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Holistic Overview Forage Quality: From Forage Production, to Ration Formulation, to Cow Performance

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Introduction

Forages are fundamental components of dairy production systems, providing a major source of energy through the microbial fermentation of structural carbohydrates in the rumen. In addition, forages supply physically effective fiber, which is essential for maintaining rumen health and promoting milk fat synthesis (Mertens, 1997). Beyond their nutritional value, forages can reduce feeding costs while constituting a substantial proportion of the dairy ration.

At the Dairy Forage Research Laboratory at Virginia Tech, our research program has focused on applied studies examining forage quality, forage management, fiber digestion, and their implications for dairy cattle nutrition and performance. The objective of this manuscript is to provide an overview of the research conducted at Virginia Tech related to forage quality, management practices, and fiber digestion. By synthesizing findings from these studies, we aim to provide dairy producers, nutritionists, and industry professionals with practical information to support informed decision-making and enhance the efficiency and sustainability of dairy production systems.

Fiber Digestibility Within and Across Species

On more than one occasion, dairy producers and nutritionists have proposed growing sorghum forages carrying the brown midrib (BMR) genotype instead of corn for silage, with the goal of utilizing forage sources that are less expensive than corn. In this context, it has been suggested that the reduction in starch concentration in sorghum could be compensated for by an increased energy contribution resulting from greater digestibility of the NDF fraction. A clear response to this proposition can be derived from applying the Lucas test of nutrient availability. According to the Lucas principle (NASEM, 2021), the digestibility of starch is assumed to be complete and uniform, whereas the digestibility of NDF is incomplete and variable. This concept implies that the energy contribution of NDF would never exceed that of starch unless the physical form of the feed limits starch utilization, as may occur in the presence of intact grain kernels (Ferreira and Mertens, 2005). Therefore, it is unlikely that the reduction in starch concentration in sorghum would be compensated for by a greater energy contribution arising from increased digestibility of the NDF fraction.

In addition, it is important to highlight that differences in NDF digestibility exist both within and across forage species (Ferreira et al., 2022). In a replicated plot study, Ferreira et al. (2022) measured the ruminal NDF digestibility of BMR and non-BMR varieties of

corn, sorghum, and pearl millet and observed that conventional corn varieties exhibited an NDF digestibility similar to those of BMR sorghum varieties and greater to those of BMR pearl millet varieties (Figure 1). These results indicate that the digestibility advantage associated with the BMR trait is relative to the forage species in which it occurs and should not be extrapolated across species. From a dairy nutrition standpoint, this means that replacing corn silage with BMR sorghum or BMR pearl millet should not be expected to increase fiber digestibility or energy supply simply because these forages carry the BMR mutation.

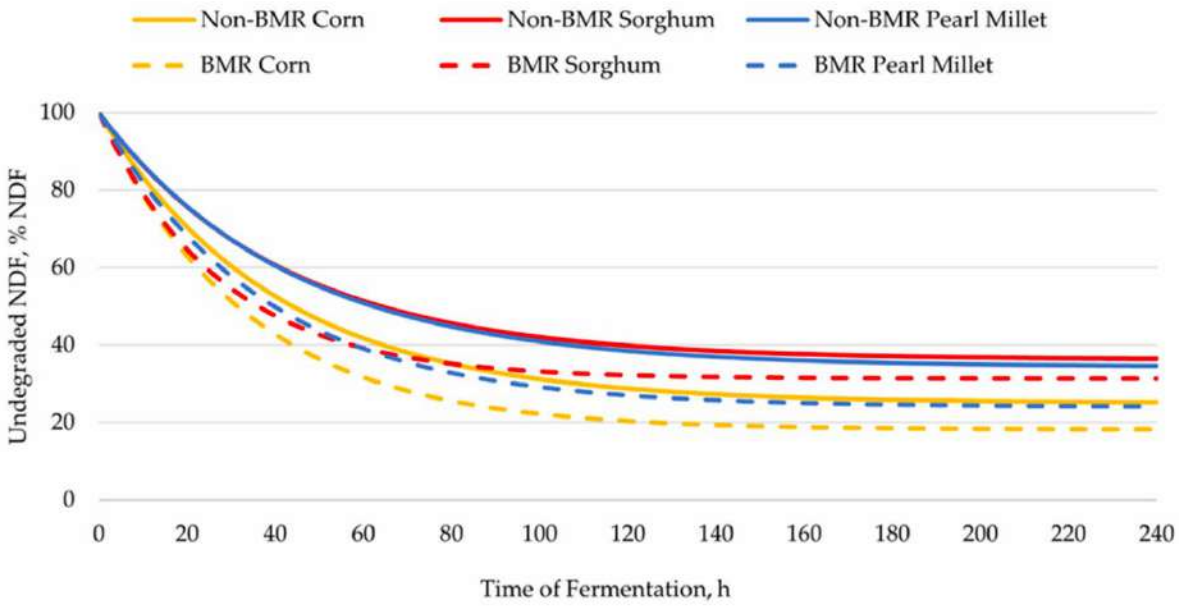


Figure 1. Degradation kinetics of whole plants of warm-season annual grasses of different forage types (corn, sorghum, or pearl millet) and genotypes (brown midrib (BMR) or non-BMR). Each curve represents the residual or undegraded neutral detergent fiber (uNDF) of each forage type by genotype combination that remained after its ruminal fermentation. Figure extracted from Ferreira et al. (2022).

Drought Stress and Forage Quality

The most obvious and direct effect of drought stress is the reduction in the yield of grain and forage crops (Lauer, 2016; Ferreira and Brown, 2016). However, at the plant tissue level, information regarding the impact of water stress on NDF digestibility remains limited and, in many cases, contradictory. There is a belief among producers, nutrition consultants, and extension personnel that drought stress improves NDF digestibility (Mahana and Thomas, 2011; Hutchens, 2017; Ferreira, 2021). Nevertheless, this belief needs more rigorous scientific evidence for at least two reasons.

Numerous classical studies evaluating the effects of drought stress on DM digestibility (Van Soest et al., 1978; Wilson, 1983; Dias Filho et al., 1991) did not specify whether the observed effects were attributable to changes in the concentration of NDF in the whole plant, changes in NDF digestibility, or a combination of both. Also, it is generally accepted that, in addition to being indigestible, lignin limits the digestibility of other cell wall components, including cellulose and hemicellulose. In this context, an agronomic report (Lauer, 2016) indicated that drought stress tends to increase fiber concentration while improving fiber digestibility because of reduced lignin production in corn. This statement implies that lignin synthesis is highly sensitive to water stress, although it is not clear whether increased lignification reflects a greater proportion of lignified tissues in the whole plant (e.g., changes in the stem-to-leaf ratio) or a greater lignin concentration within the same tissue resulting from an increased rate of lignification (Ferreira and Brown, 2016). This distinction is critical for accurately evaluating the effects of water stress on fiber digestion.

From a nutritional perspective, the effects of drought stress on fiber utilization by ruminants are still uncertain, and studies specifically evaluating the impact of water stress on corn cell wall composition or fiber utilization are scarce (Vincent et al., 2005; Ferreira et al., 2021; Ferreira et al., 2023a). This lack of information is due, in part, to the methodological challenges associated with implementing and reproducing drought conditions in controlled experiments (Farooq et al., 2009; Ferreira et al., 2021; Ferreira et al., 2023a).

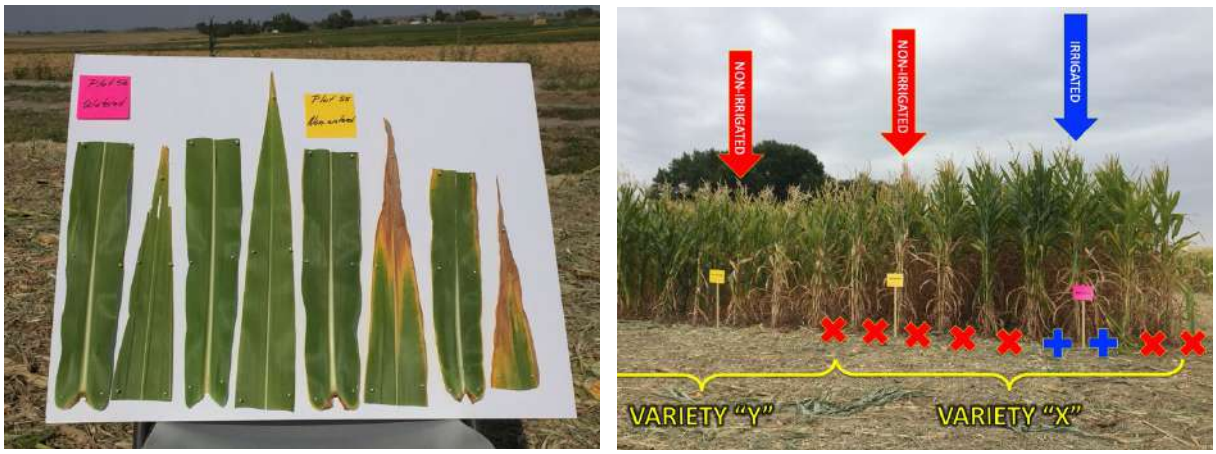


Figure 2. Effect of drought stress on the composition of corn for silage (Ferreira et al., 2021). Figure 2A depicts how water stress induced senescence in leaf blades. Figure 2B depicts the effect of water status on plant height and morphology.

Ferreira et al. (2021) induced water stress by restricting irrigation in corn plots during a growing season in Idaho. The greater whole-plant DM concentration and the evident leaf senescence (Figure 2A) suggested that plant drought stress was successfully accomplished (Figure 2B). This senescence may explain the greater NDF concentration

observed in leaf blades from non-irrigated corn compared with irrigated corn (62.8 and 61.3%, respectively). In addition, stem internodes also exhibited greater NDF concentrations in non-irrigated corn (62.5 vs. 57.2%), and this difference was even more pronounced than that observed in leaf blades. This observation may be related to cells with smaller volumes and greater surface area-to-volume ratios resulting from reduced turgor pressure.

The increase in NDF concentration in vegetative tissues under drought stress deserves special attention when discussing digestibility. Partially based on the study by Dias Filho et al. (1991), Van Soest (1994) stated that water restriction increases digestibility, without distinguishing between DM and NDF digestibility, whereas irrigation tends to decrease digestibility. Interestingly, Dias Filho et al. (1991) also reported greater crude protein concentrations under water stress, a finding that appears biologically counterintuitive when tissue senescence is considered (Figure 2). It is worth mentioning, however, that the referenced study (Dias Filho et al., 1991) separated and discarded dead tissues before sample analysis. As tissue senescence is likely to increase under drought conditions (Figure 2), the study of Dias Filho et al. (1991) has little merit to arrive to the conclusion that restrictions of water increase digestibility.

Contrary to the common belief that water stress improves NDF digestibility, Ferreira et al. (2021) showed that drought stress reduced NDF digestibility in stem internodes (42.2 vs. 39.1% NDFD), although no effect was observed in leaf blades (53.6% NDFD). Furthermore, although it has been suggested that drought stress reduces lignin concentration, Ferreira et al. (2021) found that water stress slightly increased lignin concentration in leaves (14.1 vs. 16.1%) and had no effect on stem internodes (26.6%). Molecular studies support these findings, showing that water stress can increase the activity of phenylalanine ammonia-lyase, a key enzyme in the lignin biosynthesis pathway (Le Gall et al., 2015). In addition, increases in cell wall-bound phenolic compounds have been reported under water stress conditions (Hura et al., 2012), which could further reduce cell wall digestibility. Therefore, statements indicating either greater or lower lignification under water stress are inherently ambiguous, as they may refer either to a greater proportion of lignified tissues within the whole plant or to a greater lignin concentration within individual tissues. Distinguishing between whole-plant and tissue-level responses is essential when evaluating forage quality under drought stress conditions.

In the same study, Ferreira et al. (2021) evaluated the composition and NDF digestibility of corn tissues among hybrids and within the plant, comparing lower and upper phytomers. They observed that in vitro NDF digestibility varied more among hybrids than because of drought stress, and no significant interaction was detected between hybrid and irrigation treatment. These findings suggest that the impact of water stress on fiber digestibility may be smaller than commonly assumed and that additional research is needed to better understand the interactions between fiber composition and fiber digestibility under water-stress conditions.

After considering the currently available evidence, it is reasonable to ask what facts support the widespread belief among farmers that drought stress improves forage quality and, consequently, enhances milk production. Several plausible hypotheses may help explain the origin and persistence of this perception. First, drought years often lead producers to reduce the forage-to-concentrate ratio of dairy diets to stretch limited corn silage inventories. Consequently, any increase in milk production observed under these circumstances may be confounded with the greater inclusion of concentrates rather than with an improvement in the nutritive value of drought-stressed silage itself. A second hypothesis relates to what could be described as a *panicking effect* at harvest. During drought conditions, particularly when accompanied by high temperatures and very low relative humidity, corn leaves often become dry, brittle, and brown (Figure 3), prompting some producers to harvest earlier than originally planned out of concern for declining forage quality. However, an earlier harvest inevitably results in a crop harvested at a less advanced phenological stage, with fewer accumulated growing degree days.



Figure 3. Drought-stressed corn fields showing folding and senescent leaf blades.

Ferreira et al. (2023b) highlighted maturity at harvest as one of the major determinants of NDF digestibility. In the study, corn harvested at the kernel milking (R5) stage exhibited substantially lower in vitro NDF digestibility than corn harvested at tasseling (VT), with reductions observed in upper leaf blades (67.9 vs. 78.0% NDFD), upper leaf sheaths (62.6 vs. 76.6% NDFD), and lower stem internodes (36.8 vs. 56.3% NDFD). These declines were generally accompanied by increases in cell wall lignin concentration, indicating that continued lignification during plant maturation contributes to reduced NDF degradation. Importantly, the effects of maturity on NDFD were greater and more consistent than those associated with agronomic environment (i.e., planting population and N fertilization), demonstrating that harvest timing exerts a stronger influence on fiber quality than moderate environmental stress. Therefore, when evaluating differences in NDF digestibility among corn silages, maturity at harvest should be considered a primary factor because later-harvested crops tend to contain more lignified and less digestible cell walls than earlier-harvested crops. In conclusion, because NDF digestibility generally declines with plant maturity, any improvement in NDF digestibility observed in drought-affected silages may simply reflect earlier harvest timing rather than a direct physiological effect of drought stress.

Maturity at Harvest and Forage Quality

Questions from farmers regarding the optimal harvest timing for small-grain silages have frequently motivated research examining the relationships among forage maturity, yield, and nutritive value. In this line, Ferreira et al. (2025) evaluated the optimal harvest timing of small-grain cereal forages, including barley, rye, and triticale, intended for silage production in dairy systems. Field experiments were conducted in Blacksburg, Blackstone, and Orange, Virginia, during the 2020 and 2021 growing seasons. Eight cultivars were evaluated, including two barley, two rye, and four triticale varieties. Plots were harvested either at the boot (BT) stage, when the developing grain head remains enclosed within the leaf sheath, or at the soft dough (SFT) stage, when kernels are visible and contain soft starch. For each species and cultivar, biomass yield, nutrient composition, and NDF digestibility were determined. In addition, silage production costs and ration costs were estimated under multiple commodity price scenarios. Harvesting at the SFT stage increased dry matter yield by approximately two- to threefold compared with BT harvest, resulting in a lower silage cost per unit of DM. However, SFT forage exhibited lower concentrations of crude protein and energy, greater concentrations of NDF, and reduced NDF digestibility. In contrast, BT forage was characterized by superior nutritive value but substantially lower biomass yield. Ration formulation simulations indicated that when commodity prices were low, neither small-grain cereal silage was selected in least-cost rations, which instead relied primarily on corn silage, likely due to its more favorable nutrient profile. Under scenarios characterized by elevated commodity prices or limited corn silage availability, small-grain cereal silages were incorporated into rations; however, no consistent preference was observed between BT and SFT forages. These findings suggest that harvest stage has only a limited effect on ration formulation outcomes. Nevertheless, delaying harvest from BT to SFT postponed the establishment of the subsequent crop by 24 to 44 days, potentially reducing overall cropping system productivity. Collectively, the results demonstrate a tradeoff between forage yield and nutritive value. Harvesting at SFT stage maximizes DM production and modestly reduces silage cost but decreases forage quality and delays planting of the next crop. Conversely, harvesting at the BT stage improves forage nutritive value while reducing yield. Therefore, the optimal harvest strategy will depend on individual farm objectives, feed inventory requirements, cropping system considerations, and prevailing market conditions.

Based on the results of the plot study, harvest timing at the BT stage or SFT stage appeared to have limited implications for ration formulation. Consequently, our subsequent objective was to determine whether dairy cows would respond differently when fed silages produced from these two harvest maturities. To address this question, a feeding trial was conducted to evaluate the effects of triticale silage harvested at different maturities on milk production, nutrient digestibility, and milk fatty acid composition in high-producing dairy cows (Schultz et al., 2025). Triticale was cultivated on a 25-acre field, with approximately half of the area harvested at the BT stage and the remaining half harvested at the SFT stage. Harvests occurred 26 days apart. Following ensiling, the silages were incorporated into diets formulated to contain either a high-forage concentration (HF; 55% forage) or a low-forage concentration (LF; 38% forage). Contrary to expectations, the two silages exhibited remarkably similar nutrient compositions,

differing only slightly in CP and NDF concentrations. Nevertheless, BT silage was characterized by a faster rate of digestion of pdNDF (2.30 vs. 1.43%/h) and lower concentrations of uNDF (22.9 vs. 39.2% uNDF; NDF basis) than SFT silage. Cows fed BT silage produced slightly more milk (95.7 vs. 93.3 lb/d) and greater yields of milk protein and lactose compared with cows fed SFT silage; however, energy-corrected milk yield did not differ between treatments. Total-tract nutrient digestibility was largely unaffected by silage harvest stage, and milk fatty acid profiles were generally similar between treatments. Only minor differences were observed in selected fatty acids associated with ruminal lipid metabolism. The unexpectedly small differences in silage composition, likely attributable to spatial variability in soil characteristics between field sections (Schultz et al., 2025), limited the extent to which the original hypothesis could be rigorously tested. Overall, the results indicate that, under the conditions of the study, triticale harvest stage exerted minimal effects on dairy cow performance, nutrient utilization, and milk composition. These findings are consistent with the ration modeling results and suggest that management factors such as forage yield, silage inventory, and the timing of subsequent crop establishment may be more influential than harvest maturity when evaluating the practical advantages of harvesting triticale at BT versus SFT.

The results the previous feeding study (Schultz et al., 2025) were limited by the unexpectedly small differences in nutrient composition between the BT and SFT silages. Consequently, the study was unable to provide definitive guidance regarding the optimal harvest stage of triticale for dairy producers. To overcome this limitation, a second feeding trial was conducted in which harvesting and silage production procedures were modified to ensure substantially greater differences in forage quality between the two maturity stages (Galyon et al., 2026a). The objective of the study was to further investigate the effects of triticale harvest maturity on milk production, nutrient digestibility, and enteric methane emissions in lactating dairy cows. Silages harvested at the BT stage and SFT stage were incorporated into diets containing either a high forage concentration (HF; 52% forage) or a low forage concentration (LF; 37% forage). As intended, marked compositional differences were obtained between the two silages. The BT silage exhibited substantially greater nutritive value, containing nearly twice the CP concentration (16.7 vs. 8.7%), lower NDF concentrations (51.1 vs. 62.6%), faster pdNDF degradation rates (2.33 vs. 1.53 %/h), and lower uNDF concentrations (13.4 vs. 35.8% uNDF, NDF basis) than the SFT silage. These differences in forage quality translated into meaningful production responses. Cows fed BT silage produced more milk (103.2 vs. 95.5 lb/d) and greater milk protein yield than cows fed SFT silage. Likewise, cows receiving LF diets produced more milk than those fed HF diets. Despite these differences in production, feed efficiency and energy-corrected milk yield did not differ among treatments. In contrast, BT silage improved the apparent total-tract digestibility of DM (69.3 vs. 67.3%) and NDF (59.4 vs. 54.5%), indicating enhanced nutrient utilization relative to SFT silage. A particularly noteworthy finding was the effect of harvest maturity on enteric methane production. Contrary to expectations, cows consuming BT silage emitted less methane despite exhibiting greater DM intake and higher NDF digestibility. Daily methane emissions were approximately 6% lower for cows fed BT silage, and methane yield was reduced by approximately 11% on a DM intake basis compared with cows fed SFT silage. Low-forage diets also decreased methane emissions; however, these reductions were accompanied

by lower nutrient digestibility. Overall, the results demonstrate that harvesting triticale at the BT stage can simultaneously improve animal performance and reduce enteric methane emissions relative to harvesting at the SFT stage. These findings suggest that earlier harvest may provide dairy producers with a practical management strategy to enhance milk production while contributing to environmental sustainability, potentially reducing reliance on costly methane-mitigating feed additives. Nevertheless, the biological mechanisms underlying the reduction in methane emissions remain uncertain.

Fiber Passage and Degradation Kinetics

Dietary uNDF has been proposed as an indicator of the filling effect of the diet and, consequently, as a potential factor limiting DM intake. Fustini et al. (2017) fed alfalfa-based diets differing in uNDF concentration (10.9 vs. 9.5% of DM) to dairy cows, achieved by varying the inclusion of soybean hulls, a feed ingredient characterized by a low uNDF concentration. Contrary to their expectations, increasing dietary uNDF did not affect DMI or NDF intake. Furthermore, although greater dietary uNDF would be expected to increase ruminal retention time and thereby enhance total-tract NDF digestibility, diets with lower uNDF concentrations resulted in greater NDF digestibility (63.0 vs. 57.5%). This improvement in digestibility was likely attributable to the inclusion of soybean hulls rather than to differences in ruminal retention time. Therefore, the findings of this study do not provide conclusive evidence regarding the role of dietary uNDF in regulating digesta retention time or passage rate.

Kammes and Allen (2012) fed diets containing either alfalfa silage or orchardgrass silage as the only forage source to lactating dairy cows. The alfalfa silage contained 42.3% NDF of which 54.5% was uNDF, whereas the orchardgrass silage contained 58.2% NDF of which 27.7% was uNDF. While the alfalfa diet included ~60% alfalfa silage (DM basis) and the grass diet included ~43% orchardgrass silage, both diets contained 25% forage NDF. Intuitively, one might expect the orchardgrass diet to be a better diet based on the lower uNDF of the silage. However, neither milk yield, DM intake, or NDF intake differed between diets. These results highlight that there is more to learn about fiber metabolism beyond uNDF concentration.

In a study from our laboratory (Galyon et al., 2024), we observed that cows consuming diets containing alfalfa hay had a faster ruminal passage rate (5.02 vs. 4.03 %/h) and a shorter mean retention time of uNDF than cows consuming diets containing orchardgrass hay, and this occurred despite the greater concentrations of dietary uNDF in the alfalfa-based diet (9.8 vs. 8.3% uNDF, DM basis). In addition, cows consuming alfalfa-based diets had a greater total tract NDF digestibility than cows consuming grass-based diets (40.4 vs. 35.9% NDFD). In agreement with Fustini et al. (2017), dietary uNDF did not seem to drive ruminal retention time and rate of passage.

In a recent study from our laboratory (Galyon et al., 2026b), cows fed triticale silage harvested at the BT stage of maturity consumed similar amounts of DM as cows fed triticale silage harvested at the SFT stage of maturity, despite the substantially greater concentration of uNDF in the SFT diet (12.1 vs. 7.2% of DM). Interestingly, the ruminal

passage rate of uNDF was similar between diets (4.15 and 3.91%/h for BT and SFT, respectively), whereas NDF digestibility was markedly greater for the BT diet (51.8 vs. 43.7%). These results indicate that rumen fill cannot be explained solely by the accumulation of uNDF. Rather, the extent of digestion of pdNDF fraction appears to be an important determinant of rumen fill and feed intake. Greater pdNDF digestibility in cows fed the BT diet likely increased the rate of NDF disappearance from the rumen, thereby alleviating physical constraints to intake despite the absence of differences in uNDF passage rate. Collectively, these findings suggest that ruminal digestion and passage kinetics influence NDF utilization and feed intake in ways that extend beyond dietary uNDF concentration or traditional measures of forage quality.

Take-home Summary

Forage quality is a complex concept that cannot be adequately defined by any single parameter. Continued efforts to characterize the intrinsic composition and digestibility of forages across species, maturities, and environmental growing conditions remain essential for understanding differences in feeding value. However, the effects of forage quality on whole-rumen metabolism may be less straightforward than traditionally assumed. Fiber utilization in the rumen reflects a dynamic balance between the digestion and passage of both the pdNDF and uNDF fractions. Consequently, forage responses cannot be fully explained by isolated measures of NDF concentration, digestibility, or uNDF concentration.

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Strategies to Improve Nutrient Digestibility and Optimize Microbial Protein Synthesis

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Introduction

The rumen has sometimes been described as a “superorganism”. “The rumen and its microbes” (the title of Robert Hungate’s 1966 seminal book) are so critical to all ruminants, especially the high-producing dairy cow. One of the dairy cow’s “superpowers” is rumination, as in Rick Grant’s recent article in Hoard’s. During my Master’s program in the 1980’s, I became interested in both subjects, and I’m still amazed that so much of the cow’s rumen is geared toward optimizing fiber degradability (measured as NDF degradability, NDFD). Again, the challenge is especially for the high-producing cow because increasing feed intake increases ruminal outflow, which would increasingly push out potentially degradable NDF (pdNDF), whereas fortunately forage genetics also have improved pdNDFD. Increased intake of degraded NDF has simultaneously diluted the cow’s maintenance energy while also diluting the proportion of energy used by microbes in parallel, which was the theme of my doctoral thesis. Both optimizing NDFD and passage of microbes increase supply of ruminal microbial protein (MicP) production to the small intestine. However, despite their importance, optimizing NDFD and MicP outflow are still hard to predict reliably because of so many challenges we face in measurement and in differences among cows.

Many of our main research and feeding management gains have been made as a result of fixing problems. One of the first problems was bloat, which stimulated the first Rumen Function Conference, which made rapid progress and moved primarily into gut microbiology. Today, metagenomics gut (and rumen) microbes are highlighted at the biennial Congress on Gastrointestinal Function, often alluding to but not measuring ruminal NDFD and MicP. Negative associative effects of starch suppressing NDFD had large “discounts” of digestible energy for NRC publications through 2001. Now, we have improved our usage of fibrous byproducts to dilute starch and lessened this discount in NASEM (2021). However, I will emphasize that these byproducts also are susceptible to negative associative effects.

Many of you are sieving manure, TMR, and feed refusals. You are measuring starch and its in vitro disappearance plus undegraded NDF (uNDF). Despite the importance of these empirical practices related to my topics, I will focus on what you often cannot see. We can measure starch in feces, but why aren’t we evaluating NDF relative to starch? For example, besides stimulating copious ensalivation to buffer ruminal pH, the process of rumination opens up considerable substrate that should be the cow’s bread and butter. Although uNDF is valuable, we are not evaluating the plant’s infrastructure and its relationship to a far more diverse microbial community than Hungate could have

imagined in 1966. I think we have focused considerably on the microbes we can easily see: the protozoa. They clearly have pros and cons I will discuss. We are considering the archaea produce methane that we are increasingly measuring; but they are doing so on the shoulders of fiber degrading bacteria and fungi adherent to plant particles and not just the protozoa. In my presentation, I will describe processes unfolding about the microbial interactions between the fibrolytic specialists and the generalists that can work together but sometimes do not (and why not?).

We have learned new things about the rumen through feeding practices. For example, optimizing stearic and oleic acid for optimal fat digestibility and milkfat production probably has a secondary role in improving NDFD. Through conditions that optimize the usage of isoacids, we also can improve NDFD in general. We tend to focus on degraded carbohydrate to support the amount of MicP, but we also should be studying how the amount is dictated by the efficiency of microbial protein synthesis (EMPS; i.e., MicP divided by energy available to support microbial growth). Some of these processes are generally well modeled and adapted to computer software. However, some processes, particularly the negative associative effects of increasing starch on NDFD and EMPS still are not as well modeled. My goal will be to provide you with my overall view of mechanisms behind changes and to emphasize when to look for conditions in which NDFD and MicP supply likely are not being optimized. Some of this paper will cover aspects that are available in more detail from the proceedings of the 2025 Florida Ruminant Nutrition Symposium ANSAssistant@ifas.ufl.edu and Tri-State Dairy Nutrition Conference https://www.tristatedairy.org/_files/ugd/36a444_5d9fe4e77e6543869cda55f117eca0b5.pdf.

A Brief Overview of What We Have Learned About Rumen Microbiology

Compared with when I started in this business, we have learned the most about diversity of bacteria and, secondarily, archaea. Luckily, key cellulolytics could be isolated and characterized even in Hungate's book. Some are critical to rumen function, and some probably have been overemphasized. The first genomes put on display for us all through high-throughput sequencing projects like the Hungate 1000 (Seshadri et al., 2018), which is rapidly being updated. Their vast ability of microbes to produce interconnecting enzymes has been demonstrated. To encode those enzymes, key cellulolytic specialists left out of their genomes the ability to independently provide their own nitrogen and other building blocks needed for their own growth. Their ability to adhere to plant fiber took on new roles, including for "cellulose-binding domains" for ruminococci and "fibroslime complexes" for *Fibrobacter* (Moraïs and Mizrahi, 2019). Apparently because of limited space, early sequencing projects documented how some archaea (methanogens) invested in genes to allow them to flip flop between encoding genes for adhesins to adhere to plant matter or even other microbes versus critical (Leahy et al., 2013). Often, this ability to adhere to plants or to protozoa has been suggested teleologically to allow them to grow fast enough to be in high density in the rumen, whereas a more likely reason for their adhesion is that close proximity allows direct cell-to-cell transfer of H_2 because otherwise H_2 is poorly soluble and more likely to escape to the ruminal headspace and

be emitted (Mackie et al., 2024). Although recycling of microbes is wasteful, it apparently provides microbes with key growth factors such as coenzyme M (for methane).

Protozoa have long been cast as unwelcome pests (even by me) because of intraruminal recycling of MicP and emphasis on H_2 production to stimulate methanogenesis. In contrast, their predation of bacteria to provide for their own nucleic acids and much of their amino acids is probably less in the lactating cow because of her increased feed intake and faster ruminal passage rate compared with the conditions for most defaunation (protozoa eliminated) models (Firkins et al., 2020). Dihydrogen production is most associated with the isotrichid populations (Dai et al., 2022), probably because they distribute hydrogenobodies more fully across their fully ciliated landscape (Xie et al., 2026), but all too often all are being targeted.

Although small entodinia are most predatory against bacteria, the isotrichids most likely are the most detrimental to MicP supply to the cow. These large, fully ciliated cells store considerable quantities of glycogen from which fermentation (after glycogen breakdown) is about 25% less efficient in net ATP generation than direct fermentation of glucose. These isotrichids need all of this glycogen to support their migratory lifestyle between the ventral rumen and reticulum only to return to the ruminal mat to await the next ingested feed, particularly from grazed forages (Firkins et al., 2020). Because of this partial sequestration in the rumen, they provide less MicP supply for the cow relative to their consumed carbohydrate (small starch granules and sugars except lactose). In contrast, the majority population of entodiniomorphids can take in small amounts of sugars but are far more adept at engulfing starch granules and fiber particles. Particularly the larger ones are being actively researched because of their rather outsized role in degradation of fiber (Firkins et al., 2025), and some of them do indeed metabolize sugars (Oelker et al., 2009), although those authors noted the relative lack of understanding of sugars for these larger fibrolytic protozoa. This group does not sequester but likely swims around within the milieu of degrading particles and passes in that phase (Firkins et al., 2020), thus taking themselves and their ingested bacteria with them. Among other positives are their consumption of fatty acids attracted to the waxy layer of plant particles, thus reducing the biohydrogenation pool and passing essential fatty acids to the intestine.

In a group effort, Firkins et al. (2025) explained the role of microbial communities to “share” resources to best decompose the complex fibers that are consumed by dairy cattle. Some cellulolytic gram-positive bacteria such as *Ruminococcus albus* and especially *R. flavefaciens* have unique cellulose-binding domains as part of their intricate cellulosomal complexes used to break down cellulose and hemicellulose. *Fibrobacter succinogenes* has a gram-negative cell wall that allows it to release satellites that help form pits on fibers and therefore allows the cell to embed into that particle. Both of these genera have a complete orchestra of individual enzyme instruments that allow them to decompose even crystalline cellulose and yet require at least one branched-chain volatile fatty acid (BCVFA) and phenylalanine derivatives (ruminococci) just to adhere to that cellulose. As stated previously, they also lack the enzymatic capacity to produce these growth factors but rely on sharing partially decomposed hemicellulose to the consortium.

In contrast with these relatively well described (because they are culturable) cellulolytic specialists, current research is documenting more diverse cellulases spread across other less well characterized (uncultured) groups such as those only being able to annotated to families Lachnospiraceae and Prevotellaceae or even to the order Bacteroidales. That is, they are not yet classified into genera and species, yet their fingerprints are revealing highly abundant cells and associated enzymes that have major roles in fiber degradation. The unfolding story is that some appear to work well as a team, and some perhaps have a genomic lifestyle that lends itself to shifting gene expression to fend for itself as the ingested particle matures through its process of breakdown and passage from the rumen (Firkins et al., 2025).

Many sequencing efforts have characterized roles of particular groups of microbes associated with high or low feed efficiency of beef and dairy cattle. These roles have been described many times by various authors and yet with some differences among studies. In my own assessment (Firkins, 2021), I think we are relying too much on canned procedures that basically are doing multiple correlations. The correlations with individual taxa are not consistent across studies perhaps because they are singled out as individual correlations rather than being assessed in groups using more complicated network analyses. Thus, a lower diversity of microbes that work together is probably optimum. Another obstacle to cumulative progress is that short amplicon sequencing techniques have greatly spread the utility of these techniques for researchers, but these high-throughput approaches cannot discriminate subtle differences among closely related strains or species that can have important differences in function. These issues are being sorted out with ever-advancing sequencing technology and will no doubt be used in future studies. One lingering concern I have is to make sure that NDFD is more consistently evaluated along with feed efficiency or methanogenesis projects (Firkins et al., 2025).

Improving Digestibility

Digestibility of feed is critical to providing energy and the MicP resulting from that degradation. In addition, higher NDFD aids the high-producing dairy cow to achieve the feed intake that her mammary gland is pulling to support production of milk components. To support milk fat production, we can rely on supplemental dietary fats but also on de novo synthesis, which largely comes from acetate and therefore from optimized NDFD. Some researchers are describing butyrate as part of the de novo equation, but butyrate can only serve as the primer, not the elongator (which is from acetate). Even with this caveat, many fiber degraders also are relying on butyrate to yield their energy. Because butyrate fuels the gut and signals the host in other ways, both butyrate and acetate produced from NDFD are useful.

Digestibility should be decomposed into fatty acids (small intestine) and fiber (primarily in the rumen), whereas site of starch digestibility would ideally be suited both the rumen for microbes and the small intestine, where it is energetically more efficient than the rumen. However, most processing techniques that improve total tract (i.e., overall) digestibility also tend to increase ruminal digestibility. I am in favor of maximizing starch digestibility of grain and the kernels in corn silage in the rumen because I think it

lessens the variability associated with total tract starch digestibility. Then, if the variability is decreased, we can more reliably provide the moderate amount of rumen-degraded starch to lessen negative effects on NDFD and still help fuel the animal and lessens fill restriction of feed intake (Zebeli et al., 2012). A more moderate amount of starch likely provides more optimal conditions for networks of microbes to optimize NDFD during the time that forage and nonforage fibers are in the rumen of the high-producing cow (Firkins, 2021; Firkins et al., 2025). I have repeatedly emphasized that part of the negative depression of NDFD resulting from high starch is a result of decreased ruminal pH, and part of the response is independent (but covarying) of low pH. This so-called "carbohydrate effect" might be just as important as low pH.

If we start with the needs for adequate chewing by the cow to buffer the rumen, the "OG" of ADSA symposia describing physically effective NDF (peNDF) was presented at ADSA in Cornell in 1995 and published in a series of symposia papers in Journal of Dairy Science in 1997. Although the peNDF concept springing from Dave Mertens' paper has been highlighted (rightfully so) for decades and has vast importance, both forage and nonforage fiber are negatively impacted by high dietary starch. The lignification of forages decreases NDFD, but lignin is not involved in the negative associative effect because both forage and nonforage NDFD are suppressed. Moreover, the negative associative effect of NDFD is worsened with higher feed intake. The studies of Mike Allen at Michigan State routinely emphasized that cows with more "pull" for higher intake are the ones most affected by both physical and chemical factors that suppress feed intake (Allen et al., 2019). Long forage is critical to proper mat formation to keep the smaller fibrous byproducts from passing from the rumen too fast (Grant, 1997) and lessen NDFD from small byproducts compared with their pdNDF (Firkins, 1997). Many studies have shown that byproduct NDF substitution for forage NDF has improved NDFD, but that shouldn't be the endpoint for evaluation, which should be the NDF digestibility of pdNDF (Erickson et al., 2024). Please note that there still is risk in that an improvement in NDFD is being measured but not being compared against its pdNDF—and what is formulated by your computer. Byproducts vary, but those like soyhulls and corn gluten feed can be approaching 90% or higher pdNDF. Because of their small particle size and high density, they are prone to passage from the rumen and therefore passage into manure without a firm enough rumen mat to retain them. I will not spend more time on this concept in this paper because managerial practices have lessened this problem over the years. However, you should be aware that this problem still pops up.

