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Balancing Dietary Amino Acids for Optimum Milk Fat Synthesis and Implications of Dietary Fatty Acid Supply and Profile – A Modeling Exercise

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With the implementation of genomic selection, the genetic capacity for milk fat and protein synthesis has dramatically increased. The rate of change for milk fat is nearly exponential, given the selection pressure on genes and alleles such as SCD1 and DGAT1, and the rate of change for milk protein yield is similar. These changes suggest, at least in Holsteins, that the potential content and yield of milk fat and protein is extraordinarily high with reports of first lactation Holstein cattle with milk fat contents of >6.5% and true protein contents >4.0%, and combined component yields approaching 9 pounds per day. These changes are analogous to creating a new genotype, similar to reevaluating new generations of growing swine to match the essential amino acid (EAA) supply to the protein accretion rate of the animal (https://webconnect.uscdcb.com/#/summary-stats/genetic-trend).

Dietary strategies that allow for increases in the yields of milk and milk components are important to increase profitability for dairy producers, the dairy industry's sustainability, and to meet the ever-increasing genetic capacity. In previous studies conducted in our lab, the focus has been on formulating diets that optimize milk component synthesis by providing adequate dietary aNDFom content and digestibility, starch, sugar, fatty acids (FA), nitrogen, and amino acids (AA), among other essential nutrients. LaPierre et al. (2020) and Higgs et al. (2023) evaluated the relationship between metabolizable energy (ME; Mcal/d) and AA (g/d) supply to determine the AA requirements of histidine (His), methionine (Met), and lysine (Lys) to be 1.19g His/Mcal ME, 1.19g Met/Mcal ME, and ~3.20g Lys/Mcal ME (or 2.7 times Met) as modeled in CNCPS v6.55 and 6.56. After balancing the supply for these AA, it is important to recognize that there may be limitations in the supply of other EAA, such as Leucine (Leu) and Isoleucine (Iso) (NASEM, 2021), and possibly non-essential AA (NEAA) such as proline (Pro) or alanine (Ala). The BCAA are essential for biological functions and have been reported to be important upregulation signaling molecules for mammalian target of rapamycin (mTOR) and other downstream cascades involved in milk component synthesis (Arriola Apelo et al., 2014; Pszczolkowski et al., 2020). Casein protein is a three-dimensional molecule comprised of ~20% BCAA, and contains many Pro molecules which, due to the three-side chain structure, allows for adequate turning and folding of the side chains in an energetically unfavorable configuration (Kumosinski et al., 1993). Accordingly, the Pro content of casein is about 10%, making it one of the most used AA in the structure of the protein. In high-producing dairy cattle that might be resource-limited, it is possible that NEAA-like Pro can become limiting, especially in early lactation. In diets where the supply of EAA are limited compared to their requirements, biological function and milk production are constrained to the most-limiting AA even as the supply of non-limiting AA increases. Thus, it is crucial to meet the requirements of all AA to sustain many biological processes while maximizing productive functions such as growth, reproduction, and lactation.

It is also critical to recognize other end-products of metabolism that can confound our estimations of N utilization, mainly when we apply a reductionist approach to N metabolism where the utilization of N appears intuitive but might not produce a direct outcome. For example, when considering the EAA requirements of lactation in cattle, many calculations are made solely based on the EAA requirements for milk protein synthesis and yield (NASEM, 2021; Lapierre et al., 2005). This is partially true, as EAA, although essential and contributing to the production of milk protein, is functional in the mammary gland outside of milk protein synthesis. Lactose and fat synthesis are EAA-intensive processes that also involve metabolic regulation through protein and enzyme synthesis. When discussing EAA requirements, all metabolic processes in the mammary gland for the yields of milk and milk components are protein synthetic pathways (Bionaz and Loor, 2008; Mu et al., 2021; Osario et al., 2016; Palmquist and Harvatine, 2020) and calculating efficiencies of use for EAA must incorporate all uses of the EAA integrated with diet energy allowable productivity – from a CNCPS perspective this is ME. The study of Higgs et al. (2023) and review by Reed et al. (2014) suggested the concept of relating N efficiency to the ME supply and energy status of the animal and expands on many of the approaches previously used to improve N efficiency. When considering available ME, all available energy sources, including carbohydrates, lipids, and proteins, should be well described. In the context of nitrogenous compounds, such as AA, many EAA and NEAA are glucogenic, providing necessary precursors for cellular energetics and metabolism. Discrete predictions for EAA supplies have been commonplace in our diet formulation systems, yet, until discrete NEAA supply is fully described, MP will still be used as a proxy of total AA sufficiency, for both protein synthesis and energy metabolism.

The requirements of FA for lactation are not as well defined as the requirements of AA or other nutrients, although there is a biological requirement of FA for many productive functions. Additionally, improving our understanding of the extent of ruminal biohydrogenation of unsaturated FA and the quantification of bacterial and protozoal FA may lead to more precise predictions of the post-ruminal FA supply and profile. Milk fat is comprised primarily of FA that originates from three main sources: de novo, mixed, and preformed FA. De novo FA are short and medium-chain FA (<16 carbons) that are synthesized in the mammary gland, preformed FA are long-chain FA (>16 carbons) that originate from dietary sources, and mixed FA (16-carbon) can be synthesized de novo or absorbed from the diet. Gresti et al. (1993) quantified the most prevalent FA combinations found in milk triglycerides (TG) and determined TG containing a C4:0, C16:0, and *cis*-9 C18:1 had the greatest molar frequency compared to other combinations of three FA suggesting a balance of FA from all sources may be important to optimize milk fat synthesis. Similarly, balancing the intake or supply of dietary FA to maintain a ratio of 1.5:1:1 for C16:0, *cis*-9 C18:1, and C18:0 may improve the balance of preformed FA supply and optimize the conversion process of C18:0 to *cis*-9 C18:1 in the mammary gland. In addition, there are interactions between the amount and profile of FA being supplied to the cow. The general recommendation is to feed moderate levels of total FA and a balanced ratio of individual FA, similar to balancing for EAA.

Overall, more research is warranted to understand the requirements of FA, thus allowing for more refined diet formulation to optimize FA supply for milk component synthesis and other productive functions.

In previous studies, we observed that optimizing AA supply to meet the requirements increased milk fat and protein content. Benoit et al. (2021) fed treatment diets comprised of 15% CP, 32% aNDFom, 25% starch, 5.75% sugar, and 3.63% total FA that supplied 1.19g Met/Mcal ME, 1.24g Met/Mcal ME, and 3.09g Lys/Mcal ME to mid to late lactation dairy cows. Although the study was designed to test the effect of increasing the dietary supply of monensin, the average production across all treatments was 39.6 kg/d milk yield, 46.6 kg/d energy-corrected milk (ECM), 4.67% milk fat, 3.37% milk true protein, and 4.63% milk lactose. Interestingly, the increase in milk fat was achieved by synthesizing higher levels of de novo and mixed FA with the average FA content (g/100g milk) across treatments being 1.16% de novo FA, 1.89% mixed FA, and 1.35% preformed FA which was similar to the milk fat composition of Jersey dairy cows (Barbano et al., 2019). Additionally, Danese et al. (2024) evaluated the effect of increasing the supply of Met, while maintaining equivalent levels of His and Lys, on milk, milk components, and milk FA. The authors observed that increasing Met from 0.86g/Mcal ME to 1.19g/Mcal ME increased ECM yield, the contents of milk fat, true protein, de novo FA, and mixed FA, suggesting that optimizing the supply of Met is essential to optimize milk component synthesis and ECM yield (Tables 1 and 2). The data from these studies suggest that supplying optimal levels of Lys, His, and Met that meet the requirements for lactation increases milk fat production by increasing de novo FA synthesis. Thus, AA may play a role in enzyme activity or signaling cascades that are essential for milk FA synthesis. Interestingly, balancing for AA improves energy efficiency by overcoming limiting resources and allowing increased nutrient utilization in the mammary gland, thus increasing milk component production and ECM yield.

Table 1. Body weight, dry matter intake, milk and energy corrected milk yield and milk components in lactating dairy cows fed three levels of rumen protected methionine where lysine and histidine were formulated at 3.2 and 1.19 grams per Mcal ME using CNCPS v6.5. Danese et al. 2024. Rows with different superscripts differ P < 0.05.

	Diet, g Metabolizable Met/Mcal ME				
Parameter	0.86	1.05	1.19	SEM	*P*-value
Body Weight, kg	698	705	701	3.3	0.30
Delta BW, kg	16.4	23.9	9.80	6.8	0.35
Dry Matter Intake, kg	26.4	26.5	26.1	0.30	0.59
Milk Yield, kg	44.6	45.3	44.8	0.40	0.38
ECM, kg	48.8^a	50.2^b	50.4^b	0.44	0.02
ECM/DMI, kg/kg	1.87	1.88	1.92	0.02	0.21
Milk True Protein, g/100g	3.09^a	3.24^b	3.34^c	0.01	<0.01
Milk True Protein, kg	1.38^a	1.46^b	1.49^b	0.01	<0.01
Milk Fat, g/100g Milk	4.21^a	4.25^a	4.36^b	0.03	<0.01
Milk Fat, kg	1.88	1.92	1.94	0.02	0.16
MUN, mg/dL	11.20	11.44	11.09	0.12	0.12

Table 2. De novo, mixed and preformed fatty acid content in lactating dairy cows fed three levels of rumen protected methionine where lysine and histidine were formulated at 3.2 and 1.19 grams per Mcal ME using CNCPS v6.5. Rows with different superscripts differ $P < 0.05$.

	Diet, g Metabolizable Met/Mcal ME				
Parameter, g/100g milk	0.86	1.05	1.19	SEM	*P*-value
Denovo	1.14^a	1.17^b	1.20^b	0.010	<0.01
Mixed	1.65^x	1.67^{xy}	1.70^y	0.015	0.07
Preformed	1.16	1.15	1.19	0.013	0.20
Relative Concentration, g/100g FA					
Denovo	28.79^a	29.33^b	29.34^b	0.09	<0.01
Mixed	41.83	41.61	41.56	0.15	0.40
Preformed	29.33	29.08	29.07	0.17	0.43

Many studies have evaluated the mechanistic role of AA and FA in milk synthetic pathways by assessing their effects on mTOR and sterol regulatory element-binding protein (SREBP), as well as essential enzymes for milk fat synthesis, such as acetyl-CoA carboxylase (ACC), FA synthase (FAS), stearyl-CoA desaturase (SCD), and diacylglycerol acyltransferase (DGAT). Li et al. (2019) supplemented bovine mammary epithelial cells in vitro with Lys and a FA mix containing C16:0 and *cis*-9 C18:1 and observed that cells treated with both Lys and FA increased the expression of SREBP-1 and secretion of triglycerides (TG) compared to supplementation of only one treatment. Lys has been shown to facilitate the upregulation of FA binding protein (FABP) and SREBP, which in turn, upregulate enzymes such as acetyl CoA synthase (ACS), ACC, and FAS, enhancing de novo FA synthesis. Further, Ding et al. (2022) observed that infusing arginine (Arg) increased de novo and mixed FA yield and increased the expression of ACC, SCD, and DGAT compared to the control. Met and Leu have also been shown to be involved in upregulating SREBP(Li et al., 2018). Elongation of the FA carbon chain requires FAS and data has demonstrated the role of His, Lys, serine (Ser) and cysteine (Cys) in the expression and activity of the enzyme system, also suggesting that FAS requires both EAA and NEAA. In addition, there are interactions the amount and profile of FA supplied to the cow. To achieve increased milk FA synthesis, the requirements of EAA at a metabolizable level are generally much greater than historically supplied. Using the approach of Higgs et al. (2023), the grams per Mcal of ME provides a more precise approach for achieving the supplies required to increase the expression of these enzymes. Additionally, the level of AA supply for these processes and protein synthetic pathways reflects the increased genetic capacity of the mammary gland to achieve higher milk fat and protein production. For example, supplying at least 1.19 g metabolizable Met and His/Mcal ME, and 3.2g metabolizable Lys/Mcal ME provides adequate levels of those EAA to achieve increased milk fat synthesis. It is also important to consider dietary sources of substrates that enhance ruminal butyrate production, as butyrate is used in significant quantities to synthesize FA and triglycerides on a molar basis.

These data suggest AA are required to optimize milk FA synthesis by providing substrate for enzyme activity or acting as signaling molecules for protein synthetic cascades. Protein synthetic pathways and cascades are regulated by the amount of nutrients available for the synthesis of the end-product of interest and the signaling molecules that dictate the action of these nutrients to form a specific end-product. Mackle et al. (2000) utilized the hyperinsulinemia-euglycemic clamp technique to evaluate the effect of increased insulin supply with and without the infusion of casein and BCAA. The authors observed that insulin independently increased the yields of milk and milk protein compared to the control, whereas insulin and AA infusion further increased the yields of milk and milk protein compared to the treatments independently. This data suggests insulin may be an essential regulator of the partitioning of nutrients in the mammary gland for milk component synthesis. Insulin-induced genes (INSIG) are responsive to insulin changes and affect the translocation and activation of SREBP, affecting the downstream actions of genes involved in milk fat and protein synthesis (Bionaz and Loor, 2008).

Nutrient supplies need to increase concomitantly with the change in capacity to realize the genetic capabilities of high-producing dairy cattle. This requires a more integrated approach where both the AA and FA are described together as a function of the ME and MP supply, to better meet the mammary capacity for milk fat and protein synthesis and yield. This also requires that our nutrient requirements and supply models are more precise in describing the supplies of these nutrients to optimize productivity while reducing the environmental impact of dairy production.

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Optimizing Amino Acid Nutrition for Sustainability: Emerging Innovation Published in the *Journal of Dairy Science*

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Introduction

Dairy production plays a significant role in global food production. With a high concentration of essential amino acids, milk protein accounts for approximately 10 % of the global food supply of protein and amino acids (Boland and Hill, 2020). The dairy cow possesses a remarkable ability to convert feed nitrogen (N) into highly edible foods (Hodgson, 1971; White and Hall, 2017) but does so at variable efficiency. Specifically, 15-35% of feed N is captured in milk, a measure often referred to as feed nitrogen use efficiency (feed-NUE) (Powell et al., 2010). This is similar to estimates for pork production (10-44%) while higher than estimates for beef production (4-8 %) and lower than estimates for poultry (25-62%) (Gerber et al., 2014; Shurson and Kerr, 2023). The reported variation in feed-NUE serves as strong evidence that opportunities still exist to even further improve productive efficiency through improvements in genetics and nutrition as well as with technological advancements.

Optimizing N and amino acid nutrition has become an increasingly important focus for driving milk production while also seeking to reduce the environmental footprint of dairy operations (de Oliveira et al., 2025). An official journal of the American Dairy Science Association, the *Journal of Dairy Science* (JDS), the leading peer-reviewed dairy research journal in the world. JDS publishes original research, review articles, and other scholarly work that relates to the production and processing of milk or milk products intended for human consumption (Journal of Dairy Science, 2025). Resources such as the Web of Science (WOS) (https://www.webofscience.com/), a research database and citation index system maintained by Clarivate Analytics (formerly part of Thomson Reuters). It provides data related to peer-reviewed literature, scholarly books and some conference proceedings. It also track citations across an array of disciplines, enabling discovery of influential research and analysis of research impact. Major research metrics including Journal Impact Factor, h-index, citation counts and is widely used in academic evaluation. Using WOS, an analysis of publication trends in the *Journal of Dairy Science* reveals both a long record of impactful publications (see examples related to methionine listed in Table 1) and a marked increase in research addressing amino acid supply, utilization, and bioavailability in lactating cows over the past decade. This growth reflects the growing recognition of the role amino acid balance plays in improving feed-NUE and supporting sustainable dairy production.

This article will provide a discussion of emerging knowledge in this area with emphasis on the unique adaptations of highly efficient cows, the recent findings on the challenges posed by unbalanced amino acid supply, and the evolving understanding of bioavailability. Emphasis will be placed on how these insights can inform more precise diet formulation strategies that drive production while minimizing nitrogen excretion into the environment. By integrating bibliometric trends with cutting-edge research, this session aims to provide nutritionists with a broader perspective on where the field has been, where it is headed, and how these developments can be applied.

Adaptations of highly efficient cows

Feed efficiency (FE) can be defined simply as milk output per unit of feed, assuming no change in body tissue (NASEM, 2021). Both profitability and environmental efficiency are known to be heavily influenced by FE (VandeHaar and St-Pierre, 2006; Guinguina et al., 2020). Although the heritability of nitrogen efficiency is probably low, opportunities for genetic selection potential exist (Chen et al., 2022, 2023b; a) future gains in overall FE will reduce environmental impact of dairy production. Recent research examined differences in liver metabolism and amino acid (AA) utilization between high-efficiency (HE; low residual feed intake) and low-efficiency (LE; high residual feed intake) Holstein cows (Daddam et al., 2025) . HE cows consumed less feed per day despite producing similar amounts of milk fat and protein. Liver proteomics revealed differences in abundance of proteins between the two groups of animals. Upregulated proteins were involved in the TCA cycle, fatty acid degradation, and amino acid biosynthesis, suggesting that HE cows are more fit to extract energy from feed and conserve amino acids. Downregulated proteins were linked to ketone body synthesis and amino acid catabolism, indicating HE cows limit energy losses and minimize unnecessary AA breakdown. Practically, this may indicate that HE cows are metabolically adapted to maximize energy utilization and amino acid efficiency under reduced feed intake. From a management standpoint, feeding high- and low-efficiency cows the same diet in mixed pens may be suboptimal: HE cows risk being overfed, leading to excess nitrogen excretion, while LE cows may be underfed, limiting their production potential.

Nitrogen and amino acid supply

Precise feeding practices are an effective way to improve feed-NUE. Major factors identified and known to affect NUE include diet CP content, rumen degradability of protein, carbohydrate source, frequency of feeding, feed processing methods, and amino acid feeding strategies (Schwab et al., 2005). Nitrogen efficiency, can be improved by lowering diet N (Chowdhury et al., 2024), but if diets are formulated with too little N, they may dramatically compromise milk protein synthesis and production (de Oliveira et al., 2025). Nonetheless, clear opportunities exist for dairy nutritionists to improve feed-NUE by balancing diets for amino acids. In a systematic review of the literature published in *Livestock Science*, Robinson, (2010) reported that on average supplementation of two rumen protected amino acids (RP-AA), namely methionine and lysine, improved feed-NUE 4%. In a controlled experiment published in the *Journal of Dairy Science* reducing the oversupply of metabolizable protein in diets balanced to meet lysine and methionine requirements increased feed-NUE by 3% (Laroche et al., 2022). Similarly, diets with RP-AA have been reported to have improved marginal efficiency of supplied AA transformed into milk protein (Nichols et al., 2024). Despite these promising results, when diets are formulate for individual amino acids, improvements in feed-NUE are not always observed (Van den Bossche et al., 2023). Possible reasons for this may include the fact that even when attempts are made to correctly supply individual amino acids supplies may still fall short of requirements. This may be the case when assumptions in feed libraries lead to overestimates of digestible amino acid supply. Interestingly, supplying excessive valine has appeared to have negatively impact performance and overall feed-NUE (Weston et al., 2024). It was speculated that high valine disrupts amino acid transport and utilization and the study provided evidence that in addition to serving as "building blocks" amino acids also serve as metabolic regulators, influencing mammary signaling pathways and interactions. When practically evaluating N supply on-farm nutritionists commonly consider milk urea nitrogen (MUN) measures. A recent study published in the *Journal of Dairy Science* investigated the influenced relationships between MUN and urine N excretion and feed-NUE (Zhao et al., 2025). As expected MUN served as a positive indicator for both and affected by diet concentrations of CP and nonfiber carbohydrates. Although a good indicator of N supply before making diet adjustments it is also important to remember there are other factors than can influence MUN. These include stage of lactation, season, temperature, and other animal factors that affect N metabolism (Fatehi et al., 2012)

Digestibility and bioavailability

Predictions of metabolizable protein supply rely on estimating either the rate of protein degradation in the rumen or on the amount of protein that bypasses the rumen. This bypass protein, or rumen undegradable protein (RUP), can be determined using in vivo, in situ, and in vitro methods (Ørskov and McDonald, 1979; Krishnamoorthy et al., 1983; Vanzant et al., 1996). Although final estimates may be affected by small particle washout and microbial contamination, the NASEM (2021) model uses inputs generated by the in situ method to estimate RUP. To estimate the intestinal digestibility of RUP (dRUP), NASEM (2021) relied upon data generated by the mobile bag (MB) (Hvelplund, 1985), the three-step (TSP) and modified three-step (MTSP) procedures (Calsamiglia and Stern, 1995; Gargallo et al., 2006). While many of these methods have been used for a long period of time more recently the Ross assay has shown to be an effective assay to rapidly estimate the digestibility of feed samples (Ross et al., 2013). Similarly, accurate estimates of the bioavailability of RP-AA are necessary for the improvement of NUE in dairy cattle. Bioavailability is best defined as the proportion of a nutrient absorbed in a utilizable form (Littell et al., 1995). Post-ruminal availability of RP-AA is challenging to measure and has been evaluated by in situ and in vitro assays (Bach and Stern, 2000; Wu et al., 2012) as well as by in vivo measures of plasma appearance or milk protein response (Littell et al., 1997; Graulet et al., 2005; Borucki Castro et al., 2008; Whitehouse et al., 2017; Smith et al., 2022). More recently a new method published in the *Journal of Dairy Science* and referred to as the fecal free amino acid (FFAA) method estimates the bioavailability of RP-AA through total fecal collection to determine the amount of free amino acids excreted in feces. This is then used to calculate the proportion of amino acids digested and absorbed (Räisänen et al., 2025). In the case of rumen protected lysine and methionine it has yield similar results while also avoiding the complexities related to post-absorptive metabolism, plasma dynamics, and likely among-animal variance found in plasma responses. Although promising, developers of the assay suggest that further research and comparison of this assay to that involving isotope-labeled amino acids and multi-cannulated cows is needed.

Summary

Optimizing amino acid nutrition in dairy cows is both a challenge and an opportunity, carrying direct implications for production efficiency, environmental stewardship, and long-term sustainability. Research continues to show that precision feeding strategies such as balancing amino acids or improving bioavailability estimates can yield measurable gains in nitrogen use efficiency while safeguarding milk protein output. At the same time, emerging insights remind us that amino acids are more than just building blocks; they act as metabolic regulators that influence cow physiology in complex ways. Moving forward, integration of genetics, nutrition, and novel analytical approaches will be essential to refine feeding practices and reduce N waste and improve feed-NUE. For nutritionists, the next phase of progress lies in translating these innovations into practical on-farm strategies that simultaneously enhances profitability and reduces the environmental footprint of dairy production

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Table 1. Listing of highly cited papers published in the Journal of Dairy Science for which Web of Science has categorized “methionine” as a “topic” (defined as term appearing in searches of title, abstract, and keywords).

