). Many other ways have been detected in bacterial genomes since then by Dr. Hackmann. Thus, on the flip side of harnessing extra energy are ways to intentionally unharness and waste energy

(Hackmann and Firkins, 2015b). Other than peptides (discussed later), models do not do a good job of estimating shifting changes in efficiency of microbial protein synthesis.

Microbial protein supply in the Cornell Net Carbohydrate and Protein System (**CNCPS**, version 6.5) was particularly sensitive to rate of starch degradation in corn grain and corn silage (Higgs et al., 2015) and we will discuss in the presentation that predicted microbial protein supply responds quickly to increased degradable starch in the diet. However, derivation of this rate constant is tricky because feeds need to be dried and ground, which might not mimic actual feeding. Problems associated with saturating microbial amylases in these analyses are not accounted for. In some parts of the U.S., though, the approach is that “more starch is always better”, which assumes a high degree of cow and feeding management.

Traditional meta-analyses have documented that increasing ruminal starch degradability often is not linearly associated with measurements of microbial protein (Firkins et al., 2001). In that review, increasing ruminal starch degradability through more aggressive grain processing did not increase measurements for microbial protein production because of decreased efficiency of microbial protein synthesis. However, most of these studies were done with duodenally cannulated dairy cattle fed different types of grain or with different processing methods that were grouped within classes (i.e., discrete groupings), so one person’s “fine-ground corn” back then might not be that fine today. Corn particle size often was not even done.

Since 2001, researchers shifted from duodenal to omasal sampling for animal handling purposes. Despite the numerous measurements of ruminal NDFD with omasal sampling (Huhtanen et al., 2010), most of those earlier omasal sampling studies did not report ruminal starch digestibility. Those authors argued that their much higher proportion of total tract NDFD in the rumen (e.g., 95 to 97% of the total tract NDFD, depending on the regression) were more biologically appropriate than the lower proportion of total NDFD in the rumen for duodenal sampling. Even so, the relatively few studies with cannulas in the ileum averaged 11.9% NDFD (Gressley et al., 2011), which greater than 3 to 5%. Huhtanen et al. (2010) further argued for omasal sampling by using the example that ruminal starch digestibility, when measured, was 85% of total tract starch digestibility from barley and oats (Huhtanen et al., 2010). This argument leaves two problems: 1) corn was not well represented and 2) 85% of the apparent digestibility of starch would approach 100% on a true basis (i.e., correcting for microbial “starch”. Microbes synthesize storage carbohydrate measured as “starch”, particularly when nitrogenous growth factors limit starch usage for cellular growth (Hackmann and Firkins, 2015b).

The lack of balanced meta-data for similar types of studies across duodenal and omasal sampling for starch digestibility and even the role of starch on NDFD plagued NASEM (2021) prediction as identified by White et al. (2016). Although sadly ignored by too many people doing meta-analyses, interactions of variables with the random effect of study could only be removed by inclusion of class effects for omasal sampling being higher for both starch digestibility and microbial N than for duodenal sampling.

The resulting equation used by NASEM (2021) included a squared effect of dietary crude protein (**CP**) for predicting ruminal NDFD, and the squared term was deemed as biasing predictions for NDFD when CP is high (Martineau et al., 2023). Although those authors noted an inflection at 27% CP above which NDFD became negative, Firkins and Lapierre (2023) cautioned that 21.4% CP (mean plus 2 standard deviations in the derivation database) should be used as a maximum response to limit such a bias (the error from the squared term grows geometrically with increasing CP). Even 21% CP is excessive in practice unless animals are grazing lush pastures for which few measurements are available for modeling, anyway. Even if not a problem in normal diets, this problem with CP^2 term documents the challenges with meta-analyses when various dietary nutrients are not balanced across studies. We have limited mechanistic understanding of how dietary CP (probably a proxy for RDP, which rarely is measured) affects ruminal starch digestibility, NDFD, and microbial protein production in lactating cow studies.

Fibrolytic Bacteria Benefit from Preformed Amino Acids

Most researchers actually measuring RDP have used solvents, in vitro, or in situ methods, and most in vivo studies are using library values based on those procedures. The lack of measurement in lactating cows is in contrast with its importance. Peptide supply is a critical factor for bacteria degrading nonstructural carbohydrates (Van Amburgh et al., 2015), yet even peptides (as opposed to RDP) rarely have been measured owing to inconsistent measurements (Firkins et al., 2007). Inconsistencies were described by Reynal et al. (2007), who opted for a molecular weight filter to assess a relatively large proportion of dietary amino acids (**AA**) passing in peptides <10 kDa molecular weight. Almost nothing has been done since. Although CNCPS version 6.5 modified peptide supply according to this information (Van Amburgh et al., 2015), our unpublished work suggests that fibrolytic bacteria (and NDFD) is responsive to peptide supply in continuous culture. Thus, more attention should be given to the need by cellulolytic bacteria to use preformed AAs (Wallace et al., 2001). Three different cultures of cellulolytic bacteria increased cellular growth from small peptides and AAs versus ammonia, particularly when grown on cellobiose (the repeating disaccharide in cellulose) compared with a source of pure cellulose (Atasoglu et al., 2001). However, that cellulose source is relatively crystalline and need not represent the type of cellulose in high quality forages or fibrous byproducts. Therefore, an important takeaway is that adequate RDP from true protein is needed to optimize NDFD.

Previously, the role of peptides was discussed as stimulating the amylolytic/proteolytic microbes but also potentially for cellulolytics — either directly for AA or indirectly to be provided with ammonia and branched-chain volatile fatty acids (**BCVFA**) — but peptides probably have a more nuanced role. Peptide concentration is a net of production and usage. Probably there is an optimum supply of peptides in which too little can limit NDFD, but too much peptide supply might limit NDFD (Jones et al., 1998); moreover, diets providing such high amounts of peptides would not be economical. Some low abundance microbes can make a living off of RDP for AA both

for protein synthesis and for energy, which is inefficient and wasteful and could tilt microbial structure in a negative way. On the other hand, preformed AA provided from adequate RDP (not excessive) increases efficiency of protein synthesis in most cells.

Peptides have been studied in multiple ways over the past decades. However, studies dosing just protein in batch culture likely are biased by limiting carbohydrates to effectively convert those peptides into cells (Hackmann and Firkins, 2015b). When studied, increasing ammonia concentration usually inhibited peptidolysis as a feedback mechanism to avoid wasting AA. However, in addition to ammonia, availability of all AA is probably sensed through branched-chain AA concentration inside cells (Firkins et al., 2024). Sensing both ammonia and probably a balance of leucine, valine, and isoleucine helps queue bacteria to grow more rapidly and efficiently. Although lack of peptides or AA limited cellulose digestibility (Griswold et al., 1996), adequate ammonia is needed to support microbial growth and therefore peptidase activity (Griswold et al., 2003). Because of the central role of ammonia, its limitation must be managed first, whereas AA from peptides can then secondarily limit microbial growth.

Our unfolding research suggests ammonia limitation must always be prevented before supplemental BCVFA can be efficacious (Firkins et al., 2024). However, phenylalanine might be particularly limiting for the cellulolytic bacteria (Wallace et al., 2001) and likely is a reason why true RDP (i.e., not urea) must be adequate for optimum fiber digestibility and response to BCVFA supplementation. There are numerous papers in the literature showing inhibition of peptidases with increasing peptide supply, and supplemental BCVFA can both increase or decrease peptidase activity, depending on conditions (Firkins et al., 2024). We should consider peptidolysis to not be constant and therefore needed adequate RDP (more specifically peptide supply) to optimize microbial protein synthesis.

Peptidolysis and the Unfolding BCVFA Story

Some researchers are suggesting an average of 10% of total AA flow from the rumen being of soluble dietary origin (Reynal et al., 2007). Presumably, this 10% is coming from some unknown mix of what would be considered RDP and RUP. If the soluble peptide fraction resulted from feed protein that washed out of the soluble “A” fraction of Dacron bags (assumed to a constant 6.4% in NASEM, 2021), then outflow of peptides would only partially increase the true RUP fraction. However, what if we add the layer that peptidolysis is not a constant, nor if the AA profile is constant? Proline, phenylalanine, and the branched-chain AA are candidates for increasing outflow relative to degradation (Chen et al., 1987) and all have important roles in bacterial metabolism.

Adequate ruminal ammonia plus amino-N seem to be a predicate for the efficacy of BCVFA to stimulate NDFD (Firkins et al., 2024). Our current research in progress supports conclusion. Adding urea at 0.19% of dietary DM (about 0.5% CP basis) in diets containing 10.3 to 10.5% total RDP increased NDFD, but the response was greatest with cows fed a higher forage (20.7% forage NDF) than lower forage (16.6% forage NDF; not accounting for whole cottonseed) in the diet (Redoy et al., 2025). Milk fat yield

was increased when BCVFA were added to high forage diets, but average daily gain was increased in higher grain diets. Our unpublished research suggests that a shift toward average daily gain would be more pronounced with primiparous than multiparous cows. Using isotopes of the BCVFA, increasing forage increased isotope recovery in bacteria from continuous cultures administered higher forage diets, emphasizing the need by cellulolytic bacteria for BCVFA (Mitchell et al., 2023). Thus, for BCVFA to have the most opportunity to maximize NDFD, there must be both adequate ammonia and adequate provision of preformed AA in the RDP.

Replacing Starch with Sugars (or Oligosaccharides) and Fat

Cellulose is all glucose but varies considerably in its crystallinity and interactions with other cell wall components. Hemicellulose, pectin, and other soluble fibers vary considerably in chemical makeup and bonding. Thus, cellulolytics are specialists with respect to how they use crystalline cellulose but still hydrolyze hemicellulose primarily to gain access to the long strands of cellulose. In contrast, hemicellulolytic and pectinolytic bacteria tend to be generalists that work on a variety of substrates. Emerging evidence is that many of these generalists shift their gene expression of enzymes needed as these non-cellulose substrates are changing among diets or over time since the last meal (Firkins et al., 2025). Some of these generalists can also use starch, and many are active protein degraders. Thus, a balanced consortium of microbes fosters cellulolytics (which do not degrade protein) at the base, which opens up room for others and even "shares" oligosaccharides. Hemicellulolytics share in this niche for carbohydrate and help to provide protein degradation end-products such as BCVFA and ammonia. In contrast, an unbalanced population, especially with an overly high abundance of starch degraders, allows excessive growth of "selfish" bacteria. Low pH and lack of other growth factors further tilt abundance toward the selfish ones. The cycle progresses with high starch or poor feedbunk management as unbalanced populations lose potential NDFD — even before a dysbalanced population would be promoting subacute acidosis.

Pectin has long been ignored in research because it has been assumed to be nearly completely degradable. However, pectin differs in its chemical structure, proximity in the plant cell, and in its role in the microbial consortium. Growing evidence suggests that pectinolytic bacteria include generalists and specialists. Some generalists are closely related to hemicellulolytics (e.g., *Prevotella* or *Butyrivibrio*). Other pectinolytic specialists include the corkscrew-shaped *Treponema,* which vortexes non-motile cellulolytics into newly ingested fiber particles and often shares their requirement for BCVFA as growth factors (Firkins et al., 2024). The latter have been routinely characterized as partners with the specialist cellulolytic bacteria. Soluble fiber from citrus pulp has been studied even less than that from forage but probably fall into a similar camp. Those microbes growing on soluble fiber probably also benefit from having adequate peptides or BCVFA derived from them to maximize efficiency of microbial protein synthesis.

Although there are procedures for estimating the soluble fiber concentration, the most common approach in research is by residual organic matter (**rOM**). This pool is

derived by difference of organic matter – CP – NDF – lipid – (starch + sugar). If done right, rOM is repeatable and very highly degradable (Tebbe et al., 2017). In NASEM, the rOM was somewhat also an error pool i.e., inflating variation by combining errors from all of the analyses. We could not derive an equation using rOM, but plotting rOM alongside the predicted microbial protein had no residual patterns. In other words, rOM is probably baked into the equation in normal diets, although diets low in NDF and starch (i.e., high in soluble fiber) might predict lower microbial protein than actual.

Sugars can be from many different sources. Both citrus pulp or byproducts from sugar processing (both cane and sugar beet) contain free sugars and so are categorized as sugar sources. However, they also can contain significant oligosaccharides measured as soluble fiber. Is it the sugar or the soluble fiber? We do not really know why sugars sometimes improve NDFD (Firkins et al., 2025). However, any potential improvement would likely be negated if provided with high inclusion of starch, and moderate starch diets likely are needed for any benefit in increased milk fat secretion (Oba, 2011). There are few studies, and they are inconsistent with respect to microbial N production from supplemental sugars. Feeding sugars can decrease ammonia or BCVFA concentrations (Firkins et al., 2006). Part of the sugar response could be through maintaining a basal prevalence of microbes using lactic acid; some of these might be able to use or benefit from increased AA availability.

Firkins et al. (2025) emphasized potential roles for moderate amounts of palmitic and oleic acids. A paper from our lab and one from Dr. Batistel at the University of Florida were highlighted to emphasize how lipids can be negative, neutral, or positive for microbial growth. The fibrolytic bacteria have unique and important enzyme complexes on their exterior. However, those enzymes must be synthesized intracellularly before being translocated through the cytosolic membrane prior to assembly into multi-enzyme complexes. In a “Goldilocks” approach, membranes must be fluid enough for transferring intact proteins yet rigid enough to anchor those complex factories to the cell wall. Cellulolytic bacteria vary in how they use these extracellular proteins, but there is no doubt that they are specialists because of them. However, even some bacteria degrading hemicellulose and resistant starch have critical assemblies that are on the exterior of the cell or in between the two membranes of gram-negatives.

We do not know but assume membrane fluidity is sensed and fatty acid synthesis adjusted in response to changes in ruminal conditions (Hackmann and Firkins, 2015a; Firkins et al., 2025). Because bacteria cannot desaturate FA anaerobically, they must add more fluidizing (unsaturated or branched-chain FA) as needed to be in a proper ratio with the stiffening saturated FA. The fibrolytics need much more branched-chain FA than those degrading starch (Firkins et al., 2024). However, the most well characterized biohydrogenating bacteria in the butyrivibrio group (genera *Butyrivibrio* and *Pseudobutyrivibrio*; primarily using hemicellulose) are particularly enriched in branched-chain FA (Hackmann and Firkins, 2015a). They also have low concentrations of stearic acid, and some have higher palmitic acid (opposite of most bacteria). Strains of the cellulolytic bacteria *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* also are high in palmitic and branched-chain FA but low in stearic; oleic was not recorded

(Saluzzi et al., 1993). Thus, supplemental palmitic and oleic acids could interact with membranes and get activated (esterified to coenzyme A). Anaerobes do not store and use FA as fuel as do the aerobes. Palmitic acid supplements are typically combined with some oleic acid, which aids in intestinal absorption. Providing palmitic acid in the free form probably helps intestinal absorption but also makes this fat source more rumen-active. Oleic is far more soluble in the rumen; inconsistent responses of oleic acid on ruminal NDFD (Firkins et al., 2025) could be a result of over-supplementation on an amount basis rather than on a rumen-available basis.

The role of fats on microbial protein supply has been considered in multiple ways (Hanigan et al., 2021). Twenty years ago, fat was thought to dilute degradable carbohydrate and decrease dietary energy to support microbial protein synthesis. After studies showed no decrease in microbial protein production, fat was assumed to improve efficiency of microbial protein synthesis either by sparing carbon or through decreased protozoal numbers and intraruminal recycling of microbial protein. More recently, those authors noted neither benefit nor detriment from dietary fat. In meta-analyses, each factor is always accounted for at the average of each other factor in a model. Fat can sometimes decrease DMI, which obviously would decrease microbial protein production. Moreover, grouping FA without considering FA profile and as free versus esterified FA could prevent any potential benefit. Except for excessive doses of medium chain FA (lauric and myristic), fat probably has a minimal negative effect on NDFD (and therefore microbial N) unless fed at high enough inclusion rates to promote milk fat depression or depress DMI (Weld and Armentano, 2017), which should be avoided anyway. That meta-analysis suggested no benefit for palmitic supplements, but many of those palmitic supplements were from triglycerides. In contrast, after excluding all triglyceride sources, the free palmitic acid supplements averaged a 4.5% unit increase in NDFD (dos Santos Neto et al., 2021).

Microbial Protein for Dairy Cattle

Microbial protein is the main protein source in most dairy cattle rations. Optimization of microbial protein as a percentage of total metabolizable protein likely is quadratic because a low % contribution could reflect high RUP supplementation, whereas a high contribution could reflect limiting essential AA in the ration. Although assumed to be constant, uncertainties still remain in the profile AA from microbial protein depending on how much particulate- versus fluid-phase bacteria and protozoal outflow from the rumen (Gresner et al., 2022). Perhaps because of these different techniques, the AA composition of microbial protein is assumed to be constant with respect to diet. Its non-protein N is relatively constant (15-20% of N, and it has consistently high digestibility in the small intestine (NASEM, 2021). Microbial true protein has a well-balanced AA profile compared with milk protein except for being low in histidine. Decreasing RUP in the diet, especially high bypass sources such as blood meal, runs the risk of dropping metabolizable histidine supply too low. Isoleucine also might be lower in RDP compared with isoleucine in bacteria, which is another reason to monitor this AA. Supplementation of BCVFA containing its precursor, 2-methylbutyrate, but this isoacid is racemic, and only about half is used (Firkins et al., 2024). With so many feedstuffs low in isoleucine,

other essential AA could be over-supplemented when dietary CP is increased to match the recommendations for isoleucine or even phenylalanine. Even more than normal diets, BCVFA should be supplemented only when diets are adequate in true protein RDP.

NASEM (2021) predicts microbial protein synthesis using predicted ruminal starch and NDFD and RDP. All of these were predictions from standard dietary analyses, which has limitations because some of these were predicted (including all of the RDP). As discussed previously, approaches to predict the carbohydrate digestibility and RDP are limited because in vitro or in situ approaches do not consistently represent in vivo responses, and even in vivo approaches are limited by methods that prevent derivation of accurate outputs. Drying and grinding clearly shift kinetics of degradation by ruminal microbes, so the conditions that affect ruminal digestibility on dairy farms are generally also limited (understandably) by analyses done by commercial laboratories. That said, all models allow relative comparisons and simulations that are very useful for dietary changes prior to on-farm evaluation. Some of the reasons limiting generalizing across different conditions will be discussed subsequently.

Many models are being evaluated against in vivo data, but users need to understand differences. For example, both the 2001 NRC (Broderick et al., 2010) and CNCPS version 6.5 (Van Amburgh et al., 2015) were evaluated against omasal flow data. However, NRC (2001) had no source data using omasal sampling. The evidence against duodenal sampling using a single marker is, in fact, not particularly robust (Firkins et al., 1998). However, when collecting samples from the omasal canal, the more complicated triple marker approach is needed. Almost no studies include both duodenal and omasal sampling; of those, there is a clear distinction in the stable isotope of nitrogen (N; i.e., ^{15}N) that is associated with the higher microbial N flow for omasal sampling than for duodenal sampling (Martineau et al., 2023). Therefore, is it the flow method and its markers or is it the microbial sampling and microbial markers? If there is no known “gold standard” approach to measuring microbial protein supply (and therefore the nonammonia-nonmicrobial N supply that standardized RUP measurements), we are relying on relative comparisons. For NASEM (2021), the intercept shift for higher microbial and lower nonmicrobial-nonammonia N for omasal sampling (~17.6% of treatment means) must be fixed in time. As more papers are published using omasal (or reticular) sampling, the NASEM (2021) model will continue to be increasingly “biased” compared with this changing evaluation database.

Other key differences remain among models or their evaluation. If models are relying on a ruminal passage rate, there is no good marker approach as a gold standard. Because of these problems, the passage rate was derived iteratively such that RUP on average had no bias from all of the protein sources in all of the studies compared with measured nonammonia-nonmicrobial flows (corrected for endogenous protein) in the database (Hanigan et al., 2021). Another complicating factor is that a number of studies used in NASEM (2021) were excluded for experimental reasons (Roman-Garcia et al., 2016), whereas other researchers might be keeping these studies. We weighted results for SEM, which often has not been done by others; using

residuals analyses to assess bias cannot account for the weighting factor. As we explained in that paper, when using standard fitting procedures, residuals analysis from the same source data can NOT yield a mean bias. Any suggestion otherwise is a data issue, not a model issue. All protein models have unknown errors from derivation or evaluation using imperfect meta-data. Each is wrong to whatever degree the data are biased, whereas standardization and adjustment allow them all to be useful.

Models assume constant digestibility of AA within the digestible RUP (**dRUP**). In contrast, some source data would have higher or lower lysine digestibility depending on heating of protein sources. Combining the constant factoring of AA with repeatedly using library values because so few studies have measured AA flows to the duodenum and ileum, we are constantly using predictions as observations, which centralizes the variation and introduces interdependences in predictions (Tedeschi, 2019). Thus, prediction error increases as conditions move outside of the centralized data in derivation databases.

Practical Examples for Microbial Protein Compared with RUP

In high-producing dairy cows, we count heavily on feedstuffs high in RUP. Many speakers have discussed variation in RUP and the dRUP, which influence the real value in the diet. Many users just take the library values with the knowledge they are incorrect with each lot of feed but correct on average. Alternatively, an assay developed at Cornell University in 2013, the “Ross Assay” or “multi-step protein evaluation” incubates a feedstuff subsample in rumen fluid at 8 h or 16 h, and then assesses dRUP using in a cocktail of intestinal enzymes. However, the original intention of the assay was to target unavailable N computed in the last step—a value with higher repeatability. Many feeds have been assessed using 12- or 16-h in situ degradability (Liebe et al., 2018). Certainly, the passage rate of protein varies and is not known, as described above. However, using NASEM’s constant passage rates of 5.28 and 4.87%/h for concentrates and forages (Hanigan et al., 2021) for usage in NASEM (2021) would yield 18.9 and 20.5 h. If factoring in soluble protein having a faster passage, 16 h would appear to be a more reasonable endpoint than 8 h in the Ross Assay.

Many RDP sources, such as soybean meal, have consistent degradability such that library values are fairly reliable, and little screening needs to occur. However, animal protein byproducts can vary greatly in RDP and dRUP. In one of the few studies reported, feather meal varied considerably using the Ross assay compared with other standard approaches used in NASEM’s database (Buse et al., 2022). Evaluating both accuracy and precision, Estes et al. (2022) noted that up to 14 submissions would be needed for statistical certainty, with some differences among labs. However, no nutritionist will send in the same sample 14 times—they need to rely on a single result to make ration balancing decisions. Best practices include sending samples in at the same time to mitigate the run (batch) effect and maintaining a consistent lab to improve understanding of how protein digestibility can change with different sources.

Using anonymized commercial data shared from Cumberland Valley Analytical Services (Matt Michonski, personal communication, 2025) from the past 2 years, we will show in the presentation for the conference the variability of some of the more unpredictable RUP sources. The purpose of this exercise is to support our premise that microbial protein supply should be given strong credence to be able to make best usage of the more expensive RUP sources.

Starting with the variability in current blood meal supply, CP is predictably reliable, with averages at 101% on a DM basis (± 2.8% standard deviation). Total tract digestibility averaged 84.9 ± 14.0%), with 10% of the samples submitted over the past 2 years ranging between 30 and 70% unavailable N. The reason for including a protein like blood meal is to boost metabolizable essential AA such as histidine that will be limiting in other ingredients and must complement microbial protein's AA profile.

Canola meal samples submitted averaged 39% CP on a DM basis, possibly driven lower than expectations by nearly half of the samples as-received being low enough in CP to fail trade specifications. There were some (<15% of total) canola samples with poorer payload of digestible bypass protein (<10% dRUP on a DM basis). However, this appears to be because of differences in RUP, not its digestibility (i.e., dRUP). Estimates for RUP of canola meal as a % of CP averaged 49% ± 5.5%. In contrast with the high consistency of soybean meal as a commodity, we hoped to capture the real variability experienced in local crushing or home/custom crushing that can influence these measurements and their feeding value, especially as producers consider implementing a roasted high oleic bean in the future. Although CP and total tract digestibility are fairly consistent, the RUP portion is widely variable and should influence the value placed on roasted soybeans. Applying an extrapolated $0.80/lb value on dRUP, the protein feeding value of these soybeans ranges from $100 to 480/ton. This range in value is real money being left on the table in cow performance.

Finishing with distillers grains, the distribution of data is more limited by sample size. Even so, the current dataset indicates a greater %RUP than the NASEM (2021) book values on DDGS and likely reflects differences in analytical approaches discussed previously. The inference could be that more sampling should be included on farm for variation in bypass protein concentration for distillers grains (implied here by variable dRUP on a DM basis, ranging from 16 to 50% among submitted samples). The on-farm supply of distillers grains could be so variable load to load that sampling could be a vain effort. Instead, these data help direct us to consider instead the value of maximizing microbial protein supply and improving rumen efficiency before adding more expensive and variable RUP sources.

Dietary Application of Microbial Protein Value in CNCPS 6.55

As an illustration maximizing intake, digestible CHO, and N-source availability for microbial growth, we simulated several diets within NDS, which is a CNCPS 6.55 software platform. We used a basic diet heavy in corn silage (22 lbs DM), ground corn (10 lbs), soybean hulls (8 lbs), and soybean meal (6.5 lbs), complemented with canola

meal (5 lbs), sorghum silage, straw, mineral mix, and some other bypass soy, blood, fat, and methionine (8 lbs total). We then compared the effects of different changes to the estimated microbial protein without changing the nutrients delivered to the cow. The microbial protein supply only increased 1% for each 1 lb of additional corn. However, increasing the 7-h starch digestibility estimate by 10 points yielded 3% more microbial protein. As explained previously with respect to a degradation rate for starch, this assay probably is far more variable than is measuring starch per se, and we should be considering if extra starch decreases NDFD and efficiency of microbial protein synthesis.

We can simulate a more dramatic change by lowering dietary RUP (cutting blood meal and canola meal) and bypass fat by raising starch about 2 percentage points with ground corn and increasing soybean meal, with 0.01 lb more bypass methionine. The simulation resulted in a $0.15/cow/d savings yet increased the percent of MP from bacteria by 2.5 percentage points because we decreased dietary CP from over 17 to 16.2%. Considering the mass of AA yield increased from microbial origin, we added the equivalent of 1 lb soybean meal in EAA while decreasing the CP of the diet. The CNCPS model predicts a 3.5 lb increase in ECM. The point here is that we should be maximizing microbial protein prior to supplementing and evaluating these higher quality protein sources.

There seem to be systematic issues for NASEM and CNCPS 6.5.5 for essential AA when compared against observed data (Martineau et al., 2024). Some of these factors are being worked out. Authors suggested that the mean bias for lysine in NASEM (2021) could be a result of the addition of protozoal lysine outflow to the “microbial” fraction. However, this assumption can be countered. Only one main protozoal group avoids ruminal outflow, and it is relatively low in the lactating dairy cow (Firkins et al., 2020). Even more papers since then have documented protozoal outflow from the rumen — perhaps even more than that used in NASEM (2021). Instead, we would suggest that factoring of constant dRUP without consideration to how overheating depresses digestible lysine more than the other AA. In fact, increasing the supply of rumen-undegraded lysine increased milk protein linearly in a recent meta-analysis, perhaps because its intestinal supply was limited by heating of basal protein supplements (Arshad et al., 2024).

Conclusions

As the adage goes, “all models are wrong, but some are useful”. Our point throughout has been that all models can be more useful when the user has more mechanistic background. Feeding high corn is not necessarily a cheap way to provide protein to the cow because of decreased NDFD and efficiency of microbial protein synthesis. If corn is cheap and high RUP protein sources are not, then maybe this would be a good option. However, if high RUP protein sources are relatively expensive per unit of delivered metabolizable AA, we argue that we should be maximizing NDFD, DMI, and microbial protein before the more expensive protein pays off. Microbial protein is limited in histidine but could provide more lysine than traditionally thought (because of

protozoa). Maintaining ruminal pH while considering sugars (and soluble fiber) and sources of palmitic/oleic FA could help boost microbial protein. Hopefully, this paper will help field nutritionists to optimize supply of AA from microbial protein and dRUP for high producing dairy cows.

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Session Notes

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Improving Fiber Utilization in Dairy Cows: Technologies Available to Influence Production Outcomes

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The Need to Enhance Fiber Digestion

Forages contribute between 40% and 100% of dairy cow rations (Bargo et al., 2002) and are essential for sustaining animal productivity and overall health. Beyond being a key energy source, fiber promotes chewing, salivation, rumination, and gut motility, supports digestive health, buffers against ruminal acidosis, regulates feed intake, and serves as a precursor for milk fat synthesis. Additionally, fiber provides the structural framework for the ruminal raft, which is essential for digesting solid feed particles in the rumen. This paper highlights the strategic importance of enhancing forage fiber utilization and evaluates different technologies aimed at improving fiber digestion, discussing their mechanisms of action, cost-effectiveness, benefits, and potential drawbacks. Improving fiber digestion is critical for enhancing productivity, profitability, and environmental sustainability in dairy production. Incomplete fiber digestion limits dry matter intake (**DMI**) reduces milk yield, and increases manure output, ultimately diminishing farm profitability. For instance, in corn cell walls, every 1% increase in lignin concentration—the primary inhibitor of fiber digestion—reduces cell wall degradability by 2% (Grabber, 2005). Similarly, a one-unit increase in forage neutral detergent fiber digestibility (**NDFD**) has been linked to increases of 0.17 kg/day in DMI and 0.25 kg/day in milk production (Oba & Allen, 1999). In perennial ryegrass (*L. perenne*), a 5–6% increase in digestibility has been estimated to boost milk production by as much as 27% (Smith et al., 1998). Consequently, elevated lignin levels in forage cell walls impose significant constraints on DMI and milk production. In addition, enhancing fiber digestion maximizes the energy derived from fibrous feed resources that are not suitable for human consumption. While grains are energy-dense and widely used in livestock diets, their availability is increasingly constrained by competition with human food production, non-ruminant animal feed, and biofuel industries, leading to price volatility. In contrast, fibrous feedstuffs for ruminants face less direct competition but remain underutilized due to their complex lignocellulose structure. Effective strategies are therefore needed to improve the efficiency of forage fiber digestion and unlock its full energy potential.

Key Factors Influencing Forage Fiber Utilization

Preharvest factors

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The NDFD values across different seasons and years, as depicted in the **Figure 1**, highlight the significant impact of genotype and environmental conditions on fiber digestibility in hybrid corn. The observed variability in NDFD across years and between spring and summer seasons underscores the influence of genetic differences among hybrids, which directly affect plant composition, fiber structure, and digestibility. Additionally, environmental factors such as temperature, rainfall, soil conditions, and growing season duration further contribute to fluctuations in NDFD by influencing plant maturity and fiber composition at harvest. The wide range of NDFD values within each season and year suggests that selecting appropriate corn hybrids with superior fiber digestibility is crucial for optimizing ruminant nutrition. These findings reinforce the importance of hybrid selection and environmental adaptation in improving forage quality and feed efficiency in livestock production systems. Brown-midrib (**BMR**) mutants have been widely studied as a genetic approach to improving fiber digestibility in forages by reducing lignin or indigestible neutral detergent fiber (**NDF**) concentrations (Grant and Ferraretto, 2018). BMR variants of corn, sorghum, and pearl millet have consistently exhibited lower lignin concentrations than their conventional counterparts, leading to enhanced nutrient digestibility and increased milk production in dairy cows (Sattler et al., 2010). Studies have shown that BMR corn silage improves DMI, passage rate, and NDF digestion compared to conventional corn silage, ultimately leading to higher milk and protein yields (Oba and Allen, 2000; Ferraretto and Shaver, 2015). BMR sorghum also demonstrates superior fiber digestibility compared to conventional sorghum, with evidence supporting its potential as an alternative forage for dairy production, particularly in regions unsuitable for corn cultivation (Dann et al., 2008). Pearl millet, another alternative, has shown promise due to its adaptability to low-fertility and drought-prone soils, with BMR varieties displaying greater digestibility than conventional millet (Harper et al., 2018). However, despite these benefits, BMR hybrids often yield less biomass than conventional hybrids, requiring careful consideration of their cost-effectiveness and strategic use in feeding programs. Establishing guidelines for targeted feeding, such as prioritizing BMR forages for high-producing cows in early lactation, can maximize their benefits while balancing economic and agronomic constraints (Bernard and Tao, 2015). Further long-term studies are needed to assess the full impact of BMR forages on lactation performance and farm profitability.

Healthy plants play a crucial role in maintaining fiber digestibility, increasing starch yield and biomass production, and allowing for a wider and more flexible harvest window. These factors are influenced by hybrid genetics, specific traits, fungicide application, and soil fertility. According to 2023 data from Michigan on hybrids yielding 23–30 tons per acre at 35% dry matter (**DM**), for every one percentage point increase in silage DM, NDFD at 30 hours decreases by 0.2 percentage points, while starch content increases by 0.6 percentage points, equivalent to an additional five bushels of dry grain per acre. In healthy plants, increasing DM from 32% to 37% results in an additional 25 bushels per acre of dry grain equivalent, a three-percentage-point increase in starch content, and a one-percentage-point reduction in NDFD30. However, in diseased plants, delaying harvest by a week can significantly reduce NDFD30 from 60.9% to 46.4% (Personal communication: Bill Mahanna).

Postharvest factors

Mechanical processing: Mechanical processing is a critical aspect of forage production due to its impact on forage physical properties, which influence gut fill and feed intake in dairy cattle. The primary objective of mechanical processing is to optimize forage particle size to ensure adequate levels of physically effective neutral detergent fiber (**peNDF**), which is essential for stimulating chewing activity, promoting salivation, and maintaining ruminal function (Hall and Mertens, 2017). Particle size can be adjusted through chopping, which balances the benefits of coarse particles—such as improved ruminal mat formation and pH stability—with the disadvantages of reduced passage rate and increased gut fill (Allen, 1997). Although manipulating particle size has been studied extensively, research findings remain inconsistent, as fiber digestion is influenced by multiple factors, including microbial attachment and passage rate (Johnson et al., 1999). A meta-analysis by Ferraretto and Shaver (2012) found that digestibility of dietary NDF, DMI, and milk production were not significantly affected by corn silage chop length, suggesting that the trade-offs between short and long particle sizes may balance out.

Shredding is another processing technique that alters the physical characteristics of silage, particularly in whole-plant corn silage. Corn shredlage, produced using forage harvesters equipped with cross-grooved processing rolls and longer theoretical lengths of cut (22–26 mm), has been shown to increase milk yield by 1.0 to 1.5 kg/cow per day compared to conventionally processed silage (Ferraretto and Shaver, 2012; Vanderwerff et al., 2015). These improvements are attributed to enhanced kernel breakage and increased ruminal starch digestibility. However, despite its apparent advantages, feeding corn shredlage has been associated with a slight reduction in total tract NDFD, likely due to its higher starch digestibility (Vanderwerff et al., 2015). Further analysis of commercial samples also indicated that 30-hour NDFD was lower in corn shredlage (55.0% vs. 53.4% of NDF) compared to conventionally processed corn silage (Ferraretto et al., 2018). These findings suggest that while corn shredlage enhances starch digestibility and milk production, its effects on fiber digestion require further investigation to fully understand its implications for ruminal fermentation dynamics. Despite the demonstrated benefits of corn shredlage in enhancing starch digestibility and milk production, the economic feasibility of adopting this technology warrants further evaluation. The additional costs associated with acquiring and maintaining a shredlage processor, as well as potential changes in fuel consumption and roll wear, must be considered when determining its cost-effectiveness relative to conventional processing methods. Currently, there is a lack of published data on these economic aspects, highlighting the need for future research to assess the long-term benefits and trade-offs of adopting shredlage processing (Ferraretto and Shaver, 2012a; Vanderwerff et al., 2015). Understanding the in vivo NDF digestion kinetics of corn shredlage could further clarify its role in dairy cattle nutrition and assist producers in making informed decisions about its implementation.

Silage *inoculation:* Some silage inoculant preparations contain fibrolytic enzymes, mainly cellulases or xylanases and studies have reported increased NDFD due to

application of such products (Queiroz et al., 2012). However, except for ferulic acid esterase (**FAE**), the enzyme secreting ability of inoculant bacteria are rarely declared and in recent meta-analyses, although no effects on NDFD were observed when bacterial homofermentative and facultative heterofermentative inoculants were applied to forages, milk yield was improved (Oliveira et al., 2017). Addah et al. (2011) reported that using a mixed bacterial culture containing *L. buchneri* LN4017 that produces FAE, and contains *L. plantarum* and *L. casei,* increased in situ NDF disappearance after 24 and 48 h of incubation by 40.5 and 14.5 %, respectively. Yet in their later study, feeding finishing steers barley silage using the same inoculant did not affect the growth performance and intake of steers (Addah et al., 2014). Other studies did not detect increases in NDFD when such inoculants where applied to alfalfa (Lynch et al., 2014) or corn silage (Lynch et al., 2015). The inconsistency in effects may be because expression of FAE depends on forage type and ensiling conditions (Muck et al., 2018) or because the targeted ester linkages are obstructed by the lignin polymer (Jung and Allen, 1995).Recently, Nino de Guzman et al. (unpublished; Figure 3) observed tendency towards greater total-tract NDFD in early lactation dairy cows fed TMR with corn silage and ryegrass silage inoculated with *L. lactis* and *L. buchneri.* More information is needed on the enzyme activities produced by inoculant bacteria, as this may lead to development of inoculants that are more potent at increasing fiber digestion.

Feeding factors

Trace minerals: The source, quantity, and solubility of trace minerals likely plays a key role in their effects on fermentation and microbial populations, which may, in turn, influence nutrient digestibility. Faulkner and Weiss (2017) observed greater NDFD in cows fed hydroxy minerals when compared with sulfate sources of Cu, Mn, and Zn (Figure 4). The improved NDF digestibility observed in cows fed hydroxy minerals in this study may be attributed to lower concentrations of soluble Cu, which could reduce its inhibitory effects on ruminal bacteria. Another potential explanation is that increased soluble Zn enhanced microbial activity, thereby promoting NDF digestion. However, reported differences in Zn solubility between hydroxy and sulfate sources have been inconsistent. Similarly, Miller et al. (2020) observed greater NDFD and DMI in cows fed corn-silage based diet with hydroxy source of trace minerals.

Novel technologies

Expansin treatment: Expansins and expansin-like proteins are a group of non-hydrolytic proteins that induce cell wall loosening, enhancing cellulose hydrolysis by cellulases and hemicellulases. These proteins are structurally characterized by a double-psi beta-barrel (**DPBB**) domain, related to glycosyl hydrolase 45 but lacking β-1,4-glucanase activity (Georgelis et al., 2011). While plant expansins are classified into α- and β-expansins, only α-expansins exhibit cell wall loosening activity (Cosgrove, 2015). Expansin-like proteins have also been identified in bacterial and fungal genomes, albeit lacking conserved plant expansin motifs (Liu et al., 2015). BsEXLX1 from *Bacillus subtilis* serves as a model for studying their role in cellulose hydrolysis due to its ease of

expression in *E. coli* (Silveira and Skaf, 2016). These proteins synergize with exogenous fibrolytic enzymes (**EFE**), significantly increasing fiber hydrolysis, with BsEXLX1 improving cellulose digestion by over fivefold in synergy with EFE (Bunterngsook et al., 2015). Recent studies demonstrated that BsEXLX1 has high affinity for lignin-rich substrates, which could enhance EFE efficacy in digesting highly lignified forages like C4 grasses and legumes (Kim et al., 2013). While research has primarily focused on biofuel applications, preliminary studies suggest that BsEXLX1 can improve fiber digestion in ruminant diets. A pilot study using bermudagrass silage found that combining BsEXLX1 (165 μg/g DM) with EFE improved IVDMD and IVNDFD by 4% and 16%, respectively, compared to EFE alone (Pech-Cervantes et al., 2017a). However, excessive BsEXLX1 doses (>400 μg/g DM) reduced EFE efficacy and volatile fatty acid production (Pech-Cervantes et al., 2017b). Further research is needed to optimize BsEXLX1 production for large-scale application, with transgenic plants offering a promising strategy for cost-effective expansin production (Yoon et al., 2016).

Yeast or Yeast Culture or Yeast Fermentation Product Supplementation: Yeast products, including live yeast and yeast cultures derived from *Saccharomyces cerevisiae*, are widely used as feed additives to enhance fiber utilization and animal performance, though their effects vary. Studies have demonstrated that yeast supplementation improves nutrient digestibility and milk production (Jiang et al., 2017a), while others report no significant effects on digestibility, dairy cow performance, or ruminal microbiome composition (Ouellet and Chiquette, 2016). Meta-analyses indicate that live yeast supplementation increases organic matter digestibility, DMI, and milk yield (Poppy et al., 2012). Yeast effects are more pronounced in high-grain diets due to their role in stabilizing rumen pH, reducing lactate accumulation, and enhancing microbial activity (Chaucheyras-Durand et al., 2005). Yeast also facilitates the growth of cellulolytic bacteria such as *Ruminococcus albus* and *Fibrobacter succinogens* by providing essential nutrients (Callaway and Martin, 1997), which has been confirmed through metagenomics studies (Jiang et al., 2017b). Additionally, yeast supplementation influences ruminal fungi and protozoa, further improving fiber digestion (Ding et al., 2014). However, the response to yeast varies depending on diet composition, lactation stage, and environmental factors, necessitating further research to optimize yeast formulations for consistent benefits in fiber digestibility and dairy cow performance.