Despite our lessening of the starch discount against NDFD over the past decades of research, I think the major hidden loss of potential NDFD still remains a problem with high inclusion of starch in dairy diets. Adding high starch might be improving milk production, but is it improving it as much as it could? Especially with current drifts toward milk components, are we optimizing NDFD and the milk fat it supports or the MicP supporting milk protein? I have focused on the negative effects of starch on NDFD in my review (Firkins et al., 2025) and at 2025 Florida and Tri-State conferences that are readily available to you. Therein, I explained more fully why I think a more optimal starch concentration has potential to lessen negative associative effects of starch on NDFD,

particularly when attention is paid to provision of adequate resources such as rumen-degraded protein (RDP) and BCVFA.

I have reviewed isoacids (Firkins et al., 2024) and spoke at CNC on the subject in 2023, so I do not want to dwell here. However, I think the study by one of the speakers of this conference profoundly emphasizes their role for NDFD (Redoy et al., 2025). Cows fed their high forage diet had the largest improvement in NDFD from isoacids. Even this diet was not that high in forage NDF, so one must question if the lesser (but still significant 4.9% unit) improvement in NDFD from isoacids in their low forage diet did not reach its potential because of faster passage of nonforage fiber, as discussed above. I will be emphasizing the point that we should be optimizing NDFD based on pdNDF.

As cited above in feed conferences and in a review (Firkins et al., 2025), I have emphasized the role of moderate sugar to optimize NDFD. We still do not really know the full reason for such improvements, but sugars probably provide a variety of benefits. They sometimes improve butyrate formation to fuel the rumen epithelium and appear to be stimulating a base of microbes that are consuming lactate to prevent its buildup. Sugars could be influencing feeding behavior, although this area is not as well characterized. I think some sugars could be stimulating some members of highly fibrolytic communities, although future network analyses are needed to support this theory. Clearly more work is needed to explain why sugars sometimes do not stimulate NDFD to more reliably formulate diets to optimize conditions in which they do increase NDFD.

Also as cited above, I have emphasized the role of palmitic (16:0) and oleic (18:1) acids to be taken up by key cellulolytic bacteria and their partners. These bacteria that are forming cellulose and hemicellulose degradation factories need to produce all of these proteins and then translocate them through the membrane. Our work has demonstrated the critical nature of the BCVFA, particularly 2-methylbutyrate, in diglyceride and plasmalogen fractions of the bacterial cell wall to provide adequate membrane integrity (firm enough but not too firm). Along with the BCVFA precursors, cells can take up 16:0 and 18:1 into their membranes. The 16:0 provides firmness, and the 18:1 and branched lipids provide flexibility. A mix of these fatty acids is needed for membranes that anchor proteins and maintain ion gradients yet are flexible enough for cellular functions and growth. Probably based on centuries of selection, bacteria seem to require the branched lipids emanating from BCVFA even when in the presence of unsaturated fat for direct incorporation. An increasing body of evidence suggests that palmitic acid products, which also contain a small amount of 18:1 that optimize fat digestibility and milk fat production also optimize the benefit to rumen bacteria. That is, the same feeding practices that stimulate transfer of fatty acids into milk fat are also stimulating acetate production for de novo synthesis.

Despite the benefits of sugars and certain fats on NDFD, their supplementation cannot fix underlying problems. They help to dilute starch but both probably are less helpful if starch is too high. Properly balanced forage and nonforage NDF with adequate effectiveness and with moderate starch and RDP are likely working with sugars and certain fats to improve NDFD and the MicP arising from that degradation. Moreover,

excessive amounts of PUFA likely will depress feed intake before depressing NDFD, yet such negative responses will not be removed just by adding sugars. With the increasing role of high-oleic soybeans, if properly heated and rolled, they can provide moderate amounts of RDP and RUP and still have a slow enough release of the oil to limit negative effects. There is some evidence that 18:1 without 16:0 will sometimes improve NDFD, but I think 16:0 still is the more important fatty acid. One 16:0 directly transferred displaces about 7 precursors that can now be used for acetate production.

An important unfolding point about RDP is that its individual components are not equal. Clearly, we must have a base of RDP that provides adequate ruminal ammonia. Research has variable estimations for optimal ruminal ammonia. This point can be confusing because an empirically derived “optimum” when assessed as the peak of a quadratic equation is really a “maximum”. Perhaps the actual optimum with respect to economics of protein sources relative to milk protein prices might be a sliding scale. Therefore, breaking down RDP into its components as done in the Cornell model does provide a potential benefit both in research and modeling. That said, when assessing feeds, assuming complete peptidolysis all the way to ammonia is not a foregone conclusion because ruminal bacteria change gene expression in response to intracellular ammonia and amino acids (Firkins and Mackie, 2020). Ruminal ammonia varies across the feeding cycle, and there are large unexplained differences in ruminal ammonia concentration among studies (Firkins et al., 2007). Moreover, a key point also made in that review is that adding peptides could stimulate EMPS enough to increase ammonia incorporation into MicP. Biologically, there might not be an optimum ammonia concentration independent of an optimum peptide concentration. Perhaps the large range in estimated quadratic optima (generally between 5 and 10 mg/dL) for peak NDFD and MicP is because we just measure ammonia far more than peptides, and peptides are challenging to measure, anyway.

Optimizing microbial protein

Microbial protein has long been known to be the main protein source of the cow. As I have reviewed (Firkins, 2021; Firkins et al., 2025) and discussed in nutrition proceedings at Gainesville and Ft. Wayne, I have repeatedly emphasized the need for adequate RDP to support MicP. In fact, such a repeated emphasis has been made both historically (Schwab and Broderick, 2017). As stated previously, we must always prevent an insufficient ruminal ammonia concentration, but peptides work along with ammonia. In accord with NASEM (2021)’s equation, RDP intake has been linearly (not quadratically) described in other meta-analyses (Ipharraguerre and Clark, 2014; Hanigan et al., 2021). Most of us agree that RDP should have some asymptotic role, so a key question remains why is linearity of RDP so stubborn? Moreover, is this linearity a result of our inability to dissociate RDP into its components (peptides and ammonia). When RDP in source diets in the derivation datasets was increased, the proportion of RDP derived from protein flowing through peptides relative to non-protein N (not just from urea but also from legume silages) was also likely increasing.

Before attempting to answer this question of how to optimize MicP, let's go back to the basics (Walker et al., 2005). Microbes grow primarily from carbohydrates. An amino acid might provide 0.5 to 1 ATP through fermentation, whereas a sugar mostly provides around 4 ATP. Many authors using pure cultures have documented that proteolytic bacteria prefer to use amino acids to stimulate protein synthesis rather than use amino acids to produce ATP during fermentation; they only ferment those amino acids after carbohydrates were exhausted. There are some low abundance bacteria that ferment amino acids, but typically they are not very proteolytic and so are at the end of the food chain. Thus, providing moderate RDP needed to support MicP might be less efficient because of these AA fermenters, but the cost of this RDP supplied even in a moderate safety factor still can be cheaper than limiting RDP's promotion of MicP and therefore relying on more expensive RUP sources to make up the shortfall.

Starch and fiber provide about the same energy to microbes on a degraded basis, but of course starch is much more degradable in the rumen than is forage fiber. Pectins are perhaps as degradable (or more) than starch, but they are not recovered in NDF and so are not well described in papers. Thus, the NASEM (2021) MicP prediction equation does not include this residual organic matter from soluble fiber and, instead, averages the coefficients for these degradable carbohydrates over their average composition in source diets because the models are fitted against MicP measurements. Even though NDFD is typically less than rumen-degraded starch in the rumen, though, the undegraded forage fiber is thought to be the vehicle that helps to move microbial protein out of the rumen (Sauvant and Nozière, 2016) and therefore both supply the cow but also minimize microbial recycling. This is a key concept. The same forces (e.g., fragility) that promote passage rate of particles (including with pdNDF that has not been degraded) also increase passage of those resident adherent microbes and thereby dilute out their maintenance energy relative to energy used for growth.

The role for passage of MicP with increasing feed intake and therefore increasing undegraded fiber is not actually a new concept (I evaluated it in my thesis) and is highly likely because about ¾ of the bacteria are thought to be associated with the rumen particulate fraction; because the fluid passes faster than the particles, the proportion of particulate-phase bacteria was assumed as 60% of the total bacteria (NASEM, 2021). Moreover, except for the isotrichids, most of the entodiniomorphid protozoa are likely not adherent but still chemotactically stimulated to pass with pdNDF (Firkins et al., 2020). My point of reference is that pdNDF lies within the physical structure of the plant. A more active fibrolytic consortium should help in the breakdown process but also exposes those cells to newly ingested particles. The particle passes partially intact in physical nature, so let's optimize NDFD and more adherent microbes with the faster passing feed particles in the high-producing cow!

The primary literature done with duodenally cannulated beef and dairy cattle in the 1980's through 2000's has supported a higher EMPS with higher forage in the diet, and chemostat studies with pure cultures mostly done by Jim Russell prior to or at Cornell suggested the same effect. Recently in a novel and elegant study, Lingga et al. (2026) suggested that glucose versus cellulose resulted in similar EMPS to counter this long-

held notion. I think this emphasizes that bacteria are bacteria and have been highly selected to thrive in the same rumen. However, glucose can be used by many bacteria and not just amylolytics. Moreover, cellulolysis likely requires cellulosomes and extracellular complexes, thus increasing maintenance energy. In contrast, the glucose treatment did not enforce similar structures that would be needed for starch (at least resistant starch). For example, *Ruminococcus bromii* is a noted starch degrader that relies on an extracellular amylosome to help it degrade starch (Mukhopadhya et al., 2018).

I have often noted in presentations that shifting cellulolytics to breaking down cellulose compared with glucose involves thickening in bacterial membranes, emphasizing BCVFA based on shifts in substrate (Burnet et al., 2015). Presumably, such extracellular machinery would increase the maintenance energy of amylolytic bacteria had those mixed cultures been presented with starch rather than glucose. Even with the above caveat, I think the biggest aspect that likely decreases EMPS is that increasing starch increases competitiveness and lack of sharing of resources in a mixed community with both starch and fiber as substrates. For example, the gram-positive and amylolytic *R. bromii* also requires BCVFA and sometimes appears to compete with the cellulolytic *R. flavefaciens* for BCVFA, with local concentrations of BCVFA directing their uptake (Mitchell et al., 2023). Moreover, many gram-negative starch users that increase in abundance in higher starch diets have complicated approaches for funneling starch oligosaccharides into their periplasm to use or preserve for themselves (Firkins et al., 2025). Thus, I think increasing starch does decrease the EMPS such that less MicP would be supplied to the dairy cow compared with the potential expected from a more completely degraded starch versus fiber. That is, increasing starch probably does increase the relative maintenance energy expenditures for mixed ruminal microbes.

With this background, the NASEM (2021) MicP equation has dry matter intake as the major driver, including being very sensitive to RDP intake. Although RDP intake includes dry matter intake as a key part of its derivation and has been described as a confounding effect (Hristov et al., 2026), the NASEM equation was derived in a Bayesian hierarchical approach that limited confounding. In fact, predicted NDFD and rumen-degraded starch intakes were embedded in a common equation using a substitution method that also limited confounding. After the initial MicP derivation prediction was based on rumen-degraded starch and NDFD, then RDP intake was hierarchically predicted using a within-study Bayesian approach. During fitting, a statistical assessment was consistently applied to verify minimal collinearity (i.e., common factors in equations that cannot be distinguished).

In contrast, if the model is oversensitive to RDP, the unbalanced nature of studies is a more likely explanation. Few researchers (perhaps a handful) intentionally varied RDP while keeping other factors constant within those studies such that the major range in RDP was a result of among-study variation. Thus, the effect of RDP can be hard to separate from the random effect of study, including in our own approach to minimize this issue. In contrast with hardly any studies intentionally varying only RDP, varying crude protein (CP) has often been done within individual studies. From these studies, the large

difference among studies was noted in a meta-analysis on crude protein (Zanton, 2016). They noted that shorter term crossover studies to lower protein could be buffered by mobilized skeletal muscle, which was reinforced in a subsequent study with cows in late lactation (Erickson et al., 2024). Some important points can be gleaned from that study. Cows were in late lactation, when lower protein should be more possible than for cows in earlier lactation. Diets had a high contribution of alfalfa silage; although mean ruminal ammonia and BCVFA concentrations declined linearly with decreasing CP, both concentrations might have been high enough to prevent a drastically decreased NDFD. As typical for many other studies, soybean meal was substituted for soybean hulls to lower dietary protein from 16.2 to 11.9%. When they evaluated the digestibility of pdNDF (accounting for increased potential NDFD of soyhulls), the digestibility of pdNDF declined linearly with decreasing protein. Unfortunately, this measurement is rarely reported.

The form of the NASEM (2021) equation was chosen based on prior attempts to attenuate the RDP response—that is, to find a plateau response. Studies conducted prior to the 2001 NRC almost never measured or even reported RDP when MicP was measured. Even when it was reported, it was with an older system. Therefore, during the building of the NASEM (2021) protein model, RDP was estimated in feeds within each study using an equation that forced RUP (i.e., 100% - RDP % of CP) to have unbiased estimation compared against measured nonammonia-nonmicrobial N (NANMN) flows (Hanigan et al., 2021). Those authors reported an empirical MicP prediction equation that also did not have a significant squared term for RDP. Although allowing a peak response, squared RDP also can suffer a limitation that a quadratic model wants to be umbrella-shaped. Therefore, quadratic models inherently want to fit RDP percentage to reach a maximum above which increasing RDP would then depress MicP production. That is, such a quadratic maximum might be hard to fit for plateauing responses. In the prior approach using Michaelis Menten integrated form (White et al., 2017), the RDP intake could be modeled to be asymptotic (i.e., plateauing) with respect to RDP percentage, which we considered as an advantage. In the final form (NASEM, 2021), though, the response to RDP intake is quite linear within ranges of RDP percentages typically fed, so the response was truncated at 12% RDP. Although this truncation is clearly stated in the NASEM report and is used in the model, some researchers have evaluated the model's prediction equation against actual data without truncation at 12% RDP. I will address this question of RDP's role more in an upcoming review article as our current research gets completed.

Finally, I want to clarify that the NASEM approach had errors in measuring MicP and RDP that were independent of each other. In contrast, using this NANMN flow derived for treatment means within a study (Martineau et al., 2023) directly forces potential correlation of measurement error. For example, if a treatment mean in a study has an overpredicted MicP flow because of microbial marker issues, then the NANMN will automatically be underestimated; this response then will overestimate RDP when using the NANMN as an "independent" assessment of RDP. As a mirror image, underestimated MicP will coincide with underestimated RDP, and vice versa. Predicting MicP based on NANMN-derived RDP will therefore potentially bias the response in simulation studies.

Another key issue is that supplementation of NASEM data with new data will almost certainly increase the representation using omasal flows, which will increase the prediction of MicP and appear to show bias of the model derived from studies with fewer omasal flow studies. However, we must remember that if these studies were added to NASEM's database to rederive an equation, they are comparing apples to oranges. Anyone using or challenging NASEM (2021)'s metabolizable protein model needs to be aware that the derivation data are fixed in time and, even if wrong, affected the prediction of both MicP and RUP (therefore RDP) the same in a system in which any errors from these two predictions tend to cancel because metabolizable protein supply was evaluated against actual milk protein production from those studies.

Because of animal usage issues, future studies will almost exclusively be done with omasal sampling (through the rumen cannula). Only a couple of studies evaluated omasal sampling coinciding with duodenally cannulated cows. As documented in one individual study (Ipharraguerre et al., 2007) and in the meta-data almost exclusively with only one technique per study (Roman-Garcia et al., 2016), omasal sampling almost always increases the measurement of MicP. The microbial marker seems the likely reason for this discrepancy. Although some authors have criticized using a single flow marker with duodenally cannulated cow (as has typically been done), I think the original support was not nearly as strong as the dogma suggests (Firkins et al., 1998). Omasal sampling requires multiple flow markers, but that does not mean that cows with properly placed duodenal cannulas did. The best we have is that, when compared flows from studies with invasive blood cannulations, duodenal cannulations were suggested as more appropriate than omasal sampling for metabolizable amino acid supply (Martineau et al., 2025).

Conclusions

I have tried to provide some fundamental basis for nutrition advisors to understand limitations we still face in research and therefore when model predictions might not always be realized. I have emphasized that palmitic acid blends of fatty acids are in fact unlikely to depress NDFD and more likely to improve it. High unsaturated fat is more likely to suppress feed intake than NDFD. These trends to improve NDFD probably relate to MicP, although MicP has not been measured much in those studies. Sugars are likely improving NDFD, at least on average; whether they improve MicP has not been addressed satisfactorily for me to make a clear projection. Isoacids should increase NDF if provided in appropriate diets with moderate RDP and in conditions in which NDFD appears less than expected and yet can be improved under those conditions. There are some indications for increased MicP with isoacids. However, adequate forage NDF both for maintaining ruminal function (adequate pH) and a consistent rumen mat is required along with adequate RDP.

Microbial protein production will always be a prediction for most of you, not something directly measured. That said, we can base projections for improvements in MicP coinciding with improved NDFD and a likelihood that overfeeding starch will decrease NDFD to be less than its potential (remember byproduct NDF is more degradable than forage NDF) but also likely decrease the EMPS. In the same cases that

higher starch is suggestive of poor NDFD based on sieving approaches, a decreased EMPS also should be expected. Prediction of MicP still needs refinement and yet with fewer studies measuring it in vivo. Duodenal versus omasal flow results in different MicP absolute values (i.e., omasal > duodenal), which makes it a challenge for prediction in the first place and then with challenging a model with new data mostly derived from omasal flow studies. There are some studies comparing models, but I think some of the approaches have lingering issues that need qualification. Therefore, I will describe in the presentation some nuances and considerations as you continue trying to optimize the nutrition and actualized MicP for tomorrow's dairy cow.

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Using Branched-Chain Volatile Fatty Acids to Enhance Nutrient Digestibility, Rumen Performance, and Sustainability

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Introduction

Branched-chain volatile fatty acids (BCVFAs), commonly referred to as isoacids (ISO), are ruminal fermentation products derived primarily from branched-chain amino acids. The major BCVFAs include isobutyrate, 2-methylbutyrate, and isovalerate. Although present at relatively low concentrations in the rumen, these compounds can function as growth factors and carbon skeletons, particularly for rumen cellulolytic bacteria. Consequently, BCVFA supply may influence fiber degradation, microbial protein synthesis, and nutrient utilization, resulting in improved animal performance. A recent review concluded that responses to ISO supplementation depend strongly on dose, dietary composition, ruminal nitrogen availability, and the microbial consortium present in the rumen (Firkins et al., 2024). Our recent work in lactating dairy cows provides a useful example of this diet-dependent response (Redoy et al., 2025a,b). Our recent in vitro work further extends this concept by testing ISO in combination with anti-methanogenic feed additives (AMFA), with the goal of achieving methane (CH_4) mitigation while maintaining or improving digestibility. Together, these findings support viewing BCVFAs as nutritional strategies that can complement diet formulation and CH_4-mitigation strategies.

BCVFAs and Rumen Microbial Function

BCVFAs are produced during microbial degradation of branched-chain amino acids and can be incorporated into microbial cell components or converted into longer-chain branched fatty acids. They are particularly important for cellulolytic microorganisms, which have limited capacity to synthesize branched-chain carbon skeletons de novo. Supplementation can therefore stimulate growth of fiber-degrading bacteria when other nutrients, especially ruminally available nitrogen, are adequate (Stewart et al., 1997; Vlaeminck et al., 2006; Firkins et al., 2024). The magnitude of response is related to the balance among individual ISO and the microbial community. Isobutyrate and 2-methylbutyrate appear more likely to be limiting than isovalerate under typical dairy diets. Continuous-culture studies have shown that BCVFA supplementation can alter microbial fermentation and nutrient flows, supporting the concept that ISO affect the rumen microbial ecosystem rather than acting only as direct energy substrates (Mitchell et al., 2023a,b,c). Thus, adequate BCVFA supply may improve the efficiency with which dietary carbohydrates and nitrogen are converted into microbial biomass and fermentation products.

Effects on Productivity and Nutrient Utilization

Redoy et al. (2025a) studied 64 mid-lactation Holstein cows in a 10-wk randomized complete-block-design 2 × 2 factorial experiment. Cows received either a high-forage fiber diet (approximately 21% forage NDF) or a low-forage fiber diet (approximately 17% forage NDF), with or without isobutyrate (7.85 mmol/kg DM) plus 2-methylbutyrate (3.44 mmol/kg DM; Table 1). The high-forage diet contained 65% forage on a DM basis, whereas the low-forage diet contained 52% forage. In the high-forage diet, BCVFA supplementation increased milk yield by approximately 7%, accompanied by marked improvements in apparent nutrient digestibility (Table 2). In the low-forage diet, milk yield did not change, but average daily gain increased by approximately 0.40 kg/d and milk urea nitrogen declined by approximately 9-10% (Table 2). These results suggest that BCVFAs can improve nutrient capture and nitrogen utilization without producing the same production response across diets.

Table 1. Chemical composition of experimental diets used by Redoy et al. (2025a,b).

Nutrient	Diets	
	High-forage fiber	Low-forage fiber
DM, %	49.3	55.5
Forage DM, %	65.1	51.5
ADF, %	18.1	19.7
NDF, %	26.4	28.0
Forage NDF, %	20.7	16.6
Non-forage NDF, %	5.70	11.4
Starch, %	32.4	30.7
CP, %	15.8	16.2
RDP, %	10.3	10.5
RUP, %	5.53	5.67
Ether extract, %	4.34	4.37
NE_l, Mcal/kg DM	1.58	1.58

Effects on Fiber and Nutrient Digestibility

The clearest response to BCVFA supplementation occurred in nutrient digestibility, particularly when forage fiber supply was high. In high-forage diets, dry matter, organic matter, neutral detergent fiber (NDF), and crude protein (CP) digestibility increased from 68 to 75%, 69 to 76%, 52 to 64%, and 69 to 77%, respectively. In low-forage diets, digestibility did not differ between ISO supplemented and non-supplemented group (Redoy et al., 2025a; Table 2). The stronger response in the high-forage diet is consistent with the proposed role of BCVFAs in supporting cellulolytic bacteria. When more fermentable fiber is available, additional microbial growth may translate into greater fiber degradation and nutrient recovery. Firkins et al. (2024) similarly summarized evidence that ISO supplementation can increase fiber digestibility by several percentage units under appropriate ruminal conditions. Therefore, diet formulation should consider both BCVFA supply and the substrate available to the microbial population.

Table 2. Effects of isoacids supplementation under high- and low-forage diets (adapted from Redoy et al., 2025a,b)

Variable	High forage fiber		Low forage fiber		SEM	P-values		
	WIA	IA	WIA	IA		FL	ISO	FL × ISO
DMI, kg/d	25.7	26.2	27.5	28.1	0.81	<0.01	0.13	0.87
ADG, kg/d	0.64^{c}	0.69^{c}	0.93^{b}	1.33^{a}	0.08	0.03	0.09	<0.01
Yield, kg/d								
MY	34.7^{b}	37.2^{a}	37.5^{a}	38.4^{a}	1.68	<0.01	<0.01	0.04
FPCM	35.6^{b}	38.2^{a}	39.1^{a}	38.4^{a}	1.14	<0.01	0.06	<0.01
Enteric methane (CH_4)								
CH_4 production, g/d	340^{b}	375^{a}	400^{a}	364^{b}	21.1	0.03	0.99	<0.01
CH_4 yield, g/kg DMI	14.0^{a}	15.5^{a}	14.8^{a}	13.1^{b}	0.94	0.11	0.77	<0.01
CH_4 intensity, g/kg FPCM	9.4^{a}	10.1^{a}	10.4^{a}	9.3^{b}	0.73	0.99	0.71	<0.01
Digestibility, %								
DM	67.7^{b}	74.8^{a}	73.3^{a}	74.6^{a}	0.92	<0.01	<0.01	<0.01
OM	69.1^{b}	75.7^{a}	74.2^{a}	75.4^{a}	0.87	<0.01	<0.01	<0.01
NDF	51.9^{b}	64.4^{a}	63.4^{a}	68.3^{a}	2.51	<0.01	<0.01	0.07
CP	68.6^{b}	76.5^{a}	72.2^{a}	74.0^{a}	1.46	0.73	<0.01	0.06

Notes: ADG = average daily gain; CP = crude protein; DM = dry matter; DMI = dry matter intake; FPCM = fat- and protein-corrected milk; FL = forage fiber level; IA = with isoacid supplementation (7.85 mmol/kg DM isobutyrate + 3.44 mmol/kg DM 2-methylbutyrate); ISO = isoacid supplementation; MY = milk yield; NDF = neutral detergent fiber; OM = organic matter; WIA = without isoacid supplementation.
a–c Within a row, mean values with different superscripts differ significantly ($P < 0.05$).

Effects on Enteric Methane (CH_4) Emissions

The CH_4 response to BCVFAs was strongly dependent on dietary forage level as well (Redoy et al., 2025b; Table 2). In high-forage diets, daily CH_4 production increased from 340 to 375 g/d, whereas in low-forage diets it decreased from 400 to 364 g/d. Note that total NDF was almost similar between high and low-forage diet (26.4 vs 28.0%). CH_4 yield did not change in the high-forage diet but was reduced from 14.8 to 13.1 g/kg DMI in the low-forage diet. Similarly, CH_4 intensity was unchanged in the high-forage diet but declined by approximately 18% in the low-forage diet (11.5 vs. 9.4 g/kg milk). These contrasting responses illustrate why CH_4 should be evaluated together with production and nutrient utilization. In the high-forage treatment, improved fiber digestibility increased daily CH_4 production; however, CH_4 intensity did not differ, most likely due to the associated increase in milk production. In the low-forage treatment, the lower CH_4 intensity was most likely associated with greater propionate production, which may have favored alternative hydrogen sinks under the lower-forage diet and contributed to improved growth performance in the ISO supplemented group.

Isoacids as a Complement to Anti-Methanogenic Feed Additives (AMFA)

Our recent in vitro studies (Redoy et al., unpublished) directly evaluated whether ISO can complement AMFA to achieve simultaneous CH_4 mitigation and co-benefits, including improved nutrient digestibility and animal performance. In a pairwise experiment using a mid-lactating Holstein diet (high-forage fiber diet as shown in Table 1), red seaweed such as *Asparagopsis taxiformis* (AT), nitrate (NO3), tannin (TAN), essential oils (EO), and ISO were tested individually and in pairs using a 3 × 3 factorial dose combinations. Combining AT with NO3, EO, TAN, or ISO reduced CH_4 by 53-68% relative to the control, but did not further reduce CH_4 beyond AT alone. Importantly, combinations with AT increased DM and NDF digestibility by 4-9% relative to AT alone, with AT + ISO providing a particularly relevant example of adding a potential digestibility co-benefit to strong CH_4 suppression. The EO + ISO combination was especially notable because it simultaneously reduced CH_4 and increased both DM and NDF digestibility compared with the control and EO alone. In a subsequent three-additive study, AT-containing triples reduced CH_4 yield by 52-74% relative to control, while the AT + NO3 + ISO combination increased DM digestibility from 52.5 to 54%. Non-AT triples reduced CH_4 yield by 40-53% and, except for NO3 + EO + TAN, generally increased DM digestibility. Collectively, these findings indicate that ISO may be most valuable not as a stand-alone CH_4 inhibitor, but as a complementary additive that helps maintain or improve fermentation and digestibility while another AMFA additive provides the primary methanogenesis-suppressing effect. A mechanistic rationale is that strong methanogenesis inhibition can alter ruminal hydrogen balance. Because methanogenesis is a major hydrogen sink, substantial CH_4 suppression may increase hydrogen pressure and constrain hydrogen-producing, particularly fibrolytic fermentation pathways (Ungerfeld, 2020). Alternative electron sinks such as nitrate or shifts toward propionate can relieve some of this pressure, whereas ISO may provide carbon skeletons needed by cellulolytic microbes. Thus, combining an AMFA with ISO could address both thermodynamic and kinetic constraints. The in vitro results also show that interactions are not uniformly beneficial: nitrate and tannin combinations tended to reduce CH_4 but could decrease NDF digestibility. This reinforces the need to evaluate additive combinations rather than assuming that greater numbers of additives will produce additive benefits.

Conclusions and Future Direction

BCVFAs can influence rumen microbial growth, fiber degradation, nutrient digestibility, animal performance, and enteric CH_4 emissions. Evidence from recent dairy cow studies demonstrates that these effects are strongly diet dependent. New in vitro evidence further suggests that ISO can complement selected AMFA, particularly when the objective is to reduce CH_4 while maintaining or improving digestibility. The practical value of BCVFAs therefore lies in their potential use as precision nutritional tools within integrated rumen-management strategies. Future work should emphasize interaction-driven, multi-objective optimization to test, identify and validate supplementation strategies that simultaneously improve nutrient utilization and animal performance while reducing undesirable outputs such as enteric CH_4 emissions.

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What's Under the Hood? Gastrointestinal Health in Dairy Cattle

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Introduction

The phrases "gut health" and "rumen health" are ubiquitous when walking onto dairy farms or working with dairy nutritionists. Maintaining healthy gastrointestinal tracts in high-producing cattle is a huge concern. Entire nutrition programs are planned or drafted with the intention of maintaining gut health. We decide how much forage is fed in the diet, how often cows are fed each day, how consistent the feeding time is each day, and target consistently mixed rations that are uniform across the bunk with rumen and gut health in mind. Each is an important consideration, and the best-run dairy farms execute precise and consistent feeding programs. But, we actually know surprisingly little about gut health, how to define it, or what truly regulates the gut at the tissue level. Progress on understanding and improving gut health is occurring, which we will review, but gaps in our knowledge and our ability to suggest management practices supported by concrete data still exist.

Defining gut health is part of the challenge. Traditionally, rumen and reticular pH have been the primary factors defining gut health. That is for good reason; reducing ruminal pH reduces NDF digestibility and alters the rumen microflora by increasing *E. coli*, specifically more pathogenic strains of *E. coli* (Khafipour et al., 2011; Krajcarski-Hunt et al., 2002). Additionally, reductions in rumen pH are often associated with reductions in milk fat concentration (Plaizier et al., 2008). But, reducing rumen pH does not cause, nor is it always associated with, reduced milk fat production. In some cases, milk fat yield is maintained or increased as rumen pH declines (Krogstad et al., 2025; Li et al., 2020). Thus, rumen pH alone may not be an adequate description of gut and rumen health. Rumen VFA are another indicator of subacute ruminal acidosis and rumen health, propionate and valerate being highly sensitive markers (Golder et al., 2023; Golder and Lean, 2024).

Another target that has been investigated for impacts on rumen health is lipopolysaccharide (LPS). Cows subject to acute grain acidosis challenges have dramatic increases in ruminal LPS concentrations (Khafipour et al., 2009b). The use of LPS as a marker for rumen health is intriguing as it is able to bind toll-like receptor 4, which has been detected at the mRNA level in rumen tissue and increases with ruminal acidosis (Pederzolli et al., 2018), which may cause an inflammatory cascade. In some cases, inflammation has occurred following an acute grain-based acidosis that also resulted in increased ruminal LPS, plasma LPS, and acute phase protein concentrations (Gozho et al., 2006; Khafipour et al., 2009b). Interestingly, rumen LPS also dramatically increased under an alfalfa-pellet acidosis challenge without increases in plasma LPS or

inflammatory markers, which, again, demonstrates that LPS alone is insufficient as a determinant of gut health (Khafipour et al., 2009a).

As rumen health research progressed, measuring acute phase proteins and inflammatory markers in plasma has been utilized as another marker of gut health challenges, usually by measuring haptoglobin (Hp) and LPS binding protein (LBP). In some cases, Hp has been demonstrated as a discriminating factor in severe ruminal acidosis (Khafipour et al., 2009c). Multiple experiments, usually grain challenges meant to cause a ruminal disturbance, have observed that acute phase proteins may increase in plasma following the feeding challenge (Gozho et al., 2005; Gozho et al., 2006; Khafipour et al., 2009b). A meta-analysis suggested that once dietary forage concentration is reduced below 56% or NDF is <39.2% of diet DM, inflammatory markers begin to increase (Zebeli et al., 2012). This could be hugely problematic as most high-producing, lactating cows are fed diets that may be considered 'at risk' of inflammatory challenges (de Souza et al., 2018). We conducted a literature review to further evaluate the role of diet on inflammatory biomarkers and focused on dietary starch concentration. We discerned that in regular feeding, or non-challenge scenarios, increasing dietary starch concentration, usually by reducing dietary NDF, did not consistently increase inflammatory biomarkers in plasma (Krogstad and Bradford, 2023). Furthermore, we conducted a mild rumen acidosis challenge in late-lactation dairy cows and observed a reduction in ruminal pH without any change in systemic inflammatory markers (Krogstad et al., 2025). The gap in the literature is how the tissue is being regulated or responding during these scenarios. We postulate that measuring tissue responses directly, and understanding their regulation would be a more sensitive indicator of changes within the rumen. Our ultimate goal is to create a sensitive and specific cow-side measure of gut or rumen health that can be utilized on dairy farms to improve nutrition and management of dairy cattle.

The rumen tissue—a resilient and highly regulatory structure

The rumen is a complex architecture that is composed of 4 distinct layers: the stratum corneum, granulosum, spinosum, and basale, which is adjacent to the basal lamina (Steele et al., 2016). The corneum is composed of dead keratinocytes that interact with the rumen contents and serve as a protective barrier for the living layers of tissue that lie beneath it. The corneum is also populated by a distinct microbial population, termed the epimural microbiome (Steele et al., 2011; Wetzels et al., 2017). The granulosum contains tight junctions and desmosomes that create an additional layer of protection for the more metabolic spinosum and basale. The spinosum and basale are populated with mitochondria and are the more metabolic layers within the rumen tissue (Baldwin et al., 2004; Graham and Simmons, 2005).

A healthy rumen structure exhibits each of the clearly defined layers and an organized and tightly adhered stratum corneum. Diet can substantially alter the organization of these layers. In a severe acidosis challenge, where cows went from a high-forage diet with 7% starch to a high-grain diet with 40% starch coming from wheat, barley, and corn grain, the rumen epithelium was remarkably degraded. The stratum

corneum was disorganized and no longer providing protection to the layers beneath it. The basal layers also became less discernible, further demonstrating the disorganization. Similar responses are observed after calving as the tissue becomes less organized and more visible degradation of the stratum corneum is observed (Steele et al., 2015). More modest changes across the transition period have also been observed with epithelial thickness reducing by 11% as lactation progressed (Dieho et al., 2016). These data demonstrate what we often suspect, that the rumen is at greater risk when feeding additional grain and during times of stress, such as during the peripartum period. One of the studies also measured plasma markers of inflammation, but none evaluated the tissue's inflammatory response or regulation.

To build on this research, we conducted a subacute ruminal acidosis challenge experiment with 9 ruminally cannulated cows. Cows were fed a baseline high-forage diet (24% forage NDF, 27% starch) for 14 d, then cows were switched to an acidosis induction diet (AID; 16% fNDF, 32% starch) for 7 d. During this experiment, we measured ruminal pH and collected rumen biopsies for histological, mRNA, and flow cytometry analysis (Krogstad et al., 2025). Our resulting ruminal acidosis was mild; ruminal pH was reduced from 5.9 to 5.7, and fecal pH was not affected (Figure 1). Plasma biomarkers of inflammation were also unaffected by AID, which was counter to our expectation and confirmed the mild nature of the challenge. We also observed a 30% reduction in stratum corneum thickness and a 66% increase in the interstitium within the rumen tissue within 3 d of the diet change (Figure 2). One of our primary objectives, though, was to evaluate tissue-level inflammatory responses, so we evaluated 5 cytokine genes; only *IL1B* was affected and it tended to be less for AID cows, suggesting a reduced inflammatory status or a counter-regulatory response to dampen inflammation during this challenge.