Citation	Title	Citations
(DePeters and Cant, 1992)	Nutritional factors influencing the nitrogen composition of bovine milk: a review	340
(Schwab et al., 1976)	Response of lactating dairy cows to abomasal infusion of amino acids	286
(Van Amburgh et al., 2015)	The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5	273
(Gustafsson and Palmquist, 1993)	Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields	232
(Schwab et al., 1992)	Amino acid limitation and flow to duodenum at four stages of lactation. 1. sequence of lysine and methionine limitation	215
(Schwab and Broderick, 2017)	100 Year review: Protein and amino acid nutrition in dairy cows	207
(Lee et al., 2012)	Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet	207
(Santos et al., 1998)	Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review	174
(Osorio et al., 2013)	Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function	170
(Higgs et al., 2015)	Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs	167

Multifaceted Roles of Lysine in Dairy Cattle

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Introduction

Lysine (Lys) and methionine (Met) are consistently identified as the first-limiting essential amino acids in dairy cow diets. Both are critical for milk protein synthesis and play emerging roles in regulating immune and antioxidant function. While these amino acids (AA) are traditionally recognized as a limiting amino acid for milk protein synthesis in dairy cows, there is an expanding body of evidence highlighting their broader role in regulating hepatic metabolism, mammary gland metabolism, antioxidant defense, and immune function.

A recent meta-analysis of 12 studies with 40 treatments confirmed that increasing Lys supply improves milk and milk protein production, with responses influenced by stage of lactation and duration of feeding Lys in the postpartum period (Arshad et al., 2024a). Beyond its role as a substrate for protein synthesis, Lys contributes to metabolic regulation through carnitine production and interactions with other amino acids, particularly methionine. Precision supplementation with rumen-protected Lysine (RPL) has therefore emerged as a practical tool to optimize nutrient efficiency, reduce reliance on variable protein sources, and support both production and environmental goals in modern dairy systems.

Previously, studies in bovine hepatocytes demonstrate that different forms of Met and its analogs, such as 2-hydroxy-4-(methylthio)butanoic acid (HMB), modulate genes controlling transmethylation, transsulfuration, and regeneration pathways, with consistent reductions in methionine cycle activity as Met supply increases (Zhang et al., 2016; Zhang and White, 2017, 2019) . Beyond its metabolic functions, Met-derived compounds such as S-adenosylmethionine and glutathione are central to redox balance and the inflammatory response, linking dietary supply to immunometabolic resilience. These mechanistic insights provide a foundation for understanding recent in vivo findings on how Met supplementation influences hepatic bioenergetics and production responses during the transition period in dairy cows. Interestingly, these prior in vitro studies revealed metabolic support for the Met:Lys ratio commonly referenced in the field when it was identified that the ratio of Met:Lys, not just the amount of Met, was important to mitigate the inflammatory response to LPS challenge in vitro (Zhang and White, 2017).

Replacing Porcine Blood Meal with RP Lys

Given the importance of ensuring that Lys and Met needs are met within dairy cattle diets, rumen-protected (RP) amino acids offer a practical alternative, allowing nutritionists to target limiting AA directly; however, the extent to which a complex metabolizable protein source (e.g. porcine blood meal, PBM) can be replaced with only the most limiting, or two most limiting AA, is not yet fully elucidated. In a recent study, we investigated (1) the effects of partially or fully replacing PBM with RPL on production and nutrient partitioning (Arshad et al., 2025a) and (2) the effects of MP adequacy on $CD4^+$ T lymphocyte bioenergetics (Arshad et al., 2025b). Traditionally used as a source of AA, PBM has a valuable AA profile but its use is increasingly limited due to variability, cost, and regulatory restrictions. In this study, we replaced increasing amounts of PBM with RPL, while maintaining metabolizable Met, without supplementing other AA. Sixty-four mid-lactation Holstein cows were assigned to one of four diets for 12 weeks: PBM (control), or diets where 33% (RPL33), 66% (RPL66), or 100% (RPL100) of PBM-derived Lys was replaced with RPL. Methionine supply was maintained across treatments with RP-Met. Data collected included dry matter intake (DMI), milk yield and composition, nitrogen (N) balance, gas exchange, and blood metabolites. Nutrient partitioning was assessed via respiratory quotient, carbohydrate and fat oxidation, and heat production.

Replacing Lys from PBM with RPL did not alter body weight, body condition score, or estimated body energy balance, suggesting that overall energy status was maintained regardless of the source of Lys, despite a linear tendency for reduced (P = .06) DMI with increasing RPL replacement. Milk yield was similar for cows fed the PBM, RPL33, and RPL66 treatments although it was significantly reduced in the cows fed the RPL100 diet (49.6 kg/d vs. 45.9 kg/d, PBM vs. RPL100, respectively). Milk fat percentage increased linearly (P = 0.02) with RPL inclusion, whereas protein percentage tended to be reduced (P = 0.09) linearly. Overall, the total milk solids increased linearly (P = 0.04) and energy-corrected milk yield only tended to be reduced linearly (P = 0.08).

Nitrogen intake decreased linearly with RPL inclusion due to lower dietary crude protein content. Milk nitrogen output also declined, but fecal and urinary nitrogen excretion were not affected. Consequently, nitrogen balance and nitrogen use efficiency (milk N as a proportion of intake N) were similar across treatments, although cows on higher RPL diets partitioned less nitrogen toward milk protein. Milk urea nitrogen concentrations decreased with RPL, consistent with reduced nitrogen intake.

Gas exchange measurements showed that enteric methane and carbon dioxide emissions were not influenced by diet; however, oxygen consumption was reduced at RPL66 and RPL100, suggesting a decrease in oxidative metabolism. Methane yield (g/kg DMI) and intensity (g/kg ECM) remained similar across treatments. Blood metabolite concentrations, including glucose, fatty acids, β-hydroxybutyrate, and liver enzyme activities, were not altered by treatment. Blood urea nitrogen declined slightly with RPL inclusion, again consistent with reduced nitrogen intake.

In order to investigate the impact of metabolizable protein on immune function, cows (n=32) from the two extreme treatment groups were used for additional analysis. Blood was collected from cows fed the PBM diet (MP-adequate; 3,111 g/d MP) or 100% RPL diet (MP-deficient; 2,983 g/d MP) to isolate $CD4^+$ T lymphocytes at week 11 and analyzed for mitochondrial and glycolytic function using a Seahorse extracellular flux analyzer. Cells were studied under resting (non-activated) and stimulated (activated) conditions. Cows fed the MP-deficient diet had reduced maximal respiration and spare respiratory capacity in both resting and activated $CD4^+$ T cells. Glycolytic activity (extracellular acidification rate) declined progressively over time in MP-deficient cows. Mitochondrial and glycolytic ATP production rates were unchanged, but immune persistence appeared compromised.

These studies highlight the dual importance of amino acid nutrition for both production and immune resilience. While RPL can effectively substitute PBM to meet up to 66% of the Lys requirement, complete replacement reduces total MP supply and impairs milk yield. This demonstrates the need to preserve MP adequacy while balancing limiting AAs. Additionally, modest MP deficiency reduced the metabolic fitness of $CD4^+$ T lymphocytes, which may compromise immune responses. This contributes to growing evidence that Lys, Met, and their relative availability, play an important role in immune function.

Conclusions

Maintaining adequate MP supply while balancing Lys and Met is essential for optimizing production, nutrient efficiency, and immune competence. This research indicates that partially replacing PBM with RPL, while maintaining metabolizable Met but allowing a modest reduction of MP, maintained overall production, ECM to feed rations, and nitrogen use efficiency. In contrast, full replacement of PBM with RPL resulted in reduced SMI, milk yield, milk protein yield, and a tendency for reduced ECM. Fully replacing PBM with RPL also linearly decreased nitrogen intake and manure nitrogen excretion without influencing overall nitrogen balance. Take together, these results suggest that partial substitution of PBM with RPL is practical as long as total metabolizable protein supply is maintained. Precision nutrition strategies should ensure MP adequacy, while fine-tuning Lys and Met balance, especially during periods of metabolic or immune stress.

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Designing Feeding Programs for Dairy Profitability

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Introduction

With feed costs comprising over 50% of the total cost of milk production, feeding programs are central to dairy farm profitability. Although attendees at this conference certainly recognize the importance of careful diet formulation to optimize use of nutrients, efficient logistics, shrink avoidance, and feedbunk management are also critical to profitable feeding. We will dig into the keys to effective feeding programs, drawing on insights from Michigan State University Extension's assessments of dairy farms ranging from 180 to 3,500 cows. The goal is to provide actionable recommendations for dairy farm experts to enhance efficiency, safety, and productivity.

Components of a Comprehensive Feeding Program Assessment

Beginning in 2021, our team of MSU Extension colleagues developed a list of feeding program characteristics that could be feasibly evaluated on a commercial dairy by two experts in the course of a day. Our list of factors centered around the optimal use of resources – feed, equipment, time, and human resources, all while supporting optimal cow productivity. Importantly, given the substantial amount of time that nutrition consultants typically spend on ration formulation, we avoided this aspect entirely.

We developed a list of 104 management factors to evaluate, each rated on a 1 to 5 scale, allowing for detailed analysis. We then organized a score sheet (Figures 1 and 2) such that criteria are grouped by the physical location of the factor to be evaluated (in a typical dairy layout), supporting an efficient assessment process. However, we also recognized that bringing a list of 104 scores to a management team would be unlikely to lead to action but rather would overwhelm them. To help focus the discussion on the most problematic areas, we assigned each assessment factor to a broader category: safety, mixing, hygiene, shrink, efficiency, or production. Together, these categories covered the gamut of factors that support efficient resource use and cow productivity.

As of summer 2025, the MSU Extension team has carried out Feeding Program Assessments on 18 farms in Michigan, with a wide range of scales, management strategies, and facility design. Let’s dig into the categories assessed and what we’ve learned in the process.

AREA OF FOCUS			Score (5 = best)					Category	Photo?	Notes
Equipment										
	Mixer wagon & tractor / truck									
		Wagon appearance overall	1	2	3	4	5	Mixing		
		Clean-out	1	2	3	4	5	Mixing		
		Auger condition	1	2	3	4	5	Mixing		
		Knives	1	2	3	4	5	Mixing		
		Kicker plate	1	2	3	4	5	Mixing		
		Liner	1	2	3	4	5	Mixing		
		Forage restrictors (out)	1	2	3	4	5	Mixing		
		Discharge buildup & seal	1	2	3	4	5	Mixing		
		Auger timing & RPM (running)	1	2	3	4	5	Mixing		
		Are scale calibrated regularly?	1	2	3	4	5	Mixing		
		Lights	1	2	3	4	5	Safety		
		Hazard sticker	1	2	3	4	5	Safety		
		PTO cover	1	2	3	4	5	Safety		
		Tractor cleanliness, esp. radiator	1	2	3	4	5	Safety		
		Tractor and wagon size	1	2	3	4	5	Mixing		
Feed center										
	Loader									
		Lights	1	2	3	4	5	Safety		
		Cleanliness - esp. bucket	1	2	3	4	5	Hygiene		
		Bucket	1	2	3	4	5	Mixing		
		Size	1	2	3	4	5	Efficiency		
	Feed storage									
		Labeling	1	2	3	4	5	Mixing		
		Cleanliness	1	2	3	4	5	Hygiene		
		Lighting	1	2	3	4	5	Mixing		
		Mirrors for blind corners	1	2	3	4	5	Safety		
		Ingredient contamination	1	2	3	4	5	Mixing		
		Ingredient hazards (fire)	1	2	3	4	5	Safety		
		Dry forage processing	1	2	3	4	5	Mixing		
		Shrink	1	2	3	4	5	Shrink		
		Pest mitigation	1	2	3	4	5	Hygiene		
		Ingredient exposure to elements	1	2	3	4	5	Hygiene		
	Diet mixing									
		Layout (ingredient locations)	1	2	3	4	5	Efficiency		
		Mixer on level ground	1	2	3	4	5	Mixing		
		Exposure to wind / weather	1	2	3	4	5	Shrink		
		Efficiency of process / routing	1	2	3	4	5	Efficiency		
		Order of ingredients	1	2	3	4	5	Mixing		
		Load size (relative to wagon size)	1	2	3	4	5	Mixing		
		Use of cheater piles	1	2	3	4	5	Efficiency		
		Ingredient loading position in mixer	1	2	3	4	5	Mixing		
		Liquid position and distribution	1	2	3	4	5	Mixing		
		Mixing time during / after loading	1	2	3	4	5	Mixing		
		People wearing high-visibility clothing	1	2	3	4	5	Safety		
Forage area										
	Defacing									
		Appropriate equipment	1	2	3	4	5	Efficiency		
		Face	1	2	3	4	5	Hygiene		
		Blending at defacing?	1	2	3	4	5	Mixing		
		Plastic management and tires	1	2	3	4	5	Hygiene		
		Spoilage	1	2	3	4	5	Hygiene		
		Carryover day-to-day	1	2	3	4	5	Hygiene		
	Infrastructure									
		Pad	1	2	3	4	5	Hygiene		
		Cover	1	2	3	4	5	Hygiene		
		Walls	1	2	3	4	5	Shrink		
		Mirrors for blind corners	1	2	3	4	5	Safety		
		Signage	1	2	3	4	5	Safety		
	Silage									
		Density	1	2	3	4	5	Shrink		
		Smell	1	2	3	4	5	Hygiene		
		Evidence of mold?	1	2	3	4	5	Hygiene		
		Temperature	1	2	3	4	5	Shrink		
		Shrink	1	2	3	4	5	Shrink		
		Height of pile relative to equipment	1	2	3	4	5	Safety		
		Parking relative to pile	1	2	3	4	5	Safety		

Figure 1. Feeding program assessment score sheet for use on farms (page 1).

AREA OF FOCUS			Score (5 = best)					Category	Photo?	Notes
Bunks / barns										
	Infrastructure									
		Rail / headlocks	1	2	3	4	5	Production		
		Concrete	1	2	3	4	5	Shrink		
		Environment at bunk for cows	1	2	3	4	5	Production		
		Water - adequate amount and quality	1	2	3	4	5	Hygiene		
	Refusals									
		Well cleaned out	1	2	3	4	5	Hygiene		
		Separate blade (not used for manure)	1	2	3	4	5	Hygiene		
		Particle size - evidence of sorting?	1	2	3	4	5	Mixing		
	Feed drop									
		Speed	1	2	3	4	5	Safety		
		Feed distribution	1	2	3	4	5	Production		
		Running over feed?	1	2	3	4	5	Hygiene		
		Proximity of wagon to cows or rails	1	2	3	4	5	Safety		
		Tire cleanliness	1	2	3	4	5	Hygiene		
	TMR assessment									
		Visual appearance - uniformity	1	2	3	4	5	Mixing		
		Particle size uniformity in lactating TM	1	2	3	4	5	Mixing		
		Particle size uniformity in dry cow TMF	1	2	3	4	5	Mixing		
	Cows									
		Come to the bunk?	1	2	3	4	5	Production		
		Aggressiveness?	1	2	3	4	5	Production		
		Bunk space	1	2	3	4	5	Production		
Software / office / people										
	Management									
		Calibration of scales (TMR wagon)	1	2	3	4	5	Mixing		
		Written SOPs for maintenance	1	2	3	4	5	Mixing		
		Written SOPs for feeding	1	2	3	4	5	Mixing		
		Maintenance schedule	1	2	3	4	5	Mixing		
		Use of refusals	1	2	3	4	5	Efficiency		
		Are refusals weighed?	1	2	3	4	5	Shrink		
		How are bunk reads/feed calls manage	1	2	3	4	5	Production		
		SOP for feed sampling and analysis	1	2	3	4	5	Mixing		
	Feeder									
		Maintenance of TMR wagon	1	2	3	4	5	Mixing		
		First in / first out inventory	1	2	3	4	5	Hygiene		
		Plastic removal process	1	2	3	4	5	Safety		
		Silage sampling process	1	2	3	4	5	Safety		
		Knowledge	1	2	3	4	5	Mixing		
	Feed software									
		Deviations by feeder	1	2	3	4	5	Mixing		
		Deviations by ingredient	1	2	3	4	5	Shrink		
		Timing of feed drops	1	2	3	4	5	Production		
		Push-outs as a % of fed (if available)	1	2	3	4	5	Shrink		
		DM updates	1	2	3	4	5	Mixing		
		Inventory management	1	2	3	4	5	Shrink		
		Shrink (if available)	1	2	3	4	5	Shrink		
Camera assessment										
	Bunk									
		Feed availability	1	2	3	4	5	Production		
		Cow activity at feed drop	1	2	3	4	5	Production		
		Cow activity at feed push-ups	1	2	3	4	5	Production		
		Push-up frequency	1	2	3	4	5	Production		
		Feed redistribution	1	2	3	4	5	Production		
		Feed drops (if not in software)	1	2	3	4	5	Production		
		Time without feed	1	2	3	4	5	Production		
		Lock-up time	1	2	3	4	5	Production		

Figure 2. Feeding program assessment score sheet for use on farms (page 2). Download at: https://www.canr.msu.edu/dairymetabolismgroup/Decision-Tools/.

Insights from Assessments

Although a full explanation of the scoring criteria for each factor is beyond the scope of this paper, we expected gold-standard practices to earn a score of 5 for any factor, with a score of 1 representing a missing practice or one highly divergent from best practices. As not every factor is relevant on every farm, the scoring system was designed to report the category score relative to the possible score on a given farm. With this approach to normalize assessments across farms, we were able to provide benchmarks by category for each farm (Figure 3). This report demonstrates that scores for most categories ranged from around 65 to 95% (e.g., average scores from 3.25 to 4.75), but that the production category had both the lowest average score and the widest range of scores. Let's consider what we can learn from assessment in each of these categories.

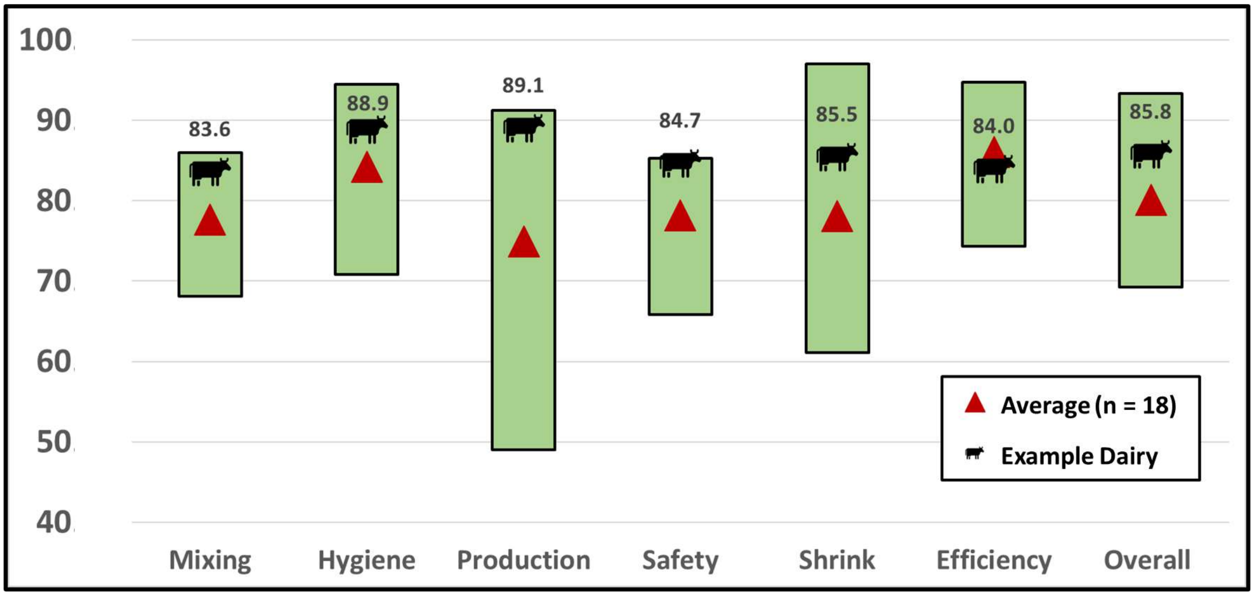

Figure 3. Example benchmarking report showing assessment results by category compared to peer farms.

Safety

Although it may seem strange to lead off with safety when the assessment program is focused on efficient use of resources, valuing and safeguarding people is the most important thing any farm can do. Nothing is more disruptive to workflow than injuries (or worse). Overlooking this aspect is not only a risk to our human resources but can also result in both social and economic burdens on the operation. Key areas for improvement across farms included mitigating the risk of silage pile face collapse, maintenance of power take-off shields, providing appropriate lighting for feed center operations in the dark, and use of high-visibility clothing for employees. One farm conveyed to us that their insurance company now mandates high-visibility clothing, and we anticipate that this will become widespread across the industry in the near future.

Mixing

This category included assessment of particle size uniformity across the bunk for several diets on the farm using the Penn State Particle Separator. It also included evaluation of feeding equipment fit for task and mixer maintenance, including both the status of mixer parts on the day of the visit and standard procedures for ongoing maintenance. We also utilized feed management software to determine ingredient loading accuracy. In general, we were impressed by the ability of most feeders to consistent hit ingredient loading targets with less than 2% deviation, but we did find an example of an individual feeder who fed more than $7,000 in excess soybean meal over the course of a single month (a 33% deviation).

Hygiene

Feed consumption by pests or discarded due to spoilage is a source of shrink (more below) but also can lead to health problems in cows. The most common problems in this category were exposure of feed to the elements (e.g., commodity barns with ingredients overflowing beyond the roof line), excessive bird populations in and above stored feeds, and suboptimal silage face management. Although some of these problems are nearly impossible to eliminate, the farms we evaluated were conscientious about keeping the feed center clean and eliminating silage mold.

Production

We evaluated a number of feeding-related factors that directly impact the ability of cows to maintain a high level of milk production. We assessed the quality of feedbunk environment, but relied heavily on 4-5 days of video recorded in two areas of each farm. Trail cameras (set to take pictures every 15 seconds) were placed with a view of a mid-lactation feed line as well as a close-up or fresh cow pen, and videos of these images were reviewed to assess feed delivery (timing and distribution), push-ups and redistribution, cow lock-up, feed availability over the course of the day, and feed clean-out. Although most farms were excellent at consistent feed delivery and pushed up feed 6 or more times per day, we found a few common gaps across many farms. First, initial feed pushes often occurred 5 or 6 hours after feeding. Although this seems logical due to the large amount of feed in front of cows at that time of day, cows also eat very aggressively in the hours after feeding, often consuming nearly half of the feed allocated within a few hours. As a result, an obvious feed ridge can develop within 2 hours of feeding, with at least some cows having difficulty reaching feed in this window of time; these observations point to the need for an earlier feed push on many farms. Secondly, a surprising number of farms repeatedly ran out of feed 4-6 hours prior to feeding (e.g., midnight – 2 am). Although cows typically eat fewer meals at that time of day, there were nonetheless many images of cows seeking feed and failing to find any. Feeding for limited refusals is important to avoid wasted feed, but the reality is targeting 2-3% refusals is not sufficient to ensure that cows are not running out of feed, as distribution issues and sorting can result in the last 3% of feed being ignored for many hours, while cows go hungry.

Managing for low refusals necessitates a system to evaluate feedbunk conditions in the middle of the night, using employees, technology, or both.