White and Brown-Rot Fungi: White-rot fungi achieve lignin depolymerization through ligninolytic enzymes, including lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccase, as well as extracellular reactive oxygen species that initiate lignocellulose decay (Sindhu et al., 2016). These fungi can selectively degrade lignin while preserving cellulose, making them beneficial for improving fiber digestibility in ruminants (van Kuijk et al., 2015). Studies have shown increased in vitro digestibility of fiber fractions in wheat straw treated with certain strains of white-rot fungi, such as *Ceriporiopsis subvermispora* (Nayan et al., 2018). Feeding trials in ruminants have demonstrated improved DMI, digestibility, and performance when white-rot fungi-treated forages were included in the diet (Shahzad et al., 2016). However, the widespread application of white-rot fungi is limited by long pretreatment times and potential carbohydrate degradation (van Kuijk et al., 2015). Brown-rot fungi, in contrast, modify

rather than remove lignin, employing reactive oxygen species to depolymerize cellulose and hemicellulose, thereby increasing accessibility to enzymatic hydrolysis (Kaffenberger and Schilling, 2015). Although brown-rot fungi have been primarily studied in biofuel applications, limited studies have demonstrated improvements in fiber digestibility when used in ruminant diets (Gao et al., 2012). Despite the potential of fungal treatments to enhance fiber utilization, further research is needed to identify strains that maximize lignin degradation while minimizing losses of fermentable carbohydrates.

Conclusions

Enhancing fiber utilization in dairy cow diets is essential for improving productivity, profitability, and sustainability in the dairy industry. Forage fiber plays a crucial role in rumen health, digestion, and milk fat synthesis, making its efficient utilization a priority. However, factors such as lignin content, genetic variability, environmental conditions, and dietary strategies influence fiber digestibility. Brown midrib hybrids have shown consistent success in increasing fiber digestion, though their lower biomass yields necessitate strategic implementation. Mechanical processing methods, such as shredding and particle size manipulation, offer potential benefits but require a balance between physical effectiveness and digestibility. Biological treatments, including bacterial inoculants, fibrolytic enzymes, and yeast supplementation, have demonstrated varying degrees of success in improving fiber digestibility and milk production, with meta-analyses supporting their efficacy in certain contexts. Emerging technologies such as expansins and fungal treatments offer promising avenues for breaking down lignin and enhancing fiber digestion, though further research is needed to refine these approaches and assess their economic viability. Overall, a combination of genetic, mechanical, chemical, and biological strategies is necessary to optimize fiber utilization in dairy production. Continued research is required to develop cost-effective and practical solutions that maximize the energy potential of forages while maintaining environmental and economic sustainability. Future advancements in omics technologies and precision feeding strategies may further enhance the efficacy and consistency of fiber utilization techniques, ultimately benefiting dairy producers and improving livestock performance.

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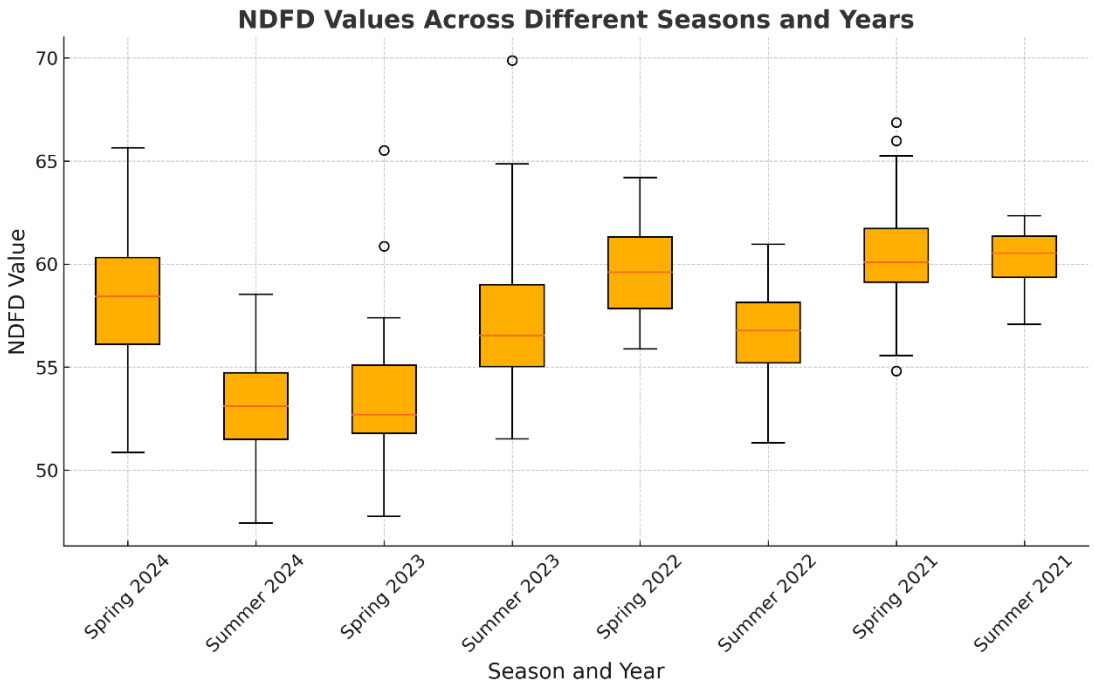

Figure 1. Comparison of NDFD values across seasons and years in hybrid corn trials.

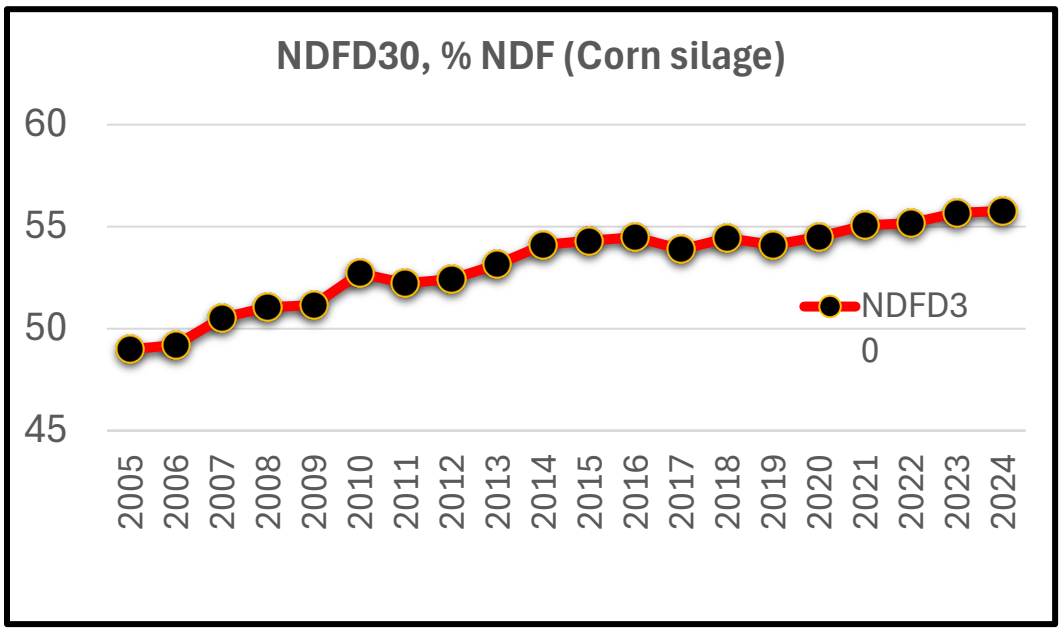

Figure 2. Trends in NDFD30 of silage: insights from the DairyOne feed composition library (2005–2024)

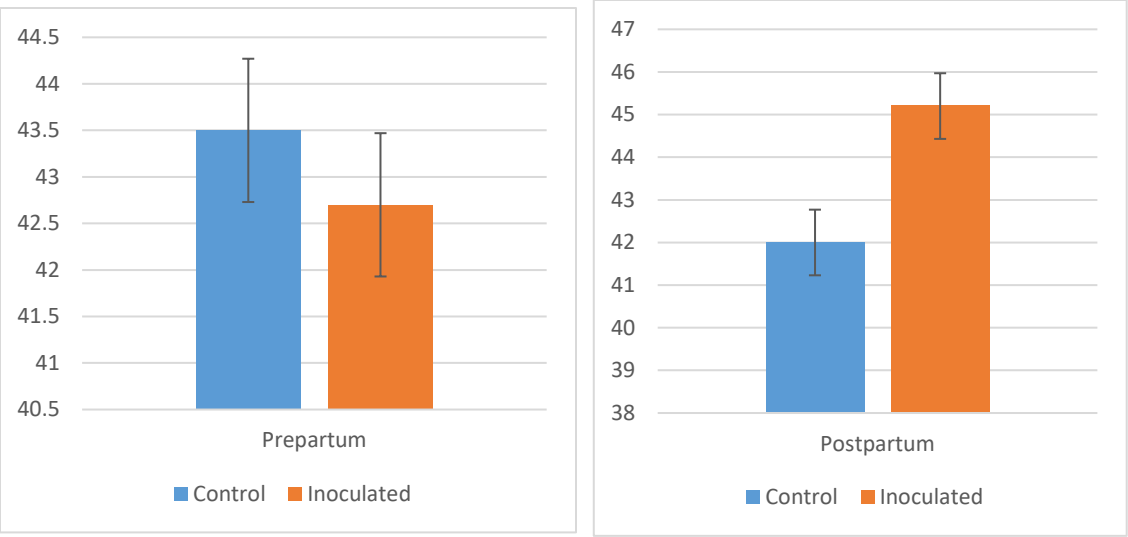

Figure 3. Effects of feeding silage-based total mixed ration containing corn silage and ryegrass silage ensiled with (INO) or without (CON) microbial inoculant on the total-tract apparent nutrient digestibility of transition dairy cows (Prepartum: Treatment *P*-value = 0.48; Postpartum: Treatment *P*-value = 0.06; Nino de Guzman et al., unpublished)

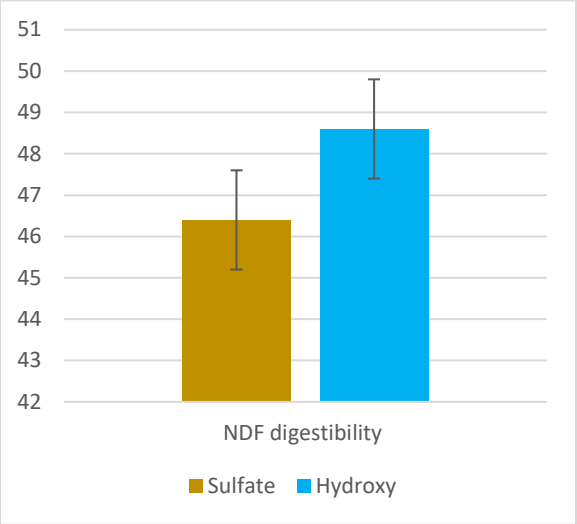

Figure 4. Effects of feeding diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources on neutral detergent fiber digestibility (Mineral source P value: 0.02; Results adapted from Faulkner and Weiss, (2018).

Session Notes

The Role of Buffers and Alkalinizers to Improve Rumen Function and Animal Performance

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Introduction

Dairy cows typically digest more than 50% of the feed consumed in the rumen. Some nutrients are degraded in the rumen, such as proteins, and then fully digested (either in the form they were ingested or in the form of bacterial protein) and absorbed in the intestine, whereas other nutrients, such a fraction of carbohydrates consumed by the cow are partially digested, and their fermentation end-products and absorbed directly in the rumen. Most nutrients consumed by cows could be digested and utilized in the intestine (i.e. starch, protein, and fat), but ruminants, and non-ruminant animals, lack the ability to synthesize cellulases and other enzymes involved in the breakdown and digestion of fiber; thus, ruminants depend on the enzymes produced by rumen bacteria to digest and extract energy from the rations they consume. Typical dairy rations contain about 27- 30% fiber (cellulose, hemicellulose, and lignin), which represents ~25% of the gross energy of the diet. Whether this gross energy is converted into net energy for maintenance and lactation depends mainly on quality of the ingredients containing fiber and the conditions of the rumen and the fermentation activity by rumen bacteria.

In an attempt to provide sufficient energy to high-producing cows, the proportion of nonfiber carbohydrates (**NFC**) in the diet is commonly increased at the expense of fiber or forage. These rations are effective in promoting a vigorous fermentation in the rumen and thus have potential to sustain high levels of milk production, but they can also overwhelm the rumen with the accumulation of large amounts of fermentation end-products, mainly volatile fatty acids (**VFA**), and in some instances lactic acid, which may cause a reduction in rumen pH. Rumen acidosis can be classified as chronic, with an average rumen pH about 5.6, acute with an average rumen pH around 5,2, and subacute with an average rumen pH between 5.2 and 5.6. However, in practice, it is difficult to find all these forms of rumen acidosis, mainly because cows that experience reductions in rumen pH rapidly reduce the amount of feed they consume, and thus rumen conditions return to normal, although that may be accompanied by a reduction in milk production or other physiological functions such as reproduction. The mechanisms behind this reduction of intake when rumen pH decreases are multiple. One of the reasons is a stimulation of the immune system and its concomitant release of cytokines into the blood stream. When pH of the rumen fluid is low, it may damage the rumen epithelium and make it permeable to some molecules. For example, under low rumen pH, several Gram negative bacteria may die and fragments of their cell wall, such as

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lipopolysaccharide, may translocate into the bloodstream if the rumen wall is damaged. This translocation may elicit an immune response (Plaizier et al., 2012); which may lead to increased circulating concentrations of haptoglobin and TNF-α in blood (Albornoz et al., 2020). Cytokines, such as TNF-α, are potent inhibitors of feed intake (Kushibiki et al., 2003; Bradford et al., 2009; Yuan et al., 2013) and they also reduce gastric motility (Plaza et al., 1997, Lippolis et al., 2017). However, it seems that continuous exposure to high concentrations of TNF-α hinder the negative effect on intake (Martel et al., 2014), and thus, it could be speculated that overtime, cows might become less sensitive to episodes of low rumen pH and continue to eat under those situations (although, as seen below, their digestive capacity may be hampered).

In addition to the potential loses in milk production due to reductions in intake when rumen pH decreases, digestion may also be compromised (Beauchemin, 2000; Bach et al., 2005), further contributing to an impairment of milking performance and altered milk composition (Nocek, 1997). Fibrolytic bacteria are sensitive to pH. When rumen pH falls below 5.8; most rumen bacteria lose the capacity to attach to feed particles and fiber (Sung et al., 2007) and thus, their fibrolytic enzymes cannot reach their substrate and fiber degradation is stopped. If these conditions of low ruminal pH persist, most fibrolytic bacteria are washed out from the rumen through passage rate and replaced by other bacteria that survive and continue to digest nutrients other than fiber in the rumen at low pH. This situation compromises the ability of cows to fully extract energy from the ration consumed resulting in lowered milk production along with concomitant changes in milk composition.

Prevention of Rumen Acidosis: Ration Formulation

When nutritionist perceive a risk of rumen acidosis, one of the first interventions they tend to take consist of increasing the amount of fiber in the diet, hoping that this will stimulate mastication, rumination, and saliva production, and that this saliva will increase rumen pH due to its supply of phosphate and bicarbonate. However, as discussed above, it could be argued that the real cause of rumen acidosis, is not an inadequate supply of fiber (and insufficient saliva production), but an excessive supply of rapidly fermentable carbohydrates (**CHO**). Thus, when nutritionists add more fiber to the diet, they basically reduce the proportion (and supply) of NFC, and thus, they indirectly correct the root of the problem (i.e., an excess of rapidly fermentable CHO). But, another way of 'correcting' the problem, would be to substitute part of the NFC by slowly degradable CHO sources (such as coarse corn), or increasing the particle size of the grains used in the diet, as this would reduce the rate of acid being produced. Another option could be to substitute part of the dietary NFC by fat, which is not fermented in the rumen and does not contribute to the acid load of the ruminal fluid. Also, regarding fiber, to prevent rumen acidosis, it is commonly suggested to provide a minimum of physically effective fiber (**peNDF**) in the ration. Physically effective fiber is defined as the proportion of dietary NDF contained in particles above 1.8 mm according to Mertens (1997) and above 8 and 19 mm according to Lammers et al. (1996). It is still unclear which of these two approaches best predicts chewing times, saliva production, and more importantly rumen buffering capacity (Einarson et al., 2004). A number of

studies have been conducted to evaluate the effects of peNDF, but the results are not conclusive. Several authors have reported a relationship between peNDF and rumen pH (Soita et al., 2000; Krause et al., 2002; Beauchemin et al., 2003; Calberry et al., 2003), whereas others have not (Yang et al, 2001; Knonoff et al., 2003; Kononoff and Heinrichs, 2003; Einarson et al., 2004). Likely, the discrepancies in the literature stem from 1) sorting activity of cows, 2) small differences in saliva secretion, and most important 3) the rate and type of fermentation of entire diet but especially that of the non-forage components of the ration.

Prevention of Rumen Acidosis: Direct Fed Microbials

Some feed additives based *on* direct fed microbials (**DFM**) either yeast- or bacteria-derived have been proposed and used in the field to control rumen acidosis.

Most yeast are unable to proliferate in the absence of oxygen, although there are some yeasts, such as some strains of *Pichia* that divide in anaerobiosis and are found in the rumen of cows. Another yeast able to multiply in anaerobiosis is *Saccharomyces cerevisiae*, although this species is not part of the natural rumen microbiome, it is capable of growing the in the rumen provided there is ergosterol produced by other bacteria (Krause, 2023). Reports in the literature are inconsistent in terms of outcomes in rumen pH when supplementing dairy cows with live yeast. For example, Chung et al. (2011) evaluated the effects of two live yeast strains on rumen fermentation *in vivo* and reported either no change with a strain previously reported to elicit an increase in rumen pH (Bach et al., 2007; Thrune et al., 2009) or a reduction in rumen pH with a strain previously reported to increase fiber degradation *in vitro* (Chaucheyras-Durand et al., 2008). But other studies have reported no changes in rumen pH when supplementing live yeast (Ambriz-Vilchis et al., 2017; Cattaneo et al., 2023). Part of the variable response to live yeast is likely caused by the strain (Chaucheyras et al., 2008), the type of ration (Dias et al., 2018; Ferreira et al., 2019), and the dose used.

In ruminants, most bacterial DFM have targeted intestinal health of young animals, mainly calves, although some studies have explored the use of bacteria to modulate rumen pH. But in the last decade, there has been an interest in using bacteria-based DFM to control rumen pH. Philippeau et al. (2017) reported an increase in rumen pH when supplementing *Propionibacterium* spp. and *Lactobacillus* spp. to dairy cows. Aikman et al. (2011) reported a positive response in ruminal pH when supplementing cows with *M. elsdenii*/d. But Zebeli et al. (2012) reported no changes in ruminal pH when supplementing cows with the same DFM. As it seems to be the case for yeast, the strain combination and the dose at which bacteria-based DFM are supplemented may also play a role behind the discrepancy in the results.

Prevention of rumen acidosis: Buffers and Alkalinizers

Another alternative to control rumen pH consists of using buffers and alkalinizers.

Buffers are substances that prevent large variations in pH of a solution; whereas alkalinizers are substances that counteract or neutralize acidity. One of the most common feed additives to control rumen pH is sodium bicarbonate (SB). Sodium

bicarbonate is a buffer. Most of the research studies reporting increases in rumen pH when adding SB to the rations use dietary inclusions >0.7% (Erdman et al, 1982; Rogers et al., 1985; Neville et al., 2019). When smaller doses of SB are used ruminal pH fails to be kept above acidosis level, with a range between 0.7 and 1.2% being considered optimum (Hu and Murphy, 2005). This means that if we aim to control rumen pH using SB, a minimum of about 200-250 g/d of SB should be fed to a lactating cow. Furthermore, it seems that SB, even when fed at 1% of the DM is not effective in modulating rumen pH when the TMR has no corn silage. Hu and Murphy (2005) conducted a metanalysis and concluded that SB had no effect on TMR that did not contain corn silage. Economically, supplementing TMR with about 250 g of SB carries a direct cost of about 10 cents/cow/d. But, in practice, the cost of adding 1% of SB to a dairy ration is much greater than the direct cost of SB. If SB is added, now there is less space in the ration to provide all the required nutrients to sustain milk production which means that the nutrient density of the diet must be increased (i.e., increase the percentage of protein, increase the concentration of energy) and these indirect costs may be greater than the direct costs. For example, in a ration costing 10.37 €/cow, the addition of 270 g of SB, would raise the cost to 10.62 € cow/d (10 cents from SB, and 0.15 cent to the increase in nutrient density of the diet).

One can estimate the theoretical change in rumen pH when supplementing cows with SB using the following adapted Henderson-Hasselbalch equation proposed by Kohn and Dunlap (1998):

$$\text{Rumen pH} = 7.74 + \log([HCO_3^-]/\text{partial } CO_2 \text{ pressure})$$

Imagine an initial rumen pH of 5.8, which implies, assuming a partial CO_2 pressure in the rumen of 0.7 atm (Barry et al., 1977), that the concentration of HCO_3^- in the rumen would ~0.008 m*M*. An addition of 250 g of SB is equivalent to about 2.98 mols, as SB has a mas of 84 g/mol. These additional 2.98 mols of HCO_3^- to the 150 l of rumen fluid would increase rumen pH to ~6.3. However, in the field and in the literature, is not uncommon to find SB supplementations in the TMR around 100 g of SB. In this case, the impact on rumen pH would be lower, as only 1.19 mol of SB would be available to buffer rumen acidity rendering a rumen pH ~ 6.1 (assuming the same conditions as above). Another alternative would be to supplement alkalinizers, with a stronger action on rumen pH which would allow to use smaller doses in the ration and lower both direct and indirect costs. A common alkalinizer used in dairy nutrition is magnesium oxide (**MgO**). This mineral, per se, is not a base, but it can be converted to magnesium hydroxide, which is a relatively powerful base, when MgO is solubilized following this reaction:

$$MgO + H_2O \rightarrow Mg(OH)_2 \rightarrow Mg^{2+} + 2OH^-$$

Then, OH^- can react with CO_2 in the rumen fluid generating carbonate or bicarbonate:

$$CO_2 + OH^- \rightarrow HCO_3^-$$

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$$

For every mol of MgO solubilized there is a release of 2 mols of OH^-. Thus, considering a molecular mass of 40.3 g/mol, 70 g of MgO would provide 3.48 mols of OH^- if all MgO was fully solubilized in the rumen fluid, which is not typically the case. Given that pH is expressed in log scale, a solution with pH~5.8 implies that it has a concentration of about 1.58×10^{-6} mol of H^+/l ($10^{-5.8}$). This means, we have a solution containing a total of 2.37×10^{-4} mols of H^+. Thus, the mols of OH^- (3.48) would clearly outnumber the mols of H^+ (2.37×10^{-4} mols) present in 150 l of rumen fluid with a pH of 5.8, and theoretically, rumen pH would increase significantly (>7.50). However, because the rumen is an anaerobic environment with high concentrations of CO_2, a large proportion of OH^- will bind to carbonic acid (derived from the CO_2 dissolved in the rumen) to produce bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), limiting a bit the rise in pH as these compounds act as buffers preventing large shifts in pH. Typically, concentrations of CO_2 dissolved in the rumen fluid are about 40 m*M*, and using numerical solvers it can be estimated that the final pH after adding MgO, considering the role of CO_2, would be about 6.5, equivalent, if not greater than what it would be obtained with 250 g of SB (at a greater cost). However, differently from SB, MgO may not easily dissolve in the rumen fluid. The solubility of MgO depends greatly on the source and the process of the product. In general, the greater the calcination temperature and the lower the particle size of MgO the greater its solubility. Interestingly, the solubility of MgO is negatively correlated with rumen pH (Martens et al., 2018), that means that as rumen pH decreases a greater proportion of MgO is solubilized and may contribute to hinder further reductions in acidity.

The stronger action of MgO than SB on rumen pH has been recently demonstrated in vitro by Darwin (2019), who showed that after adding glucose to rumen fluid *in vitro*, the pH of the rumen fluid was greater in MgO than in SB, and the production of lactic acid was virtually non-existent with MgO (Figure 1). Two other recent studies (Bach et al, 2018; 2023) compared, *in vivo*, the evolution of rumen pH through the day when supplementing SB or MgO to cows exposed to a rumen acidosis challenge consisting of sequentially removing forage and substituting by barley starting at forage to concentrate ratio (**FCR**) of 48:52, then 44:56, then 40:60, and finishing at 36:64. In the first study (Bach et al., 2018), when cows were fed the TMR with the lowest FCR, only those supplemented with MgO maintained rumen pH stable and had greater average rumen pH (5.93±0.04) than unsupplemented cows (5.83±0.04) or cows supplemented with SB (5.85±0.04). Furthermore, MgO cows spent less time (32.3±6.1%) with rumen pH ≤ 5.8 when fed the lowest FCR than unsupplemented cows and cows supplemented with SB (50.7±5.02%). In a follow-up study (Bach et al., 2023), using the same experimental protocol to induce rumen acidosis, unsupplemented cows and cows fed SB consumed less DM (23.5 kg/d) than cows supplemented with MgO (25.1 kg/d) when fed dietary FCR of 44:56 and 40:60 (Figure 2). Most studies report an increase in DMI when the

dietary FCR decreases (Kargar et al., 2010). This reduction in DMI, was most likely due to a reduction in rumen pH. The reduction of dietary FCR resulted in a decrease in rumen pH, and rumen acidosis has been shown to hamper DMI (Gao and Oba, 2016). Interestingly, as shown in Figure 3, cows fed MgO were able to maintain rumen pH even though DMI was greater (i.e. their rumens were filled with greater amounts of fermentable material). Lastly, rumen bacterial population of fibrolytic bacteria, even though it was reduced as FCR decreased, was greater in cows fed MgO than in cows unsupplemented or supplemented with SB.

Lastly, some nutritionist may be concerned about reducing the dietary action anion differences (DCAD) of the diet if SB is removed or not included in the TMR. Dietary cation anion difference describes the relative balance between the most important cations (K and Na) and the most important anions (Cl and S) in dairy rations. It is expressed in mEq per kg or per 100 g, and mEq are determined as (mass x valence)/molecular weight. The DCAD equation cited by Ender et al. (1962) and used by Block (1984) is the one most applied in ruminants. This equation considers the four main anions and cations:

$$\text{DCAD, mEq/kg} = (Na^{+} + K^{+}) - (Cl^{-} + S^{2-})$$

But, later, Horst et al. (1997) and the NRC (2001) recommended to account for the effect of bivalent cations to calculate DCAD with the following equation to account for the strength and bioavailability of Ca, Mg, and S:

$$\text{DCAD, mEq/kg} = (0.15\ Ca^{2+} + 0.15\ Mg^{2+} + Na^{+} + K^{+}) - (Cl^{-} + 0.6\ S^{2-}),$$

This equation was later amended by Goff (2000) accounting for the contribution of P3−.

Including the multivalent minerals such as Ca, Mg, P, and S in the DCAD calculation faces the challenge of including large variation from the bioavailability of these ions compared with that of Na, K, and Cl. Thus, nutritionist need to be aware of the drastic difference in DCAD values that are reported in formulation programs (based on the way DCAD is calculated). Also, caution should be placed when looking at DCAD values because if DCAD does not account for Ca, Mg, and P it provides an incomplete picture of the acid-base status of the animal, but even if it does, the figures are likely to be incorrect because of uncertainties about actual bioavailability of some cations and anions.

Tucker et al. (1988) was the first to evaluate DCAD in lactating dairy cows and reported that milk yield was increased by 9% when a diet with DCAD of 200 was compared with a ration with a -100 mEq/kg of DM. This led to the hypothesis that manipulating DCAD might benefit milk production. In fact, subsequent studies (West et

al., 1991, 1992; Delaquis and Block, 1995a,b) also reported improvements in milking performance. Hu and Murphy (2004) conducted a metanalysis using data from 1984 and 1997 and concluded that maximum milk yield and maximum feed intake were reached when DCAD was between 340 and 400 mEq/kg of DM, respectively. However, level of milk yield and intake were relatively low compared to current production standards. Average milk yield and feed intake of the studies included in the meta-analysis were 23.0 and 18.5 kg/d, respectively. A more recent meta-analysis conducted by Iwaniuk and Erdman (2015) using studies between 1980 and 2010 with an average of 19.5 kg/d of dry matter intake and 27.6 kg/d of milk yield (with the majority of studies increasing DCAD by providing more SB to the diet) reported that increasing DCAD tended to result in a nonlinear increase in milk yield and dry matter intake increased in a curvilinear fashion. Their most relevant conclusion was that the tendency towards increased milk yield and the increase in feed intake were most likely due to changes in ruminal pH, feed digestibility, and ruminal volatile fatty acid concentrations.

Most studies evaluating the impact of DCAD on intake and milk yield include a treatment with a negative DCAD. It is well known that negative DCAD cause a depression in feed intake, which has been proposed to be due to low acceptance by cows for the salts use to provide anions (West et al., 1991). Maynou et al. (2018) recently demonstrated that, independently of a potential negative effect on palatability, feeding anionic salts containing calcium sulfate, calcium chloride, and ammonium chloride exerts a drastic negative effect on intake. These authors provided anionic salts via a bolus delivered directly into the rumen of the cows, and thus any potential effect on palatability was discarded. In that study, feed intake of lactating cows decreased by 2.9 kg within 24 h of administration and it took about 4 days to fully recover intake following a single dose of anionic salts (Figure 4). Thus, when assessing the impact of acid-base balance on intake of lactating cows, it would be desirable to exclude treatments with negative DCAD.

Several studies indicate that DCAD above 250-300 mEq/kg of DM have no further benefits on feed intake. For example, Sanchez et al. (1994) estimated that milk yield and dry matter intake were optimized at a DCAD of approximately 150 to 200 mEq/kg of DM. Sanchez and Beede (1996) suggested that both milk yield and DMI were maximized at a DCAD concentration equal to 280 mEq/kg of DM. Feed efficiency was improved by 7.7% when the DCAD increased from 250 to 340 mEq/kg of DM using K_2CO_3 supplementation (Erdman et al., 2011). Martins et al. (2016) reported that when DCAD increased from -70 to 290 mEq/kg of DM, both NDF digestibility and blood pH also increased, but milk yield was maximized at 190 mEq/kg of DM. Also, Chan et al. (2005) reported that 230 and 330 mEq/kg of DM is adequate to optimize milk yield, whereas DCAD of 500 mEq/kg of DM may be excessive and decreases dry matter intake. Similarly, Roche et al. (2005) drenched post-partum cows with various combinations of minerals to alter DCAD from 320 to 880 mEq/kg of DM and concluded that DMI was not affected by DCAD; again, supporting the concept that DCAD above 250-300 mEq/kg of DM provides no benefits to cows. A relatively recent study (Iwaniuk et al., 2015) assessing the effects of feed DCAD ranging between 165 and 600 mEq/kg of DM concluded that optimum milk production was obtained around 300 mEq/kg of DM

(Figure 5). Furthermore, Iwaniuk and Erdman (2015) summarized 63 studies and, as it can be seen in Figure 6, feed intake from 18.5 to 19.8 kg/d between a DCAD ca. 0 mEq/kg and a DCAD ca. 300 mEq/kg; but then increasing DCAD from 300 to 600 mEq/kg of DM, only resulted in a modest increase of 0.3 kg (from 19.8 to 20.1 kg/d), which was much lower than the variation in intake observed with a DCAD of 300 mEq/kg of DM (ranging from 18.8 to 21.8 kg/d). The fact that the variation in the Y axis (daily intake) is greater than the variation in the X axis (DCAD) in Figure 6 further supports the concept that the relationship between DCAD and intake in the literature is mainly driven by the inclusion of values derived from negative DCAD. In fact, Iwaniuk and Erdman (2015) showed that DCAD above 225 mEq/kg of DM had negligible impact on milk yield (Figure 7). Also, it is interesting to note that in the study by Bach et al. (2023), cows had a greater DMI with lowest FCR when supplemented with MgO than when fed SB, despite the fact that DCAD of the ration with MgO was 147 mEq/kg and that of the ration with SB was 228 mEq/kg; however, when DCAD was calculated taking into account the contribution of Mg, the difference in DCAD was minor: 319 vs 371 mEq/kg, respectively.

Summary

Due to the relatively low impact of SB at lose inclusion rates (<250 g/d), rations containing such low doses of SB would benefit from entirely removing it from the ration and use the available 'space' in the ration to provide nutrients to cows (i.e., amino acids, carbohydrates, vitamins, etc...). When feeding rations with adequate levels of SB (e.g., >220 g/d), then it may be worth to explore whether a partial substitution of SB by magnesium sources may be beneficial. Magnesium oxide is more effective in increasing (and controlling) rumen pH than SB, and this allows for lower dietary inclusion rates making it easier (and less expensive) to satisfy the nutritional needs of the cows within the limitation of total feed intake. If SB is removed the ration, DCAD is likely to decrease, but as long as it is maintained above 280 mEq/kg of DM there should not be negative consequences on intake or performance.

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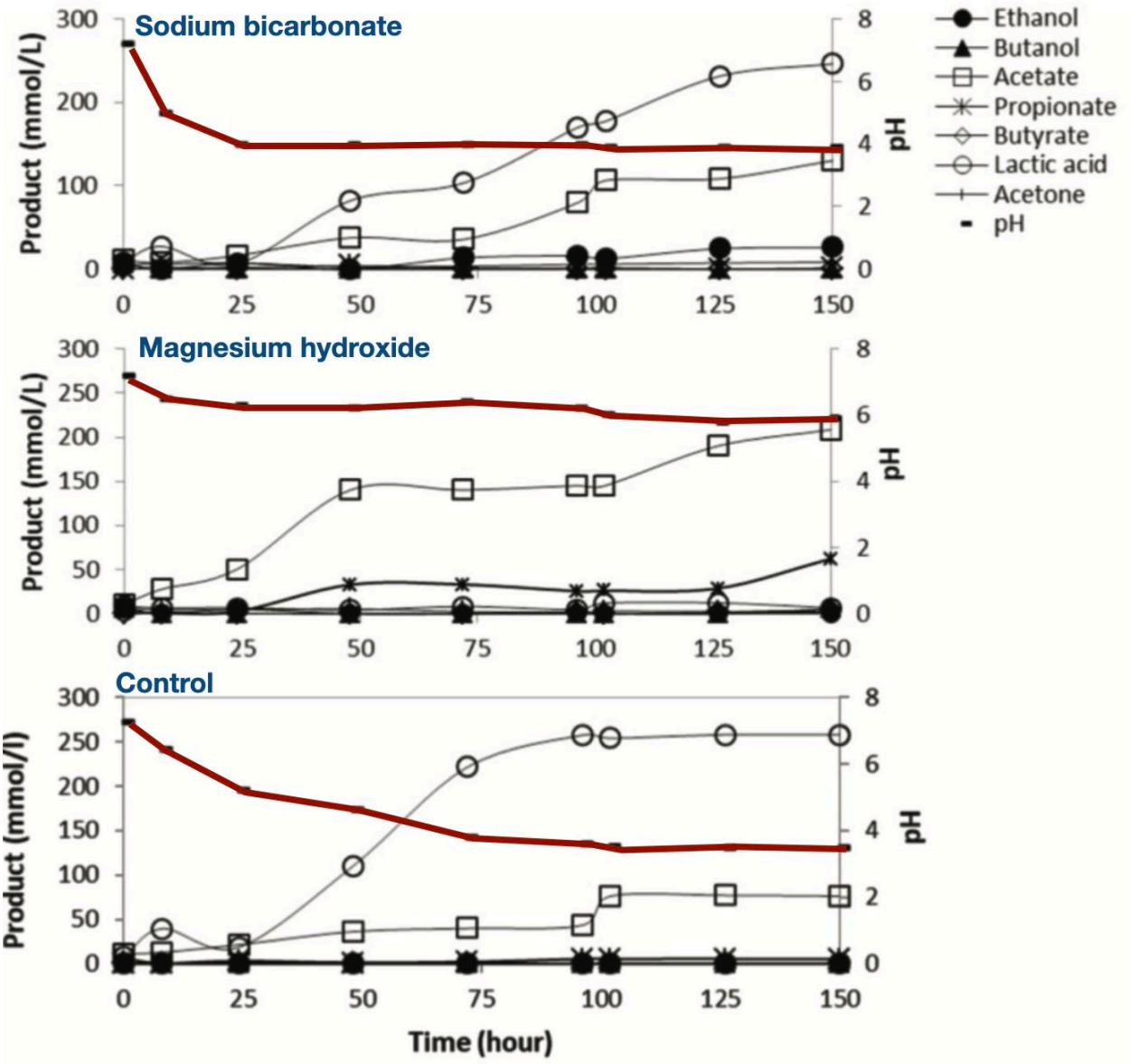

Figure 1. Evolution of pH and concentration of fermentation end-products as affected by sodium bicarbonate or magnesium hydroxide. Adapted from Darwin (2019).

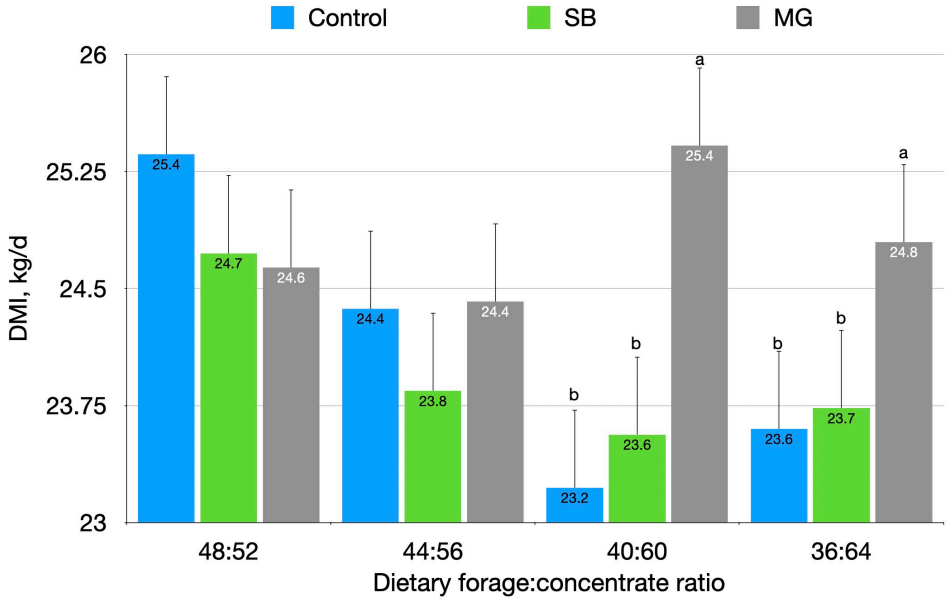

Figure 2. Dry matter intake (DMI) and rumen pH in dairy cows fed different forage:concentrate ratios and 170 g of sodium bicarbonate (SB), 90 g of MgO (MG), or no supplementation (Control). Adapted from Bach et al. (2018).

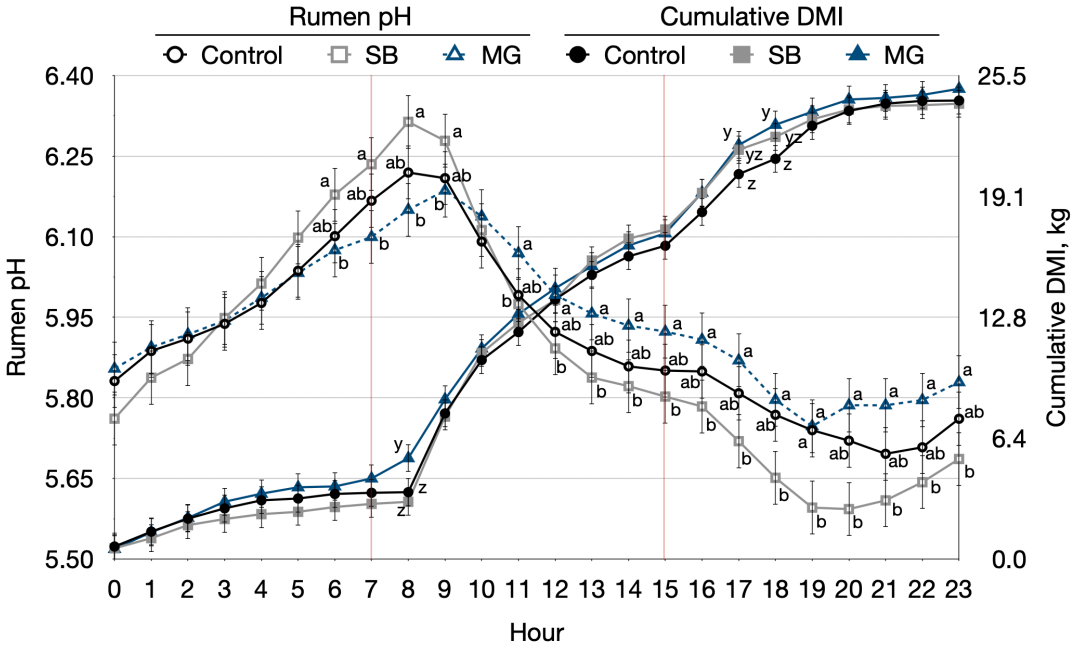

Figure 3. Evolution of dry matter intake (DMI) and rumen pH in dairy cows fed a 36:64 forage:concentrate ratio and 200 g of sodium bicarbonate (SB), 60 g of MgO (MG), or no supplementation (Control). Adapted from Bach et al. (2023).