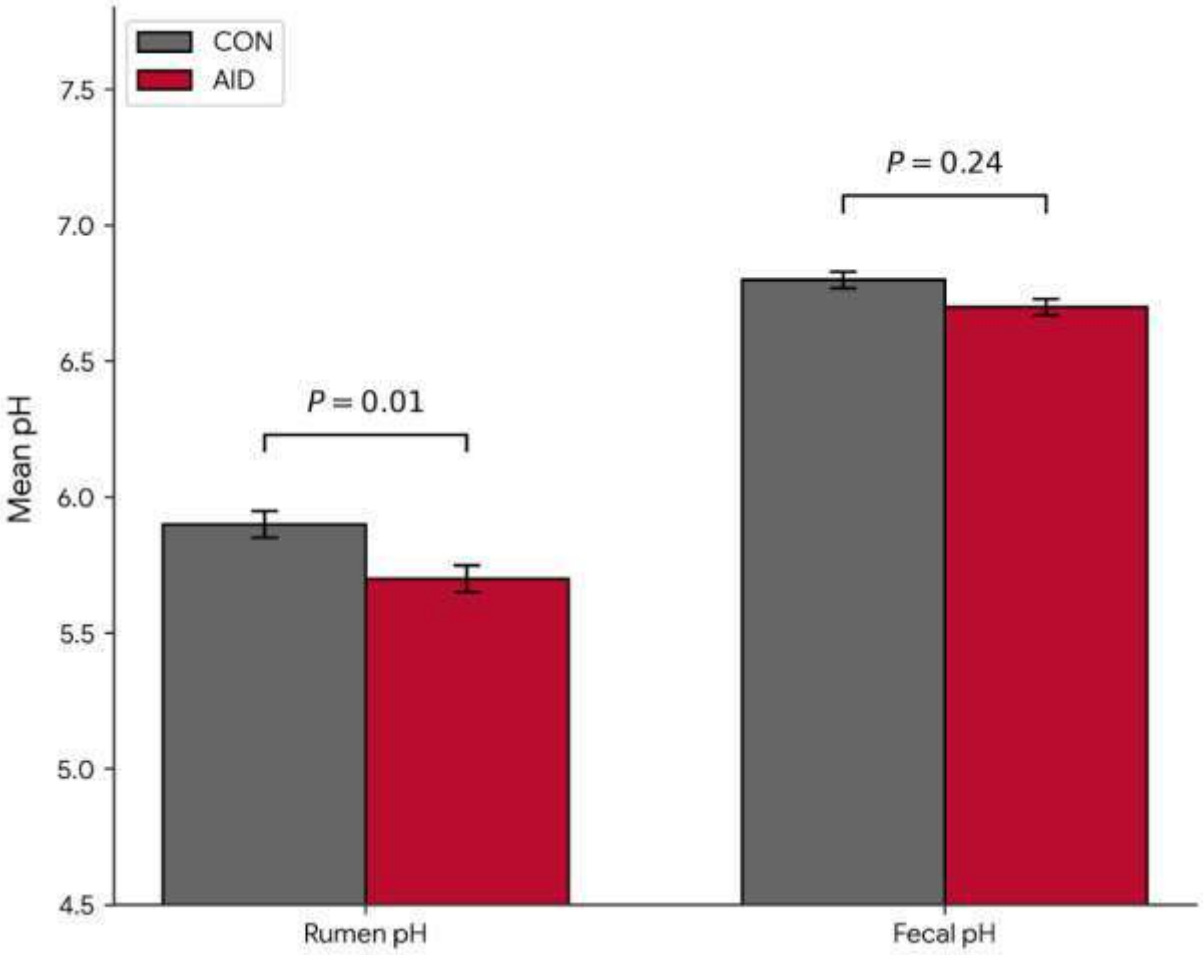


Figure 1. Overall ruminal and fecal pH of lactating dairy cattle fed a high-forage control diet (CON) or an acidosis induction diet (AID). Bars represent treatment means, and error bars indicate the standard error of the mean (SEM). Brackets and values denote the main effect of dietary treatment. Data adapted from Krogstad et al., 2025.

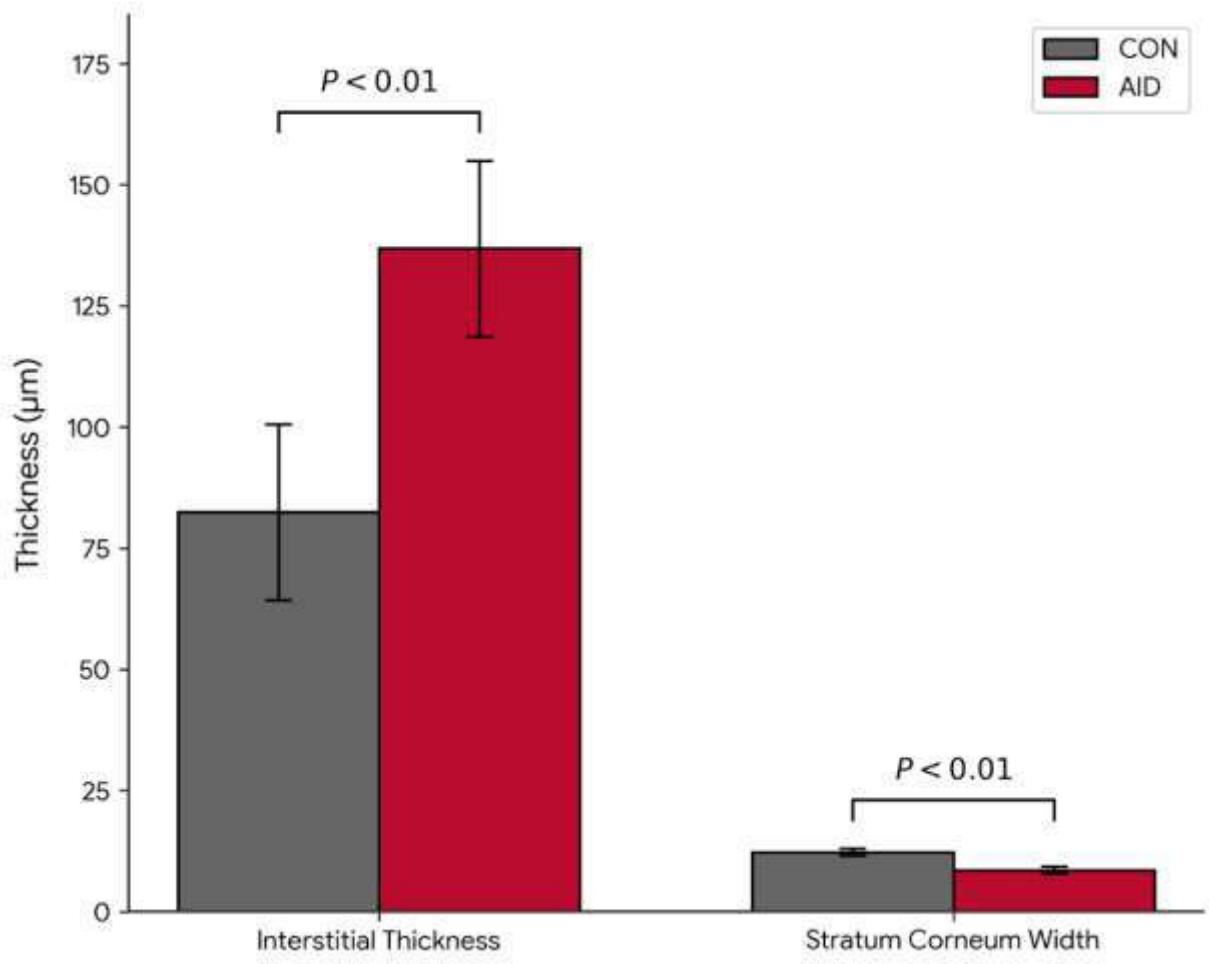


Figure 2. Rumen papillae fixed interstitial thickness and stratum corneum width in lactating dairy cattle fed a high-forage control diet (CON) or an acidosis induction diet (AID). Bars represent treatment means, and error bars indicate the standard error of the mean (SEM). Adapted from Krogstad et al., 2025.

In this pilot study, we also investigated whether there are immune cells present within rumen tissue and what their phenotype may be. Tissue leukocytes are critical in the regulation of many tissues (Bradford and Contreras, 2024), and we suspect the rumen is no different. Previous research suggested that T cells are present within rumen tissue (Jonsson et al., 2020), and so we conducted flow cytometry to evaluate the presence of T cells (CD3+) as well as myeloid cells (CD172a+). Our initial research suggests that 85% of the leukocytes within rumen tissue are T cells, while 6-7% are or myeloid origin, with the remaining being undefined. The proportion of immune cells was not affected by diet in our initial investigation. We also conducted immunohistochemistry, and the results aligned with the flow cytometry evaluation. We did not assess how the function of these ruminal immune cells was affected by diet, and this remains an area ripe for future research across ruminant species.

Our first experiment established and confirmed that the rumen wall's structure is rapidly responsive to diet, but we did not observe the severe changes that other research has documented when increasing grain by feeding barley and wheat. We also established that inflammatory pathways may change in response to diet, but perhaps not by increasing inflammation as we may have expected. Finally, we confirmed the presence of immune cells in the rumen tissue, mostly in the basal lamina and basal layers of the tissue. Our next objectives were to characterize the phenotype of the T cells present and evaluate a broader range of inflammatory biomarkers in both plasma and rumen tissue.

In our first follow-up experiment, we used 4 cannulated, clinically healthy lactating Holstein cows (Vandevoorde and Krogstad, 2026). We collected rumen tissue biopsies from the ventral rumen and evaluated them for T cell phenotypes with flow cytometry. We demonstrated, again, that the majority of immune cells are T cells (84%). Within that T cell population, we also observed that γδ T cells were the most prominent T cell subset in rumen tissue (66% of all immune cells, 78% of T cells). The strong presence of γδ T cells in rumen tissue is intriguing as these cells are considered to have regulatory functions, like IL-10 secretion, which may promote resolution and dampen inflammatory activity (Guzman et al., 2014). These results suggest that the rumen is a highly resilient tissue populated by regulatory immune cells, which we suspect aid in dampening inflammatory responses in a critical tissue.

Following identifying the presence of γδ T cells, we wanted to assess if their expression responded to differences in the diet fed to dairy cattle. Thus, we conducted a research experiment with 6 cannulated lactating Holstein cows where we fed diets with 12% fNDF and increasing concentrations of dietary starch from 24% to 32% of diet DM by increasing corn grain inclusion (Moussiaux et al., 2026). Our results confirmed again that T cells dominate the rumen tissue. The γδ T cells were still the largest proportion of T cells, but at much lower proportions than previously observed. Also, diet did not affect T cell proportions, but γδ T cells were reduced from 63% of T cells in the 28% starch diet to 38% of T cells in the 32% starch diets. The stratum corneum also reduced from 5.2 μm to 2.3 μm when going from 28 to 32% dietary starch. In this experiment, we also assessed both systemic and local rumen tissue markers of inflammation. The systemic markers, acute phase proteins and cytokines, were not affected by dietary starch concentration. We observed that 4 cytokines (IFN-γ, IL-1α, IL-4, and IP-10) linearly increased in the rumen tissue with increasing dietary starch concentration. Importantly, these changes in rumen tissue inflammation occurred without any changes in ruminal pH, which further demonstrates the limitation of rumen pH, alone, to assess rumen health. These data suggested to us that rumen tissue inflammatory regulation shifts in response to diet and does so before we measure changes in traditionally measured plasma biomarkers of inflammation. Also, the proportion of γδ T cells was negatively correlated with concentrations of IP-10 ($r = -0.54$) and MCP-1 ($r = -0.57$), suggesting that increasing γδ T cells in rumen tissue is associated with reduced inflammatory tone in rumen tissue.

One bottom-line takeaway across these three experiments is the prominence of T cells and γδ T cells within rumen tissue (Figure 3). Our current data suggests that T cells are 84-88% of ruminal immune cells, with γδ T cells comprising 38%-78% of those T cells

(Krogstad et al., 2025; Moussiaux et al., 2026; Vandevoorde and Krogstad, 2026). Further investigations into the phenotypes and T cell functions will continue to clarify the role of these cells in the rumen tissue.

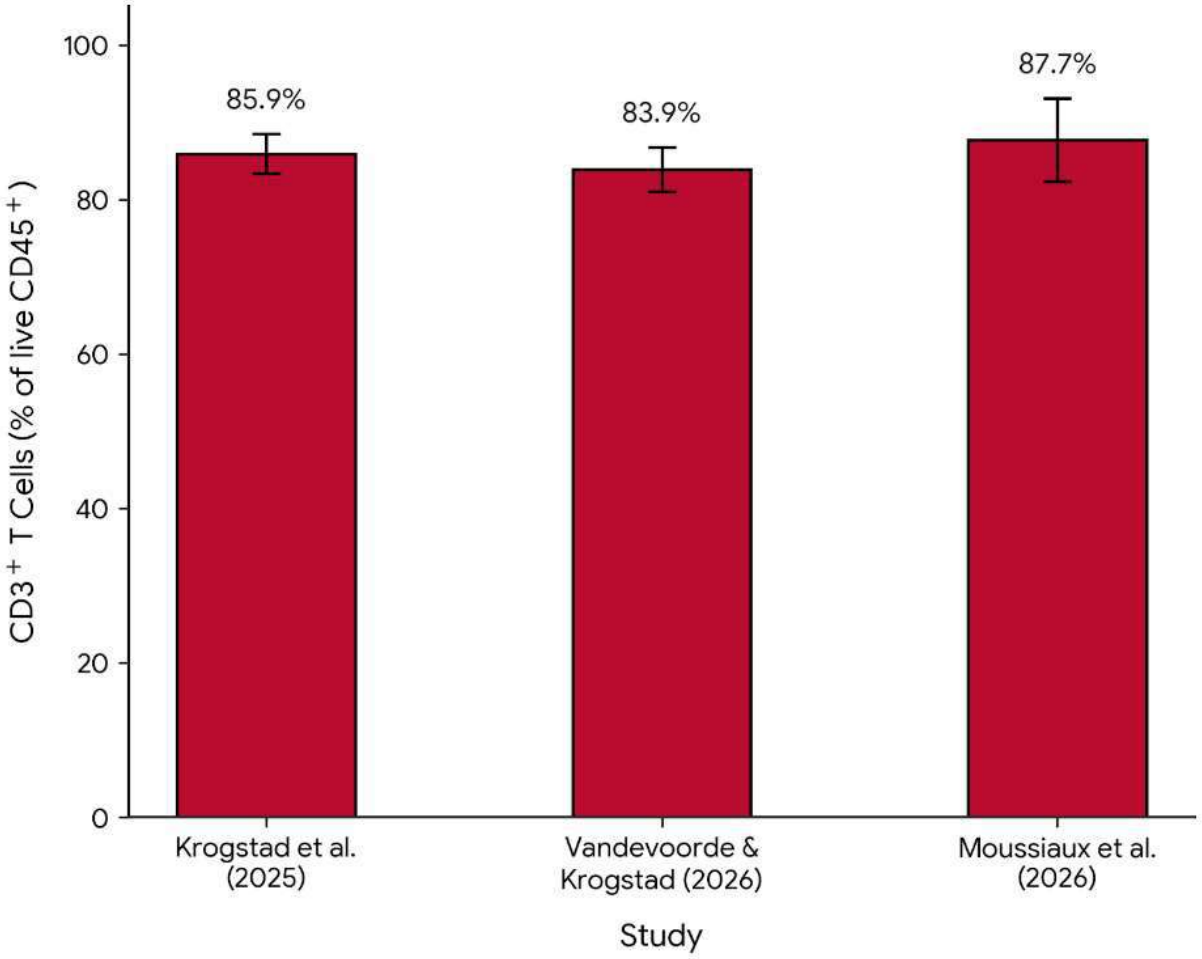


Figure 3. Ruminal $CD3^+$ T lymphocytes (as a % of viable $CD45^+$ leukocytes) in control/baseline lactating dairy cows across three experiments. Control groups represented are CON (high-forage diet) from Krogstad et al. (2025), baseline rumen tissue from Vandevoorde and Krogstad (2026), and the LS diet (24% starch control) from Moussiaux et al. (2026).

Moussiaux et al. (2026) assessed only corn grain (particle size: 839 µm) in a very low-forage scenario, which may have resulted in an already stressed rumen environment. Our next goal was to assess the effects of dietary starch source and starch concentration on rumen tissue physiology when feeding diets with adequate forage (Vandevoorde et al., *In review*). In a Latin square with 8 ruminally cannulated cows, we assessed the effect of feeding corn (792 µm) or barley (832 µm) as the primary grain at 22% or 27% dietary starch concentration. The diets contained 20% fNDF, which is within the ideal range outlined by NASEM (2021). Feeding barley in place of corn reduced the reticulorumen pH, measured using a rumen bolus (SmaXtec), from 6.54 to 6.41. Within this study, much

like Moussiaux et al. (2026), we observed limited and modest shifts in plasma cytokine concentrations. As dietary starch increased, rumen tissue IFN-γ was reduced by 20%, which contrasted with our previous observations. Also, feeding barley in place of corn grain reduced both IL-1β and IL-10 within the rumen tissue ($P \leq 0.07$). Perhaps the changes in inflammatory signals were more modest due to adequate fNDF inclusion in the diets or the reduced starch concentration. In Moussiaux et al. (2026), the most dramatic changes in rumen tissue inflammatory tone occurred when the diet contained 32% dietary starch. Perhaps 27% starch from moderately ground grains present a low risk for rumen tissue inflammatory responses.

Another critical finding of our research thus far is the importance of the IL-1 family of cytokines within the rumen tissue. In our most recent study, we observed that the relative proportions of IL-1α and IL-36Ra were 6- to 7-fold greater in rumen tissue than in plasma (Vandevoorde et al., *In review*). Moussiaux et al. (2026) also observed increased relative proportions for IL-1α, but the degree of increase for IL-36Ra could not be assessed as it was greater than the detection limit of the assay when evaluating rumen tissue. The presence of these cytokines alone is not a suggestion of more or less inflammation. IL-1α is generally considered to be a pro-inflammatory alarmin (Garlanda et al., 2013), but it is constitutively expressed in cells, especially those at the epithelium (Di Paolo and Shayakhmetov, 2016). Upon cell death and cell lysis, the IL-1α can be released and bind to IL-1R on neighboring cells and initiate an inflammatory cascade. Thus, within the epithelia, the location of IL-1α is critical. If it is extracellular, it may indicate a brewing inflammatory response. IL-36Ra, on the other hand, is anti-inflammatory by blocking IL-36 signaling (Mantovani et al., 2019; Ngo et al., 2021). Even more important is that IL-36 signaling appears to play a critical role at epithelial barriers, including the gut (Hovhannisyan et al., 2020). It appears that the rumen is similar in that IL-36 may be an important regulator of tissue function and health. We also observed that IL-17A, a member of the IL-17 family, is 1.5- to 10-fold greater in rumen tissue than in plasma, further demonstrating the unique inflammatory milieu within the rumen tissue. The investigation of these cytokines in rumen tissue is just beginning, and understanding their dynamics may open new opportunities for creating gut-health-promoting solutions for ruminant livestock.

Managing gut and rumen health with what we know

Given what we know and do not know about the rumen, how can we manage 'rumen health' on the dairy farm? At this point, there are three primary considerations with *some* evidence as to their importance for the rumen health of dairy cattle. The main management points are starch profile of the diet (both concentration and type), forage concentration, and ration consistency. Each of these topics has some evidence of affecting rumen health, but often only in the most extreme scenarios, and the evidence is not concrete.

If rumen health is a concern on a dairy farm, dietary starch concentration is one of the first culprits under consideration. Veterinarians consider dietary starch concentration a risk factor for Jejunal Hemorrhage Syndrome (a.k.a. hemorrhagic bowel syndrome;

HBS) (Godden et al., 2001). The epidemiological evidence for such an association is limited; time of year, size of the herd, and level of production are modestly associated with HBS incidence (Berghaus et al., 2005; De Jonge et al., 2024). In a narrative review, Krogstad and Bradford (2023) evaluated both acidosis challenge experiments and experiments that increased dietary starch in non-challenge experimental conditions. They concluded that increasing dietary starch outside of challenge conditions did not consistently cause or alter acute phase protein concentrations. Inflammation occurred most often when a portion of the forage in a diet was replaced with readily degradable grains, like barley and wheat. When feeding dry ground corn and corn silage to dairy cattle, keeping total dietary starch ≤32% of diet DM reduces the risk of increasing systemic biomarkers of inflammation (Krogstad and Bradford, 2023). The cutoffs we proposed in this review do align with results of Moussiaux et al. (2026) such that plasma biomarkers of inflammation were unchanged as starch increased, but each diet starch was ≤32% of diet DM. What the Moussiaux et al. (2026) study demonstrated, though, is that rumen tissue stress or inflammatory shifts are probably occurring prior to measurable changes in systemic markers.

Grain source is another factor to consider. Unfortunately, in the literature assessing rumen health, grain source and starch concentration are often confounding factors. As mentioned previously, in ruminal acidosis challenges, wheat and barley are often used to increase dietary starch concentration (Gozho et al., 2007; Khafipour et al., 2009b; Krogstad et al., 2025). Wheat and barley present greater risk for rumen upset because they are more degradable than ground corn (Ferraretto et al., 2013; Huntington, 1997). Feeding small grains like barley or wheat also reduce pH of the rumen when compared to feeding corn grain (Yang et al., 1997). High-moisture corn is another high-risk grain source. Feeding high-moisture corn in place of ground corn reduces ruminal pH and increases ruminal starch digestibility (Oba and Allen, 2003a, b). When fed during the postpartum period, high-moisture corn in rations with 22% or 28% dietary starch reduces energy-corrected milk yield by 4.95 kg/d and slightly increases plasma Hp (Albornoz and Allen, 2018; Albornoz et al., 2020). Feeding moderately degradable starch sources, like dry ground corn, instead of rapidly degradable sources reduces the risk of gut-associated inflammation.

Grain particle size is likely another consideration. Reducing grain particle size increases digestibility (Ferraretto et al., 2013). Results from Shipandeni et al. (2023) concur, as reducing particle size from 600 μm to 250 μm increased starch digestibility and reduced rumen pH when feeding sorghum, but not corn grain. Unfortunately, we are not yet aware of an investigation regarding rumen health and ruminal inflammation when feeding reduced particle size. Research in swine and poultry has explored this and identified a tradeoff such that reducing particle size may increase performance, but increases gastrointestinal stress (Kiarie and Mills, 2019; Vukmirović et al., 2017). The effects of grain particle size on gut health are an urgent area of need in the ruminant nutrition and physiology literature.

Forage concentration should be considered when thinking about rumen and gut health. The effect of forage NDF (fNDF) is usually confounded by dietary starch concentration in the rumen health literature (Gozho et al., 2007; Krogstad et al., 2025). There is one intriguing example that demonstrates the potential impact of fNDF on rumen health and inflammation. Engelking and Oba (2024) conducted an experiment where they offered free-choice hay for 5 days postpartum. 1.3 kg/d of hay intake reduced Hp by 38% during the 1st week after calving. We cannot definitively say this is related to the rumen or the gut, but it is reasonable to posit improved rumen health as the cause of the reduced Hp from supplying the grass hay.

One final area to manage is the bunk—both the consistency of the feed and ingredient mix as well as the consistent access to feed in the bunk. There is some modest evidence that feed consistency may be important for rumen health and an animal's stress response, but the evidence is mixed. In a set of ruminal acidosis challenge experiments (Khafipour et al., 2009a, b), investigators utilized a sudden grain challenge or a step-up-based alfalfa-pellet challenge to cause ruminal pH reductions. Both experimental models reduced ruminal pH and increased ruminal LPS, but only the sudden grain-based challenge resulted in an inflammatory response (Figure 4). Of course, the sudden vs. long-term diet change effect here is confounded by dietary strategy. An additional experiment also assessed the effect of bunk management by investigating cows' response to chronically greater starch concentrations (29% of diet DM) or a sudden increase in dietary starch concentrations to 32% of diet DM for 2 d, 1 week apart. The sudden increase in dietary starch concentrations caused a 78% increase in serum amyloid A, a positive acute phase protein, compared to the control diet where starch was 24% of diet DM. The chronically greater starch diet did not cause changes in serum amyloid A, suggesting that the sudden diet change was more the culprit than the dietary starch concentration (Gott et al., 2015). In another study, starch was alternated from 21% to either 28%, 25%, or 42% of diet DM over the course of a week. Production measures were largely unchanged, but rumen pH declined with greater dietary starch variation during the alternating period. Fat concentration and energy-corrected milk yield also had notable declines, although they did not reach statistical significance (Hernández-Castellano et al., 2021). In their review, Weiss and St-Pierre (2024) noted that cows can handle some degree of diet variation if the diets average to a reasonable mean over the short term. Variation in dietary composition and cows' response to it is challenging to investigate and likely needs to be investigated in different settings, like at varying stocking densities or when mixing parities, which may cause additional challenges for the cows. One other note on bunk management is that reducing access to feed can reduce the ability of the rumen to absorb volatile fatty acids, so maintaining constant access to feed is advisable (Zhang et al., 2013).

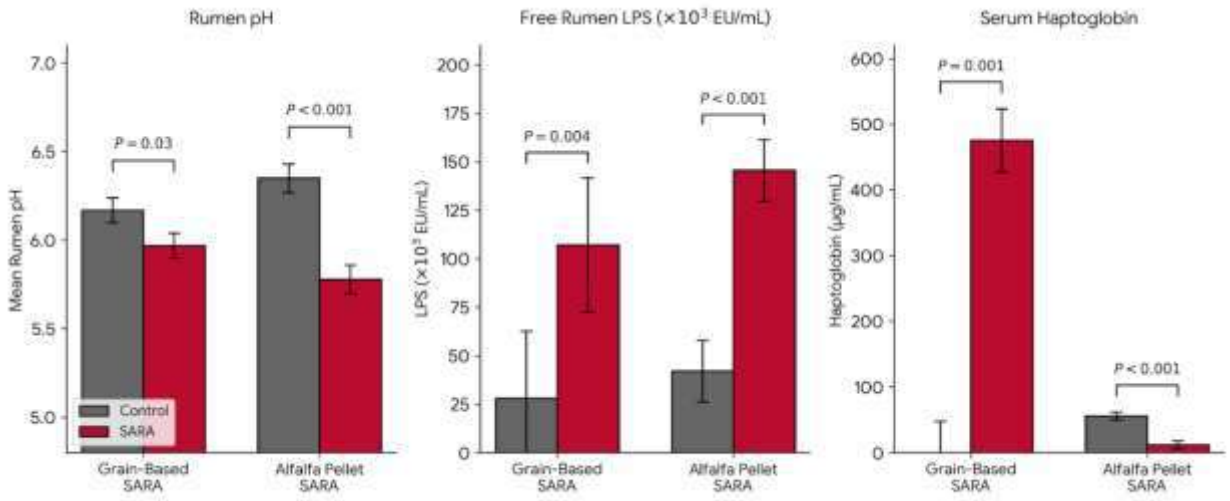


Figure 4. Comparison of mean rumen pH, free rumen lipopolysaccharide (LPS), and serum haptoglobin (Hp) in lactating dairy cows undergoing either a grain-based subacute ruminal acidosis (SARA) challenge or an alfalfa-pellet-induced SARA challenge. Data adapted from Khafipour et al. (2009a; alfalfa-based challenge: wk 1 hay control vs. wk 6 40% alfalfa pellet substitution) and Khafipour et al. (2009b; grain-based challenge: 21% wheat-barley pellet substitution). Brackets denote statistical significance between dietary treatments ($P < 0.05$).

Where are the gaps in the literature?

Substantial progress has been made on understanding rumen dynamics, rumen health, and rumen metabolism in the last decade, but more work needs to be done. On the applied side, additional longer-term investigations (≥12-week feeding periods) of how different diets affect the health of the gastrointestinal tract are needed. Most experiments are short-term or cross-over studies that limit the external validity of the findings. Assessment of grain particle size and its effects on dairy cattle gastrointestinal health are also needed. We've learned that in poultry and swine, excessively small feed particles can stress the gut. Reducing forage in the diet reduces pH and may cause rumen upset, but many experiments evaluating dietary forage concentration are confounded by dietary starch concentration (Li et al., 2020; Yang and Beauchemin, 2004); additional investigation of fNDF while maintaining dietary starch concentration will be helpful to our understanding of rumen health.

The next frontier in rumen health research will involve more direct investigations of the rumen tissue and its physiology. This includes evaluating the signaling networks through proteomic or transcriptomic techniques. The investigation of the epimural, or tissue-adhered, microbiome ought to occur alongside our growing knowledge of rumen tissue physiology. Integration of tissue dynamics with microbiome shifts will open new opportunities for improving rumen health, feed digestion, and animal performance.

Summary

In summary, we have learned a great deal about rumen health and rumen physiology, but much more needs to be learned. Rumen pH has been the traditional metric of rumen health, but more recent research has demonstrated that rumen tissue physiology may not directly correlate with rumen pH and thus requires more direct investigation. Our team has also observed that the rumen's epithelium is a dynamic structure that responds rapidly to diet and appears to be heavily enriched with the IL-1 family of cytokines. In preliminary research, we have observed that pro-inflammatory cytokines may increase as dietary starch increases when feeding reduced forage rations, but follow-up research is needed to confirm these findings. We've also observed that the rumen tissue is populated by T cells, but we have yet to learn how they get there, what their function is, and whether they are responsive to dietary or management changes. At this point, managing dietary carbohydrates, feed consistency, and access to the feed bunk may improve dairy cattle performance outcomes, but more investigation is needed to develop concrete recommendations or products that promote a resilient rumen and gastrointestinal tissue.

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Advancing Dietary Formulation Techniques for Optimal Ruminal and Cattle Performance

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Introduction

The productivity of modern dairy cattle has increased substantially because of genetic selection, improvements in management, and advances in nutrition. These changes have increased the importance of accurately defining nutrient requirements and developing dietary formulation approaches that account for the biological supply and utilization of nutrients by both ruminal microorganisms and the animal. The Cornell Net Carbohydrate and Protein System (CNCPS) was developed as a mechanistic framework to describe nutrient supply and requirements and to support precision diet formulation (Van Amburgh et al., 2015). Continued evaluation and development of the system are necessary to ensure that it remains appropriate for contemporary high-producing dairy cattle. In addition, there is increasing pressure to reduce nitrogen (N) excretion from dairy cattle to reduce the environmental impact of milk production. Thus, strategies that improve partitioning of N to the mammary gland through enhanced energy-collected milk (ECM) yield, and reduce urinary N excretion while reducing N intake are needed.

Thus, one area where greater precision in formulation may have substantial environmental and economic benefits is dietary N. Dairy cattle are frequently supplied with more dietary N than what is ultimately captured in milk and tissue, resulting in urinary and fecal N losses (Ipharraguerre and Clark, 2005; Higgs et al., 2012; Van Amburgh et al., 2015). Reducing dietary N intake can therefore improve N use efficiency and reduce environmental losses. However, simply reducing dietary N may negatively affect microbial protein synthesis, ruminal fiber digestion, and milk or milk component production if the supply of ruminally available N and metabolizable amino acids (AA) are not adequately maintained. (Reynolds and Kristensen, 2008). The relationship between ruminal N supply and microbial growth is particularly important. Ruminal microorganisms require ammonia (NH_3) to support microbial protein synthesis, but the amount required depends on the availability of fermentable carbohydrates, the microbial population, and the efficiency of microbial growth (Firkins, 1996). Thus, dietary crude protein (CP) concentration alone is an inadequate indicator of whether the rumen is receiving sufficient N.

Isoacids, or branched-chain volatile fatty acids (BCVFA), provide a potential strategy for improving ruminal microbial function under reduced-N conditions. Isoacids contribute to the synthesis of branched-chain amino acids (BCAA) by ruminal microorganisms and may support microbial growth and fiber digestion without directly increasing dietary N intake (Firkins et al., 2024). Their use provides an opportunity to investigate whether ruminal NH_3 supply can be reduced while maintaining animal productivity by employing BCVFA as a N management tool. Importantly, this also requires

consideration of metabolizable AA supply relative to metabolizable energy (ME), as previous work demonstrated the importance of balancing ruminal N and essential AA relative to energy supply (LaPierre et al., 2020; Higgs et al., 2023). Therefore, the current work evaluated strategies to reduce ruminal N supply while maintaining productive performance and investigated whether isoacid supplementation could support production and nutrient utilization under low-N feeding conditions. Collectively, these studies provide an opportunity to advance CNCPS-based dietary formulation for improved ruminal and animal performance.

Reducing ruminal ammonia supply while maintaining production

Our initial study evaluated the effects of decreasing ruminal NH_3 supply in lactating dairy cows receiving isoacid supplementation (Study 1). A total of 144 lactating Holstein cows averaging 88 ± 35 d in milk (DIM) were enrolled in an 84-d experiment. Cows were randomly assigned to 9 free-stall pens containing 16 cows per pen, with 12 multiparous and 4 primiparous cows per pen. Pens were balanced according to DIM, milk yield, and parity. Three diets were formulated using CNCPS version 6.5.6 (Van Amburgh et al., 2015) to provide 130, 120, or 110% of predicted ruminal NH_3 requirements. Urea was used to establish the different ruminal NH_3 supplies. All diets contained an isoacid product (Zinpro® IsoFerm®, Zinpro Corporation, Eden Prairie, MN) at 60 g/d. The diets were formulated to provide the same amount of true rumen-degradable protein relative to fermentable carbohydrate (177 g/kg fermentable carbohydrate), while varying the total N supply. Diets contained approximately 6.3% sugar, 27.8% starch, 31.1% aNDFom, and 4.9% total fat. Metabolizable methionine and histidine were supplied at 1.19 g/Mcal metabolizable ME, and lysine at approximately 3.20 g/Mcal ME.

Reducing predicted ruminal NH_3 supply from 130 to 110% of requirements did not affect DMI, milk yield, ECM, fat-corrected milk, or ECM feed efficiency ($P > 0.05$; Table 1). Cows maintained approximately 44 kg milk/d and 50 kg ECM/d across treatments. Body weight and body condition score were also unaffected. Milk protein, fat, and lactose concentrations and yields were not affected by treatment. Milk fatty acid groups, including de novo, mixed, and preformed fatty acids, were similarly unaffected. These results demonstrate that ruminal NH_3 supply can be reduced substantially when the diet is formulated to maintain appropriate relationships among fermentable carbohydrate, rumen-degradable protein, rumen-undegradable protein, and metabolizable AA. Importantly, reducing dietary N did not result in a measurable loss of productivity. The primary response to reduced ruminal NH_3 supply was observed in MUN. Milk urea N declined from 13.4 to 12.3 to 10.4 mg/dL as NH_3 supply decreased from 130 to 120 to 110% of predicted requirement, respectively ($P < 0.01$). The reduction in MUN occurred without a corresponding change in milk yield or milk protein yield. Therefore, the additional N supplied in the higher-N diets did not appear to be necessary for maintaining production under these dietary conditions. Importantly, reductions in urinary N excretion were observed, with urinary N reduced by as much as approximately 47 g/d at the lowest ruminal NH_3 supply evaluated. These findings suggested that the ruminal NH_3 requirement for productive dairy cattle may be lower than values commonly used in practical diet formulation when adequate sources of fermentable carbohydrate and

Table 1. Productive performance of dairy cows fed diets with isoacids and varying rumen NH_3 requirements.

Item	130% NH_3	120% NH_3	110% NH_3	SEM	Treatment	Week	Treatment × Week
Body weight, kg	693	688	687	3.37	0.31	< 0.01	0.99
Body weight gain, kg/d	0.11	0.10	0.13	0.13	0.98	< 0.01	0.98
Body condition score	2.96	2.99	2.97	0.02	0.50	< 0.01	0.59
Dry matter intake, kg/d	27.2	27.6	26.8	0.34	0.35	< 0.01	0.02
Milk yield, kg/d	43.8	44.4	44.6	0.51	0.48	< 0.01	< 0.01
Energy corrected milk, kg/d	50.8	50.2	50.1	0.80	0.81	< 0.01	0.05
Fat corrected milk, kg/d	52.0	51.6	51.5	0.94	0.92	< 0.01	0.15
Feed efficiency (ECM/DMI)	1.86	1.85	1.86	0.02	0.84	0.02	0.21
Protein, %	3.20	3.19	3.16	0.02	0.26	< 0.01	0.99
Fat, %	4.67	4.50	4.52	0.05	0.27	< 0.01	0.27
Lactose, %	4.62	4.60	4.57	0.02	0.14	< 0.01	0.97
Protein, kg	1.40	1.41	1.40	0.01	0.89	< 0.01	0.02
Fat, kg	2.04	2.00	1.99	0.05	0.74	< 0.01	0.24
Lactose, kg	2.02	2.04	2.04	0.02	0.82	< 0.01	0.04
MUN, mg/dL	13.4[a]	12.3[b]	10.4[c]	0.14	< 0.01	< 0.01	< 0.01
De novo FA, %	1.21	1.20	1.21	0.02	0.95	< 0.01	0.32
Mixed FA, %	1.55	1.54	1.53	0.02	0.76	< 0.01	0.20
Preformed FA, %	1.28	1.29	1.31	0.02	0.46	< 0.01	0.04

[a,b] Least squares means in the same row with different superscripts differ ($P \leq 0.05$).

[x,y] Least squares means in the same row with different superscripts tend to differ ($0.05 < P \leq 0.10$).

isoacids are available. Overall, these results provide evidence that precision formulation can reduce N losses by matching N supply more closely to biological requirements rather than maintaining excess dietary N as a safety margin.

Evaluation of low-nitrogen diets with different sources of ruminally available nitrogen

Study 2 was conducted in a similar design to Study 1, where 144 lactating Holstein cows (114 ± 41.2 DIM) assigned to 9 free-stall pens containing 16 cows per pen for a 70-d period. The study was designed to further explore the lower limits of dietary N supply in lactating dairy cows evaluated three practical dietary strategies: a control diet, an isoacid plus urea diet (Iso + U), and an isoacid plus slow-release urea diet (Iso + SRU). All diets were formulated using CNCPS (v. 6.5.6 and v.7) and were formulated to contain approximately 30% aNDFom, 6.6% sugar, 29% starch, and 4.9% total fat.

As shown in Table 2, milk yield was not affected by treatment (40.3, 40.8, and 40.4 kg/d for control, Iso + U, and Iso + SRU, respectively; $P = 0.59$). However, ECM was greater for cows receiving the isoacid treatments compared with control cows (50.1, 52.2, and 52.2 kg/d; $P = 0.04$). Milk protein yield increased from 1.38 kg/d in control cows to 1.44 kg/d in both isoacid treatments ($P < 0.01$). Milk protein concentration also tended to increase from 3.46% in control cows to 3.50 and 3.51% for Iso + U and Iso + SRU, respectively (P = 0.06). The increase in ECM and milk protein yield without a reduction in milk yield or milk component concentrations is particularly relevant when evaluating low-N feeding strategies. These results indicate that reducing dietary protein concentration to less than 14% true protein did not prevent cows from maintaining high levels of milk component production when isoacids were included in the diet.