Shrink

Shrink refers to feed loss due to spoilage, spillage, or mismanagement, potentially accounting for up to 10% of feed costs. Sometimes the magnitude of this number gets lost because we are used to it – but we should stop and think about it. If a farm loses 10% of its feed to shrink and feed costs are 55% of total production costs, shrink accounts for 5.5% of the cost of milk production, which may be larger than the profit margin on the farm in a given year! Zero shrink is not a reasonable goal (the perfect ensiling process will generate at least 5-6% shrink), but proper silage storage and handling as well as investments in commodity handling equipment and facilities can make a big dent in shrink costs. We found a wide range of positive and negative practices related to shrink. A substantial number of farms have invested in grain handling systems to limit shrink from wind and precipitation. On the other hand, one farm was pre-batching a protein, vitamin, and mineral mix in the evenings for overnight storage on an uncovered concrete pad, with thousands of dollars of expensive nutrients washing away during every nighttime rain event. On-farm corn milling has become increasingly popular for medium-sized dairies, and savings from not using a commercial mill can help to justify investment in new commodity handling equipment.

Efficiency

On many farms, feeding operations occupy at least one full-time employee, and in some cases, much more. Given the pressures on staffing on most dairies, it’s not surprising that farms generally excelled at optimizing use of employee time. Having equipment scaled appropriately to pen sizes, efficient routing, and use of “cheater piles” to save time in returning excess feed after loading are examples of practices that were generally on point across the farms we evaluated. We also assessed use of refusals, which were utilized as a feed resource on most farms, although increasing practice of raising heifers elsewhere is limiting options for use of refusals on some farms.

How Can Farm Advisors Bring More Value?

Our Feeding Program Assessment has overlaps with many excellent evaluation processes developed by companies over time, with particular credit to Tom Oelberg and colleagues at Diamond V (Oelberg and Stone, 2017). It is our view that every dairy can benefit from external review of these feed management processes, in the same way that AI companies now routinely provide breeding program assessments or crop input companies offer crop scouting. Use of cameras offers a particularly powerful means to get insight into cow behavior and protocol compliance over 24 hours of dairy operations each day, with the caveat that employees may not behave the same with cameras in place. A spreadsheet version of the assessment tool is available for free download at: https://www.canr.msu.edu/dairymetabolismgroup/Decision-Tools/.

Surveys returned from evaluated farms indicated that 100% learned new information regarding their feeding program and intended on making changes to their current program. Reported financial impacts of farm changes initiated through these assessments ranged from $300 to $50,000 per year. These producers also highly recommend the assessment to their peers.

In addition to assisting farms with assessment of feeding practices, farm advisors could bring value by helping farms to make better use of feed management software. These are powerful tools, offering opportunities for employee accountability and process management, but also the potential to carefully track feed shrink. Unfortunately, only a small minority of farms input sufficient information into these systems to generate accurate shrink estimates. The lack of this information, in turn, makes it very difficult to determine a return-on-investment estimate for new feed infrastructure.

Finally, employee expertise varies widely across farms. Training programs for feeders, similar to those for milkers, enhance understanding of feed ingredients, mixing protocols, and equipment maintenance. Farm advisors, in partnerships with Extension, can have a significant impact on motivation of feeders to comply with protocols by helping them to understand the “why” underlying recommended practices.

Summary

Designing feeding programs for dairy profitability requires a multifaceted approach. Safety must be a top priority, accompanied by efficient and consistent feed delivery, effective bunk management, and systems that limit shrink. Employee training and evaluation technology are key for maintaining high standards over time. Infrastructure investments, when strategically planned, can yield significant returns. By focusing on these areas, dairy farms can enhance productivity, reduce costs, and ensure long-term sustainability.

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Building Better Transitions That Lead to Efficient Lactations: Connections Between Immune and Energy Systems

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Introduction

The transition to lactation period in dairy cows is of great interest, not only because of the potential challenges that can arise but also because of the many opportunities for nutritional or managerial interventions to have positive effects. Increased attention to immune response and relationships between comorbidities has continued to provide insight into responses during the peripartum period and how they can impact the subsequent lactation cycle.

Metabolic Disorders

Postpartum metabolic disorders such as fatty liver (FL) and hyperketonemia (HYK) often develop as comorbidities and may have compounding negative effects on dry matter intake and milk composition. In our past research, we observed changes in liver gene expression profiles at calving in cows that would subsequently develop HYK (Weld et al., 2020). In that study, cows were overfed energy prepartum and although the incidence of HYK postpartum was consistent with industry averages, it is possible that the change in liver gene expression was due to prepartum starch intake and not fully an indicator of subsequent HYK onset. In another study, we found that while some cows were resistant or susceptible to HYK onset when induced or not, cows that developed FL also developed HYK (Pralle et al., 2021). To follow up on this in a situation of only natural onset, we recently completed a peripartum study with retrospective assignment of cows (n=28) to treatment groups based on natural onset of metabolic disorders. Cows were followed from 28 days before calving to 56 days after calving and grouped by the absence of FL, presence of FL (liver triglycerides (TG) ≥ 13.9% DM), or the presence of both HYK (plasma BHB ≥ 1.2 mM) and FL (Praisler et al., 2025). In this study, there was only one cow identified with HYK in the absence of FL; therefore, that cow was removed from the analysis. Comorbid cows had similar milk yield but had lower milk protein and SNF, altered liver enzyme profiles, and elevated glucose postpartum compared to cows without metabolic disorders or FL alone. An interesting finding in this study was the absence of a cohort that developed HYK without FL; however, it should be noted that this was a small retrospective study and should be repeated with more cows.

During the retrospective study described above, we were able to analyze postpartum $CD4^+$ T cells to determine immune system metabolic function. Immune cells were isolated from blood samples collected at -28, +3, and +28 days relative to calving and analyzed on a Seahorse XF using a T Cell Metabolic Profiling Kit (described in Arshad et al., 2024b). When not activated in situ, the oxygen consumption rate of $CD4^+$ T cells was only greater in cells collected at +28 compared to those collected at -28 and +3 days relative to calving (Fernández Wallace et al., 2025). Interestingly, when activated, cells collected at +3 and +28 had similar oxygen consumption rate, both greater than that of cells collected at -28 days relative to calving. Postpartum CD4$^+$ T cells showed increased proliferation, glycolysis, and oxidative phosphorylation. Production of inflammatory cytokines production (e.g. IL-1 α, IL-1 β and IL-6) was also greater in cells collected at +3 and +28 compared to -28 days relative to calving. These findings suggest that T helper cell metabolism and proliferation capacity are upregulated after calving, and that these cells express a more pro-inflammatory phenotype, indicating that after parturition, immune function on this subset of cells does not appear to be suppressed, rather activated which is consistent with other recent findings in the literature. When metabolic activity of these CD4$^+$ cells was analyzed by the retrospective HYK categories, cows with HYK had lower CD4$^+$ metabolic activity postpartum. Specifically, glycolytic ATP in non-activated CD4$^+$ T cells was lower in HYK cows compared with non-HYK cows (50.4 ± 1.058 vs. 58.46 ± 1.059; $P = 0.018$) at 28 days postpartum (Fernández Wallace et al., unpublished data).

Influences on Lactational Success

Metabolic and immune responses to the peripartum period are interesting in their own right, but are potentially more impactful when we think about how they may influence the rest of the lactation in terms of production and feed efficiency. Previous research supports that RFI does not influence subsequent lactation HYK onset (Rathbun et al., 2017) and that having HYK or other health disorders in a peripartum period does not influence RFI as a cow continues into mid-lactation (Martin et al., 2021). In the above study, when analyzed by their previous lactation phenotype of high or low residual feed intake (RFI), high efficiency (-RFI) cows tended to have greater oxygen consumption rate of activated CD4$^+$ T cells at +28, but not at -28 or +3, days relative to calving (Fernández Wallace et al., unpublished data). This aligns with exploration of CD4$^+$ T cell metabolic capacity in mid-lactation cows determined to be high or low RFI where high FE (-RFI) cows had greater mitochondrial and glycolytic activity in both resting and activated CD4$^+$ T cells (Arshad et al., 2024a). Cells from high FE cows also had enhanced spare respiratory capacity, ATP production, and expression of CD62L$^+$ markers. Although we do not think about high FE cows as having an activated immune system, this work may suggest an immune readiness phenotype where there is more efficient capacity to respond during an infection in HE cows. Together with another cohort of mid-lactation cows, high FE (-RFI) cows were noted to have less methane and CO_2 emissions. Liver biopsy samples were interrogated for oxidative capacity and although expression of uncoupling, mitochondrial, or tricarboxylic acid cycle genes were similar across RFI status, mitochondrial complex IV had lower oxygen consumption rates in -RFI cows when quantified with complex I. Together with altered blood metabolites (e.g., fatty acids, insulin, glucose), these data suggest a potential for differences in hepatic nutrient use between high and low FE cows.

Summary

Metabolic and immune health during the peripartum period continues to garner attention, both because of the immediate impacts and increasingly because of our understanding of the long-term impacts of this critical period. Recent research supports that immune function during the peripartum period is likely excessively activated immediately after calving, and some of these differences can be tracked as differences in mid-lactation. It is incredibly challenging to tease apart causality in such complex relationships, but it is becoming apparent that immune responsiveness is a key part of transition success and likely an underlying influencer of lactational feed efficiency. The relationship between immune activation and peripartum metabolic incidence and feed efficiency should continue to be explored in larger studies.

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Does Extreme Weight Cycling Contribute to Poor Disease Resilience in Older Dairy Cows?

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Introduction

Adipose tissue (AT) plays a critical role in energy storage, endocrine signaling, and immune regulation. Mobilization of stored nutrients – particularly from adipose tissue, muscle, and bone – is a key adaptation to support the start of lactation in many species, including dairy cattle. The nutrients are typically restored in later lactation, ideally returning the cow to a similar body condition as before lactation. From a nutrient flux standpoint, this balances out over the production cycle and seems to align with the biology of lactation.

Recent evidence, though, has provided some nuance to this view. While most cows do lose body weight in early lactation and regain in later lactation, the magnitude of these changes varies dramatically between cows. Concurrently, new research in other species has shown that *both* rapid AT lipolysis and AT lipogenesis can promote accumulation of immune cells in AT that change the function of the tissue. We present a hypothesis that a circular relationship between extreme weight cycling and excessive AT immune cell infiltration disrupts immune and metabolic homeostasis, leading to increasingly fragile health as these cows age.

What is Weight Cycling and What Does It Have to Do with Dairy Cattle?

Weight cycling refers to repeated loss and gain of body weight. In different species, this can be driven by seasonal availability of feed, migration-induced energy expenditure, or even social pressure (i.e., humans). Because AT is the body's primary rapidly available energy reserve, its mass fluctuates more dramatically than other tissues with shifts in energy balance.

In dairy cattle, weight cycling is a recurring phenomenon associated with lactation. Many cows experience significant body condition loss postpartum followed by repletion later in lactation (Roche et al., 2009). In a rare study where tissue mass was quantified in early lactation, approximately 30% of AT mass was lost in the first 6 weeks of lactation (Akter et al., 2011). This sort of tissue mobilization, however, is not unique to domesticated species selected for high milk yields. In fact, marine mammals are famous for their ability to transfer dramatic quantities of nutrients via milk, often while fasting, leading to similar 20 – 30% depletion of AT mass and also protein (from muscle and organs) during lactation (Oftedal et al., 2000).

These high-level explanations of tissue mobilization to support lactation, however, mask a dramatic range of outcomes with a population of dairy cows. Figure 1 shows data from 809 cows on a single commercial herd tracked during a 1-year study, demonstrating that change in BCS over 4 to 5 weeks around calving ranges from a gain of 0.25 units in a small subset of cows to a loss of 1.25 units or more in a different subset. The range of outcomes among cows *in the same herd* is astounding, especially considering the short period of time in which these changes are taking place. Similar observations have been reported by others using very different methods (Siachos et al., 2022).

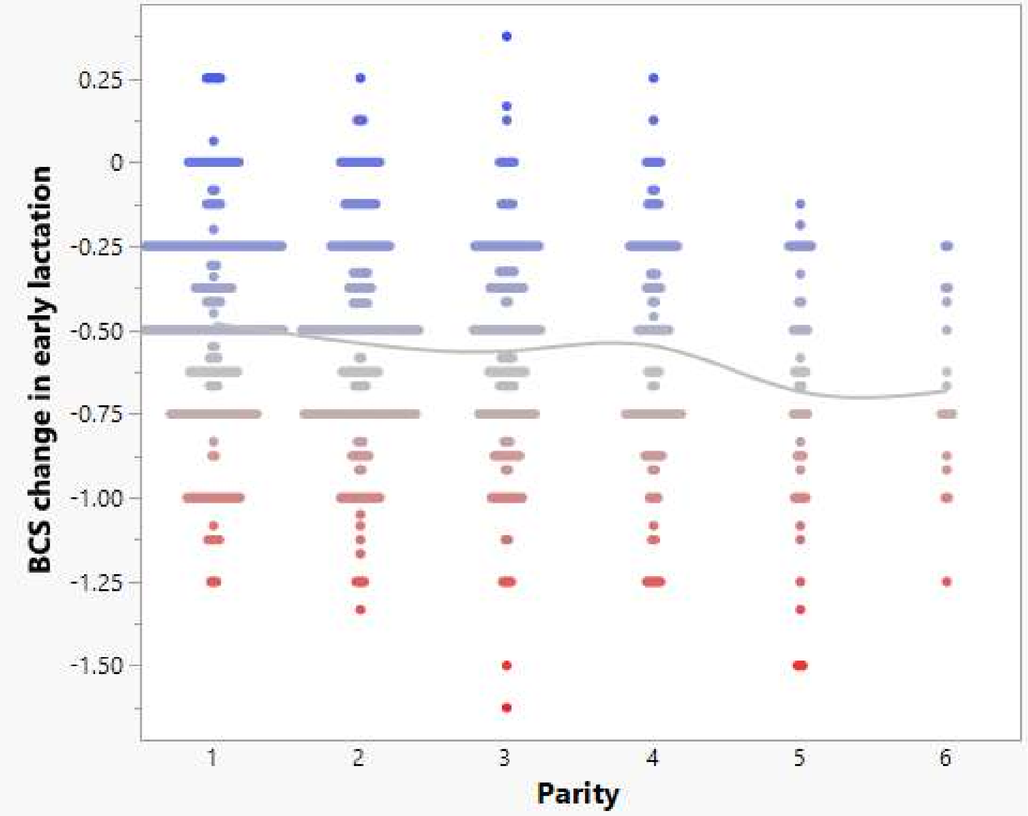

Figure 1. Change in body condition score (BCS) of 809 cows from 14 d before expected calving through 14-21 days postpartum. Parity 5 cows had significantly greater BCS loss than parity 1 (Tukey's HSD, *P* < 0.05), but no other contrasts are significant. Data from Krogstad and Bradford, 2025.

Extreme BCS loss, sometimes defined as loss of more than 0.75 units in early lactation, is associated with reduced pregnancy risk (Manríquez et al., 2021) and increased risk of culling (Krogstad and Bradford, 2025), while greater body weight loss is genetically and phenotypically associated with greater risk of both metabolic and infectious diseases (Frigo et al., 2010). Focusing on the inverse of the same relationship, Middleton and colleagues (2019) reported that cows that gained BCS in the transition period had reduced disease pressure and improved reproductive performance relative to cows that lost substantial body condition. Clearly, dramatic loss of BCS in early lactation has negative associations with health and reproductive success, although in such studies it is difficult to disentangle BCS from clinical and subclinical diseases that can drive BCS loss.

Zachut and Moallem (2017) made an important observation by tracking body weight changes in 92 cows across their first 5 lactations. Cows that lost more than 6% of body weight over the first 5 weeks of lactation (on average) showed progressively greater weight loss across lactations than the low weight loss group. By lactation 5, the high weight loss group shed 10% of body weight over 5 weeks, whereas the low weight loss group lost less than 4% of body weight during that time. The groups did not differ in milk yield over the first month of lactation, but the high weight loss group took progressively longer to get pregnant with advancing parity, in contrast to the low weight loss group. Furthermore, two separate cohorts of high-weight-loss cows showed reduced AT responsiveness to a glucose bolus (insulin resistance) in the prepartum period, demonstrating that altered AT physiology is at least associated with this phenotype (Zachut et al., 2013; Zachut and Moallem, 2017).

Unfortunately, large data sets with cow body weight or BCS data over time and across lactations are almost non-existent in a U.S. production system context. Based on the limited data available, there appear to be phenotypic differences in the extent of tissue mobilization in early lactation that widen with increasing parities. Furthermore, limited evidence points to altered AT signaling as a feature of these high-weight-loss cows.

Adipose Tissue is Far More Complex Than Once Appreciated

Much of the traditional debate about the merits and risks of relatively stable vs. dynamic BCS over the course of a production cycle was built around the concept of AT as a simple energy bank, where calories were just deposited or withdrawn in the form of fatty acids. It is now widely recognized that this understanding of AT is too simplistic (McNamara and Huber, 2018). Recent advances in single-nuclei sequencing have enabled a much more sophisticated view of the cellular composition of bovine adipose tissue (Michelotti et al., 2022). These analyses revealed diverse subtypes of adipocytes, adipocyte progenitors, and immune cells that appear to undergo dynamic shifts during different stages of lactation. Furthermore, these cell types engage in crosstalk within the tissue and also release a variety of endocrine and immune signals that can act on other organs throughout the body (Häussler et al., 2022). As a result, AT plays a critical role not only in energy storage, but also in endocrine signaling and immune regulation in dairy cattle.

It's also worth noting that different AT depots can act differently in response to both immune stimulation and changes in energy balance. Several studies have shown that abdominal AT accounts for more tissue accumulation in the dry period and more mobilization in early lactation than subcutaneous AT (Szura et al., 2020). As abdominal AT is more difficult to visualize and is typically more immune-active (Michelotti et al., 2022), this suggests that changes in BCS may sometimes underestimate key AT dynamics that influence signaling.

Inflamed Adipose Tissue is Linked to Transition Cow Problems

During periods of negative energy balance, such as early lactation, AT undergoes lipolysis, releasing free fatty acids and inflammatory mediators. This process recruits macrophages and other immune cells, leading to a transient pro-inflammatory environment (Bradford and Contreras, 2024) that resolves as negative energy balance is reduced. During disease states the process becomes chronic, leading to a drastic influx of immune cells into AT that limits the organ’s response to antilipolytic stimuli (Contreras et al., 2015; Contreras et al., 2016). The mechanisms driving the immune cell trafficking into AT include oxidized fatty acids (oxylipins; Contreras et al., 2017) and proteins secreted by adipocytes and resident macrophages such as osteopontin (Abou-Rjeileh et al., 2023).

Although the tools to carefully study adipose tissue immune cell infiltration and inflammation are still relatively new, several reports in dairy cow populations support the concept that problematic transition periods are associated with enhanced AT immune cell accumulation. De Koster et al. (2018) reported that cows with high BCS loss in the 9 days after calving had ~5 times the proportion of AT macrophages (type of immune cell) relative to cows with little or no loss of BCS. Newman and colleagues (2019) also reported that AT macrophage abundance was more than 50% greater at 4 and 21 days postpartum in cows that lost more than 0.5 BCS units compared to those that lost 0.25 units or less, reflecting a clear increase in the macrophage population over time, with a greater rate of accumulation in cows with greater BCS loss. Additionally, evidence of enhanced AT inflammation was detected in cows with clinical mastitis (Zandkarimi et al., 2018) and displaced abomasum (Contreras et al., 2015) in early lactation compared to healthy herdmates.

Collectively, these observations led us to develop a formal hypothesis that excessive weight cycling promotes susceptibility to disease as dairy cows age, via progressive infiltration of inflammatory immune cells into adipose tissue (Figure 2). In this model, inflamed adipose tissue promotes excessive lipolysis and systemic inflammation in response to either metabolic or infectious disease challenges, which ultimately makes the cow less resilient to these disruptions (Bradford and Contreras, 2024).

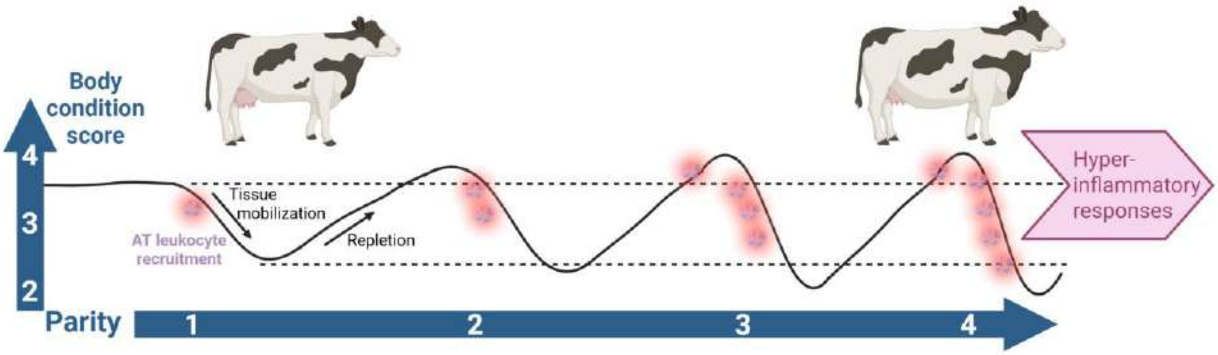

Figure 2. Over time, repeated cycles of lipolysis and repletion may result in persistent immune cell infiltration and altered AT function, in turn driving altered immune responses to disease challenges. Adapted from Bradford and Contreras, 2024.

Strategies for Mitigating Excessive Weight Cycling

Although we want to emphasize that the proposed links between extreme weight cycling and poor disease resilience are not proven, it's worth considering what interventions might be feasible in this scenario. The crux of this concept is that the weight cycling itself contributes to immune cell accumulation in AT, although transition disease could also contribute to the development of an inflamed AT phenotype.

The first and most obvious strategy to reduce the excessive mobilization of AT after calving is limiting AT lipogenesis prior to calving by dietary management. Many studies have shown that cows that calve with greater BCS have greater lipid mobilization after calving (e.g., Szura et al., 2020). This aligns with well-established recommendations in favor of controlled energy diets in the dry period as well as BCS monitoring and management in late lactation.

A recent randomized study of ketosis interventions points to a more novel strategy that may disrupt extreme weight cycling. Cows with clinical ketosis were randomized to treatments including propylene glycol (PG; standard treatment) or PG with the addition of the anti-lipolytic nutrient niacin and the anti-inflammatory drug flunixin meglumine (Banamine). This combined treatment, intended to suppress inflammatory lipolysis in adipose tissue, reduced blood free fatty acids and inflammatory markers compared to other treatments, and improved the proportion of cows returning to normal blood ketone levels by day 7 after treatment (Chirivi et al., 2023). Likewise, this sort of intervention may hold promise for short-circuiting the rapid lipolysis that may drive inflammatory immune cell accumulation in AT, even before symptoms of clinical ketosis.