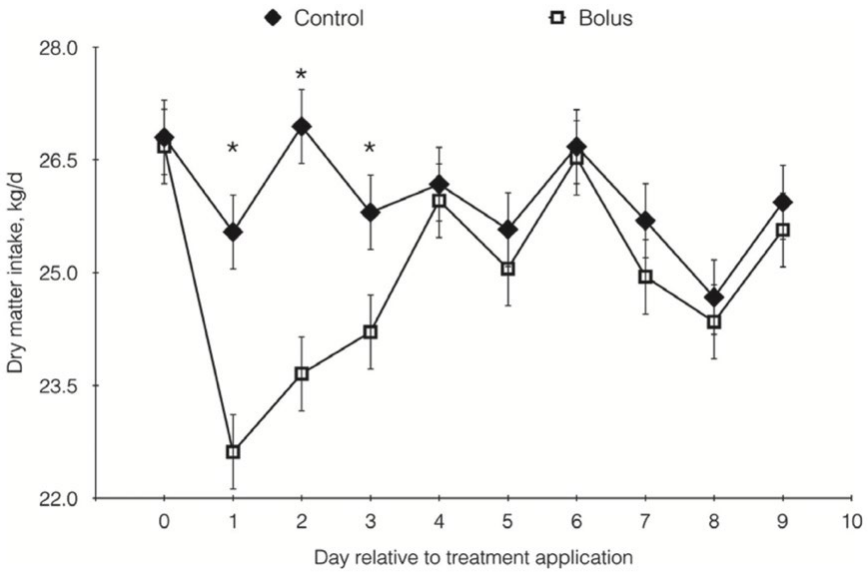

Figure 4. Effect of intra-ruminally administration of anionic salts containing calcium sulfate, calcium chloride, and ammonium chloride (bolus) on dry matter intake in lactating dairy cows. Adapted from Maynou et al. (2018).

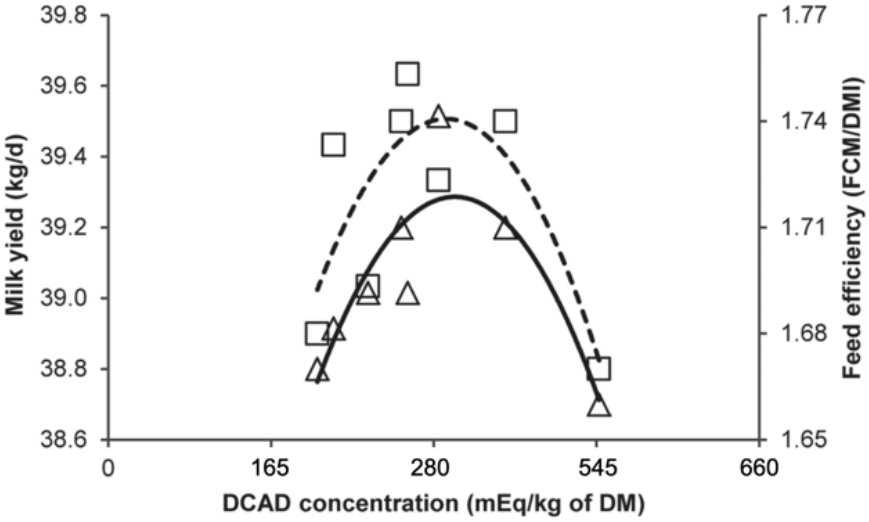

Figure 5. Relationship between DCAD (Na + K - Cl - S) and milk yield (solid line) and feed efficiency (dashed line). Adapted from Iwaniuk et al. (2015).

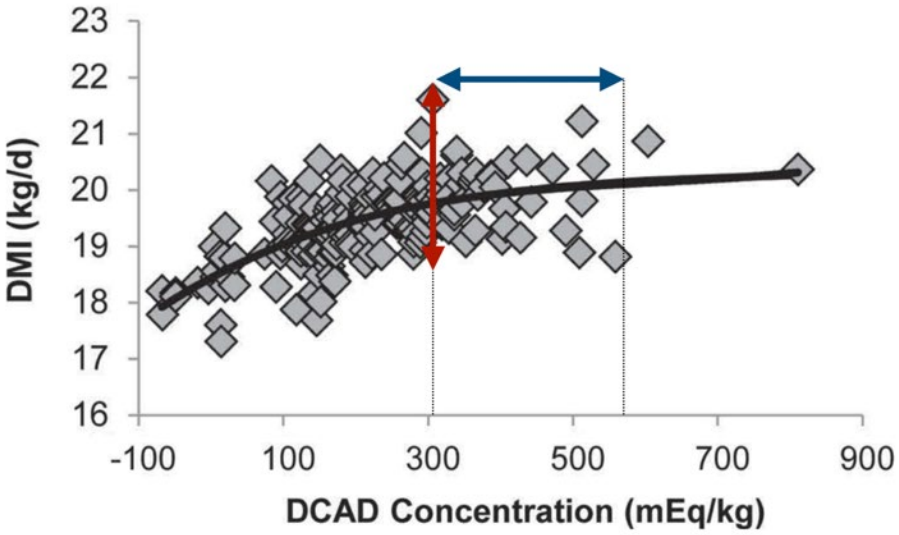

Figure 6. Relationship between DCAD and feed intake. Adapted from Iwaniuk and Erdman (2015). The red line depicts the variation in dry matter intake with a DCAD of ~300 mEq/kg, whereas the blue line shows a range in DCAD equivalent to the variation in dry matter intake with a DCAD of ~300 mEq/kg.

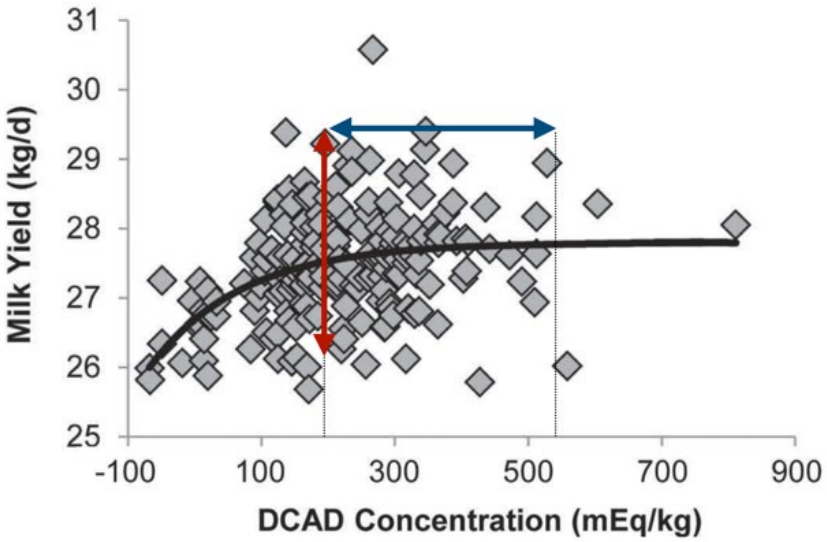

Figure 7. Relationship between DCAD and milk yield. Adapted from Iwaniuk and Erdman (2015). The red line depicts the variation in milk yield with a DCAD of ~200 mEq/kg, whereas the blue line shows a range in DCAD equivalent to the variation in milk yield with a DCAD of ~200 mEq/kg

Session Notes

Importance of Gut Health to Drive Animal Performance and Health

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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 result in hindered productivity including heat stress, ketosis, rumen and hindgut acidosis, feed restriction, and psychological stress associated with normal animal practices (i.e., pen changes, weaning, shipping). Although these insults have different origins, a commonality among them is increased production of inflammatory biomarkers and markedly altered nutrient partitioning. We and others have generated convincing data strongly implicating intestinally derived lipopolysaccharide (**LPS**) as sometimes being the culprit in these situations.

Heat Stress

During heat stress (**HS**), blood flow is diverted from the viscera to the periphery to dissipate heat, and this leads to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013), resulting in increased passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as LPS, is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which is abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Immune system activation occurs when LPS binding protein (**LBP**) initially binds LPS and together with CD14 and TLR4 delivers LPS for removal and detoxification, thus LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration into the bloodstream during HS, which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS, such as an increase in circulating insulin (Lim et al., 2007).

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Intramammary LPS infusion increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009; Kvidera et al., 2016, 2017c). Interestingly, increased insulin occurs prior to increased inflammation and the temporal pattern agrees with our previous *in vivo* data and a recent *in vitro* report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via GLP-1 (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of HS agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin during both HS and immunoactivation is energetically difficult to explain as feed intake is severely depressed in both experiments.

Ketosis and the Transition Period

Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Bionaz et al., 2007; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders (i.e. the inflammation was not apparently due to mastitis or metritis). In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LBP, serum amyloid A, and haptoglobin were also increased (Abuajamieh et al., 2016a). However, even seemingly healthy cows experience some degree of inflammation postpartum (Humblet et al., 2006). 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). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis) and mammary gland (mastitis) (Mani et al., 2012). Additionally, we believe intestinal hyperpermeability may also be responsible for periparturient inflammation in dairy cows as many of the characteristic responses (rumen acidosis, decreased feed intake, and psychological stress) occurring during this time can compromise gut barrier function.

As aforementioned, mild inflammation is observed even in cows which seemingly complete the transition period successfully, suggesting that some level of inflammation plays an important role in cow health. In fact, previous reports have demonstrated that blocking endogenous inflammation (via administration of non-steroidal anti-inflammatory drugs [**NSAID**]) can increase the incidence of negative health outcomes (i.e., fever, stillbirth, retained placenta, metritis) and reduce productivity (Schwartz et al., 2009;

Newby et al., 2013, 2017). Beneficial effects of NSAIDs have been observed on production performance (Carpenter et al., 2016a), but inconsistencies exist (Priest et al., 2013; Meier et al., 2014) including how NSAIDs seemingly work better in specific parities (Farney et a., 2013) and interfere with fiber digestion (Carpenter et al., 2016b) and compromise feed intake (Carpenter et al., 2017). Although NSAIDs may be an effective prophylactic strategy during the periparturient period, further research is necessary to determine the timing of administration and type and dose of NSAID that is most effective at improving health. Alternatively, administrating a chemokine (anti or even pro-inflammatory) may hold promise in improving transition cow performance.

Rumen and Hindgut Acidosis

A transitioning dairy cow undergoes a dietary shift from a high forage to a high concentrate ration post-calving. This has the potential to induce rumen acidosis (**RA**) as increases in fermentable carbohydrates and DMI stimulate the buildup of short chain fatty acids and lactic acid (Nocek, 1997; Enemark, 2008). Rumen acidosis has direct and ancillary consequences accompanied by various production issues (decreased DMI, reduced milk yield, milk fat depression) and health challenges such as laminitis, liver abscesses, and potentially death (Nocek, 1997; Kleen, 2003). The mechanisms linking RA and the development of health disorders are not entirely clear, however, recent literature has indicated that inflammation associated with epithelial damage and consequential LPS translocation are at least partially responsible for production losses associated with RA (Gozho, et al., 2005; Khafipour, et al., 2009). Although many hypothesize LPS translocation occurs at the rumen epithelium directly (Guo et al., 2017; Minuti et al., 2014), others point towards LPS translocation in the hindgut to be a potential source of peripheral inflammation (Li et al., 2012). Interestingly, when RA was induced using either alfalfa pellets or high-grain diets, increased peripheral inflammation was only observed in the high-grain group, irrespective of rumen acidotic conditions being similar between the two treatments (Khafipour et al., 2009a,b). It was hypothesized that the grain supplemented group likely had increased starch flow to the hindgut, and therefore, increased fermentation that could potentially lead to hindgut acidosis and LPS translocation across the large intestine. However, we were unable to recreate production losses and systemic inflammation when we abomasally infused 500 g/d of resistant starch (Piantoni et al., 2018) or even 4 kg/d of purified corn starch (Abeyta et al., 2019). Both of our aforementioned experiments were accompanied with marked reductions in fecal pH so it is unlikely that large intestinal acidosis per se is the specific reason for systemic inflammation described in the previous reports (Li et al., 2012, Khafipour et al., 2009a,b). Regardless, we recently reported that cows with the largest decrease in fecal pH post-calving consumed less feed, produced less milk, had a larger acute phase protein response, and had increased NEFA and BHB compared to cows that had a mild decrease in fecal pH following parturition (Rodriguez-Jimenez et al., 2019). Clearly, our current understanding of how hind-gut acidosis impacts the immune system and ultimately periparturient productivity is woeful.

Feed Restriction and Psychological Stress

Stress associated with feed restriction along with several other regular production practices (e.g., heat stress, weaning, transportation, overcrowding, restraint, social isolation/mixing) is frequently encountered in animal agriculture (Chen et al., 2015) and is associated with gastrointestinal hyperpermeability. In fact, we have repeatedly reported reduced intestinal barrier integrity in pigs pair-fed to their HS counterparts (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Furthermore, we recently demonstrated shortened ileum villous height and crypt depth (Kvidera et al., 2017d) as well as increased appearance of the intestinal permeability marker Cr-EDTA (Horst and Baumgard, unpublished), indicating reduced intestinal health in cows fed 40% of ad libitum intake. Recent literature indicates that the corticotropin releasing factor (**CRF**) system may be the mechanism involved in stress-induced leaky gut (Wallon et al., 2008; Vanuytsel et al., 2014). The CRF and other members of the CRF signaling family including urocortin (1, 2, and 3) and their G-protein couple receptors CRF1 and CRF2, have been identified as the main mediators of the stress-induced intestinal changes including inflammation, altered intestinal motility and permeability, as well as shifts in ion, water, and mucus secretion and absorption (as reviewed by Rodiño-Janeiro et al., 2015). These alterations appear to be regulated in large part by intestinal mast cells (Santos et al., 2000). Mast cells are important mediators of both innate and adaptive immunity and express receptors for the neuropeptides CRF1 and CRF2, which may in part explain the association between emotional stress and intestinal dysfunction (Smith et al., 2010; Ayyadurai et al., 2017). Furthermore, mast cells synthesize a variety of pro-inflammatory mediators (i.e., IFN-γ and TNF-α) that are released upon activation, mainly via degranulation (de Punder and Pruimboom, 2015). Excessive mast cell degranulation plays an important role in the pathogenesis of different intestinal inflammatory disorders (Santos et al., 2000; Smith et al., 2010). A better understanding of the role psychosocial stress plays on the initiation of different intestinal disorders in livestock is of obvious interest for multiple animal agriculture systems.

Metabolism of Inflammation

LPS-induced inflammation has an energetic cost which redirects nutrients away from anabolic processes that support milk and muscle synthesis (see review by Johnson 1997, 1998) 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.

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 (Figure 1;

Wannemacher et al., 1980), and hypertriglyceridemia (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005) occur. 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.

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, 2017b,c, 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 (Spitzer et a., 1985; Waldron et al., 2003b) 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 particular consequences during lactation as it requires ~72 g of glucose for synthesizing 1 kg milk (Kronfeld, 1982).

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.). We and others have now demonstrated that HS, rumen acidosis, and psychological stress increase circulating markers of endotoxin and inflammation. We believe that the circulating LPS originates from the intestine (small or large) and initiates an immune response. This activated systemic immune response reprioritizes the hierarchy of glucose utilization and milk synthesis is consequently deemphasized.

Conclusion

There are various situations in an animal's life that hinder production performance (i.e., heat stress, feed restriction, rumen acidosis, etc.) and we suggest, based upon the literature and on our supporting evidence, that LPS (of intestinal origin) may be the

common culprit in these circumstances. Immune activation in response to LPS markedly alters nutrient partitioning as a means of fueling the immune response. More research is still needed to understand the mechanisms and consequences of intestinal permeability and associated inflammation in order to provide foundational information for developing strategies aimed at maintaining productivity.

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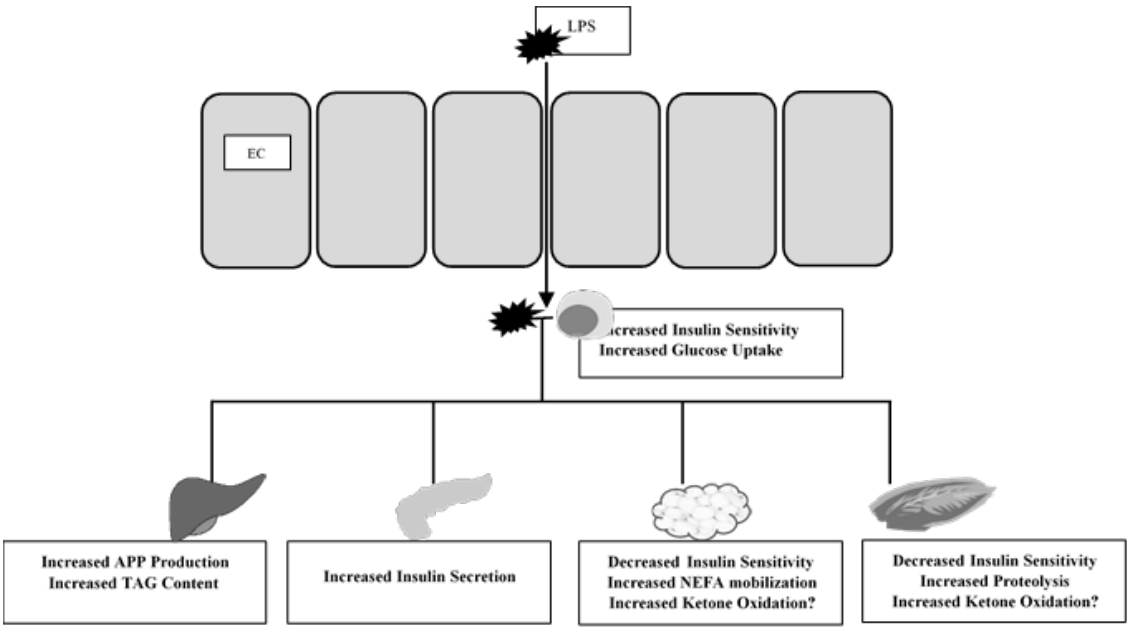

Figure 1. LPS induced alterations in peripheral metabolism.

Session Notes

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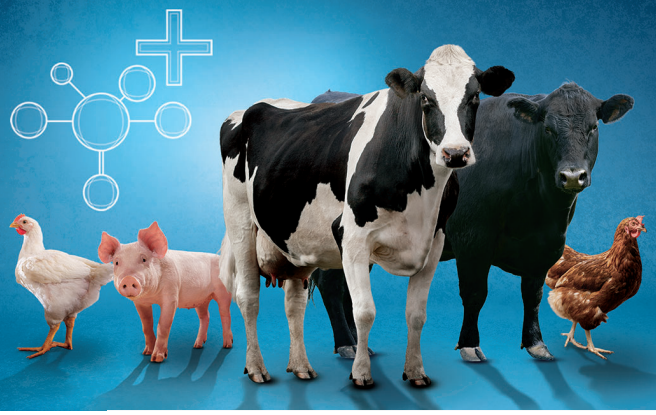
IntelliBond® is Different



IntelliBond gives you more for your money.

IntelliBond advantages	Other hydroxy disadvantages
1 Product characteristics	
• A more consistent hydroxychloride product • IntelliBond Z and C typically deliver 95% hydroxychloride molecular form • Patented OptiSize® Large Particle Technology • More even distribution in the feed • Non-hygroscopic, non-caking, non-clumping properties	• No guarantee of molecular form; not a consistent product • Less of the hydroxychloride form you're paying for—sometimes much less - 81.02% avg. zinc hydroxychloride form; as low as 30.60% - 10.00% avg. zinc oxide form; as high as 30.10% - 8.98% avg. "other contaminants"; as high as 64.10% • Small, inconsistent particle size; lacking uniformity • More dust = decreased worker safety • More hygroscopic, behaves like a sponge • Prone to caking and clumping, prohibiting use and creating problematic issues for handling, in pre-mix, and in the feed
2 Supply chain	
• Made in Indiana • Approximately 48-hour transit time from plant site to customer delivery (average of 1,319 miles) • Reliable and consistent delivery when you need it • Produced in a certified food facility with SQF, FAMI-QS, ISO, and SF/SF certifications • 100% traceability from raw material to final packaged product	• Made outside the U.S. • Shanghai to San Francisco by ship: 14-21 days, 6,904-7,480 miles, plus additional days for truck or rail delivery - Longer wait times—overseas shipping delays, longer customs process - Less secure, unknown traceability, lower regulatory standards • Increased potential for contaminants • Other hydroxy suppliers are chemical trading companies
3 Animal performance	
• Enhanced mineral delivery to the animal • 150+ animal studies over 25 years	• Very limited study data
4 Sustainability	
• Independent verification of IntelliBond C, Z, and M's carbon footprint (CO_2 eq.) per metric ton of final product; the first and only trace mineral to offer this verified reduction in cows' carbon footprint • Reduced freight due to U.S.-based manufacturing	• No approved LCAs; shipping methods = less sustainable carbon efforts • More waste and energy consumption = less sustainable

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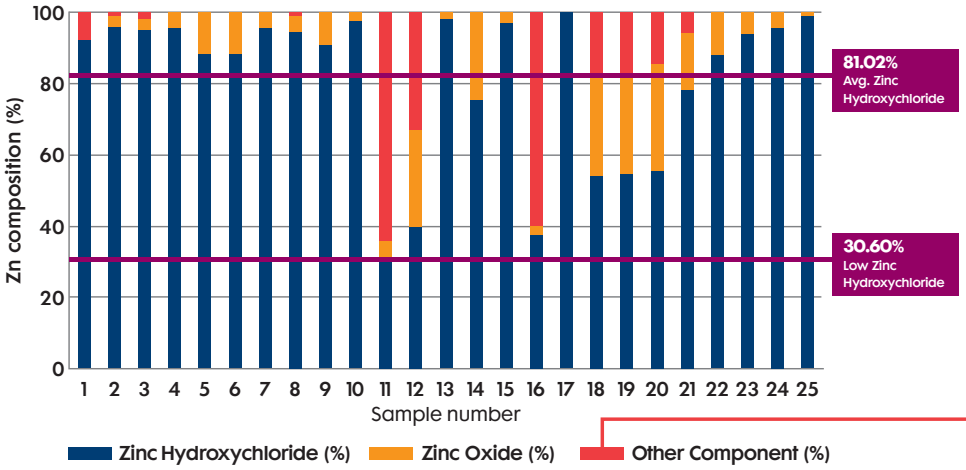
Smart Minerals. Smart Nutrition. Smart Decision.

Zinc composition analysis: Other hydroxy sources



An analysis of other hydroxy sources of zinc collected over an 8-year period shows an average zinc hydroxychloride concentration of 81.02%, with levels dropping as low as 30.60%. IntelliBond Z typically delivers 95.00% zinc hydroxychloride.

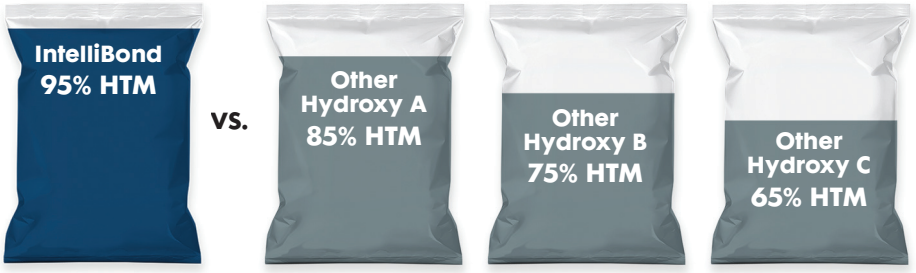
"Other contaminants" found in sample analysis include:

- **Dioxin-like PCBs** - toxic chemicals that persist in the environment and accumulate in the food chain
- **Zinc perchlorate** - oxidizing agent and potential explosive under extreme heat
- **Basic copper carbonate** - blue/green coloring agents used in pyrotechnics and fireworks

Economic analysis: The cost of saying "no" to IntelliBond advantages

The real cost (zinc) example:

Other hydroxy trace minerals cost more when they do not deliver the hydroxychloride form you think you're buying.



You get more with IntelliBond

- Highest quality product in the market
- Greater efficiency and better feeding precision
- Consistency of OptiSize® Large Particle Technology

Other hidden costs:

- **Handling, flowability issues/ non-uniformity of particle distribution**
  - Cost of lost efficiency/time in the feed mill
  - Cost of over formulating
  - Cost of animal performance loss due to "hot" and "cold" spots in feed
- **Potential animal performance loss due to inconsistent molecular form**
- **Long lead times, supply chain delays**
- **Supplier sustainability concerns**
- **Lack of U.S. regulatory oversight of non-domestic manufacturing**
  - Costs/risks of contamination
  - Costs/risks of food and feed safety concerns

Ask your Selko representative how IntelliBond can deliver more advantages.

www.selko-us.com

Mechanisms of Absorption, Transport, and Homeostasis of Zinc in Animals

Robert J. Cousins[1]
University of Florida

Introduction

The past quarter century has provided many new insights into zinc absorption mechanisms by the gastrointestinal tract and practical outcomes. Zinc-related outcomes involves the three basic functions of zinc in biology. Those are structural: required for protein structure, including DNA binding proteins, catalytic: required for activity of zinc-dependent enzymes including gene modifying enzymes, and regulatory: facilitate cellular signaling pathways (King and Cousins, 2014; Ryu and Aydemir, 2020). Extensive research has documented how one or more of these functions are needed for proper immune functions, maximizing growth potential, reproductive capacity and resistance to cellular and environmental stressors.

It is generally assumed that the absorption process is similar at the molecular genetic level in monogastrics and ruminants. Obvious differences occur in anatomy, absorptive surface area and impact of the gastrointestinal microbiome composition. Overall, it is assumed that the absorption process is a linear reflection of dietary zinc content, but is saturable at high concentrations of ingested bioavailable zinc. Hence, both facilitated (transporter mediated) and passive (non-saturable) components exist Hoadely et al.,1987; Steel and Cousins, 1985). At a more global level, the abundance of zinc-binding ligands from various dietary components (Cousins, 1988) and metabolites produced by resident intestinal microbiota (Cheng et al., 2024) as sources of bioavailable zinc are currently receiving increased attention.

Zinc metabolism and homeostasis in all animal species are regulated by cellular transporter proteins from two gene families: *ZIP (**SLC30A***) and *ZnT (**SLC39A***). Their functions are to decrease or increased intracellular zinc levels, respectively (Eide, 2020: Lichten and Cousins, 2009). For efficient zinc absorption a functional transporter, Zip4 (**SLC39A4**) is required. Zip4 is located at the apical surface of mucosal epithelial cells of the intestinal tract (Liuzzi et al., 2004: Wang et al., 2004). There appears to be an absolute requirement for functional Zip4 transport activity as it serves as the gatekeeper for absorption. Mutations of the *Zip4* gene in humans leads to the disease *Acrodermatitis enteropathica* (Eide, 2020) and mutated bovine *Zip4* leads to *Adema disease (Lethal trait A 46)*, an inherited bovine zinc deficiency (Weismann and Flagstad,1976) in cattle. Both present as skin abnormalities, including dermatitis and gut intestinal inflammation and secondary infections related to depressed immunity. Both conditions are corrected through supplemental zinc therapy. Another inherited bovine

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skin disease with similar skin lesions, PLD4 deficiency, is not related to atypical *Zip4* (Landenmayer et al, 2018). Zip4 is most highly expressed in the proximal small intestine, which corresponds to estimates of zinc absorptive capacity (King and Cousins, 2014), including ruminants (Kincade et al., 1967; Miller et al., 1967). Nevertheless, in both monogastrics and ruminants, zinc is absorbed to some extent along the entire gastrointestinal tract. A unique feature of Zip4 expression is that the *Zip4* gene is upregulated when the dietary zinc supply is below normal, i.e., below the requirement. Both transcriptional (involving the transcription factor KLF4) and post-transcriptional mechanisms (influencing *Zip4* mRNA translation) are involved in *Zip4* gene regulation. Zinc depletion increases Zip4 expression and repletion increases Zip4 expression (Liuzzi et al., Wang et al., 2004). In that regard, this zinc responsive gene is not regulated by the metal responsive element (MRE) transcription factor that monitors the zinc status of animals and activates specific genes upon the need for zinc-dependent functions. Release of zinc at the basolateral surface of enterocytes is dependent upon the export transporter, ZnT1 (McMahon and Cousins, 1998). ZnT1 is zinc-regulated, so with increased intracellular zinc levels, zinc is transported into the systemic circulation for body distribution.

In contrast to the Zip4-dependent pathway for zinc absorption from regular diets/rations of natural components, zinc absorption via amino acid chelates may be quite different mechanistically. A substantial literature base has documented the advantage of zinc chelates over inorganic zinc salts for productive outcomes (Arthington and Ranches, 2021; Heiderscheit and Hansen, 2022; Marres and Hasse, 2020; Sauer et al., 2017; Spears, and Kegley, 2002: Spears, 2003). These tend for be situation specific as all studies are not in agreement. There are at least three explanations for the enhanced absorption of amino acid-chelated zinc. 1).the chelated zinc could be transported via amino acid transporters at the apical surface of the enterocytes, 2) zinc from these chelates may be released in a way that allows for more efficient absorption and/or 3) the zinc acid chelates may be absorbed via the paracellular (saturable) pathway, i.e. between intestinal epithelial cells. Most studies looking into the mechanisms involved are in vitro, hence many conditions of the normal intestinal tract, e.g. microbiota and digestive enzymes are not present. Nevertheless, in vitro experiments using reporter systems, of ZnT1 expression and intracellular zinc detection support a mechanism where the amino acid chelate is absorbed from the apical surface (Marres and Hasse, 2020; Sauer, 2017).

Zinc requirements for cattle for growth and gestation are 30 mg/kg ration, using NRC guidelines and 40 mg/kg in the UK. Substantial evidence indicates the intestinal microbial population utilizes a significant amount of the zinc that is absorbed (one estimate is 20% of ingested zinc in a study with rats). Some studies with ruminants suggest that the gut microbial diversity is refractory to changes in zinc content of the ration (Duffy et al.,2023). Intestinal microbial composition is receiving attention, both from a role in controlling to zinc availability for absorption, but also how ingested zinc may influence the microbial composition (Cheng, et al., 2024: Jimenez-Rondan et al., 2022: 2025).

Key to any discussion of zinc absorption and homeostasis is the role played by endogenous secretion of zinc returned to the gastrointestinal tract. That relationship holds for ruminant animals and humans alike. Studies with ruminants have shown that dietary zinc status influences the amount of endogenous zinc returned to the small intestine. Studies by Miller et al using the radioactive tracer, ^{65}Zn, helped delineate these pathways for cattle (Kincade et al., 1967; Miller et al., 1967). As with other species, in cattle, endogenous zinc loss is into the small intestinal lumen primarily from the pancreatic fluids. The magnitude of that zinc being of pancreatic origin is not clear. This is in contrast to other animals and humans where endogenous loss is via the pancreatic route and controls homeostasis during a variable dietary zinc intake. In mice where the role of zinc transport proteins for endogenous zinc loss has been documented. Activity of zinc exporters, ZnT1 and ZnT2, in the exocrine pancreas are extremely important route of controlled zinc loss (Guo et al., 2010). Comparable *ZnT1* and *ZnT2* genes have been identified in cattle (Elsik et al. 2009). Another major loss of zinc in lactating cows is from zinc in milk. This source of zinc is controlled through the activity of the ZnT2 transporter which facilitates movement of zinc ions across mammary gland. Of note, mutated ZnT2 in humans causes a zinc deficiency in nursing infants (Chowanadisai et al., 2006). A corresponding mutation in cattle could influence calf development.

Once absorbed, zinc is rapidly taken up by all tissues, again this is dependent on the expression of individual zinc transporters and the stimuli they respond to. These stimuli could be dietary, hormonal or environmental. For example, stress of infectious disease produces a marked transient increase in zinc uptake by the liver. The transporter Zip14 is a primary component responsible for this metabolic change, responding to stress and numerous mediators of immunity and inflammation (Aydemir and Cousins, 2018). In humans, little zinc is released into the urine under normal conditions. That may increase during certain conditions, such as cachexia and acute illness (King and Cousins, 2014). In cattle, by contrast, urinary zinc loss is an important route for endogenous zinc loss. In addition, urinary zinc is increased transiently following an acute viral infection (Arthington,1996). Prolonged urinary zinc loss is likely to lead to dysfunctional zinc-dependent pathways and may represent important outcomes in cattle.

Recent papers originating from my laboratory at the University of Florida describe how a deletion of a zinc transporter gene, coding for a zinc importer produces measurable changes to pathways. These include effects on both zinc and manganese metabolism that impact processes in brain, adipose tissue, bone, intestine and gut microbiota (Aydemir and Cousins,2018). Key to these defects is a traceable to loss of zinc targeted to specific cellular sites. Included is a loss of zinc needed for zinc-requiring enzymes that influence genes expression through epigenetic regulation (Jimenez-Rondan, 2022, 2025). Such a loss could result in subtle (silent) effects of zinc deficiency that have been described since the essentiality of zinc for animal health was discovered a century ago.

Summary and Conclusions

A number of references are available that describe the latest knowledge of zinc metabolism and function in production animals and humans are cited here, but represent only a fraction of the robust literature available on these topics.

Mechanisms of zinc absorption and homeostasis are currently receiving attention because of practical considerations and for relevance to health and disease. As a Type II nutrient deficiency, zinc deficiency is characterized by growth conservation, hidden symptoms, reduced growth and impaired immune functions. While beyond the scope of this brief overview, use on model systems where specific genes, particularly those for zinc transport, are deleted from expression coupled with global gene sequencing and proteomics, have revealed important zinc dependent functions that are not outwardly obvious, but can influence overall health and productivity. Hence, it is most encouraging that these mechanistic concepts with model systems are becoming integrated into research on production animals (Anas et al., 2023: Franco et al., 2024). Future exciting possibilities for zinc supplementation for ruminants may include epigenetic changes to gene expression influencing phenotype and productivity, alteration in microflora of the gastro-intestinal tract including the rumen, maintenance of gut barrier function and mechanisms to optimize innate and adaptive immunity.

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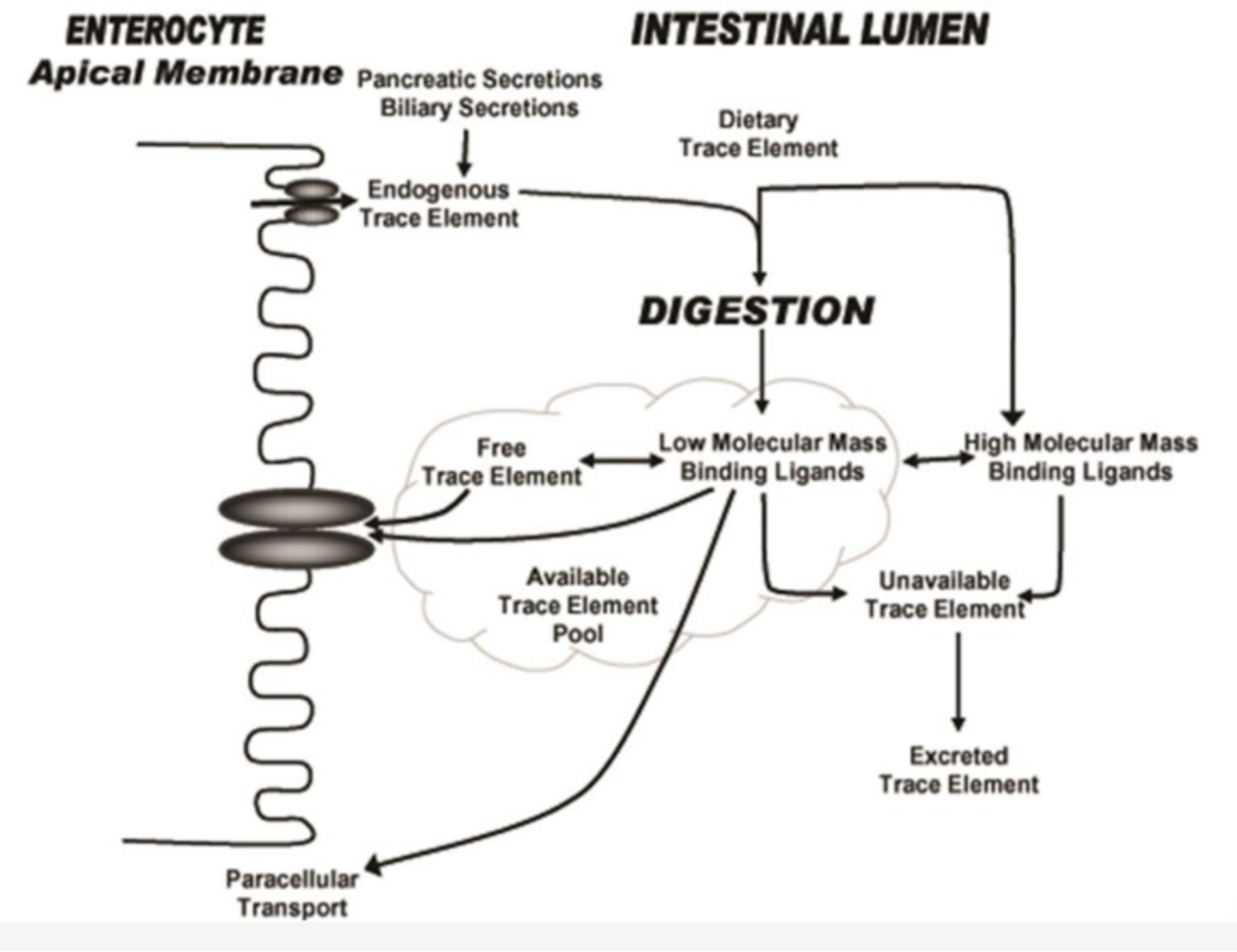

Figure 1. Interactions of ingested zinc in the intestinal lumen. Dietary zinc is introduced to the small intestine as a variety of biological ligands including polynucleotides, carbohydrates, metalloproteins, low molecular weight molecules including zinc chelates, zinc salts and a variety of organic matrices. Many of these molecules are of microbial origin. Zinc in the intestinal lumen is believed to always be bound to some ligand and can exist as free zinc ion only as a transient state during transfers between ligands. The amount of free zinc is dependent on pH of the micro-environment where it exists. Most of the zinc available for absorption via the transcellular route first interacts with transporter molecules located at the apical surface of enterocytes after traversing the mucus layer of the epithelial surface. When the amount of zinc provided in diet is high zinc and not bound to macro molecules it can also be transported via the paracellular route (non-saturable), i.e., between epithelial cells. Depending upon the species involved, the lumen will contain zinc of endogenous origin secreted into the intestinal lumen via the pancreas. (Adapted from Cousins, 1989).

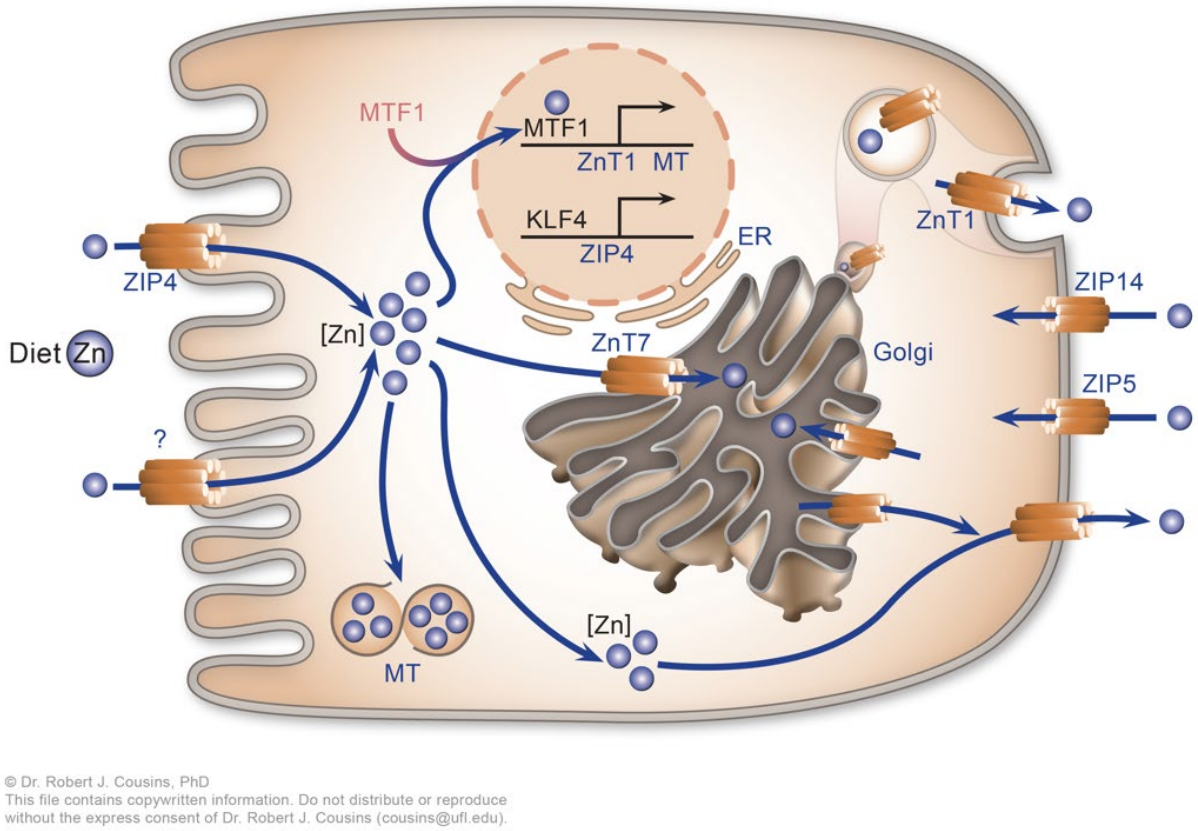

Figure 2. Mechanisms involved in the transcellular phase of zinc absorption. The main route of zinc is from transport across the apical membrane of enterocytes lining the gastrointestinal tract. Zip4 is the main transporter involved. Defects in Zip4 expression led to hereditary zinc deficiency, e.g., Adema Disease in cattle. Transcellular zinc movement involves numerous transporter-related mechanisms, including ZnT7. When dietary zinc levels are less than needed to meet body requirements, Zip4 expression is increased. High levels of dietary zinc induce expression of both the intracellular metal buffer, metallothionein and the efflux zine transporter, ZnT1, located at the enterocyte basolateral membrane. Zip5 and/or Zip14 may act as sensors of body zinc status. Zip14 is a metal importer that helps maintain zinc-dependent process in enterocytes using endogenous zinc sources. (Cousins, 2010).