The MUN was slightly greater for the isoacid treatments than for control cows (7.27, 7.64, and 7.66 mg/dL for control, Iso + U, and Iso + SRU, respectively; $P = 0.02$). This was expected since both treatments had the inclusion of additional urea (20 g) in the diets. But importantly, the MUN concentrations across all treatments were substantially lower than those observed in Study 1 at the highest ruminal NH_3 supply.

CNCPS predictions around nitrogen utilization and methane emissions

The CNCPS framework provides an opportunity to evaluate the potential partitioning of N among productive and excretory pathways. Current model estimates from both studies are in Table 3. Reducing ruminal NH_3 supply in Study 1 substantially decreased predicted N losses while maintaining productive N. As ruminal NH_3 supply decreased from 130 to 110% of requirement, predicted N intake declined from 719 to 653 g/d, whereas productive N remained relatively constant (234, 235, and 236 g/d, respectively). The reduction in N intake was primarily reflected in lower predicted urinary N, which decreased from 230 to 177 g/d, while fecal N decreased from 267 to 253 g/d. Consequently, total manure N decreased from 498 to 430 g/d, and the proportion of N captured in productive output increased from 33 to 36%. The productive N to urinary N ratio also increased from 1.02:1 to 1.34:1. These predictions are consistent with the

observed reduction in MUN and indicate that lower ruminal NH_3 supply can reduce predicted N losses without reducing milk or ECM production when the diet is appropriately balanced.

In Study 2, compared with the control diet, the addition of isoacids to the treatments increased predicted N intake by approximately 22 to 24 g/d in accordance with the increase in dietary urea, and productive N by 6 to 11 g/d. Predicted urinary N also increased numerically from 190 g/d in the control to 196 and 200 g/d for Iso + U and Iso + SRU, respectively, while fecal N increased by approximately 6 to 7 g/d. As a result, total manure N was numerically greater with isoacid supplementation. However, it is important to note that these changes occurred concurrently with greater ECM and milk protein production. The proportion of N captured in productive output remained similar among treatments (i.e., 34 to 35%), suggesting that the additional N supply was accompanied by greater productive output rather than a major change in the overall partitioning of N.

Completion of feed chemistry analyses and integration of the final dietary composition with the animal performance data will be important for interpreting these responses. In particular, the relationship among dietary N concentration, ruminal N supply, fermentable carbohydrates, isoacid supply, microbial protein synthesis, and milk protein production needs to be considered when evaluating the potential for further reductions in dietary N.

The CNCPS estimates also provided an opportunity to evaluate the environmental implications from a methane (CH_4) perspective. In Study 1, predicted CH_4 production remained similar across ruminal NH_3 treatments (i.e., 642 to 648 g/d), while the reduction in N excretion was achieved without compromising production. In Study 2, predicted CH_4 production increased numerically from 652 g/d in the control group to 666 to 668 g/d with isoacid supplementation. However, because ECM production was greater with the isoacid treatments, predicted CH_4 intensity remained relatively similar, ranging from 12.95 g/kg ECM in the control group to 14.28 and 14.48 g/kg ECM for Iso + U and Iso + SRU, respectively. Predicted CO_{2eq} intensity was likewise similar among treatments. Importantly, the present estimated values (0.34 – 0.35 kg CO_{2eq}/kg ECM) were much lower than previously reported cow-level values across farms in Australia, New Zealand, Ireland, and the U.S. states of Pennsylvania, New York, and Idaho (approximately ranging from 0.42 – 0.70 kg CO_{2eq}/kg ECM; Rotz, 2018; Dida et al., 2025). This suggests an opportunity to achieve greater alignment between N utilization and greenhouse gas (GHG) efficiency, whereby reducing unnecessary N excretion while maintaining or increasing productive output may also improve the environmental efficiency of milk production. In this context, precision formulation strategies that optimize nutrient supply relative to animal requirements could provide simultaneous benefits for N-use efficiency and GHG intensity. However, these environmental estimates should be interpreted as model predictions rather than direct measurements of farm-level emissions, and further validation using measured emissions is warranted.

Table 2. Productive performance of dairy cows fed a control diet and diets with isoacids and urea (Iso +U), and Iso and slow-release urea (Iso + SRU).

Item	Control	Iso + U	Iso + SRU	SEM	Treatment	Week	Treatment × Week
Body weight, kg	696	695	696	3.13	0.90	< 0.01	0.06
Body weight gain, kg/d	0.15	0.10	0.14	0.12	0.25	< 0.01	0.07
Body condition score	2.92	2.95	2.95	0.01	0.10	< 0.01	0.01
Dry matter intake, kg/d	28.5	29.2	29.6	0.38	0.22	< 0.01	0.01
Milk yield, kg/d	40.3	40.8	40.4	0.35	0.59	< 0.01	< 0.01
Energy corrected milk, kg/d	50.1^{b}	52.2^{a}	52.2^{a}	0.68	0.04	< 0.01	0.72
Fat corrected milk, kg/d	51.4^{y}	53.5^{x,y}	53.7^{x}	0.81	0.08	< 0.01	0.48
Feed efficiency (ECM/DMI)	1.76	1.80	1.78	0.03	0.76	< 0.01	0.53
Protein, %	3.46^{y}	3.50^{x,y}	3.51^{x}	0.02	0.06	< 0.01	0.01
Fat, %	5.22	5.27	5.35	0.08	0.46	< 0.01	0.59
Lactose, %	4.62	4.63	4.64	0.01	0.38	< 0.01	0.14
Protein, kg/d	1.38^{b}	1.44^{a}	1.44^{a}	0.02	< 0.01	< 0.01	< 0.01
Fat, kg/d	2.10	2.18	2.20	0.04	0.15	< 0.01	0.92
Lactose, kg/d	1.86^{b}	1.92^{a}	1.91^{a}	0.02	0.03	< 0.01	< 0.01
MUN, mg/dl	7.27^{b}	7.64^{a}	7.66^{a}	0.11	0.02	< 0.01	< 0.01
De novo FA, %	1.39^{b}	1.43^{a,b}	1.46^{a}	0.01	< 0.01	< 0.01	0.09
Mixed FA, %	1.93^{b}	1.97^{a,b}	2.00^{a}	0.02	0.01	< 0.01	0.02
Preformed FA, %	1.42^{b}	1.45^{b}	1.47^{a}	0.01	0.05	< 0.01	< 0.01

^{a,b} Least squares means in the same row with different superscripts differ ($P \leq 0.05$).

^{x,y} Least squares means in the same row with different superscripts tend to differ ($0.05 < P \leq 0.10$).

Table 3. Estimated N, CH_4 and CO_2-equivalent (CO_{2eq}) excretion values calculated using CNCPS for experiment diets in Study 1 and 2.

Item	130% NH_3	120% NH_3	110% NH_3	Control	Iso + U	Iso + SRU
N intake, g	719	703	653	675	699	697
Productive N, g	234	235	236	233	244	239
Urinary N, g	230	214	177	190	196	200
Fecal N, g	267	266	253	265	272	271
Total manure, g	498	480	430	455	468	471
Productive N/Total N	0.33:1	0.34:1	0.36:1	0.35:1	0.35:1	0.34:1
Productive N/Urinary N	1.02:1	1.14:1	1.34:1	1.23:1	1.25:1	1.20:1
CH4, g/d	643	648	642	652	668	666
CH4 intensity, (g/kg ECM Pen level)	12.65	12.92	12.81	12.95	14.28	14.48
CO_{2eq} (kg/d)	17.35	17.51	17.32	17.62	18.03	17.98
CO_{2eq} intensity (kg/kg ECM Pen level)	0.34	0.35	0.35	0.35	0.34	0.34

Variation among cows and opportunities for herd-level improvement

An additional observation from the current work is the substantial variation among individual cows in milk component production and ECM yield. Several cows have consistently performed well above their pen and treatment averages, particularly in milk components production. For example, the highest-performing cows produced an average of 58.0, 74.9, and 64.8 kg ECM in the Control, Iso + U, and Iso + SRU treatments, respectively, compared with treatment averages of approximately 50 - 52 kg/d (as shown in Table 2). These top-performing cows also demonstrated greater ECM efficiency (e.g., 1.96 - 2.16 vs. 1.76 - 1.80) and lower predicted CO_{2eq} intensity (0.26 - 0.31 vs. 0.34 - 0.35 kg CO_{2eq}/kg ECM) than their respective treatment averages.

These animals demonstrate that considerable productive capacity exists within commercial dairy herds, even when cows are managed under the same dietary and environmental conditions. Understanding the nutritional and biological factors associated with these high-performing cows may provide an additional opportunity to improve herd-level efficiency. If a greater proportion of cows could express higher milk component production while maintaining similar nutrient intake and excretion, as well as methane production, the environmental impacts could be diluted over a greater quantity of fluid milk and milk components especially as N intake is reduced to reduce urinary N excretion. This concept emphasizes the importance of evaluating nutritional strategies not only by their effect on average cow performance but also by their ability to allow a greater proportion of the herd to achieve high levels of productive efficiency.

Conclusions

Modern dairy cattle have the genetic capacity to produce substantially greater quantities of milk and milk components than the cows used to establish many traditional nutrient requirements. Consequently, feeding strategies must increasingly focus on matching nutrient supply to the biological requirements of high-producing animals while minimizing excess nutrient intake. Results from our studies indicate that isoacid supplementation may support lactational performance when dietary N and ruminal ammonia supply are constrained. In Study 2, isoacid supplementation increased ECM and milk protein yield while feeding lower N containing diets. Current CNCPS estimates indicate similar methane and CO_{2eq} emission intensities among treatments despite greater ECM production, suggesting an opportunity to dilute environmental impacts per unit of milk produced. Continued evaluation of N partitioning, economic returns, and herd-level methane inventories will be necessary to determine how these strategies can be translated into practical feeding recommendations for dairy producers. Ultimately, improving dairy sustainability requires an integrated approach that considers nutrient supply, milk component production, economic performance, and environmental impact simultaneously.

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Innovations in Corn Silage

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Introduction

Whole-plant corn silage (**WPCS**) is the predominant forage used in dairy cattle diets worldwide. About 110 million Mg of fresh corn forage is harvested per year in the United States. Although WPCS is an important feed for dairy cows, nutritive value varies widely. Starch and fiber are the main constituents of WPCS and usually have the largest influence on nutritive value and subsequent performance of dairy cows. Starch in WPCS is very digestible and provides energy to support maintenance and lactation (NASEM, 2021). Although not as digestible as starch, fiber also provides energy to dairy cows (Oba and Allen, 1999) but also stimulate chewing and salivation, rumination, gut motility and health, regulate feed consumption and are the structural basis of the ruminal mat, which is crucial for ruminal digestion. Even though new technologies targeting corn silage starch utilization were developed and studied in recent years, fiber utilization garnered more attention. Therefore, the objective of this article is to summarize recent innovations in corn silage fiber utilization.

Corn silage fiber utilization

Under certain conditions, corn silage fiber utilization may have greater biological significance than starch as it impacts intake, rumen dynamics and energy value. Ferraretto et al. (2015), for example, compared hybrids differing in digestibility of fiber (brown midrib) and starch (floury) on lactation performance. Despite the 5-percentage-units greater total tract starch digestibility, cows fed the floury hybrid had lower intake and milk yield than cows fed brown midrib silage.

Incomplete fiber digestion reduces the profitability of dairy production by limiting intake and hence, animal productivity, and increasing manure production. A 1-unit increase in forage NDF digestibility (**NDFD**) is associated with 0.17 and 0.25 kg/d increases in DMI and milk production, respectively (Oba and Allen, 1999). Lignin is the key obstacle to fiber digestion as it obstructs enzyme access to the digestible fiber fractions, cellulose and hemicellulose. In addition, rumen microorganisms cannot breakdown lignin. Due to its importance to animal performance, this association between lignin and other fibrous fractions (i.e. cellulose and hemicellulose) is considered in many diet formulation models. This undigested or indigestible NDF fraction is estimated with lignin or quantified as the proportion of NDF remaining after in vitro or in situ ruminal incubations (i.e. 240 h uNDF). Thus, the reduction of lignin or indigestible NDF fractions in forages improves fiber digestibility.

Short corn

Hybrid selection is an important management decision and largely influences WPCS nutritive value (Ferraretto and Shaver, 2015). With the removal of brown midrib hybrids from the market by the larger seller, other hybrids improving fiber digestibility would be of great interest. Other hybrid types with different stalk characteristics also exist, including leafy hybrids, although these have less effect on nutrient composition and dairy cow productivity (Ferraretto and Shaver, 2015). Corn hybrids carrying the *brachytic2* (*br2*) mutation are garnering a lot of interest. These hybrids have reduced plant height due to the shortening of internodes and more erect leaves than near-isogenic hybrids (Bourdoncle et al., 2023). The *br2* mutation affects the lignin distribution within the stem of whole-plant corn, increasing NDFD in comparison to conventional hybrids (Bourdoncle et al., 2023; Mastroeni et al., 2026). Interestingly, starch concentration was greater for br2 than conventional hybrids in these studies. A similar DM yield in the study by Mastroeni et al. (2026) must be considered cautiously, however, as plant population was greater for br2 hybrids (13 plants/m^2 vs. 9 plants/m^2) and recommendations may vary for different regions and climates. Feeding trials with br2 hybrids are still limited. Table 1 has a summary of the effects of feeding br2 hybrids to lactating cows. The initial studies (Sarmikasoglou et al., 2025; Catellani et al., 2026) observed increased milk yield (but not ECM), despite the lack or marginal effects on intake. Sarmikasoglou et al. (2026) evaluated a br2 hybrid in diets with approximately 35% or 55% of corn silage inclusion. Overall results were greater intake and ECM, but these results were largely driven by the high corn silage inclusion diet. Initial studies are promising and differ in response to brown midrib trial which often reported increase intake. Further studies are needed to better understand the potential advantages of br2 corn silage, and its effects on rumen dynamics, energy balance, metabolic status, and performance.

Table 1. Effect of feeding whole-plant corn silage harvested at different chop heights to dairy cows.

Trial	DMI, kg/d	Milk, kg/d	ECM, kg/d	ECM Feed Efficiency	Apparent Total Tract NDFD, % NDF
Sarmikasoglou et al., 2025	NS	+1.0	NS	NS	NS
Sarmikasoglou et al., 2026	+0.9	NS	+1.8	NS	+4.4%-units
Catellani et al., 2026	NS	+1.8	NS	NS	NS

Silage hygiene

Discerning the changes occurring after aerobic exposure at feed-out is critical for nutrient preservation and silage hygiene and aerobic deterioration patterns are well-defined in the literature (Borreani et al., 2018). A recent study from our laboratory (Cruz et al., 2025) underscores the need to further understand the effects of yeast counts at ensiling on corn silage quality and nutritive value. Briefly, a meta-analysis was performed

with up to 886 treatment means from 107 peer-reviewed manuscripts to elucidate the effects of yeast counts prior to ensiling on silage quality and nutritive value. Despite having similar pH, lactic and acetic acid concentrations, corn silage with increased yeast counts at ensiling had poorer aerobic stability and reduced NDFD compared with silage ensiled with lower yeast counts. Increased yeast counts and ethanol concentration at opening support our premise that yeast counts at ensiling are concerning for dairy production and suggest a potential direct negative effect on fiber digestibility.

Tabacco et al. (2011) evaluated the relationship of estimates of milk yield per ton of harvested silage DM to mold count after air exposure in corn and sorghum silages. A reduction in estimated milk production was observed as mold count exceeded 4 log cfu/g of silage. Moreover, when the mold count reached or was greater than 8 log cfu/g of silage, milk production was reduced by 50%. These effects were based only on the nutritive value of these forages. Santos et al. (2015) inoculated ruminal fluid with three doses of a pure culture of *Issatchenkia orientalis* (previously known as *Candida krusei*) to determine the effects of yeast contamination on rumen fermentation and reported reduced fiber digestibility. Using a similar approach to Tabacco et al. (2011) with the data by Santos et al. (2015), the milk/ton estimates are reduced by 40 kg/Mg in high compared with low yeast counts (Cappellozza et al., 2025). Therefore, further research is needed to evaluate the effects of other prevalent yeasts on fiber digestibility and other carbohydrate fractions.

Ferulic acid esterase bacterial inoculant

Ferulic acid is the primary inhibitory phenolic acid that impairs ruminal fiber digestion in forages by establishing cross-linkages to polysaccharides through ester bonds and to lignin components mainly through ether bonds. The ferulic acid esterase (FAE) can partially or completely break the ester linkages between lignin and hemicellulose; hence, improving ruminal digestion or increasing the susceptibility of cell walls to ruminal degradation (Nsereko et al., 2008). Silage inoculants that produce FAE could be another option to improve forage quality.

We (Pupo et al., 2026) evaluated the effects of dietary forage concentration and microbial inoculation of corn silage on lactation performance and total tract nutrient digestibility by dairy cows. Cows were allowed 1 wk to acclimate to pen and feeding gates, followed by a 3-wk covariate period. Following the covariate period, cows were enrolled in an 8-wk treatment period during which cows were randomly assigned to 1 of 4 treatments consisting of forage inclusion in the diet, either 48% or 59% of DM, and the microbial inoculation of corn silage containing ferulic acid esterase-producing bacteria (strains of *L. buchneri* and *L. plantarum*) or without microbial inoculation. Regardless of forage concentration, microbial inoculation of corn silage tended to increase component-corrected milk yields in dairy cows. In addition, improved actual and component-corrected feed efficiencies and NDF digestibility were also observed.

Summary

Feeding brown midrib hybrids has been among the most consistent strategies to increase corn silage fiber digestion and milk production by dairy cows. In this context, short-stature corn represents a promising new technology for silage systems, but its full potential has yet to be defined. Among the biological treatment technologies, inoculants may help mitigate issues with silage hygiene and a novel bacterial inoculant containing ferulic-acid esterase increased fiber digestion and milk production by dairy cows in a recent study and may be another tool to aid farmers improve corn silage fiber utilization.

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Diet, Dose and Duration: Practical Nutrition for Mastitis Control

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Introduction

Mastitis remains the most common disease affecting dairy cattle in the United States despite decades of improvement in dairy herd management. On today's dairy farms, the incidence rate of clinical mastitis is 25% (Gonçalves et al., 2022; USDA, 2014). Current mastitis control practices include using intramammary antibiotics at dry off, employee training, and ensuring proper milking procedure. In a survey of 628 dairy producers in Pennsylvania, Florida and Michigan, 94% of producers surveyed believed improving milk quality was important or very important (Kayitsinga et al., 2017). Although clinical mastitis incidence remains at 25%, the widespread adoption of mastitis control practices has shifted the distribution of mastitis pathogens from predominantly contagious (*Staphylococcus aureus*) to environmental pathogens. A study of 8 commercial dairy farms in New York observed that the most common pathogen results from more than 8,000 clinical mastitis cases were culture negative (29.6% of cases) followed by *Escherichia coli* (17.9%), *Streptococcus uberis* (9.9%), *Streptococcus dysgalactiae* (8.7%), *Klebsiella spp.* (6.7%), and *Staphylococcus aureus* (3.1%) (Alanis et al., 2022). The consequence of the shift in mastitis pathogens on dairy farms is that antibiotic treatment is recommended for <30% of non-severe clinical mastitis cases (Ruegg, 2023).

When a cow experiences a clinical case of mastitis, the costs vary depending on the treatment protocol. In an observational study from 37 dairy herds in Wisconsin and 20,625 mastitis cases, the average direct costs (milk discard, antimicrobials) of a clinical mastitis case were $192; however, the costs ranged from $118-$337, which was driven by days of milk discard which is directly related to the duration of antibiotic therapy (Leite de Campos et al., 2023). With costs of mastitis cases increasing, there is sustained interest in alternative approaches to managing mastitis. However, to date, there is little evidence of effective alternatives to antimicrobial therapy and no FDA-approved alternative intramammary treatments for clinical mastitis (Debruyn et al., 2025; Ruegg, 2025). Another avenue for mastitis management on dairy farms is nutritional modulation of the immune system. We are beginning to understand more fully the role nutrition may play in supporting the mammary immune system and the animal's response to disease challenges (Kolar et al., 2025; Krogstad et al., 2024; Swartz et al., 2026).

Nutrition can be a tool for mastitis management, but it must be viewed in context with the cow's situation. We propose a framework where cows are on a gradient that spans from susceptible to resilient (Figure 1). Cows with negative nutrient balance, experiencing more stress, of poorer genetic merit, and greater parity are at greater risk of disease. Nutrition alone cannot mitigate these risk factors, but it may shift cows along this gradient to a more resilient phenotype. This approach demonstrates that no single

mitigation method is a silver bullet or will eliminate mastitis, but some approaches will reduce risk for more animals. Furthermore, specific nutrients affect the immune system in different ways. Some may accelerate or increase immune activity ('gas pedal') while others may dampen these activities ('brake pedal'). Our gas-brake dichotomy is an oversimplification but is an appropriate starting point for understanding the nutritional options at your disposal when considering animal health and mastitis mitigation. This cursory review will introduce nutrients and strategies to manage mastitis in dairy cattle considering nutrients within both the 'gas' and 'brake' categories.

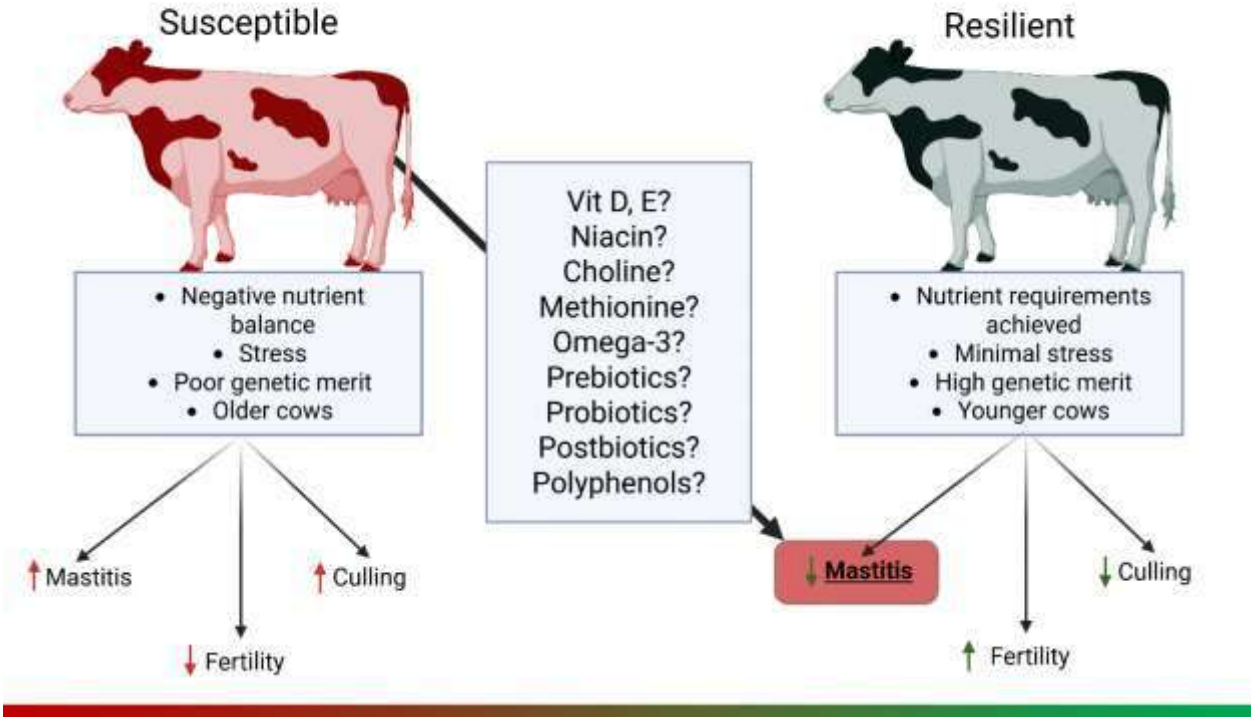


Figure 1. The resilient cow framework when utilizing nutrients to manage animal disease, like mastitis. Cows' risk for disease is a continuous gradient based on risk factors like nutrient balance, stress, genetic merit, and age. Nutrition cannot prevent these risk factors from occurring or prevent mastitis directly, but, perhaps, nutrition can shift cows from a susceptible to a resilient phenotype.

Nutrient Candidates for Modulating Mastitis

'Gas Pedal' Nutrients

Increasing immune activity and functionality is one way to reduce clinical mastitis incidence. The premise for gas-pedal nutrients is that if the cow's immune system is better able to phagocytize cells, perform oxidative burst, and release extracellular traps, it will be better able to rapidly contain mastitis-related bacteria that enter the mammary gland.

Thus, this improved immune functionality, or competence, will increase the response to an initial pathogen exposure, reduce the ability of a pathogen to grow and cause damage, thus reducing the incidence of clinical mastitis cases.

Vitamin E is one of the prominent 'gas pedal' nutrients we feed to dairy cattle. Vitamin E improves immune function by acting as an antioxidant and aiding in maintaining cell membranes and cellular function. Vitamin E is a generic term that includes tocopherol and tocotrienols (NASEM, 2021). RRR-α-tocopherol is the plant-made form and most active form of vitamin E. Forages and oilseeds contain appreciable α-tocopherol, but concentration varies depending on harvest and processing. Heating, wilting, ensiling, and oxygen exposure reduce the α-tocopherol concentrations of feeds (NASEM, 2021). Fresh forages, like pasture, can have substantial, but variable, concentrations of α-tocopherol, which reduce the need for supplemental vitamin E for dairy cows consuming fresh pasture grass as part of their daily diet. NASEM (2021) reduces the required supplemental vitamin E by 50 IU/d for every kg of fresh pasture intake. Adequate intakes of Vitamin E, as outlined in NASEM (2021), are 1.6 IU/kg of BW for dry cows, 3 IU/kg of BW for cows within 3 weeks of calving, and 0.8 IU/kg of BW during the postpartum.

When fed during the peripartum period, Vitamin E increases immune cell antimicrobial functions like oxidative burst (Hogan et al., 1992) and reduces mastitis incidence (Smith et al., 1984; Weiss et al., 1997). Supplementing vitamin E during the peripartum period also reduces SCC (Politis et al., 1995). In studies where vitamin E reduced mastitis, supplemental feeding rates during the prepartum were <3,000 IU/d. When ≥3,000 IU/d of supplemental vitamin E were provided, and successfully reduced mastitis, it was only for the final 2 or 3 weeks prepartum. Bouwstra et al. (2010) supplemented 3,000 IU/d for the entire prepartum period (approximately 8 weeks) and observed an increase in mastitis incidence. Persson Waller et al. (2007) supplemented approximately 3,220 IU/d of vitamin E from 4 weeks prepartum until 2 weeks postpartum and observed no effect on mastitis. The bottom line is that just because some is good, does not mean that more is better. Both vitamin E dose and duration are important considerations in your dietary strategies. Supplementing between 2,000 and 3,000 IU/d for TMR-fed cattle for the final 3 weeks of gestation and meeting the lactation requirement according to NASEM (2021) (approximately 500 IU/d of supplemental Vitamin E) during the initial postpartum period is a reasonable starting point.

Methionine has also received attention and investigation regarding its immunological implications. Methionine alters immune cell function through its role as a methyl donor and ability to modulate mTORC1 signaling (Kelly and Pearce, 2020). Supplementing rumen-protected methionine to dairy cattle increases immune cell phagocytosis and oxidative burst (Osorio et al., 2013; Zhou et al., 2016; Zhou et al., 2018). On the opposite end of the spectrum, research in mice demonstrates that depleting methionine reduced IFN-γ and IL-17 production from T cells, further demonstrating methionine's critical role in the immune response, especially T cell signaling (Roy et al., 2020).

Our team recently conducted an experiment with 36 lactating multiparous cows and supplemented 12 g of metabolizable rumen-protected methionine from 2 different sources during summer heat stress. The control ration provided approximately 86% of estimated metabolizable methionine requirements, while the supplemental diets met requirements (CNCPS v 6.5.5). Cows supplemented with rumen-protected methionine had greater milk protein and reduced milk urea nitrogen concentrations ($P < 0.05$). We also observed that supplying rumen-protected methionine increased somatic cell score (SCS) by 0.50 in mid-lactation cows, further suggesting that methionine supplementation increases immune cell competence (Majewski et al., *In preparation*; Figure 2). After observing increased SCS, we evaluated systemic inflammatory signals, Hp and cytokines, to determine whether a heightened inflammatory status accompanied the increased SCS. The markers of systemic inflammation were similar across treatment groups demonstrating that, although methionine supplementation increased SCS, it did not result in a generalized inflammatory response. We interpreted this response as increased immune competence during heat stress when cows were supplemented with rumen-protected methionine.

Evaluating immune cell functions and inflammatory markers is useful, but eventually, we need to assess actual morbidity and mortality in response to dietary interventions like methionine supplementation. Multiple experiments evaluated how rumen-protected methionine affects disease and culling outcomes; supplementing methionine pre- and postpartum reduced culling of lactating dairy cattle following calving (Leão et al., 2024; Toledo et al., 2023). In these experiments, prepartum supplementation was 5 and 7 g/d of metabolizable methionine and postpartum supplementation was 9 and 16 g/d of supplemental metabolizable methionine. Based on these responses, and the meta-analysis evaluating peripartum rumen-protected methionine supplementation (Zanton and Toledo, 2024), rumen-protected methionine supplementation should begin prepartum at approximately 9 g of metabolizable methionine/day and continue postpartum at 11 g/d to obtain the production and health benefits described herein.

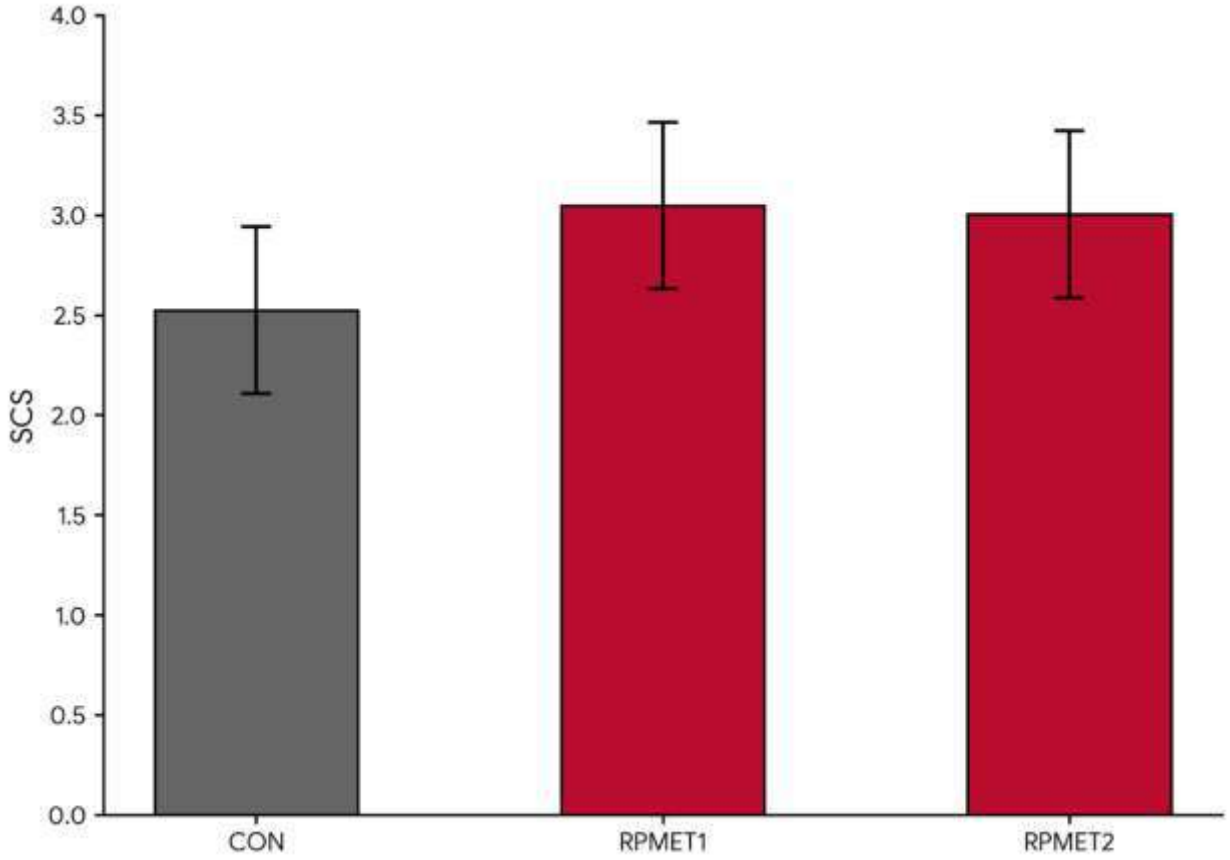


Figure 2. Supplementing rumen-protected methionine to mid-lactation cows experiencing heat stress increased SCS ($P < 0.01$), which may suggest increased immune cell activity or competence. Data from Majewski et al., *In progress*.

In addition to nutrients like vitamin E and methionine, microbials and plant extracts have shown promise in modulating immune responses. Different *Saccharomyces cerevisiae* products have been researched during the peripartum period or during other challenges and demonstrated generally positive effects on animal health outcomes (Kolar et al., 2025; Maina et al., 2024; Rients et al., 2021). Kolar et al. (2025) provided a *Saccharomyces cerevisiae* fermentation product (SCFP) at 19 g/d to lactating dairy cows prior to and during a *Streptococcus uberis* challenge. Supplementing SCFP increased DMI, milk yield, and milk protein from cows with bovine leukemia virus prior to the challenge and had limited effects during the mastitis challenge. The SCFP-supplemented cows tended to have reduced pathogen load in milk ($P = 0.11$) following challenge, which suggests improved antimicrobial functions of the immune system, but this result is tenuous on its own. We also observed that CD14+ cells increased in multiparous cows supplemented with SCFP (Figure 3), suggesting an increase in highly phagocytic immune cells in milk prior to mastitis (Kolar, 2023). The reduced pathogen load in milk and increased phagocytic cell population in milk both suggest enhanced antimicrobial activity in milk when feeding SCFP. On their own, these results are interesting but become more compelling when viewed in context with other literature. Yuan et al. (2015) observed that

providing a hydrolyzed yeast product (0, 30, 60, or 90 g/d) to transition cows increased IgG following an ovalbumin challenge and increased fecal IgA, suggesting an improved humoral and mucosal immune response. The mRNA abundance of myeloperoxidase and neutrophil elastase increased in uterine samples when supplementing the hydrolyzed yeast, further demonstrating the immune-promoting effects of yeast products. In calves, Mahmoud et al. (2020) observed that SCFP-supplemented calves had reduced clinical disease scores and improved lung pathology following a respiratory disease challenge. They also observed enhanced phagocytosis of lung macrophages and increased inflammatory cytokine production from peripheral blood mononuclear cells when supplementing SCFP. *Saccharomyces cerevisiae* and its derivatives have demonstrated promise for increasing immune activity; effects in calves are compelling and effects in dairy cows are more modest, but still potentially beneficial. Additional, replicated on-farm research investigating morbidity and mortality is needed to demonstrate external validity of these research findings.

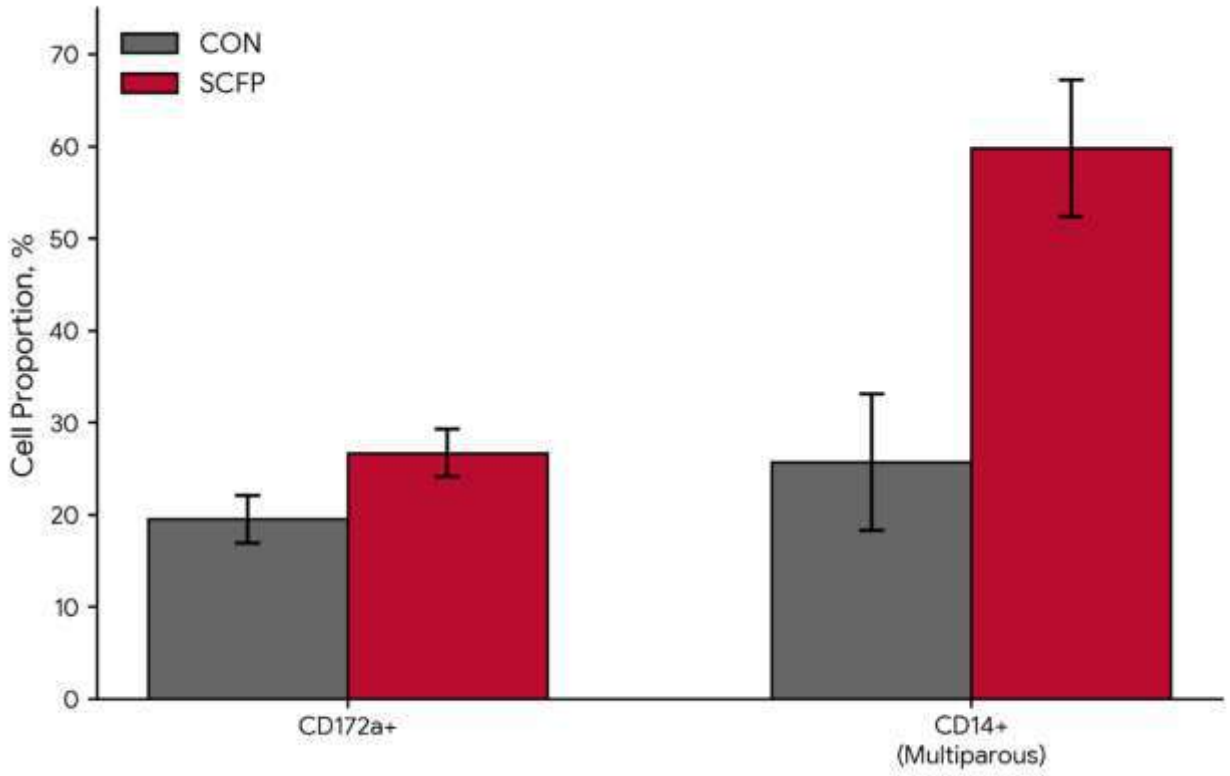


Figure 3. Effects of supplementing *Saccharomyces cerevisiae* fermentation product (SCFP) on milk immune cell proportions in lactating dairy cows. SCFP increased CD172a$^+$ cells (P = 0.07) and CD14$^+$ cells in multiparous cows (Lactation ≥ 2; P = 0.03) prior to an *S. uberis* mastitis challenge.