Additionally, dietary oleic acid may serve to limit lipolysis and enhance lipogenesis in early lactation, thereby limiting AT inflammation. Feeding studies in early lactation cows have documented dose-dependent reductions in body weight loss in cows fed more oleic acid in blended fatty acid supplements (de Souza et al., 2021). This insight was then extended when abomasal infusions of oleic acid in early lactation cows demonstrably altered AT physiology, reducing lipolysis and increasing insulin sensitivity (Abou-Rjeileh et al., 2023). Fatty acid supplements are widely fed, and the increasing popularity of high-oleic soybeans provides another practical means to supply oleic acid to transition cows. As we learn more about the impacts of oleic acid in early lactation and about AT immune cell dynamics, perhaps these exciting areas of research will intertwine.

Summary

In dairy cattle, weight cycling is a recurring phenomenon associated with lactation, where cows experience significant body condition loss postpartum followed by repletion later in lactation. Recent findings provide initial evidence to support a hypothesis that weight cycling in dairy cattle is not merely a metabolic adaptation to support lactation but also – in its extreme versions – a potential driver of chronic AT inflammation and disease risk. Understanding and mitigating these effects could lead to improved animal welfare and performance. However, innovative research is needed to test this hypothesis in a way that isolates weight cycling from many factors that confound most observational studies.

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Dietary Strategies to Optimize Calcium Metabolism and Maximize Postcalving Performance

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Introduction

Hypocalcemia, in both clinical and subclinical forms, has been recognized as a gateway disorder for many years (Horst et al., 1997). Over the past 5 to 10 years, our understanding of the dynamics of calcium metabolism and relationships with outcomes of interest has been refined. Neves et al. (2018) studied the epidemiology of hypocalcemia during the first 4 days postpartum and determined that circulating total calcium concentrations during 1 day in milk **(DIM)** were not associated with greater risk of postpartum health disorders, and multiparous cows with lower total calcium concentrations at 1 DIM actually made more milk than cows with higher total calcium concentrations. On the other hand, cows that had lower circulating total calcium concentrations on days 2, 3, and 4 postpartum were generally more at risk for health disorders and multiparous cows with low blood calcium at 4 DIM made less milk than counterparts with higher blood calcium. In follow up work, McArt and Neves (2020) classified cows as normocalcemic, transiently hypocalcemic, persistently hypocalcemic, or delayed hypocalcemic. Multiparous cows with transient hypocalcemia had higher risk for adverse health events than normocalcemic cows, but the risk was much greater in cows with persistent or delayed hypocalcemia. Furthermore, both primiparous and multiparous cows with transient hypocalcemia made more milk during the first 10 weeks of lactation than cows in the other three groups. Consistent with these results, Seely et al. (2021) determined that normocalcemic and transiently hypocalcemic cows had higher dry matter intakes **(DMI)** than cows with persistent or delayed hypocalcemia, and transiently hypocalcemic cows produced more milk than cows categorized into the other groups.

Collectively, these results suggest that prevention of hypocalcemia, particularly hypocalcemia that persists beyond or develops after the first day postpartum, is critical for transition period success. There are currently two prepartum dietary strategies available that are effective in decreasing postpartum hypocalcemia – decreasing the dietary cation-anion difference **(DCAD)** and using a compound that binds calcium and phosphorus to decrease absorption of these minerals from the digestive tract, creating a situation that activates the homeostatic mechanisms to maintain calcium status. At least at this point in time, these are generally considered to be distinct strategies, although research has not been conducted to determine otherwise.

Decreasing the DCAD During the Prepartum Period to Decrease Hypocalcemia

The concept of managing prepartum DCAD [(Na+ + K+) - (Cl- + S-2)] for improved health and performance of dairy cattle has existed for nearly 50 years (Horst et al., 1997; NRC, 2001). It is well-established that decreasing the dietary cation-anion difference in the diet fed during the prepartum period improves calcium status and decreases risk of hypocalcemia during the immediate postpartum period (Goff, 2014). Several mechanisms are likely responsible for this, including increased calcium flux related to increased urinary calcium excretion and increased sensitivity of tissues to parathyroid hormone for cows fed acidogenic diets (Wilkens et al., 2020). Research from our laboratory demonstrated that further decreasing the DCAD of a low K+ control diet using anionic supplements linearly increased plasma calcium levels and linearly increased postpartum DMI and milk yield (Leno et al., 2017). Researchers at the University of Florida fed either positive or negative DCAD diets with two different sources of Vitamin D (cholecalciferol or calcidiol) during the prepartum period (Martinez et al., 2018; Rodney et al., 2018). Feeding negative DCAD prepartum increased postpartum circulating concentrations of total and ionized calcium, but did not affect postpartum DMI or milk yield; source of Vitamin D did not affect circulating calcium concentrations, but calcidiol supplementation prepartum increased postpartum milk yield. More recently, Poindexter reported that replacing 3 mg/d of cholecalciferol with calcidiol in negative DCAD prepartum diets increased both colostrum and milk yield during early lactation.

Subsequent work by Lopera et al. (2018) sought to determine whether relationships existed between degree of acidification with anions and duration of feeding with outcomes. They fed diets with DCAD of -7 mEq/100 g DM (actual urine pH ~6.5) or -18 mEq/100 g DM (actual urine pH ~ 5.6) for either 21 or 42 days prepartum. Feeding the more negative DCAD diet decreased prepartum DMI, increased blood ionized Ca concentrations on the day of calving and did not affect postpartum performance. Extending the duration of feeding did not affect blood calcium but decreased milk yield by 2.5 kg/day. These results differ from those of Weich et al. (2013) and Wu et al. (2015) who reported that extending the duration of feeding negative DCAD diets up to 42 days before calving did not affect postpartum outcomes; however, in both of these studies the urine pH were more similar to the cows fed the -7 mEq/100 g DM diet in the Lopera et al. study.

Two meta-analyses have been published in the past 5 to 10 years that provide updates to previously conducted meta-analyses exploring the effects of DCAD and other macrominerals on hypocalcemia and performance. Santos et al. (2019) assembled a dataset including 42 experiments with 134 treatment means and 1,803 cows (including 5 experiments with 15 treatment means and 151 nulliparous cows) and evaluated relationships with outcomes. They developed a model to compare the estimated effects of decreasing the DCAD from +20 to -10 mEq/100 g of DM. Using this model, decreasing the DCAD resulted in a predicted decrease in DMI of 0.7 and 0.4 kg/day for nulliparous and parous cows, respectively. Importantly, however, the model predicted increased postpartum DMI of about 1 kg/d for cows fed negative DCAD prepartum. An interaction of parity and prepartum DCAD was present such that parous cows fed the negative DCAD produced 1.7 kg/day more milk postpartum; whereas milk production in nulliparous cows was not affected by prepartum DCAD. The more negative prepartum DCAD was predicted to increase postpartum blood Ca, decrease postpartum beta-hydroxybutyrate concentrations, and decrease incidence of milk fever, retained placenta, and metritis.

Lean et al. (2019) assembled a dataset including a maximum of 31 experiments, 58 comparisons, and a total of 1,571 cows with the objective of exploring the effects of reducing DCAD intake on outcomes. Treatments reflecting the lower DCAD intake had lower urine pH, lower DMI, increased postpartum DMI, and increased milk yield, although an interaction for parity existed for milk yield. Consistent with the Santos meta-analysis, treatments reflecting the lower DCAD intake decreased risk for clinical hypocalcemia and retained placenta and lowered the odds of metritis. Both meta-analyses highlighted the relative lack of data on the relationships of prepartum dietary DCAD on outcomes in nulliparous cows.

Dietary calcium supplementation strategies in conjunction with low DCAD diets fed during the prepartum period continues to be an active area of discussion and debate in the industry. Moore et al. (2000) reported that concurrently decreasing the DCAD (+15, 0, -15 mEq/100 g) and increasing dietary calcium concentration (0.44, 0.97, 1.50% of DM) improved blood calcium status postpartum; however, the effects of DCAD and calcium supplementation cannot be separated in their experiment. Diehl et al. (2018) fed cows either moderate (~ -2.4 mEq/100 g) or low (~ - 21 mEq/100 g) DCAD diets with either 1.3% or 1.8% calcium during the prepartum period. Few differences in circulating Ca concentrations or performance were observed, except that cows fed 1.8% Ca had higher circulating Ca concentrations at d 1 postpartum and cows fed low DCAD made more milk after 45 DIM. Glosson et al. (2020) fed cows either a non-acidogenic positive DCAD diet (+6 mEq/100 g; average urine pH ~ 8.1), or two negative DCAD diets (-24 mEq/100 g; average urine pH ~5.75) fed with either low dietary calcium (0.40% of DM) or high dietary calcium (2.0% of DM) for the last 28 d prior to calving. Feeding negative DCAD slightly decreased prepartum DMI, increased both ionized and total calcium concentrations in blood directly after calving and 24 h after calving, and increased total calcium concentrations at 48 h postcalving. Postpartum DMI (% of body weight) tended to be increased for cows fed negative DCAD prepartum, but milk production was not affected by treatment.

Amundson et al. (2018) created an experimental model of hypocalcemia in nonlactating, nonpregnant cows and evaluated three different calcium feeding levels (0.45, 1.13, 2.02% of DM) in conjunction with low DCAD (-18 mEq/100 g DM). Hypocalcemia was induced by intravenous infusion of a Ca-specific chelator, EGTA. Cows fed the highest calcium concentration maintained higher circulating concentrations of ionized calcium during the challenge period, took longer to reach 60% of baseline circulating calcium concentrations, and required more EGTA to reach 60% of baseline concentrations, suggesting that cows fed higher calcium levels had better ability to maintain calcium homeostasis.

Recently, our group published results from a study conducted to look at the degree of DCAD implementation and prepartum dietary Ca levels (Graef et al. 2025). We fed cows diets targeting partial acidification (DCAD -2.6 mEq/100 g; urine pH 6.7) or full acidification (DCAD -10.3 mEq/100 g; urine pH 5.6) with either 0.7% dietary calcium or 1.5% dietary calcium. Neither prepartum nor postpartum DMI were affected by degree of acidification and overall differences in DCAD strategy were minimal. However, feeding higher dietary Ca prepartum increased DMI by 0.8 kg/d and energy-corrected milk yield by ~ 2 kg/d over the first 9 weeks of lactation.

Feeding a Binder of Calcium/Phosphorus During the Prepartum Period to Decrease Hypocalcemia

The concept of decreasing the absorbable calcium in the prepartum diet to below requirements in order to trigger mechanisms related to calcium metabolism is not new; however, diets typically are not low enough in calcium supply to achieve this. One way to functionally achieve a calcium deficiency is to include a calcium binder in the diet. Thilsing-Hansen and Jorgensen (2001) and Thilsing-Hansen et al. (2002) fed a synthetic zeolite during the prepartum period and determined that feeding the zeolite essentially eliminated postpartum hypocalcemia; milk yield was not reported by Thilsing-Hansen and Jorgensen (2001) and Thilsing-Hansen et al. (2002) reported that differences in accumulated 105-day milk were not significant, although both studies were limited in cow numbers for reporting production results. More recently, Kerwin et al. (2019) fed 500 g/day of synthetic zeolite A for the last 3 weeks prepartum. Similar to the previous research, cows fed the synthetic zeolite A had markedly lower prevalence of hypocalcemia during the postpartum period. Differences in milk yield were not significant; however, cows fed the synthetic zeolite A tended to have a higher hazard of becoming pregnant within 150 DIM. Roche et al. (2018) and Crookenden et al. (2020) reported similar responses of blood calcium concentrations as well as performance and reproduction to supplementation with synthetic zeolite A in grazing cattle. Most recently, Wisconsin researchers fed prepartum cows either a positive DCAD (+19 mEq/100 g) control, a negative DCAD diet (-6.5 mEq/100 g), or a diet with 500 g/day of synthetic zeolite A (Frizzarini et al., 2024a; 2024b). They determined that cows fed synthetic zeolite A had higher blood ionized calcium concentrations from days 0 to 2 postpartum and higher IgG concentrations in colostrum than cows fed the other two treatments; however, overall milk yields were not affected by treatment.

The studies above were conducted under the auspices that the main mode of action of synthetic zeolite A related to its ability to bind calcium; however, in recent times the discussion has focused more on the potential that the mode of action is occurring through binding of dietary phosphorus. All of the studies that measured blood phosphorus concentrations reported marked decreases in prepartum blood P for cows fed synthetic zeolite A. This concept is supported by work conducted in Germany in which cows fed 0.16% P vs. 0.30%P during the last 4 weeks before calving had markedly lower circulating blood P during the prepartum period and higher total blood calcium concentrations (Wachter et al., 2022). Cows fed the low P diet also had increased concentrations of markers related to bone resorption. Although my perspective is that this mechanism is plausible, it has not been determined definitively. Nonetheless, the patterns of response of synthetic zeolite A on calcium status in cows postcalving are very consistent.

Most recently, Francisco Somare (Somare, 2025) in our research group conducted a study focused on comparing a negative DCAD approach with synthetic zeolite A (Calbal®9[Pro], Chemlock Nutrition, Cincinati OH). Within each treatment, cows also received either 0 or 3 mg/d of calcidiol (Nutrivit® Puri-D®, Chemlock). The DCAD of the two negative DCAD treatments averaged -8 mEq/100 g and urine pH averaged ~6.5. The synthetic Zeolite A was fed to target 300 g/d. Despite the lower feeding rate compared to previous studies, cows fed synthetic zeolite A had significantly lower blood P levels prepartum and higher blood calcium concentrations postpartum than cows fed the negative DCAD diets. These results are consistent with previous work at the higher feeding rates. Dyscalcemia (blood calcium ≤ 2.2 mmol/L at day 4 postpartum) was much lower for cows fed synthetic zeolite A (11%) than cows fed negative DCAD (38%). Postpartum DMI and milk yields were not affected by treatment.

Summary

Both manipulating the DCAD of the prepartum diet and use of a synthetic zeolite A to bind dietary calcium and/or phosphorus are effective strategies to prevent postpartum hypocalcemia in transition dairy cows and synthetic zeolite A appears to be very effective at decreasing dyscalcemia. Feeding negative DCAD diets prepartum consistently increases postpartum milk yield compared to low K controls without added anions. Although initial work suggested that use of synthetic zeolite A in the prepartum diet does not increase milk yield compared to low K controls without added anions, two recent studies suggest similar postpartum performance for cows fed negative DCAD or synthetic zeolite A prepartum.

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Metabolizable Energy (ME) in the Lactating Cows: Improving How We Represent Supply and Requirements

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Introduction

Governed by the laws of thermodynamics, the field of bioenergetics seeks to understand how energy is captured, transformed, and then routed to support cell function, growth, lactation, reproduction, and survival (Weiss and Tebbe, 2019). The study of bioenergetics is arguably the most thoroughly documented and most storied pursuits in all of animal nutrition. Biochemists, physiologists, and nutritionists have all joined the journey with each bringing their unique perspective and important contributions. Nutritionists have historically sought to measure and describe the animal's requirements for energy and as well as the feeds which supply it (Blaxter, 1967). Although there are a number of different energy evaluation systems in the world they are unified in the fact that each estimates metabolizable energy (ME) supply by subtracting energy losses from gross energy (GE) (Azarfar et al., 2025).

Metabolizable energy is a pool that represents the energy that is available to the animal for all biological functions and physical activity. In modern dairy cows, over 60% of the ME consumed each day is used to synthesize milk, making the mammary gland the single most energy-demanding organ in her body. From a feeding standpoint, this underscores why even small imbalances in energy supply can rapidly show up in milk production, much before they can be seen in changes in body weight or body condition. In dairy farming productive efficiency can be defined as the yield of milk and milk components yield to the energy expended on maintenance, lactation and of returning the cow to the level of body condition that existed before the onset of lactation (Bauman et al., 1985). For nutritionists seeking to help dairy producers maximize productive efficiency of energy, understanding how ME is estimated, assumptions used, and knowing the key physiological and nutrition factors that influence it can provide valuable insights for making formulation adjustments. This paper will discuss factors that influence the supply and use 1) how the supply of ME is estimated and the factors that affect both ME supply and use, 2) some key assumptions in representing ME that may not always apply and how resulting challenges may be overcome, and 3) potential modifications to improve predictions and formulation practices.

Supply of Energy

Flow of Energy Through the Cow

Figure 1 outlines the flow of energy through the dairy cows. Data in the figure stem from a study aimed to estimate the among-animal variance in energy utilization in lactating Jersey cows (Carroll et al., 2024). The study included data from 15 energy balance experiments in which indirect calorimetry and total fecal and urine collection were used to measure energy intake, digestion, and the partition of energy. In all, 115 Jersey cows were used and they supplied a total of 560 animal-period observations, covering a range of days in milk, milk production, and dietary composition. Figure 2 also illustrates the contributions of among-animal variance observed in this study on key measures related to energy utilization. To generate this figure, statistical analyses were conducted to partition variance among animals, dietary treatments, and experiments, highlighting the role of inherent animal differences in energy metabolism.

Characterization of energy flow begins with gross energy (GE) which is measured by determining the heat produced when a feed is completely oxidized (Pond et al., 1995). Once the total GE consumed is measured, the energy lost in the feces can be quantified and subtracted from the GE consumed and the remaining energy is described as digestibility energy (DE). This is usually the single greatest loss of energy (Carroll study). Urinary and gas energy losses can then be subtracted from DE, giving us a measure of the remaining ME.

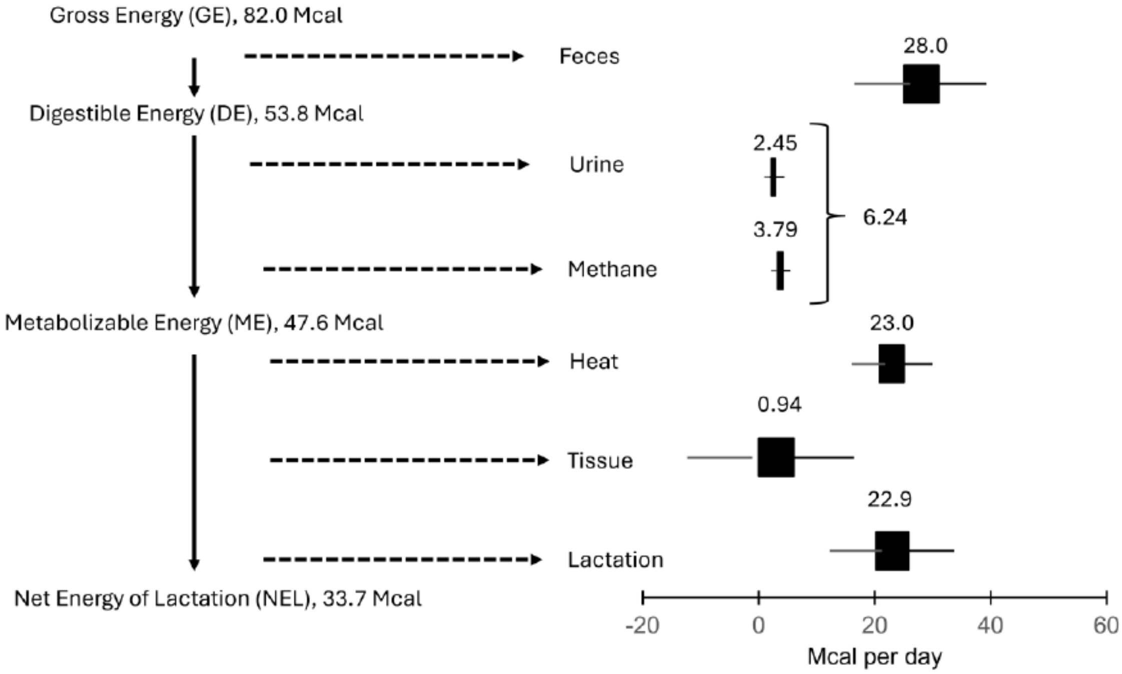

Figure 1. Illustration of energy flow and quantification of energy losses in lactating Jersey cows, values represent median while boxes represent 25th and 75th quartile and whiskers represent minimum and maximum observations (data from Carroll et al., 2024).

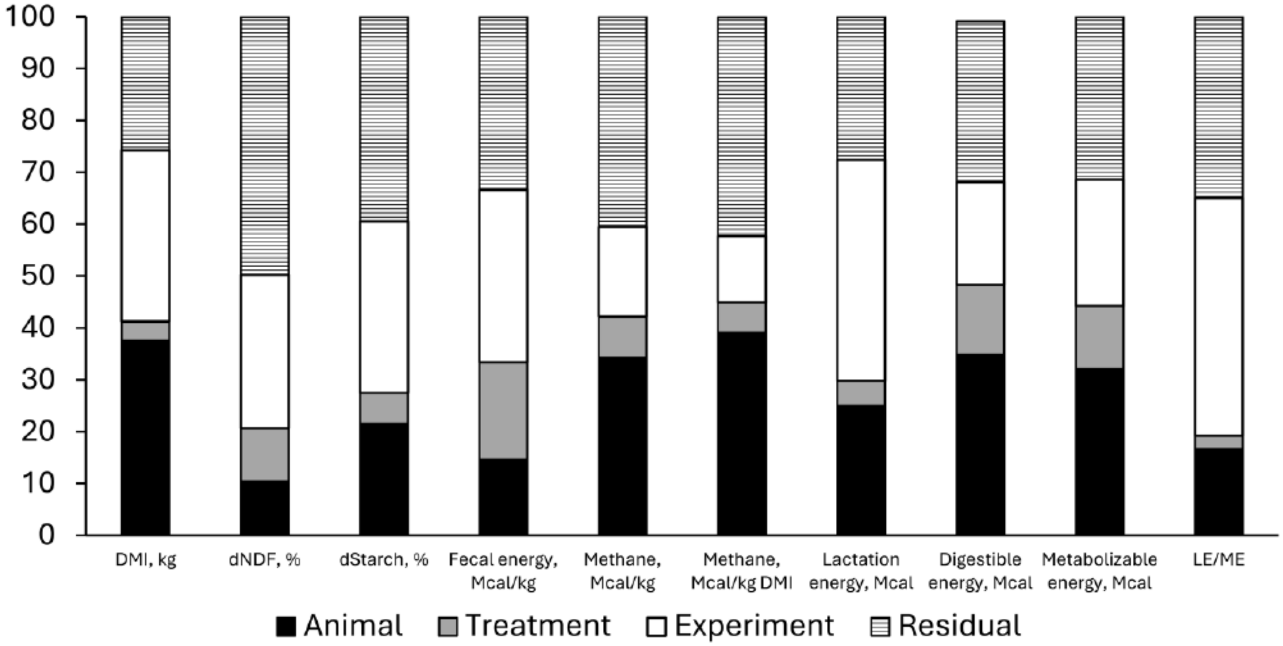

Figure 2. Analysis of sources of variance reported as the proportion (%) of total variation from differences sources. Sources was variation was analyzed for DMI, NDF digestibility (dNDF), starch digestibility (dStarch), fecal energy, methane energy, lactation energy, digestibility energy, metabolizable energy and conversion of metabolizable energy to lactation energy (LE/ME) (data from Carroll et al., 2024).