Session Notes

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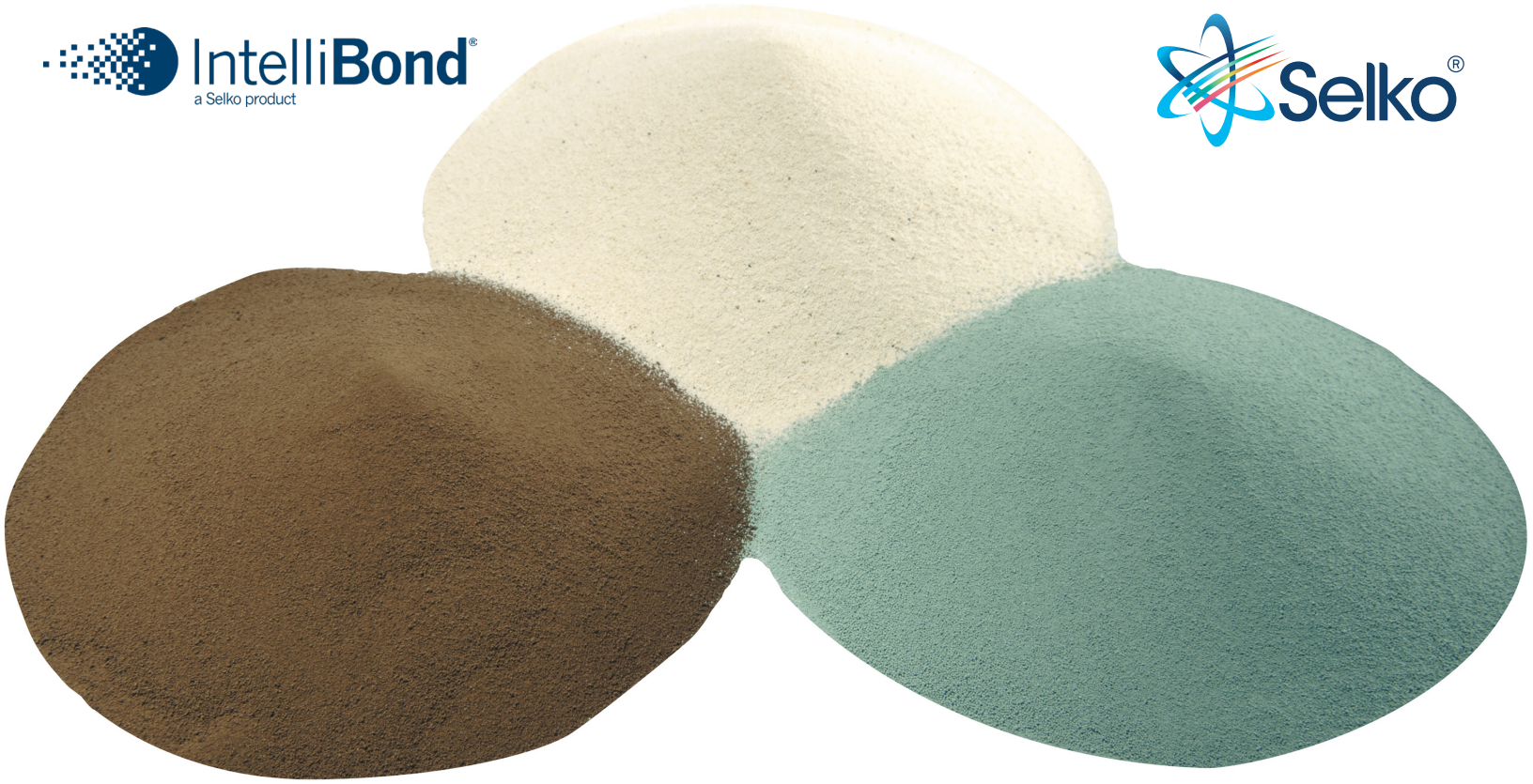

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IntelliBond is the original hydroxy trace mineral that takes basic nutrition to a higher standard in the feed, in the animal, and in the food chain.

In the feed

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- Decrease in free metal ions/oxidative stress
- More available minerals, vitamins, and lipids vs. SO_4

High mineral concentration
- Leads to cost savings in labor, convenience, and storage
- More formula flexibility: Leaves room for other feed additives

OptiSize® technology
- Large particle technology improves mixability for uniform distribution of minerals
- Superior handling characteristics: Low dust, no clumping

In the animal

Improved source
- Strong covalent bonds allow for slow release, minimizing interactions with antagonists
- Allows minerals to be present at higher concentrations further down the GI tract

Demonstrated performance
- 100+ research trials in swine, poultry, and ruminants since 1996
- Each product in the IntelliBond portfolio provides performance benefits

Solid economics
- Delivers economic return based on sound science and research

In the food chain

U.S. made products exceeding the highest standards of six certification organizations
- Dedicated team monitors multiple critical control points in manufacturing to assure quality

U.S. based supply chain
- 100% control of traceability, process, and quality

Trusted results
- Quality and safety are non-negotiable

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The chemical structure of IntelliBond hydroxy trace minerals means low solubility and reactivity, for better bioavailability and absorption in the animal.

Comparison of trace mineral sources

Inorganic salts
- Sulfates
- Highly soluble/reactive in the feed and the animal's digestive tract
- Low absorption
- Low cost
- Carbonates, chlorides, and oxides

Organic chelates
- Metal is complexed with AA(s), proteins, polysaccharides
- Moderate solubility/reactivity
- Higher absorption
- Highest cost

Hydroxy trace minerals
- Metal is complexed with hydroxy groups within a defined crystalline structure
- Very low solubility/reactivity
- Higher absorption
- Moderate cost

Other hydroxy trace minerals
- ✗ Unpredictable composition and quality
- ✗ Fragmented supply chains

IntelliBond
- ✓ Predictable and controlled composition, quality, and supply
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Mechanisms of Ca Absorption in the Gastrointestinal Tract of Ruminants

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Introduction

Much of our knowledge about the mechanisms of Ca absorption in the intestine of terrestrial vertebrates comes from research on laboratory rats and mice (Christakos, 2021). Studies in rats and in humans have also indicated that colon absorption of Ca can contribute as much as 10% of total Ca absorbed (Beggs et al., 2022). However, the mechanisms and role of the rumen in obtaining dietary Ca can only be discovered from studies of rumen tissue or from in vivo research on different ruminant species. Although the mechanisms of Ca absorption from the rumen are still not fully understood, studies in vivo in sheep indicate that up to about 50% of Ca obtained from the diet can be absorbed in the rumen (Schröder et al., 1997).

In the small intestine and the colon, Ca is absorbed by both transcellular and paracellular routes across the mucosal epithelium with its villous structure and cells with apical microvilli (Deluque et al., 2024). The mechanisms here are under endocrine regulation, particularly by 1,25-dihydroxyvitamin D [**1,25(OH)$_2$D**] (Christakos, 2021). The very different mucosal structure of the rumen, with its stratified squamous epithelium and a keratinized outer luminal layer (Lavker et al., 1969) indicates that the Ca absorption mechanism is inevitably different from those in the small and large intestine. The efficiency of the absorption mechanism is highest in the duodenum and jejunum where the transit time for the ingesta is short. Whereas in the rumen and the colon the luminal contents reside for longer so substantial Ca absorption can occur by less efficient mechanisms. Nevertheless, at times of challenge for Ca homeostasis, as at the onset of lactation in dairy cows, the role of Ca absorption from the rumen contents is critical for avoiding clinical hypocalcemia.

Ca Absorption in the Small Intestine

The transcellular absorption of Ca in all segments of the small intestine can be stimulated by 1,25(OH)$_2$D which acts by binding to the Vitamin D Receptor protein (**VDR**) and promoting the production of 3 cell proteins involved in the active absorption of Ca. These proteins are a microvillous membrane calcium channel, the transient receptor potential vanilloid channel type 6 (**TRPV6**), a cytoplasmic calcium binding protein (**CaBP$_{D9K}$**) and a basolateral plasma membrane Ca^{2+}-ATPase isoform-1b (**PMCA1b**). Ca from the intestinal lumen can enter the endothelial cells through the TRPV6 calcium channel and then by binding to CaBP$_{D9K}$ it can diffuse to the basolateral

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membrane without increasing the cytoplasmic Ca^{2+} concentration above the normal low levels of 10^{-7} mM. The active transport of Ca to the extracellular fluid is then mediated by PMCA1b. Another route of transferring Ca from the intracellular to the extracellular fluid is by means of a sodium/calcium exchange protein (**NCX1**) (Deluque et al., 2024; Wilkens and Muscher-Banse, 2020) (Figure 1).

Passive paracellular absorption of Ca occurs when the luminal Ca concentration is >6mM, such as after oral supplemental Ca intake or when the luminal Ca becomes concentrated as luminal water is absorbed (Wilkens and Muscher-Banse, 2020). Permeability to Ca of the tight junctions between the mucosal cells is facilitated by two tight junction proteins, claudin-2 and claudin-12 (Garcia-Hernandez, et al. 2017). In mature, non-lactating monogastric animals on a standard dietary Ca intake, Ca absorption is predominantly by this passive, paracellular process (Deluque et al., 2024). The paracellular transit of Ca is bidirectional and allows Ca to flow from the circulation into the small intestinal lumen as well as being absorbed from the lumen, depending on how the concentration and electrical gradients favor the passive movement of Ca.

Although in monogastric animal studies, the stimulation of transcellular Ca absorption by dietary calcium restriction or by increases in circulating $1,25(OH)_2D$ is quite pronounced, the results of studies in different ruminant species have been variable both in vivo, and in in vitro mucosal preparations in Ussing chambers. For example, when dairy goats are fed a calcium restricted diet, jejunal calcium absorption is stimulated more than that in the duodenum, whereas in dairy sheep on such a diet, no apparent change in intestinal calcium absorption was seen in either segment (Wilkens et al. 2012). This suggests that variations in calcium absorption to meet changes in requirements of ruminant animals may be quantitatively greater in regions of the alimentary tract other than from the duodenum to the ileum.

Ca Absorption in the Colon

The calcium transport mechanisms found in the small intestine are also present in the colon of monogastric species (Beggs et al. 2022).The apical TRPV6 calcium channel, the $CaBP_{D9k}$ cytoplasmic Ca buffering protein and the basolateral PMCA1b energy-requiring Ca delivery to the submucosal fluid are all found in the colon mucosa and are also all promoted by increased production from the action of $1,25(OH)_2D$. Likewise, the passive diffusion of Ca through the paracellular route into or out of the lumen is mediated by the claudin tight junction proteins. The presence of these absorption mechanisms and their ability to be enhanced by endocrine stimulation, suggest that some residual Ca could be retrieved during the slow transit of large intestinal contents.

However, studies in humans and in rats found that increasing the supply of fermentable dietary fiber enhanced the absorption of Ca from the large intestine by both paracellular and transcellular routes. The mechanism appeared to be related to the increased production of short-chain fatty acids that both increased the availability of Ca ions and also promoted the Ca absorption pathways.

Ca Absorption in the Rumen

Studies in vitro in Ussing chambers, with rumen mucosa from different species have demonstrated that Ca can be actively absorbed from the luminal to the serosal compartments. The rate of absorption increases with increasing luminal Ca concentration. With no electrochemical gradient across the rumen mucosa, Ca transport is further enhanced by the presence of short chain fatty acids on the luminal side. It has therefore been proposed that uptake of Ca into the rumen epithelial cells may be via H^+/Ca^{2+} membrane exchange (Schröder et al. 1997, 1999). The actual chemical mechanism of the Ca absorption process and its control has been difficult to identify. When a calcium channel blocker, verapamil, was added to the luminal medium there was no effect on Ca transit across the mucosa. Likewise, when vanadate was added, as an inhibitor of a Ca-ATP-ase pump, there was no effect on the active luminal to serosal passage of Ca. Although a low level of the VDR was detected in the rumen mucosa of sheep, when rumen mucosa from sheep with elevated plasma $1,25(OH)_2D$ concentrations was studied in Ussing chambers, there was no stimulation of the Ca absorption process. Furthermore, no TRPV6 or $CaBP_{D9K}$ was detected in the cells of the rumen mucosa of sheep and goats (Schröder et al. 2001) or in bovine rumen mucosa (Schröder et al. 2015). These various findings have been summarized in Figure 2.

One technique to study Ca absorption in vivo is to measure the appearance in blood of strontium ions (Sr) after an oral dose of strontium chloride. Ca absorption mechanisms in humans have been shown to absorb Sr as a Ca analogue (Milsom et al. 1987). This was tested in sheep using an oral dose of 1 g Sr as $SrCl_2$ combined with 50 mg Ca as radioactive $^{45}CaCl_2$ in 25 ml water. Blood was then collected at intervals over 48 h to measure the plasma concentration of Sr and ^{45}Ca (Hyde and Fraser, 2014). There was a rapid appearance of both cations in plasma after dosing, with a correlation coefficient of 0.98 in the first 8 h, indicating that Sr was being absorbed by the same mechanism as Ca from the rumen (Figure 1). Direct injection of aqueous $SrCl_2$ through the abdominal wall into the rumen also showed the same rapid increase in Sr concentration in blood, confirming that absorption was occurring through the rumen mucosa.

To test whether $1,25(OH)_2D$ could stimulate this absorption mechanism in the rumen, sheep were continuously infused via subcutaneous osmotic minipumps, with $1\alpha\text{-}OHD_3$ at the rate of 12 µg/day for 6 days and rumen and intestinal Sr absorption was determined before and after this 6-day continuous infusion with $1\alpha\text{-}OHD_3$. As expected, absorption from the intestine was increased by this treatment. However, in contrast to the absence of an effect of $1,25(OH)_2D$ on Ca transit through rumen mucosa in vitro (Schröder et al. 2001), the sheep infused with $1\alpha\text{-}OHD_3$ showed a doubling of the absorption rate of Sr from the rumen (Hyde and Fraser, 2014). One substantial difference between these two studies would have been in the plasma concentration of $1,25(OH)_2D$. In dairy cows at the onset of lactation, the plasma $1,25(OH)_2D$ concentration rises from about 25 to 50 pg/ml up to between 75 to 175 pg/ml (Barton et

al. 1981); about a 3-fold increase. In the sheep infused with 1α-OHD_3 over 6 days, the plasma 1,25$(OH)_2$D concentration rose from about 25 to about 450 pg/ml, about an 18-fold increase.

Both in vitro and in vivo studies indicate that the rumen has a Ca uptake mechanism that allows a considerable proportion of dietary Ca to be absorbed. However, the absence in the rumen of the absorption mechanism components found in the small intestine and colon which can be regulated by 1,25$(OH)_2$D, and the conflict between in vitro and in vivo findings of response to raised circulating 1,25$(OH)_2$D concentrations, indicates that the rumen Ca absorption mechanism has yet to be defined.

One feature of Ca absorption in the small intestine of monogastric animals is the presence of a membrane receptor protein that binds 1,25$(OH)_2$D. The effect of this binding is a rapid non-genomic action which increases Ca uptake into the mucosal cells, resulting in increased Ca absorption across the mucosa. This receptor protein is known variously as 1,25D-membrane associated rapid response steroid-binding protein (**1,25D-MARRS**), and also as ERp57 and PDIA3 (Nemere et al. 2012). There is no published evidence of this protein having been investigated in rumen mucosa, but if it were present in rumen epithelial cells, it might explain the discrepancy between the lack of an effect of 1,25$(OH)_2$D on Ca transport in sheep rumen in vitro studies (Schröder et al. 2001) and the marked stimulation by 1,25$(OH)_2$D in vivo on Sr absorption from the rumen (Hyde and Fraser, 2014). The lack of any clear increase in Ca absorption in sheep in response to dietary Ca deficiency (Wilkens et al. 2011, 2012) indicates that the promotion of Ca absorption from any region of the alimentary tract in sheep is minimal compared to the stimulated absorption seen in goats (Wilkens et al. 2012) and dairy cows (Hyde at al. 2019; Hernández-Castellano et al. 2020) when there is a challenge to Ca homeostasis. It could be postulated that the extremely high plasma levels of 1,25$(OH)_2$D in the 1α-OHD_3 infusion study in sheep (Hyde and Fraser, 2014), could have promoted rumen Ca absorption by a 1,25D-MARRS rumen mechanism, that remains inactive to the lower levels of 1,25$(OH)_2$D in sheep.

One further endocrine factor that might provoke increased rumen Ca absorption at the onset of lactation in dairy cows is prolactin. This pituitary hormone has been shown to promote Ca absorption from the alimentary tract of monogastric animals (Wongdee and Charoenphandhu, 2013). Although it is clear that rumen Ca absorption is stimulated at the onset of lactation in dairy cows (Hyde et al. 2019; Hernández-Castellano 2020), this process is blocked when there is rumen stasis from hypocalcemia (Jørgensen et al. 1998; Hyde et al. 2019). Therefore, whatever the mechanism controlling the absorption of Ca from the rumen and its endocrine control factors, Ca uptake from the lumen ceases with hypocalcemia causing ruminal stasis. The rapid response of enhanced Ca absorption from the rumen of dairy cows when rumen motility is restored by correction of hypocalcemia with intravenous Ca infusion, indicates that the hypocalcemia of parturient paresis is not caused by some critical defect in the rumen Ca absorption mechanism and its regulation.

Conclusions

The Ca absorption mechanisms identified in the small intestine and colon of ruminants and their stimulation by 1,25$(OH)_2$D binding to VDR and activating the genes producing the protein components of those mechanisms, are similar to those in more thoroughly studied monogastric animals. In contrast, the mechanism of Ca absorption and its stimulation in the rumen of ruminants is less well defined. Further research could identify the molecular components in the rumen epithelial cells that enable the transit of Ca from the lumen into the circulation. A particular challenge is to determine whether the increase in rumen Ca absorption of dairy cows at the onset of lactation is influenced by the increased concentration of 1,25$(OH)_2$D in the circulation at that time.

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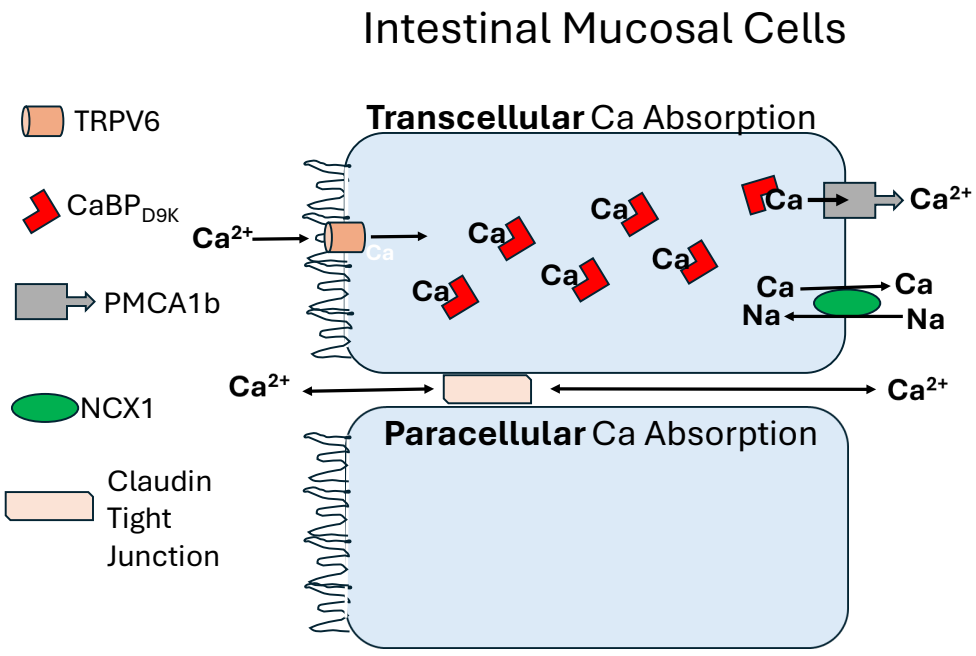

Figure 1. Schematic representation of small intestinal absorption of calcium by transcellular and paracellular mechanisms. In transcellular absorption, Ca enters the mucosal cells through the microvillous membrane calcium channel, TRPV6, and immediately links to the cytoplasmic calcium-binding protein CaBP$_{D9k}$. Ca is actively extruded across the basolateral membrane either by the calcium-ATPase, PMCA1b, or by a calcium/sodium exchange protein, NCX1. Paracellular Ca transport can occur through the claudin tight junction proteins either from lumen to the submucosal fluid or in reverse, depending upon the Ca gradient.

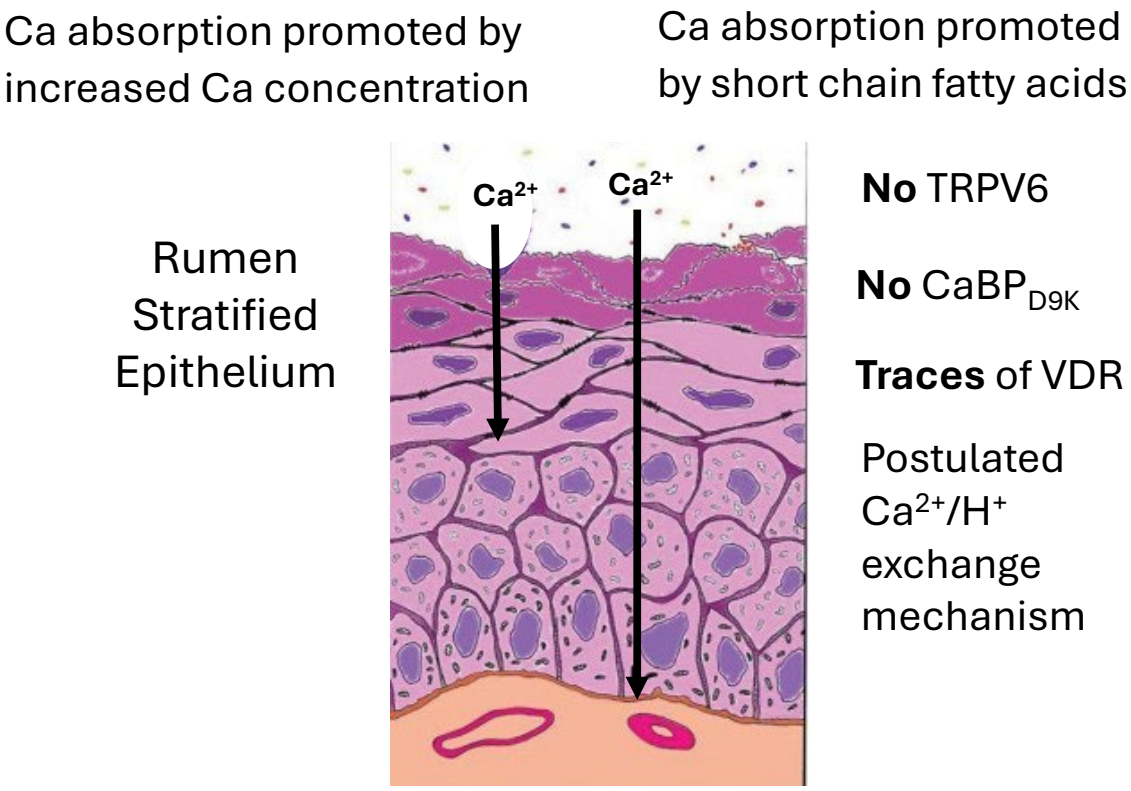

Figure 2. Schematic representation of active Ca absorption through the rumen stratified squamous epithelium mucosa, without the 1,25$(OH)_2$D-controlled Ca transport proteins found in the mucosal cells of the small intestine and colon. The principal absorption mechanism is postulated to be a Ca^{2+}/H^+ exchange across the squamous epithelial cell membranes.

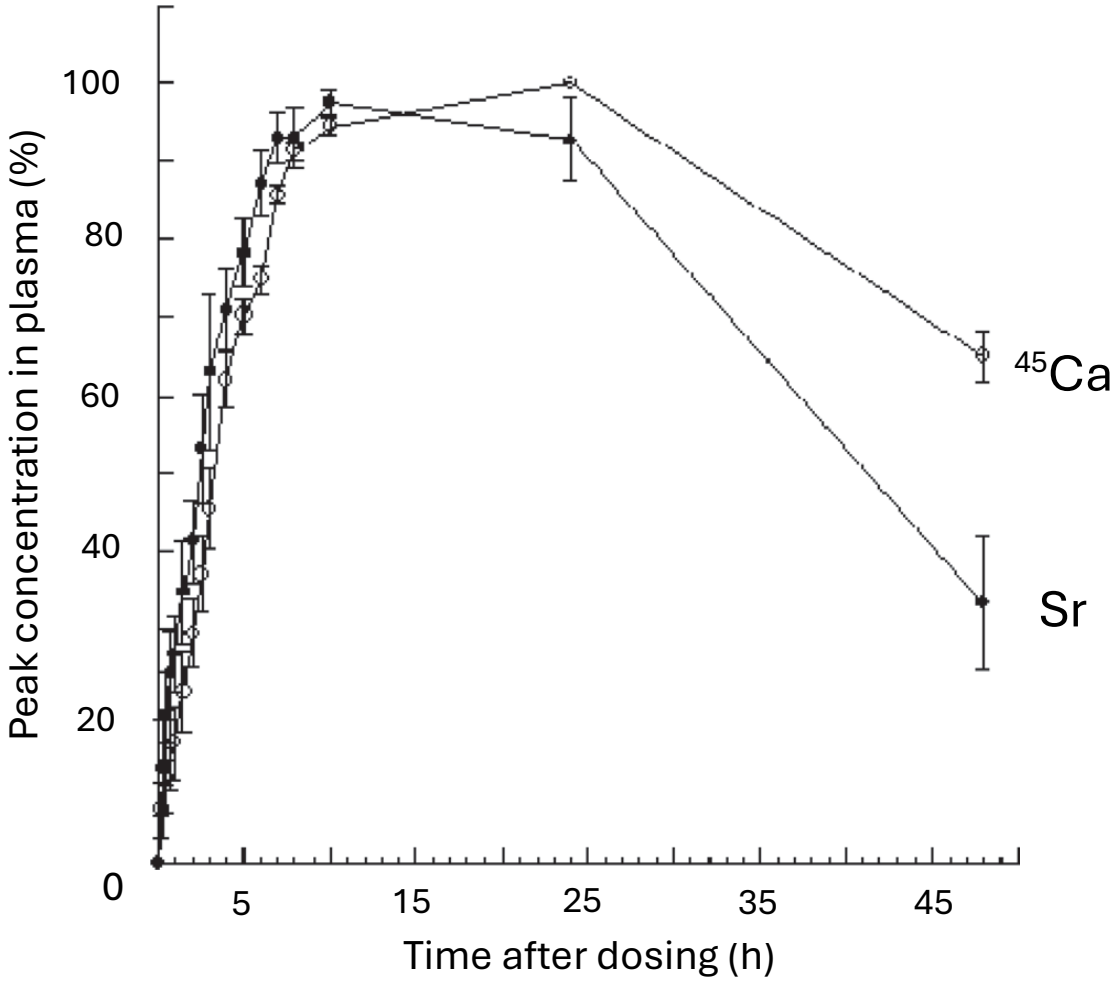

Figure 3. Changes in radioactive ^{45}Ca and Sr concentrations in plasma over 48 h after simultaneous oral administration of 50 mg ^{45}Ca and 1 g Sr in 25 mL H_2O. The profile of these two cations in plasma reflect their immediate absorption from the rumen and the subsequent absorption from the small intestine. Values are means of 4 sheep with standard errors indicated by vertical bars (after Hyde and Fraser, 2014).

Session Notes

Use of Hydroxychloride Trace Minerals in Diets of Cattle

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Introduction

Most essential trace minerals are generally supplemented to beef and dairy cattle diets to ensure that their requirements are being met. Inorganic trace mineral sources (primarily sulfate and oxide forms) started being supplemented to cattle to a limited extent in the 1930's, and are widely used today. Supplementation of inorganic trace minerals has been effective in correcting as well as preventing trace mineral deficiencies in cattle. However, in the presence of certain antagonist, bioavailability of inorganic trace mineral sources can be low. Various feed-grade sources of a particular metal (oxide, sulfate, etc.) can also differ in purity, and other factors that can affect bioavailability of the mineral.

Organic trace minerals are complexed or chelated to organic ligands (generally amino acids or polysaccharides). Use of organic trace mineral sources has increased greatly in the past thirty-five years. Because of their cost, organic trace minerals are generally added to replace a portion (20 to 50%) of the supplemental inorganic trace minerals. In theory the covalent bonds formed between the metal and ligand (s) should allow organic trace minerals to resist many of the interactions encountered by inorganic trace mineral sources. Organic sources of trace minerals have been found to be more bioavailable than inorganic sources in some studies (Hansen et al., 2008). However, results have been variable and some studies have reported no differences in bioavailability between inorganic and organic sources (Cao et al., 2000).

Hydroxychloride trace minerals represent a fairly new category of trace minerals. Micronutrients USA LLC (Indianapolis, IN) developed hydroxychloride trace minerals and introduced basic copper chloride (IntelliBond® C) to the market in 1995. Zinc and manganese hydroxychloride (IntelliBond® Z and M) were introduced by Micronutrients in 2012. In contrasts to sulfates where the metal is bound to sulfate via weak ionic bonds, the metals in hydroxy trace minerals are covalently bonded to multiple hydroxy groups. Hydroxy trace minerals are relatively insoluble in water but become soluble under acidic conditions typical of those found in the abomasum of ruminants (Spears et al., 2004). The low solubility at neutral pH results in hydroxy trace minerals being non hygroscopic and less reactive in feeds and premixes than sulfate forms, which may improve vitamin stability and cause less oxidation of lipids (Luo et al., 2005).

Bioavailability of Hydroxychloride Trace Minerals

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Bioavailability of copper hydroxychloride relative to copper sulfate was 132% based on plasma copper and 196% based on liver copper in steers fed diets high in the copper antagonists, molybdenum and sulfur (Spears et al., 2004). In a later study, hydroxy copper had a relative bioavailability of 112% compared with copper sulfate in steers fed diets high in molybdenum (Van Valin et al., 2019). Copper hydroxychloride has been shown to be similar in bioavailability to organic copper lysine in lambs (Cheng et al., 2011) and steers (Van Valin et al., 2019). Apparent absorption and retention of zinc was higher in steers supplemented with 25 mg Zn/kg from hydroxy zinc compared with those receiving zinc sulfate (Shaeffer et al., 2017). Bulls supplemented with hydroxy trace minerals had greater concentrations of liver copper and zinc than those receiving sulfate forms at the end of a 71-d feeding trial (Geary et al., 2021). Beef cows receiving a free choice mineral supplement containing zinc and copper hydroxychloride had greater liver zinc and copper concentrations than cows offered a mineral containing a combination of 75% sulfates and 25% amino acid complexes of zinc and copper (Jalali et al., 2020).

Rumen Metabolism of Hydroxychloride Trace Minerals and Effects on Rumen Fermentation and Digestibility

The metabolism of zinc and copper hydroxychloride in the rumen differs greatly from sulfate forms in cattle fed diets that result in a rumen pH of 6.2 or higher after feeding (Caldera et al., 2019; Guimaraes et al., 2021a, 2022). Steers dosed with sulfate trace minerals had much higher ruminal soluble zinc and copper concentrations after dosing than steers receiving hydroxy sources. In steers fed a high concentrate diet, that resulted in a rumen pH of approximately 5.5 after feeding, differences between sulfate and hydroxy forms in ruminal soluble concentrations of zinc and copper were less dramatic (Guimaraes et al., 2021b). Zinc and copper concentrations in ruminal solid digesta were much higher in steers dosed with hydroxy trace minerals (Caldera et al., 2019). Ruminal soluble and solid digesta concentrations of manganese were less affected by trace mineral source than copper and zinc (Caldera et al., 2019; Guimaraes et al., 2021a, 2022). Based on their release following dialysis against a chelating agent (**EDTA**) copper and zinc from hydroxychloride forms were less tightly bound to ruminal solid digesta than sulfate forms (Caldera et al., 2019; Guimaraes et al., 2021a,b, 2022). The greater bioavailability of hydroxy forms of zinc and copper may relate to their weaker binding to ruminal solid digesta. The weaker binding of copper and zinc to the solid digesta likely relates to hydroxy forms interacting less with antagonists in the rumen environment.

Studies that utilized a total fecal collection to determine neutral (**NDF**) and acid detergent fiber (**ADF**) digestibility in cattle supplemented with hydroxy or sulfate trace minerals are summarized in Table 1. Beef steers or lactating dairy cows were used to measure digestibility. All studies used *Bos taurus* cattle except for the Abreu et al. (2024) study that used *Bos indicus* cattle. Diets used included medium quality grass hay, tropical forage hay, corn–corn silage, dairy total mixed rations (**TMR**), and high concentrate finishing diets. Cattle fed hydroxy trace minerals had greater NDF digestibility than those fed sulfate forms in five of the six studies that have reported NDF digestibility. Improvements in NDF digestibility in cattle receiving hydroxy versus sulfate

sources have ranged from 2.1 to 4.6 percentage units with an average increase of 2.9 percentage units across the six studies. Acid detergent fiber digestibility has been reported in four studies. Digestibility of ADF was greater in cattle supplemented with hydroxy vs. sulfate trace minerals in three of the studies. Studies using internal markers to estimate digestibility have also observed improvements in NDF digestibility in beef heifers (Kuijk et al., 2022) and lactating dairy cows (Daniel et al., 2020; Miller et al., 2020) fed hydroxy compared to sulfate trace minerals. It is unclear if hydroxy trace minerals enhance fiber digestibility or sulfate forms negatively affect fiber digestibility. The greater fiber digestibility in cattle receiving hydroxychloride trace minerals may relate to their low solubility in the rumen relative to sulfate sources. High concentrations of soluble zinc and copper in the rumen can negatively affect fiber digestion (Durand and Kawashima, 1980). The improved fiber digestion in cattle receiving hydroxy trace minerals was associated with greater total volatile fatty acid (**VFA**) concentrations post feeding in steers fed medium quality hay (Guimaraes et al., 2021a) and a lactating dairy type diet (Guimaraes et al., 2022).

In steers fed a high concentrate diet, low in fiber, NDF and ADF digestibility were not affected by trace mineral source (Guimaraes et al., 2021b). However, molar proportion of ruminal propionate was greater in steers supplemented with hydroxy compared to those fed sulfate trace minerals.

Effect of Hydroxychloride Trace Minerals on Cattle Performance

Performance of feedlot steers and lactating dairy cows supplemented with hydroxy trace minerals has generally been similar to those receiving a combination of sulfate and amino acid complexes. Milk production and 3.5% FCM were similar for lactating dairy cows supplemented with hydroxy forms of Zn, Cu, and Mn, and those receiving a combination of 75% sulfate and 25% amino acid complexes during the first 84 days of lactation (Yasui et al., 2014). Performance and carcass characteristics of finishing steers supplemented with hydroxy Zn and Cu were similar to those receiving a 75% sulfate, 25% organic combination of Zn and Cu (Wagner et al., 2016). In large scale feedlot studies (n = 568, Heldt and Davis, 2019; n = 735, Hilscher et al., 2019) performance and health were not affected by treatment in steers fed a sulfate (65-67%), amino acid complex (33-35%) combination of Zn or hydroxy Zn. Finishing steers supplemented with hydroxy Zn had similar performance but greater longissimus area than those receiving a sulfate (67%), organic combination (Budde et al., 2019).

A cow-calf study conducted over a 2-year period compared a free choice mineral with Zn, Cu, and Mn supplemented from a combination of 75% sulfate and 25% amino acid complexes or hydroxy forms (Jalali et al., 2020). Cow body weight changes and reproductive performance, and calf weaning weights were not affected by treatment.

Performance of steers supplemented with Zn sulfate or hydroxy Zn was similar (Budde et al., 2019). However, hot carcass weight and longissimus muscle area tended to be greater for steers receiving hydroxy Zn (Budde et al., 2019). Performance and carcass characteristics did not differ among finishing steers supplemented with hydroxy

Zn, Cu, and Mn and those supplemented with sulfate forms (Caldera et al., 2017). However, in a recent study with greater numbers and cattle uniformity, finishing steers supplemented with hydroxy Zn, Cu, and Mn had greater hot carcass weight, dressing percentage, longissimus muscle area, and yield grade, and tended (P = 0.12) to have greater average daily gain (**ADG**) than steers receiving sulfate forms during a 154-day study (Table 2; Spears et al., 2024).

Milk production of Holstein cows supplemented with sulfate or hydroxy Zn, Cu, and Mn for 21 days pre to 84 days postpartum was affected by a treatment x week interaction (Yasui et al., 2014). Cows fed hydroxy trace minerals had greater milk yield during week 5 of lactation. In a recent Florida study 70 cows per treatment were provided hydroxy or sulfate trace minerals from 28 days prepartum to 105 days postpartum (Adeou et al., 2024). Colostrum and total solids yield tended (P = 0.08) to be greater for cows receiving hydroxy trace minerals. During the first 105 DIM, 3.5% fat-corrected milk yield was greater for cows in the hydroxy treatment compared to the sulfate group. Milk protein and total solids yield were also greater for cows supplemented with hydroxy trace minerals. Percent cows pregnant by 305 DIM also tended (P = 0.08) to be higher for cows receiving hydroxy trace minerals (Sarwar et al., 2024).

A recent 3-year study at Colorado State University compared performance of beef cows and their calves provided hydroxy or sulfate trace minerals (Farmer et al., 2024). Cows were provided free choice minerals formulated to provide 100% of the NASEM (2016) requirement for Zn, Cu, and Mn from sulfate or hydroxy sources. In year 1 pregnancy rate following artificial insemination (**AI**) and calf weaning weights were not affected by treatment. However, in years 2 and 3 pregnancy rate following AI and calf weaning weights were greater for hydroxy than sulfate supplemented cows. At the end of years 2 and 3 cows provided hydroxy trace minerals had higher liver Zn, Cu, and Mn concentrations than cows receiving sulfates. In Brazil, Nellore bulls supplemented with hydroxy trace minerals had greater gain when grazing topical pastures than those receiving sulfate forms (Cidrini et al., 2020).

Health

Dairy cows supplemented with hydroxy trace minerals from 28 days prepartum to 105 DIM had a lower incidence of retained fetal membranes and overall morbidity than cows receiving sulfate sources (Sarwar et al., 2024). Calves receiving hydroxy trace minerals had similar morbidity to those fed sulfate or organic forms during a 45-day receiving study (Ryan et al., 2015). Morbidity and mortality were similar in finishing steers supplemented with Zn hydroxy or Zn sulfate (Budde et al., 2019). Studies suggest that lactating dairy cows supplemented with hydroxy Zn had greater gut barrier function during feed restriction (Horst et al., 2020) or heat stress (Rodriguez-Jimenez et al., 2022). Lactating dairy cows supplemented with hydroxy Zn had lower fecal populations of *Treponema spp.* than cows supplemented with Zn sulfate (Wenner et al., 2022). Bovine digital dermatitis has been associated with *Treponema* bacteria in dairy cows.

Conclusions

Hydroxychloride trace minerals are covalently bonded to multiple hydroxy groups and are relatively stable in the rumen at a pH above 6.0. Copper and zinc hydroxychloride have been shown to be more bioavailable than sulfate forms in cattle. Studies in steers and lactating dairy cows have reported greater NDF digestibility in cattle supplemented with hydroxychloride trace minerals compared to those receiving sulfate sources. Replacing sulfate sources with hydroxychloride trace minerals has increased pregnancy rate to AI in beef cows, and improved hot carcass weight and longissimus muscle area in finishing steers. Lactating dairy cows supplemented with hydroxychloride trace minerals had greater 3.5% fat-corrected milk yield and lower morbidity rate than cows receiving sulfate sources.