'Brake Pedal' Nutrients

As an alternative to the 'gas pedal', we can also use the brakes. Brake pedal nutrients may reduce the extent or severity of immune cell functions, thereby avoiding the worst damage resulting from an immune response. It also may regulate an overactive

immune response. Brake pedal nutrients are also nutrients that increase anti-inflammatory and resolution-promoting signals, which heal tissues.

Omega-3 fatty acids are a promising brake-pedal nutrient. Omega-3s receive attention in both human and animal health literature for their potential health-promoting effects. They reduce translocation of transcription factors that promote inflammation, thereby reducing cytokine signaling; they also are upstream metabolites for anti-inflammatory lipid molecules known as oxylipids (Sordillo, 2018). It is challenging to provide omega-3 fatty acids to dairy cows because of the biohydrogenation in the rumen. Still, omega-3s can be provided through Ca-salts of fatty acids or oilseeds like flax.

In a pen-study, supplementing flaxseed, at 5% of diet DM, and reducing dietary omega-6:omega-3 ratio from 12.6 to 1.7 in diets fed to dairy cattle reduced SCC and overall mortality following calving (Moallem et al., 2020). The immune-modulating functions of omega-3 were also observed in a series of studies which demonstrated that abomasal infusion of up to 6 g/d of omega-3 fatty acids reduced SCC in healthy cows, increased anti-inflammatory oxylipids, and altered leukocyte functions (Hoorman et al., 2026; Myers et al., 2026; Reisinger et al., 2026). Interestingly, the effects of omega-3 supplementation on leukocyte function differed for gram-negative and gram-positive bacteria. Increasing omega-3 fatty acids increased phagocytosis when cells were stimulated with gram-negative, but not gram-positive, bacteria. The gram-stain-specific effects require closer scrutiny. These data also demonstrate that our gas-brake dichotomy is imperfect: although omega-3 may reduce some markers of the immune response, it may increase some immune cell activities, like phagocytosis of gram-negative bacteria.

Although the immune-modulating effects of omega-3 are promising, production responses need further evaluation. Researchers supplementing a fish oil Ca-salt (enriched with eicosapentaenoic and docosahexaenoic acid) to dairy cattle starting at 14 DIM observed increased milk yield and milk fat yield with increasing omega-3 intake from 63 g to 77 g/d. In this experiment, they replaced an omega-6 fatty acid source (Ca-salt of safflower oil) with a Ca-salt of palmitic acid and a Ca-salt of fish oil. Overall, this strategy reduced the omega-6:omega-3 ratio from 5.9 to 3.9 (Greco et al., 2015). Their dietary approach of slightly increasing palmitic acid while increasing omega-3 must be taken into account as supplying palmitic acid increases milk fat yield during the postpartum period (de Souza and Lock, 2019). Other investigators observed that increasing omega-3 by feeding 5%-10% of the diet DM as flaxseed (reduced omega-6:omega-3 ratio from 8-12 to 1-1.7) reduced milk fat yield by 100-140 g/d (Moallem et al., 2020; Zachut et al., 2010). Most of the omega-3 delivered by flax seed is linolenic acid (C18:3), while the other studies focused on longer-chain and more unsaturated fatty acids such as docosahexaenoic (C22:6) and eicosapentaenoic acid (C20:5). Supplementing an algae product, targeting 10 g/d of supplemental docosahexaenoic acid, starting at 27 d postpartum, increased milk and milk protein yield by 0.9 kg/d and 20 g/d, respectively. In the same study, milk fat yield was reduced by 40 g/d when supplementing the algae product (Sinedino et al., 2017). The reduction in milk fat production was also observed by Miller (2024), who targeted 18 g/d of supplemental omega-3 prepartum and 26 g/d postpartum. They observed a 180 g/d reduction in milk fat yield and a 0.25% reduction in

milk fat concentration when supplementing omega-3 from a Ca-salt. Miller (2024) also reduced palmitic acid supply by approximately 20 g/d and oleic acid supply by 5 g/d when feeding the omega-3-enriched supplement, which challenges the interpretation of the milk fat response.

The dose of omega-3 may be critical and a potential example of when less is more. Co-supplementation of rumen-protected methionine and omega-3 fatty acids, through a Ca-salt fed at 1.8% of diet DM (targeted 10 g/d prepartum and 18 g/d postpartum of supplemental omega-3; achieved 1 g/d and 6 g/d of supplemental omega-3 based on fatty acid analysis), increased DMI by 1.2 kg/d, energy-corrected milk yield by 2.4 kg/d, and increased milk fat yield by 120 g/d after calving. In a companion study, they observed that providing omega-3 altered oxylipid profiles, phospholipid profiles, and the liver functionality index (France et al., 2026). Supplementing omega-3 fatty acids may improve milk yield and reduce inflammatory responses, but it also may reduce milk fat yield. The timing and dose of omega-3 supplementation require further investigation to optimize both immune and production responses from lactating dairy cows.

Niacin, or vitamin B3, is nearly ubiquitous across metabolism as it is part of the NAD and NADH coenzymes which are part of glycolysis, the Krebs cycle, and the electron transport chain. The rumen provides approximately 2.5 g/d of niacin to a cow consuming 25 kg/d of DM (NASEM, 2021). Additional niacin, beyond physiological amounts, provides benefits to the cow like reducing inflammation by reducing cytokine secretion and reducing lipolysis around calving (Lipszyc et al., 2013; Wanders et al., 2013; Yuan et al., 2012). Therefore, niacin has the potential to improve the health of dairy cows by reducing inflammatory responses to diseases like mastitis.

We conducted an experiment where we supplemented 26 g/d of a rumen-protected niacin product (Microtinic PP; Vetagro), targeting 6 g/d of bioavailable niacin, during an LPS mastitis challenge. Supplementing rumen-protected niacin increased circulating niacin metabolites by 40%, indicating our dose of niacin increased the circulating pool of niacin or its metabolites. Prior to the LPS challenge, SCS was reduced from 1.24 to 0.05 ($P < 0.01$) when supplementing rumen-protected niacin; similar SCS reductions have been observed previously by other researchers, demonstrating that supplementing rumen-protected niacin may reduce immune cell trafficking into healthy mammary glands (Morey et al., 2011; Yuan et al., 2012). We also observed 30% reductions in plasma Hp and LBP (Figure 4) for niacin-supplemented cows following the LPS challenge, while SCS in the LPS-challenged quarter was similar for both control and niacin-supplemented cows (Krogstad et al., 2024). Our data suggest that the systemic response to LPS-induced mastitis was dampened, while the local response in the mammary gland was maintained with rumen-protected niacin supplementation. We conducted a follow-up experiment where an identical dose of niacin from the same product was supplemented during the first 21 d of lactation. Neither Hp nor SCS was affected by niacin supplementation, but milk yield increased in 1st and 2nd parity cows by 670 kg over the course of their lactation (Krogstad et al., 2025). The findings of a recent meta-analysis concur with our results as rumen-protected niacin supplemented during lactation increased energy-corrected milk yield by 0.7 kg/d (Arshad et al., 2025). The current literature demonstrates that providing

approximately 6 g/d of bioavailable niacin during lactation may reduce inflammation and SCS, while slightly increasing milk yield.

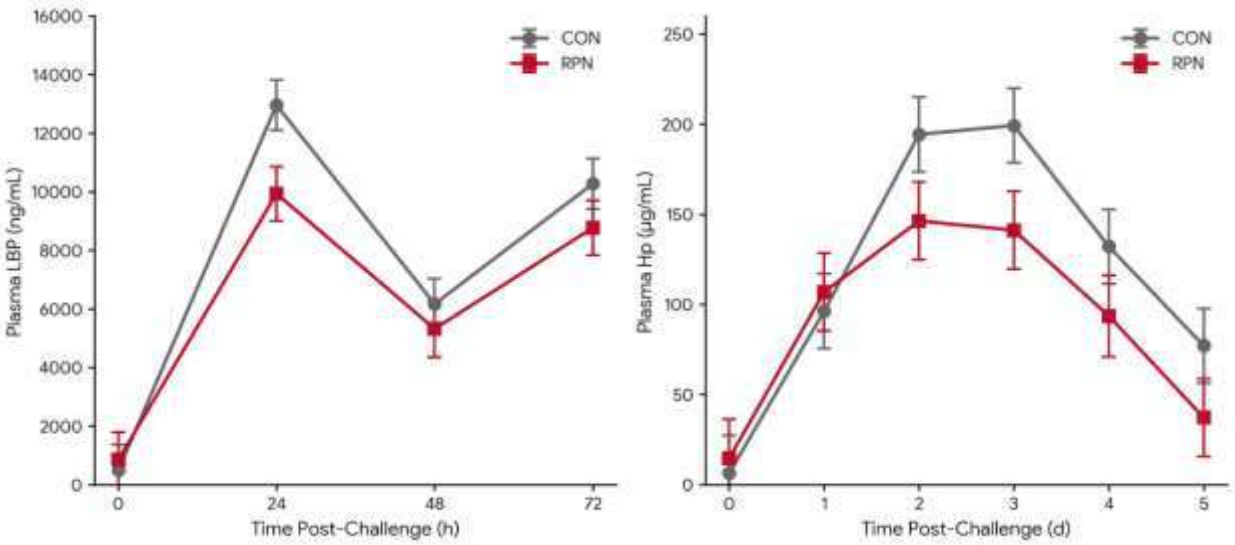


Figure 4. Plasma acute-phase protein responses in dairy cows supplemented with rumen-protected niacin (RPN) during an intramammary lipopolysaccharide (LPS) challenge. The RPN tended to reduce LBP concentrations at 24 h post-challenge (P = 0.08). Plasma haptoglobin (Hp) concentrations tended to be reduced by 30% following an intramammary LPS challenge (P = 0.08). Adapted from Krogstad et al., 2024.

Plant bioactive molecules can modulate inflammatory responses, but there are thousands of unique compounds, some of which have data to support their effects and some that do not have rigorous data (Olagaray and Bradford, 2019; Zachut et al., 2022). β-caryophyllene (BCP) is one compound that has garnered interest. BCP modulates the immune system through binding with the cannabinoid 2 receptor (CB2), which is part of the endocannabinoid system that modulates immune and metabolic physiology of dairy cattle (Zachut et al., 2018; Zachut et al., 2025). BCP binds to this receptor with similar affinity to endogenous cannabinoid molecules and reduces LPS-stimulated IL-1β and TNF-α in human peripheral blood (Gertsch et al., 2008). It also may reduce cell adhesion and migration, which may reduce excess inflammatory responses (Andrade-Silva et al., 2016), and modulate immune pathways in dairy cattle (Butenko et al., 2026). We evaluated BCP supplementation at a rate of 5 mg/kg of BW to lactating cows undergoing an LPS-induced mastitis challenge. Supplementation of BCP reduced Hp area under the curve and tended to reduce composite SCS (Figure 5; Krogstad et al., *In review*). The LPS-challenged quarter SCS was not affected by BCP, indicating that BCP did not affect the mammary gland's acute response to challenge. Additionally, BCP reduced body temperature area under the curve following the 1st LPS challenge. One potential limitation for use of BCP is that it tended to reduce DMI following the first LPS challenge. The mechanism for this deserves greater investigation before widespread adoption of BCP as an immunomodulatory feed additive.

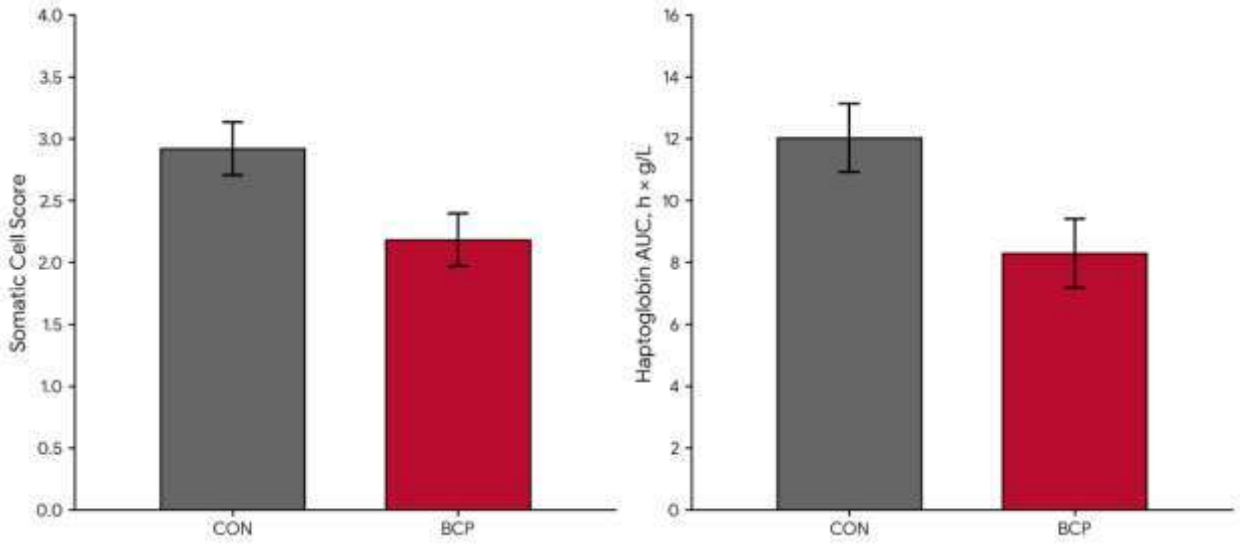


Figure 5. Effects of β-caryophyllene (BCP) supplementation on composite somatic cell score (SCS) and plasma haptoglobin area under the curve (AUC) in dairy cows following an intramammary lipopolysaccharide (LPS) challenge. Supplementation with BCP reduced SCS post-challenge and the total area under the curve for plasma haptoglobin (h × g/L; $P \leq 0.03$).

Summary

Nutrition does not prevent mastitis, but, as we proposed, it may increase the resilience of cows to clinical mastitis. The nutrients that reduce mastitis risk come in different flavors; some nutrients increase immune functions and activities, while others may reduce it or divert immune function to a different path. This dichotomy is an oversimplification but serves as a useful starting point for understanding these bioactive nutrients and compounds. Understanding how these nutrients impact the immune system is critical to understanding when they may be most useful for dairy cattle. Methionine and Vitamin E have strong evidence for increasing immune function and altering mammary health, but, for vitamin E, dose and duration of supplementation must be considered. Vitamin E responses for TMR-fed cattle are best when 1,000-3000 IU/d are provided in the final 2-3 weeks prior to calving and 500-1,000 IU/d are supplied after calving. Providing 9 g/d of metabolizable methionine prepartum and 11 g/d postpartum may improve immune competence and increase milk production. Yeast products, whether pro- or post-biotics, may enhance immune function, especially in pre-weaned calves, but responses are more subtle in mature cows. Review literature closely if you're considering these yeast-based products and feed them according to label instructions. Omega-3 fatty acids and niacin reduce inflammatory signals, SCC in milk, and may improve milk production. The risk of omega-3 supplementation is reduced milk fat production, especially at greater doses or when provided through flaxseed supplementation. Providing 10 g/d or less of omega-3 fatty acids through a Ca-salt may mitigate the risk for milk fat reductions. Another strategy may be supplementing omega-3 fatty acids during the prepartum only, which may provide anti-inflammatory benefits without reducing milk

fat yield once lactation begins. Niacin appears most beneficial when provided postpartum at a rate of 6 g of bioavailable niacin per day. Follow-up investigations evaluating the timing of both omega-3 fatty acids and niacin would be useful in confirming these hypotheses. Plant-derived compounds are numerous, but very few have data in support of, or against, their use to mitigate mastitis. Early data for BCP, a plant-derived cannabinoid, when supplemented at a rate of 5 mg/kg are positive, but the reductions in DMI when supplementing BCP require further investigation. The bottom line is that nutrition is a tool to manage or mitigate mastitis challenges on dairy farms, but you must get the details right—ensure your dose and duration are optimized for the supplement you're feeding.

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Practical Assessment of TMR Mixing Quality in Dairy Farms

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Introduction

Total mixed rations (TMR) are designed to provide dairy cows with a consistent supply of nutrients by combining forages, concentrates, minerals, vitamins, and other feed ingredients into a single mixture. Conceptually, the objective is straightforward: every portion of feed delivered to the bunk should approximate the diet the nutritionist formulated. In practice, however, achieving this objective is challenging because a TMR contains ingredients that differ substantially in particle size, density, moisture, shape, inclusion rate, and other physical characteristics. Consequently, formulation accuracy alone does not guarantee that cows receive the diet that was formulated.

This distinction is particularly important when considering the progression from the formulated diet, to the diet prepared and delivered on the farm, and ultimately to the diet consumed by the cow. Adequate TMR mixing is required to minimize differences among these diets and provide a consistent nutrient supply to the rumen (Sova et al., 2014; Santos et al., 2026). Inadequate mixing may create spatial variation in nutrient concentration within a load, meaning that cows eating from different locations along the feed bunk may effectively be offered diets with different nutrient compositions.

Mixing quality should therefore be viewed as part of precision feeding rather than simply as a mechanical characteristic of the mixer. Precision nutrition requires not only an appropriately formulated diet but also management practices that translate that formulation into the feed consumed. On commercial farms, differences between formulated and delivered diets may originate from variation in forage composition and dry matter, ingredient weighing errors, loading sequence, mixer condition, load size, mixing time, and feed delivery (Sova et al., 2014; Turiello et al., 2018) .

The feed-manufacturing literature provides a useful conceptual foundation for understanding these challenges. The objective of mixing is to distribute ingredients and nutrients uniformly, and the central measurement in a mixer test is therefore the variation among samples of the finished mixture. Mixer performance can be influenced by ingredient particle size and shape, density, sequence of ingredient addition, mixer design, mixing time, fill level, cleanliness, and equipment wear (Baker and Herrman, 2002) . Large differences in particle size among ingredients can additionally promote segregation after mixing.

These principles become considerably more complicated in dairy TMR than in conventional dry feeds. A dairy TMR may simultaneously contain long forage particles, finely ground grain, wet fermented feeds, dry concentrates, liquids, minerals, and

ingredients included at very low concentrations. Thus, mixing does not necessarily involve particles with similar physical properties, and achieving uniformity for one component of the ration does not necessarily demonstrate uniformity for every other component.

Many mechanical and management factors can contribute to TMR variation. Previous TMR audits have identified equipment wear, inadequate mixing time after adding the final ingredient, inappropriate load size, an unlevel mixer, loading position, forage processing, loading sequence, liquid distribution, auger speed, and mixer settings as potential sources of variation (Stone and Oelberg, 2016). Importantly, mixing time cannot be assumed from a generic recommendation because the time required to produce a homogeneous mixture depends on mixer design, load characteristics, and operating conditions (Groesbeck et al., 2007; Stone and Oelberg, 2016). Mixer performance testing therefore remains an important means of establishing whether the mixing process produces the desired degree of uniformity (Herrman and Behnke, 1994).

The practical difficulty is determining how to measure mixing quality. Traditionally, dairy consultants have relied heavily on physical evaluation of TMR particle distribution. The Penn State Particle Separator (PSPS; Lammers et al., 1996), for example, provides a practical method for characterizing the proportions of feed retained on different sieves. Fresh TMR samples can be collected before cows have a chance to sort the ration, and particle distribution can then be compared across locations along the bunk.

Stone & Oelberg (2016) proposed collecting 10 TMR samples along the feed bunk and evaluating variation in PSPS fractions as an on-farm assessment of mixing consistency. Their approach highlights an important principle: a mixer evaluation should not depend on a single grab sample. Instead, samples should represent the progression of the load as it is discharged and delivered. Similar principles have long been used in feed-manufacturing mixer evaluations, where multiple samples collected at predetermined locations or intervals during mixer discharge are used to quantify variation in the finished mixture (Herrman and Behnke, 1994; Groesbeck et al., 2007).

However, the PSPS also illustrates a fundamental challenge in evaluating TMR mixing: the measurement itself can contribute to variation. Particle-separation results may be influenced by shaking intensity, stroke length, operator, sample moisture, and other procedural factors (Spanghero, 2002). Santos et al. (2026) noted that coefficients of variation (CV) as high as 30 to 35% have been reported for material retained on the upper PSPS sieve. Consequently, variation among samples may reflect a combination of true TMR heterogeneity and variability introduced by the measurement procedure itself (Turiello et al., 2018). This issue becomes even more important when CV is used to classify mixer performance. In dry-feed manufacturing, a CV below approximately 10% has traditionally been interpreted as evidence of adequate mixing, whereas progressively greater CV values may indicate the need to evaluate mixing time, ingredient addition, equipment condition, or mixer fill (Herrman and Behnke, 1994). Salt has historically been used as a tracer in mixer tests because it is normally included at a

relatively low concentration and chloride can be measured relatively inexpensively (Herrman and Behnke, 1994).

Yet a universal CV threshold assumes the marker behaves consistently. That assumption may not always be valid. A marker may exhibit high variability because the ration is poorly mixed, but it may also exhibit high variability because of its concentration, physical characteristics, natural occurrence in other ingredients, analytical error, or sampling methodology. Therefore, comparing CV values among different markers without considering their characteristics may lead to incorrect conclusions about mixer performance. This distinction is particularly relevant for TMR because the feed matrix is substantially more heterogeneous than a conventional dry concentrate mixture. Consequently, the question on dairy farms is not simply “What is the CV?” It is first “What should we measure?” A practical method for assessing TMR mixing requires a marker that responds to spatial heterogeneity in the ration while contributing as little intrinsic sampling and analytical variation as possible. This distinction provided the rationale for evaluating nutritional components as potential markers of TMR mixing quality.

A First Assessment of Nutritional Markers for TMR Mixing on Dairy Farms

Santos et al. (2026) addressed this question directly by evaluating nutritional markers that could be used to characterize mixing quality under commercial dairy conditions. The study was based on the premise that a useful marker should capture variation associated with the distribution of feed along the bunk while remaining relatively insensitive to systematic variation among days, feedings, or diets.

Experimental approach

The experiment was conducted on a commercial dairy farm over five consecutive days. Three TMR were evaluated, representing high-producing lactating cows, mid-lactation cows, and dry cows. Each diet was evaluated during three daily feedings, and 10 incremental samples were collected from equidistant positions along the feed bunk immediately after feed delivery and before cows had access to the feed (Santos et al., 2026).

Rather than simply calculating a CV for one conventional marker, the study evaluated several nutritional components. Potential markers included trace minerals, macrominerals, and major ration components. The statistical approach partitioned variation into systematic sources associated with diet, day, and feeding, and residual variation associated primarily with sampling locations along the feed bunk. The underlying rationale was that an effective marker should have a high proportion of its total variance represented by residual variation—the variation that could reveal differences in ingredient distribution among locations—while showing relatively little variation associated with day or feeding (Santos et al., 2026).

We then used the CV of each nutrient within diet as a complementary measure of consistency. This distinction is important. A nutrient could theoretically vary substantially among sampling locations and therefore be sensitive to mixing, but still be a poor practical marker if it also showed excessive intrinsic variability. Consequently, Santos et al. (2026) established criteria combining a high proportion of residual variance, preferably greater than 90%, with a relatively low CV, ideally less than 10%.

Which markers worked?

The evaluated components behaved very differently. The proportion of total variance represented by residual variation was 99.1% for potassium (K), 96.0% for crude protein (CP), 95.7% for ether extract (EE), 94.3% for zinc (Zn), 93.0% for phosphorus (P), 92.8% for neutral detergent fiber (NDF), 91.3% for magnesium (Mg), 81.9% for manganese (Mn), 79.3% for iron (Fe), 77.9% for calcium (Ca), and 77.4% for sodium (Na) (Santos et al., 2026).

However, looking only at the proportion of residual variance would have produced an incomplete conclusion. Zinc, for example, had a high residual-variance proportion but a relatively high CV. Manganese and Fe also presented characteristics that reduced their usefulness as practical markers. In contrast, Mg combined a high residual-variance proportion of 91.3% with a CV of 9.5%, making it the most consistent mineral marker evaluated (Santos et al., 2026)

Potassium also exhibited a very high proportion of residual variance (99.1%), supporting its potential value as a marker. However, its practical application is complicated by the fact that K occurs naturally at relatively high and variable concentrations in forage ingredients. Consequently, changes in forage K concentration could contribute variation unrelated to mixer performance. Santos et al. (2026) therefore considered Mg more attractive from a practical perspective.

Interestingly, conventional feed-analysis measurements also performed well. NDF and CP had residual-variance proportions of 92.8 and 96.0%, respectively, together with relatively low CV values of approximately 9%. Their performance was therefore comparable to that of Mg (Santos et al., 2026). This observation has substantial practical relevance because commercial feed laboratories routinely measure NDF and CP, and dairy nutritionists are already familiar with them.

These findings suggest that a dairy nutritionist troubleshooting TMR mixing may not necessarily need an unusual external tracer. Nutrients already routinely measured in feed samples may contain useful information about mixing consistency, provided that sampling is conducted systematically and the intrinsic characteristics of the marker are considered.

Practical interpretation

The study therefore shifts the interpretation of a TMR mixing test from simply asking whether the CV is below an arbitrary threshold to asking whether the marker itself is appropriate for the matrix being evaluated. The conventional 10% CV threshold can provide a useful reference, but CV should not be interpreted independently of the marker, sampling strategy, and characteristics of the ration.

Santos et al. (2026) ultimately identified Mg as the most promising mineral marker, with CP and NDF also showing strong potential as practical indicators of TMR mixing quality. These results should nevertheless be interpreted within the scope of the experiment. The evaluation was conducted on a single well-managed commercial dairy using one horizontal mixer over a five-day sampling period. The study also did not deliberately create poor-mixing conditions. Therefore, additional validation is necessary across mixer designs, ration types, mixing times, loading procedures, and farms before universal thresholds can be established (Santos et al., 2026).

This last limitation is particularly important. Identifying a marker that behaves consistently in a well-mixed TMR is the first step. A useful diagnostic marker must ultimately also be sensitive to differences in mixing quality. In other words, it should remain relatively stable when mixing is adequate but respond when the ration becomes less homogeneous

The work of Santos et al. (2026) provides an important first step toward a more objective assessment of TMR mixing on dairy farms. However, it also raises another practical question. What if the TMR mixer is not the original source of the variation? Many dairy TMR contain a grain or concentrate mixture prepared separately, either on the farm or by a commercial feed mill. If that concentrate is already heterogeneous when it enters the TMR mixer, even excellent TMR mixing may not completely compensate for inconsistent nutrient distribution upstream.

What About Grain-Mix Quality?

A TMR mixing investigation should therefore not automatically begin and end with the mixer wagon. You must also consider the consistency of the ingredients entering the mixer. This is particularly important for grain and concentrate mixtures containing minerals, vitamins, amino acids, feed additives, or other low-inclusion ingredients. If these components are not uniformly distributed during concentrate manufacture, the TMR mixer receives an ingredient that is itself heterogeneous.

The principles governing concentrate mixing are similar to those for TMR, but the physical system is different. Dry-feed mixer evaluation has traditionally relied on a low-inclusion tracer and multiple samples collected throughout the batch or mixer discharge. Salt, for example, has commonly been used because of its relatively low inclusion rate and the ease of measuring chloride (Herrman and Behnke, 1994). Nevertheless, marker

selection remains critical, and commonly used CV thresholds do not necessarily account for the inherent behavior of different markers.

Vidal et al. (2025) addressed this issue by developing a new protocol specifically for evaluating concentrate-ration homogeneity in commercial feed mills. Their objectives were threefold: to identify suitable mineral markers, establish a simplified but reliable sampling protocol, and develop a practical statistical approach for evaluating marker dispersion.

Experimental approach

The study was conducted under routine operating conditions in a commercial feed mill in Brazil. Four commercially available horizontal mixers were evaluated. Samples, referred to as increments, were collected sequentially during mixer emptying over five consecutive days. Ten increments were obtained from each batch, resulting in 200 increments across the experiment (Vidal et al., 2025).

Nine minerals were evaluated as potential markers: Ca, P, K, Na, Mg, Fe, Zn, copper (Cu), and Mn. Mineral concentrations were determined using established analytical procedures, and the authors evaluated variation at three levels: among mixers, among batches within a mixer, and among increments collected during mixer discharge (Vidal et al., 2025).

These sources of variation provide information about different characteristics of the mixing process. Variability among mixers can indicate reproducibility, whereas variability among batches within the same mixer represents repeatability. Variation among increments during discharge reflects the uniformity of marker distribution within the mixture itself (Vidal et al., 2025). This framework highlights a critical characteristic of a useful mixing marker. When the mixing process is known or assumed to be adequate, an ideal marker should show little variation between mixers, between batches, and between increments. A marker with substantial intrinsic variation could falsely suggest poor mixing even when the mixer is functioning properly.

Potassium and magnesium emerged as the strongest markers

The minerals behaved differently across these three dimensions of variability. Several performed adequately in individual dimensions, but only K and Mg simultaneously showed low variability among mixers, among batches, and among sampling increments (Vidal et al., 2025). Consequently, these two minerals were selected as the most suitable markers for evaluating concentrate-ration homogeneity.

Measures of location supported this interpretation. Although the overall concentration range across mixer-emptying positions did not indicate significant differences for the evaluated minerals, K and Mg had particularly narrow ranges and low variability. Phosphorus also showed a relatively narrow range but greater between-

increment variability, reducing its value as a marker relative to K and Mg (Vidal et al., 2025).

This finding is particularly interesting when considered alongside Santos et al. (2026), because Mg emerged as a strong marker in two substantially different feed matrices. Santos et al. evaluated wet, forage-based TMR delivered along a dairy feed bunk, whereas Vidal et al. (2025) evaluated dry concentrate mixtures manufactured in commercial horizontal mixers. Despite these differences, Mg demonstrated characteristics desirable in a mixing marker in both studies. Vidal et al. (2025) additionally identified K as an effective marker for concentrate mixtures.

The study also questioned the assumption that routine mixer evaluation always requires a large number of samples. Conventional homogeneity-testing protocols commonly rely on approximately 7 to 10 increments per batch. Vidal et al. (2025), however, noted that these recommendations have limited empirical support and may not adequately account for marker characteristics, sampling dimensionality, or interactions between the marker and the sampling procedure.

A practical two-sample protocol

Based on the observed behavior of K and Mg, Vidal et al. (2025) proposed a substantially simplified procedure requiring only two increments from each concentrate batch. The samples are collected at the second and eighth tenths of the mixer-emptying time.

For example, if complete discharge of a mixer requires 600 s, samples would be collected approximately 120 and 480 s after discharge begins. These sampling points are symmetrical relative to the beginning and end of mixer emptying and are sufficiently separated to represent early and late portions of the discharge (Vidal et al., 2025). Instead of calculating a CV from numerous samples, the concentration of K or Mg is determined in the two increments, and the ratio between the higher and lower concentrations is calculated

$$Homogenity\ ratio = \frac{highest\ marker\ concentration}{lowest\ marker\ concentration}$$

A ratio <1.26 was proposed as evidence of an adequately homogeneous concentrate mixture. When the ratio is ≥1.26, the concentrate cannot be considered adequately mixed according to the proposed protocol, indicating that the mixing process or equipment should be reevaluated (Vidal et al., 2025).

The simplicity of this approach is particularly attractive for commercial applications because analytical cost and sampling effort are major limitations to routine mixer testing. Reducing the number of samples from approximately 10 to two while

maintaining a defined sampling strategy could make periodic evaluation of concentrate mixing considerably more feasible.

However, important boundaries limit its application. Vidal et al. (2025) emphasized that the procedure is based on one-dimensional sampling during mixer discharge. It should therefore not simply be transferred to three-dimensional sampling of material inside a mixer compartment or storage bin. In addition, the authors recommended that the protocol not be applied to batches exceeding 2.5 metric tonnes without additional validation.

Connecting concentrate quality back to the dairy TMR

Together, the studies of Santos et al. (2026) and Vidal et al. (2025) suggest that troubleshooting feed uniformity should be viewed as a sequence rather than as a single mixer test. Variation observed along the dairy feed bunk can originate during TMR preparation and delivery, but variation may already exist in an ingredient before it reaches the TMR mixer. This is particularly relevant for grain mixtures containing minerals and other low-inclusion nutritional components.

Thus, when a TMR mixing audit identifies unexpected variability, the practical question should not immediately be “What is wrong with the mixer wagon?” Instead, the investigation should move systematically through the feeding system. Are the ingredients themselves consistent? Is the grain mix homogeneous? Are ingredients being weighed accurately? Are they being loaded appropriately? Is mixer fill appropriate? Is mixing time adequate? Is the mixer mechanically functioning as intended? And, finally, is the finished TMR distributed consistently throughout discharge and along the feed bunk?

An important limitation of the available information is that marker consistency under adequate mixing does not necessarily demonstrate sensitivity to inadequate mixing. Therefore, the next step was to deliberately challenge the mixing process and determine which measurements distinguish adequately from inadequately mixed TMR.

Can We Detect the Difference Between Poorly and Adequately Mixed TMR?

The studies described above provided a framework for selecting potential markers and evaluating their variability, but one important question remained unresolved: can these measurements distinguish inadequately mixed TMR from adequately mixed TMR? This is particularly relevant because Santos et al. (2026) conducted their study under normal management conditions and did not deliberately impose poor mixing. Thus, although Mg, CP, and NDF emerged as promising markers based on their variance characteristics and CV, their sensitivity to a controlled change in mixing quality was not directly evaluated.

We therefore conducted a subsequent experiment at the William H. Miner Agricultural Research Institute to deliberately generate TMR with contrasting degrees of

mixing. The study incorporated concepts developed from Santos et al. (2026) and Vidal et al. (2025), while expanding the evaluation to include both chemical composition and physical particle distribution. We also aimed to distinguish two related but different characteristics of an effective mixing marker: its ability to detect systematic changes in composition during mixer discharge and its ability to detect differences in the variance of that composition.

Experimental design and sample collection

We evaluated two diets representative of common dairy feeding situations: a lactating-cow TMR and a dry-cow TMR. We prepared each diet using two mixing times: a deliberately short mixing time intended to produce an inadequately mixed ration and a longer mixing time representing adequate mixing. Thus, unlike the previous observational work, the experiment was designed to create a controlled contrast in mixing quality.

The procedure was repeated over five days to account for day-to-day variation in the mixing process. For each diet, mixing time, and day, 10 samples were collected directly from the conveyor at evenly spaced intervals during TMR discharge, resulting in 200 original TMR samples (2 diets × 2 mixing times × 5 d × 10 sampling positions).

Each original sample was divided into two subsamples that were independently processed by two operators, resulting in 400 analytical subsamples. This step was included because operator technique may contribute to variation in PSPS measurements (Spanghero, 2002; Santos et al., 2026) . Each subsample was evaluated using the Penn State Particle Separator (PSPS) for particle-size distribution and a portable near-infrared spectroscopy system (NOA) for chemical composition.

Because paired subsamples originated from the same TMR sample, operator measurements were not considered independent experimental replicates. We instead evaluated potential operator-related variation descriptively by plotting paired differences between operators against processing time for each day and response variable. Overall mean responses were generally similar between operators, with paired differences centered near zero and no strong systematic directional bias. However, the magnitude of variation differed between operators, with one operator generally producing more variable measurements than the other, particularly for PSPS fractions. In addition, interoperator differences tended to become larger later in the daily processing sequence for some variables. Although the cause of this pattern cannot be determined from the present experiment, it may reflect changes in operator consistency as repeated measurements accumulated, potentially associated with fatigue or other time-dependent aspects of sample processing. This consideration may be particularly relevant for PSPS measurements, which require repeated manual shaking and therefore depend on maintaining a consistent shaking intensity, stroke, and technique throughout the analytical session. These observations emphasize that operator training alone may not be sufficient; standardized technique and appropriate working conditions should also be maintained throughout sample processing to minimize operator-associated variance.

Accordingly, when large numbers of samples are evaluated, attention should be given to operator training, consistency of the shaking procedure, workload, and the potential for measurement precision to deteriorate during prolonged analytical sessions. Despite these differences in measurement variability, the absence of a substantial overall directional bias between operators supported averaging the paired measurements and treating the original TMR sample as the observational unit in subsequent analyses.

Statistical approach: separating changes in means from changes in variance

Lactating- and dry-cow diets were analyzed separately because preliminary evaluation indicated differences in the magnitude and structure of variation between diets. The statistical approach expanded upon Santos et al. (2026) and Vidal et al. (2025) and consisted of two sequential analyses evaluating (1) changes in mean concentration or particle fraction during discharge and (2) variation around those expected means.