Nutrient Digestibility and Digestible Energy (DE)

In the study of (Carroll et al., 2024) fecal energy losses represented the single greatest loss of energy (28.0 Mcal/d or 34 % of the total GE intake); this also holds on commercial farms. This lost energy is in the form of nutrients in undigested feed. Of the nutrients reported, NDF not only had the lowest digestibility (46.6 ± 3.1 %) but the greatest variation. With NDF frequently often suppling as much as 20 % of the DE these data underline a long standing challenge and opportunity to increase energy availability by improving fiber digestion (Van Soest, 1994). Many of our nutrition models such as NASEM (2021), assume that digested NDF contains 4.2 Mcal/kg but this assumption may not always stand. In fact Stypinski et al. (2023) directly measured the energy content of NDF in a small number of feeds and observed it to be 4.0 Mcal/kg. In a follow-up, whole-animal energy balance study by Stypinski et al. (2024) NDF was observed to contain slightly more energy (4.1 Mcal/kg) but still less than assumed by NASEM (2021). Thus, when comparing NASEM (2021) predictions to live animal observations, it was not surprising that NASEM (2021) overpredicted both GE and DE. Although these studies observed the energy of content to be less than assumed by NASEM, in reality some variation even greater than what is assumed is also likely. One example is a feed containing a high concentration of lignin, a component that contains 6.0 Mcal/kg of energy (Voitkevich et al., 2012). Thus, in cases in which feeds contain a high concentration of lignified fiber, the energy measured may be greater than what is actually available to the animal. Overall, future nutrition models should consider representing energy on an ash, lignin-free, possibly even CP-free basis.

In the United States, starch commonly supplies more the 35% of the total DE intake of dairy cattle. Compared to NDF, starch possesses much higher digestibility and also has a lower variation in digestibility. Starch digestibility is affected by factors like grain type (e.g., barley vs. corn) and processing methods (e.g., dry-rolled vs. high-moisture corn). Average starch digestibility often ranges from as low as 77% to over 96%, and when formulating a diet, incorporating feed-specific starch digestibility coefficients can improve DE estimation accuracy. Although sugar is considered in the NASEM model it is accounted for in the in the residual organic matter (ROM). Representing sugars, organic acids, glycerol, and soluble fiber, this fraction is assumed to have an enthalpy of 4.0 Mcal/kg which is less than starch (4.2 Mcal/kg). In some diets sugar can provide a large amount of energy but on a per gram basis, starch supplies more than sugar (Azarfar et al., 2025). Directly speaking, the longer chains of glycosidic bonds found in starch result in a higher concentration of energy than sugar. In practice the energy contributed by these sources also depends on how they affect the nature of ruminal fermentation and the overall diet composition (Heldt et al., 1999). For example, starch also favors the production of the more propionate, a key gluconeogenic precursor, while sugars tend to favor butyrate production in the rumen (Oba and Kammes-Main, 2023; Piantoni and VandeHaar, 2023). Overall, the efficiency of energy capture is generally greater when feeding starch. In NASEM (2021) sugar containing ROM is assigned a constant digestibility of 96%, but its enthalpy is estimated at 4.0 Mcal/kg, which is lower than the assumed enthalpy of starch (4.2 Mcal/kg). This adjustment accounts for the nutritional differences between starch and ROM.

Urine and Gas Energy

Metabolizable energy is estimatedby subtracting model predictions of energy losses in urine and methane from DE. Carroll et al. (2024) reported that together, these sources of energy losses represented 6.24 Mcal/d (7.6% of the total GE intake). Energy losses from urine averaged 2.45 Mcal/d (2.99 % of the total GE intake).The equation used by NASEM to predict urinary energy losses was adopted from Morris et al. (2021b) in which urinary nitrogen was used to predict urinary energy. This approach was deemed as an accurate way to predict ME losses, especially when dietary protein varies. Moe et al. (1972) noted that when assessing energy utilization of diets, the supply of protein is also considered. This is because when protein is fed in excess, energy is required to process and excrete N to support metabolic transformations, synthesis of urea, and excretion by the kidneys (Reed et al., 2017). However, relative to popular belief energy needed to excrete N is small, approximately 14.6 kcal/g of N (Morris et al., 2021b).

According to Carroll et al. (2024), energy losses from methane averaged 3.79 Mcal/d (4.62 % of the total GE intake). Interestingly, the among animal variation in methane production is large (Figure 2), and even increased when methane is expressed per unit of DMI. When estimating gas energy losses to derive ME, the current NASEM

(2021) only accounts for methane. This is generally appropriate because other energy containing gasses such as hydrogen production are normally found only in trace amounts. However, this is not the case when some additives are used to reduce enteric methane production. For example, 3-Nitrooxypropanol has been shown to reduce enteric methane production but also result in a small increase hydrogen gas production (Lopes et al., 2016). Energetically, although gaseous energy losses are reduced when methane production is reduced, increases in hydrogen gas represents small but measurable losses of energy. For example, in a study designed to test the effect of a bromoform-containing feed additive, methane energy losses were reduced from 4.06 to 3.26 Mcal/d but hydrogen gas losses increased from 0.0 to 0.17 Mcal/d (Sherwood et al., 2025). When taken together, feeding the additive resulted in a net reduction of gaseous energy losses and this reduction was observed to have a positive effect on ME supply and also the efficiency of converting DE to ME. Consequently, when trying to reduce gas energy losses by feeding supplements that reduce methane, future nutrition models should probably consider accounting losses in hydrogen when predicting ME.

Utilization of Metabolizable Energy for Maintenance and Milk

The energy left after the losses of fecal, urinary, and gas energy, called ME is either lost as heat or incorporated into "products" which in the case of the dairy cow, is milk, conceptus, and body tissues (Reynolds, 2000). In the NASEM (2021), efficiency of how ME is converted into these products is denoted by k with different subscripts. Those discussed here include the conversion of ME into NE for maintenance (k_m =0.66) and lactation (k_L= 0.66) (Moraes et al., 2015; NASEM, 2021). For information related to the conversion of ME into NE for lactating cow tissue gain (k_g = 0.75), and milk production from tissue energy loss (k_T = 0.89) readers are referred to NASEM (2021).

Use of metabolizable energy for maintenance

Metabolizable energy for maintenance (MEmaint) represents is the energy required to support 1) nutrient digestion and absorption and 2) internal work for existence. This is equal to the heat produced when an animal is fed in the state of maintenance (Moe et al., 1972; Carroll and Kononoff, 2024). Net energy maintenance (NEmaint) refers to energy only needed to support internal work for existence and is equal to the heat produced while an animal is in a fasted state. The efficiency of converting MEmaint to NEmaint, denoted as k_m and in NASEM (2021) assumed to be the same and k_L (0.66), and NELmaint is assumed to be 0.1 Mcal × kg body weight$^{0.75}$. This was increased from 0.08 Mcal × kg body weight$^{0.75}$ in the NRC (2001) report. Recent evidence supports the change in NEmaint (Morris and Kononoff, 2021; Carroll and Kononoff, 2024) and these studied also serve as evidence that the current requirements are equally applicable to Jersey's. However, it is also thought that NELmaint in cows may vary as much as 8 – 10 % (NRC, 1989).

Use of metabolizable energy for milk synthesis

In NASEM (2021) the efficiency of use of ME for milk production is denoted as k_L. and assumed to be 0.66. In a respective analysis of data collected at Beltsville, (Moraes et al., 2015) reported that efficiency of converting ME into NE for milk synthesis (k_L) has increased over time (0.60, 0.62, and 0.69 in 1963 to 1973, 1974 to 1983, and 1984 to 1995). While the observed increase were most likely and largely driven by genetic improvement, these authors also noted that k_L was also positively correlated to milk yield and dietary fat. The positive association with dietary fat has long been known (Andrew et al., 1991) but not accounted for in the current NASEM (2001) model. In the evaluation of modern Jersey cows (Carroll et al., 2024) observed k_L to be substantially higher (0.73). In addition to the reasons listed above, the higher k_L could also be higher because the Jersey cows were producing a higher concentration of milk fat (5.49%) and the synthesis of this milk component requires less energy than milk protein (Morris et al., 2021a). Moe et al. (1972) reported that k_L varied among animals by as much as 9 %, while more recently Carroll et al. (2024) also observed that k_L varied substantially among animals. It should be noted that mathematically when NEmaint is increased, k_L will also increase because these coefficients are inherently positively correlated (Moe, 1981). In a recent study evaluation residual feed innate (RFI), k_L was observed to increase from 0.601 to 0.69 in low and high feed efficient cows. Overall, there does appear to be substantial evidence to increase k_L even more than 0.66 in our models to better represent contemporary animals. Alternately, nutrition models could be modified to compute variable efficiencies which could be based upon animal and/or feed-based factors known to affect k_L.

Final Thoughts

Future advances in the energy systems should attempt to shed further light on how the variation in nutrient enthalpies affects estimates of predicted and observed outputs in digestible energy. This is particularly important when feeding heterogenous chemical components such as NDF which support a large portion of DE and ME supply. Likewise, recent interest and attention related to cattle feeding and the environment has spurred application of methods to reduce gaseous losses, and these effects may have direct implications on energy utilization of dairy cattle. Thus, factors such as differences in the conversion or DE to ME or increases in hydrogen gas production should be integrated into future energy systems. Finally nutrients may be used with variable efficiencies to support lactation. Although nutrients such as fat appear to enhance the efficiency of k_L the notion has not been directly integrated into models, and other nutrients remain relatively unexplored.

Summary

- Milk production drives ME demand. In modern dairy cows, over 60% of daily ME intake goes directly toward milk synthesis. Small imbalances in ME supply can rapidly impact milk yield before changes in body weight or condition become apparent.

- Fiber digestibility is critical. NDF has the lowest digestibility and usually has the greatest variation among nutrients. Improving fiber digestibility offers one of the largest opportunities to enhance digestible energy (DE) and overall ME supply on-farm.

- Starch vs. sugar energy contribution. Starch provides more energy per gram (4.2 Mcal/kg) than sugar (4.0 Mcal/kg) and tends to favor propionate production in the rumen, improving efficiency of energy capture. Diet formulation should account for grain source, processing, and starch digestibility coefficients to improve accuracy.

- Methane mitigation can enhance ME supply. Enteric methane typically accounts for ~4.6% of gross energy losses. Feed additives that reduce methane losses may also slightly increase the efficiency of converting DE into ME but future models should also account for associated hydrogen production.

- Reconsidering efficiency coefficients. Current NASEM (2021) models assume kL = 0.66 (efficiency of converting ME to milk energy), but recent studies in modern genetics and diets suggest in some cases this may be closer to 0.73. Incorporating variable efficiencies based on animal factors, genetics, and diet composition could improve diet formulation accuracy and milk production predictions.

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Effect of Different Nutritional Strategies on Replacement Heifers in the Postweaning Period

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Introduction

The growth rate for replacement heifers should consider cost, number of nonproductive days, appropriate body size and composition, and productive lifetime (Van Amburgh et al., 2019). The most advantageous time to economically, with feed cost and feed efficiency in mind, grow an animal is right after weaning until ~200 days of life (Bach et al., 2021). Targets for growth should incorporate mature body weight of the heard and set targets of 55% of MBW at pregnancy and postcalving BW of 82-85% (Fox et al., 1999; Van Amburgh et al., 2019). In the early postweaning period the rumen and the gastrointestinal tract are the tissues gaining the most mass as the heifer transitions to solid feed (Baldwin et al., 2004), which requires a significant amount of protein (Van Amburgh et al., 2019). The composition of gain is dependent on the stage of maturity and as the animal moves toward puberty the energy retained per unit of empty body weigh increases (Van Amburgh et al., 2019).

Focusing on protein nutrition is important for optimizing growth, feed efficiency, and future lactation potential in young calves and continues to be a factor for growing heifers after weaning (Ghaffari et al., 2025). Stamey Lanier et al., (2021) established that high protein starters (26% CP) optimized composition of empty body weight grain and increased reticulorumen weight at 10 wk of age during the postweaning period. A balance of RDP and RUP is important for optimizing microbial protein supply, but this could be somewhat dynamic in the postweaning period where the heifer is still developing functionality of the reticulorumen. The relative contributions of rumen degradable protein (RDP) and rumen undegradable protein (RUP) to metabolizable protein shifts from 30 to 600 d of age which is connected to changes in body weight, growth rate, and rumen development (NASEM, 2021). Appropriate RUP to RDP balance enhances N retention by providing bypass protein for direct absorption, especially when microbial synthesis is limited (Ghaffari et al., 2025).

The proportion of RDP in CP increases from 47.6 to 78.7% (100 to 600 d of age), which indicates the increased reliance of MCP as heifers age. Current recommendations from NRC (2001) and NASEM (2021) recommend RDP of 60-70%, however previous studies challenge this when average daily gain is above 1 kg/d. Silva et al. (2018) reported that a RUP ratio of 51% of dietary crude protein during the pre- and post-pubertal stages increased body weight and nitrogen retention without excessive fat accumulation. Response to higher RUP diets may be dependent on the energy density of the diet to maximize body weight gain, feed efficiency, and nitrogen utilization. As a result, there have been suggestions that the ratio of RDP:RUP should be adjusted based on heifer age, physiological state, and dietary composition (Silva et al., 2018), yet limited evaluation in the immediate postweaning period has been evaluated.

Is there a benefit of higher RUP in the early postweaning period? Does this depend on the crude protein supplied by the diet?

Miner Institute Study: Evaluating Protein Content and Rumen Undegradable Protein Supply in Post-weaned Heifers

The study was conducted at the William H. Miner Agricultural Research Institute (Chazy, NY, USA) in the Charles J. Sniffen Dairy Research and Education Complex. All experimental procedures involving heifers were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee prior to the start of the study (2024AUR10).

Experimental Design and Management of Heifers

Heifers that were 67 ± 2 d of age at enrollment were used in a randomized block design study with repeated measures over an 11-week treatment period. The experimental unit was pen (5 heifers per pen) with 4 pens per treatment. Heifers were enrolled into a pen based on age and body weight, and the pens were randomly assigned to one of three dietary treatments. A total of sixty heifers were used.

Three compound pelleted grain treatments were used to evaluate the effect of crude protein (CP) content and rumen undegradable protein supply:

- 22%-LRUP: 22% CP starter, with a low rumen undegradable protein (RUP), ~35% of dietary CP.
- 26%-LRUP: High CP starter (~26%) with a low RUP, ~35% of dietary CP.
- 26%-HRUP: High CP starter (~26%) with a high RUP, ~50% of dietary CP.

Pelleted grains were formulated using a commercial ration formulation platform (AMTS.Cattle.Professional, Agricultural Modeling & Training systems, LLC, Groton, NY; version 4.22) with CNCPS biology (v 6.5.5 Cornell University, Ithaca, NY). Inputs used for dietary formulation included 3.2 kg/d DMI, 0.9 ADG, and BCS of 3.0. The ingredient inclusion and formulated chemical composition are shown in Table 1. The pelleted grains were formulated to have varying crude protein dependent on the treatment (22 vs 26% of DM). As a result, we varied aNDFom content between the 22 and 26% CP diets. However, we aimed to keep fat, starch and sugar similar across diets.

Sources of rumen undegradable protein (i.e. heat-treated canola and soy) were used to vary the ratio of RUP to RDP between the 26% CP treatments. The targeted ratio for the treatments with LRUP was ~65% of CP RDP and ~35% of CP RUP while the diet with HRUP was ~50% of CP RDP and 50% of CP RUP.

Table 1. Ingredients (% DM inclusion) and formulated chemical composition of experimental compound pelleted grains used to evaluate the effect of crude protein (CP) content and source in post weaned dairy heifers.

	Pelleted Grain[1]		
Item	22%-LRUP	26%-LRUP	26%-HRUP
Ingredient			
Wheat middlings	48.65	28.33	31.93
Fine corn meal	21.38	25.04	25.40
Amino max PGI[2,3]	14.18	11.20	31.56
Distillers grains	6.34	12.81	3.25
Cane molasses	4.28	4.29	2.63
Soybean meal	2.48	15.65	2.52
Other	2.68	2.69	2.73
Chemical Composition			
CP, % of DM	22.1	26.0	26.4
RDP[4], % of DM	14.0	17.2	13.8
RDP, % of CP	63.4	66.3	52.0
RUP, % of DM	8.1	8.8	12.7
RUP, % of CP	36.6	33.7	48.0
aNDFom, % of DM	25.2	19.7	20.1
Starch, % of DM	24.3	24.0	24.3
Sugar (ESC), % of DM	6.8	7.2	6.6
Ether extract, % of DM	5.0	4.9	4.8
ME Mcal/ kg	2.88	3.02	3.03

[1] 22%-LRUP is a pelleted grain that contains 22% CP and has a low ratio of rumen undegradable protein (RUP). 26%-LRUP contains 26% CP and has a low ratio of RUP. 26%-HRUP contains 26% CP and has a high ratio of RUP. [2] Afgritech, LLC, Watertown, NY. [3] Poulin Grain Inc., Newport, VT. [4] RUP and RDP reported values are based on ingredient feedbank values and predictions from a commercial ration formulation platform (AMTS.Cattle.Professional, Agricultural Modeling & Training systems, LLC, Groton, NY; version 4.22) with CNCPS biology (v 6.5.5 Cornell University, Ithaca, NY).

Heifers were fed the diets in the form of pelleted grain and hay separately for ad libitum intake, which was measured daily on a pen basis. Water was offered for ad libitum consumption, but intake was not measured. Heifers were housed in small, bedded pack bedded with sawdust (5 x 4.4 m) with a feed alley (3.1 x 4.4 m) group pen (five heifers per pen). There was an individual water trough per pen. Heifers were moved through an animal handling area for body weight determination weekly.

Feed efficiency (kg/kg) was calculated and expressed as gain/DMI by week during the 11-week treatment period. The body weight (Stockway SW3300; Digi-Star, Ft. Atkinson, WI, USA with a Avery Weigh Tronix Model 640M indicator; Fairmont, MN) of individual heifers was measured, and body condition was assigned in 0.25-unit increments on a 1 to 5 scale (Ferguson et al., 1994) weekly during the 11-week treatment period. The body weight and BCS of each animal was averaged across the pen.

Blood was collected ~6 h after feeding during wk 2, 6, and 10 of feeding the treatment diets (wk 12, 16, and 20 of age). Serum samples were analyzed for concentrations of urea N, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), total protein, glucose, triglyceride, cholesterol, aspartate aminotransferase (AST) at the Animal Health Diagnostic Center at Cornell University (Ithaca, NY) using automated enzymatic analysis procedures. Serum was analyzed for insulin using radio immunoassay (RIA) at the Animal Health Diagnostic Center at Cornell University. Insulin growth factor 1 (IGF-1) was measured in serum using an Bovine Insulin-like growth factor 1 Elisa kit following manufacturer's instructions (AGF Scientific, Northbrook, IL, USA).

Back fat thickness was measured by ultrasound at during wk 2, 6, and 10 of feeding the treatment diets (wk 12, 16, and 20 of age) using a 6.2 mHZ linear probe and an ibex Pro Ultrasound (E.I. Medical Imaging, Loveland, CO, USA). The images were measured for back fat thickness using ImageJ by two individuals independently of each other (twice per image per person) and the average of was determined.

Results and Discussion

Table 2. Analyzed chemical composition of experimental pelleted grains fed with a grass hay used to evaluate the effect of crude protein (CP) content and rumen undegradable protein supply in post weaned dairy heifers.

Item	22%-LRUP[1]	26%-LRUP	26%-HRUP	Grass hay
n	4	5	5	5
Dry matter, %[2]	86.7±0.2	87.0±0.3	86.4±0.5	83.2±1.2
Crude protein (CP), %	21.3±0.2	25.3±0.9	25.0±0.1	14.8±0.5
Soluble protein, % CP	36.8±1.5	30.6±2.4	31.8±3.2	33.2±0.6
Acid detergent fiber, %	10.2±0.2	8.8±0.2	10.4±0.3	33.2±0.6
Neutral detergent fiber (aNDF[3]), %	24.2±0.9	19.3±1.3	20.1±0.3	55.1±0.5
Acid detergent lignin, %	3.1±0.4	2.0±0.3	3.2±0.1	3.5±0.4
Nonfiber carbohydrates, %	44.7±0.8	45.7±1.4	45.7±0.7	21.8±0.6
Starch, %	25.6±0.7	25.3±0.2	26.4±0.7	0.7±0.3
Sugar (ESC[4]), %	6.9±0.4	8.7±0.7	8.2±0.2	5.0±0.5
Ether extract, %	4.7±0.1	4.5±0.1	4.1±0.2	4.3±0.1
Ash, %	6.8±0.1	6.5±0.2	6.9±0.2	8.0±0.5
7-h starch D, % of starch	71.3±0.5	72.5±1.9	71.7±2.3	-
30-h NDF D, % of NDF	-	-	-	61.4±1.0

[1]22%-LRUP is a pelleted grain that contains 22% CP and has a low ratio of rumen undegradable protein (RUP). 26%-LRUP contains 26% CP and has a low ratio of RUP. 26%-HRUP contains 26% CP and has a high ratio of RUP. [2]Dry matter (105°C) for ingredients; Hay n=21, TMR n=13, 22% LRUP n=17, 26% HRUP n=21, 26% LRUP n=21. [3]NDF with residual ash using α-amylase and without sodium sulfite. [4] Ethanol soluble carbohydrates.

Table 2 presents the analyzed chemical composition of the pelleted grains and dry hay fed to heifers ad libitum for 11 wk in the postweaning period. The analyzed CP content of the grains was ~1% lower than formulated for all treatments (21 vs 25% of DM). The NDF was close to formulated for the 22%-LRUP grain (24.2%) and the 26% grains (19.7%). Starch and fat content were similar across treatments and averaged 25.8% and 4.4%, respectively. Sugar was more varied than formulated across treatments; the 22%-LRUP grain was 6.9%, the 26%-LRUP was 8.7%, and the 26%-HRUP pellet was 8.2%. The grass hay averaged 14.8% CP, 55.1% NDF, and 61.4% 30 h NDF digestibility.

Table 3 presents DM, CP, and starch intake of grains, hay, and total for heifers fed the three grain treatments. Dry matter intake of the experimental grains did not differ (*P* ≥ 0.12) and averaged 4.32 kg/d. Intake of dry feed is similar to reported values by Quigley et al., (2021) from a large dataset of calves from 3 to 114 d of age, where the average intake was 4.3 kg/d at 114 d of age which would have been about wk 7 of the current trial. There was a significant treatment by time interaction (*P* = 0.001) and treatment effect (*P* = 0.04) for hay intake with heifers fed the 22%-LRUP grain consumed less (0.49 kg/heifer/d) than either of the 26% CP grains (0.62 kg/heifer/d). There was a significant (*P* < 0.001) treatment by time interaction for grain to hay intake ratio with heifers fed the 22%-LRUP diet consuming less hay and more grain, especially in the first two weeks of the study compared to heifers fed 26%-LRUP (tendency) and significantly less than 26%-HRUP. The overall treatment effect for grain to hay intake was significant (*P* = 0.02) with heifers fed the 22%-LRUP averaging 9.8 kg/kg and the heifers fed the 26% grains averaged 7.42 kg/kg. Heifers on this study were allowed to consume both grain and hay free choice however, intake of hay remained between 7 and 10% of the total diet after the second week of the study. The differences in intake of both grain and hay seems to be driven by the differences in the NDF content of the pellet, with heifers fed the lower NDF pellet consuming more NDF from hay (discussed below). Intake as a percentage of body weight was not different among treatment groups and averaged 3.18%. This range of intake as a percentage of body weight is also comparable to values reported by Quigley et al., (2021).