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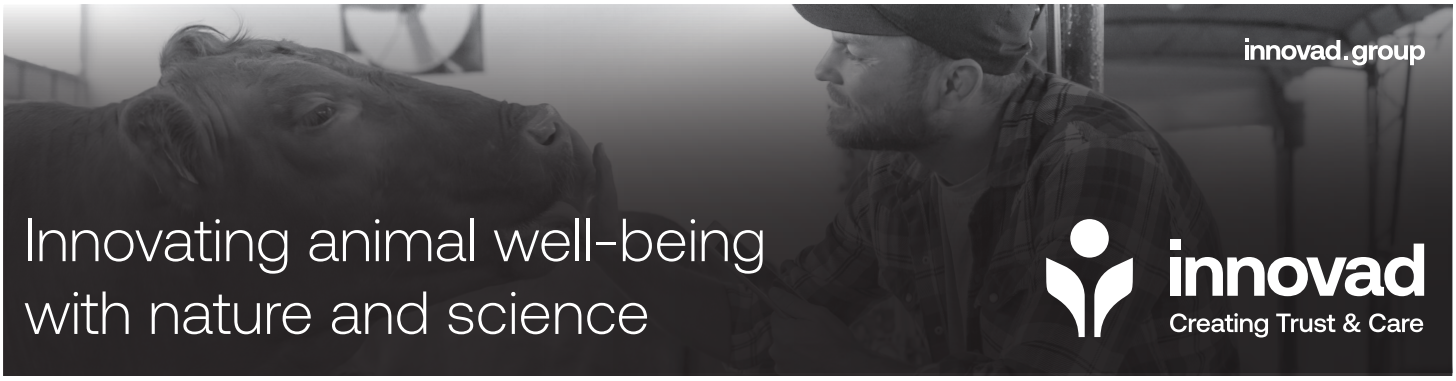
Table 1. Summary of NDF and ADF digestibilities in studies comparing sulfate and hydroxychloride trace minerals

Reference	Diet type	Sulfate	Hydroxy-chloride	P-value
Faulker and Weiss, 2017	Dairy TMR			
NDF dig, %		46.4	48.6	0.02
Caldera et al., 2019	50% corn - 50% corn silage			
NDF dig, %		37.8	41.2	0.07
Guimaraes et al., 2021a	Forage based			
NDF dig, %		40.4	42.7	0.04
ADF dig, %		32.4	34.1	0.05
Guimaraes et al., 2021b	High concentrate			
NDF dig, %		41.2	41.0	0.71
ADF dig, %		27.2	30.5	0.15
Guimaraes et al., 2022	Dairy TMR			
NDF dig, %		43.0	47.6	0.05
ADF dig, %		29.8	32.4	0.05
Abreu et al., 2024	Forage Based			
NDF dig, %		59.4	61.5	0.05
ADF dig, %		61.3	63.9	0.05

Table 2. Effect of trace mineral source on performance of finishing steers. (Spears er al., 2024)

	Sulfate	Hydroxy	P-value
n	200	200	
ADG, kg/d	1.72	1.77	0.12
DM intake, kg/d	9.9	10.1	0.27
Gain:feed	0.174	0.176	0.59
Hot carcass wt, kg	402.1	411.1	0.04
Dressing, percentage	62.4	63.3	0.01
Longissimus muscle area, cm^2	89.4	91.3	0.03
Yield grade	2.53	2.70	0.04

Session Notes



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Nutrient Requirements of Preweaning Calves

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Introduction

The nutrient requirements of dairy calves have changed substantially over the years with different versions of the National Research Council (**NRC**) publications of *Nutrient Requirements of Dairy Cattle.* The latest version, now the National Academies of Science, Engineering and Medicine (**NASEM**) published in 2021, is the most accurate and comprehensive look at calf requirements to date. The computer model has been updated and can be downloaded at no cost at: https://nap.nationalacademies.org/catalog/25806/nutrient-requirements-of-dairy-cattle-eighth-revised-edition. This article aims to present the major concepts of the NASEM (2021) system.

During the preweaning stage, the rumen is underdeveloped and the calf largely relies on the liquid diet of milk or milk replacer to receive its protein, carbohydrates, and fat, along with minerals and vitamins. Research over the last 25 years has shown the benefits of feeding calves more milk than historical convention. Calves should be encouraged to consume starter feed at an early age to develop the rumen in size and function. Fermentation of starter carbohydrates produces the volatile fatty acids (**VFA**) butyrate and propionate that stimulate development of the ruminal epithelium or papillae. Calves fed greater amounts of milk will have delayed starter intake, and thus our management systems need to be adapted to allow smooth weaning. Because the rumen and its microbial population are immature at this stage and the pH is lower than the minimum of 6.0 for optimal fiber fermentation, the calf is unable to make use of the nutrients found in forage. Calves have limited ability to use forages until well after weaning.

Several major changes were incorporated into the NASEM (2021) requirement system. 1) Empty body weight (**EBW**) is used for all calculations. This is the live BW minus the rumen and gut contents. 2) An equation to estimate starter intake was developed. 3) Energy requirements were updated using a dataset from experiments that measured body composition changes in dairy calves. Maintenance requirements differ for different classes of calves and environmental conditions, including both heat stress and cold stress. Equations were developed to estimate body composition and efficiency of use of metabolizable energy (**ME**) for growth. 4) New equations for estimating feed ME values were developed. 5) A metabolizable protein (**MP**) system was adopted and protein requirements were updated. 6) The mineral requirement system was updated and some vitamin recommendations were revised. 7) For purposes of estimating

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nutrient requirements, calves are considered to be cattle less than 18% of mature BW, and heifers those >18%.

Body Weight and Empty Body Weight Interconversions

Based on literature values, the empty BW of calves fed milk only was set to 94% of live BW, whereas when calves begin to eat starter the proportion is 93% of BW. For weaned calves, the EBW is only 85% of BW. This means that gut fill is increasing as the rumen develops. The decrease in proportion of EBW/BW occurs due to the rapid expansion of the rumen and gut size as starter intake increases.

Dry Matter Intake

For calves fed milk for *ad libitum* intake, the milk solids intake is approximately 2.25% of BW, increasing to 2.5% of BW in calves > 65 kg (143 lb). In contrast, for weaned calves the dry matter intake (**DMI**) is about 3% of BW.
The NASEM committee developed equations to predict voluntary starter intake. Body weight, daily ME intake from the liquid diet, average daily gain (ADG), and time since first offer of starter were used as independent variables. A data set of 3,491 observations from 853 calves in Florida, Georgia, and Brazil was used. For calves in semi-tropical environments such as Florida, the resulting equation was:
Starter DMI (g/d) = 600 × (1 + 14,863 × (exp(-1.553 x age of starter)))^-1 + (9.95 × BW) – (130 × ME from liquid diet)

Although this is a complex equation, users can have the computer model calculate the estimate. Users can also use known DMI if it is available.

Energy Requirements of Calves

The NASEM (2021) system uses an ME-based system, although some of the calculations use net energy (**NE**) values. Energy requirements were established for calves consuming milk only, those consuming milk and starter, and weaned calves. Recommendations and equations were developed from a database of 255 calves from 7 comparative slaughter experiments (Diaz et al, 2001; Tikofsky et al., 2001; Meyer, 2005; Bartlett et al., 2006; Bascom et al., 2007; Mills et al., 2010; Stamey Lanier et al., 2021). In these experiments, the body composition (and thus body energy) was determined at baseline (close to birth) and then some weeks later so that the changes in body composition and energy could be calculated.

Heat production (**HP**) by the calves was calculated as ME (**ME)** intake minus retained energy (**RE**). The final equation was:

$$HP, Mcal/EBW^{0.75} = 0.077 \times e^{(3.3426 \times MEI, Mcal/EBW^{0.75})}$$

where EBW is empty body weight.

Since the net energy (**NE**) for maintenance equates to HP at zero intake, from the intercept of this equation the NE for maintenance is 77 kcal/$EBW^{0.75}$, which is lower than NRC (2001) values. However, the maintenance ME value (101 kcal/$BW^{0.75}$) is nearly identical to the previous value.

Based on limited data for Jersey calves, the maintenance requirement may be somewhat greater than for Holstein calves. However, the committee concluded there were insufficient data to model a separate equation for Jerseys, and the same equation was used for both small and large breeds.

An adjustment of the maintenance value is possible for both heat stress and cold stress, defined as 2.01 kcal/kg $BW^{0.75}$ above or below the upper and lower critical temperatures for calves. The upper critical temperature, the temperature where calves must expend energy to dissipate excess heat, was set at 26°C (79°F), whereas the lower critical temperature, which is the temperature below which calves have to expend energy to maintain body temperature, is set at 15°C (59°F). Thus, calves in Florida experience both heat and cold stress, and the NASEM model calculates the additional maintenance requirements to deal with those stressors.

The current system (NASEM, 2021) for calf growth is based on ME-allowable growth and MP-allowable growth. Establishment of the energy requirements for growth relies on accurate estimation of the amount of RE per unit of growing tissue, as determined by relative amounts of fat and protein deposited. The amount of protein deposited per unit of BW gain generally is quite invariable if MP is sufficient, whereas tissue fat deposition is variable depending on total ME intake or limitation of growth by MP supply (Van Amburgh et al., 2019).

The database of calves from slaughter studies was used to derive equations to predict RE from EBW and EBW gain. The final equation was:

$$RE = (EBG^{1.100}, kg/d) \times (EBW, kg^{0.205})$$

To predict RE and growth from a quantity of ME available to the calf from milk, the efficiency of ME use for RE must be known. The NASEM committee set this value at 55%. The efficiency of use of ME from starter feed is lower than that for milk, and this is determined by a separate equation. The combined efficiency is then a weighted average of efficiencies for milk and starter use based on relative amounts of milk and starter consumed.

Rearrangement of this latter equation results in the ability to predict ME-allowable EBW gain and then ADG:

$$\text{EBW gain (kg/d)} = RE, Mcal/d / (EBW^{0.205}, kg)^{1/1.1}$$

Protein Requirements

Dietary protein is needed to furnish amino acids for growth, and to provide substrates for microbial protein synthesis in the developing rumen. The committee declined to develop an amino acid-based system because of limited data on amino acid requirements in young calves.

The MP requirement for maintenance is relatively small, and consists of requirements for scurf (hair and skin cells shed), endogenous urinary N loss (excretion of N from breakdown of body proteins and amino acids in metabolism), and metabolic fecal crude protein (**CP**), which is the endogenous loss of N in secretions, cells, and microbial protein in feces.

The equations are as follows;

Scurf CP, g/d = $0.22 \times BW^{0.60}$

Endogenous urinary CP, g/d = $2.75 \times BW^{0.50}$

Metabolic fecal crude protein, g/d = (11.9 × LFDMI, kg/d) + (20.6 × SFDMI, kg/d)

where LFDMI is liquid feed DMI and SFDMI is starter feed DMI. These calculations are on a net protein basis, and to convert to MP the assumed efficiencies are 0.68 for scurf and metabolic fecal protein and 1.0 for endogenous urinary protein.

Net protein for growth is calculated as the amount of CP retained in the EBW gain and is calculated as a function of rate of gain and energy content of the gain. The equation was derived from the database of the 7 slaughter experiments:

Net protein gain = (166.2 × EBW gain, kg/d) + (6.1276 × (RE, Mcal/d / EBW gain, kg/d)

This is different from NRC (2001) where body protein gain was calculated as a fixed amount per unit of tissue. Efficiency of converting net protein to MP decreases with age of the calf, and so an equation was developed to calculate the correct efficiency based on the proportion of mature body size:

Efficiency of MP for gain = 0.70 - 0.532 × proportion of mature BW

Conversion of CP to MP uses a factor of 0.95 for milk or milk-derived ingredients, 0.75 for dietary proteins digested post-ruminally in the young calf fed both milk and starter, and a value of 0.70 for conversion of CP to MP for calves with a functioning rumen.

Calculating ME Values of Feeds

The ME values of liquid feeds are calculated similarly to the previous edition with modifications. First, the gross energy (**GE**) of the feed is calculated by multiplying the

percentage composition on a DM basis by the respective heats of combustion, according to the following formula:

GE, Mcal/kg DM = ((FA × 9.3) + (Protein × 5.7) + (100- Protein - FA - Ash × 4)) / 100

where values are on a DM basis. Fatty acid (**FA**) concentration is better than crude fat for nutritional characterization of feeds, however, many feed labels such as those on milk replacer are based on crude fat. Crude fat from ingredients commonly used in milk replacers can be converted to FA by multiplying crude fat by 0.945. Feeds can contain other organic compounds such as partially hydrolyzed starch, dextrins, glucose, or glycerol that may be incorporated in small amounts (usually less than 10% of DM in aggregate) into milk replacer (**MR**). This fraction is assumed to have the same heat of combustion as lactose (4 Mcal/kg). Values for whole milk are determined similarly after converting the composition to a DM basis.

Ash content normally is not listed on feed tags but generally will be 6 to 12% of total MR DM. Because ash has no energy it affects the ME value and should always be determined analytically. Users are cautioned that feed tag values for MR components are given on an “as fed” or air-dry basis, which for MR is usually 95 to 97% DM. Failure to account for this residual moisture will introduce error into the calculation of ME.

The ME values for MR then are derived by multiplying the gross energy content by 0.91, which is the product of the average digestibility (0.95) and metabolizability of the digestible energy (0.96) for MR. For whole milk, the gross energy (**GE**) is multiplied by 0.93 because of the slightly higher digestibility for milk (0.97; NRC, 2001).

The digestible energy (**DE**) values for solid feeds are calculated as for adult ruminants with the exception that the digestibility coefficient for fat is assumed to be 0.81 rather than 0.74 as for older cattle. The coefficient of 0.81 for fat digestibility represents the average of studies that measured digestibilities for crude fat in weaned calves. The DE was calculated without discounting for intake or starch concentration (i.e., intake was set at 3.5% of BW and dietary starch was assumed to be 25% in equations). The efficiency of converting DE to ME by young calves fed MR and various starters varied from 0.91 to 0.95; therefore, the ME of dry feeds was set at DE × 0.93. To derive accurate estimates, starter should be analyzed for CP, FA, NDF, 48-hour NDF digestibility, starch, sugars, and ash.

Validation and Visualization of Model

The new model for requirements of ME and MP was validated in NASEM (2021) by comparing the estimated growth using the model and the actual reported values in the literature. The committee assembled a database of 416 treatment means from 94 published articles that represented older and newer studies, and a range of milk or milk replacer intakes, starter intakes, and ADG. Figure 1 shows the plot of observed values (Y-axis) versus predicted values (X-axis), along with a plot of the residual values (observed minus predicted). The relationship between observed and predicted values is

strongly linear, with the tight distribution demonstrating that the model produces quite precise estimates. The residuals should have a mean of zero and lie in a tight distribution along the X-axis without slope. The results show these qualities, indicating that the equations have minimal bias and are precise. Values predicted by the NASEM (2001) system are more accurate in predicting actual growth than the NRC (2001) system.

To demonstrate the resulting requirements for ME and MP, Table 1 shows the results for milk-fed calves at various growth rates. As desired rate of ADG increases, the calf must consume more DM. The ME requirements approximately double over this range, whereas CP requirement approximately triple. This relationship between ME and CP means that the milk replacer must contain more CP as growth rate increases. Similar concepts apply to the calf fed starter as well.

Mineral and Vitamin Requirements of Calves

The NASEM committee took a more quantitative approach to establishing minerals recommendations than in the past (NASEM, 2021). Requirements (adequate intakes) were calculated using a factorial approach where possible, which has not been done previously for young calves. Required mineral concentrations to meet adequate intakes of minerals for calves of different size and growth rates were then calculated (Table 2). Compared to recommended concentrations in the previous edition (NRC, 2001), recommended Ca concentrations are lower for MR but similar for starter and grower. Recommended concentrations of P are about 15% lower for MR, starter and grower. Recommended K concentration in MR is about 70% higher but similar for starter and grower and recommended Na concentrations are similar to the previous edition. Recommended concentrations of Cu are about half the previous value, Fe is about 15% lower for MR but similar for starter and grower. Recommended concentration of Mn is higher for MR but similar to the previous edition for starter and grower. Zinc concentrations are about 40% greater than those in the previous edition. The Co requirement has been removed from milk replacer since cobalt is only needed in the rumen to form vitamin B_{12}.

Recommended fat-soluble vitamin concentrations are found in Table 3. Similar to minerals a factorial approach was taken to establish requirements, which were then equated to concentrations required in feeds. Requirements for vitamins A and D are largely unchanged from NRC (2001). Vitamin E has been increased substantially based on recent research. Calves require the B-vitamins and choline in the milk replacer, but recommended concentrations have not changed since NRC (2001).

Conclusions

The NASEM (2021) calf chapter is a major step forward in predicting the nutrient requirements of preweaned (and weaned) dairy calves. The NASEM committee made many changes relative to the previous edition. The model is ME-allowable growth based, with protein requirements calculated to meet the needs of the growth allowed by

ME intake. The equations are robust and produce unbiased predictions of growth with both accuracy and precision. Mineral and vitamin recommendations are now based on methods used for older cattle, representing a major improvement. Vitamin E concentration was increased but vitamins A and D are unchanged. The publication and associated computer model are valuable tools to estimate nutrient requirements or predict ADG from a given diet.

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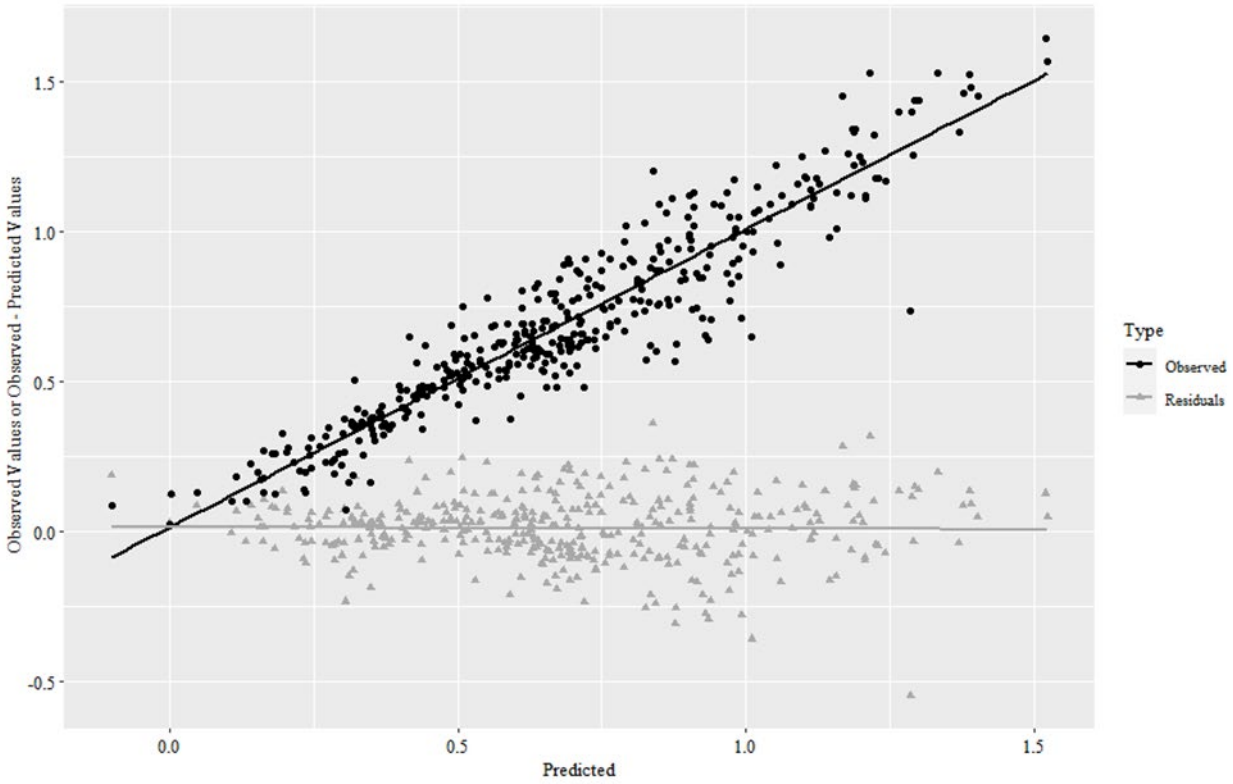

Figure 1. Observed minus predicted values for ADG from 401 literature treatment means, with residuals plotted. From NASEM, 2021.

Table 1. Requirements for DMI, ME, and CP at different rates of gain for a 110-lb calf under thermoneutral conditions based on NASEM (2021).

ADG (lb/d)	DMI (lb/d)	ME (Mcal/d)	CP (g/d)	CP (% of DM)
0.44	1.23	2.56	102	18.2
0.88	1.58	3.29	156	21.6
1.32	1.96	4.05	209	23.7
1.76	2.33	4.85	262	24.7
2.20	2.73	5.66	315	25.5

Fed milk replacer containing 2.08 Mcal ME/lb DM

Table 2. Recommended concentrations of minerals in milk replacer, starter, and grower based on NASEM (2021).

Mineral	Milk replacer	Starter	Grower
Ca, %	0.80	0.75	0.65
P, %	0.60	0.37	0.33
Mg, %	0.15	0.15	0.16
K, %	1.10	0.60	0.60
Na, %	0.40	0.22	0.20
Cl, %	0.32	0.17	0.15
Co, ppm	NA	0.2	0.2
Cu, ppm	5	12	12
I, ppm	0.8	0.8	0.5
Fe, ppm	85	60	55
Mn, ppm	60	40	60
Se, ppm	0.3	0.3	0.3
Zn, ppm	65	55	50

Table 3. Recommended concentrations of fat-soluble vitamins in milk replacer, starter, and grower based on NASEM (2021).

	IU/kg BW	IU/kg DM		
		Milk replacer[2]	Starter[3]	Grower[4]
Vitamin A	110	11,000	3700	3700
Vitamin D	32	3200	1100	1100
Vitamin E	2.0	200	67	67

Session Notes

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The Untapped Opportunity of Early Life Nutrition

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The discovery of long-term effects of early life nutrition has come to question traditional objectives for calf rearing and set completely different ones. However, despite the increase in academic research and industry focus, there is still a massive mismatch between current scientific understanding of this opportunity and current feeding practices, even in the most technically developed segments of the global dairy industry.

Nowadays, the long-term benefits of improved growth for long-term productivity are generally accepted in our industry. This clear association (Soberon and Van Amburgh 2013), in several cases confirmed as causal, is often discussed using terms such as “epigenetics” and “metabolic programming”, linking this phenomenon to other, more deeply studied, biological examples such as human metabolic syndrome (Fernandez-Twinn and Ozanne 2010). However, although dairy calves could represent one of the best examples of biological thrifty phenotype cases, dairy nutrition science has rarely delved deeper than the value driving connection between calf growth and health and future lactation performance.

Strictly speaking, metabolic programming refers to long-term alterations of metabolic processes caused by early life factors. In the same way, epigenetic effects are restricted to those that change the expression of genes, not any other phenotypic change, even if its cause may have been much earlier in time, for example, the consequences of different morphological development. For example, a dairy heifer that calves at a higher body weight because it grew faster earlier in life, would exhibit a lower rate of growth during the first lactation, thereby competing less with resources available for lactation. We should not refer to such effect either as metabolic programming or epigenetics. There are, nevertheless, plenty of reasons to propose that the opportunity in dairy cows is a case of true metabolic programming, even if the supporting body of evidence is still limited.

Biology has come to accept a third term in the classic “phenotype = genotype + environment” equation, the perinatal environment. This third factor is neither truly genetic, nor truly environmental, because although it is caused by the environment, its consequences persist over time, even into new generations (Laporta et al. 2020). It is as if Lamarck’s theory of evolution were to prove a small angle of truth, reminding us once again that biology is more complicated than we imagined.

Metabolic programming is about adaptation for survival in a future that can be predicted by environmental signals sensed today. Seeds germinating in a dry environment are configured for a future of drought (Aswathi et al. 2022), and nutrient-deprived babies configure their future hunger regulation mechanisms for

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eventual periods of food scarcity (Prentice 2005). This ability of organisms to configure their future metabotype to be fit for survival has an obvious evolutionary advantage.

Dairy science has described that dairy cows can set their heat resistance metabotype in response to perinatal heat stress (Tao and Dahl 2013). Early life nutrient availability and future lactation performance also fit this biological reasoning.

Dairy calves as a thrifty phenotype case

The thrifty phenotype hypothesis proposes that early life nutrient deprivation results in a permanent adaptation or damage to energy metabolism. Calves born from a dairy dam have been nutrient deprived to a greater or lesser extent since the onset of human consumption of bovine milk. Domestication of cows has benefited the human and bovine species. Dairy has been and is essential to our food security (FAO 2018), and bovines are among the most successful animal species because of their relationship with humans (McTavish et al. 2013). Nonetheless, even if modern dairy cows yield severalfold the amount of milk their offspring could consume, nutrient competition between calves and human consumption persists.

Unlike most domestic animals, dairy calves are not fed ad libitum until weaning. A young calf roughly requires half a kilogram of milk solids for maintenance, and about another half a kilogram to achieve a natural growth rate. Historically, the role of domestic bovines has been to create food from non-food resources. Consequently, restricting calves to a minimum supply of valuable milk and delaying growth until they can consume food solids follows a clear, human-focused food security logic. This practice also persisted in modern dairy systems driven by simple economic logic.

Modern dairy bovines are genetically and phenotypically very different from the originally domesticated bovine species and other current bovine forms. Similarly, the perinatal environment of dairy calves is certainly a major deviation from any imaginable bovine biological reference. Nutrient supply is a major element of these differences, but not the only one.

Biologically, we should view a newborn animal that is fed just above its maintenance energy needs at the expense of sacrificing growth. This creates an extreme perinatal nutritional environment that is followed by the extreme homeorhetic challenge of energy metabolism of the dairy cow during every lactation cycle.

Ethically, traditional feed restriction should also be questioned (Costa et al. 2019). Hunger is an unacceptable form of compromised welfare, and a major deviation from dairy consumer expectations. This is certainly an aspect of the untapped opportunity of early life nutrition.

Economically, the opportunity to spare milk resources by restriction may come at long-term hidden costs. This is especially true when one of the main challenges of modern dairy production is homeostatic competence of dairy cows in regulating energy in the lactation cycle (Ghaffari et al. 2023). Still our industry views increased

nutrient supply simply as an opportunity to improve productivity and health. Biologically, the thrifty phenotype hypothesis proposes an induced and sustained damage to energy regulation competence associated with the feed restriction of calves.

The dual biological role of milk: nutrient transfer and signaling

Milk is a unique reproductive feature of mammals. It serves to sustain parental support for the offspring after birth, which allows for incomplete development at birth. This is especially relevant in ruminants, because their full digestive competence requires the gradual establishment of the ruminant ecosystem.

Milk shares features across mammals, such as, for example lactose, a sugar that meets the specific osmolality needs to supply water and nutrients at a certain ratio. It is also able to supply large amounts of glucose with a moderate glycemic signal (Schaafsma 2008). Other milk characteristics are unique to each species or unique to the lactation stage, as colostrum and milk composition during the first days of lactation.

Bovine milk composition is variable in its multiple characteristics. However, there are well-defined boundaries for this variation (Costa et al. 2019). Not only are there boundaries for the environmentally driven variation, but more interestingly, there are hard boundaries for genetic variation and, therefore, limitations on the possibilities for genetic selection for these parameters within the species. Genetic selection boundaries are typically connected with parameters that are critically connected with selection pressure, in particular reproductive success and survival of the offspring (Berry et al. 2024), which are both tightly connected with the utility of milk.

An example of milk components being well-preserved within a species and diverse across mammalian species is milk oligosaccharides. Interestingly, their role is not understood to be nutritional, but rather as an ecological signal to configure the gastrointestinal ecosystem of the offspring.

Another interesting example is found in the field of infant formula composition. The mismatch between the amino acid profile of bovine dairy proteins and that of human milk proteins led to the dilemma of matching the amino acid supply or matching total protein content in the formulations. For this reason, infant formulas traditionally contained higher protein than human milk. However, this slightly higher protein signal has been linked to childhood obesity (Weber et al. 2014), which has led to reconsideration of protein levels in infant formulas.

Milk is generally accepted to have a dual role as a nutrient carrier and signaling element between the mother and the offspring (Power and Schulkin 2013). Many examples beyond the above illustrate this role. This should not surprise us if we consider that milk is a much more ancient reproductive feature in mammals than placental reproduction. Marsupials are born in an extreme state of incomplete development and rely exclusively on milk for nutrient supply and development signals. Even more ancient forms of mammals, such as monotremes, although still oviparous, supply milk to their offspring.

The opportunity of early life nutrition for dairy calves

The association between improved feeding, health or growth in early life and future productivity was suggested long before the topic gained its current attention (Bar-Peled et al. 1997). In the first decade of the century this topic gained attention, and multiple publications documented these correlations, which soon were followed by several datasets proving causality in this relationship. However, still today the actual mechanism is insufficiently described, although increasing evidence indicates that this is a case of metabolic programming.

Understanding the mechanism of action is essential, because there are multiple ways to increase nutrient supply, enhance growth and prevent disease in calves, and not all of them may lead to improving their productive performance and health. The infant nutrition case described above is an excellent example in which greater nutrient supply and growth early in life do not lead to improved health later in life. Metabolic programming seems very sensitive to the nature of its causal signals and their intensity.

The study of these mechanisms has required a greater toolset beyond that of classic calf nutrition and dairy performance studies. It required measuring the development of tissues and their functionality. It also required the study of the modulation of metabolic pathways and the dynamic responses to energy metabolism signals postprandially and in challenge models.

Calves fed higher amounts of nutrients not only grow faster, but also alter their organ sizes in absolute terms and in relation to their body size (Soberon and Van Amburgh 2017). Among these tissues, mammary parenchyma development received much attention because of its intuitive link with lactation competence. Subsequently, the study of gene expression of these tissues unveiled wide differences in the metabolic configuration in relation to nutrient supply (Hare et al. 2019). Among these, the tissue presenting the greatest differences at weaning was the adipose tissue (Leal et al. 2018). These differences, related to energy utilization pathways, hint at a potential analogy with early signals leading to metabolic syndrome in humans.

An adequate scrutiny of these mechanisms requires a lifelong, longitudinal study, with prospective, randomized treatments, and a detailed study of the short-term effects of the intervention, and the eventual persistence of differences over time, or phenotypic differences that may arise later. Accordingly, our group carried out a 10 yearlong study starting in 2014, aiming to expand our understanding of these mechanisms.

Heifer calves were blocked at birth by dam parity and an identical colostrum supply, on the basis of which two levels of milk replacer supply were randomly assigned (Leal et al. 2021). Phenotypic parameters, serum metabolomics and insulin sensitivity were sequentially studied throughout their life, including blind culling decisions made during their lactation cycles. To avoid confounding effects of body weight and rate of growth at the onset of their first lactation, heifers were inseminated upon estrus expressed after reaching a certain body size, instead of age.

As expected, calves receiving a greater nutrient supply exhibited greater growth and broad differences in their serum metabolomic profiles. Upon glucose tolerance tests, in contrast to earlier suggestions (Bach et al. 2013), lifting nutrient restriction did not impair insulin sensitivity (MacPherson et al. 2016) as independently confirmed (Yunta et al. 2015). Post-weaning growth and development was apparently unaffected by treatments, but differences in metabolic profiles and glucose tolerance test responses persisted to some extent as heifers developed into adulthood (Leal et al. submitted), which indicates persistent metabolic effects long after the early life nutritional intervention.

As expected, lactation performance improved with the intervention, leading to better feed intake, higher milk yields, and improved body condition scores. Also, fertility and culling rates were improved by early life nutrition. Most interestingly, serum metabolomic profiling at 60 days after the first calving, indicated significant differences two years after the discontinuation of the nutritional treatments (Leal et al. submitted).

Dairy cows that calved for the first time with the same body weight, and whose external phenotype is apparently similar, can consume more feed, yield more milk, preserve their body condition better, and are more fit to remain in the herd through successive lactations. This is a direct consequence of an increase in nutrient supply in their first two months of life. It seems that early life nutrition acts on the Achilles heel of dairy metabolism and energy regulation. This is supported by direct energy metabolism contrasts in the study, and it is consistent with the thrifty phenotype hypothesis applied to the feed restriction of dairy calves.

Nutritional restoration of the biological reference

Having discussed nutrient deprivation as an etiological causal factor in metabolic disease in dairy cows, from an alternative perspective we could discuss the teleological purpose of nutrient sufficiency in connection with dairy cow productivity and health.

Lactation has a utility shaped by evolutionary selection pressure; this is the survival of the offspring until successful reproduction. Lactation comprises colostrum, transition milk, and lactation yield over time, including milk composition. Some of these factors we have been able to select for in the new dairy cow phenotype, but others are highly conserved and unresponsive to selection, indicating their critical value for survival. For similar reasons, all traits have boundaries beyond which selection is ineffective.

For example, cows have been successfully selected to produce massive amounts of milk. They seem, however, not susceptible to selection to producing milk with compositions beyond certain boundaries that seem set for the bovine species, just like they are not susceptible to selection to increasing their prolificacy beyond twin calvings.

An adult cow that naturally yields only 4 liters of milk for only 2 months after calving would have a competitive advantage for her survival, but knowing that such a phenotype does not exist, indicates something about the effect it would have on the

survival of the offspring. Following this reasoning, also colostrum yield, while variable, seems to have a very well-defined minimum.

Dairy cows clearly deviate from any definition of “wild type” that we could imagine. However, dairy calf rearing has traditionally severely deviated from the basic biological reference of the bovine species. The biological reference in this context can be defined as the basic phenotypic frame from which the species does not biologically deviate.

The ladder to restoration of the early life biological reference

Feeding greater amounts of milk to dairy calves should be seen as lifting a feed restriction situation. Analogously, positive responses should be understood as indications of restored health or productive potential.

Dairy replacement calves are proving to be to some degree responsive to every step we have attempted to get them closer to that biological reference. The offspring from heifers seems to perform better in association to a perinatal environment not coupled to a highly demanding lactation (Gonzalez-Recio et al. 2012). They clearly respond to restoring colostrum supply and general restoration to health in early life (Soberon et al. 2012), in the same way as they benefit from greater milk supply, from lifting the restriction all the way to ad libitum feeding. Restoring milk composition closer to whole milk has benefits in the short term (Amado et al. 2019) and potentially in the long-term. The list goes on, with positive experiences with paired housing (Costa et al. 2015) and delayed weaning (Meale at al. 2015). A good example of an unexpected response to restoring nutrient supply is found in the response to the supply of inert fats in solid feeds for early weaned calves (Berends et al. 2018), which could be seen as the restoration of fat supply from milk in that phase of development.

Tapping into the opportunity of early life nutrition in practice

Restoring the biological reference opens an opportunity for short- and long-term health and welfare improvements and animal performance. Nevertheless, dairy farming is a food production system in which the resources invested are expected to result in tangible value. Increasing levels of attention to calves, from colostrum to weaning, require increasing levels of resources invested: housing, labor, feed, and although less tangible, the investment of personnel’s attention.

Specifically, improving pre-weaning calf feeding involves complex considerations including nutritional quality, quantity invested, feed safety (pasteurization), protocols of preparation and supply, labor required, health, growth and weaning objectives. To this already long list, now we also intend to add the expectation of long-term benefits.

Whole milk is available on farm; it has unquestionable nutritional quality, but it presents feed safety risks that require precise labor and equipment-demanding feed management. Additionally, combining its intrinsic nutritional quality with our new quantity targets makes this option costly compared to the alternatives.

Milk replacer is much easier to handle and to prepare on farm, but its nutritional quality is directly related to its cost and to the extent of milk components replaced by alternative sources. This exercise of replacement is done to different extents, and to different degrees of nutritional success. Generally, this allows providing greater amounts of nutrients, with a lower investment of dairy food resources, opening an opportunity to restore nutrient supplies in early life, without compromising overall dairy system efficiency. However, the nutritional quality of milk replacers is unfortunately inversely proportional to the circular use of non-food and non-dairy milk replacer ingredients. Milk replacer allows for a resource-efficient supply of high quantities of nutrients. This, however, is only an effective option if the quality does not compromise short-term health and if its quality aims to match as much as possible the nutrients and signals of bovine milk.

A noteworthy aspect of milk replacer is its typically lower fat content as compared to whole milk. This is understandable following a resource-sparing logic because milk fat is the most valuable component of milk. Feeding whey to calves is probably as old as cheese production. Furthermore, the growth response (feed-to-gain ratio) of calves is greater with additional protein compared to additional lactose, and lowest with additional fat (Amado et al. 2024). However, low-fat milk replacer fed at high levels can exceed tolerable levels of lactose (Wilms et al. 2020), causing digestive problems (Wilms et al. 2019) and giving substantially different glycemic (Wilms et al. 2020) and amino acid signals that deviate from the biological reference.

On-farm mixing of milk waste streams with additional ingredients or milk replacers, has an unquestionable element of resource circularity worth considering. In practice this option combines the feed-safety risks and inconvenience of whole milk, with the nutritional disadvantages of milk replacement by alternative ingredients. Theoretically, the costs of this option would allow for high levels of liquid feeding at moderate cost. In reality, health is compromised by high levels of liquid feeding if the quality is suboptimal, making this not a suitable option to tap into the opportunity of early life nutrition.

Long-term economic and resource efficiency benefits associated with the restoration of milk supply in quantity and quality are much greater than the cost and resource investment required to realize them. A generous, ad libitum or close to that level, supply of milk or high-quality milk replacer is an economic opportunity, a dairy sustainability opportunity, and additionally an opportunity for calf welfare and to meet societal expectations of dairy production.

Calf nutrition changes with changing objectives for calf nutrition

Before our recent understanding of the long-term damage caused by feed restriction in calves, minimizing liquid feed supply and the use of highly valuable dairy ingredients in this liquid feed was justified to improve the food production efficiency of the dairy system. Food production is the main objective of dairy farming. Calf health and the transition to solid feeds were the main objectives. Meanwhile, the main limiting factor for dairy production efficiency is lifetime daily production, which is composed of cow productivity, age at first calving, fertility, and cow longevity. Reducing age at first calving has traditionally been a calf rearing objective, but productivity, fertility and longevity seemed independent of calf rearing.

Feeding calves in a way that minimizes resource use in that period can have adverse effects on resource efficiency in later productive phases. A cow phenotype that does not realize its production potential, fertility or does not effectively regulate energy balance in the challenging lactation cycle of the highly productive dairy cow, is limiting our ability to produce dairy food efficiently and profitably. Restoring the perinatal nutritional environment can unlock lifetime daily yield, and take dairy production efficiency to a higher level.

We have an opportunity to manage and feed calves differently, to take one more step to make dairy farming that is better for calves and cows, for the dairy industry and for the environment. A dairy production system that generates more value and that will be more valued by dairy consumers.