For the first analysis, we used a mixed model with mixing time and sampling order nested within mixing time as fixed effects. Day was included as a random effect, and sampling order was treated as a repeated measurement within mixing-time × day. This analysis determined whether the overall mean differed between mixing times and, more importantly, whether marker concentration changed during discharge. An effective marker of mixing homogeneity should show little or no sampling-order effect after adequate mixing but greater variation across sampling positions when the TMR is intentionally undermixed.

Because markers differed substantially in units and magnitude, LSMeans at each sampling position were standardized relative to the overall mean of the corresponding marker:

$$Relative\ mean = \frac{LSMean\ at\ sampling\ position}{overall\ mean}$$

A standardized value of 1 represents the overall mean. Thus, values should remain near 1.0 throughout discharge under adequate mixing, whereas larger departures would be expected under inadequate mixing.

To evaluate variation not captured by changes in mean concentration, residuals from the first-stage model were squared:

$$e_i^2 = (Y_i - \hat{Y}_i)^2,$$

where [y] represents the observed value and [ŷ] its expected value from the first-stage model. Squared residuals therefore represented observation-level deviations from the expected response. Because they were non-negative and right-skewed, squared residuals were analyzed using a gamma distribution with a log link. The model included mixing time and sampling order nested within mixing time, with sampling order treated as a repeated measurement within mixing-time × day. Day was not included as an additional random effect because day-to-day variation had already been incorporated into the first-stage model.

This second analysis determined whether residual variance was reduced by adequate mixing and whether the magnitude of that variance remained consistent throughout mixer discharge

Defining the characteristics of an effective TMR mixing marker

These analyses allowed potential markers to be evaluated against a predefined biological expectation rather than selected solely from P-values or CV. Under adequate mixing, an effective marker should remain relatively stable throughout discharge, with standardized means near 1.0 and relatively low residual variance. Under short mixing, the same marker should reveal the imposed heterogeneity through changes in mean concentration, greater departures from 1.0, increased residual variance, or a combination of these responses. Thus, the most useful marker is not simply the least variable, but one that remains stable under adequate mixing while responding to inadequate mixing.

Which markers best identified mixing quality?

The response depended substantially on diet, reinforcing the decision to evaluate lactating- and dry-cow TMR separately. More importantly, no single marker met every desired criterion in both diets. This suggests that the ability of a nutrient or physical fraction to indicate mixing quality depends partly on the composition and physical characteristics of the TMR in which it is measured.

Lactating-cow TMR

For the lactating-cow diet, DM emerged as the clearest overall indicator of the contrast between short and adequate mixing. Under adequate mixing, DM concentration did not change across sampling order, whereas a clear sampling-order effect occurred after short mixing ($P < 0.001$). Thus, inadequate mixing produced a systematic change in DM concentration during discharge that was largely eliminated by adequate mixing.

The variance analysis supported the same interpretation. Residual variance for DM was substantially lower following adequate mixing than following short mixing ($P = 0.002$). Standardized DM means also remained more closely centered around 1.0 during adequate mixing, whereas greater deviations were observed during short mixing. Together, these responses indicate that DM captured both the systematic changes in composition throughout discharge and the greater dispersion associated with inadequate mixing.

CP provided a secondary indicator, particularly through its variance response. Residual variance was lower under adequate mixing than under short mixing ($P = 0.031$), and standardized means were somewhat more tightly distributed around 1.0 following adequate mixing. However, CP concentration itself did not exhibit a clear

sampling-order response under short mixing. Therefore, it provided less complete discrimination between mixing conditions than DM.

Several PSPS fractions also responded to mixing conditions, confirming that particle distribution can detect aspects of TMR heterogeneity. However, some PSPS fractions continued to vary across sampling order even after adequate mixing, and differences in residual variance between mixing times were less consistent. Thus, although PSPS remains useful for characterizing physical distribution of the ration, the present results illustrate why variation in PSPS measurements should not automatically be interpreted as evidence of inadequate mixer performance.

Overall, for the lactating-cow TMR, DM provided the most internally consistent response to the experimentally imposed difference in mixing quality, with CP and selected PSPS fractions providing complementary information.

Dry-cow TMR

The dry-cow diet produced a different pattern, with several markers responding to the imposed difference in mixing conditions. Among the chemical components, ADF provided one of the strongest and most consistent responses. ADF changed across sampling order following short mixing ($P < 0.001$) but remained relatively stable throughout discharge following adequate mixing. Residual variance was also substantially lower after adequate mixing ($P = 0.002$), and standardized means were more closely centered around 1.0.

Several physical fractions measured using the PSPS also responded strongly. In particular, the material retained in the bottom fraction (PSPS4) showed considerably greater variance following short mixing, with variance declining markedly under adequate mixing ($P = 0.001$). The upper PSPS fraction (PSPS1) also showed the desired pattern in mean distribution: sampling order affected the short-mixed TMR but not the adequately mixed TMR, while the reduction in variance with adequate mixing approached statistical significance ($P = 0.054$).

NDF also provided useful discrimination. NDF concentration remained relatively stable throughout discharge after adequate mixing but changed markedly across sampling order after short mixing ($P < 0.001$). Standardized NDF means were correspondingly more tightly distributed around 1.0 after adequate mixing, although the overall difference in residual variance between mixing times was not statistically significant.

DM similarly showed evidence of improved homogeneity with adequate mixing. The sampling-order effect was weaker under adequate mixing than under short mixing, and residual variance was significantly reduced by adequate mixing ($P = 0.028$). Starch and CP also clearly detected changes in mean concentration throughout discharge under short mixing while remaining comparatively stable following adequate mixing, although their variance responses were less informative.

Taken together, these findings indicate that ADF was the strongest chemical indicator for the dry-cow TMR, while selected PSPS fractions provided strong complementary physical indicators. Importantly, this differs from the lactating-cow diet, where DM was the clearest marker.

What Does This Mean for Practical TMR Evaluation?

These results reinforce that no single marker may capture TMR mixing quality. Santos et al. (2026) identified Mg, CP, and NDF as promising markers under commercial TMR conditions, whereas Vidal et al. (2025) identified K and Mg as consistent markers in dry concentrate mixtures. In the present controlled challenge, however, the markers most responsive to inadequate mixing differed between lactating- and dry-cow TMR. These findings are not contradictory but reflect differences in feed matrices and study objectives, particularly whether the goal is to identify a consistently distributed marker or one sensitive to deliberately imposed changes in mixing quality.

The results also demonstrate the limitations of evaluating mixing quality solely from an overall CV. Similar CV or average nutrient concentrations can occur despite substantially different patterns during mixer discharge. Evaluating changes in mean concentration together with residual variance therefore provides a more complete assessment of TMR homogeneity.

Overall, marker performance depends on the feed matrix and the purpose of the evaluation. In the present experiment, DM provided the clearest response in the lactating-cow TMR, whereas ADF and selected PSPS fractions were particularly informative in the dry-cow TMR. Thus, the most useful indicator is not simply the marker with the lowest variation, but one that remains stable throughout discharge when mixing is adequate and responds when mixing is inadequate.

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What Works on Real Dairy Farms: Lessons from the Low Carbon Dairy Project

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Introduction

Dairy supply chains are central to the climate goals of many food companies because milk production represents a large share of agricultural greenhouse gas emissions. For Ben & Jerry's, dairy represents one of the largest portions of the company's supply chain emissions, making farm-level progress essential to meeting its science-based climate goals. Ben & Jerry's has committed to reducing scope 3 FLAG greenhouse gas emissions by 45.5% by 2035. The Low Carbon Dairy project was launched to test whether meaningful emissions reductions could be achieved on commercial dairy farms while also supporting productivity, resilience, animal care, and long-term farm viability.

The project was intentionally practical. Rather than evaluating strategies only under research conditions, the global pilot worked with 17 farms, including seven dairy farms in northwestern Vermont. The farms operated under real business, labor, infrastructure, weather, and management constraints. The purpose was to understand not only which interventions could reduce modeled greenhouse gas emissions, but also which strategies farmers were willing and able to implement, maintain, and adapt over time.

Project Background

The Low Carbon Dairy project began in 2022 as a pilot within Ben & Jerry's Caring Dairy program. The original ambition was to pursue a 50% reduction in farm level greenhouse gas emissions from the 2015 Caring Dairy baseline, while identifying practices that could be scaled through the broader dairy program. The pilot combined annual carbon footprinting, farm specific planning, technical assistance, financial support, and continuous improvement.

Participating farms agreed to engage across seven intervention areas: enteric solutions, manure management, regenerative agriculture, nutritious homegrown feed, animal welfare and longevity, renewable energy, and nature-based solutions. The U.S. farms received an annual stipend, paid through the milk check in quarterly installments, to compensate for time, recordkeeping, meetings, data transparency, implementation effort, and the management attention required to test new approaches. Technical experts also supported data collection, farm planning, intervention assessment, and implementation.

From the start, the work was framed as a whole-farm effort rather than a single practice carbon project. Feed, manure, crops, energy, animal performance, biodiversity, and farmer-led innovation were considered together. This structure reflected a central hypothesis: emissions are interconnected with daily farm management, and durable climate progress is most likely when environmental improvements also support the farm business.

Approach

The project used annual, farm-specific plans rather than prescribing a uniform package of practices. Each farm had different facilities, land resources, cropping systems, forage programs, herd performance, labor availability, capital needs, and business goals. Carbon footprint information, farm records, farmer priorities, and technical input were used to identify opportunities that were both relevant and feasible.

Annual “kitchen table” meetings brought together the farm management team and its trusted advisors, Ben & Jerry's staff, University of Vermont Extension personnel, and other technical specialists as needed. These meetings created a recurring cycle of planning, discussion, implementation, measurement, and adjustment. They also provided a practical setting in which modeled reduction potential could be weighed against cost, timing, labor, infrastructure, farmer confidence, and operational value.
The project considered a broad range of interventions. This breadth allowed the project to compare low barrier management changes, capital intensive infrastructure, emerging technologies, and improvements that reduce emissions indirectly through greater efficiency.

Table 1. Interventions considered or implemented in the Low Carbon Dairy project.

Intervention area	**Examples considered or implemented**	**Practical relevance to emissions reduction and farm management**
Feed, forage, and milk production	• Forage-quality improvements • Increased homegrown forage inclusion • Feed-loss reduction • Feed-storage upgrades • Robotic feed pushers • Increased fat and protein corrected milk (FPCM)	• Supported milk production efficiency • Feed utilization • Supported increased milk components • Lower emissions intensity per unit of saleable milk solids.
Cropping and nutrient management	• Perennial grass renovation • Cover cropping • Crop rotation • Minimum or no-till planting • PSNT and CSNT utilization	• Improved crop productivity and soil function, increased forage supply and nutrient-use efficiency while reducing unnecessary inputs and losses

	• Nutrient-management improvements	
Manure management	• Manure separation and treatment • Anaerobic digesters • Lagoon covers	• Targeted manure methane emissions, but often required significant infrastructure, technical support, capital investment, and operational fit.
Enteric methane solutions	• Enteric methane reducing feed additives • Lower emission related feeding strategies	• Offered meaningful theoretical reduction potential, but implementation depended on regulatory approval, product cost, and farmer confidence.
Animal welfare, longevity, and herd performance	• Cow comfort assessments • Herd health and performance improvements • Management changes supporting productive life and consistent milk production	• Connected emissions reduction to practical herd outcomes by supporting productivity, animal care, longevity, and efficient use of farm inputs
Energy	• Solar and renewable energy projects	• Reduced energy-related emissions
Nature-based and biodiversity solutions	• Biodiversity assessments	• Supported broader environmental benefits, farm resilience, and alignment with whole-farm sustainability goals beyond carbon alone

Results and Key Learnings

Across participating U.S. Low Carbon Dairy farms, modeled milk emissions declined from approximately 1.152 kg CO2e/kg FPCM in 2021 to approximately 0.996 kg CO2e/kg FPCM in 2025. This represents a 13.5% reduction over the project period (2021-2025). When compared with the 2015 Caring Dairy baseline, the reduction was approximately 21%. The project did not achieve the original 50% target, but it demonstrated meaningful progress under commercial conditions and clarified which pathways appear most practical and scalable.

The results require careful interpretation. The carbon footprinting platform and greenhouse gas accounting guidelines changed over the course of the project, and some farm structures also changed. Participating operations experienced herd growth or contraction, changes in acreage, movement of cows, feed, or manure between connected farm sites, facility construction, management transitions, and changing production strategies. For this reason, project results are best understood through a combination of modeled numbers and the management narrative behind them.

FPCM and Production Efficiency

One of the most important early learnings was the effect of FPCM on emissions intensity. Producing more saleable milk solids from the same or similar set of farm inputs can reduce emissions per unit of FPCM. This insight shifted the project away from viewing discrete emissions-reduction technologies as the only path forward. Cow productivity, forage quality, feed efficiency, milk components, and overall herd performance became central to understanding modeled outcomes.

Several participating farms improved FPCM while navigating significant operational changes. These results reinforced that milk production efficiency can be a practical climate strategy when gains are achieved through sound nutrition, forage management, cow comfort, and herd management rather than simply through increased inputs.

Practical Beats Perfect

The project initially expected large reductions from enteric methane inhibitors and manure technologies. These approaches continue to offer important potential, but implementation was constrained by regulatory approval, product cost, feed mill logistics, infrastructure, farm scale, technical reliability, measurement limitations, and uncertainty about the farm level business case. The intervention with the greatest theoretical reduction was not always the most practical solution for a particular farm.

Management-based changes were often more readily connected to immediate farm value. Improvements in forage preservation, feed access, nutrient management, cropping, perennial grass renovation, cow comfort, and energy awareness could reduce losses, support production, improve daily work, or build resilience. Farmers were more likely to maintain a climate-related practice when it also addressed a familiar operational need.

Whole-Farm Systems

The project reinforced the connection between soil and crop efficiency, forage production efficiency, and milk production efficiency. Healthy soils and productive crops support higher quality homegrown feed. Better forage can increase diet quality, support milk component production, and reduce reliance on purchased feed. Better nutrient management can improve crop response while reducing unnecessary losses and inputs. Cow comfort and consistent feed access can further support productive efficiency. These outcomes interact, and the most durable progress occurred when interventions reinforced one another.

This whole-farm framing changed how success was understood. A strong modeled result is not sufficient if an intervention is too costly, complex, or disconnected from farm priorities. Conversely, a collection of smaller improvements may create a durable reduction when those practices strengthen the overall production system.

Technical Assistance as an Intervention

Technical assistance and the annual planning process became interventions in their own right. Farmers valued the education, outside perspective, benchmarking, and non-judgmental discussion created through the project. The majority of farms responding to exit interview questions reported a greater understanding of on-farm emissions and potential solutions. Farmers also identified trusted expertise and cost sharing as important factors that helped move projects from interest to action.

The “kitchen table” meetings converted farm data into decisions. It created a place to review results, identify inefficiencies, test assumptions, consider costs and barriers, and determine next steps that fit the operation. This disciplined learning cycle was one of the most scalable outcomes of the pilot because it can support different practices on different farms while maintaining common program expectations.

Implications for Scaling

The Low Carbon Dairy project suggests that scaling dairy emissions reductions requires more than a list of approved practices. Programs need common direction, but farm specific plans are necessary to determine feasibility. Trusted technical assistance, credible measurement, financial support, risk sharing, and farmer-to-farmer learning help create the best conditions for adoption.

Interventions should not all be scaled in the same way. Practices that are understandable, auditable, and linked to familiar management outcomes may be appropriate for broad program expectations. More complex, costly, or technically uncertain interventions may require higher program tiers, targeted incentives, demonstration projects, or additional research. Enteric and manure technologies remain important opportunities, but their adoption will depend on a workable business case and implementation support.

For nutritionists and consultants, the project highlights the value of connecting emissions work to measures already used in farm management. Feed efficiency, forage quality, milk components, cow comfort, nutrient utilization, crop productivity, and feed loss provide practical entry points for sustainability discussions. When carbon outcomes are connected to these measures, climate action becomes less abstract and more useful to farm decision-making.

Summary

The Ben & Jerry's Low Carbon Dairy project demonstrated that meaningful greenhouse gas reductions are possible on commercial dairy farms, but durable progress depends on aligning climate ambition with farm practicality. Participating U.S. farms achieved a 13.5% reduction in modeled emissions intensity from 2021 to 2025 and a 21% reduction relative to the 2015 baseline. These gains were achieved largely through

combinations of management, forage, feed, nutrient management, energy, manure, and animal performance improvements rather than blanket adoption of a single technology.

The most important lesson was that there is no single pathway to lower carbon dairy. Progress lasts when farm specific solutions are supported by trusted expertise, financial support, credible data, and a clear value proposition for the farm. The project moved climate work out of abstraction and into everyday dairy management, providing a practical foundation for future program design and broader scaling.

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Advancing Dairy Cattle Nutrition with Cutting-Edge Microbiome Research

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Introduction

Rumen fermentation plays a central role in milk production by supplying short-chain fatty acids and microbial protein, which provide energy and precursors for the synthesis of milk components (NASEM, 2021). Microbial metabolism also generates compounds with potential effects on the host. For instance, research on the human gut microbiome has highlighted the role of microbial metabolites in host metabolism, physiology, health, and disease (Sasso et al., 2023). In dairy cows, rumen microbes, metabolites, and microbial proteins have been widely reported to be associated with key production traits, such as feed efficiency (Sasson et al., 2022; Shabat et al., 2016; Xue et al., 2022). Nutrients available to the cow depend partly on which dietary components escape ruminal degradation and which products microorganisms generate from the substrates they ferment. Nutritional models represent important aspects of these transformations, including the relationships between fermentable energy, N availability, and microbial protein supply (Van Amburgh et al., 2015). However, molecular techniques used for microbiome research now allow these processes to be examined in greater detail by characterizing the genetics of microbes present in the rumen, genes they are expressing, and individual proteins they are producing. Connecting such microbial information with nutrient supply could help explain variation in the nutritional value of diets and identify specific mechanisms to target when developing feeding strategies.

Advances in microbiome research also support the development of interventions directed at specific rumen functions. Microbiome technologies allow microbial feed additive discovery to begin with a desired rumen function and the identification of organisms that perform it (Nehme Marinho et al., 2024). This expands the search beyond familiar, readily cultivated microorganisms to include rumen microbes selected for their metabolic capabilities and associations with animal performance. Candidate strains can then be evaluated for both their nutritional effects and their suitability for commercial production. Emerging microbiome editing approaches, including the use of phages to target specific strains, offer further possibilities for more precisely modifying fermentation. This article discusses how these developments could improve nutritional models, guide microbial feed additive development, and support targeted interventions to improve dairy cattle nutrition, feed efficiency, and environmental sustainability of dairy farms.

Applying Microbiome Technologies to Nutritional Models

Sequencing and mass spectrometry offer opportunities to refine the characterization of microbial protein used in nutritional models, including its quantity and AA composition. Urinary purine derivatives have been widely used to estimate microbial

protein supply, although their interpretation depends on endogenous purine excretion and the recovery of absorbed purines (Gonzalez-Ronquillo et al., 2003). These estimates do not resolve the AA composition of microbial protein, which differs among bacterial fractions and protozoa (Sok et al., 2017). Consequently, high-resolution molecular approaches are required to overcome these analytical constraints and provide the resolution needed to refine microbial protein representations in nutritional models.

Our group has developed a molecular approach that reconstructs protein-coding expressed microbial genetic sequences *de novo* from rumen metatranscriptomes and combines their predicted AA sequences with abundance of expressed genes to estimate microbial AA profiles. Preliminary analyses generated approximately 4.6 million predicted protein sequences, expanding characterization to include microbes not previously accounted for in nutritional models, such as archaea (e.g., methanogens), fungi, viruses, and sequences of unresolved taxonomic origin. Predicted AA profiles differed among microbial groups and between cows differing in feed efficiency (Arshad et al., unpublished data), with the new fractions resolved by these molecular approaches accounting for over 50% of overall protein abundances. Feed-efficient cows also had greater lysine content than feed-inefficient cows in archaeal ($P < 0.001$), bacterial ($P = 0.01$), and uncharacterized proteins ($P = 0.04$). The resulting microbial protein AA profile from the de novo approach was strongly correlated with the NASEM (2021) reference profile ($r = 0.89$; $R^2 = 0.79$) and other adjustments performed in other studies assessing microbial protein AA profile. These findings provide a basis for investigating how variation in microbial protein composition could affect predictions of AA supply. Because transcript abundance does not directly measure protein abundance or flow from the rumen, integration with metaproteomics and measurements of microbial protein flow with appropriate markers and intestinal digestibility will be necessary to evaluate the contribution of these profiles to predicting absorbable AA supply.

Metaproteomics, the large-scale identification and quantification of proteins in complex microbial samples, offers opportunities to refine the characterization of rumen undegraded protein (**RUP**) from feed ingredients. Differences in ruminal degradation among individual dietary AA can alter the AA profile of protein escaping the rumen relative to that of the original ingredient (Gargallo et al., 2022). Our laboratory is developing approaches to distinguish dietary proteins from microbial and host proteins in digesta using peptides specific to individual feed ingredients. These measurements could identify dietary contributions to RUP and characterize the persistence of specific feed proteins during fermentation. For example, preliminary RUP analyses from our laboratory using metaproteomics has recovered 2,808 distinct soybean meal proteins post-rumen fermentation. These proteins allow the reconstruction of the AA profile of dietary ingredients after rumen fermentation, which in soybean meal revealed that histidine and methionine are the two AA most extensively utilized by rumen microbes. Combining these analyses with digesta flow measurements *in vitro*, such as dual-flow continuous culture systems, would support systematic screening of dietary formulations and evaluation of how diet composition and passage rate affect RUP quantity and composition. Incorporating intestinal digestibility assessments could further support refinement of feed library values and improve predictions of digestible AA supply in nutritional models.

A further application is integrating microbial measurements with genetic selection of dairy cows to improve predictions of nutritional responses. Analyses combining host genotypes, rumen microbial profiles, and production records have identified relationships among genetic variation, microbial populations, and feed efficiency (Martinez-Boggio et al., 2024). These findings provide a basis for testing whether microbial and genetic information explains variation in nutrient utilization beyond that captured by existing nutritional models. For example, microbial functional profiles could be evaluated alongside diet composition, feed intake, passage rate, and stage of lactation to improve predictions of microbial protein synthesis and dietary protein degradation. Statistical and machine learning approaches allow assessment of the additional predictive value of these measurements. Validation across independent herds and controlled feeding trials will be critical to establish whether the resulting predictions improve selection of protein ingredients, level of supplementation of protected AA, and other dietary interventions.

Advancing Strategies to Modify Rumen Fermentation

Microbiome research can guide feed additive development by identifying microbial functions associated with efficient nutrient utilization and selecting organisms for further evaluation. Integrating DNA and RNA sequencing with metabolite measurements helps connect microbial populations with expressed pathways and fermentation products associated with feed efficiency (Xue et al., 2020, 2022). These findings can guide targeted isolation and selection from resources such as the Hungate1000 Collection, which provides cultured rumen organisms and their reference genomes (Seshadri et al., 2018). Candidate strains can be evaluated individually or in combinations for their effects on digestion, fermentation, and microbial protein synthesis. Their nutritional requirements may also identify dietary substrates that support their activity. Supplementation with specific combinations of rumen-native microbes characterized using this workflow (Gaffney et al., 2021) has improved milk production and feed efficiency in a controlled feeding trial, demonstrating the potential of this approach (Nehme Marinho et al., 2024). Selecting microorganisms for defined metabolic functions could make supplementation a more targeted means of modifying rumen fermentation, with specific goals such as increasing fiber degradation, propionate formation, or microbial protein synthesis.

Microbiome editing extends these strategies by selectively modifying microbial populations or their genetic functions. Rumen viruses (e.g., phages) can infect and potentially suppress susceptible microbes, including bacteria, methanogenic archaea, and rumen protozoa (Gilbert and Ouwerkerk, 2020). While phages infecting rumen bacterial genera have been isolated and characterized (Gilbert et al., 2017), less is still known about those infecting other microbial groups in the rumen, such as rumen methanogens and protozoa. A second approach is to modify microbial functions through genetic delivery and editing tools, which once successful it may be able to selectively alter the activity of targeted microbes towards desirable traits. Plasmids and engineered phage particles can carry genetic material into susceptible cells, while Clustered Regularly Interspaced Short Palindromic Repeats (**CRISPR**) systems and transposons can enable targeted genetic changes. Rubin et al. (2022) demonstrated targeted DNA insertion into

selected bacteria within laboratory-grown microbial communities using CRISPR-associated transposases, while Brödel et al. (2024) used engineered phage-derived particles to deliver DNA encoding base editors and modify *Escherichia coli* directly within the mouse gut. This work highlights how modifying microorganisms in the rumen may allow improvements in substrate utilization, redirection of fermentation toward nutritionally useful products, and reduce nutrient losses.

Among the main differences observed in rumen microbial metabolism between cows with differences in fermentation and feed efficiency are propionate synthesis and hydrogen utilization (Shabat et al., 2016; Xue et al., 2022), which are key intervention targets. Studies of rumen bacteria, including *Prevotella ruminicola* and *Prevotella brevis*, have clarified pathways of propionate and succinate formation and demonstrated the role of the *Rnf* enzyme complex in balancing electron carriers during fermentation (Hackmann et al., 2017; Zhang et al., 2023). These findings identify genes and reactions for testing how changes in electron transfer affect fermentation products. While direct chemical inhibitors persistently decrease enteric methane emissions by targeting methanogenesis (Hristov et al., 2015), emerging microbiome strategies focus on redirecting accumulating hydrogen into alternative metabolic sinks. For example, Zhang et al. (2025) identified an uncultured *Duodenibacillus* species with increased hydrogenase expression following red seaweed supplementation and the genomic capacity to couple hydrogen oxidation to fumarate reduction. Manipulating these pathways would aim to increase the recovery of fermented substrates in nutritionally useful products while maintaining fiber digestion and microbial growth. This work highlights how these technologies are being used for the development of microbial solutions that simultaneously target improvements in the productivity and sustainability of dairy cattle.

With the advancements of artificial intelligence (**AI**), especially genome language models, the genetic changes investigated through these approaches may be fully synthesized in the lab to improve effectiveness (King et al., 2026). These AI models learn patterns in DNA and protein sequences and can predict effects of sequence variation or generate candidate sequences. One of the most promising software, *Evo 2*, demonstrated these capabilities across scales ranging from individual genetic elements to genomes (Brixi et al., 2026). Potential rumen applications include prioritizing changes in genes and regulatory regions involved in propionate formation, hydrogen utilization, and microbial AA synthesis, as well as designing viral genomes for genetic delivery. These applications remain to be established experimentally. Linking computational predictions with anaerobic cultivation, dual-flow continuous culture, and feeding trials would allow measured responses to guide subsequent designs. The value of an intervention would ultimately depend on its effects on digestion, nutrient flow, milk component production, and feed efficiency.

Conclusions and Future Perspectives

Advances in microbiome research provide complementary opportunities to improve dairy cattle nutrition through more accurate characterization of nutrient supply, development of microbial feed additives, and selective modification of microbial functions.

Integrating metatranscriptomics and metaproteomics with measurements of protein flow and intestinal digestibility could refine predictions of microbial protein, RUP, and digestible AA supply. In parallel, microbial discovery and microbiome editing could support interventions directed toward improved fermentation and reduced methane and N losses. Progress will depend on connecting molecular findings to reproducible changes in nutrient utilization and animal performance across diets and herds. The practical goal is to incorporate validated microbial functions into nutritional models and feeding strategies that improve milk production, feed efficiency, and resource utilization.

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What Gaps are Molecular Approaches Revealing about the Protein Nutrition of Dairy Cows

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Background and objectives

Protein nutrition supports milk production and efficient nitrogen utilization through both metabolizable protein (MP) supply and its amino acid (AA) profile (Schwab and Broderick, 2017). Microbial protein (MiP) and rumen-undegraded protein (RUP) are the major pools underlying MP supply. Traditional MiP AA profiles incompletely represent rumen microbial diversity (Sok et al., 2017). Owing to differences in AA composition among microbial groups, diet and host-associated shifts (Henderson et al., 2015) in community composition may influence MiP AA profiles. Rumen fermentation might alter RUP AA profiles (Edmunds et al., 2013). However, separate bag incubation may not fully represent feed degradation within mixed rumen contents, while conventional AA analysis does not identify individual remaining proteins. The objectives were to use RNA sequencing to characterize MiP AA composition across microbial groups and cows divergent in feed efficiency, and proteomics to explore potential changes in RUP AA composition during fermentation.

Microbial protein

Metatranscriptomics identifies expressed microbial genes through RNA sequencing; transcript assembly and translation of coding regions enable inference of protein sequences and AA composition. Rumen samples collected by stomach tubing from 24 lactating Holstein cows divergent in residual feed intake (12 feed-efficient and 12 feed-inefficient) underwent ribosomal RNA depletion and ultra-deep dual RNA sequencing. *De novo* assembly and *in silico* translation yielded approximately 4.6 million inferred protein sequences assigned to bacteria, archaea, eukaryotes, and viruses, with 39.3% remaining uncharacterized (Arshad et al., under review). The overall AA profile correlated strongly with the Sok et al. (2017) reference after correction for incomplete AA recovery using Lapierre et al. (2019; $r = 0.91$; $R^2 = 0.83$). All evaluated AA differed among microbial groups ($P < 0.001$). Feed-efficient cows had greater relative Lys proportions in archaeal, bacterial, and uncharacterized protein profiles ($P \leq 0.04$).

Dietary protein

Proteomics identifies proteins by analyzing smaller fragments, called peptides, using mass spectrometry and matching them to reference protein sequences. Feed samples and 32 reticulum samples were digested with trypsin to generate peptides, analyzed using label-free DIA-PASEF proteomics, and processed with FragPipe (Yu et al., 2023). AA profiles were calculated from detected peptide sequences, with each

peptide's contribution weighted by its measured signal. The soybean meal sample yielded 2,808 reported protein identifications. Across all five plant-source comparisons, the median shares of cysteine, histidine, lysine and methionine in reticulum AA profiles were lower than in the corresponding feed profiles. The largest median reduction in AA share occurred for histidine in the soybean meal comparison (47.3%), cysteine in corn (26.1%) and almond (77.1%), lysine in wheat (29.9%), and methionine in alfalfa (24.4%).

Implications and future work

These studies reveal microbial and feed-associated AA profile differences. Future work will combine omasal sampling, the triple-marker technique, and separation of microbial and dietary protein contributions to quantify protein and AA flow leaving the rumen. These data and intestinal digestibility estimates will inform Cornell Net Carbohydrate and Protein System (CNCPS) simulations assessing effects on predicted AA supply and protein supplementation needs.

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How Does Altering the Dietary Supplies of Amino Acids and Fatty Acids Effect Milk Composition in Lactating Dairy Cattle?

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Introduction

Improving the productive efficiency of lactating dairy cattle can be achieved by optimizing nutrient partitioning and utilization in the mammary gland to increase milk component synthesis. Determining the requirements of amino acids (AA) and fatty acids (FA) and how different dietary strategies affect their supply and whole-body utilization are integral to optimizing milk fat and protein synthesis in the mammary gland. More data are warranted to understand the dynamic interactions between these nutrients; thus, the objective of this study was to evaluate the interactions between altering the supplies of AA and FA on milk, milk component, and milk FA yields in lactating dairy cattle. The hypothesis was that independently AA and FA would increase milk protein and fat, whereas increasing the supply of both nutrients would further increase milk fat and protein yields simultaneously.

Methodology

Sixty-four, individually fed lactating dairy cows were used in a randomized complete design with a 2x2 factorial arrangement of treatments with a 10-d covariate period and 56-d experimental period. The treatment diets were: a control diet formulated to supply 3.8% total FA (intake ratio of 1.5 to 1.0 to 0.5 of C16:0 to *cis*-9 C18:1 to C18:0) and 90% of essential AA (EAA) requirements (CON), a diet formulated to supply 4.5% total FA with the same FA intake ratio and 90% of EAA requirements (FA), a diet formulated to supply 3.8% total FA and 105% of EAA requirements (AA), and a diet formulated to supply 4.5% total FA and 105% of EAA requirements (FA+AA). Diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS) v6.5.6 and supplies of AA were evaluated relative to requirements using CNCPS v7 (LaPierre et al., 2020; Higgs et al., 2020). Adjustments were made to ingredient inclusion levels to maintain similar chemical composition (28% aNDFom, 6.1% sugar, and 27% starch) among treatment diets excluding FA and AA supplies.

Dry matter intake and milk yield were recorded daily. Milk samples were collected at three consecutive milkings and were analyzed at the Department of Food Science at Cornell University (Ithaca, NY) for fat, fatty acids (FA), true protein, anhydrous lactose, and milk urea nitrogen (MUN) using a FTIR spectrophotometer (Lactoscope model FTA, Delta Instruments, Drachten, the Netherlands). Fatty acid sources were analyzed by FTIR using PLS prediction models described by Woolpert et al. (2016). All data were analyzed using SAS PROC MIXED and included the random effect and covariate adjustment of cow nested within treatment, and the fixed effect of AA, FA, week, and their interactions.

Table 1. Ingredient composition of the treatment diets.

	Treatment[1]			
Ingredient, % DM	CON	AA	FA	FA+AA
Corn silage	40.7	40.7	40.7	40.7
Grass silage	11.1	11.1	11.1	11.1
Corn grain	17.9	17.9	17.9	17.9
Soybean meal	7.14	3.46	4.96	2.14
Concentrate mix	23.2	24.1	25.4	25.4
Protein concentrate mix	-	2.82	-	2.82

[1]CON = 3.8% total FA and 90% EAA requirements, AA = 3.8% total FA and 105% EAA requirements, FA = 4.5% total FA and 90% EAA requirements, and FA+AA = 4.5% total FA and 105% EAA requirements

Table 2. Formulated nutrient composition of the treatment diets.

	Treatment[1]			
Item, % DM	CON	AA	FA	FA+AA
DM	45.3	45.3	45.3	45.3
CP	15.3	15.7	14.9	15.7
aNDFom	28.5	28.8	28.5	28.4
Starch	27.1	27.0	27.1	27.0
Sugar	6.44	6.24	6.34	6.17
Ether extract	4.80	4.80	5.42	5.43
ME, Mcal/kg[2]	2.75	2.70	2.76	2.74
MP supply, g/d[2]	3,091	3,249	3,085	3,255
FA, % DM				
Total	3.88	3.88	4.46	4.45
C16:0	1.15	1.14	1.46	1.46
C18:0	0.32	0.32	0.35	0.35
c*is*-9 C18:1	0.78	0.77	0.98	0.98
c*is*-9, 12 C18:2	1.24	1.23	1.24	1.23
c*is*-9, 12, 15 C18:3	0.22	0.23	0.23	0.23
AA, g/Mcal ME[2]				
Lysine	2.88	3.30	2.88	3.30
Methionine	1.05	1.30	1.05	1.30
Histidine	1.13	1.30	1.14	1.30

[1]CON = 3.8% total FA and 90% EAA requirements, AA = 3.8% total FA and 105% EAA requirements, FA = 4.5% total FA and 90% EAA requirements, and FA+AA = 4.5% total FA and 105% EAA requirements
[2]Model-predicted using CNCPS v6.5.6

Preliminary Results

Table 3. Preliminary milk performance of cows fed the treatment diets.

	Treatment[1]					Main Effects				*P*-value				
Item	CON	AA	FA	FA+AA	SEM	-FA	+FA	-AA	+AA	FA	AA	FA×AA	Week	FA×AA ×Week
Yield, kg/d														
Milk	45.0[a]	46.7[b]	46.4[b]	45.7[ab]	0.36	45.9	46.0	45.7	46.2	0.66	0.14	<0.01	<0.01	0.60
ECM	50.8[a]	53.6[b]	51.8[a]	51.6[a]	0.60	52.2	51.7	51.3	52.6	0.41	0.03	0.01	<0.01	0.71
Fat[2]	1.93	2.04	1.94	1.96	0.03	1.98	1.95	1.94	2.00	0.30	0.03	0.14	<0.01	0.95
Denovo, g/d	531	564	514	532	8.90	548	523	523	548	0.01	0.01	0.40	0.01	0.92
Mixed, g/d	717	747	717	716	11.3	732	717	717	732	0.17	0.19	0.15	<0.01	0.92
Preformed, g/d	585	596	581	587	10.9	590	584	583	591	0.52	0.45	0.84	<0.01	0.50
Protein	1.46[a]	1.55[b]	1.49[a]	1.49[a]	0.02	1.50	1.49	1.48	1.52	0.27	<0.01	0.01	0.86	0.40
Lactose	2.13[a]	2.19[b]	2.21[b]	2.12[a]	0.02	2.16	2.16	2.17	2.15	0.99	0.39	<0.01	<0.01	0.87
Content, g/100g milk														
Fat	4.31	4.40	4.19	4.38	0.05	4.36	4.29	4.25	4.39	0.20	0.01	0.34	<0.01	0.97
Denovo	1.17	1.23	1.11	1.19	0.02	1.20	1.15	1.14	1.21	<0.01	<0.01	0.48	<0.01	0.93
Mixed	1.59	1.62	1.55	1.60	0.02	1.60	1.58	1.57	1.61	0.17	0.07	0.50	<0.01	0.95
Preformed	1.29	1.29	1.27	1.32	0.02	1.29	1.29	1.28	1.30	0.88	0.36	0.19	<0.01	0.62
Protein	3.24	3.32	3.21	3.31	0.02	3.28	3.26	3.23	3.32	0.16	<0.01	0.57	<0.01	0.07
Lactose	4.72	4.67	4.74	4.66	0.01	4.70	4.70	4.73	4.67	0.97	<0.01	0.34	0.02	0.39
MUN, mg/dL	6.44	8.08	7.23	8.56	0.22	7.26	7.90	6.84	8.32	<0.01	<0.01	0.46	<0.01	0.06
BCS	2.95	2.95	2.88	2.93	0.02	2.95	2.91	2.92	2.94	0.04	0.26	0.42	<0.01	0.28
BW, kg	737[a]	746[b]	745[b]	744[b]	2.40	742	745	741	745	0.18	0.08	0.05	<0.01	0.70

[a-d]Means within a row differ with different superscripts ($P < 0.05$).