There was a tendency for a treatment by time interaction (*P* = 0.06) for CP intake from grain among treatments. Intakes of grain were not different, therefore difference in the CP intake was highest for heifers fed the 26% CP grains (1.10 kg/d), as expected from the formulation, compared to heifers fed the 22%-RUP (0.92 kg/d). There were significant treatment by time (*P* = 0.001) and treatment differences from CP from hay but the overall supply of this was low compared to the grain contribution to the diet. The total intake of CP was significantly different (*P* = 0.004) among treatments and was 1.00 kg/heifer/d for heifers fed the 22%-LRUP, 1.23 kg/heifer/d for 26%-LRUP, and 1.16 kg/heifer/d for 26%-HRUP.

There were no differences in starch intake for grain, hay, or total intake ($P \geq 0.11$). Starch intake as a percentage of body weight was also not different and averaged 0.73%.

Table 3. Intake of free choice experimental pelleted grains, grass hay, and total intake in postweaned dairy heifers for 11 wk. Experimental grain formulations containing varying crude protein content (22 vs. 26%) and supply of rumen undegradable protein (low; LRUP vs. high; HRUP).

	Treatments				*P*-values		
Variable	22%-LRUP	26%-LRUP	26% -HRUP	SEM	Trt	Time	Trt × Time
Grain, kg/heifer/d	4.34	4.44	4.27	0.13	0.66	<0.001	0.12
CP, kg/heifer/d	0.92^{b}	1.13^{a}	1.06^{a}	0.03	0.005	<0.001	0.06
Starch, kg/heifer/d	1.11	1.12	1.12	0.03	0.90	<0.001	0.12
aNDF[1], kg/heifer/d	1.04^{a}	0.85^{b}	0.86^{b}	0.03	0.003	<0.001	0.04
Hay, kg/heifer/d	0.49^{y}	0.62^{x}	0.62^{x}	0.04	0.04	<0.001	0.002
CP, kg/heifer/d	0.07	0.09	0.09	0.01	0.06	<0.001	0.001
Starch, kg/heifer/d	0.003	0.004	0.004	0.004	0.36	<0.001	0.50
aNDF, kg/heifer/d	0.27^{y}	0.34^{x}	0.34^{x}	0.02	0.04	<0.001	0.002
Total, kg/heifer/d	4.79	5.05	4.87	0.13	0.36	<0.001	0.28
CP, kg/heifer/d	1.00^{b}	1.23^{a}	1.16^{a}	0.04	0.004	<0.001	0.14
Starch, kg/heifer/d	1.11	1.13	1.13	0.03	0.89	<0.001	0.11
aNDF, kg/heifer/d	1.31	1.19	1.20	0.03	0.05	<0.001	0.16
Intake, % of BW	3.12	3.20	3.21	0.04	0.27	<0.001	0.30
aNDF intake, % of BW	0.85^{x}	0.76^{y}	0.78^{y}	0.01	0.003	<0.001	0.08
Starch intake, % of BW	0.73	0.72	0.74	0.01	0.20	<0.001	0.14
Grain:hay, kg/kg	9.80^{ax}	7.70^{y}	7.13^{b}	0.55	0.02	<0.001	<0.001

[1] NDF with residual ash using α-amylase and without sodium sulfite. ^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$). ^{x,y}Means within a row with different superscripts tend to differ ($0.05 < P \leq 0.10$).

There were significant treatment by time interactions ($P \leq 0.04$) for grain and hay aNDF intake. The DMI of grain did not differ among the treatments but the content of aNDF did, and as a result the aNDF intake from grain was highest (1.04 vs 0.86 kg/heifer/d) for heifers fed the 22%-LRUP grain compared to heifers fed either of the 26% grains. Furthermore, aNDF intake from hay was lowest for heifers fed the 22% grain compared to either of the 26% grains (0.27 vs 0.34 kg/heifer/d, respectively) because of the lower hay intake. There was a tendency ($P = 0.08$) for a treatment by time interaction for total aNDF intake and a significant effect of treatment ($P = 0.003$). Heifers that were fed the 22%-LRUP grain consumed more total aNDF but this started to become more pronounced in the last three weeks of the study. Overall, heifers fed the 22%-LRUP grain consumed 1.31 kg of aNDF/d compared to 1.20 kg/d for heifers fed either of the 26% CP grains. There was a tendency for a treatment by time interaction ($P = 0.08$) for aNDF intake as a percentage of body weight with heifers fed the 22%-LRUP grain consuming 0.85% compared to heifers consuming either of the 26% CP grains consuming on average 0.77 % of BW. There was no difference in the first week of the treatment period among treatments for NDF Intake (% of BW) but there was a tendency in week 2 for heifers fed the 22%-LRUP grain to consume more compared to the 26%-HRUP fed heifers. Generally, after the first week heifers fed the 22%-LRUP grain consumed more NDF intake (% of BW) but it became more pronounced during week 9 to 11 where those heifers consumed the most, while heifers fed 26%-LRUP consumed the least and heifers fed 26%-HRUP were intermediate.

Ration NDF concentrations have been shown to increase dry matter intake with increasing concentrations in the diet (Quigley et al., 2021). Work done with feeding varying levels of NDF from the forage indicated that heifers will consume more NDF with higher concentrations, while not necessarily displacing grain intake (Khan et al., 2012; Antúnez-Tort et al., 2023). Yet, dry matter intake is usually constrained by NDF at a level of 1.0% of BW (Hoffman et al., 2008). The heifers on this study did not reach that upper limit, which may be an indication that for these younger heifers, less than 140 days of age, have a lower limit which is more realistic (i.e. < 0.9% of BW as NDF intake) at least under these feeding circumstances. Interestingly, we are unaware of other studies that demonstrate varying forage intake based on the NDF provided by the grain.

Table 4. Body weight, stature, body condition score (BCS), and back fat thickness of heifers fed experimental compound pelleted grains formulated to contain varying crude protein content (22 vs. 26%) and varying ratios of rumen undegradable protein (low; LRUP vs. high; HRUP) during the postweaning period for 11 weeks.

	Treatments				*P*-values		
Variable	22%-LRUP	26%-LRUP	26%-HRUP	SEM	Trt	Time	Trt × Time
Initial BW, kg	91.1	92.8	90.4	0.8	0.17	-	-
Mean BW, kg (wk 1-11)	151.4	154.4	151.4	1.5	0.40	<0.001	0.67
Final BW, kg (wk 11)	203.3	207.0	199.7	3.2	0.32	-	-
ADG, g/d	1.46	1.47	1.47	0.04	0.99	0.03	0.31
Gain:feed, kg/kg	0.33	0.32	0.33	0.01	0.39	<0.001	0.47
Mean BCS	3.15	3.12	3.10	0.02	0.18	<0.001	0.65
Back fat thickness, mm	1.99	2.08	2.02	0.05	0.42	<0.001	0.79
Withers height, cm	103.9^{y}	104.6^{x}	104.4^{xy}	0.2	0.07	<0.001	0.23
Hip height, cm	108.0	108.4	108.3	0.2	0.33	<0.001	0.16
Heart girth, cm	119.1^{y}	120.5^{x}	119.1^{y}	0.4	0.05	<0.001	0.87
Body length, cm	108.1	109.9	109.2	0.6	0.15	<0.001	0.70
Hip width, cm	33.5^{b}	34.1^{a}	33.8^{ab}	0.1	0.02	<0.001	0.35

^{a,b}Means within a row with different superscripts differ ($P \le 0.05$). ^{x,y}Means within a row with different superscripts tend to differ ($0.05 < P \le 0.10$).

Table 4 presents body weight, gain, efficiency, and body stature of heifers fed experimental grains. There was no difference ($P \ge 0.17$) among treatments for initial, mean, or final (11 wk) body weight of heifers during the trial. Average daily gain for the 11 wk of the treatment period was not different ($P \ge 0.31$) and averaged 1.47 kg/d for all treatments. Postweaning growth has been linked to future milk production, and achieving fast growth (>0.9 kg/d) has been proposed to be desirable (Bach et al., 2021). There was no difference in body condition score (3.12) or back fat thickness (2.03 mm; $P \geq 0.18$). Previous work with increasing dietary protein (from 14.9 to 16.9%), supplied as RUP, was associated heifers having increased growth rates without negative consequences on mammary gland development or first lactation milk. However, there does not appear to be a benefit for the age and supply that was provided in the current study.

Feed efficiency, represented as gain to feed, was also not different among treatments and averaged 0.33 kg/kg. These values are similar to other reported values for this aged heifer with a range of 25 to 42% (Khan et al., 2007; Dennis et al., 2019; Castells et al., 2015; Terré et al., 2015). It has been proposed to maximize ADG after weaning until breeding because of its advantageous economic return (Bach et al., 2021) Furthermore, there were limited differences for body stature measurements or stature gain between treatments. There was a tendency ($P = 0.07$) for treatment for heifers fed the 26%-LRUP grain to have a higher wither height with an average of 104.5 cm compared to 103.9 cm for heifers fed the 22%-LRUP grain. There was a significant difference ($P \leq 0.05$) for larger heart girth and hip width for heifers fed the 26%- LRUP grain compared to the other two treatments. There appears to be little benefit of additional CP in the grain of these postweaned heifers except very small differences in body stature of heifers fed the 26%-LRUP grain.

Blood serum concentrations of metabolites are shown in Table 5. There was a treatment effect ($P = 0.001$) for serum urea N with heifers fed either of the 26% CP grains having higher (14 mg/dL) concentrations compared to heifers fed the 22%-LRUP (11 mg/dL). The elevated urea N may indicate suboptimal AA profile or insufficient energy intake relative to protein supply (Stamey Lanier et al., 2021). There was a significant treatment by time interaction ($P = 0.004$) for NEFA with heifers fed 26%-HRUP having the highest concentration in wk 2 of the study compared with heifers fed the 22%-LRUP grain. There was a significant effect ($P = 0.02$) of treatment on triglycerides with heifers fed the 22%-LRUP grain having the highest (21 mg/dL) compared to heifers fed 26%-HRUP (18 mg/dL) and tended to be different from heifers fed the 26%-LRUP grain (19 mg/dL). There was a significant treatment by time interaction ($P = 0.05$) for insulin, but when multiple mean comparisons were done within treatment week there were no differences among treatments. There was an overall treatment effect on insulin with heifers fed 26%-LRUP grain tending to have the highest while heifers fed the other two grains were lower. Serum insulin concentrations tended to be higher in diets with 51% RUP (Silva et al., 2018), which goes against what was observed in the current study. There was a significant effect of treatment ($P = 0.05$) on IGF with heifers fed the 26%-HRUP grain having the highest, 26%-LRUP intermediate, and 22%-LRUP with the lowest.

Summary and Perspectives

Heifers in the postweaning period were fed from ~ 63 until 140 days of age ad libitum pelleted grains that contained either 22 or 26% and varying contributions of rumen undegradable protein in the higher CP grains and a dry grass hay. Heifers grew well (1.47 kg/d) with little differences in growth, feed efficiency, or body condition amoung the treatments. There does not appear to be a benefit of changing the RUP:RDP ratio at the current level of CP, however, it may be interesting to evaluate at lower CP levels. When heifers in the postweaning period were fed ad libitum grain and hay the NDF content of the grain influenced intake of hay and total NDF intake, which appeared to stabilize around 0.9% of BW for this heifer age.

Table 5. Blood metabolites of heifers fed experimental compound pelleted grains formulated to contain varying crude protein content (22 vs. 26%) and varying ratios of rumen undegradable protein (low; LRUP vs. high; HRUP) during the postweaning period for 11 weeks.

	Treatments				*P*-values		
Variable	22%-LRUP	26%-LRUP	26%-HRUP	SEM	Trt	Time	Trt × Time
Urea N, mg/dL	11^{b}	14^{a}	13^{a}	<1	0.001	<0.001	0.83
Albumin, g/dL	4	4	4	<1	0.89	0.02	0.14
AST[1], U/L	101	112	102	4	0.22	0.86	0.51
BHB[2], mg/dL	4.1	3.7	3.9	0.1	0.38	0.01	0.48
NEFA[3], mEq/L	0.13	0.13	0.16	0.01	0.10	0.01	0.01
Triglycerides, mg/dL	21^{ax}	19^{y}	18^{b}	<1	0.02	0.14	0.41
Cholesterol, mg/dL	74.82	70.02	69.94	2.58	0.36	<0.001	0.88
Total protein, g/dL	6.41	6.52	6.45	0.06	0.53	0.01	0.60
Glucose, mg/dL	95.10	97.28	94.01	1.69	0.45	0.01	0.16
Insulin, uIU/mL	21.30^{y}	25.92^{x}	21.10^{y}	1.19	0.04	<0.001	0.05
IGF[4], ng/mLc	33.58^{b}	36.69^{ab}	38.54^{a}	1.22	0.05	0.62	0.61

[1] Aspartate aminotransferase. [2] Beta-hydroxybutyrate. [3] Nonesterified fatty acids. [4] Insulin-Like Growth Factor-1. ^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$). ^{x,y}Means within a row with different superscripts tend to differ ($0.05 < P \leq 0.10$).

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Enteric Methane Mitigation and Monitoring: Updates Regarding Sensor Technologies, Fatty Acids, and Bromoform

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Introduction

Enteric methane (CH_4) is a major greenhouse gas associated with ruminant livestock, representing both an environmental challenge and an energetic loss to the animal. Recent advances in dairy cattle nutrition have focused on strategies to mitigate CH_4 production while maintaining animal health, productivity, and efficiency. Dietary fatty acids (FA) and halogenated compounds such as bromoform have emerged as promising feed-based interventions. Fatty acids can influence ruminal fermentation by altering microbial populations and hydrogen flow, while bromoform, a bioactive compound found in red seaweeds such as *Asparagopsis* spp., directly inhibits rumen methanogens. Although mechanistically different, both approaches illustrate the potential of feed additives to shift fermentation toward reduced methanogenesis while sustaining or enhancing nutrient utilization and performance.

Equally critical is the accurate quantification of enteric emissions under production conditions. Respiration chambers remain the reference standard for CH_4 measurement, yet practical on-farm technologies are increasingly adopted to evaluate mitigation strategies. The integration of innovative feed additives with robust measurement technologies provides an opportunity to validate dietary interventions and support evidence-based recommendations for the dairy industry. This paper presents results from three complementary studies: (1) a comparison of CH_4 measurement systems in lactating cows, (2) an evaluation of dietary FA supplementation on milk production and gas emissions, and (3) an assessment of the stability and dose-response efficacy of bromoform-based additives.

Evaluation of Methane Measurement Systems in Lactating Dairy Cows

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Accurate and scalable measurement tools are critical for quantifying enteric CH_4 and implementing effective mitigation strategies in dairy production. We aimed to evaluate the accuracy of a head-chamber system and a modified sniffer system for measuring enteric CH_4 emissions in lactating dairy cows, using open-circuit respiration chambers as the reference.

Methodology

Twelve multiparous lactating Holstein cows (202 ± 12 days in milk, 46 ± 6.5 kg of milk/day, and 710 ± 30 kg of body weight) were enrolled in a replicated 3 × 3 Latin square to compare 3 measurement technologies: (1) respiration chambers (RC; No Pollution Ltd., Leicester, UK), (2) the GreenFeed system (GF; C-Lock Inc., Rapid City, SD), and (3) the Agscent Air GHG × Optiweigh system (AO; (Agscent Ltd., Carwoola, NSW, Australia). Cows were blocked by parity, days in milk, and baseline CH_4 emission (determined previously with GF), and randomly assigned within blocks to one of the systems during each 3-d period. Animals were transported from the Cornell Dairy Research Center (Harford, NY) to the Cornell Large Animal Research and Teaching Unit (Ithaca, NY), housed in tiestalls, and acclimated to facilities and measurement devices for 2 weeks before data collection. For GF and AO, cows remained in their stalls and were led to the respective units at 3-h intervals across 8 time points (08:00, 11:00, 14:00, 17:00, 20:00, 23:00, 02:00, and 05:00 h). For RC, cows were individually housed in 1 of 4 chambers for 72 h with continuous gas exchange monitoring. All cows received a basal total mixed ration (DM basis: 55% corn silage, 12% haylage, and 33% concentrate) formulated to meet or exceed requirements (AMTS.Cattle.Professional v. 4.14; Agricultural Modeling and Training System LLC, Groton, NY). Feed was offered once daily (09:30 h) with ad libitum access, and an equal amount of pelleted bait (Purina Animal Nutrition, Shoreview, MN) was provided across systems. Cows were milked at 06:00 and 17:30 h using portable milking units, either in tiestalls or inside the RC.

Specifications and operation of the RC units followed Machado et al. (2016) and Keller et al. (2022). Chambers maintained controlled conditions (18°C, 55% relative humidity). Calibration was conducted monthly using certified CH_4 and carbon dioxide (CO_2) standards (Airgas USA, Radnor, PA), with zero calibration via nitrogen. Gas recovery tests using CO_2 were performed regularly to verify accuracy. Total gas production (g/d) was calculated from inlet-outlet concentration differences, corrected for airflow rate, pressure, and temperature. The mean recovery was 99.6% for CO_2 and 99.4% for CH_4. The AO system collected exhaled breath through a vacuum-driven, open-circuit setup and analyzed samples in real time using a tunable diode laser spectroscopy sensor for CH_4 (0 - 40,000 ppm, 0.01 ppm resolution) and a nondispersive infrared sensor for CO_2 (0 - 20,000 ppm, 5 ppm resolution), with integrated temperature, pressure, and humidity probes. Measurements were recorded every second. The GF system used an open-flow design with nondispersive infrared sensors for CH_4 and CO_2, with automatic calibration using certified span and zero gases (Airgas USA). A CO_2 recovery test at the start and end of the trial yielded 99.6% recovery (SD = 2.5).

Ambient temperature and humidity were monitored with HOBO data loggers (model LMX2300; Onset Computer Corp., Bourne, MA). Samples of the total mixed ration were collected 3 times per week for DM determination. Feed ingredients were sampled twice weekly, composited by week, dried at 55 °C for 48 h, ground through a 1-mm screen (Wiley mill; Thomas Scientific, Philadelphia, PA), and stored in sealed bags until analysis. Milk yield was recorded daily. Milk samples were collected every 3 days over 2 consecutive milkings (n = 9) into vials containing 2-bromo-2-nitropropane-1,3-diol (Broad

Spectrum Microtabs II; Advanced Instruments Inc.) and stored at 4 °C. Analyses for fat, true protein, lactose, and milk urea nitrogen were conducted by Dairy One DHIA Laboratory (Ithaca, NY) using Fourier-transform infrared spectroscopy (Milkoscan FT+; Foss Inc.). Data were analyzed using the MIXED procedure of SAS (v9.4; SAS Institute Inc., Cary, NC). The model included measurement method, block, repetition, and their interaction as fixed effects, with cow nested within square as a random effect. Least squares means were separated with Tukey's adjustment. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Preliminary Results

During the experimental period, ambient temperature and relative humidity in the facilities were comparable to those in the RC, averaging 18 ± 0.5°C and 57 ± 7.4%, respectively. Dry matter intake, milk yield, energy-corrected milk, and 3.5% fat-corrected milk averaged 24.6, 33.3, 42.0, and 35.6 kg/d, respectively, with no differences among measurement systems. Milk fat content was also unaffected (mean = 5.27%). True protein concentration was slightly higher for GF (3.43%) than AO (3.36%) or RC (3.33%; $P < 0.01$), and total solids tended to be greater for GF (14.7 vs. 14.4%; $P = 0.07$), although these differences were not biologically relevant. Yields of milk components, milk urea nitrogen, and feed efficiency were similar across systems. Despite reports that chamber housing can reduce intake, no differences in DM intake were observed, suggesting that the acclimation protocol effectively minimized housing effects.

Daily CH_4 production differed by method ($P < 0.01$), averaging 394 g/d (AO), 403 g/d (GF), and 546 g/d (RC). Respiration chambers measured ~38% higher emissions than spot-sampling approaches. Carbon dioxide showed a similar pattern (8.2, 13.3, and 15.6 kg/d for AO, GF, and RC; $P < 0.01$). Methane yield (g/kg DMI) and intensity (g/kg milk or ECM) were also greatest for RC, reflecting its continuous capture of emissions.

Methane estimates from AO were moderately correlated with RC (r = 0.57), whereas GF showed weaker agreement with RC (r = 0.36). The two spot-sampling systems were moderately correlated (r = 0.41). Concordance with RC was poor for both spot methods (CCC ≤ 0.09). Methane production was positively associated with DMI, strongest for RC (r = 0.77), intermediate for AO (r = 0.62), and weak for GF. Relationships with ECM were moderate for RC and AO but not significant for GF. Continuous chambers provided the most robust intake-emission associations, whereas spot-sampling methods tended to underestimate absolute emissions and attenuate correlations.

Overall, RC yielded higher CH_4 and CO_2 values and stronger relationships with intake than either spot-sampling system, reflecting their complete diurnal coverage and controlled environment. Spot methods (AO and GF) produced comparable but lower emission estimates and weaker associations with productivity, emphasizing the need to account for methodological differences when interpreting or comparing enteric gas measurements.

Effects of Dietary FA on Milk Production and Ruminal Greenhouse Gas Emissions in Lactating Dairy Cows

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Supplementing dairy cow diets with lipids has been explored as a strategy to mitigate enteric CH_4 emissions. Meta-analyses report reduction in CH_4 production (g/day) and intensity (g/kg of milk) when fats are incorporated, although responses vary with lipid type, inclusion level, and diet composition (Beauchemin et al., 2008; Patra, 2013; Arndt et al., 2022). Our objective was to investigate the effects of alternative dietary FA supplementation strategies, substituting either fiber or starch (non-isoenergetic and isoenergetic, respectively) on milk production and composition, nutrient digestibility, and enteric gas emissions in lactating Holstein cows.

Methodology

Forty-eight multiparous Holstein cows (94.4 ± 20.7 days in milk; 46.3 ± 6.5 kg milk/day) were housed at the Cornell Dairy Research Center (Harford, NY). Following a 3-week acclimation period and GreenFeed^{TM} training, cows were allocated to 1 of 2 main plots (24 cows per plot): non-isoenergetic (NONISO) and isoenergetic (ISO). Within each plot, cows received 1 of 4 treatments in a replicated 4 × 4 Latin square with 21-day periods: (1) no FA supplementation (CON), (2) palmitic acid (PA; 85% C16:0; PeliPalm85; Pelican Solutions, Inc. Novi, MI), (3) calcium-salts of FA (CSFA; 59% C16:0, 25% oleic acid [C18:1], and 3% eicosapentaenoic acid plus docosahexaenoic acid; Virtus Nutrition, Corcoran, CA), or (4) whole cottonseed plus canola oil (OIL). Diets supplied ~2% supplemental FA (DM basis). The feeding level for canola oil was targeted at 0.5% of ration DM with the remainder of FA derived from whole cottonseed. Diets were primarily composed of corn silage, almond hulls, cornmeal, alfalfa hay, and a vitamin-mineral premix. Diets were formulated using AMTS.Farm.Cattle (Pro). Total mixed rations were offered once daily at 0700 h and cows were milked 3 times daily at 0600, 1400, and 2200 h. Feed refusals were maintained between 5 and 10% of total feed offered.