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Session Notes

SCIENTISTS SAY:

Choline is a **Required Nutrient** for Essentially Every Cow

> *Even in very high-producing cows, we saw a milk response of approximately 5 lbs/cow/day after supplementation.*
>
> **-Dr. Heather White,**
> *Tri-State Dairy Nutrition Conference, 2023*

> *Choline dramatically increased colostrum yield – an 85% increase in our study.*
>
> **-Dr. Barry Bradford,**
> *Tri-State Dairy Nutrition Conference, 2023*

> *Is choline essential or required? I think it's required and we should be framing out a requirement in our nutrition models.*
>
> **-Dr. Mike Van Amburgh,**
> *Cornell Nutrition Conference, 2022*

> *Choline plays an important role in metabolic health. Multiple studies have shown ReaShure's impact on transition cow health.*
>
> **-Dr. Marcos Zenobi,**
> *Research Study from 2018*

> *Certainly, Rumen-Protected Choline appears to have some new opportunities to be placed in high-producing dairy cow rations and may impact animal health during the transition.*
>
> **-Dr. Mike Hutjens,**
> *Mikehutjens.com*

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Feeding Rumen-Protected Choline during the Peri-conceptional Period Programs Postnatal Phenotype of Calves

Masroor Sagheer, McKenzie L. Haimon, Daniella Heredia, Federico Tarnonsky, Mauro Venturini, Angela Gonella-Diaza, Nicolas DiLorenzo, Joseph W. McFadden, and Peter J Hansen

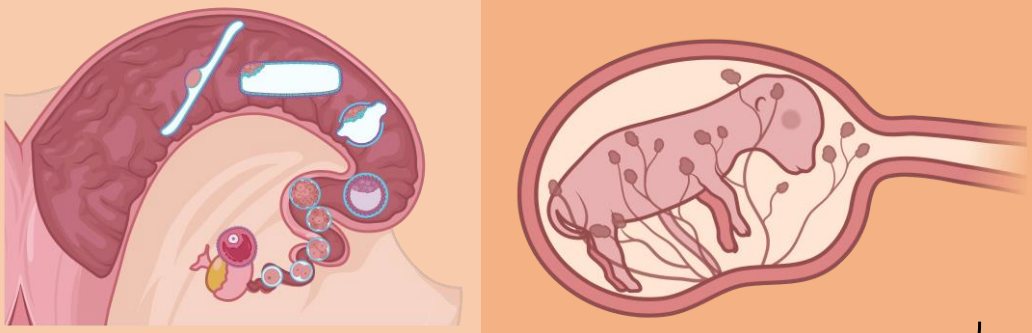
Department of Animal Sciences, University of Florida
Department of Animal Science, Cornell University

1

Developmental programming

Low protein diet | **OCM deficient diet** | **Heat stress** | **Over/undernutrition**

Early pregnancy | **Late pregnancy**

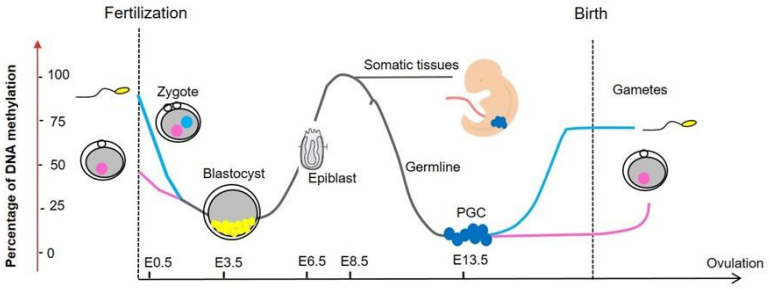


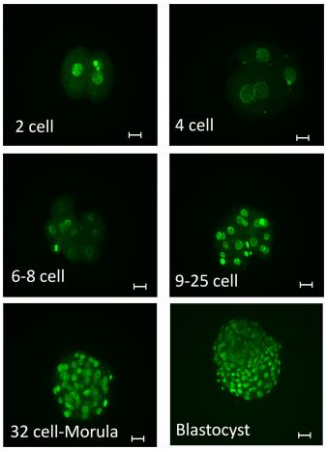
Effects on offspring
Birth weight, growth rate, organ development/function, and hormone concentrations

(Sinclair et al, 2007; Watkins et al, 2011; Laporta et al 2020; Odhiambo et al 2020)

2

Epigenetic reprogramming during embryonic development





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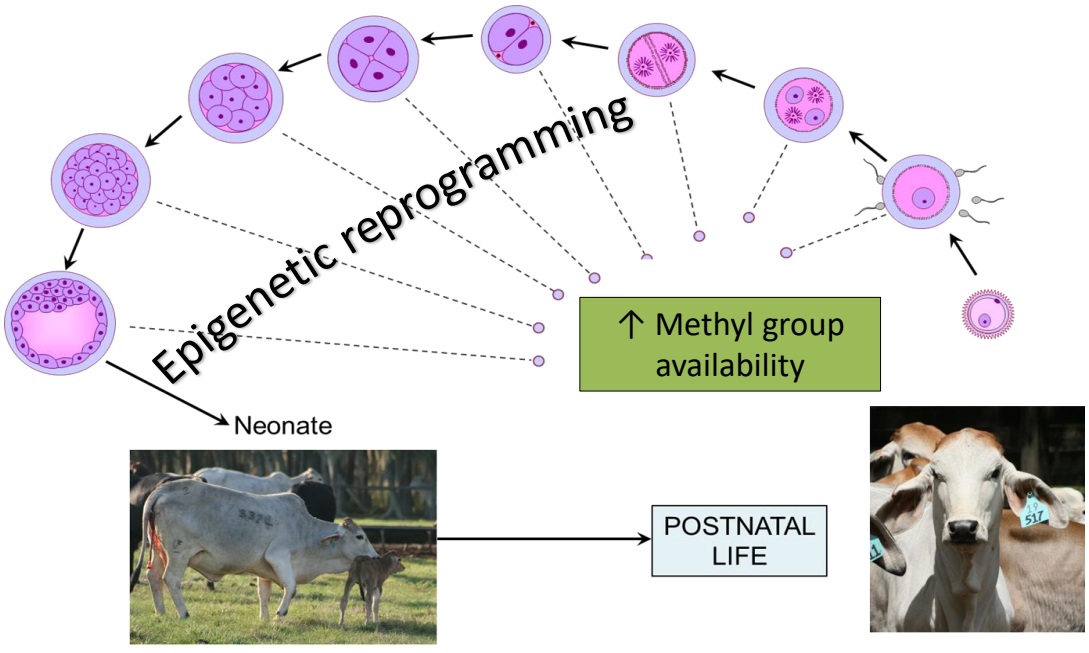
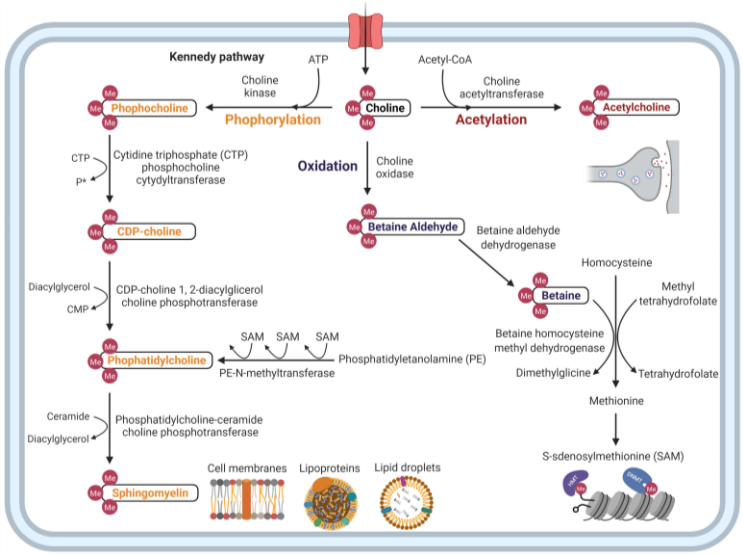


4

Choline metabolism



(Estrada-Cortes, 2021 adapted from Fisher et al., 2002)

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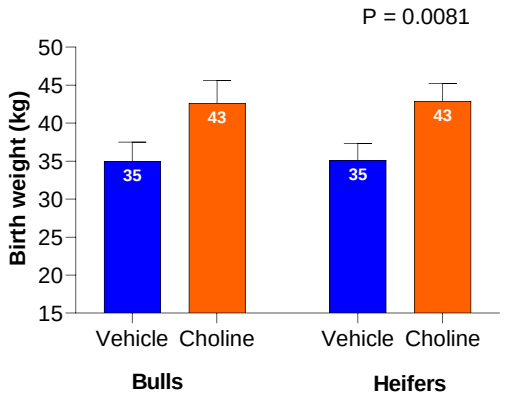
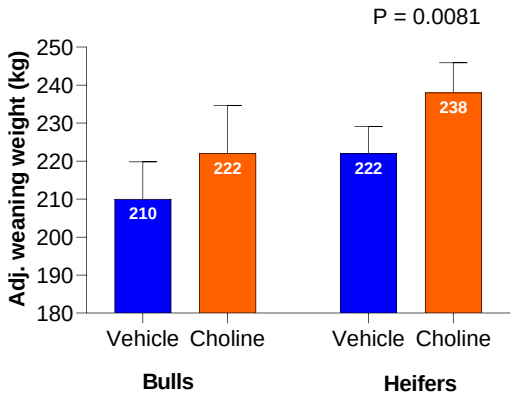
5

Choline chloride treatment increased birth weight and weaning weight





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6

Objectives

To determine;

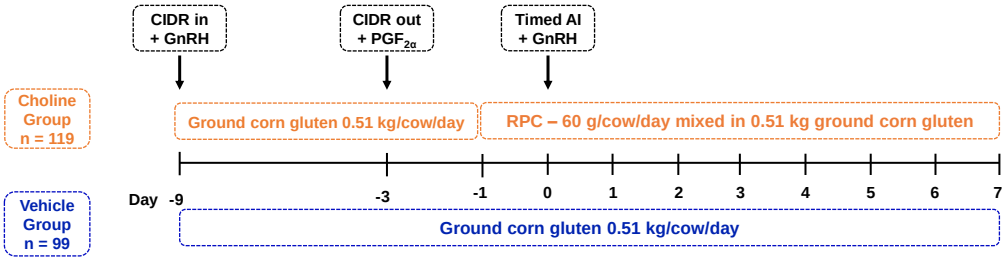
- Whether feeding rumen protected choline (RPC) affects pregnancy rate, embryonic loss, and programs postnatal phenotype in beef cows subjected to timed artificial insemination (TAI)

Hypothesis

- Feeding RPC during the periconceptional period will alter the postnatal phenotype in beef cattle

Experimental design

- ✓ Postpartum beef cows (n = 218)
- ✓ Blocked by breed and body weight
- ✓ Individual feeding by Super SmartFeed (SSF)
- ✓ ~ 21 days training period with SSF
- ✓ Timed AI using 6 beef sires
- ✓ 3 replicates – n = 67, 82, 69

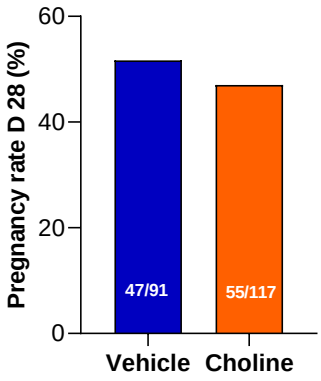


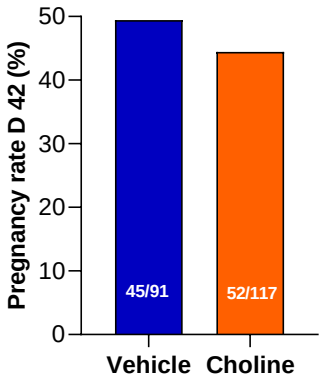
Source of choline – ReaShure® (28.8% choline chloride)

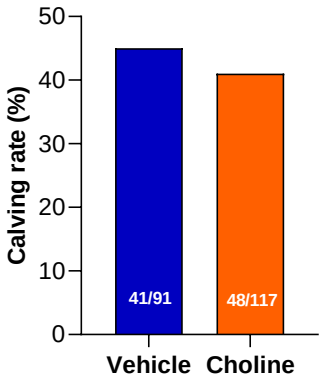
9

RPC did not affect pregnancy outcomes

P > 0.05







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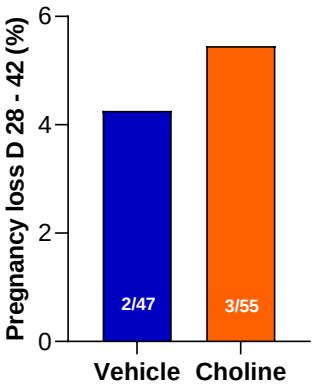
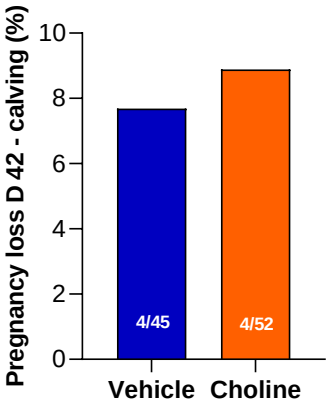
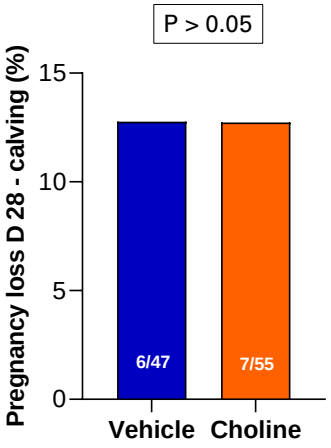
RPC did not affect pregnancy outcomes







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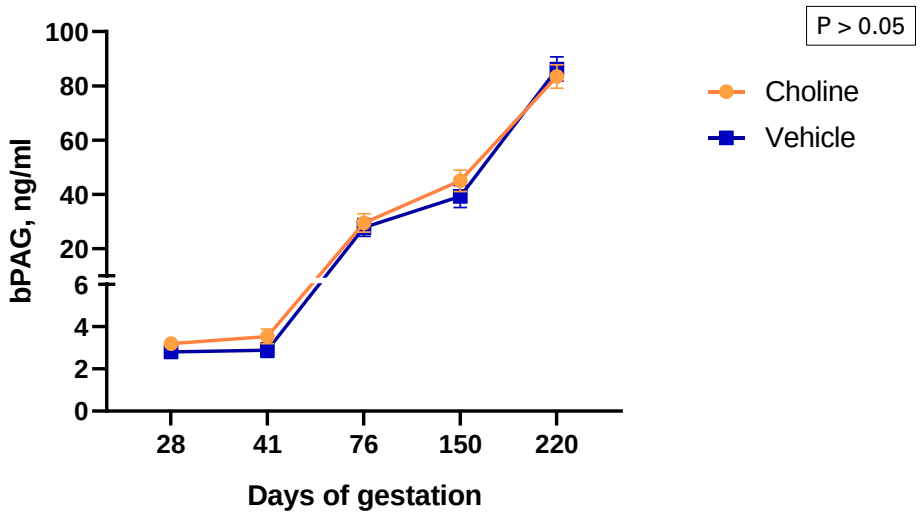
11

RPC did not affect plasma PAG concentrations



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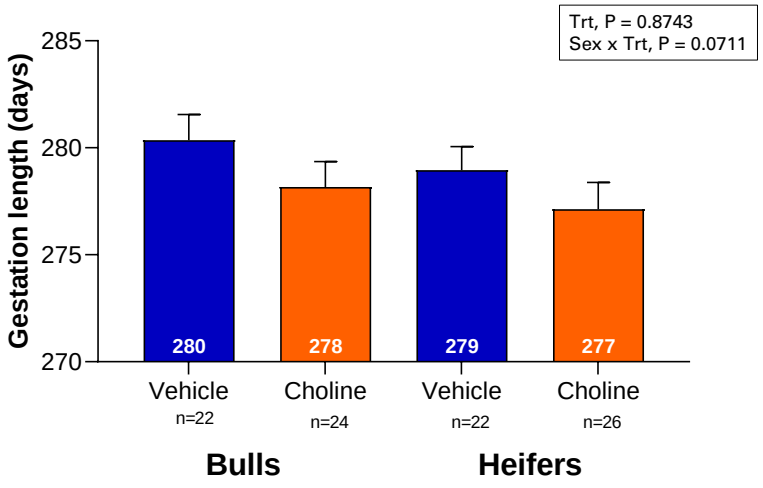
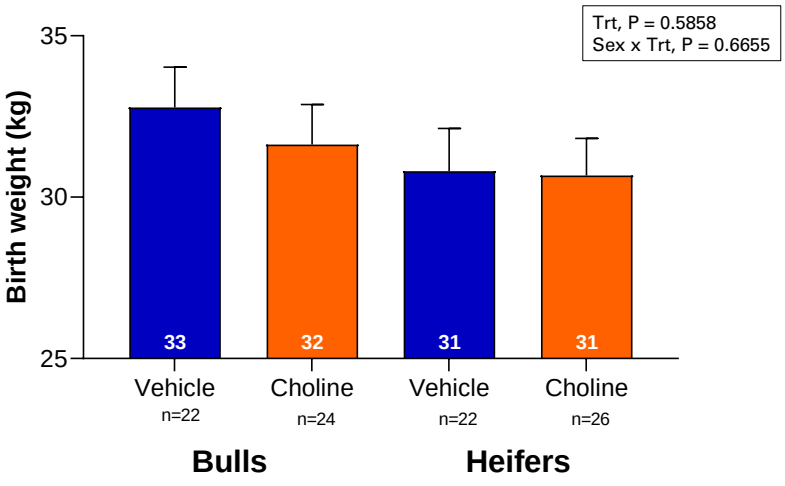
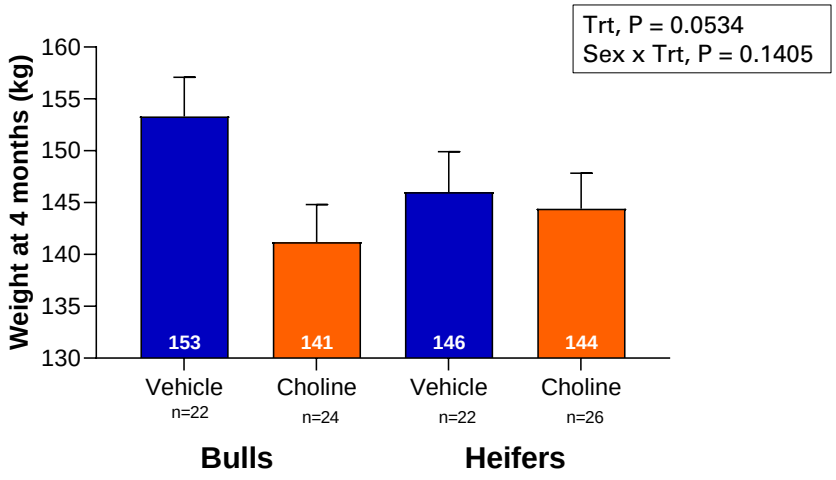
RPC did not affect gestation length



RPC did not affect birth weight of calves



RPC reduced body weight at 4 months of age



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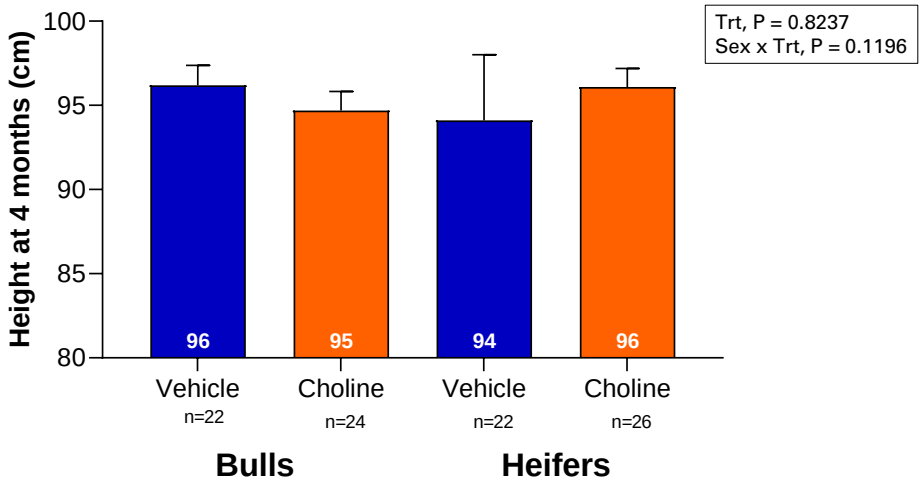
15

RPC did not affect height at 4 months of age



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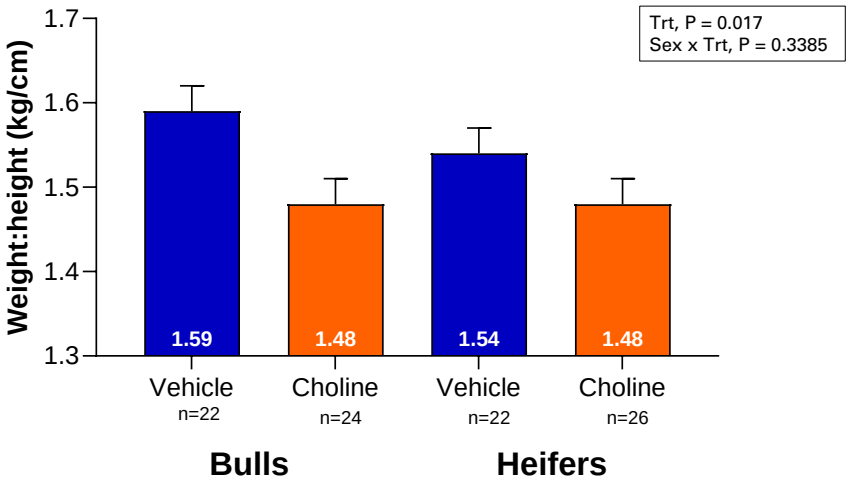
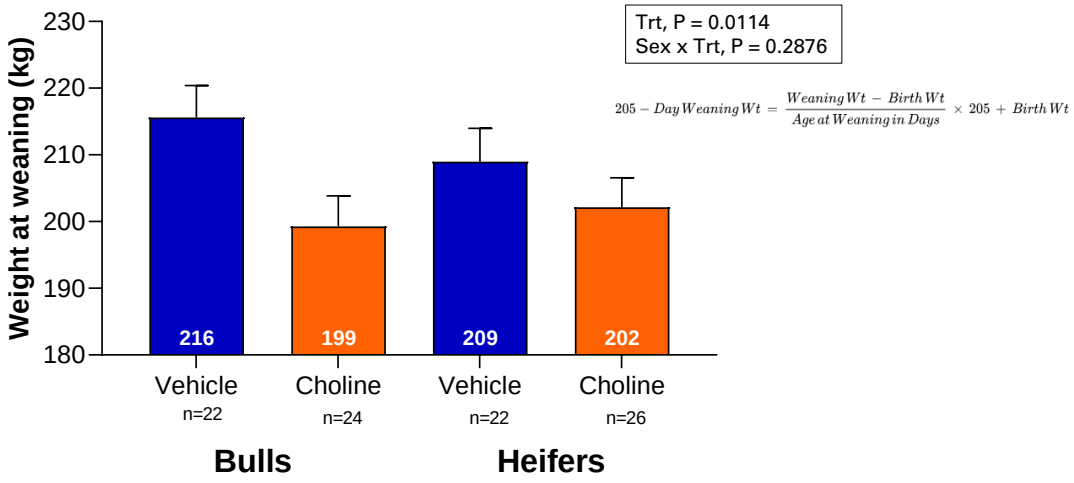
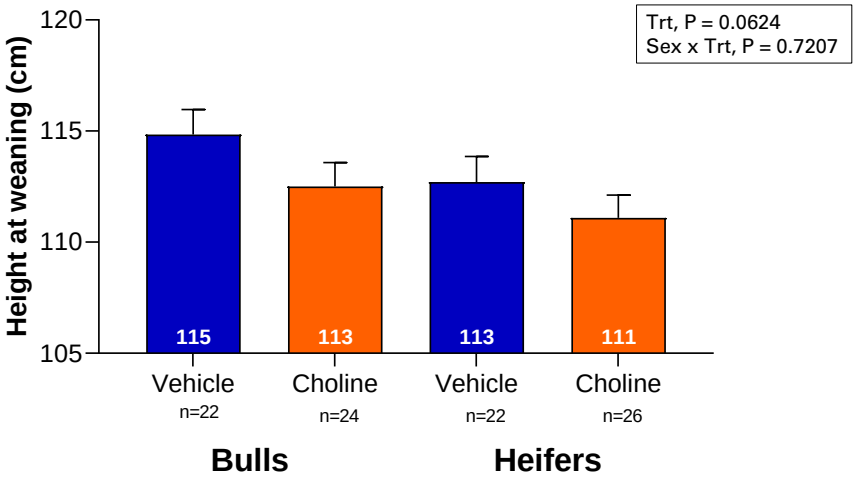
RPC reduced weight to height ratio at 4 months of age



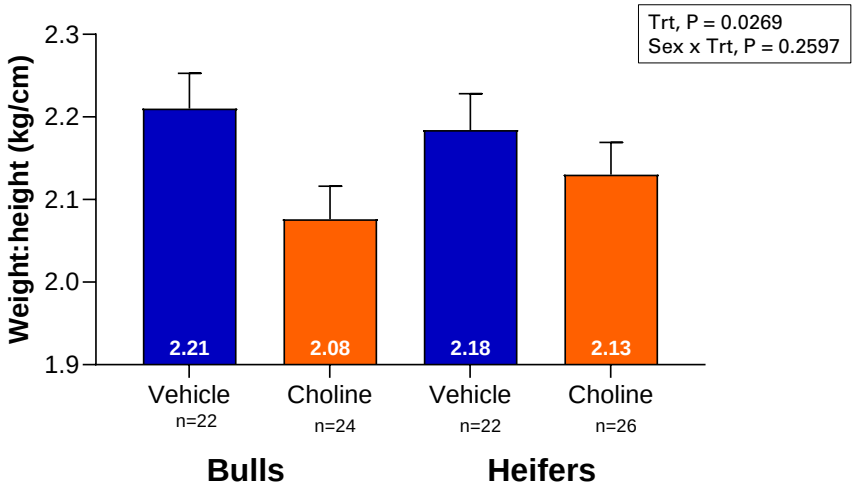
RPC reduced body weight at weaning



RPC did not affect height at weaning



RPC reduced weight to height ratio at weaning



Conclusions

- The periconceptional period is plastic and can be modified through maternal feeding to alter the postnatal phenotype of calves.
- RPC feeding during periconceptional period reduces the body weight, hip height, and weight to hip ratio at 4 months of age and weaning.
- Actions of choline feeding on the calves are opposite to what was observed in in vitro supplementation.

Future research

- Understanding the mechanisms by which choline supplementation alters the phenotype of calves.
- Does feeding RPC alter the uterine microenvironment?

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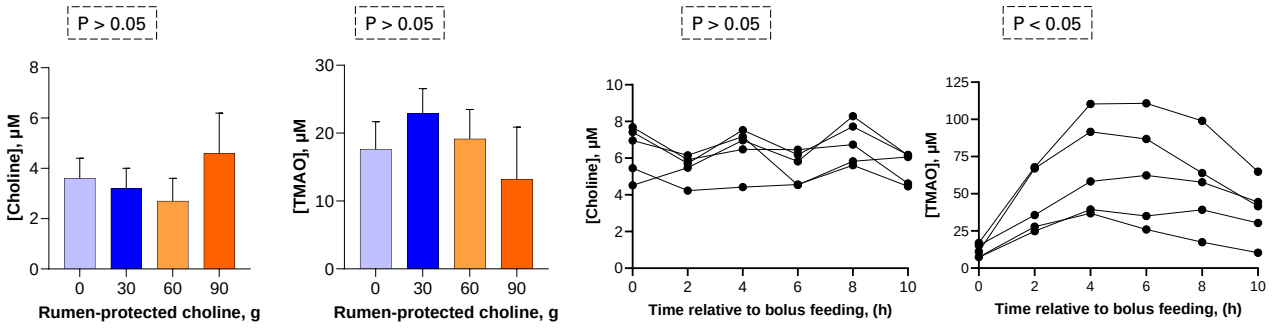
Tentative Explanation for Differences in Actions of Feeding Choline vs Co-culture of Embryos

Feeding choline does not cause a measurable increase in circulating choline concentrations in blood in cows and ewes



Source of choline – ReaShure® (28.8% choline chloride)

Source of choline – ReaShure-XC® 5g/ewe (60% choline chloride)

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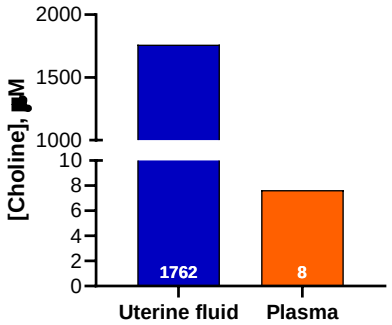
Tentative Explanation for Differences in Actions of Feeding Choline vs Co-culture of Embryos

Choline concentrations in uterine fluid are much higher than in blood- ~1800 micromolar vs 4 - 8 micromolar (Sagheer et al., unpublished)



Dairy cows fed RPM at 50 ± 5 DIM

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Acknowledgements

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Quinn Hoorn
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NFREC crew, UF
McFadden Lab, Cornell
Pohler Lab, Texas A&M

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24

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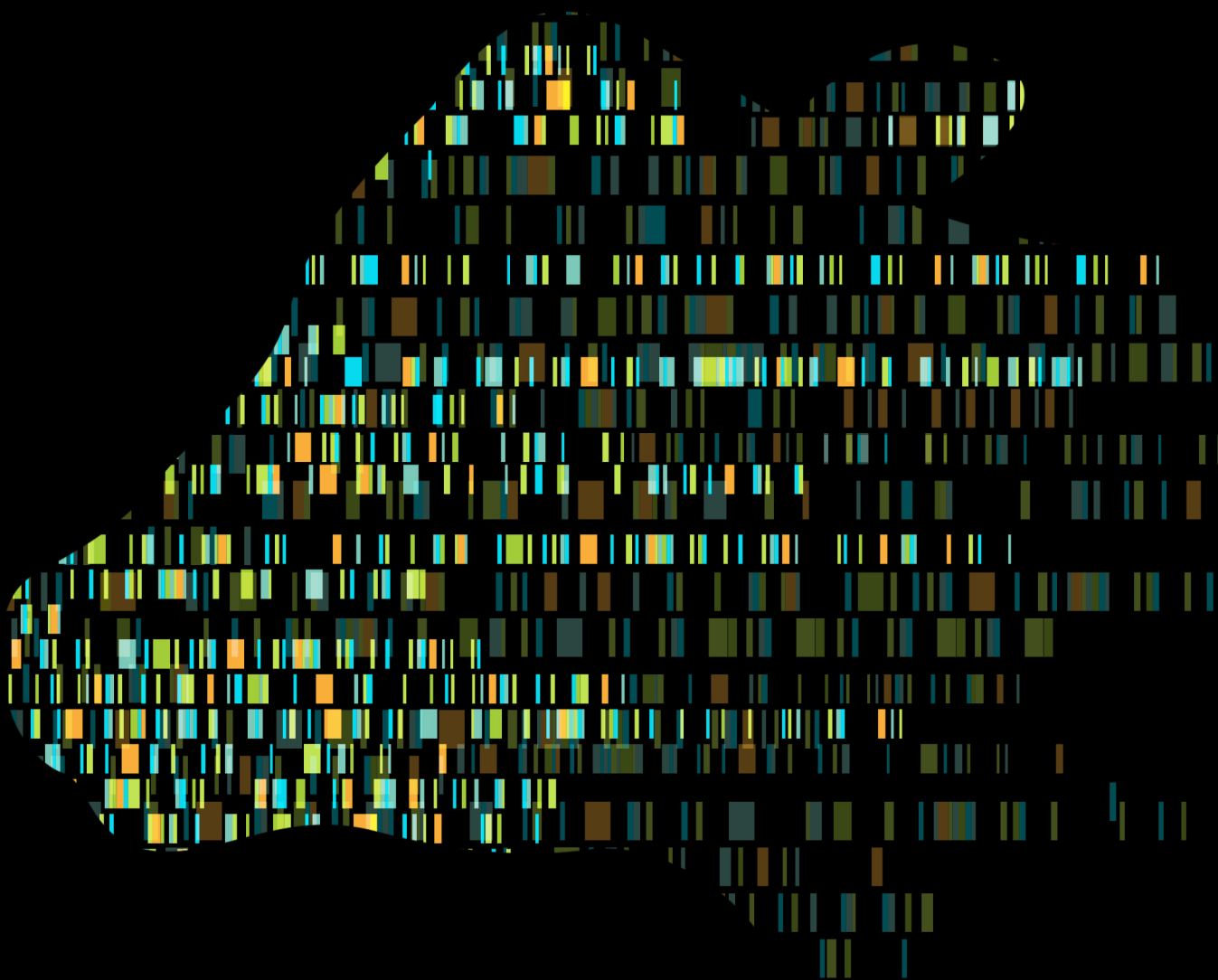

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Exhaled Breath Approaches to Assess Rumen Fermentation and Metabolic Changes in Dairy Cows

Mutian Niu[1] *and Usman Arshad*
ETH Zürich, Switzerland

Introduction

Rumen fermentation is central to nutrient metabolism in dairy cows. Ruminal microbial fermentation produces volatile fatty acids (**VFA**) for the host as energy sources while simultaneously generating greenhouse gases, primarily methane (**CH_4**). Improving our understanding of these processes is key to enhancing dairy productivity and promoting environmental sustainability. Traditional methods for assessing ruminal fermentation, such as fistulation, oro-ruminal tubing, and rumination boluses, have advanced our understanding of ruminant physiology (de Assis Lage et al., 2020; Larsen et al., 2020; Indugu et al., 2021). However, these techniques are invasive, labor-intensive, and impractical for real-time, large-scale monitoring of rumen function, nutrient utilization, and metabolic changes related health (Duffield et al., 2004; Muizelaar et al., 2020). In contrast, exhalomics, the study of volatile organic compounds (**VOCs**) in exhaled breath, offers a non-invasive alternative for monitoring both rumen fermentation (Islam et al., 2023; Islam et al., 2024a) and health status (Haddadi et al., 2022) in dairy cows. Leveraging cutting-edge analytical technologies, such approach has been used to detect metabolic signatures (Lan et al., 2020; Haddadi et al., 2022). This review examines historical perspectives and recent advancements in exhaled breath analysis, focusing on key biomarkers, technological developments, and the challenges in assessing rumen fermentation and metabolic changes in dairy cows.

Evolution of Exhaled Breath Analysis to Assess Rumen Fermentation

The study of exhaled breath as a tool for assessing rumen fermentation began with an interest in non-invasive techniques to monitor metabolic processes in ruminants. Early studies focused on measuring headspace gases, such as CH_4 and carbon dioxide (**CO_2**), in fistulated animals, where these gases served as proxies for ruminal fermentation efficiency and microbial activity (All et al., 1980). Throughout the 1980s and 1990s, scientific investigations primarily centered on the relationship between specific VOCs in ruminal headspace gases and the overall fermentation process in the rumen, although technological limitations restricted detailed analyses (Dobbelaar et al., 1996; Elliott-Martin et al., 1997).

Advances in gas chromatography and mass spectrometry in the early 2000s improved the precise quantification of compounds such as VFA, CH_4, hydrogen (**H_2**), and other fermentation by-products. For example, Dewhurst et al. (2001) employed selected-ion-flow-tube mass spectrometry (**SIFT-MS**) to measure gases such as hydrogen sulfide, methyl sulfide, and ammonia (**NH_3**) in the rumen headspace of fistulated Holstein cows. They observed that sulfur compounds decreased after

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feeding, a reflection of sulfur amino acids fermentation, while NH_3 concentrations were pH-dependent and could help diagnose subacute ruminal acidosis. On the contrary, the profile of VFA in the headspace of rumen gas showed slight differences from rumen liquor. Following this, researchers began to explore the potential of using ruminal headspace gases to assess dietary and microbial shifts during the rumen fermentation (Cai et al., 2006; Dewhurst et al., 2007). Specifically, Cai et al. (2006) investigated rumen gas composition in three rumen-cannulated Angus steers using solid-phase microextraction (**SPME**) and gas chromatography-mass spectrometry-olfactometry. The authors identified 50 VOCs, including sulfides, VFA, ketones, and phenolic compounds in rumen gas samples. The abundance of these VOCs and odors, particularly sulfides and VFA, was higher after feeding, indicating that rumen gases have the potential to be used to monitor digestion and fermentation patterns in cows.

Until 2018, most studies focused on cannulated animals, by investigating headspace gases and compounds. A major innovation occurred in 2018 when Oertel et al. (2018) introduced a face mask coupled with proton transfer reaction-time of flight-mass spectrometry (**PTR-TOF-MS**) to capture real-time eructation gases. In a pilot study involving cattle, goats, and sheep, the researchers detected up to 36-fold increase in certain VOCs during eructation events in cattle compared to other ruminant species. They also developed a "*Burb Tracker*" algorithm to effectively separate eructation events from normal breathing cycles, to enable more accurate VOC analysis. More recently, Islam et al. (2023) used GreenFeed, a head-chamber system, to monitor CH_4 during eructation events of dairy cows in real-time (Figure 1). This method leveraged CH_4 as a marker to precisely identify burping of cows for the collection of exhalome (total volatile metabolites contained in exhaled breath) samples. The exhalome samples were analyzed off-line via secondary electrospray ionization high-resolution mass spectrometry (**SESI-HRMS**) primarily to quantify ruminal VFA profile. They were able to characterize the diurnal patterns of exhaled VFA in relation to ruminal CH_4 emission pattern from 4 Brown Swiss and 3 Holstein cows (Islam et al., 2023). While a total of 1,298 features were recorded (649 features in positive ion mode and 649 features in negative ion mode), the authors observed that concentrations of exhaled acetate, propionate, and butyrate peaked after feeding and followed a pattern similar to CH_4 production. Moreover, the acetate was the most abundant VFA, followed by propionate and butyrate, reflecting the known ruminal VFA composition.

Subsequent work by Islam et al. (2024a) validated potency of exhalomics as a non-invasive modality for assessing ruminal fermentation dynamics in dairy cows. Using a switch-back design with 4 cannulated Original Brown Swiss cows subjected to two distinct diets (high-starch vs. low-starch), the authors compared the molar proportions of VFA in exhalome samples with direct rumen samples obtained through the cannula, as well as to the Henry's Law constant predicted VFA profile in the gas phase of the rumen. Exhalome samples was collected over 8 sampling events spanning 24 hours to represent every 3-h of a day, alongside simultaneous rumen fluid sampling from the cannulated cows. The study found no interactions between diet and VFA measurement methods and strong correlations between exhaled and ruminal VFA, with diurnal patterns closely mirroring each other, both peaking post-feeding (**Figure 2**). These investigations revealed no significant interactions, implying consistent daily VFA molar proportion trajectories, regardless

of dietary input. The applicability of exhaled breath samples to detect dietary effects was further demonstrated by Eichinger et al. (2024), who utilized solid-phase extraction cartridges to capture volatiles from the exhaled breath of cows fed hay- and silage-based diets. These analyses revealed a strong correspondence between exhalome profiles and rumen fluid composition, showcasing the capacity of exhalomics to detect dietary-induced changes in rumen metabolism. In a complementary approach, Jorge-Smeding et al. (2023) applied SIFT-MS to distinguish cows on high-fiber and high-starch diets. Their work identified exhalome biomarkers linked to sub-acute ruminal acidosis and other diet-induced dysfunctions, further demonstrated that exhalome biomarkers can reflect dietary-induced changes in rumen metabolism.

A more comprehensive perspective on microbial activity was provided by Islam et al. (2024b), who compared the metabolomic profiles of rumen fluid and exhalome samples. Their study not only identified consistent diet-induced changes in metabolite abundances but also pinpointed alterations in key carbohydrate metabolism pathways, such as galactose, and starch and sucrose metabolism pathways. Based on current knowledge, exhalomics holds potential as a non-invasive tool for assessing rumen fermentation. Despite these promising findings, several methodological challenges remain – including the standardization of breath collection, variability across analytical platforms, and the limited availability of comprehensive spectral libraries for accurate metabolite annotation – that must be overcome before exhalomics can be widely adopted as a non-invasive tool for assessing rumen fermentation.

Evolution of Exhaled Breath Analysis for Health Assessment

The evolution of exhaled breath analysis for health assessment has progressed from the basic detection of a few key gases to a refined method for monitoring metabolic and physiological states. Early research focused on measuring major gases like CO_2 and NH_3 to diagnose infectious and metabolic disorders. As analytical techniques improved, researchers began detecting a wider range of VOCs linked to specific biological processes, greatly enhancing the potential for non-invasive disease diagnosis and metabolic monitoring.

One of the first applications was in detecting ketosis in dairy cows. Dobbelaar et al. (1996) examined the relationship between breath acetone levels and ketone body concentrations in blood and milk. In a study involving 4 healthy lactating cows, ketosis was induced by reducing feed intake, and gas chromatography-mass spectrometry (GC-MS) analysis revealed that elevated breath acetone corresponded with higher levels of serum β-hydroxybutyric acid and milk acetoacetate. These results established breath acetone as a promising indicator for ketosis. Building on this foundation, subsequent studies refined the approach. Elliott-Martin et al. (1997) and Mottram et al. (1999) also contributed to advancing the use of breath analysis for early detection of ketosis in dairy cows, where each study addressed distinct aspects of the methodology and validation process. While Dobbelaar et al. (1996) focused on establishing a direct correlation between acetone levels in breath and ketone bodies in blood and milk, Elliott-Martin et al. (1997) introduced a portable breath sampling device and explored a gas sensor array for real-time breath analysis, demonstrating the feasibility of on-farm, non-invasive health monitoring.

Mottram et al. (1999) extended these findings by focusing on induced hyperketonemia, revealing that breath acetone levels rose more rapidly than changes in milk, suggesting breath analysis could serve as an earlier marker for ketosis than traditional methods.

In the early 2000s, the scope of breathomics expanded to include broader health assessments. Spinhirne et al. (2004) investigated the use of breath samples to detect respiratory disease by identifying compounds such as acetaldehyde and decanal as potential biomarkers in steers. Fend et al. (2005) furthered this work with an electronic nose to analyze VOC profile from cattle serum, successfully distinguishing infected animals from healthy ones at an early stage. In a subsequent field study, Burciaga-Robles et al. (2009) sampled 183 heifer calves, focusing on gases like nitrous oxide and carbon monoxide. Although increases in VOC such as acetone were observed with the severity of bovine respiratory disease (**BRD**), the authors concluded that these biomarkers alone were insufficient for reliable disease prediction, highlighting the need to combine breath markers with clinical observations for a more comprehensive diagnostic approach.

While earlier studies focused on identifying specific biomarkers in exhaled gases, more recent work has emphasized refining these techniques for broader applications, including real-time monitoring and multi-sample approaches. Ranade et al. (2014) assessed oxidative stress in dairy heifer calves by measuring hydrogen peroxide in exhaled breath condensate, highlighting respiratory stress during weaning. Gierschner et al. (2019) then used an online PTR-TOF-MS system to profile VOC in real time, identifying significant shifts associated with postpartum metabolic changes and paratuberculosis infections. Similarly, Brebu et al. (2023) compared VOC profiles from breath, fecal, and skin samples in healthy and sick dairy cows to explore potential biomarkers for bovine tuberculosis (**bTB**), demonstrating the advantages of a non-invasive, multi-sample diagnostic approach.

Collectively, these studies demonstrate how exhaled breath analysis has evolved into a sophisticated tool for early disease detection and comprehensive health monitoring in cattle. While challenges remain – such as standardizing sampling methods and integrating data from diverse analytical platforms – the continued advancements in this field underscore its promise for improving livestock health management.

Challenges, Future Directions, and Concluding Remarks

Although exhaled breath analysis have emerged as innovative fields for assessing rumen fermentation (e.g., VFA, sulfides) and health markers (e.g., ketosis, BRD, or bTB) in dairy cows, several challenges limit their current application. One major hurdle is the variability in sampling methods – whether using head chambers (e.g., GreenFeed), electronic noses, or face masks – which can lead to inconsistent sample collection. In addition, the mixing of target breath with ambient exhaled air complicates the accurate isolation of specific compounds. Another challenge lies in the diversity of analytical platforms employed, ranging from online techniques like PTR-TOF-MS to offline methods such as GC-MS, SIFT-MS, or SESI-MS. Although these platforms are sensitive enough to detect hundreds of features, their effectiveness is restricted by the lack of comprehensive spectral libraries and robust

annotation frameworks. For instance, SESI-MS produces data as mass-to-charge ratios; without proper annotation, linking these values to specific metabolites or metabolic pathways remains difficult. Even when exhaled air is condensed for untargeted metabolomic analysis, many detected metabolites do not correspond to entries in major biochemical databases (e.g., HMDB, KEGG), which hampers pathway enrichment analysis and limits insight into underlying metabolic processes.

To address these issues, researchers are exploring hybrid methodologies that integrate condensed breath analysis with high-resolution untargeted metabolomics. Although this approach can improve metabolite annotation, many features remain unidentified in standard databases, complicating the connection to known biological pathways. In parallel, network-based methods have also been proposed to visualize complex interrelationships among metabolites, but these require advanced computational tools and expertise due to the non-linear nature of the networks. Therefore, there is a clear need for improved analytical tools, curated spectral libraries, and integrative frameworks. Developing targeted annotation workflows specifically for livestock and incorporating artificial intelligence-based predictive models may bridge these gaps, enhancing both annotation and interpretation of breathomics data in relation to cow health and metabolism.

Looking ahead, future research should focus on integrating breath analysis with rumen microbiome and metabolomic studies to provide a holistic view of cow metabolism. The development of affordable, portable analytical devices will facilitate on-farm implementation, making these techniques more accessible to farmers and researchers alike. Longitudinal studies are essential for establishing baseline biomarker profiles and assessing their predictive value over production cycles. Moreover, leveraging advanced artificial inteligence methods can help decipher complex datasets, identify novel biomarkers, and ultimately deepen our understanding of cow physiology. By overcoming current challenges and adopting innovative solutions, exhaled breath-based tools can play a pivotal role in enhancing dairy cow health and welfare, productivity, and sustainable livestock management practices.