[1]CON = 3.8% total FA and 90% EAA requirements, AA = 3.8% total FA and 105% EAA requirements, FA = 4.5% total FA and 90% EAA requirements, and FA+AA = 4.5% total FA and 105% EAA requirements

[2]FA sources classified as de novo < 16 carbon FA, mixed = 16-carbon and 17-carbon FA, and preformed ≥ 18 carbon FA

Conclusion

Fatty acids and AA interacted to affect the yields of milk, ECM, protein, and lactose. The 105% EAA treatment increased the yields of ECM, fat, de novo FA, and protein, and the contents of fat, de novo FA, protein, lactose, and MUN compared to the 90% EAA treatment. This data suggests that meeting AA requirements are important to enhance both milk fat and protein production. Evaluating the interactions between nutrients such as FA and AA are important to understanding milk component synthesis, and how dietary changes can be made to increase either fat or protein yield.

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The effect of dietary phosphorus intake in prepartum Holstein cows fed a negative DCAD diet on peripartum mineral metabolism and lactation performance

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Introduction

Feeding a negative dietary cation anion difference (DCAD) diet before calving is a widely adopted strategy for preventing hypocalcemia (Block, 1984). By inducing a mild compensated metabolic acidosis, a negative DCAD diet increases tissue responsiveness to parathyroid hormone (PTH), improves calcium (Ca) homeostasis, and reduces the risk of hypocalcemia (Charbonneau et al., 2006; Lean et al., 2006; Santos et al., 2019). Nevertheless, subclinical hypocalcemia remains common, prompting investigation of additional nutritional strategies. Because Ca and phosphorus (P) are interconnected through hormonal regulation, including PTH and 1,25 $(OH)_2D$ (Reinhardt et al., 1988), changes in P intake can affect Ca metabolism around calving when Ca demand increases. Increasing prepartum dietary P from 0.3 to 0.4% has been reported to increase hypocalcemia risk by 18% across a range of studies (Lean et al., 2006). Although it remains unclear how changes in P intake combined with a fully acidogenic diet fed to periparturient cows may influence mineral metabolism and lactation performance. Therefore, the objective of this study was to determine the effects of prepartum dietary P intake within a fully acidogenic (i.e., negative DCAD) diet on periparturient mineral metabolism and subsequent lactation performance of mature Holstein cows. We hypothesized that lower P intake would improve mineral metabolism and lactation performance.

Experimental Design

Late pregnant multiparous Holstein cows (n = 53) were used in a randomized complete block design study, blocked by parity, expected calving date, body weight, and previous lactation performance (305ME), and assigned randomly within block to 2 prepartum dietary treatments (Tables 1 and 2). Both dietary treatments had a negative DCAD (-8.2 mEq/100 g) achieved by using an anionic mineral supplement (AMS; Animate, Phibro Animal Health Corporation, Teaneck, NJ), while one treatment supplemented additional P to target a higher P intake (59 g/d (**AMS-HP**; n = 28 cows) and the other did not supplement additional P and had a lower P intake (42 g/d, **AMS-LP**; n = 25 cows). The dietary treatments were fed from 24 d before expected calving to calving. After calving, cows were fed a fresh diet through 10 days in milk (DIM) followed by a high diet through 56 DIM (Tables 1 and 2).

Table 1. Ingredient composition [% dry matter (DM) basis] of diets.

	Prepartum Diet[1]		Postpartum Diet	
Item	AMS-HP	AMS-LP	Fresh	High
Corn silage	42.834	42.834	37.879[2]	44.254
Haycrop silage: dry cow	21.417	21.417	-	-
Haycrop silage: lactating cow	-	-	18.940	12.069
Straw	14.827	14.827	-	-
Animate[3]	3.039	3.039	-	-
Calcium carbonate	2.797	3.046	-	-
Dicalcium phosphate	0.656	-	-	-
Soy hulls	0.097	0.504	-	-
Beet pulp	-	-	8.618	7.691
Urea	-	-	0.152	0.154
Grain mix				
Corn meal	-	-	13.389	13.298
PGI Amino Max[4]	8.596	8.596	9.460	3.264
Soybean meal	-	-	4.073	6.764
Canola meal	-	-	-	4.296
Distillers grain	-	-	-	1.909
Sugar	-	-	1.089	1.480
Cane molasses	-	-	0.852	0.579
Fat – calcium salt	-	-	1.722	1.561
Urea	-	-	0.206	0.105
Sodium sesquicarbonate	-	-	1.160	0.788
Calcium carbonate	-	-	0.812	0.709
Soy hulls	5.125	5.125	-	-
Dicalcium phosphate	-	-	0.403	0.048
Salt	0.274	0.274	0.574	0.390
Magnesium oxide	0.165	0.165	0.418	0.315
Trace minerals/vitamins[4,5]	0.091	0.091	0.138	0.101
Smartamine[5]	-	-	0.059	0.063
MetaSmart[5]	-	-	0.045	0.153
Vitamin E, 88,000 IU/kg	0.042	0.042	-	-
Selenium yeast 0.06%	0.025	0.025	-	-
Rumensin 90[6]	0.015	0.015	0.012	0.007

[1] AMS-HP = a fully acidogenic diet with an anionic mineral supplement and a higher P target; AMS-LP = a fully acidogenic diet with an anionic mineral supplement and a lower P target.
[2] Mean ± standard deviation.
[3] Phibro Animal Health Corporation, Teaneck, NJ. Animate was added or removed (typically in 45 g (0.1 lb) as fed increments based on formulated intake) from the AMS-HP and AMS-LP diets as needed to adjust the dietary cation-anion difference to maintain a urine pH in the range of 5.5 to 6.0.
[4] Poulin Grain Inc., Newport, VT.
[5] Micronutrients USA LLC, Indianapolis, IN.
[5] Adisseo USA Inc., Alpharetta, GA.
[6] Elanco Animal Health, Greenfield, IN.

Cows were fed individually daily and their body weight and body condition score were determined weekly. Their blood was sampled from the coccygeal vein or artery at enrollment, weekly during the prepartum period, and on d 1, 2, 3, 4, and 7 ± 1 relative to calving. Serum was analyzed for anion gap, total bicarbonate (CO_2), Ca, chloride (Cl), magnesium (Mg), P, potassium (K), and sodium (Na) concentrations, and Na to K ratio (Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI). Cows were retrospectively classified into 1 of 4 calcium dynamic groups based on blood Ca concentration at 1 and 4 DIM (Seely et al., 2021). Urine was sampled at enrollment, -14 ± 3, -7 ± 3, and -1 (or the closest to -1) d relative to calving and 24 ± 6 and 48 ± 6 h relative to calving and analyzed for Ca, Cl, creatinine, P, K, Na, and Na to K ratio (Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI).

After calving, colostrum was sampled and analyzed for immunoglobulin G content by refractometry (Quigley et al., 2013). Cows were milked 3x/d and milk yield was recorded. Milk samples from 6 consecutive milkings from each cow were collected weekly and analyzed for fat, true protein, lactose (anhydrous), solids nonfat, urea nitrogen, and de novo, mixed, and preformed fatty acids by mid-infrared procedures (Wojciechowski and Barbano, 2016; Wojciechowski et al., 2016; Woolpert et al., 2016).

Statistical computations were performed using the Statistical Analysis System (version 9.4; SAS Institute Inc., Cary, NC). Prepartum and postpartum data were analyzed by period. Statistical analyses were conducted using analysis of variance with the MIXED procedure in SAS (Littell et al., 1996). Fixed effects included treatment, time, block, and the treatment × time interaction. Cow was defined as the experimental unit and random effect. For blood, a covariate was added to the model based on data collected at enrollment. For data that violated the assumption of normality, the GLIMMIX procedure was used and the distribution option selected was based on data visualization.

Results and discussion

Mineral metabolism

As expected, cows fed the AMS-HP had a greater ($P < 0.01$) prepartum serum P concentration than cows fed AMS-LP (2.17 vs 1.93 mmol/L, respectively; Table 3). This response was similar to Peterson et al. (2005), who observed increased serum P with increasing P intake. In their study, cows received 34, 48, or 67 g/d with a slightly negative DCAD (-2.0 to -1.3 mEq/100g DM), with serum P concentrations of 1.63, 2.07, and 2.13 mmol/L, respectively. In both the current study and the Peterson et al. (2005), the lower P intake diets lowered serum P concentration but maintained it within the normal range (1.45-2.1 mmol/L; Abramowicz et al., 2026). Other studies have explored restricting P intakes to below maintenance requirements (Cohrs et al., 2018 and Wächter et al, 2022; 19 and 17g/d, respectively) which resulted in serum P concentrations indicative of subclinical hypophosphatemia (< 1.3 mmol/L; Abramowicz et al., 2026). Both dietary treatments in this study met the needs for adequate intake according to NASEM, 2021. Despite differences in prepartum serum P concentration,

postpartum serum P concentration was not affected by treatment and averaged 1.70 mmol/L (Table 3), suggesting that cows were able to recover quickly after transition to the common postpartum diet.

Table 2. Analyzed composition of diets fed to Holstein cows.

	Prepartum Diets[1]		Postpartum Diets	
Item	AMS-HP	AMS-LP	Fresh	High
Dry matter, %	42.8±2.2[2]	42.8±2.3	42.9±2.1	44.1±2.5
CP %	12.6±0.5	12.8±0.7	17.3±0.9	16.8±0.6
ADF, %	30.2±0.9	30.6±0.5	19.0±1.5	18.3±2.2
NDF, %	46.3±1.7	46.6±1.7	29.5±1.4	28.1±1.8
peNDF, %	34.3±1.3	34.3±1.4	20.7±1.4	18.9 ± 1.6
Starch, %	15.0±1.8	14.9±1.7	25.7±1.2	27.5±1.4
Sugar[3], %	4.2±0.7	4.7±0.3	5.2±0.6	5.2±1.1
Ether extract, %	2.8±0.5	2.7±0.4	4.2±0.2	3.93±0.3
Ash, %	6.77±0.69	7.15±0.8	8.29±0.46	7.14±0.96
Calcium, %	1.19±0.14	1.21±0.18	0.85±0.07	0.70±0.07
Phosphorus, %	0.38±0.03	0.26±0.02	0.41±0.02	0.34±0.03
Magnesium, %	0.40±0.03	0.40±0.03	0.45±0.03	0.37±0.03
Potassium, %	1.04±0.08	1.05±0.07	1.23±0.1	1.06±0.08
Sulfur, %	0.28±0.02	0.29±0.02	0.20±0.01	0.20±0.01
Sodium, %	0.13±0.01	0.13±0.01	0.54±0.04	0.38±0.02
Chloride ion, %	0.80±0.04	0.81±0.05	0.68±0.05	0.49±0.03
DCAD, mEq/100 g	-8.2±2.6	-8.2±2.6	23.1±3.2	17.5±1.6
NEL Mcal/kg	1.45±0.03	1.43±0.3	1.66±0.02	1.68±0.03

[1] AMS-HP = a fully acidogenic diet with an anionic mineral supplement and a higher P target; AMS-LP = a fully acidogenic diet with an anionic mineral supplement and a lower P target.
[2] Mean ± standard deviation.
[3] Ethanol soluble carbohydrates.

Urine P concentration was low with many samples below the detection limit of 1 mg/dL during the prepartum (>70% of samples) and postpartum (>80% of samples) periods. For samples below the detection limit, 0.99 mg/dL was used. Using this approach, prepartum urinary P concentration and excretion were greater for cows fed AMS-HP (Table 4). There was no difference during the postpartum period. The low values were typical for cows in early lactation given the increased metabolic demand from production (Grünberg, 2014).

Serum Ca concentration was not different between treatments during the prepartum (2.37 and 2.38 mmol/L; $P = 0.59$) or postpartum (2.10 and 2.12 mmol/L; $P = 0.56$) periods for AMS-HP and AMS-LP, respectively (Table 3). Based on serum data from d 1 and 4 after calving, 29% of AMS-HP cows were classified as normocalcemic regarding their Ca dynamics, and 53%, 7%, and 11% were classified as transient, persistent and delayed, respectively. Forty percent of AMS-LP cows were classified as normocalcemic, 36% were classified as transient, 4% as persistent, and 20% as delayed. This distribution, and large proportion of cows classified as transient, is

consistent with previous reports describing Ca dynamics in healthy, high-producing cows (McArt et al., 2020). The concentration of Ca in the urine (Table 4) was not affected by treatment during the prepartum period and >90% of postpartum samples analyzed for Ca concentration were below the detection limit (<2 mg/dL). Other serum minerals (Mg, Na, K, and Cl; Table 3) were not affected by treatment or time during the postpartum period.

Cow performance

Treatment did not affect DMI ($P \geq 0.22$) and averaged 15.8 kg/d (1.92% of BW/d) and 26.0 kg/d (3.55% of BW/d) during the prepartum and postpartum periods, respectively (Table 5). There was no difference in BW, or rumination time (Table 5) during the prepartum or postpartum period. Body weight averaged 828 and 739 kg during the prepartum and postpartum periods, respectively ($P \geq 0.30$). Prepartum and postpartum rumination averaged 576 and 597 min/d, respectively ($P \geq 0.36$). Cows fed AMS-LP had a higher BCS than cows fed AMS-HP (3.20 vs 3.12; $P = 0.04$) during the prepartum period; however, this difference is likely attributed to the wk -4 measurement, when fewer cows contributed to observations due to differences in calving dates. After calving, BCS was not affected by treatment (2.97; $P = 0.67$).

Calf birth weight, colostrum yield, and colostrum quality were not affected by treatment (45.0 kg, 4.9 kg, and 81.9 g IgG/L, respectively; $P \geq 0.15$). Given the substantial metabolic demands of colostrum synthesis, changes in prepartum mineral metabolism have the potential to interfere with colostrogenesis (Westhoff et al., 2024). However, these results suggest that the lower P supply in this study was sufficient to support colostrogenesis and colostrum quality.

Milk yield increased rapidly after calving and averaged 53.2 kg/d over the first 8 weeks of lactation. This was not affected by treatment ($P = 0.19$) and was consistent with previous studies investigating prepartum P intake reporting no effects on subsequent milk yield (Keanthao et al., 2021; Wächter et al., 2022a). However, in contrast to previous literature, energy corrected milk (ECM) was greater for AMS-LP cows and averaged 61.3 kg/d overall ($P = 0.03$; Figure 1), with a significant time and treatment by time effect ($P \leq 0.01$ and $P = 0.01$). This difference was driven primarily by greater milk fat yield in AMS-LP cows than AMS-HP cows (2.38 kg/d vs 2.25 kg/d; $P = 0.03$), despite no treatment effect on postpartum DMI or actual milk yield. Content of milk fat averaged 4.45% and was not affected by treatment ($P = 0.13$). The milk true protein content (3.21%) was not affected by treatment ($P=0.42$), although there was a tendency for cows fed AMS-LP to have greater milk true protein yield than cows fed AMS-HP which may have also contributed to the increased ECM (1.69 kg/d vs 1.64 kg/d; $P = 0.08$ Feed efficiency expressed as ECM/DMI was not affected by treatment ($P = 0.59$) and averaged 2.34, consistent with early lactation cows experiencing negative energy balance. Energy balance averaged 78.8% of NEL requirement and was not affected by treatment (P = 0.72). To our knowledge, an improvement in component-corrected milk has not previously been reported with cows receiving lower prepartum P intake. The greater ECM and milk fat and protein yields observed in cows fed AMS-LP

warrant further research to determine how prepartum P intake may influence milk component synthesis, or if supplementing dietary P in the prepartum period may restrict or inhibit milk component synthesis.

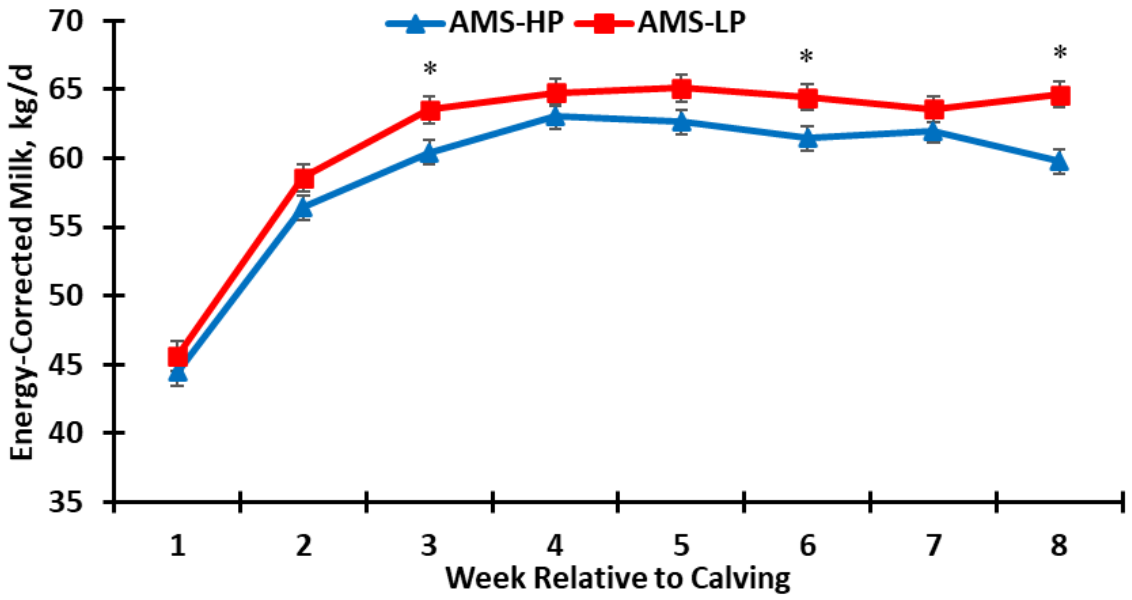


Figure 1. Least squares means of energy-correct milk (ECM) yield from wk 1 to 8 of lactation for Holstein cows (n = 53). AMS-HP = a fully acidogenic diet with an anionic mineral supplement and a higher P target; AMS-LP = a fully acidogenic diet with an anionic mineral supplement and a lower P target. Error bars represent standard error. The *P*-values for treatment (TRT), time, and TRT x time were 0.03, <0.01, and 0.01, respectively. Time points labeled with an asterisk (*) denote significant treatment differences ($P \leq 0.05$).

Conclusions and Implications

Lower prepartum P intake (42 vs 59 g/d) within a fully acidogenic diet lowered the concentration of P in the serum during the prepartum period. These changes had no carryover effect into the postpartum period, as mineral metabolism, DMI, BW, BCS, milk yield and energy status were not affected by treatment. However, cows fed the lower P diet produced greater milk fat and tended to produce greater protein yield, contributing to greater ECM. The underlying mechanism of this response remains unclear and warrants further research. Overall, feeding phosphorus at lower recommended amount supported mineral metabolism without compromising lactation performance during the transition period. These findings suggest that supplemental dietary phosphorus is unnecessary when cows are fed a fully acidogenic close-up diet.

Table 3. Least squares means of serum-based chemistry (mineral and electrolyte focused) data from the prepartum period and first week of the postpartum period of Holstein cows (n = 53). Time represents week or day relative to calving.

	Treatment (TRT)[1]			*P*-value		
Item	AMS-HP	AMS-LP	SE	TRT	Time	TRT x Time
Prepartum: wk -4 to -1						
Ca, mmol/L	2.37	2.38	0.01	0.59	0.17	0.80
P, mmol/L	2.17	1.93	0.03	<0.01	<0.01	0.82
Mg, mmol/L	0.96	0.92	0.10	0.01	<0.01	0.48
Na, mmol/L	141.5	141.1	0.3	0.29	<0.01	1.00
K, mmol/L	4.8	4.5	<0.1	<0.01	<0.01	0.02
Na:K ratio	30	31	<1	<0.01	0.34	<0.01
Cl, mmol/L	102	101	<1	0.22	<0.01	0.77
Anion Gap, mmol/L	17	16	<1	0.52	0.93	0.95
Total CO_2, mmol/L	28	28	<1	0.83	0.64	0.72
Postpartum: d 1, 2, 3, 4, 7						
Ca, mmol/L	2.10	2.12	0.03	0.56	<0.01	0.50
P, mmol/L	1.70	1.69	0.06	0.94	<0.01	0.31
Mg, mmol/L	0.99	0.97	0.02	0.31	<0.01	0.61
Na, mmol/L	144	144	<1	0.67	<0.01	0.14
K, mmol/L	5.0	4.9	<0.1	0.66	<0.01	0.85
Na:K ratio	29	29	<1	0.67	<0.01	0.56
Cl, mmol/L	101	101	<1	0.95	<0.01	0.97
Anion Gap, mmol/L	16	16	<1	0.75	0.01	0.20
Total CO_2, mmol/L	32	32	<1	0.66	<0.01	0.97

[1] AMS-HP = a fully acidogenic diet with an anionic mineral supplement and a higher P target; AMS-LP = a fully acidogenic diet with an anionic mineral supplement and a lower P target.

Table 4. Least squares means of urine chemistry data and estimates of mineral excretion from the prepartum period (-14 ± 3, -7 ± 3, and -1 (or the closest to -1, e.g., -2 or -3) d relative to actual calving) taken 24 ± 6 and 48 ± 6 h relative to actual calving time of Holstein cows (n = 53). Time represents day or hour relative to calving.

	Treatment (TRT)[1]			*P*-value		
Item	AMS-HP	AMS-LP	SE	TRT	Time	TRT x Time
Prepartum: d -14 ± 3, -7 ± 3, -1						
Urinary creatinine, mg/dL	108	108	4	0.98	0.03	0.37
Urinary Ca, mg/dL	33.6	32.3	1.7	0.60	0.03	0.48
% of sample <2 mg/dL (detection limit)	0	0	-	-	-	-
Urinary P, mg/dL	4.4	1.7	0.7	0.03	0.04	0.23
% of sample <1 mg/dL (detection limit)	72	89	-	-	-	-
Urinary Cl, mmol/L	112	109	5	0.71	<0.01	0.94
Urinary K, mmol/L	196	199	9	0.81	<0.01	0.43
Urinary Na, mmol/L	64	64	4	0.99	0.18	0.67
Calculated urinary Ca excretion, g/d[2]	7.5	7.8	0.4	0.50	0.50	0.94
Calculated urinary P excretion, g/d	1.1	0.4	0.2	0.05	0.09	0.34
Postpartum: 24 ± 6 and 48 ± 6 h						
Urinary creatinine, mg/dL	91	80	4	0.06	<0.01	0.44
Urinary Ca, mg/dL	2.5	1.8	0.3	0.14	0.31	0.31
% of samples <2.0 mg/dL (detection limit)	93	100	-	-	-	-
Urinary P, mg/dL	1.8	1.3	0.3	0.27	0.61	0.41
% of samples <1.0 mg/dL (detection limit)	84	90	-	-	-	-
Urinary Cl, mmol/L	98	83	6	0.08	0.08	0.60
Urinary K, mmol/L	183	195	11	0.44	<0.01	0.91
Urinary Na, mmol/L	115	105	9	0.41	<0.01	0.56
Calculated urinary Ca excretion, g/d	0.7	0.7	<0.1	0.38	<0.01	0.51
Calculated urinary P excretion, g/d	0.5	0.4	0.01	0.51	<0.01	0.89

[1] AMS-HP = a fully acidogenic diet with an anionic mineral supplement and a higher P target; AMS-LP = a fully acidogenic diet with an anionic mineral supplement and a lower P target.

[2] Mineral excretion was calculated based on a daily creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Table 5. Least squares means of DMI, BW, BCS, rumination, lactation performance, and feed efficiency data from the prepartum (wk -4 to -1) and postpartum period (wk 1 to 8 of lactation) of Holstein cows (n = 53 Time represents week relative to calving.

	Treatment (TRT)[1]			*P*-value		
Item	AMS-HP	AMS-LP	SE	TRT	Time	TRT x Time
Prepartum: wk -4 to -1						
DMI, kg/d	15.6	15.9	0.4	0.49	<0.01	0.24
DMI, % of BW/d	1.91	1.92	0.04	0.81	<0.01	0.07
BW, kg	821	834	9	0.30	<0.01	0.07
BCS	3.12	3.20	0.03	0.04	0.86	0.47
Rumination, min/d	571	581	8	0.36	<0.01	0.11
Postpartum: wk 1 to 8						
DMI, kg/d	25.5	26.4	0.5	0.22	<0.01	0.76
DMI, % of BW/d	3.51	3.58	0.06	0.33	<0.01	0.93
BW, kg	734	744	10	0.47	<0.01	0.03
BCS	2.96	2.98	0.03	0.67	<0.01	0.87
Rumination, min/d	597	597	7	0.95	<0.01	0.34
Milk, kg/d	52.5	53.8	0.7	0.19	<0.01	0.45
ECM, kg/d[3]	58.8	61.3	0.8	0.03	<0.01	0.01
Fat, kg/d	2.25	2.38	0.04	0.03	<0.01	0.06
True protein, kg/d	1.64	1.69	0.02	0.08	<0.01	0.07
Lactose, kg/d	2.47	2.52	0.04	0.30	<0.01	0.16
Fat, %	4.35	4.54	0.09	0.13	<0.01	0.56
True protein, %	3.18	3.24	0.05	0.42	<0.01	0.29
Lactose, %	4.68	4.66	0.02	0.46	<0.01	0.34
De novo FA[2], g/100 g milk	1.01	1.06	0.03	0.28	<0.01	0.46
Mixed FA[2], g/100 g milk	1.40	1.47	0.04	0.25	<0.01	0.17
Preformed FA[2], g/100 g milk	1.70	1.77	0.06	0.41	<0.01	0.81
Urea N, mg/dL	7.23	7.41	0.39	0.74	<0.01	0.13
SCS	0.79	1.04	0.31	0.57	<0.01	0.77
Milk/DMI	2.05	2.04	0.05	0.88	<0.01	0.61
ECM/DMI	2.31	2.36	0.06	0.59	<0.01	0.37

[1] AMS-HP = a fully acidogenic diet with an anionic mineral supplement and a higher P target; AMS-LP = a fully acidogenic diet with an anionic mineral supplement and a lower P target.
[2] Fatty acid.
[3] ECM was calculated using a formula modified to account for use of true protein instead of total protein (Tyrrell and Reid, 1965; Hall 2022): 0.327 × kg of milk + 12.95 × kg of fat + 7.65 × kg of true protein.

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How Far Can Genomic Selection for Milk Components Take the Dairy Cow?

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Introduction

Phenotypic trends for fat and protein yield in Holstein cows are shown in Figure 1. Since data began flowing into the National Cooperators Database in 1957 for fat and 1969 for protein, average fat production has increased by a factor of 2.71× and protein has increased by 1.95×. This represents an annual trend of 11.04 pounds/year for fat and 8.05 pounds/year for protein. These gains represent both increases in genetic merit and improvements in feeding, housing, and other farm management practices, and they do not operate in isolation. High-performance genetics require high-quality management to achieve their full potential.

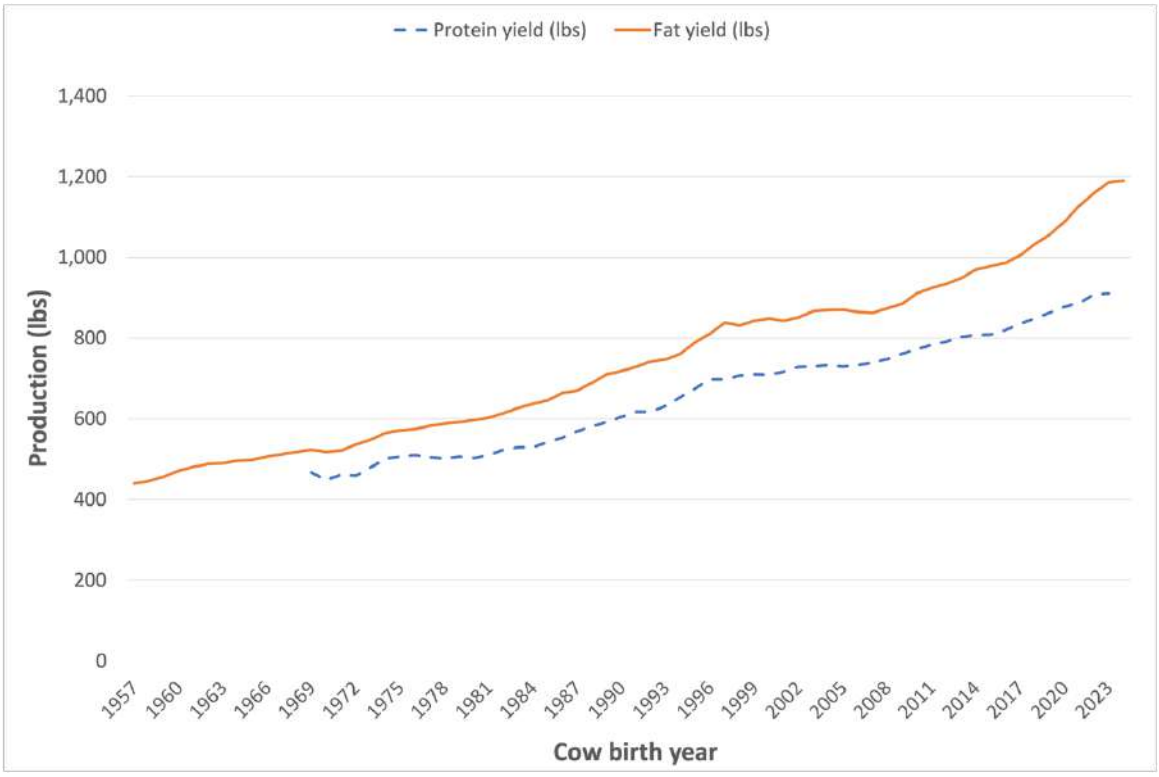


Figure 1. Fat (solid orange line) and protein (dashed blue line) production of Holstein cows in pounds (lbs) by year of birth (Source: National Cooperators Database).

Genomic evaluations were first introduced in the U.S. for Brown Swiss, Holstein, and Jersey cattle in 2009 (Wiggans and Carrillo, 2022), and evaluations for Ayrshires and Guernseys were introduced in 2013 and 2016, respectively. In January 2009 the National Cooperator Database included genotypes from 368 Brown Swiss, 1,558 Jersey, and 14,720 Holstein animals; as of May 5, 2026, the database included 111,985 Brown Swiss, 11,229,763 Holstein, and 1,272,516 Jersey genotypes. The generation interval for Holsteins in 2009 was 7.1 y, decreasing to 2.2 y for bulls born in 2017 (Guinan et al., 2023). This dramatic reduction in generation interval was accompanied by a rapid increase in genetic trends and selection intensity for many traits. Anecdotally, some top-producing herds now average >9% milk fat and >5% milk protein, far-exceeding historical norms, challenging our ideas of what the dairy cow can do, and posing difficulties for milk processors (Van Amburgh, 2026, personal communication). In light of these situations, as well as gains described by, e.g., García-Ruiz et al. (2016) and Guinan et al. (2023), the question “what are the limits to selection” is a very natural one to ask for scientists, allied industry, and dairy producers.

Materials and Methods

Haplotyping

Lower and upper bounds of selection limits were calculated for milk, fat, and protein yield, fat and protein percent, and lifetime net merit (NM$) in US Ayrshire (AY; n = 20,373), Brown Swiss (BS; n = 89,136), Guernsey (GU; n = 11,407), Holstein (HO; n = 9,119,871), and Jersey (JE; n = 928,883) cattle. Lifetime net merit is a selection index which maximizes lifetime income of cows based on the average utilization of a hundred-weight of milk (Cole et al., 2021). Genotypes were imputed to the set of 69,200 single-nucleotide polymorphisms (SNP) used for routine genomic evaluation in the U.S. and maternal and paternal haplotypes were phased using Findhap v.3 (VanRaden, 2016). Selection limits and estimates of Mendelian sampling variance were calculated with custom Python 3.12.2 scripts using the methods described in Cole and VanRaden (2011) using results from the August 2025 genomic evaluation run.

Selection limits

The process used to calculate selection limits is illustrated in Figure 2. Marker values for paternal and maternal haplotypes were summed for each genotyped animal to obtain maternal and paternal chromosomal direct genomic values (CDGV) for each of the 19 traits described above, as well as for the lifetime net merit (NM$) selection index (VanRaden et al., 2025). Chromosomes included the 29 autosomes as well as the pseudoautosomal region of the X- and Y-chromosomes, subsequently referred to as chromosome 30 for consistency with previous research (e.g., Cole and VanRaden, 2011).

Marker values were summed for each genotyped animal to obtain chromosomal estimated breeding values (EBV) for each of the 19 traits described above, as well as for the lifetime net merit (NM$) selection index (VanRaden et al., 2025), and the resulting EBV were summed to obtain direct genomic values (DGV). The EBV were those

calculated for the August 2025 genetic evaluation run and included base adjustments, polygenic effects, and information from non-genotyped relatives.

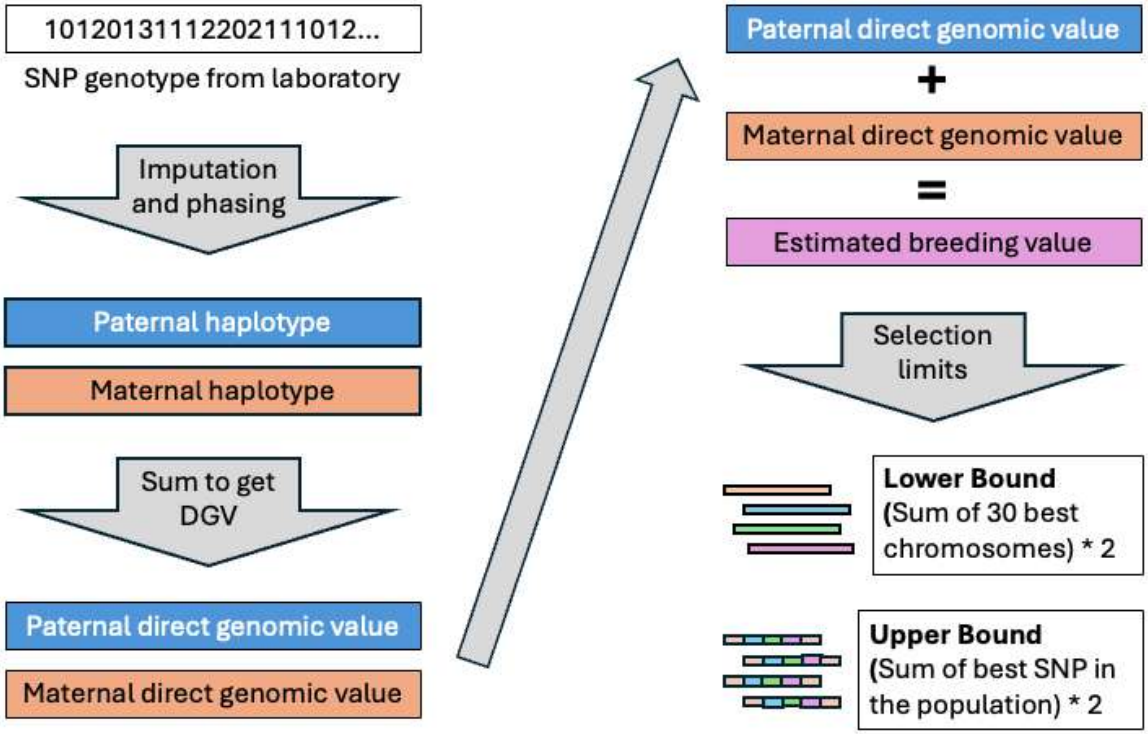


Figure 2. Process used to compute upper and lower bounds of selection limits from single-nucleotide polymorphism (SNP) genotypes. Genotypes are first imputed to a standard set of 69,200 markers and phased into maternal and paternal haplotypes. Individual SNP effects are summed to produce chromosomal direct genomic values (DGV). The DGV are summed to produce estimated breeding values (EBV) for an animal. Lower bounds are calculated by summing the DGV for the 30 best chromosomes in the population. Upper bounds are calculated by summing the best alleles for each of the 69,200 SNP in the genomic evaluation panel. The results are multiplied by 2 to produce EBV. Chromosomes include the 29 autosomes and the pseudoautosomal region of the X- and Y-chromosomes (chromosome 30).