Individual feed, total mixed ration, and orts samples were collected for DM analysis and composited every period for chemical analyses. Oven-dried samples were analyzed according to AOAC (2000) methods for DM (934.01), crude protein (990.03), ether extract (2003.05), and ash (942.05). Starch content was determined using the method described by Hall (2009). Fiber fractions were analyzed for acid detergent fiber (973.18) and amylase-treated neutral detergent fiber (aNDFom; Van Soest et al., 1991). Undigestible neutral detergent fiber was used as an internal marker to determine total apparent tract digestibility and was determined after 240 h of in-vitro fermentation following Goering and Van Soest (1970). All analyses were conducted by Cumberland Valley Analytical Services Inc. (Waynesboro, PA). Milk samples were collected during the final 3 days of each experimental period (9 milkings per period). Samples with preservative were analyzed for concentrations of fat, true protein, and lactose using Fourier transform infrared spectroscopy. Fecal samples were obtained either via rectal collection or voluntary defecation every 9 h over the final 3 days of each experimental period. Methane, CO_2, and hydrogen (H_2) emissions were measured over 3 consecutive days at the end of each experimental period using 3 GreenFeed units. A total of 3 spot measurements per animal per period were obtained at 0230, 1030, and 1830 h. A custom pelleted bait feed was offered within the GreenFeed units. A CO_2 recovery test was conducted at the beginning of each experimental period (101% CO_2 recovery, SD = 3.85, n = 4). Statistical analyses were carried out using the MIXED procedure of SAS. The mixed-effects model included treatment, plot, period, square, and treatment × plot interaction as fixed effects, and cow nested within square as a random effect. Least squares means were separated using Tukey's adjustment.

Summary of Results

In brief, DMI, milk yield, and energy-corrected milk yield were modified by treatment ($P < 0.01$). In the NONISO plot, cows fed OIL had greater milk fat concentration (4.08%), relative to CON (3.60%; $P < 0.01$). In the ISO plot, cows fed PA had higher milk fat concentration (4.49%), relative to CON (3.85%; $P < 0.05$). Milk protein concentration in the ISO plot was lower across all FA treatments (~3.33%; $P < 0.01$), relative to CON (3.50%). Milk fat yield in the NONISO plot was highest in cows fed OIL (1.82 kg/day), compared to other treatments (~1.62 kg/day; $P = 0.02$). In the ISO plot, PA increased milk fat yield (1.87 kg/day), relative to CON (1.63 kg/day; $P < 0.02$). Milk protein yield was not affected by treatment in the NONISO plot but was reduced by PA and OIL in the ISO plot (1.37 and 1.39 kg/day, respectively; $P = 0.04$), relative to CON (1.46 kg/day).

Yields of milk C16:0 and mixed FA were greatest in cows fed PA, relative to CON ($P < 0.01$; both plots). Yields of milk C16:0 and mixed FA were greatest in cows fed PA, relative to CON ($P < 0.01$; both plots). Specifically, yield of C16:0 was greatest in ISO plot when cows were fed PA, (629 g/day; treatment × plot; $P = 0.01$), relative to CON (497 g/day). Mixed FA yield in the ISO plot was greatest when cows were fed PA (644 g/day; treatment × plot; $P = 0.02$), relative to CON (515 g/day). Yields of milk C18:0 and performed FA were greatest in cows fed OIL, relative to all other treatments ($P < 0.01$; both plots). Specifically, concentration of C18:0 was greatest in NONISO and ISO plot in cows fed OIL (9.34% and 9.22%, treatment × plot; $P = 0.04$), relative to CON (~5.62%). Similarly, Yields of C18:0 was greatest in NONISO and ISO plots were greatest in cows fed OIL (172 g/day and 168 g/day; treatment × plot; $P = 0.01$), relative to CON (~90.5 g/day). Performed FA concentration was greatest in cows fed CSFA and OIL in both NONISO and ISO plots, relative to CON. In the NONISO plot, cows fed OIL (614 g/day) had greater preformed FA yield, relative to CON (435 g/day, treatment × plot; $P = 0.04$). In the ISO plot, cows fed CSFA and OIL (523 and 584 g/day, treatment × plot; $P = 0.04$) had the greatest preformed FA yields, relative to CON (439 g/day). In NONISO and ISO plots, *de novo* FA concentrations were numerically reduced across all FA treatments; however, no significant differences were observed in *de novo* FA concentration and yield across all FA treatments. In the NONISO plot, cows fed CSFA had greater yields of C18:2 *trans*-10, *cis*-12 (0.48 g/day), relative to CON (0.37 g/day; treatment × plot; $P = 0.02$). In the ISO plot, cows fed CSFA had greater yields of C18:2 trans-10, cis-12 (0.61 g/day), relative to CON (0.38 g/day; treatment × plot; $P = 0.02$).

In the NONISO plot, the apparent total-tract digestibility of DM, organic matter, and fiber (aNDFom) followed a pattern. In the NONISO plot, cows fed PA had greater digestibility of DM (71.3 vs. 66.7%; treatment × plot, $P < 0.01$), organic matter (72.8 vs. 68.8%; treatment × plot, $P < 0.01$), and aNDFom (50.2 vs. 41.5%; treatment × plot, $P < 0.01$), relative to CON. In contrast, in the NONISO plot, cows fed OIL had lower digestibility of dry matter (63.0 vs. 66.7%; treatment × plot, $P < 0.01$), organic matter (64.9 vs. 68.8%; treatment × plot, $P < 0.01$), and aNDFom (33.4 vs. 41.5%; treatment × plot, $P < 0.01$), relative to CON.

Methane emissions were notably low across treatments (328 to 385 g/d). In the NONISO diets, CH_4 was not affected by lipid source. When starch was replaced (ISO diets), all fat supplements increased CH_4 (~381 vs. 345 g/d for control; $P = 0.05$). Hydrogen emissions were greater with PA than with other treatments in the NONISO plot (1.43 vs. ~1.23 g/d; $P = 0.03$), and all fat sources increased H_2 production in the ISO plot (~1.75 vs. 1.41 g/d for CON; $P = 0.03$).

In summary, this study generated several key insights. Methane emissions were consistently low (328 to 385 g/d) in cows fed low-forage, high-concentrate diets, regardless of lipid supplementation, despite their high DM intake. For comparison, high-producing cows offered rations containing more than 50% forage typically emit 450 to 600 g of CH_4/day, highlighting the inherently low methane yield (g/kg of DM intake) observed across all animals in this trial. A moderate depression in milk fat was evident in some treatments but was alleviated when PA replaced starch, highlighting the role of dietary FA profile in maintaining milk fat under high-concentrate feeding. The results also indicate that low CH_4 emissions may occur at the expense of milk fat synthesis. Substituting starch with PA, calcium-salts of long-chain FA, or oil increased CH_4 production, whereas replacing fiber with lipids did not. Overall, these findings suggest that, in high-intake, low-forage systems, reducing dietary starch may be more effective than lipid supplementation for reducing CH_4 emissions. Furthermore, when baseline emissions are already low, the inclusion of FA alone is unlikely to deliver additional mitigation.

Stability and Dose-Response Evaluation of Novel Bromoform-Based Methane Mitigation Products in Lactating Dairy Cows

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Reducing enteric CH_4 from ruminants is a critical priority for improving the environmental sustainability of dairy production. Bromoform-containing feed additives, particularly those derived from *A. taxiformis*, have shown substantial potential to suppress ruminal methanogenesis. However, their adoption requires evidence that these products remain stable during storage and can be fed safely and effectively to lactating cows. To address these needs, we evaluated 3 bromoform-based CH_4-mitigation products, a synthetic bromoform preparation, an *A. taxiformis* pellet, and an *A. taxiformis* oil, in two studies: a 16-week shelf-life trial assessing how storage temperature and light exposure influence bromoform retention, and a pilot dose-response trial assessing efficacy, safety, and effects on intake, milk yield and composition, and residue transfer in Holstein cows.

Methodology

For the shelf-life stability study, solid formulations (synthetic bromoform and *A. taxiformis* pellet) were packaged in stand-up barrier pouches made of either clear polyethylene/nylon laminate or metallized polyethylene (3.1 × 5.1 × 2 in.; Uline, Pleasant Prairie, WI, USA). The oil formulation (*A. taxiformis* in canola oil) was dispensed into 4-mL clear or amber screw-top glass vials (15 × 45 mm; Sigma-Aldrich, St. Louis, MO, USA). Three replicate samples of each product were prepared for every storage condition and sampling time. Packages were stored for 16 weeks under combinations of temperature (-20, 4, 25, or 37°C) and light (dark at all temperatures, with continuous fluorescent illumination at 25 and 37°C). Bromoform concentration (mg/kg DM) was analyzed at 0, 2, 4, 8, and 16 weeks by gas chromatography at Bigelow Laboratory for Ocean Sciences Analytical Services, and retention was expressed as a percentage of the initial concentration.

For the pilot dose-response study, 50 multiparous Holstein cows (121 ± 27 days in milk; 2.6 ± 0.3 parity) were housed in a free-stall barn equipped with Calan gates to allow individual feed intake measurements. Cows were fed once daily a total mixed ration (52:48 forage-to-concentrate ratio, DM basis) and milked 3 times per day (05:00, 13:00, and 21:00 h). Before the experiment, cows completed a 17-d training period, consisting of 10 d of adaptation to Calan gates and 7 d to GreenFeed units for gas-flux measurement. Animals underwent a 17-d pre-trial period consisting of 10 d of adaptation to Calan gates and 7 d of habituation to GreenFeed units for gas-flux measurement. The experimental period lasted 4 weeks and followed a randomized complete block design. Treatments consisted of 4 bromoform inclusion rates (0, 15, 22.5, and 30 mg/kg of DM intake) tested separately within each of 3 formulations: synthetic bromoform (SYN), *A. taxiformis* pellet (PAT), and *A. taxiformis* in canola oil (OIL). Supplements were top-dressed onto the total mixed ration once daily.

Dry matter intake and milk yield were recorded daily. Weekly composite milk samples were submitted to the Barbano Laboratory at Cornell University for analysis of fat, true protein, lactose, milk urea nitrogen, and FA composition using mid-infrared spectroscopic methods. Iodine content was analyzed by Michigan State University, and bromoform residues by Bigelow Laboratory for Ocean Sciences Analytical Services. Enteric emissions of CH_4, CO_2, and H_2 were measured daily with 3 GreenFeed units positioned across the pen to allow individual access except during milkings. A pelleted bait feed was dispensed automatically. Each cow was allowed a maximum of 2 visits per 24 h period to each GreenFeed unit, with no more than 6 drops (~30 g pellets/drop) per visit. On average, cows completed 3.65 ± 0.29 visits per day, with mean visit duration of 4.21 min. Body weight and body condition score were assessed at the beginning and end of the study.

Data were analyzed using the MIXED procedure of SAS. The model included treatment, week, and their interaction as fixed effects, with cow as a random effect. Repeated measurements across weeks were modeled using an appropriate covariance structure selected by the lowest AIC. Least squares means were estimated for each treatment and compared using Tukey's adjustment. Significance was declared at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

Preliminary Results

Bromoform stability was significantly affected by storage conditions. After 16 weeks, all formulations retained ≥95% of their initial concentration at -20°C and >90% at 4°C. Degradation became apparent at 25°C, particularly with light exposure, where retention ranged from ~45 - 55% for the synthetic product to 75 - 80% for the oil formulation. At 37°C, degradation was highest, and the combination of heat and light reduced bromoform to <10% of its original content in the synthetic product and to ~30 - 40% in the oil. Overall, cold, dark storage preserved >90% of bromoform across formulations, with the oil matrix consistently providing the greatest protection under suboptimal conditions.

Bromoform supplementation decreased feed intake at the highest inclusion level. At 30 mg/kg of DM intake, we observed a decrease of 18% for PAT and 13% for OIL compared with control, whereas moderate doses (15 - 22.5 mg/kg) caused only minor, nonsignificant reductions (2 - 9%). Across all synthetic bromoform treatments, DMI was unaffected. Milk yield showed no significant treatment effect ($P = 0.08$), although numerical differences of ±4 kg/day were observed. Energy-corrected milk (ECM) and 4% fat-corrected milk (FCM) showed significant treatment effects ($P < 0.01$). The pellet *A. taxiformis* reduced ECM and FCM at both 15 and 30 mg/kg (~15% below control), whereas SYN at 22.5 mg/kg maintained or slightly exceeded control levels, and OIL produced intermediate responses. Body weight (~725 kg) and body condition (~3.3) were unaffected ($P > 0.40$), indicating no short-term negative energy balance.

Milk composition was largely unaffected by treatment. Fat percentage remained unchanged (4.39 - 4.82%; $P = 0.17$), while true protein content showed a modest treatment effect ($P = 0.02$), with PAT at 22.5 mg/kg of DM intake being slightly lower than the control. Fat and protein yields declined only in treatments where intake was reduced (PAT at 15 and 30 mg/kg of DM intake). Milk iodine concentrations increased by >35% in all PAT treatments and in OIL at 30 mg/kg ($P < 0.01$). Bromoform residues were dose- and time-dependent but transient. All supplemented cows had detectable residues, averaging 48 ng/mL, whereas controls remained near assay limits (~9 ng/mL). Residues peaked at week 3 (33 cows with detectable concentrations), declined to 16 detections by week 4, and were below assay limits by weeks 5 and 6 (post experimental period), indicating clearance within two weeks after dosing ceased.

Analysis of milk FA showed few treatment effects on concentration (g/100 g milk), but several differences emerged for yields (g/d). De novo FA concentration (g/100 g milk) was unaffected ($P = 0.17$) but yield differed ($P = 0.04$), cows receiving SYN at 22.5 mg/kg of DM intake produced the highest de novo yield (610 g/d), whereas PAT at 15 mg/kg was lowest (493 g/d). Mixed FA concentration showed a tendency to decline with higher bromoform doses ($P = 0.08$), and yields were reduced in PAT_15 compared with control (668 vs. 818 g/d; $P = 0.02$). Preformed FA concentrations and yields remained unchanged ($P \geq 0.17$). Among individual FA, palmitic (16:0) yield was affected ($P = 0.02$), cows receiving PAT at 15 mg/kg had the lowest output (627 g/d), whereas SYN at 22.5 mg/kg and control cows produced the highest (742 and 770 g/d, respectively). Concentrations and yields of stearic (18:0) and oleic acid (18:1 cis-9) were not different among treatments ($P \geq 0.18$). Neither average chain length nor level of unsaturation differed by treatment ($P \geq 0.25$). Weekly effects were evident for most response variables ($P < 0.01$), but no treatment × week interactions were detected.

All 3 formulations reduced methane emissions relative to control. The greatest absolute reduction occurred with OIL at 30 mg/kg of DM intake (~50%), followed by PAT at 22.5 and 30 mg/kg (~27-40%) and OIL at 22.5 mg/kg (~27%). Synthetic bromoform at 30 mg/kg of DM intake reduced CH_4 by 24%. Across formulations, the 22.5 mg/kg dose achieved a consistent ~24% reduction in CH_4 yield and intensity without compromising milk production. Carbon dioxide production remained unchanged (~15 - 16 kg/day), while H_2 increased, indicating inhibition of methanogenesis without affecting basal respiration or milk performance. Although high between-cow variability limited statistical significance in this short-term trial, the data suggest that moderate doses of bromoform products can substantially suppress methane while maintaining intake and production.

Summary

Comparisons between respiration chambers with spot-sampling systems (GreenFeed and Agscent Air × Optiweigh) demonstrated that chambers provide the most complete and precise assessment of enteric CH_4, whereas spot-sampling methods yield lower but consistent estimates suitable for large-scale monitoring when their inherent limitations are considered. Research on dietary FA showed that altering lipid source or substituting starch and fiber influenced milk fat synthesis and nutrient digestibility but produced little additional reduction in CH_4 where baseline emissions were already low, suggesting that starch management may be a more effective strategy in high-intake, low-forage diets. Research on bromoform-based additives integrated storage stability and feeding trials to support practical application. Bromoform remained stable under cool, dark conditions, with oil formulations offering the greatest protection under less favorable environments. A short-term feeding trial indicated that moderate inclusion of synthetic bromoform, seaweed pellets, or seaweed oil reduced CH_4 emissions without compromising intake, milk yield, or composition, and residues in milk were transient, clearing rapidly after supplementation ended. Overall, these findings highlight the importance of accurate emission measurement, targeted dietary strategies, and feed additives as complementary tools to reduce gas emissions while sustaining performance and milk quality in modern dairy systems.

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Harnessing Hemp: Evaluating Nutritional Value and Application in Equine and Swine Diets

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Introduction

There is a limited body of research evaluating the nutritional potential of hemp seed meal (HSM) in monogastric species, particularly pigs and horses. The most recent comprehensive survey estimated the U.S. horse population at 7,246,835 (American Horse Council Foundation, 2017). Given these numbers, the equine feed industry represents a promising market for HSM. However, data on its nutritional value and safety are essential to guide and support its adoption in horse diets. The purpose of this presentation is to outline the nutrient composition of HSM and review the current knowledge on its application in swine and equine nutrition.

Hemp Seed Meal Definition and Composition

Hemp seed meal is a byproduct of oil extraction from whole hemp seeds, followed by milling of the residual material into meal. Botanically, hemp and marijuana are both classified as *Cannabis sativa L.* but represent distinct cultivars bred for different purposes. The psychoactive cannabinoid ∆9-tetrahydrocannabinol (∆9-THC) is the thermal decarboxylation product of tetrahydrocannabinolic acid (THCA), which occurs in varying concentrations depending on plant part, being highest in the flowers, as well as cultivation practices. Total potential THC is defined as the small measurable amount of ∆9-THC plus the calculated amount of ∆9-THC that would be produced by the full decarboxylation of the THCA in the plant. Selective breeding has produced marked differences in cannabinoid profiles and plant architecture. Industrial hemp is characterized by low THC content (≤0.3% in flowers), tall stalks for fiber, and seeds for oil, whereas marijuana has been bred for high THC levels in the flowers to produce psychoactive effects. By legal definition, industrial hemp contains ≤0.3% ∆9-THC in the flowering tops on a dry weight basis (Oregon State University Extension Service, 2023). Other plant parts, such as the seeds used to produce HSM, typically contain nondetectable levels (<4 ppm). In contrast, marijuana flowers by definition contain >0.3% ∆9-THC, with some varieties exceeding 30% (Hesami et al., 2023).

Hemp seed meal contains only trace amounts of cannabinoids. Addo et al. (2023) reported concentrations of approximately 0.0001% THC and 0.0003% CBD in HSM. In industry testing of hemp-derived food products, most samples including hemp seeds, protein powders, and oils were found to contain total THC and CBD concentrations below regulatory limits (5 mg/kg for seeds and related products; 10 mg/kg for oil) (Food Standards Australia New Zealand, 2022). These concentrations, equivalent to 0.0005–0.001%, are orders of magnitude beneath the 0.3% legal threshold for THC in plant tissue and are attributed to minor surface contamination of the seed coat rather than accumulation within the seed itself (Callaway, 2004). Despite this negligible content, HSM has not yet been legally approved as a feed ingredient for poultry meat, livestock, or companion animals, including horses. Consequently, few studies have evaluated its digestibility as an alternative protein source for monogastric animals. As of August 2024, HSM has been approved solely as a feed ingredient for laying hens, and additional research is needed to assess its feasibility and nutritional value in diets of livestock and companion animals (Hemp Feed Coalition, 2024).

Whole hemp seed is characterized by approximately 20–25% crude protein, 30–35% crude fat, and 30–35% neutral detergent fiber (NDF), with negligible starch (Callaway, 2004; USDA, 2019; Bailoni et al., 2021). Interestingly, in the whole seed, fat and fiber are present in similar proportions, reflecting the oil-rich kernel and the fiber-rich seed coat. In contrast, HSM, the byproduct remaining after oil extraction, contains 28–32% crude protein, 9–10% crude fat, and 32–40% NDF, with negligible starch (Kasula et al., 2021; Bailoni et al., 2021; USDA, 2019). The relative increase in protein and fiber after pressing highlight HSM's potential as a concentrated protein and fiber source in equine diets. In addition, the fat content of hemp seed is relatively higher than that of most common equine feed ingredients and is comparable to rice bran, a popular supplement valued for its fat and fiber contribution to horse diets (NRC, 2007).

Table 1. Nutrient composition of whole hemp seed and hemp seed meal, %[1]

Item	Whole hemp seed	Hemp seed meal[2]
Crude protein	20 - 25	28 - 32
NDF	30 - 35	32 - 40
Ether extract	30 - 35	9 - 10
Starch	<2	Negligible

[1] Bailoni et al., 2021; Callaway, 2004; Kasula et al., 2021; USDA, 2019.

[2] Cold-pressed.

Current Knowledge on Hemp Seed Product Application to Pigs

Hemp seed meal contains antinutritional factors such as phytic acid, tannins, and protease inhibitors, although generally at lower concentrations than in other oilseeds; these may still influence protein bioavailability (Rizzo et al., 2023). Nevertheless, animal studies indicate that hemp proteins and HSM have favorable digestibility compared with other common feedstuffs. In a rat bioassay, hemp proteins achieved a protein digestibility-corrected amino acid score (PDCAAS) equal to or greater than that of certain grains, nuts, and pulses (House et al., 2010). Protein digestibility values ranged from 84.1 to 86.2% for whole hemp seed, 83.5 to 92.1% for dehulled hemp seed, and as high as 90.8 to 97.5% for HSM. In pigs, Presto et al. (2011) evaluated standardized ileal digestibility (SID) of protein and amino acids in HSM compared with linseed and canola meal at inclusion rates of 25–30%. Apparent ileal CP digestibility was similar between HSM- and linseed-based diets (86.5 vs. 86.7%), as was total tract digestibility (91.1 vs. 90.4%). However, SID of several essential AA, including Arg (94.0 vs. 91.1%), His (89.4 vs. 82.3%), and Tyr (88.3 vs. 80.0%), were greater in HSM than in linseed meal. Most essential AA SID values were also superior in HSM compared with canola meal. In addition, apparent ileal crude fat digestibility was higher in pigs fed HSM than in those fed linseed meal (91.9 vs. 84.7%).

We recently completed a pilot acceptability study with 60-kg growing pigs and observed that an inclusion rate of 10% HSM could partially replace soybean meal (SBM) when diets were formulated to meet the SID Lys requirement (Zhang, 2024). However, a higher inclusion level of 15% was associated with reduced growth, likely due to the relatively high fiber content of HSM, which may preclude greater dietary inclusion for the growing pig. However, HSM may have promising application in sow diets, where dietary fiber is well recognized for its benefits in gestating sows, including improved satiety, reduced stereotypic behaviors, enhanced gut health, and favorable effects on reproductive performance (NRC, 2012; de Leeuw et al., 2008; Renteria-Flores et al., 2008).