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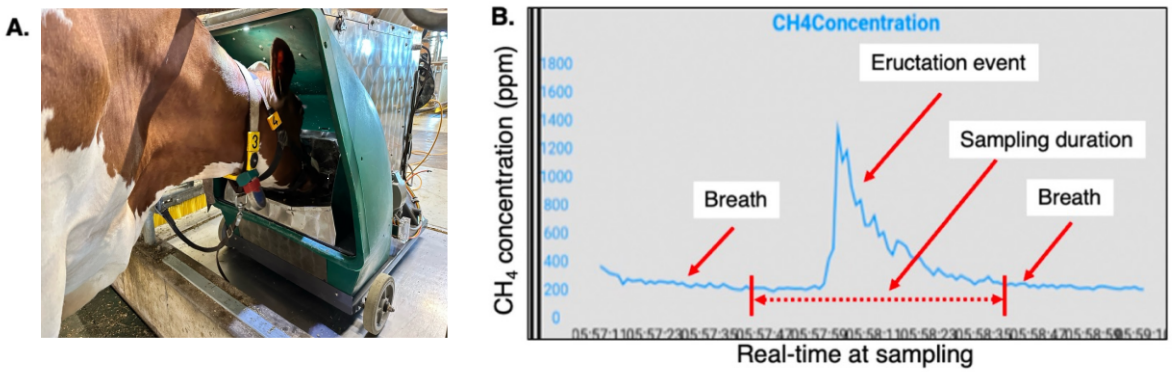

Figure 1. (A) Exhalome sampling of dairy cows using gas sampling unit of the GreenFeed system (C-Lock Technology Inc.). (B) Real-time ruminal CH_4 emission measurements (x-axis: real time of measurement; y-axis: CH_4 concentration, ppm) on the "Control Feed" mobile application (version 1.51, C-Lock Technology Inc.), showing the eructation event with an exhalome sampling duration of approximately 1 min. (Revised from Islam et al., 2023)

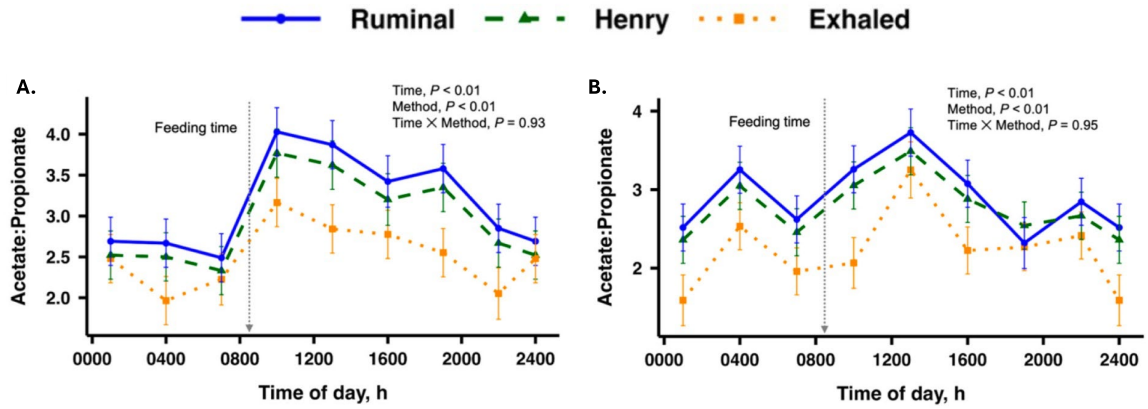

Figure 2. Molar proportions of acetate: propionate ratio measured in dairy cows fed a relatively (A) high-starch diet (16.2% of DM) and (B) low-start diet (6.31% of DM). (Revised from Islam et al., 2024a)

Session Notes

New Tools to Assess and Optimize Forage Quality and Diet Formulation

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A Total Digestible Nutrient Mindset

The concept behind milk per ton and milk per acre dates back over 30 years (Undersander et al., 1993). The milk production potential in forage is tied to both energy and intake potential, with energy being a focal point in many evaluation programs. Initial energy and intake potential estimates were driven by neutral and acid detergent fiber (aNDF and ADF) measures. In the late 1990's and early 2000's, feed energy quantification took a dramatic leap forward as Weiss (1998) put forth a summative model to estimate energy potential in feeds. Weiss' work provided the nucleus for the Nutrient Requirements of Dairy Cattle 7th rev. edition energy model (NRC, 2001) and subsequently, the MILK2006 corn silage quality index (Shaver, 2006).

More recently, NASEM (2021) published an updated dairy cattle nutrient requirements model and dramatically changed the approach for modeling energy potential in feeds. The model accounts for known interactions between feeds and nutrients in total mixed rations, such as a negative relationship between fiber digestion potential and starch concentration. MILK2024 subsequently adapted this modeling approach with modifications, to bring forth an updated MILK corn silage quality index (Diepersloot et al., 2024). The MILK2024 model allows for more current laboratory forage quality inputs, such as starch, aNDF digestibility (**NDFD**, % aNDF), undigestible aNDF (**uNDF**, % DM) and rumen starch digestibility (% starch). While many are evaluating this tool to index forage quality, the increasingly complex nature to the NASEM energy model and need for a total diet to be modeled have hastened both user understanding and implementation. Further, the model directly predicts energy and not total digestible nutrients (**TDN**) like prior MILK and energy models.

Further, the MILK model is not easily adopted into new worksheets for modifications or expanded evaluation efforts due to several factors. Hence, practical forage quality training and modeling efforts will continue to also employ a summative energy modeling approach with TDN.

Total digestible nutrients can be determined by quantifying nutrient fractions or pools and then determining the nutrient digestibility. The product of nutrient and digestibility coefficients equates to a digestible nutrient fraction (% DM), which can then be summed up to yield a total digestible nutrient content in feed. This simplistic TDN approach can provide a customizable path to evaluate feed energy density and energy yield, for making comparisons in a similar manner as to what Milk per Ton or Milk per

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Acre offer. The customizable solution is desirable in many cases, as different dairy producers or nutritionists may place emphasis on different nutrient components or digestibility coefficients in feeds corresponding to their region or diet formulation philosophies. Further, many different laboratory carbohydrate digestibility measures are now available and these can be differentially included in customized TDN models for evaluation or projection purposes.

Forage critical control points

Nutrient content and digestibility

The nutrient concentration and digestion information used to drive nutritionist models range widely. Diet formulation models make attempts to estimate in vivo nutrient digestion in different fashions. Mechanistic and empirical models are deployed within NASEM and Cornell Net Carbohydrate and Protein System models (**CNCPS**; Van Amburgh et al., 2015). Nutritionists are increasingly focused upon nutrient digestion coefficients, though accurately accounting for feed quality begins with robust nutrient content measures to match with nutrient digestion estimates.

While amino acids are now readily balanced in lactating diets, nutritionists are now realizing performance opportunities rooted in improvements in fatty acid nutrition. As we continue to increase the granularity in nutrient definition and measures, individual sugar measurements and formulations will likely be the next frontier that nutritionists explore. Currently, starch and water-soluble carbohydrates as a proxy for sugar are the core carbohydrate pools driving substantial energy yield in dairy diets.

As sugar rich ingredients or forages with substantial sugar content become available, fructose, sucrose, glucose or lactose concentrations in the diet will likely be a focal point to improve diet formulations. Different sugars are thought to ferment at different rates (Sniffen, personal communication) and for over 30 years supplemental sugar in diets has been documented to substantially improve rumen digestion and microbial protein synthesis relative to starch (Chamberlain et al., 1993).

Nutrient digestion within the rumen is a non-linear complicated process. Modeling nutrient digestion has proven exceedingly difficult, due to interactions between physical and chemical characteristics as well as the non-linear nature of nutrient digestion. Laboratory rumen fiber digestibility measures have advanced, with commercial laboratories now regularly reporting more fiber digestion information than any single model will account for. Forage quality estimates and diet formulation efforts accounting for digestible fiber will likely continue advancing with improvements in both data available and more flexible non-linear fiber degradation models. For forage quality comparisons, the Total Tract aNDF Digestibility (**TTNDFD**) model developed by Combs and the University of Wisconsin, then further validated against in vivo digestion by Lopes et al. (2015), continues to be a valuable indexing tool. This tool accounts for dynamic fiber digestion measures, but presents users with a single value to index fiber quality. The tool also permits users to compare within or between forage types. This

latter point has not been possible with traditional in vitro rumen fiber digestibility measures.

Fiber digestibility within a feed can be affected by seed genetics, plant population, soil fertility, agronomic and crop protection practices, growing conditions, plant health at harvest, harvest timing and even ensiling. Fiber digestibility in vivo is also affected by the fiber particle size and other rumen interactions, such as with starch content as noted above.

Another substantial area for growth in diet formulation and feed analysis lies in rumen fermentable starch. There is not a consensus among commercial feed analysis laboratories in measuring rumen starch digestibility. As a result, diet formulation strategies and modeling rumen, intestinal and hind-gut digestion are lagging. Starch is also digested in a relatively rapid and non-linear fashion. Though wide ranges in rumen starch digestion with commercial feeds are readily apparent (Rock River Laboratory data, not shown). Different non-linear modeling methods may be also warranted for starch relative to fiber; this area warrants further investigation. Starch digestibility within a feed is affected by seed genetics, soil fertility, agronomic practices, growing conditions, grain maturity at harvest, feed particle size and fermentation. Particle size is exceedingly important to account for in dairy diets, with particle size affecting both laboratory or in vivo starch digestion.

Grain and fiber particle size

As noted in the prior section, fiber and grain particle size are important to account for in determining feed quality and formulating diets. In my experience, there are sizable opportunities rooted in both better understanding and then managing fiber and grain particle size relative to dairy cattle performance.

Kernel processing score (**KPS**, % starch; Ferreira and Mertens, 2005), berry processing score (**BPS**, % starch; Johnson et al., 2016), and grain mean particle size (**MPS**, micron; Kalivoda et al., 2017) assessments will highlight substantial variation in feed feeding potential. The corn silage KPS continues to be a focal point with custom harvesting and dairy producer audiences alongside nutritionists. Guidelines have advanced relative to the initial benchmarks established by Ferreira and Mertens, as the industry average is now approximately 65 to 70% and the goal is now to exceed 75 to 80% KPS (upper 15th percentile; Rock River Laboratory, Inc. commercial KPS analysis data). The KPS can be affected by seed genetics as well as harvest timing and other factors (Lawrence and Kerwin, 2020). Joe Lawrence (personal communication) and I have also speculated that cob or other plant characteristics could also affect the self propelled harvester's processor ability to break corn grain kernels.

Grain particle size is recognized to affect rumen and total tract starch digestion (Ferraretto et al., 2013). Commercial dry ground corn has been documented to widely range in MPS, with MPS and surface area also having been related to in situ rumen starch digestion (Goeser and Shaver, 2020). More recent commercial data suggests for

each 100 units increase in MPS, rumen in situ 7h starch digestibility will decrease by roughly 7% units (Figure 1). This relationship can be helpful to both assess corn grain feeding potential, and formulate diets where rumen in situ starch digestibility data is not available.

With substantially more acres planted to sorghum for silage in arid and drought stricken regions, the energy potential in the sorghum berries is increasingly important to capture. New sorghum berry processors are being engineered and installed with self propelled forage harvesters. Early indications are showing these new processors can improve BPS by approximately 20 or more units (Katie Raver, personal communication). Historically, Rock River Laboratory BPS averaged around 20 percent, however with samples processed with new technologies the average has increased to around 40 percent. The BPS with 2.36 mm threshold is currently recommended and the goal is to exceed 50% of starch passing the 2.36 mm sieve (J. Pineiro, L. Ferraretto, K. Raver and Goeser, personal communication). Further research will soon be conducted and shared relating BPS to berry starch digestibility and performance potential. The BPS will be helpful for assessing commercial sorghum berry processing as well projecting sorghum silage starch feeding potential.

Fermentation compounds

Forage and grain fermentation yield acids, alcohols, esters and other compounds. Fermentation acids can stabilize feed for years to come and provide energy in dairy diets. Alcohols such as ethanol often result from less efficient fermentation by enterobacteria, yeast or other microbes. Undesirable fermentation can also produce compounds which may negatively affect feed intake and feeding potential or rumen metabolism, such as ethyl-lactate or ethyl-acetate. This is an area where further research is warranted and industry experience suggests we have much yet to learn. Numerous case studies with fermentation analyses following substantial dry matter intake depression have indicated some alcohol or ester compounds may be associated with the negative intake response, however cause and effect cannot be determined.

Feed hygiene characteristics

In addition to nutrient content, digestibility, particle size and fermentation compounds, the feed's hygienic quality needs to be accounted for. There are numerous cases where commercial feed analyses indicate great energy potential in forage, however the potential is not realized due to feed hygiene issues such as fungal or bacterial contamination. Feed hygiene assessments should consider starting with the TMR, and working backward through the feeding system to account for contamination that may happen during feed mixing and delivery. Experience has shown that substantial contamination happens outside of the feed storage and mixing area. This is an area where much work and learning needs to be done.

During feed hygiene investigations, account for mold, yeast, mycotoxin, and bacterial contamination in addition to assessing management or nutritional stressors.

Feed hygiene interpretive guidelines can be found (https://rockriverlab.com/pages/Guidelines,-Handling,-&-Methods.php). One common nutritional stressor to consider is poor starch digestibility, in which additional grain is added to the diet to increase energy density and support performance. In these cases, added grain in the diet also increases the rumen bypass starch load. Greater rumen bypass starch load is thought to negatively affect post-rumen digestive health in situations when feed hygiene is also an issue (Angie Rowson, personal communication).

Florida versus Midwestern Forage

In closing, the growing environment has been discussed to impact forage quality in different ways as described above. The appendix here provides forage quality population comparisons for forage samples submitted by dairy producers and nutritionists in Florida or the Midwestern US. Notably, nutrient content and digestibility seem to differ, with populations separating. While corn silage fiber content appears to overlap, the corn silage population from Florida seems to be numerically lower in TTNDFD relative to the Midwest. This difference may be attributable to growing conditions, with moisture and temperature during the growing season differentially affecting the crop in each respective region when summarized over numerous years.

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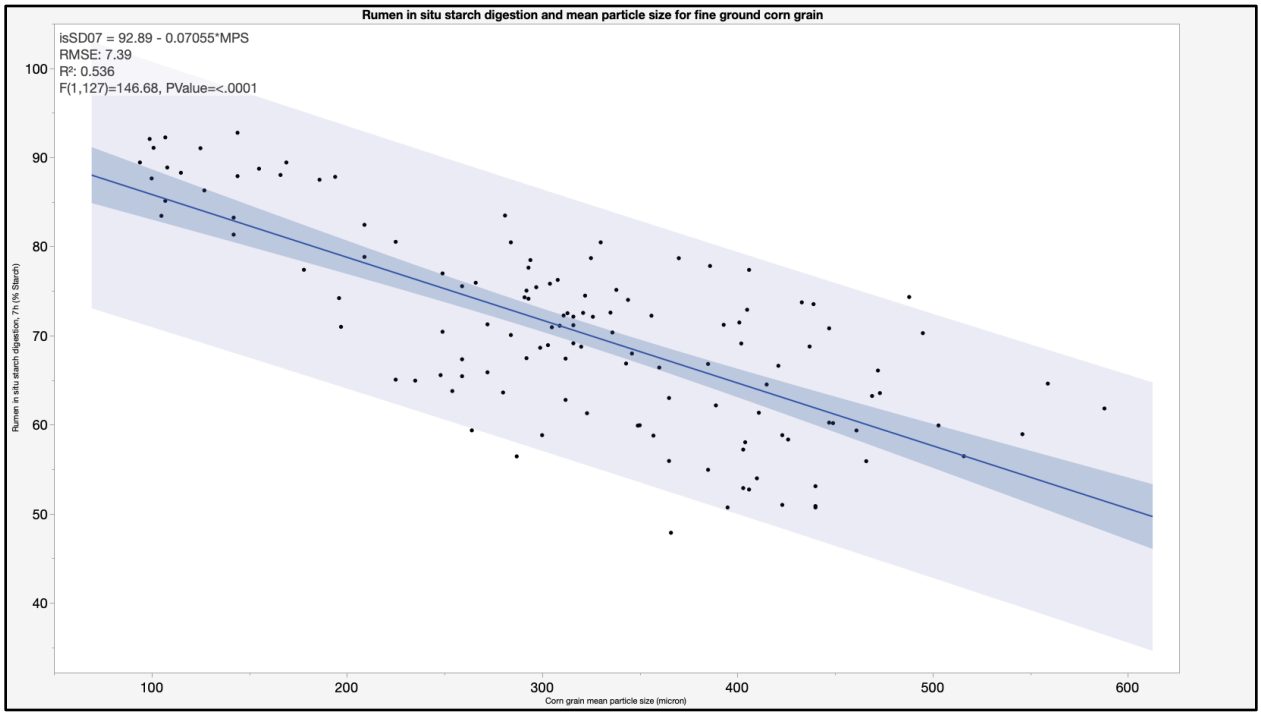

Figure 1: Commercial dry ground corn mean particle size (MPS; x-axis) and 7 hour rumen in situ starch digestibility (isSD7; % starch; y-axis) for samples submitted to Rock River Laboratory, Inc. from 2019 to 2025, with less than 18% moisture and smaller than 600 micron MPS. *The linear regression equation for isSD7 = 92.9 - 0.071 x MPS; with an unadjusted r-square = 0.54 and RMSE = 7.39 (P* < 0.0001*).*

Appendix: Florida and Midwestern Forage Nutrient and Digestibility Population Distributions for Samples Analyzed by Rock River Laboratory, Inc. (Watertown, WI, USA)

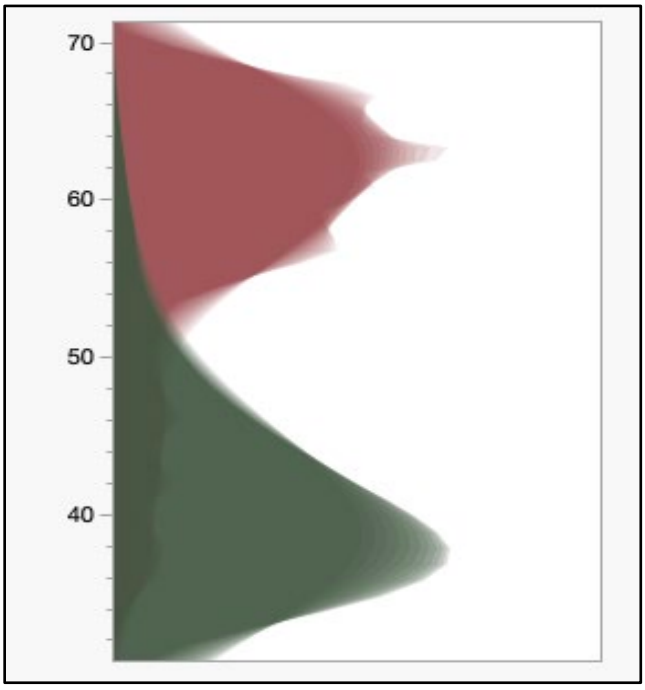

Figure 2: Haylage aNDF content (% DM) for MW (Green) versus Florida (Red) samples analyzed by Rock River Laboratory, Inc.

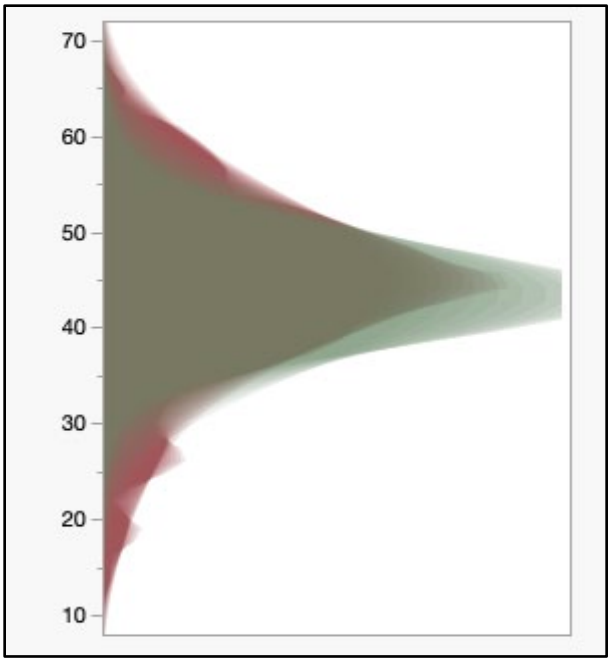

Figure 3: Haylage total tract NDF digestibility (TTNDFD-UW; % aNDF) for MW (Green) versus Florida (Red) samples analyzed by Rock River Laboratory, Inc.

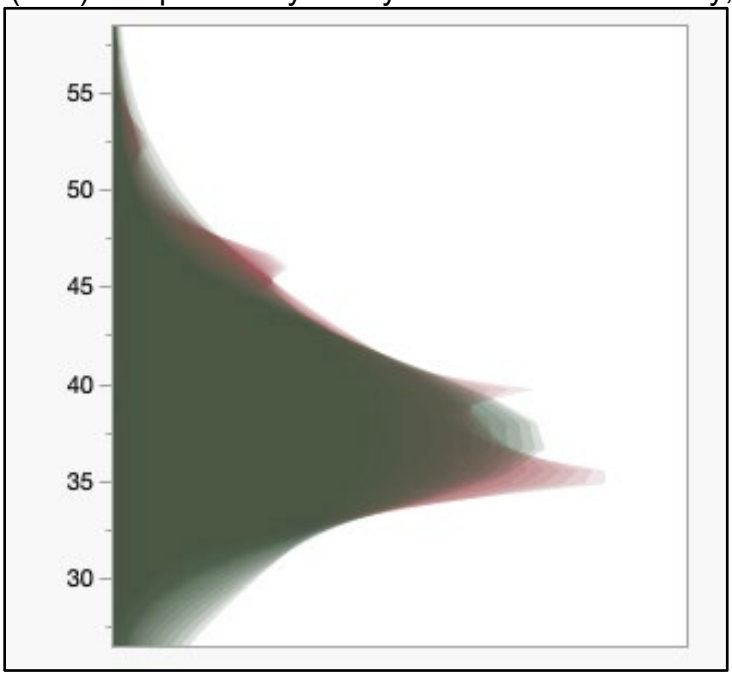

Figure 4: Corn Silage aNDF content (% DM) for MW (Green) versus Florida (Red) samples analyzed by Rock River Laboratory, Inc.

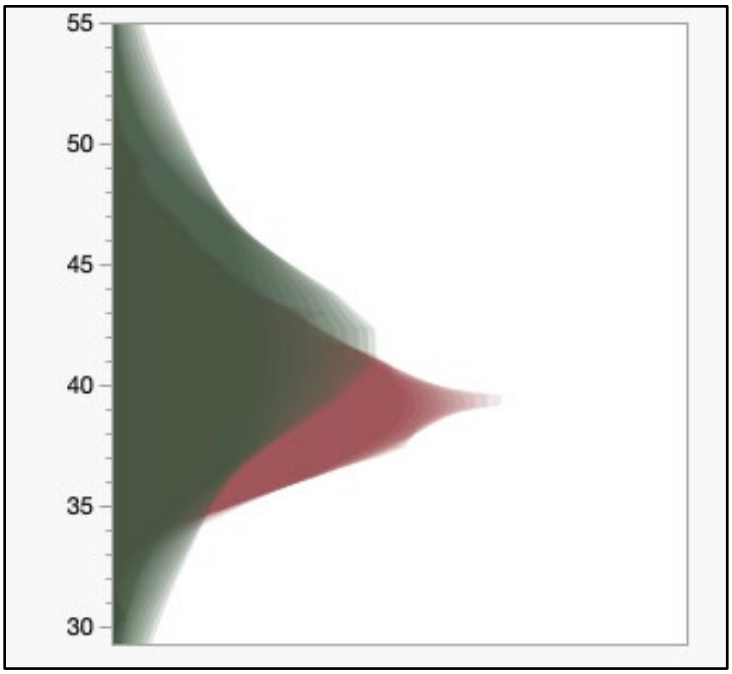

Figure 5: Corn Silage total tract NDF digestibility (TTNDFD-UW; % aNDF) for MW (Green) versus Florida (Red) samples analyzed by Rock River Laboratory, Inc.

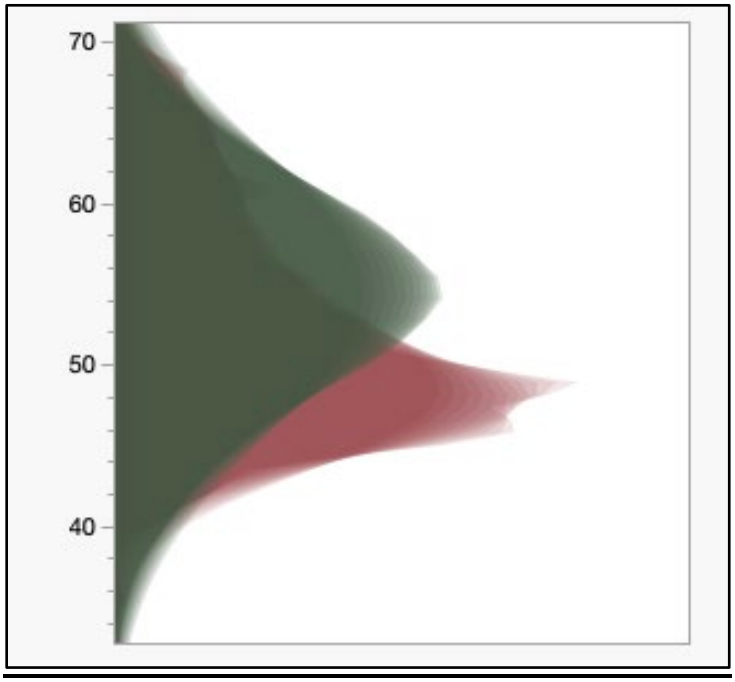

Figure 6: Sorghum or rye silage aNDF content (% DM) for MW (Green) versus Florida (Red) samples analyzed by Rock River Laboratory, Inc.

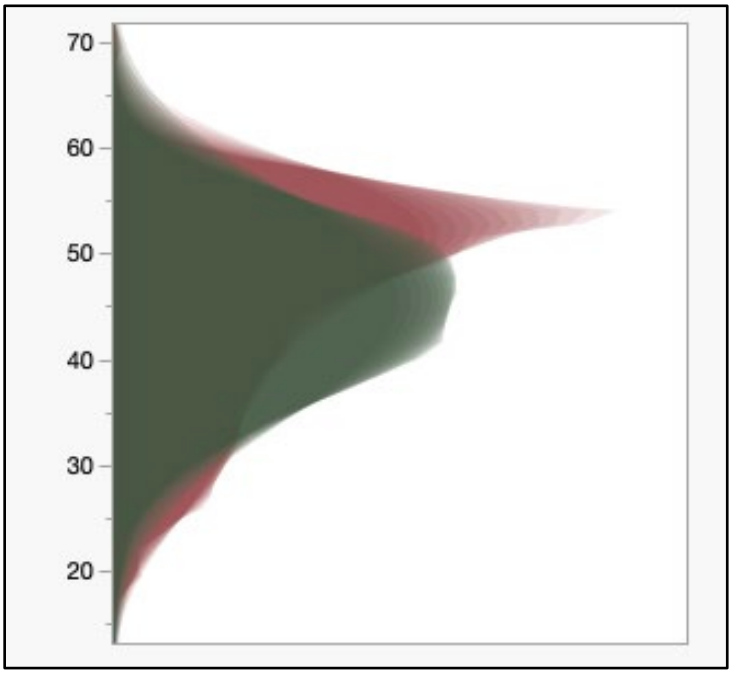

Figure 7: Sorghum or rye silage total tract NDF digestibility (TTNDFD-UW; % aNDF) for MW (Green) versus Florida (Red) samples analyzed by Rock River Laboratory, Inc.

Session Notes

A life cycle assessment of the environmental impacts of cow-calf operation in Florida

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February 2025

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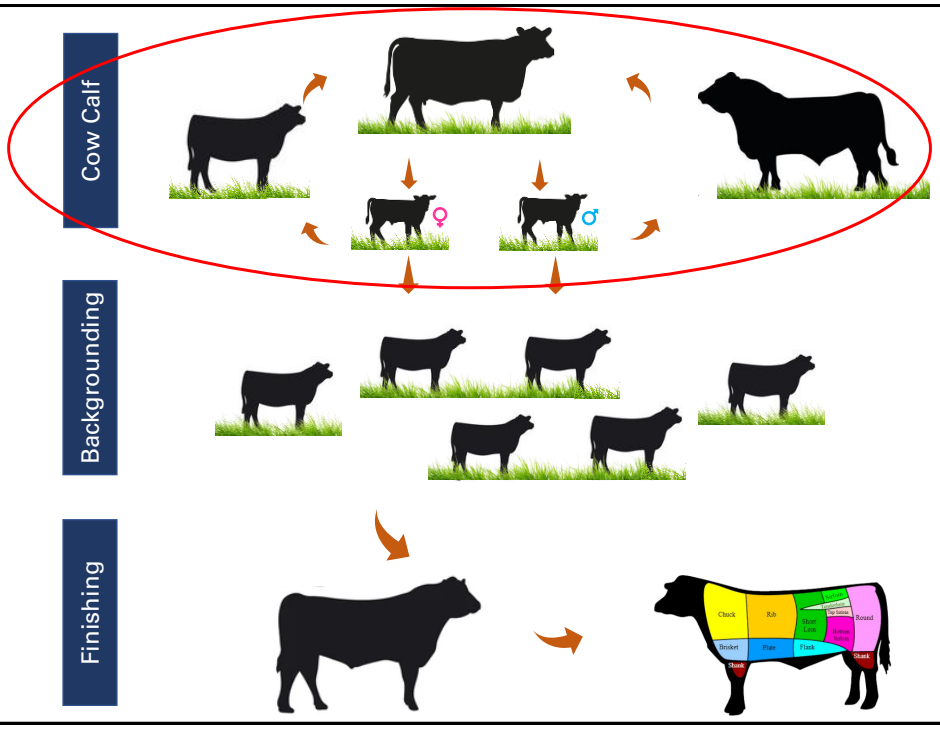

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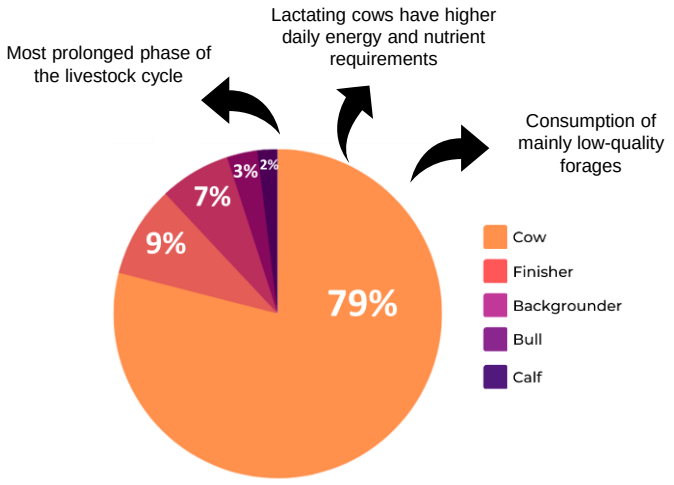
Why Cow-calf?

- Florida is predominantly a cow-calf state
- It is one of the major producers of calves in the USA, holding over 900 thousand head of cattle
- The cow-calf operation is predominantly in grazing systems with low technological input



Beauchemin et al. (2010). Agr. Syst. 103:371–379

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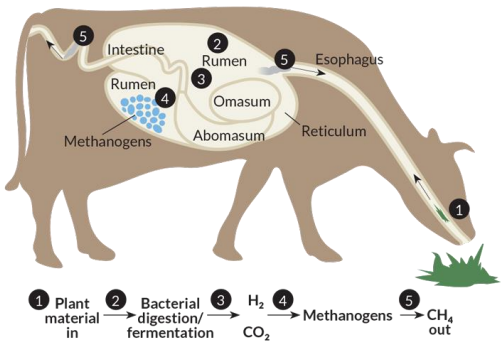

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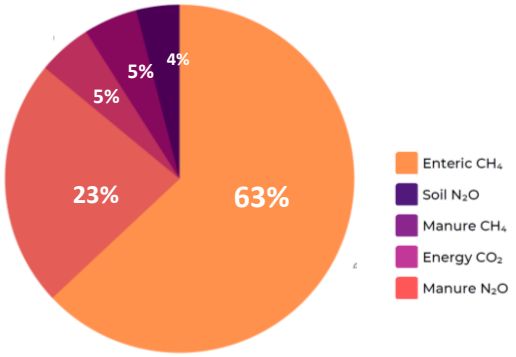

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Goal and Scope

- **Characterize** commercial beef farms in Florida with diverse management practices.
- **Assess** the environmental impacts, particularly **GHG emissions**, of beef cow-calf systems using **Life Cycle Assessment (LCA)** methodology.
- **Identify** opportunities for **environmental and productivity improvements** within livestock production systems.
- **Evaluate** the contribution of beef production to **human protein requirements** using the **Net Protein Contribution (NPC) model**.

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System boundary

Inputs, sources of emissions, and components of the life cycle assessment

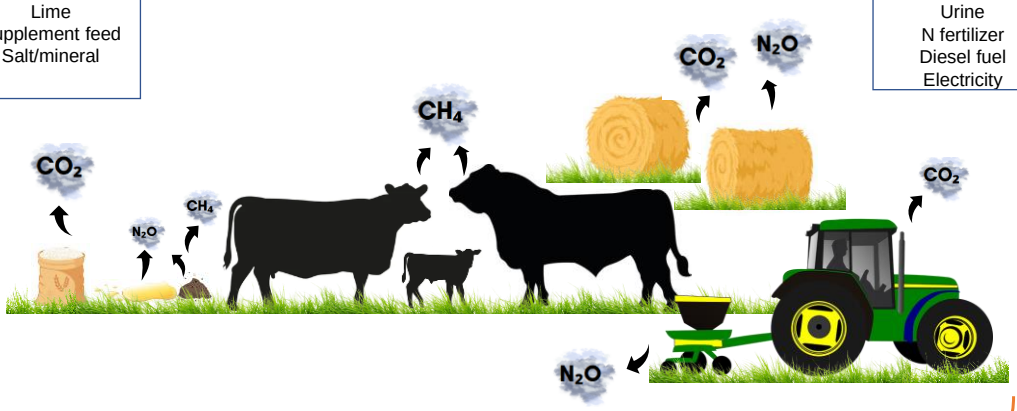
Cradle-to-gate

Off-Farm CO2:

Fertilizer
Lime
Supplement feed
Salt/mineral

On-Farm CO2:

Enteric fermentation
Feces
Urine
N fertilizer
Diesel fuel
Electricity



Functional unit: 1 kg of LW from calves weaned

CO_2e

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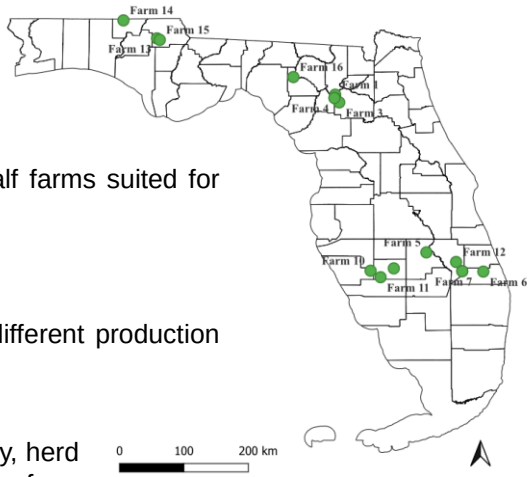
Emission Inventory



- Identification and recruitment of 16 beef cow-calf farms suited for the study
- North, central and south Florida → to identify different production systems
- Survey covering topics such as, herd productivity, herd characteristics, herd nutritional management, farm structure, forage crops, farm manure management plan and farm energy use
- The modeling data were integrated with the reviewed literature and an electronic spreadsheet based on the IPCC models, which facilitated the calculation of GHG intensities

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LCA Study - Farm selection survey (all information acquired is confidential)

Farm name	
Farm Ownew	
Location	

Farm Structure				**Comments**
Farm Size				
Forest area				
Construction area				
Grazing area				
Forage	Type:		Area:	
Liming	YES	Amount per year (lb):		
	NO			
Mineral fertilizer on pasture	YES	Type mineral:	Amount per year:	
	NO			
Fuel (machinery)	Working hours/week:			
Animal breed				
Breeding season	AI			
	Natural breeding			
Calving Season				
Crop 1	Type of crop:		Area:	
	Planting Date	Harvesting date: June	Productivity (tons/acre):	
	Fertilization		Liming:	
Crop 2	Type of crop:		Area:	
	Planting Date	Harvesting date	Productivity (tons/acre)	
	Fertilization		Liming	
Crop 3 (WINTER FORAGES)	Type of crop:		Area:	
	Planting Date	Harvesting date	Productivity (tons/acre)	
	Fertilization		Liming	
Cows in production (heads)				
Average live weight				
Forage consumption	Days grazing:			

Concentrate consumption	lb/head/day:	number of days:	
Silage/hay consumption	lb/head/day:	number of days:	
Mineral consumption	lb/head/day:	number of days:	
Heifers (heads)			
Average live weight (lb)			
Forage consumption	Days grazing:		
Concentrate consumption	lb/head/day:	number of days:	
Silage/hay consumption	lb/head/day:	number of days:	
Mineral consumption	lb/head/day:	number of days:	
Age at first heifer calving (months)			
Pegnancy rate (%)			
Calving interval (d)			
Bulls (heads)			
Average live weight (lb)			
Forage consumption	Days grazing:		
Concentrate consumption	lb/head/day:	number of days:	
Silage/hay consumption	lb/head/day:	number of days:	
Mineral consumption	lb/head/day:	number of days:	
Calves born per year (unit)			
Calves weaned per year (unit)			
Birth weight (lb)			
Weaning weight (lb)			
Forage consumption	Days grazing:		
Concentrate consumption	lb/head/day:	number of days:	
Silage/hay consumption	lb/head/day:	number of days:	
Mineral consumption	lb/head/day:	number of days:	
Calf mortality rate (%)			
Mortality rate of breeding herd (%)			

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LCIA method

- All data were converted to their 100-year global warming potential in CO_2; CO_2e values for CH_4 and N_2O are 27.2 and 273, respectively

Gas/ source	Emission factor	Reference
Methane sources		
Enteric Fermentation	23.3 g CH_4 kg^{-1} DMI	IPCC (2019)
Manure emission	0.01 kg CH_4^{-1}	IPCC (2006)
Burning	2.3 kg CH_4 ha^{-1}	IPCC (2006)

DMI = dry matter intake.

Carbon Dioxide sources		
Electricity	180.8 kg $CO_2e \cdot MWh^{-1}$	EIA (2020)
Fuel use	2.69 kg CO_2e L^{-1}	EIA (2022)
N fertilizer production	3.88 kg CO_2e kg^{-1}	Ledgard et al. (2011)
P fertilizer production	2.7 kg CO_2e kg^{-1}	Ledgard et al. (2011)
K fertilizer production	1.11 kg CO_2e kg^{-1}	Ledgard et al. (2011)
Lime	0.48 kg CO_2e kg^{-1}	IPCC (2006)
Manufacture of feed	from 0.29 to 1.29 kg CO_2e kg^{-1} DMI (depending on the type of feed)	Feedprint

MW = mega watts.

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LCIA method

Nitrous Oxide Sources		
	Direct Emissions	
Manure	Urine	
	0.0214 kg N_2O N^{-1}	Kohmann (2013)
	Feces	
	0.0002 kg N_2O N^{-1}	Kohmann (2013)
Soil N inputs	0.01 kg N_2O N^{-1}	IPCC (2019)
Burning	0.21 kg N_2O ha^{-1}	IPCC (2006)
	Indirect Nitrous Oxide	
Manure and Soil N inputs	Leaching	
	EF = 0.011 kg N_2O N^{-1}	IPCC (2019)
	$Frac_{leach}$ = 0.24	IPCC (2019)
	Volatilization	
	EF = 0.01 kg N_2O kg N^{-1}	IPCC (2006)
	$Frac_{volatilization}$ = 0.21 kg N^{-1}	IPCC (2006)
Soil N inputs	Leaching	
	EF = 0.011 kg N_2O N^{-1}	IPCC (2019)
	$Frac_{leach}$ = 0.24	IPCC (2019)
	EF = 0.01 kg N_2O kg N^{-1}	IPCC (2019)
	$Frac_{volatilization}$ = 0.21 kg N^{-1}	IPCC (2019)

EF = Emission factor, $Frac_{leach}$ = fraction leaching, $Frac_{volatilization}$ = fraction volatilization.

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Beef's Contribution to Meeting Human Protein Requirements

A summative model of NPC was used to estimate beef's contribution to meeting human protein requirements (Wilkinson, 2011; Ertl et al., 2015, 2016; Baber et al., 2018).

- *Conversion Efficiency of Beef Cattle (hePCE)*

- hePCE is a metric of comparison indicating the conversion of HeP_f into beef
- $$hePCE = \frac{hePP}{hePF}$$

NPC = net protein contribution; hePCE = human-edible protein conversion efficiency; hePP = human-edible protein produced; hePF= human-edible protein consumed in feed.