Lower bounds (SL_L) were predicted by selecting the 29 individual autosomes plus the pseudo-autosomal region of the X and Y chromosomes (chromosome 30) with the largest direct genomic values (DGV) for each breed-trait combination. Upper bounds (SL_U) were calculated by summing the alleles at each locus with the largest allele substitution effects. SL_L and SL_U were also compared to the largest observed DGV (DGV_{max}) for each breed-trait combination. The SL_L and SL_U for each animal were calculated as:

$$SL_L = \sum_{c=1}^{30} \max_{H} \left(\sum_{m=1}^{n_c} l_m \, \alpha_m \right)$$

and

$$SL_U = \sum_{m=1}^{69{,}200} \max_{L} (l_m \alpha_m)$$

respectively, where *c* indicates a chromosome, *m* denotes a marker, α_m is the estimated allele substitution effect for the m^{th} marker, *H* represents the set of all unique haplotypes in the genotyped population, n_c is the number of markers present on the c^{th} chromosome, h_m represents the m^{th} marker of an individual haplotype, *L* is the set of all marker loci in the genotyped population, and l_m represents the genotype of the m^{th} marker locus.

Results and Discussion

Selection limits for the current population were estimated assuming that either entire chromosomes or individual alleles can be selected and combined at will to produce whole genomes, as described in Cole and VanRaden (2011). Lower and upper bounds for each trait, as well as the largest DGV observed in the genotyped population, are presented in Table 1. The lower bounds represent selection limits attainable by sampling only from haplotypes already in the population, while the upper bounds are attainable under the assumption that complete haplotypes can be constructed from individual alleles in the population (i.e., Cole and VanRaden, 2011; Jenko et al., 2015; Hayes et al., 2024). In most cases, SL_C and SL_U were largest for HO, intermediate for BS and JE, and lowest for AY and GU. These results reflect, in part, the relative population sizes of each breed in the US, as selection limits are constrained by the haplotypes with the largest effects in the population. It is easier to find outlier haplotypes in large populations.

The traits included in Table 1 were selected for their relationship to milk composition; other traits, including health and fertility, are discussed in Cole and Null (2026). In all cases, the DGV_{max} is bounded by SL_L and SL_U, as expected, and the ratios of SL_L to DGV_{max} and SL_U to DGV_{max} range from ~2.3 for fat percent in HO to ~16 for milk yield in JE protein percent in HO. These results are consistent with the earlier findings of Cole and VanRaden (2011) and confirm, even as selection intensity has increased in the 15 years since that report (Guinan et al., 2023), that substantial additional progress can be made.

While it is tempting to focus on the upper bounds, those values assume you can assemble 69,200 individual genetic variants, entirely divorced from their biological context, and will see strictly additive results. That is not representative of how biology generally works. A more realistic upper bound might be found by assembling blocks of SNP that are in linkage disequilibrium with one another. The lower bounds are constructed by assembling entire chromosomes, so the context in which high-impact variants are located is preserved and they are more likely to represent achievable goals. Recurrent selection can be used to assemble desirable haplotypes (Cole and VanRaden, 2011;

Hayes et al., 2024), but the combinatorics are formidable. One alternative is to slightly loosen selection criteria and, rather than breeding for only the best haplotypes or alleles, the goal could be to assemble alleles from the top 1% or 5% in the population. This would dramatically reduce the complexity of the problem without notably affecting the outcome.

Chromosomal EBV of fat yield for hypothetical animals whose genotypes consist of two copies of each of the best haplotypes in their respective US populations are shown in Figure 3. Each row (barchart) represents the “best” possible animal of each breed that can be constructed using only the haplotypes seen in that breed. There are no obvious QTL segregating in AY, BS, or GU, but *DGAT1* is segregating in the HO population. In recent years there has been increased gene flow from HO to JE, and *DGAT1* has increased to a frequency of 0.574 (57.4%) in that breed.

Table 1. Predicted lower (SL_L) and upper (SL_U) bounds of selection limits, largest observed direct genomic values (DGV_{max}), and ratios of SL_L to DGV_{max} and SL_U to DGV_{max} for milk yield, fat yield, protein yield, fat percent, protein percent, and lifetime net merit for U.S. Ayrshire (AY), Brown Swiss (BS), Guernsey (GU), Holstein (HO), and Jersey (JE) cattle.

Trait (units)	Breed	SL_L	SL_U	DGV_{max}	SL_L to DGV_{max}	SL_U to DGV_{max}
Milk (lbs)	AY	13,976.19	28,413.70	2,776.17	5.03	10.23
	BS	24,791.63	62,493.30	6,084.35	4.07	10.27
	GU	12,796.00	27,390.79	2,130.09	6.01	12.86
	HO	35,556.94	105,347.53	11,121.06	3.20	9.47
	JE	25,831.53	89,440.44	5,585.93	4.62	16.01
Fat (lbs)	AY	520.35	1,044.91	151.61	3.43	6.89
	BS	888.27	2,158.25	238.77	3.72	9.04
	GU	571.98	1,096.32	164.82	3.47	6.65
	HO	1,266.55	3,931.46	501.99	2.52	7.83
	JE	997.04	3,357.92	339.14	2.94	9.90
Protein (lbs)	AY	370.13	747.05	101.47	3.65	7.36
	BS	670.96	1,642.04	200.27	3.35	8.20
	GU	347.88	750.09	73.03	4.76	10.27
	HO	827.17	2,680.03	324.25	2.55	8.27
	JE	676.60	2,491.61	193.36	3.50	12.89
Fat percent (%)	AY	2.55	4.62	1.11	2.88	5.90
	BS	4.29	13.43	0.94	4.15	9.74
	GU	4.48	13.20	1.61	2.29	4.16
	HO	0.89	1.87	0.29	4.58	14.35
	JE	1.95	4.22	0.47	2.78	8.19
Protein percent (%)	AY	1.14	2.24	0.36	3.08	6.45
	BS	1.64	5.34	0.34	4.12	8.91
	GU	2.29	6.81	0.80	3.15	6.17
	HO	2.55	4.62	1.11	4.86	15.82
	JE	4.29	13.43	0.94	2.87	8.53
Lifetime net merit ($)	AY	4,144.15	8,393.45	908.88	4.56	9.24
	BS	8,085.39	18,124.87	2,086.48	3.88	8.69
	GU	4,187.75	8,318.17	744.13	5.63	11.18
	HO	11,704.89	39,902.78	4,728.14	2.48	8.44
	JE	8,175.53	26,400.41	2,766.43	2.96	9.54

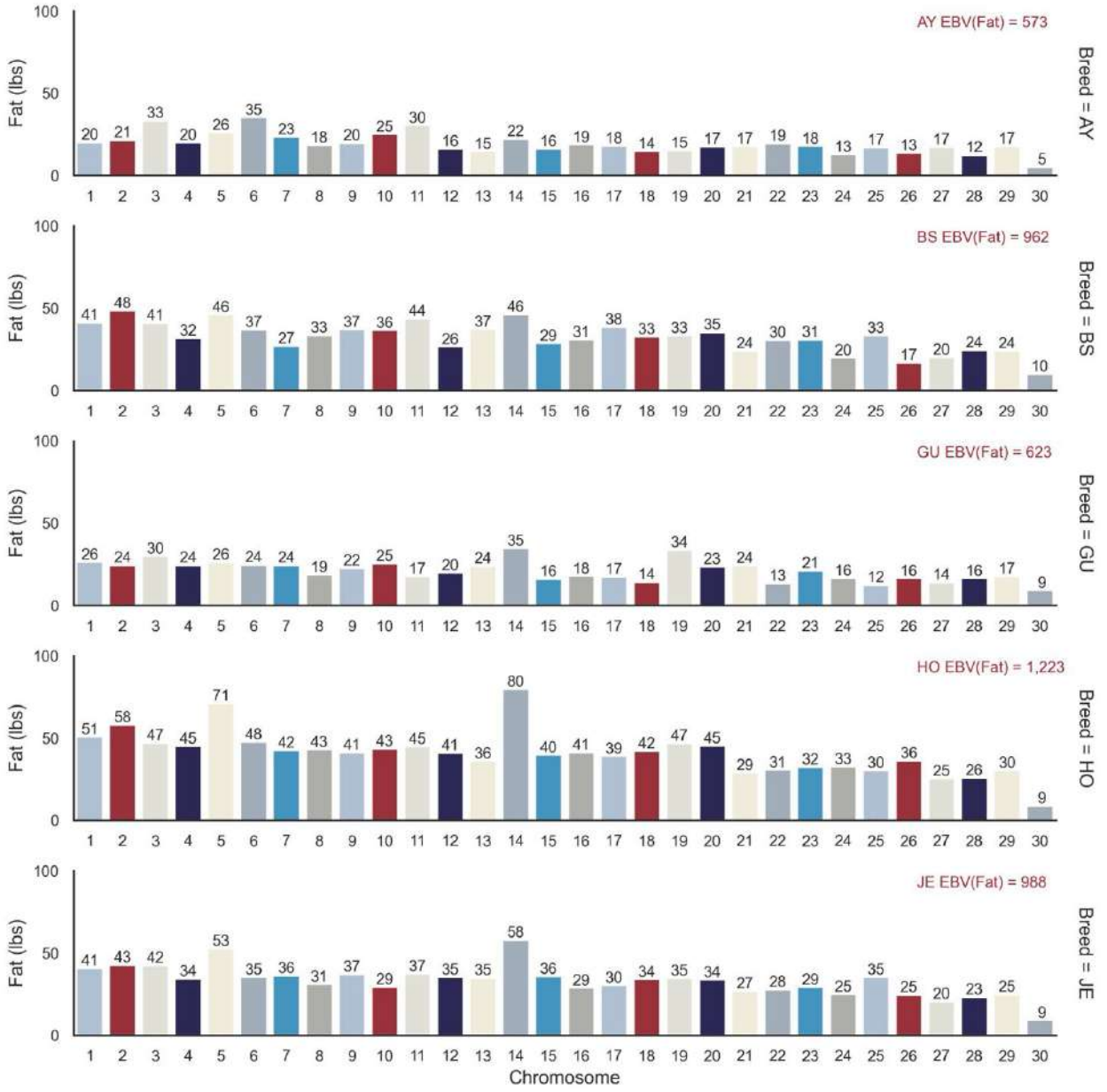


Figure 3. Chromosomal estimated breeding values (EBV) of fat yield for hypothetical Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey animals whose genotypes consist of two copies of each of the best haplotypes in their respective US populations.

As noted above, the Ayrshire and Guernsey populations in the US are small, and the gains achievable in those breeds are correspondingly modest because there are fewer opportunities to sample outlier genotypes.

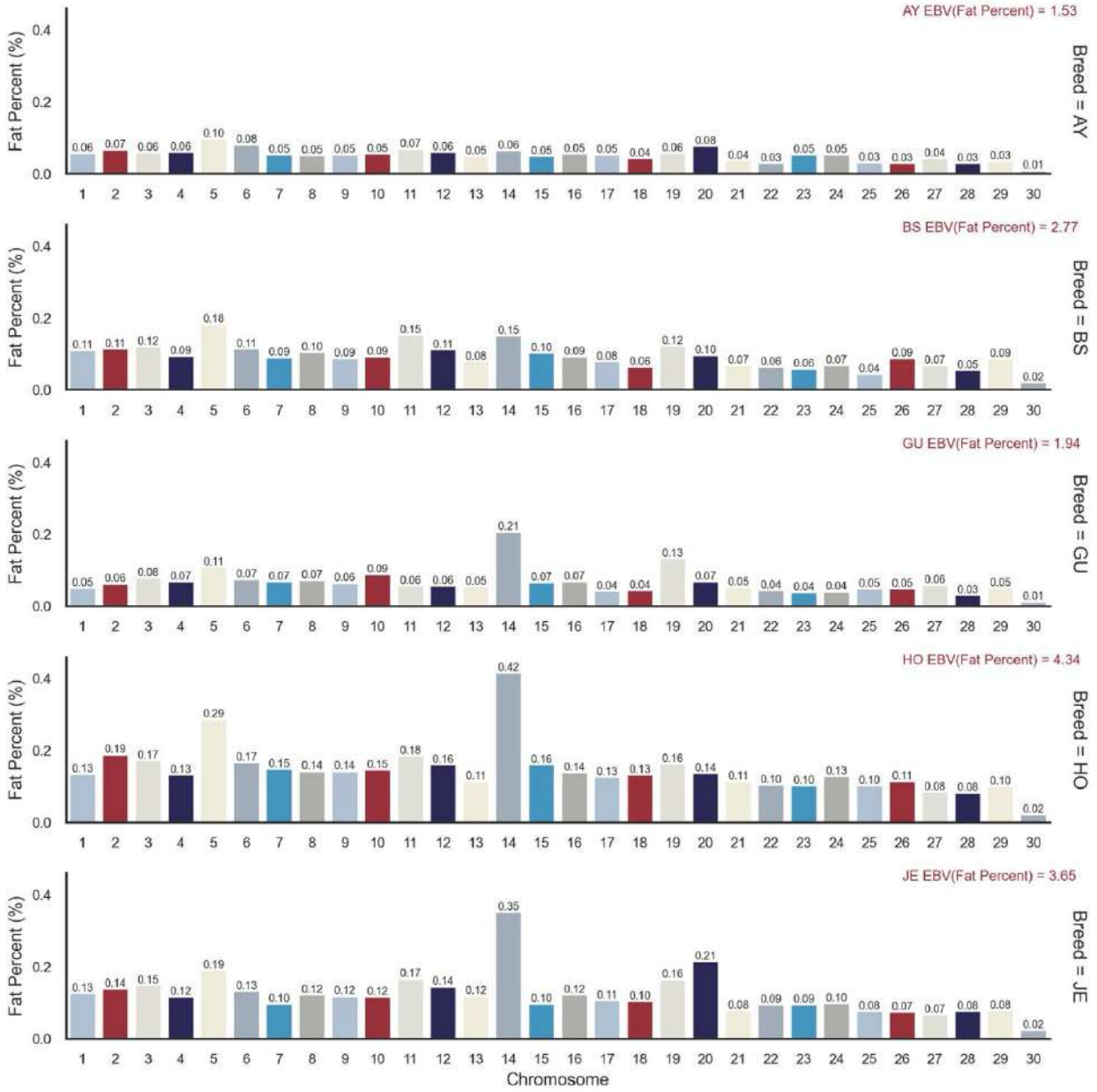


Figure 4. Chromosomal estimated breeding values (EBV) of fat percent for hypothetical Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey animals whose genotypes consist of two copies of each of the best haplotypes in their respective US populations.

If fat percent is considered instead of fat yield (Figure 4), the same general trends are observed, although absolute differences between chromosome 14 are more pronounced because of *DGAT1*'s larger effect on fat percent than fat yield. Holstein and JE have much higher potential selection gains that AY, BS, or GU, with BS occupying an intermediate position between the higher- and lower-potential groups.

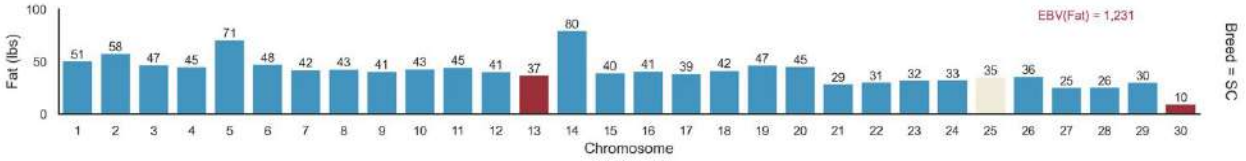


Figure 5. Chromosomal estimated breeding values (EBV) of fat yield for a hypothetical animal whose genotype consists of two copies each of the best haplotypes in the US populations. This animal's genome includes haplotypes from the Brown Swiss (red), Holstein (light blue), and Jersey (cream) breeds.

In constructing the best possible animal for fat production (Figure 5), most haplotypes would be selected from the Holstein population, but chromosomes 13 and 30 would be selected from the Brown Swiss breed, and chromosome 25 from the Jersey breeds. This calculation assumes there is no linkage across chromosomes and there is no effect of genetic background, i.e., Holstein chromosomes are not affected by admixture with Brown Swiss, and Jersey chromosomes, and *vice versa*. The *DGAT1* gene on chromosome 14 has been known for many years to have large effects on fat yield and concentration (Grisart et al., 2004). A large QTL on chromosome 5 associated with the *MGST1* gene also has been shown to affect milk composition (Cole et al., 2011; Littlejohn et al., 2016; Wang et al., 2025).

With respect to protein yield (Figure 6), the best possible animal would carry 4 Brown Swiss chromosomes (2, 17, 22, and 23), 16 Holstein chromosomes (1, 4-6, 8-11, 13, 16, 18-21. 26. And 29), 9 Jersey chromosome (3, 7, 12, 14, 15, 24, 25, 27, and 28), and 1 Guernsey chromosome (30). This calculation assumes there is no linkage across chromosomes and there is no effect of genetic background, i.e., Holstein chromosomes are not affected by admixture with Brown Swiss, Guernsey, and Jersey chromosomes, and *vice versa*. The amount contributed by each chromosome is generally proportional to its length, as is expected when large QTL are absent. Chromosome 25 includes regions associated with ketosis (Parker Gaddis et al., 2018) and milk composition (Benedet et al., 2019) in Jerseys. A search of the Animal QTL Database (Hu et al., 2022) found limited reports of QTL in Guernseys (e.g., Li et al., 2021), which remains an under-researched breed.

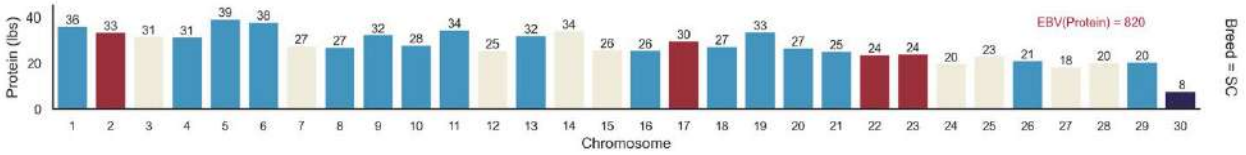


Figure 6. Chromosomal estimated breeding values (EBV) of protein yield for a hypothetical animal whose genotype consists of two copies each of the best haplotypes in the US populations. This animal's genome includes haplotypes from the Brown Swiss (red), Holstein (light blue), Jersey (cream), and Guernsey (dark blue) breeds.

Conclusions

These results suggest, despite the decrease in generation intervals and increase in selection intensity over several generations, that substantial opportunity for genetic progress remains in the US dairy cattle population. In the 15 years (approximately 6 generations) since Cole and VanRaden (2011) provided initial estimates of selection limits under genomic selection, selection limits for Brown Swiss, Holstein, and Jersey cattle are proportionally similar to those results despite continued increases in inbreeding (Cole, 2024). While genetic variation is the raw material with which the breeder works, it can also cause problems for the breeder by producing variation in selection outcomes. If we can identify all the best alleles and combine them into a single animal, we can finally produce the end-state anticipated by Jay L. Lush: “When the pure breeds finally reach equilibrium between the production of heterozygosis by mutations and the loss of heterozygosis because the effective number of animals in the breed is small, it is possible that the pure breed may support only a few scores of unfixed loci.” (Lush, 1945). While there is still a long way to go before such animals are produced, the rewards for getting there may be substantial.

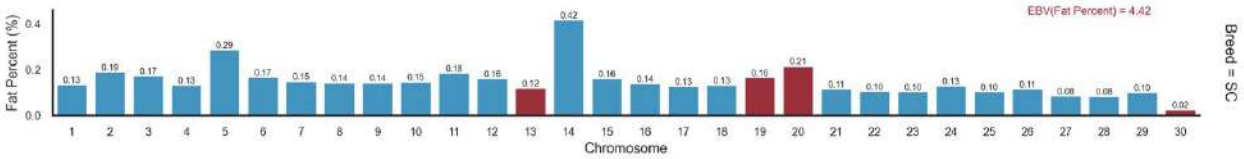


Figure 7. Chromosomal estimated breeding values (EBV) of fat percent for a hypothetical animal whose genotype consists of two copies each of the best haplotypes in the US populations. This animal’s genome includes haplotypes from the Holstein (light blue) and Jersey (red) breeds.

However, we must also ask ourselves if the potential gains described above are plausible. In all likelihood, they are not. Our calculations assume that genetic potential will continue to increase as the best alleles are assembled into individual genotypes, but selection gains are unlikely to remain additive as populations approach selection limits. In biology, systems are complex and intertwined with one another, making it difficult to predict how one change will affect overall performance. Figure 7 suggests it may be possible to breed a cow with the genetic potential to average ~4.4% more fat over the course of a lactation than average cows. In 2024, US Holsteins enrolled in milk recording programs averaged 4.27% fat, which implies that fat yields of 8.67% could be attainable. While we acknowledge that baleen whales (Oftedal, 1997) can produce milk with ~40% fat at peak lactation, it hardly seems credible that a Holstein cow will ever average 9% fat for an entire lactation. The cost of producing comparable body condition scores in Holstein cows and whales is probably economically undesirable. It is also possible that nutritional requirements may eventually exceed feed intake capacity or economic viability, such that the feed cost of producing an additional pound of fat or protein will exceed its marginal value. Homeostasis may be disrupted, particularly a cow’s ability to maintain its body temperature, which will have undesirable effects on performance. Finally, the only thing we can predict with certainty is that our predictions will ultimately fall short because there are so many things we still do not understand about high-performing dairy cows. This

does not mean we should not try; in fact, it encourages us to form new collaborations and develop new tools so that we can keep learning more.

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A Discussion about the Predictions of Net vs Metabolizable Requirements for Essential Amino Acids and the Interpretation of Milk Yield and Milk Component Responses

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Introduction

This paper will discuss different approaches to describing amino acid (AA) requirements for milk and milk component yields, the evolution of the process and what it means as we formulate diets for the current and future high genomically selected cattle. Historically, many studies have estimated net milk requirements, milk protein yield, and maintenance which ignores AA requirements for metabolic activity, such as enzyme requirements in the mammary gland, or AA use in metabolic pathways like the Krebs Cycle (Bequette and Nelson, 2006). When we combine the net and metabolism requirements, we observe a much higher AA requirement than previously described for synthesizing both milk fat and protein. Further, those requirements increase as genomic selection increases the protein synthetic capacity for milk component synthesis. Thus, we observe increased milk component yields, currently higher milk fat relative to protein, with greater essential AA (EAA) supplies that appear incongruent with our prior knowledge and training. As discussed last year (Van Amburgh and Benoit, 2025), the genetic capacity of the modern Holstein is currently unknown, and we are observing individuals, groups, and herds yielding extraordinarily high milk components while being fed their "grandmothers' diets". This likely means they have greater capacity that we do not know how to nutritionally achieve. This paper discusses the evolution of amino acid (AA) requirements for lactating dairy cattle and integrates some of the productivity observations, as the estimated requirements look unrealistic from a historical perspective; therefore, we need to continue to discuss and evaluate the predictions to gain confidence and assurance that, when supplied, the cows will respond with greater productivity.

Net versus Metabolic Approaches to Estimating Requirements

The first version of the Cornell Net Carbohydrate and Protein System (CNCPS; O'Connor et al., 1993) was the first to describe AA supply and requirements and used a factorial approach. The 1985 NRC publication (Ruminant Nitrogen Usage, NRC, 1985) laid the groundwork for much of this thinking and was co-authored by Sniffen and Chalupa, two of the authors of the 1993 CNCPS paper describing the updated model. To account for AA maintenance requirements, the model used a factorial approach and described requirements for scurf, urinary, and fecal protein, and the associated efficiencies of use for the AA. Growth requirements were determined to vary by growth stage relative to equivalent empty body weight and mature body weight (Ainslie et al., 1993). Gestation requirements were based on the net protein required for fetal growth and the efficiency of use of each AA, set at 0.85, a very high value given the amount of AA used for glucose production in the fetus. Finally, lactation requirements were

estimated based on the milk true protein content, daily milk yield, the efficiency of use of each AA for milk protein formation, and the lactation requirements. Thus, in the first attempt to model AA requirements, a factorial approach was used based on the net output of protein and AA. The AA estimates in this version of the model were a starting point providing the first estimates using the CNCPS approach and were somewhat consistent with the model of Evans and Patterson, (1985). The most important step this model developed was assigning AA to feeds and bacteria to predict AA supply and doing so with reasonably high accuracy for the data available at the time. It also laid the groundwork for further development of AA requirements, as the authors described different efficiencies for net factors, incorporating the idea that not all AA are used at the same efficiency, and that metabolic demands vary across AA and net uses.

The next evolution in AA supply and requirements in the US came from the 2001 Dairy NRC (NRC, 2001), which described AA requirements as a percentage of the metabolizable protein (MP) supply. For overall MP, NRC 2001 does use a factorial/net-requirement approach. The NRC (2001) sums net protein needs for maintenance, growth, pregnancy, and lactation (including milk protein synthesis) then converts to MP using an efficiency factor. But for methionine (Met) and lysine (Lys) specifically, the NRC (2001) didn't follow that same factorial logic. Instead, they adopted empirically derived optimal concentrations of Lys and Met as a percentage of MP (7.2% and 2.4%, respectively) based largely on dose-response work by Rulquin et al. (1993) and Schwab (1996) using data to regress graded intakes of these AA to milk protein yield responses across many production trials. This approach used a meta-analytic/dose-response rather than back-calculating a net requirement from milk protein AA composition. This was a more wholistic approach to estimating the AA requirements because it made them part of the MP supply which also includes the non-essential AA (NEAA) and indirectly links the metabolizable energy (ME) to the requirements relying on appropriate formulation that balances the ME and MP allowable milk to overcome the first limiting factor for milk or milk component yield. One of the issues that still confines our approaches to defining AA is forcing these requirements primarily on net outcomes, such as milk protein, tissue protein and fetal protein needs. That approach ignores the requirement of AA for pathways related to energy metabolism, conversion to NEAA, and enzyme activity related to every aspect of milk and milk component synthesis.

As indicated earlier, the CNCPS had some capacity to predict AA supplies and requirements, and from inception to about 2010, the CNCPS used a somewhat complicated approach to estimating the AA requirements as described above. This was called into question by some scientists and studies published by Doepel et al., (2004) and others where they identified varying efficiencies of use of AA based on supply relative to requirements. In the paper by Doepel et al., (2004) and the Cornell Nutrition Conference paper by Lapierre et al., (2007), it became apparent the AA requirements could be more directly described using a different approach, still related to the factorial system, but simplified to a combined efficiency that integrated more of the AA requirements for metabolism and not net requirements for each requirement function (e.g. maintenance, growth, lactation). In the re-development of CNCPS v6 (Tylutki et al., 2008), we adopted the combined approach of Lapierre et al. (2007), and to attempt to integrate it with energy,

we utilized the efficiencies provided when the AA supply was at 100% of the MP supply. The implication for this approach was that if the objective was to balance ME and MP supplies, then when the AA and MP were at 100% of requirement, that should match the ME, thus indirectly integrating the two requirements. This approach was useful as it allowed the CNCPS to estimate the AA requirements using the factorial approach but with a more global requirement that integrated more aspects of metabolism and metabolic fate, not just net requirements. For Met and Lys, this approach allowed for the use of the breakpoint or broken stick model as utilized in the NRC (2001) and provide similar but slightly different values. What became apparent with each incremental change in the approach of calculating the AA requirements was the increase in the gram amount of AA required to meet the demand. With this approach, the metabolizable Met requirements increased about 10 to 15 grams, which was a significant increase given the amount of rumen protected AA needed to meet that requirement. And given the relationship between Lys and Met, the increase in Lys supplementation seemed to be almost unrealistic at the time.

During the development of CNCPS v6.1 and 6.5, Ryan Higgs was developing v7 as part of his Ph.D. work and in doing so using concepts from Doepel et al (2004) and Lobley (2007), he redescribed the AA requirements based on ME supply and requirements, thus linking them directly. This was a significant departure from the factorial, breakpoint, and percent of MP approaches used by all previous models (Higgs, 2014; Higgs and Van Amburgh, 2017; Higgs et al., 2023). Once again, the most significant observation was the increase in AA requirements compared to previous estimates (Table 1). Since v7 was not going to be released quickly, the data of Higgs (2014) and LaPierre (2021) were used to estimate the metabolizable requirements for Met, Lys and His within the structure of v6.5. This resulted in the following requirement estimations: Met at 1.19 g/Mcal ME; Lys at 3.2 g/Mcal ME and His at 1.2 g/Mcal ME. And, once again, the metabolizable levels of those AA were significantly greater than previous estimates and were levels that were initially considered impractical in the field. The first use of those updated AA requirements was in the study of Benoit et al., (2026a) where all cows were fed a similar diet and the treatment was a dose titration of Rumensin. Metabolizable Met, Lys, and His supplies were calculated using the values described previously, and the gram supplies were approximately 86, 220 and 88, respectively. At the time, these levels seemed extraordinary, however, from the cattle performance on the herd diet to the treatment diets increased significantly with 0.4 and 0.2 unit increases in milk fat and protein percentages, respectively, with no change in dry matter intake or milk yield. Thus, the increase in ECM yield was about 18%, demonstrating a similar increase in ECM efficiency demonstrating that the rederived AA requirements were reasonable as cows responded positively to the supply. Similarly, increasing the supply of Met from 0.86 to 1.19 g/Mcal ME increased ECM yield by increasing milk protein and fat yields (Benoit et al., 2026b). More emerging data in vitro (Li et al., 2019; Li et al., 2019) and in vivo (Ding et al., 2020; Benoit et al., 2026b) show an essential role for AA in milk fat production primarily by enhancing de novo FA and triglyceride synthesis. Thus, this supports the need to determine AA requirements using whole body metabolism and AA use instead of just the net requirements for milk protein.

Table 1. The amino acid (AA) requirements and supply of lactating dairy cattle fed predicted from the NASEM (2021), CNCPS v6.5.6, and CNCPS v7.0 using data from Benoit et al., 2026 using the 14.5 grams per ton Rumensin treatment.

AA, g/d	NASEM Req, g[1]	NASEM Supply, g[1]	CNCPS v6.5.6 Req[2]	CNCPS v6.5.6 Supply[2]	CNCPS v7 Net Req[3]	CNCPS v7 Opt Req[4]	CNCPS v7 Supply	CNCPS v7 Gen Req[5]
Arg	73	146	148	202	160	151	181	177
His	52	67	**86**	91	80	84	80	104
Ile	101	142	142	155	174	160	176	200
Leu	173	233	270	261	265	253	285	303
Lys	145	203	**232**	225	232	224	251	266
Met	47	70	**86**	88	90	84	91	104
Phe	89	148	150	166	171	159	180	196
Thr	87	132	111	150	155	159	172	174
Trp	28	34	42	45	49	44	44	56
Val	116	160	159	182	186	184	197	212
Total MP	2,629	2,577	2,909	3,196	2,885	2,885	3,157	-

[1]AA requirements (Req) or supply predicted using NASEM (2021)
[2]AA requirements (Req) or supply predicted using CNCPS v6.5.6 using net requirements except for bolded AA that were predicted using g/Mcal ME.
[3]AA Requirements (Net Req) predicted using CNCPS v7 using the net requirements and fixed efficiency of use of each AA (Higgs et al. 2014).
[4]AA requirements (Opt Req) predicted using CNCPS v7 using the optimum g/Mcal ME and dietary ME supply (Higgs et al., 2014).
[5]AA requirements (Gen Req) predicted using CNCPS v7 using net requirements on an ECM basis. The prediction provided is an estimate of AA requirements for high genomic cattle.

In 2021, the updated National Academy of Science, Engineering and Medicine (NASEM) Nutrient Requirements of Dairy Cattle consensus report was released. The committee introduced several new recommendations and approaches to estimating the requirements of AA. They provided a modified approach that integrated some of the considerations from Higgs (2014), but they did it from a net protein perspective. They developed the requirements around the concept of target efficiency, similar to the optimums by Higgs, however the NASEM doesn't directly calculate the grams of metabolizable AA that are available for metabolism. The predictions appear to be a solution based on the net output in milk protein plus maintenance divided by the efficiency of use. This approach provides a solution based on the supply-response relationships among the experiments used to derive the relationship. Secondly, they use the target efficiency and variable efficiency which assumes, in what appears to have some priority to particular AA, that the efficiency of all AA increases or changes with overcoming the deficiency of another limiting AA. In other words, the priority of efficiency of use of the AA is focused on milk protein and does not include any utilization for metabolism. They focused on defined, measurable net outputs (milk secretion, growth accretion, gestation) and net maintenance losses (endogenous urinary loss, scurf, metabolic fecal protein)

which is a very different approach than Higgs (2014). This difference becomes apparent when evaluating the predicted requirements as in Table 1. Evaluating the data from Benoit et al., 2026 through the 2021 NASEM model, the NASEM model predicts a 47-gram requirement for Met, whereas v6.56 predicts a requirement that is 53% greater and v7 predicts a requirement that is 91% greater. Based on actual milk performance, cattle response suggests the latter is more correct.

That begs the question, what does this mean for current and future AA requirements? The cow response data generated to date at Cornell would suggest the AA requirements are going to continue to change and increase for a few reasons. First, by integrating the requirements with energy, we are, in a simplified manner, accounting for AA catabolism/oxidation, AA used for non-protein biosynthesis (glucose, other N-compounds, signaling molecules, etc.), inter-organ AA cycling, and individual variation in partitioning between mammary uptake and catabolism elsewhere. This means the requirements will be greater than any net requirement because of this accounting. Second, we have new genotypes of Holsteins, and their requirements are currently not truly understood (Van Amburgh and Benoit, 2025), and as we learn about their AA requirements, they undoubtedly will increase as currently being observed on commercial farms. As pointed out last year, there are enzymes in the mammary gland which either have more requirements for the AA or are induced by some of the AA, such as Lys and Fatty Acid Synthetase (Dudek and Semenkovich, 1995; Li et al., 2019).

Diet simulations in Table 1 were performed using the same animal and dietary inputs but utilized different models to show how different approaches change AA predictions. The requirement predictions in the table using CNCPS v7 use two different approaches: the MP requirements of AA using the demand for maintenance, milk, tissue, and endogenous proteins with efficiencies of use applied (Higgs et al., 2014), and optimum requirements calculated using g/Mcal ME optimums (Higgs et al., 2014) and ME supply. This data suggests that the optimum g/Mcal requirements for each AA likely need to be updated to reflect modern genetics and the demand for higher milk fat and protein synthesis. We roughly predict these updated requirements on an MP basis to meet the demands for protein synthesis in higher genomic dairy cattle, although this approach should be combined with re-evaluation of the optimum values to integrate the requirements with energy supply. The most obvious outcome is the significant deficiencies of AA this shows and the quantity of AA required to meet the demand. This is the opportunity and the challenge to the industry if we are going to meet the requirements for increased milk protein synthesis.

When formulating diets in our research studies, we are applying this formulation approach for AA, and the consistent outcome is greater ECM and feed efficiency. In one of our most recent studies (Fontoura et al., 2026) we were testing the lower boundaries of nitrogen (N) intake, while formulating diets to optimize productivity. The cows in this study increased milk fat by ~0.4 units and protein by at least ~0.2 units moving from the herd diet to the study diets, while maintaining milk yield. This resulted in cows at 5.2% and 5.3% milk fat and 3.45% milk protein while yielding ~117lb of ECM. Individuals on these treatments achieved up to 9.4% to 10.2% milk fat while maintaining milk yield. We

do not yet have genomic data on these cows but it appears that the enzyme systems in the mammary gland of our genomically selected cattle have greater capacity to synthesize milk fat and likely milk protein, when presented with the substrates necessary to allow them to do so and this will likely continue with each generation, suggesting that the AA requirements will continue to increase as well. And for milk fat, this is the best example of where accounting for the AA requirements of metabolism has allowed us to see these responses. On an intuitive basis, there is no net requirement for AA for milk fat synthesis, however, there appear to be a significant metabolism requirement for the enzymes that synthesize the fat and this needs to be accounted for as to not miss opportunities to take advantage of the ever-changing genetic potential of today's cows.

The modern dairy cow presents an opportunity to re-evaluate the requirements for lactation, and likely with need to be updated more frequently. Based on recent internal studies and commercial dairy production, the current AA optimum requirements more closely meet the requirements for milk fat synthesis with opportunity to further assess the AA requirements to meet the demand for higher milk protein. Other AA such as proline (Pro), valine (Val), leucine (Leu), and isoleucine (Ile) comprise a large portion of the casein, and thus increasing casein synthesis has an absolute demand for those AA. Again, the optimum AA requirements (g/Mcal ME) likely need to be re-evaluated to include the increased AA demand for greater milk protein synthesis while also capturing the demands for all metabolic functions. This also highlights the increase in energetic efficiency as the gram unit per Mcal ME increases. This is where the CNCPS v7 can provide some insight into this situation as it predicts the limiting AA and in the case of milk protein (Ile, Leu, Val, Arginine, Lys, Glutamine, Pro, Threonine, and His). Many of these are not readily available in a rumen protected form so precise supplementation is currently not possible. The amount of CP needed to meet these AA requirements would be in excess of 20%, which is not feasible as the urinary N excretion would greatly increase, there would be a significant energy loss, and N metabolism has an energy cost that would be worth pounds of milk production (Morris et al., 2021). Thus, there is a significant need for the feed industry to find a solution to meet these requirements in a manner consistent with the concept of precision nutrition.

Summary

To best describe the requirements of high producing lactating dairy cattle, the formulation systems should move away from net requirements and focus more on the metabolizable requirements of AA. When doing this, there will be a significant increase in the grams required for all AA, and based on the rate of genetic selection, this will increase every 5 to 7 years. We need to have a research system in place or a verifiable strategy that allows us to make incremental changes like the requirement system for swine nutrition. There is a significant opportunity to produce and protect a wider range of AA for use in the dairy nutrition industry to meet the requirements for milk protein synthesis and do so while moving towards commodity prices as they will become more of a commodity than a specialty product with greater genetic capacity in cows.

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