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- Assessing Protein Quality Using Digestible Indispensable Amino Acid Score (DIAAS)

- $$DIAAS = \frac{mg\ of\ indispensable\ amino\ acid\ in\ 1\ g\ of\ dietary\ protein}{mg\ of\ same\ digestible\ indispensable\ amino\ acid\ in\ 1\ g\ of\ reference\ protein}\ x\ 100$$

The output product, beef, has a DIAAS of

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PQR captures the change in biological value of HeP that occurs when plant-derived HeP is converted to beef:

- $$PQR = \frac{DIAAS\ of\ beef}{DIAAS\ of\ diet}$$

PQR = protein quality ratio.

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Net Protein Contribution

- The NPC was calculated by multiplying the ratio of HeP in beef to the HeP in feedstuffs by the PQR:

$$NPC = PQR \; x \; HePCE$$

NPC > 1 : beef value chain is positively contributing to meeting human requirements

NPC < 1 : beef value chain is competing with humans for protein

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Results

Table 1. Results from the life cycle analysis of cow-calf operation in Florida for each farm visited

Farms	Variables				
	Herd size	AU	SR	Emissions AU d^{-1}	CO_2e intensity kg weaned $calf^{-1}$
Farm-1	94.5	63.3	0.8	9.6	20.9
Farm-2	71	63.7	1.4	9.2	28.4
Farm-3	778	529.4	2	8.9	19.1
Farm-4	35	23.7	1.6	6.9	22.1
Farm-5	472.5	318.3	0.9	12.2	26.6
Farm-6	256.8	143.3	1.7	8	23
Farm-7	443.6	375.6	1.8	8.3	25.7
Farm-8	416	320.9	1.3	7.4	18.6
Farm-9	191	134.8	1.4	7.8	22
Farm-10	2822.3	2272	0.6	6.8	17.7
Farm-11	6640	4708.7	5.5	6.9	18.1
Farm-12	4087.5	2894.5	0.8	6.9	25.2
Farm-13	79	63.8	1.2	8.4	20
Farm-14	67	43.8	2.7	11.3	25.3
Farm-15	364.9	302.3	1.8	7.6	17.5
Farm-16	948.3	746.6	1.2	11.3	26.7

AU = 450 kg BW; SR= Stocking rate

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Results

Table 3. Annual methane emissions from farm production

Total kg of CH_4 farm^{-1} year^{-1}	Mean	Min	Max
Enteric emission	56462	1496	342943
Manure	999	21	6259
Burning	359	0	3909
Total	57820	1517	350570

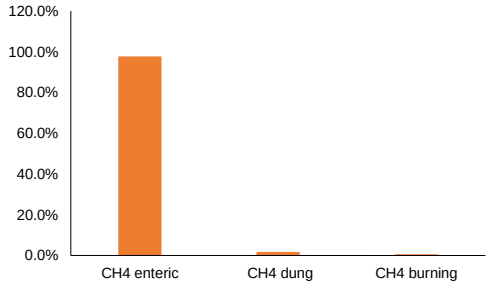
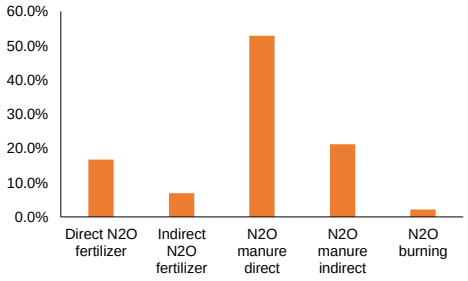


Table 4. Annual nitrous oxide emissions from farm production

Total kg of N_2O farm^{-1} year^{-1}	Mean	Min	Max
Fertilizer Direct emission	250	0	1332
Fertilizer Indirect emission	104	0	552
Manure Direct emission	789	20	4715
Manure Indirect emission	317	8	1893
Burning	33	0	357
Total	1493	29	7337



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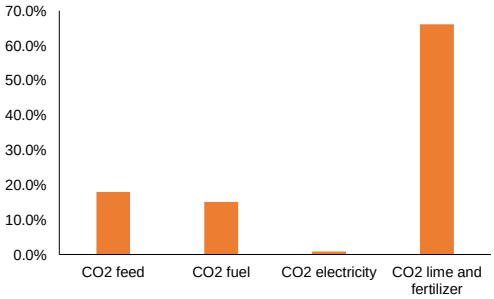
Results

Table 5. Annual carbon dioxide emissions from farm production

Total kg of CO_2 farm^{-1} year^{-1}	Mean	Min	Max
Feed and mineral	43537	499	342665
Fuel	36491	0	228574
Electricity	2078	79	10536
Manufacture and transport of lime and fertilizer	160248	0	707082
Total	242354	10502	1072671



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Results

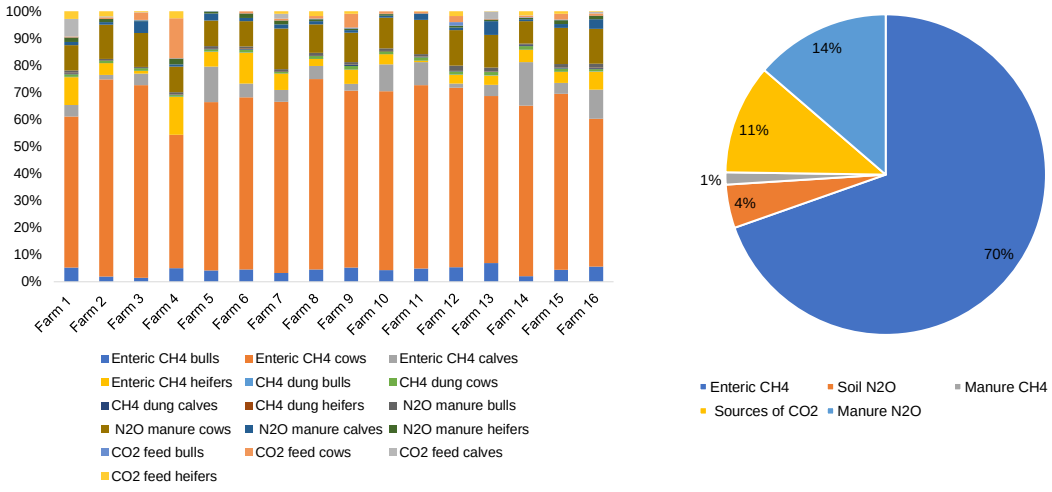

Figure 1. Contribution of each gas (CO_2e) to the carbon intensity for the studied farms

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Results

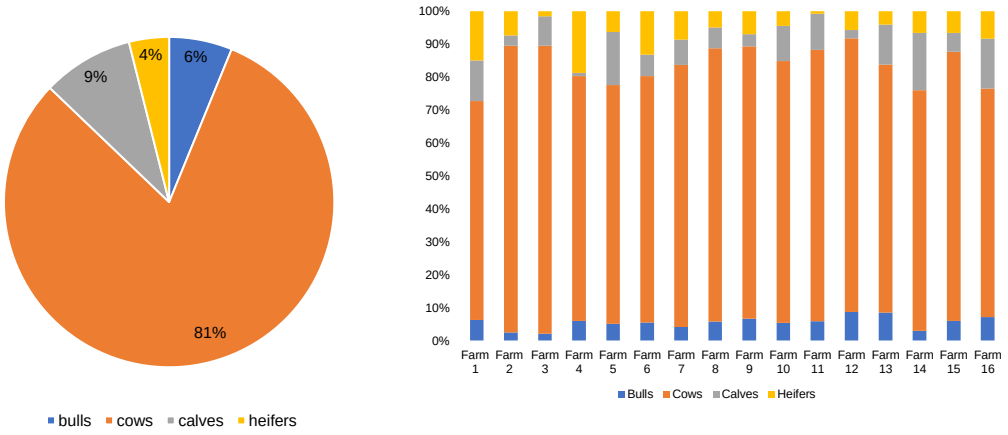

Figure 2. Breakdown of total GHG emissions (CO_2 equivalents) by component of cow-calf operation in Florida

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Results

Farms with heavier cows tend to have higher CO2e emissions intensity, while farms with higher productivity per area show lower emissions intensity

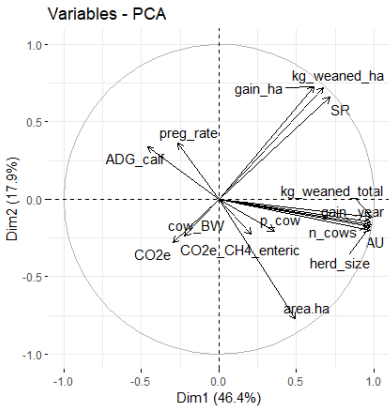

Figure 3. Principal Correlation Analysis between carbon equivalent intensity emission and farm performance variables. Dim 1 = principal component 1; Dim2 = principal component 2.

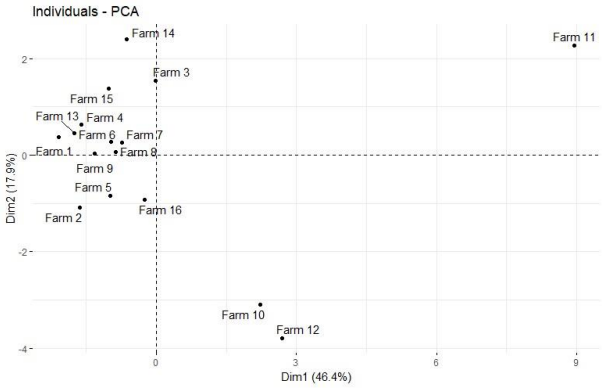

Figure 4. Principal Component Analysis of grouped farms. Dim 1 = principal component 1; Dim2 = principal component 2.

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Results

Table 2. Human-edible protein contribution and Land use efficiency of cow calf operation in Forida

Farms	Variables						
	hePF (kg total herd-1)	hePP (kg total herd-1)	DIAAS	hePCE	NPC	Pasture area (ha)	Land use eff. (ha ton hePP^{-1})
Farm-1	500.2	1712.2	54.8	3.4	7.0	80.9	47.3
Farm-2	490.4	1276.0	52.8	2.6	5.5	45.7	35.8
Farm-3	6244.6	12264.5	30.0	2.0	7.3	263.0	21.4
Farm-4	43.7	561.7	55.4	12.8	25.9	14.6	25.9
Farm-5	82.7	8112.2	56.6	98.2	194.1	364.2	44.9
Farm-6	141.1	3494.6	6.4	24.8	434.3	86.2	24.7
Farm-7	3291.6	6812.3	52.5	2.1	4.4	203.3	29.8
Farm-8	951.3	6798.0	31.7	7.1	25.3	242.8	35.7
Farm-9	734.6	2871.6	39.9	3.9	11.0	97.1	33.8
Farm-10	2776.0	46704.8	6.4	16.8	295.1	3561.2	76.2
Farm-11	4737.6	93062.2	6.2	19.6	355.6	849.8	9.1
Farm-12	14980.0	48395.2	17.0	3.2	21.2	3642.2	75.3
Farm-13	308.3	1418.0	22.5	4.6	22.9	54.6	38.5
Farm-14	456.2	1102.5	54.8	2.4	4.9	55.6	50.5
Farm-15	618.1	6875.5	32.3	11.1	38.6	170.0	24.7
Farm-16	992.3	17329.3	5.0	17.5	388.6	647.5	37.4

[1]hePF= human-edible protein consumed in feed; hePP = human-edible protein produced; hePCE = human-edible protein conversion efficiency; NPC = net protein contribution.

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Conclusions

- Enteric methane accounted for 70% of total emissions, with emission intensity ranging from 17.5 to 28.4 kg CO_2e/kg weaned calf.
- Larger cows had higher emissions, while greater productivity per hectare reduced emission intensity.
- Protein Contribution: Beef efficiently converted low-quality feed into high-quality protein, NPC>1
- Land Use Efficiency: Farms with supplementation required less land per ton of human-edible protein.

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Limitations

- Sample size
- Accuracy of the data
- Difficulty in measuring the impact of secondary processes
- Representativeness
- GWP metric

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Thank you

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Early Life Nutrition and Muscle and Adipose Tissue Deposition and Subsequent Feedlot Performance and Carcass Quality

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[†]Texas Tech University, Lubbock
[*]Utah State University, Logan

Introduction

The period time from birth through weaning of a beef calf represents a time of efficient skeletal muscle growth. These changes in skeletal muscle accretion are reflected by an increased lean tissue deposition in the young growing calf. These alterations in tissue deposition can have either a positive or negative effect on subsequent feedlot performance and carcass merit at the end of the feeding period. It is well documented that early life nutrition and management tools can dramatically alter both the rate and extent of skeletal muscle growth during the preweaning period but can also alter the rate and extent of adipose tissue accretion in the young calf and ultimately the amount of lipid deposition early in the life of the calf. These early changes can alter feed efficiency throughout the rest of the feeding period and have profound effects on carcass composition and quality. The objective of this paper and presentation is to outline how early life plane of nutrition and management tools like steroidal implants impact these processes.

Postnatal Skeletal Muscle Growth

The individual muscle fiber is considered the cellular unit of skeletal muscle tissue. The postnatal skeletal muscle fiber has several, distinguishing characteristics compared to cells that make up other tissues. The muscle fibers, as well as nuclei within each fiber, are post-mitotic, having lost the ability to divide. Additionally, muscle fiber number is fixed at birth in most meat animals. In order to sustain postnatal muscle hypertrophy, the muscle fiber needs an external source of DNA. The DNA accumulation responsible for postnatal muscle hypertrophy is highly correlated to muscle growth rate (Trenkle et al., 1978). In fact, 60 to 90% of DNA located within mature skeletal muscle fibers is accumulated during postnatal growth (Allen et al., 1979). Muscle satellite cells are now known to be the source of DNA responsible for postnatal muscle hypertrophy. By supplying more DNA to the individual fiber, there is more “machinery” available to ultimately, synthesize a greater amount of protein within each fiber. Hence, the positive relationship between DNA content in the fiber and rate of muscle growth in cattle.

Satellite cells are mononucleated cells located between the basal lamina and sarcolemma of the muscle fiber (Mauro, 1961). Moss and LeBlond (1970) determined

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there were two types of nuclei within the basement membrane of the muscle fiber that were distinguishable from one another. Following the labeling of nuclei by a single [^{3}H]-thymidine injection, male rats were sacrificed at different time intervals and the tibialis anterior muscle was removed for radioautography analysis. The results of this study showed that the true muscle nuclei were not labeled at 1 h following injection, indicating they were not actively dividing. However, the nuclei within the basement membrane were labeled, indicating the satellite cells were able to synthesize DNA and divide. Moss and LeBlond (1970) also reported that over the time course of 72 h, the number of labeled true muscle nuclei was increasing while the number of labeled satellite cells decreased. This lead to the conclusion that the source of labeled nuclei being counted within the fiber over the 72 h time frame were in fact that of satellite cells that were dividing and incorporating into the existing muscle fibers. Once the satellite cells fused with the existing fiber and donated their nuclei, they in turn lost their proliferative capacity (Moss and Leblond, 1971). These studies confirmed the postmitotic nature of true muscle nuclei and the importance of the muscle satellite cell in postnatal skeletal muscle growth.

The necessity of satellite cells in postnatal muscle growth is well understood, however, there are still limitations to the degree of DNA accretion at later stages of muscle growth. In a newborn animal, 30% of muscle nuclei are satellite cells, but the number reduces to 2 to 10% in mature animals, thus showing the actual number of satellite cells decrease with age (Cardasis and Cooper, 1975). This becomes a challenge in optimizing skeletal muscle hypertrophy in more mature cattle due to the small population of progenitor cells available to contribute to the existing fiber. Not only is there a reduction in satellite cell number, but also those cells still present withdraw from the proliferative state of the cell cycle and enter G_0 (a state of quiescence) which leads to a growth plateau (Cardasis and Copper, 1975). In order to maintain the satellite cell population necessary to support muscle hypertrophy in mature animals, the cells in quiescence must be activated to allow them to progress through the cell cycle and contribute nuclei to the existing muscle fiber. Once quiescent satellite cells have been activated, there is a need for growth factors capable of stimulating satellite cell proliferation and subsequent differentiation.

Adipose Tissue Accretion

Cattle can accumulate adipose tissue nearly indefinitely, and strong evidence exists to indicate that some portion of the increase in adiposity in the mature animal is derived from proliferation and differentiation of preexisting pluripotent fibroblasts. Beef cattle provide an especially suitable model for investigations of this process, because they are noted for vast amounts of marbling adipose tissue accumulation within their muscles. Many researchers have studied cell size in intramuscular adipose tissues. Intramuscular adipose tissue has larger cells per gram, smaller mean cell diameter, and smaller mean cell volume than subcutaneous and perirenal adipose tissue (Smith and Crouse, 1984). Smith and Crouse (1984) demonstrated that different regulatory mechanisms were present in subcutaneous (**SC**) and intramuscular **(IM)** adipose tissue. For example, acetate contributed 70 to 80% of the acetyl units for in vitro lipogenesis in SC adipose

tissue, but only 10 to 25% in IM adipose tissue. Likewise, glucose contributed 1 to 10% of the acetyl units in IM adipose tissue and 50 to 75% in IM adipose tissue. This data showed that stromal-vascular cells within IM adipose tissue were quite proliferative and used glucose for making acetyl units in adipose tissue. Also, these results contributed that the lipogenic metabolism in IM adipose tissue resembled that of myogenic metabolism in skeletal muscle tissue. Breed differences contribute to size and amounts of adipocyte in IM adipose tissue. Wagyu steers, high marbling beef, marbling adipocytes are smaller and exhibit twice the rate of DNA synthesis as marbling adipocytes from Angus steers at same physiological maturity (Chung et al., 2007). These data indicated that adipose tissue accretion is a lifelong event. The deposition of SC adipose tissue can occur at any time in the life of the beef animal is driven by excess dietary energy.

Creep Feeding

There are many nutritional and management procedures for producers to utilize, with creep feeding being popular. The need for efficient economic growth is always in high demand. Not only does creep feeding offer additional calf gain, but early exposure to grain also allows for enhanced development of the rumen (Pugh, 2023). With data meriting various results, creep feeding's impact on carcass characteristics cannot be fully determined without a simultaneous study of the weaning method and management style of each operation (Pugh, 2023). Creep feeding, when paired with a complementary weaning timeline, forage source, and management protocol, has shown to be effective in aiding the adjustment to finishing diets (Faulkner et al. 1994). Myers et al. (1999) reported that early weaned calves performed better, gaining 100% faster ($P = 0.0001$) than the average of normal weaned calves supplemented with creep feed and normal weaned calves. Faulkner et al. (1994) found that calves supplemented with corn had higher intake and gain to feed (**G:F**) in both growing and finishing periods and higher quality grade scores and larger longissimus muscle area than calves supplemented with soyhulls. Calves that were supplemented with a soyhull-based diet were leaner, with an average fat thickness of 1.16 cm and an internal fat of 2.80% compared to corn supplemented calves' 1.28 cm and 2.88%. Calves who were creep fed had higher average daily gain (**ADG**) and gain to feed G:F during the feedlot treatment (Meteer, 2011). This data also concluded that while creep fed calves had a greater degree of marbling than calves in the control group (no creep feed), ultimately early weaned calves had the greatest degree of marbling. The level of creep feed offered to calves also plays into factor for growth and carcass characteristics. Offering a limited amount of creep feed to calves led to lower intakes during the finishing period but this did not hinder their gain; with both limited and unlimited treatment groups gain being 1.03 kg/d and the limited treatment G:F being .01 higher than the unlimited treatment (Faulkner et al. 1994). This study also found calves with unlimited access to creep feed grew to have larger longissimus muscle area and higher quality grade scores. As an industry, encouragement to creep feeding can be pushed by sharing research with cow-calf operations. Producers that retain ownership or are offered incentive to creep feed can maximize advantageous rumen development and allow calves to improve their gain sooner (Pugh 2023). Regarding economical differences, creep feed calves spent more

time on their mother's side and less time in the feedlot (Shike 2007). Supplementation of feed in early life can make the transition to a new environment easier for calves. Meteer (2011) also found that creep fed calves were more profitable than early weaned calves on a fiber-based diet and the control group. This was consistent with findings by Shike et al. (2007), which showed normal weaned fiber creep fed as the most profitable followed by normal wean creep fed, normal wean no creep (control), and early wean. Lower profits of other early-life nutrition methods can be attributed to greater yardage and feed costs. Calves provided with supplemental feed and weaned later spent less days on feed (Shike et al. 2007).

Early Weaning

Weaning age is a critical management practice in beef production, influencing growth performance, feed efficiency, and carcass characteristics. Normal weaning (**NW**) occurs between six and eight months, which allows for calves to grow naturally through milk and forage consumption. Another strategy that has proven effective is normal weaning with creep feeding (**NWC**), where calves are supplemented with grain while still nursing to promote early growth and ease the transition into the feedlot. However, an alternative strategy is to early wean (**EW**) calves between 60 and 150 days of age (d). This strategy has been shown to improve feed efficiency and enhance carcass quality. EW allowed for calves to be placed on high-energy diets earlier in life, this practice could enhance marbling and improve carcass quality as they're more adapted to the high-concentrate diet and exposed to higher starch diets. The effectiveness of EW, however, depends heavily on breed, feeding system, and finishing period length.

The effect of weaning strategy on feedlot performance, specifically ADG, dry matter intake (**DMI**) and G:F, varies across studies. In Myers et al. (1999), EW steers exhibited higher ADG (1.31 kg/day) and were the most efficient (G:F = 0.170), while having the lowest DMI (7.70 kg/day) when compared to NW and NWC. These EW steers were immediately placed on a high-concentrate diet after weaning. Similarly, EW steers outperformed NW steers in feed efficiency and marbling scores (Thrift et al., 2004). Conversely, Loy et al. (1999) presented different results. In this study, 120 steer calves (Angus and Simmental-sired) were weaned at either 67 days (EW) or 147 days (late weaning). Interestingly, no significant differences in feed efficiency, ADG, or final weights were observed between early-weaned and late-weaned (LW) steers. Angus-sired steers demonstrated greater G:F (G:F = 0.178) than Simmental steers, especially in the early-weaned group. These findings suggest that while early weaning may enhance efficiency in some systems, the overall impacts depend on breed type and diet composition.

In terms of carcass characteristics, early weaning consistently improved marbling scores and carcass quality across studies. Early weaning steers had higher marbling scores (1198 vs. 1144 in NWC and 1120 in NW) and a greater percentage of carcasses grading USDA Choice or higher (Meyers et al., 1999). Loy et al. (1999) further supported these results, reporting that EW steers had greater intramuscular fat and higher marbling scores ($P < 0.05$) when compared to LW. However, Thrift et al. (2004)

found that early-weaned steers had similar or lower HCW than normal-weaned steers, suggesting that while EW improves carcass quality, it may also result in lighter carcasses. This trend was supported by Loy et al. (1999), finding that EW Angus steers were heavier than LW Angus steers at slaughter, but this pattern was inconsistent in Simmental steers. Across studies, high-concentrate diets in early weaned steers accelerated intramuscular fat deposition, suggesting that nutrient-dense feeding during early growth stages directly influences final carcass quality.

Implant Programs

Implant programs are strategic management tools designed to enhance growth rates, feed efficiency, and overall productivity. Concerns have been raised about the early use of implants and their potential negative impact on marbling, tenderness, beef quality, and feed efficiency during the finishing phase. In a research study evaluating different lifetime implant strategies and their effects on production efficiency and carcass quality. Implants did not significantly affect body weight (**BW**) at weaning or after backgrounding (Pritchard et al., 2003). However, they increased ADG by 5% compared to non-implanted controls during backgrounding (Pritchard et al., 2003). Pritchard et al. (2015) assessed the timing of suckling calf implants (**SCI**) on weaning weight (**WW**), post-weaning performance, and carcass traits. Implanting in May increased WW significantly in calves from mature cows, while August implantation benefited those from younger cows (Pritchard et al., 2015). The effects of EW versus normal weaning (NW) and the impact of aggressive versus nonaggressive implant regimens on steer performance were evaluated by Schoonmaker et al.,2001. EW steers had higher ADG, improved feed efficiency, and required a longer feeding period (Schoonmaker et al.,2001). Calfhood SCI increased WW without affecting post-weaning ADG or feed efficiency (Pritchard et al., 2015). The aggressive implant regimen further increased ADG, reduced days on feed, and led to leaner carcasses with larger longissimus muscle areas (Schoonmaker et al.,2001). During the receiving phase, implanted steers grew faster and more efficiently than non-implanted steers, while DMI remained unaffected (Pritchard et al., 2003). This study also found that stronger implant use increased carcass weight but decreased marbling scores, with no significant impact on meat tenderness. Pritchard et al. (2015) also found that carcass traits showed slight increases in HCW but had no adverse effects on marbling or yield grade. Pritchard et al. (2015) concluded that strategic implant timing based on dam age could maximize growth without compromising meat quality. Despite differences in feed efficiency, carcass quality was maintained, and EW steaks were rated more tender and juicier (Schoonmaker et al.,2001). These results suggest early weaning combined with aggressive implants can optimize growth and carcass traits while maintaining beef quality (Schoonmaker et al.,2001). Pritchard et al. (2003) concluded that aggressive implant strategies enhance efficiency but require careful management to balance growth performance and beef quality.

Conclusions

Specific early-life nutrition and management programs can have dramatic effects on the calf performance at the time of implementation. Furthermore, these programs can alter performance and ultimate carcass quality and the end of the feeding period. Some of these lifetime effects are due to alterations in skeletal muscle and adipose tissue accretion throughout the lifetime of the beef animal. Matching the right nutrition and management program to the genetics, forage resources, and marketing plan is crucial for overall profitability of the entire beef production segment.

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Table 1. Effects of weaning age, creep feeding, and type of creep on steer carcass traits from three studies

	Treatments[c]	ADG, kg[a]	ADG, kg[b]	G:F[a]	G:F[b]	DMI[a]	DMI[b]	HCW, kg	≥Avg Choice %
Meteer et al. (2011)	Control	1.20	1.60	-	.160	-	10.09	335	40.0
	EWS	1.63	1.48	.270	.140	5.96	10.63	332	78.4
	EWF	1.62	1.46	.239	.142	6.84	10.33	334	81.6
	NWC	1.53	1.58	.096	.161	3.83	9.87	334	51.4
	NWF	1.50	1.61	.096	.165	3.44	9.80	342	41.7
Myers et al. (1999)	Control	.62	1.38	-	.151	-	8.12	289	68.0
	EW	1.44	1.28	-	.170	-	7.70	301	93.0
	NWC	.82	1.38	-	.155	-	8.20	290	68.0
Shike et al. (2007)	Control	1.10	1.38	.337	.175	-	7.87	329	46.5
	EWS	1.34	1.42	.160	.177	4.01	8.01	344	72.5
	NWC	1.37	1.46	.115	.178	1.74	8.20	352	33.5
	NWF	1.29	1.46	-	.184	1.59	7.92	343	45.0

[a]During growing phase.
[b]During finishing phase.
[c]Control= normal wean-no creep, EWS= early wean- starch based diet, EWF= early wean- fiber based diet, EW= early wean, NWC= normal wean- creep fed starch based diet, NWF= normal wean- creep fed fiber based diet.

Table 2. Weaning strategy on feedlot performance and carcass characteristics

	Study[d]	Weaning Strategy[c]	no.	DMI (kg/day)	ADG (kg/day)	G:F Ratio	HCW[e] (kg)	Marbling Score[b]
Meyers et al. (1999)								
	1	EW (177 ± 9 d)	27	7.70[a]	1.31	0.170	301	1198
	1	NW (177-231 d)	28	8.12[a]	1.22	0.151	289	1120
	1	NWC (177-231 d)	28	8.20[a]	1.27	0.155	290	1144
Loy et al. (2000)								
	SIM	EW (60 d)	31	9.07	1.44	0.157	338.8	1009
		LW (147 d)	35	9.43	1.45	0.154	346.5	981
	ANG	EW (60 d)	27	8.32	1.47	0.178	343.4	1149
		LW (147 d)	27	8.51	1.41	0.165	328.4	1059
Fluharty et al. (2000)								
	1	EW (103 ± 3 d)	38	8.70[f]	1.52[f]	0.176[f]	328.7	
	1	NW (203 ± 3 d)	35	8.80[f]	1.47[f]	0.167[f]	330.7	

[a]During the feedlot phase.
[b]1000 = Small[00], 1100 = Modest[00].
[c]EW = early weaned, NW = normal weaned, NWC = normal weaned with creep feeding, LW = late weaned.
[d]SIM = Simmental, ANG = Angus.
[e]Hot carcass weight.
[f]During the finishing phase.

Table 3. Summary of studies on performance and carcass characteristics of implanted steers

	Treatments	ADG W[a] kg	ADG F[a] kg	F:G W[a] kg	F:G F[b] kg	DMI W[a] kg	DMI F[b] kg	HCW	Marbling
Pritchard et al. (2003)[x]	Control	1.21	1.40	2.05	3.23	6.0	9.97	317.5	5.68
	2	1.33	1.58	1.78	3.03	6.2	10.6	336.6	5.54
	3	1.37	1.65	1.99	2.99	6.2	10.9	341.1	5.38
	4	1.36	1.61	1.01	2.98	6.1	10.6	348.4	5.38
Pritchard et al. (2015)[y]	1	-	1.71	-	2.62	-	9.8	355.2	581
	2	-	1.69	-	2.64	-	9.8	357.9	565
	3	-	1.70	-	2.67	-	10.0	360.2	571
Schoonmaker et al. (2001)[z]	EW	1.61	1.61	3.54	4.60	5.7	7.4	332.4	444.3
	NW	1.03	1.76	-	4.83	-	8.5	331.1	440.0
	A	1.38	1.71	4.06	4.68	5.6	8.0	331.7	439.2
	NA	1.26	1.66	4.52	4.76	5.7	7.9	331.9	445.1

[a]Weaning phase.
[b]Finishing phase.
[x] 4.0 = Slight^{o}; 5.0 = Small^{o}.
[y] 400 = Slight^{o}; 500 = Small^{o}.
[z] Practically devoid = 100–199, slight=200–299, small=300–399, modest = 400–499, moderate = 500-599.

Session Notes

Beef on Dairy: Impacts on Performance and Carcass Traits

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Introduction

The growing trend of crossbreeding dairy cows with beef sires has led to the emergence of beef on dairy cattle as a significant component of the U.S. fed cattle supply. This practice, which began in 2018 due, in part, to the rising costs of dairy heifer rearing, and was exacerbated by stark downtown in demand for dairy bull calves, is now estimated to account for somewhere between 15 to 20% of the fed cattle population (Schumacher, 2024). Supplying 15 to 20% of the fed market is a relatively small when we consider that other countries, like Finland for example, fulfill 80% of their fed cattle markets with progeny from their dairy farms (Berry, 2021). One could argue the value of beef on dairy in the U.S. transition. However, the pipeline of surplus dairy calves has always helped supply beef in the United States. Prior to the beef on dairy trend, 20% of the fed cattle supply in the United States came from purebred Holsteins (Boykin et al., 2017). When Holstein calves began to lose value, the market responded, and dairy farmers rapidly adapted to using beef semen, resulting in a reduction of black and white hides at slaughter (Figure 1).

The first generations of beef on dairy were bred to have minimal impact on the dairy and priced according to buyer acceptance at the sale barn only. Thus, the initial breeding focused on achieving a black-hided calf from the dairy cow *with ease* with little emphasis placed on sire quality and the sire's potential to influence growth performance and carcass characteristics. Therefore, sires were chosen nearly at random as long as the semen was cheap and the bull was black. The preliminary variability in the calves these mating decisions generated and the regional specificity of the market space to larger dairies briefly limited the adoption of these crossbred calves in feedlots and packing plants, resulting in poor close-outs for cattle feeders that bought high and sold low.

To refocus the beef matings occurring on dairies, it was critical to address one of the dairies major concerns: Would mating dairy cows to beef sires impact relevant dairy outcomes? While the calf is a necessary outcome for milk production, it is well established that difficult deliveries can reduce productivity in subsequent lactations and, potentially, cow longevity. One of our goals at Penn State was to assess the impact of beef sires on dairy cow productivity and health postparturition. Over 75,000 records from cows across 10 dairy herds from the Northeast and Midwest were summarized (Basiel et al., 2024a).

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Results (presented during the Balchem pre-symposium) suggest that beef on dairy matings alter gestation length. In multiparous cows that carried beef-sired calves, gestation length was increased when compared to cows that carried Holstein-sired calves. Fow example, cows that carried Limousin-sired and Wagyu-sired calves carried them for 5 and 8 days longer, respectively, than those that carried Holstein-sired calves. These data suggest that dairy farms using Limousin and Wagyu semen may want to consider extending the dry-off dates of cows mated to Limousin and Waygu sires to allow for an additional week of milk production from those cows.

Additional analyses of the records suggest that the incidence of dystocia, postpartum health events and early culling decisions were not impacted by sire breed. In addition, dairy cows mated to beef sires suffered no production losses in the lactation following delivery of a beef on dairy calf. The lack of impact on dairy productivity and the increased economic return over the dairy bull calf make beef-on-dairy matings a sound investment for the dairy industry.

With the dairy challenge satisfied, programs began to emphasize the need for additional consideration on sire selection. For example, the Simmental and Holstein associations have partnered on the HOLSim™ program in 2019 to identify SimAngus sires “with genetic traits that will work for dairy producers” (Bechtel, 2019). Keep in mind, these initial 2019 programs were still focused on broad parameters (i.e. homozygous black, homozygous polled, minimum birth weight accuracy, and minimum threshold for the HOLSim Index), rather than outcomes that return profit to the cattle feeders (i.e. feed efficiency and pounds to sell). The HOLSim index now states that it includes traits of interest for both dairies (i.e. calving ease) and feeders (i.e. marbling and muscling; https://www.holsteinusa.com/holsim/).

This paper keeps returning to markets and profits because economics drive commodity markets. Therefore, some discussion of the current economics driving beef on dairy are warranted. The current market prices for day-old beef on dairy calves vary between 600 and 1,000 dollars for an approximately 45 kg calf (USDA AMS, 2024). In addition, these beef on dairy calves at 250 kg outpriced native (or full blood) beef calves at 250 kg in the spring of 2024 (USDA AMS, 2024). So, regardless of when these calves enter the beef supply chain officially, as day-olds or as feeder cattle at 250 kg, their value exceeds that of conventional, or native, beef animals. Most recent reports agree that the greatest economic advantage to mating beef semen to dairy cows still exists for the dairy farm. The economic stability (viability?) throughout the rest of the supply chain (beef farm, feeders, packers, consumers) has been less certain (Berry, 2021).

To improve cattle feeder and packer acceptance, terminal traits that yield value had to be highlighted. Again, the preliminary mating programs emphasized selection for the dairies. To this end, in 2019, Penn State wrote and was awarded a USDA CARE grant *[grant no. 2020-68008-31411]* to evaluate feedlot growth performance and carcass characteristics of beef on Holstein steers (Basiel et al., 2024b). Progeny were sired by 7 different beef breeds, including Angus, Charolais, Limousin, Hereford, Red Angus, Simmental, and Wagyu. Sires within breeds were chosen specifically based on their

rankings as terminal sires (sires that would add growth and good amounts of muscle to dairy genetics) at the time of mating, when such rankings were available. Thus, beef sires were not solely selected for hide color or the price of the semen, but intentionally selected as sires that would make BEEF. Progeny were born across 3 years to ensure that they could be housed and reared under nearly identical protocols under university oversight, eliminating the potential confounding effects of environment and nutrition.

When summarized across all 3 years of data, there were no differences in feed efficiency, measured as gain to feed, among cattle, regardless of sire breed. However, Angus, Charolais, and Simmental-sired beef on Holstein steers had the greatest average daily gain and, thus, spent the fewest days on feed. Wagyu on Holstein steers spent more days in the feedlot than any other breed.

Across all breeds in the Penn State study, there were no differences in longissimus muscle area measured after a 48 hour chill. Recently, other researchers (Foraker et al., 2022; Fuerniss et al., 2023a) have suggested that differences in muscularity between cattle breeds are best assessed distal to the longissimus dorsi; thus, our collection of standard rib-eye areas from the longissimus dorsi may have suppressed our ability to detect differences in muscularity. Due to the nominal differences in hot carcass and final body weights, by design, beef on dairy cattle had average dressing percentages of just over 61% but were largely not different. Most beef breeds will have an average dressing percentage of 63 to 64% whereas purebred Holsteins have always been challenged in this area and struggle to reach 60%; thus, these data suggest beef on dairy cattle have a greater propensity to muscle, or yield, for the packer than purebred dairy counterparts.

In addition, carcasses from Angus, Charolais, Hereford, Red Angus, and Wagyu-sired steers marbled similarly, and all averaged a USDA Choice quality grade. Although carcasses from Limousin and Simmental-sired steers did not marble *as well* as carcasses from the aforementioned breeds, the majority of those carcasses from Limousin and Simmental-sired steers still graded USDA choice on average.

Since Penn State drafted our initial grant proposal, the beef on dairy industry has gone through rapid evolution. For example, Fuerniss et al. (2023a) concluded that genetic differences were more impactful that differences due to environment and management, but went on to suggest that perhaps the current rearing practices, those that mirror dairy systems, in the early life of beef on dairy limit the phenotypic expression of their beef genetics (Fuerniss et al., 2023b). At Penn State, our study placed heavy emphasis on rearing cattle in similar environments, but those environments did largely mirror dairy-like systems.

More recent data have supported the suspicions of Fuerniss et al. (2023b) and suggest that manipulating those early life environments could improve the outcomes for beef production. Carter et al. (2025) reported that increasing the fat and protein concentrations in milk replacers can increase cross-sectional muscle fiber area and growth of beef on dairy calves in early life. Purina has been heavily vested in this area

and also suggests that our current production practice, those similar to dairy heifer rearing systems, limit the growth potential of beef on dairy calves.

There are additional concerns that beef on dairy systems still need to address. Reports have surfaced, and been widely spread across media platforms, regarding challenges in beef on dairy systems with liver abscesses. Liver abscesses are a major economic loss to the beef cattle industry, representing up to 60 million dollars of lost revenue annually (Herrick et al., 2021). While beef breed cattle have an average of about 25% of carcasses afflicted with liver abscesses, some have suggested that the dairy influence can result in up to 39% (Lawrence, 2022) or even as much as 50% of the cattle slaughtered with having liver abscesses (Grimes, 2022). The finding relative to the etiology of liver abscesses in beef on dairy remain unclear at this time.

Conclusions

The conclusions of these research studies together suggest that we have at least some additional discoveries to reveal before we optimize beef on dairy systems in the United States. The initial sire-selection emphasis on dairy traits of interest, those that would protect milk production, likely limited the first and second generations of beef on dairy progeny in beef production systems. However, the overwhelming research responses have helped drive our markets forward in a positive direction. The industry is rapidly evolving, and the pace of discovery must keep up with the commercial outcome to ensure the success of the beef pipeline from beef on dairy progeny.

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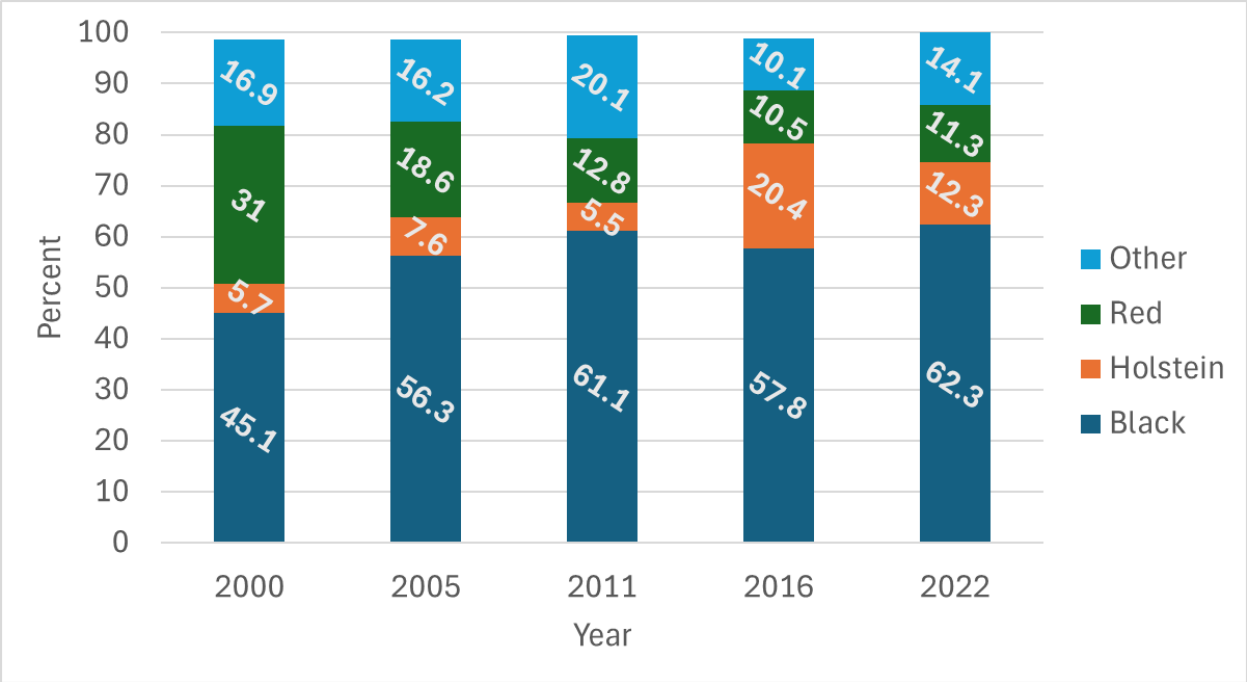

Figure 1: Adapted from the 2022 NBQA Executive Summaries.

Session Notes