While data from rat and pig studies provide useful proxies for evaluating HSM's potential in horse diets, well-controlled nutrient digestibility trials and long-term feeding studies in horses are essential to fully characterize HSM as a safe and effective dietary component.

Current Knowledge on Hemp Seed Product Application to Horses

Research on hemp use in horses has so far been limited primarily to hemp seed oil (Ely et al., 2023). Hemp seed oil is notable for its favorable omega-6 to omega-3 fatty acid ratio of 3:1 (Ely et al., 2023) and can serve as a source of essential fatty acids (Klir et al., 2019). It is also rich in gamma-linolenic acid and stearidonic acid, which have potential therapeutic applications in equine health (Ely et al., 2023). Commercial products containing CBD or full-spectrum cannabinoids from hemp extract are widely marketed for conditions such as arthritis, pain, and anxiety (Leise et al., 2023). However, the pharmaceutical use of hemp extracts or cannabinoids in equine medicine remains in its infancy. To date, only one documented case has reported their use in managing cribbing behavior in horses. More long-term, controlled studies involving oral administration are required before any conclusions can be drawn regarding the safety and efficacy of hemp extracts or cannabinoids in horses (Cunha et al., 2023).

Digestibility of nutrients and safe levels of HSM dietary inclusion have not yet been fully established in horses. The only published study to date has focused on palatability, reporting that horses showed a clear preference for and greater consumption of HSM pellets compared with soybean meal and sugar beet pulp, with palatability similar to rice bran (Springer et al., 2023). Horses also increased their intake of HSM pellets over time without a reduction in hay consumption, suggesting HSM may be an acceptable replacement for other common feedstuffs. We recently conducted a pilot study to evaluate the acceptability and total tract protein digestibility of mixed diets containing grass hay and concentrates formulated with 0 (control), 10, 20, or 40% HSM. In this study, HSM contained 14.4% lignin, which is relatively high compared with grains, soybean meal, and grass hay. Horses were offered hay and concentrate at 1% and 0.5% of body weight, respectively. No refusals of HSM-containing concentrates were observed, indicating full acceptability at inclusion levels up to 40%. Total tract protein digestibility, hay intake, and fecal scores were unaffected by HSM, demonstrating that inclusion up to 40% is safe and nutritionally adequate for horses at maintenance. Additional research with larger numbers of horses and long-term feeding periods is needed to determine HSM protein digestibility more precisely and confirm its long-term safety. Collectively, current findings suggest that HSM is a nutritionally viable and sustainable protein source with potential applications in both swine and equine diets, though systematic digestibility trials, extended feeding studies, and further regulatory approval remain critical to define optimal use in horses.

Summary

Hemp seed meal is a protein- and fiber-rich byproduct with negligible cannabinoid content. Studies in rats and pigs show high protein and amino acid digestibility, though insoluble fiber limits inclusion in growing pig diets. Its fiber, however, makes HSM promising for gestating sow diets, supporting satiety and gut health. Recent equine trials indicate good acceptability and safety up to 40% of feed concentrates. Further research is needed to establish optimal inclusion rates and long-term safety in horses.

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Precision Chewing Management: A Dynamic Approach to Forage Particle Size

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Introduction

Precision feeding management aims to optimize the rumen environment as efficiently as possible. Two management pillars support this approach: 1) feeding the right forage to the right cows, and 2) management that bolsters the cow's eating experience and optimizes feed intake (Grant, 2024). Because the rumen resides within the cow, ensuring cow well-being and her ability to practice natural behaviors is as important as optimizing ruminal fermentation. As a ruminant, nothing is more fundamental to cow health and productivity than meeting her needs for ruminating, resting, and eating as precisely and efficiently as possible.

Herd management and forage fiber are key factors given their effect on chewing activity. Within an optimized feeding environment, forage particle size can be adjusted dynamically to complement forage fiber degradability, fragility, and moisture with the goal of achieving the correct balance between chewing while eating and chewing during recumbent rumination. The goal is to make eating efficient for the cow and allow ample time for ruminating while lying down. Recent research tells us that when this occurs, cows have healthier rumens, eat more feed, and produce more milk components. With its focus on precisely managing particle size tailored to forage-fiber characteristics and feeding environment, precision chewing management provides the basis of successful dairy management systems whether we recognize it explicitly or not.

Chewing Response in the Balance

Forage fiber exerts its effect on chewing inescapably within the context of the cow's social and physical environment. Consequently, feeding management must be optimized. Key components of the management environment – such as feed availability, stall comfort, and stocking density, to name a few – profoundly affect eating, resting, and ruminating. Chewing behavior is controlled and modulated through a combination of animal factors, herd management, and physicochemical characteristics of the diet – notably fiber. Eating is affected by dietary neutral detergent fiber (NDF) content, NDF degradability, and particle size. Rumination is influenced by NDF intake, forage particle size, fragility (resistance of particles to breakdown during milling or chewing), and undegradable fiber (Beauchemin, 2018; Grant and Cotanch, 2023).

Figure 1 illustrates the requisite relationship between eating and rumination while resting, termed recumbent rumination. Albright and Arave (1997) reviewed the biological importance of sternal recumbency in that it positions the rumen optimally for the process of rumination. The goal of precision chewing management is to optimize forage-fiber and herd management inputs that modulate the balance between eating and recumbent rumination. Animal factors such as health and stress level also affect the balance between eating and recumbent rumination and are in turn influenced by this balance.

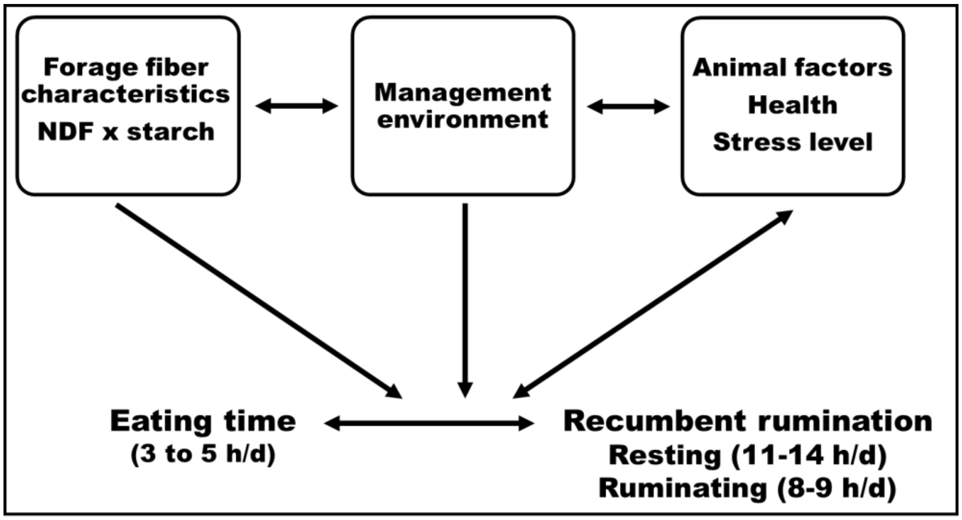


Figure 1. Balance between time spent eating and ruminating while lying down (i.e., recumbent rumination). Optimizing this chewing and resting relationship is the goal of precision chewing management.

More detail is provided in Grant and Cotanch (2023; available online at https://www.appliedanimalscience.org/article/S2590-2865(23)00027-7/pdf) but briefly, recumbent rumination is unmistakably the cow's signature behavior and super power. Cows with a greater proportion of their rumination occurring while sternally recumbent have higher ruminal pH (Campbell and Grant, 2016), consume more dry matter (DM), and produce milk with greater fat and protein content (McWilliams et al., 2022). In an unpublished study conducted at Miner Institute in 2023, Holstein cows ranging in milk fat percentage from 3.2 to 6.4% were observed for 72 h; of all behaviors recorded, the strongest positive correlation was between rumination while lying down and milk fat. Rumination time (min/d) is sensitive to diet, health, and cow well-being. As such it has been monitored extensively in precision management systems. However, it is increasingly clear that cow posture during rumination is equally important. In the future, we need to ensure that pens are monitored to observe whether individual cows are ruminating while lying down or standing. Ideally, 80% or more of daily rumination should occur while the cow is recumbent. Fortunately, the commonly measured rumination index (cows ruminating/cows lying in stalls) is well correlated with 24-h rumination time, and in fact, an index value of 50% relates to approximately 500 min/d of daily rumination time (Campbell, 2017). So, until a precision monitoring technology is available, judicious use of the rumination index will help to practically monitor recumbent rumination on-farm.

From a precision management perspective, future advances in technology need to allow monitoring of recumbent rumination given its fundamental biological importance to the cow. At the same time, eating behavior is critical for its obvious relation with DM intake (DMI) and it must be monitored as well. Research summarized by Grant and Albright (2001) indicates that, for normal feeding patterns, daily eating time should fall between 3 and 5 h for dairy cows. Eating time also needs to fall within this range to allow sufficient time for recumbent rumination. Any dietary or management factor that pushes a cow's eating time beyond approximately 5 h/d necessitates a compensatory loss of time for another behavior - often resting - hence the fundamental importance of the balance between these two behaviors. Precision management of forage and ration particle size makes eating efficient for the cow. That means chopping forages and mixing rations that are enriched with the fraction of particles retained on the second tier of the Penn State Particle Separator (more about this later). These particles, along with those on the 4-mm sieve (i.e., third tier), stimulate rumination effectively but can also be easily eaten and swallowed by the cow (Grant and Cotanch, 2023; Grant, 2024).

Fiber Content, Degradability and Dynamically Adjusting Particle Size to Optimize Chewing

Dietary NDF content, degradability, and particle size affect chewing response. As dietary forage-NDF content increases, we observe longer meal times, greater eating time, and potentially more sorting. As NDF degradability increases, or as lignin content decreases, we observe greater fragility and more rapid particle breakdown during chewing. Fragility has been positively related to 24-h NDF digestibility in a linear fashion up to approximately 60% when the relationship plateaus (Grant, 2010). Fragility may also pertain to brittleness of stems in forages such as legumes. Overall, there is less chewing with greater NDF degradability, fragility, or brittleness. As an example, Miller et al. (2021) replaced conventional with brown midrib corn silage (~10%-unit greater NDF degradability and up to 31% greater fragility) and observed approximately 30 min/d less chewing. Likewise, Grant and Ferraretto (2018) found that reduced DMI, relative to corn silage, was accompanied by longer eating time for conventional and even some brown midrib sorghums which were less degradable and less fragile. Well-accepted targets for 30-h NDF degradability are: >50% for legumes, >60% for grasses, >60% for corn silage, and >65% for brown midrib corn silage.

Over the past decade, measurement of undegraded NDF at 240 h of in vitro fermentation (uNDF240) has become commonplace in the dairy industry given its sensitivity to maturity at harvest, growing environment, and plant genetics. It is used to calculate potentially degraded NDF and is related to ruminal turnover, gut fill, and the physical effectiveness of forages (Cotanch et al., 2014; Raffrenato et al., 2018).

More recently, we have investigated the interaction between fiber (un)degradability and particle size. Researchers at Miner Institute proposed a new metric, physically effective uNDF240 (peuNDF240), that combines uNDF240 and the physical effectiveness factor (pef; Mertens, 1997). The pef is the fraction of particles retained on the 1.18-mm sieve or greater with dry vertical sieving or the 4.0-mm sieve of the Penn State Particle Separator for as-fed samples [see Grant (2023) for discussion and limitations of each method]. Farricker et al. (2022) assembled a 7-study data set and reported that, for corn silage- and haycrop silage-based diets, uNDF240 had a negative relationship with DMI (r

= -0.69), but the relationship between peuNDF240 (pef x uNDF240) and DMI was stronger (r = -0.85; Figure 2). Using peuNDF240, Smith (2018) evaluated the concept of manipulating forage particle size to complement variable dietary uNDF240. In essence, forages that had greater uNDF240 and lower NDF degradability were chopped more finely to avoid expected reductions in DMI. The results of this research led to the concept of dynamically adjusting particle size in relation to forage-fiber characteristics. For example, as forage matures (higher uNDF240, lower NDF degradability) it should be chopped finer. Or, for another example, when growing conditions enhance lignification (e.g., hot, wet weather) the forage crop may be proactively chopped finer. Importantly, Smith (2018) found that peuNDF240, since it integrated uNDF240 and pef, tracked better than uNDF240 alone with DMI, energy-corrected milk (ECM), chewing activity, and ruminal pH for lactating dairy cows. Notably, a 2.2 kg/d increase in DMI was observed when forage particle size was adjusted downward to complement higher dietary uNDF240 (Smith, 2018). A peuNDF240 of 5.9% of ration DM resulted in similar DMI, whether it was comprised of low UNDF240 chopped coarsely, or high uNDF240 chopped more finely. This DMI was similar to a diet with peuNDF240 of 5.4% and greater than a diet with peuNDF240 of 7.1%.

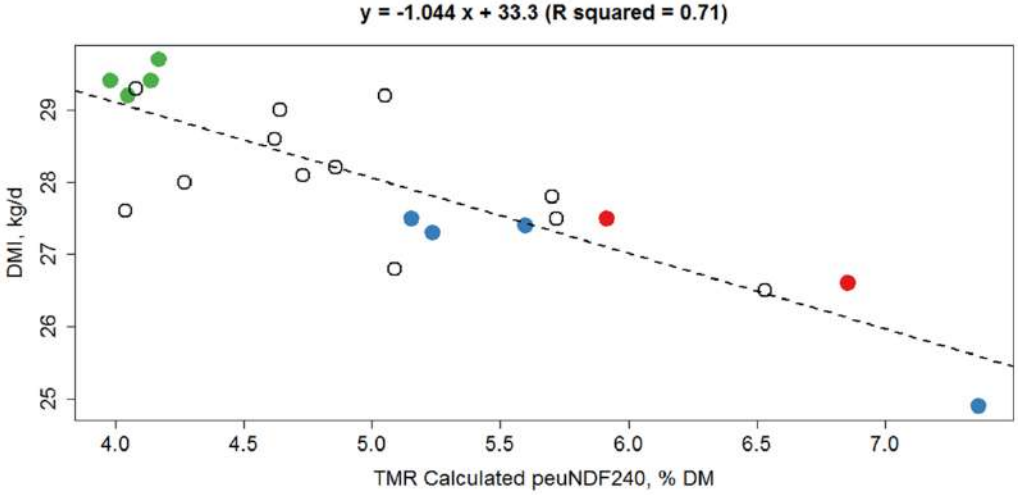


Figure 2. Relationship between dietary peuNDF240 (pef x uNDF240) and DMI from a 7-study data set based mainly on corn silage and haycrop silage diets (Farricker et al., 2022).

So there appears to be value in integrating two measures of fiber – uNDF240 and pef – when formulating rations based on corn silage, hay, and haycrop silage (perhaps alfalfa too but more work is needed). Pasture operates differently in that lush forage particles may be folded rather than comminuted during eating, so our focus is mainly on harvested and preserved forages. Forage testing laboratories report peuNDF240 in the range of 3 to 9% of ration DM based on samples received. Miner Institute data ranges from approximately 4 to 7% of ration DM, and an optimal range may be 4 to 6% of ration DM, though this certainly remains a work-in-progress. The bottom line, from a precision management perspective, is that 1) we can use this dynamic particle size concept to optimize the relationship between eating and recumbent rumination, and 2) we can use peuNDF240 as a relatively simple metric to quantitate combinations of particle size and undegradability. With the limited data to-date, a ration target of 4 to 6% peuNDF240 (DM basis) is proposed as a range where forage-fiber characteristics should allow eating time to fall within 3 to 5 h/d and with predicted DMI achieved.

One technical note: calculated peuNDF240 assumes that uNDF240 is uniformly distributed above and below the pef sieve which will not always be the case depending on dietary ingredients and forage characteristics. Directly assaying uNDF240 in the pef fraction ensures that unNDF240 in larger particles is accurately measured (Smith et al., 2020). However, this method is time-consuming as it requires an in vitro analysis on a sieved sample. We have compared the two methods, and they are highly and linearly correlated with a slope of 1.8 indicating that the directly measured peuNDF240 is 1.8x the calculated value (Farricker et al., 2022). Look for this analytical story to evolve, but at the moment it appears that, for many feeding situations, the simpler calculated value is sufficient.

Chewing and Resting Responses to Dietary Fiber: Biology Behind Precision Chewing Management

A key paper that illustrates how dietary fiber affects chewing while eating and ruminating is Jiang et al. (2017). They fed four diets ranging in forage content from 40 to 70% of DM (blend of corn silage, alfalfa, and alternative forages). As forage content increased, estimated uNDF240 content increased from 10.4 to 15.0% of ration DM (not reported in the paper but estimated based on ingredient chemical composition and known composition of similar diets). Reported pef increased from 0.42 to 0.67 using the 4.0-mm sieve of the Penn State Particle Separator. Consequently, the peuNDF240 values were estimated as 4.2, 6.2, 7.9, and 10.1% of ration DM for the 40, 50, 60, and 70% forage diets, respectively. As forage percentage increased, DMI dropped by 3.7 kg/d, especially beyond 50% dietary forage. Eating time increased by 107 min/d but rumination time crept up just 35 min/d. As we now know, this differential response in chewing is explained by the fact that cows take the time and effort during ingestive chewing to reduce dietary particle size to a relatively uniform size before swallowing regardless of dietary particle length. This concept is absolutely critical to understanding precision chewing management and the importance of not feeding too many long particles in the ration. Again, detailed discussion can be found in Grant and Cotanch (2023). The bottom line is that total chewing time increased by 141 min/d, driven by eating time, and to balance the daily behavioral time budget, resting time decreased by 141 min/d. It is noteworthy that eating time exceeded 300 min/d (i.e., 5 h/d which is the upper limit for normal eating behavior) when forage content exceeded 50% and peuNDF240 exceeded 6.2% of ration DM. For diets less than 50% forage the concept of adjusting particle size to maintain DMI is less important since there will likely be no fill constraint on intake. Rather, with lower forage diets particle size adjustment is focused on ensuring adequate chewing, rumen health, and milk composition.

Particle size of the ration is not the particle size of the swallowed bolus while eating. The bolus particle is more uniform, and Smith (2018) observed up to a 6-fold reduction in size for the longest particles during eating. Work by Schadt et al. (2018) found that particle size ranged from approximately 8 to 12 mm in the swallowed bolus while eating, which corresponds to the size of the particles retained on the 8-mm sieve of the Penn State Particle Separator. When cows were fed longer particles, they expended more chews per unit of NDF to reduce the particle size. Hence, our goal should be to mimic the cow when chopping forages for inclusion in the ration – that is the essence of precision chewing management from the cow's perspective. For corn silage and haycrop silage-based rations, this means enriching the total mixed ration with particles retained on the 8-mm sieve of the Penn State Particle Separator and minimizing particles retained on the 19-mm sieve.

We need to avoid dietary particles that are too long. Aside from potentially extending eating time beyond 5 h/d and thereby impinging on adequate time for recumbent rumination, there are several important reasons to avoid excessively long particles. Particles of total mixed ration that are retained on the 19-mm sieve of the Penn State Particle Separator are most likely to be sorted by the cow. Particles retained on the 19-mm sieve are associated with greater variation in chewing, ruminal pH, DMI, and milk yield in early lactation cows. Additionally, the percentage of particles retained on the 19-mm sieve is negatively associated with DMI, ECM, and milk fat percentage. Herds with the highest milk fat test have >50% of total mixed ration particles retained on the 8-mm sieve, and perhaps unsurprisingly, particles retained on the 8-mm sieve are more effective at stimulating chewing than particles on the top sieve (Kononoff et al., 2003; McCarthy et al., 2018; Piran Filho et al., 2023; Ferraretto et al., 2014).

What if the dietary particle size is finer than the cow would naturally swallow in the feed bolus while eating? Research shows that feed particles that are 8 mm or less tend to not be further reduced in particle size during eating. But the cow is inescapably populating her rumen with smaller than normal particles if we feed a diet chopped finer than 8 mm. One example would be a compact total mixed ration where the delivered ration has too many particles retained on the 4.0-mm sieve and pan. The particle size would be less than 8 mm, on average, in the ration and consequently feed efficiency would be low. Overall, it may be difficult to boost rumination with long particles, but we can surely depress it by chopping forages more finely than the cow would herself. Part of precision management is ensuring that the ration particle size is not less than the cow would create herself; or not chopping to particle sizes less than 8 mm. The management goal is to attain eating time between 3 and 5 h/d – not to minimize it.

Considerations with dry hay-based diets

Bauer et al. (2023) found that, when meadow hay-based rations were fed to lactating dairy cows, particle distributions of 21% retained on the 19-mm sieve, 20% on the 8-mm sieve, 20% on the 4.0-mm sieve, and 39% in the pan resulted in greater DMI, but lower total tract digestibility likely due to rapid particle passage rate. High quality alfalfa may not stimulate chewing as much as moderate quality alfalfa. Fine stems are more brittle and easily broken down as they are chewed or processed prior to feeding. In our limited experience with finely chopped alfalfa hay diets, we do see greater risk of cows going off-feed and we attempt to chop the hay coarser (i.e., 5 to 10% on 19-mm sieve; 2.5- to 4.0-

cm particle length to avoid sorting). However, Fustini et al. (2017) successfully fed finely chopped alfalfa hay with lower and higher NDF degradability with <1% retained on the 19-mm sieve and >80% on the 1.18-mm sieve and pan. The peNDF ranged from only 11.2 to 12.9% of ration DM in that study. They included chopped wheat straw in their diets, and it may have been an important part of their success because straw elicits about 1.5 times the chewing response as similarly sized alfalfa hay and it contains a high content of uNDF240 and slowly degrading NDF. Straw should be chopped finely enough so that cows cannot sort it (i.e., 20% on 19-mm sieve, 40% on 8-mm sieve, 20% on 4-mm sieve, and 20% in pan using Penn State Particle Separator; H. Dann, Miner Institute). One more point, purely observational: every good stockperson knows the value of providing long-stemmed hay to cows that are off-feed. It bears emphasizing that the particle size guidelines to be presented in the next section assume a generally healthy herd and these guidelines are intended to maintain a high level of productivity and herd health.

Particle Size Recommendations to Optimize Chewing Behavior

The following tables are adapted from Grant and Cotanch (2023) and summarize current recommendations for: 1) forage particle size that allows the ration to fall within the peuNDF240 target range, and 2) rations that allow cows to achieve the proper balance between eating and recumbent rumination. Table 1 presents recommended particle distributions for total mixed rations with interpretive comments for each particle size fraction. The original version was published by Cotanch (2017) in the Miner Institute Farm Report. Keep in mind that these recommendations, aimed at minimizing particles on the 19-mm sieve and focusing instead on the 8-mm sieve, are part of an overall feeding system that includes feed availability 24/7 and ample access to comfortable stalls.

Table 1. Recommended total mixed ration particle size recommendations using the Penn State Particle Separator (PSPS) to optimize balance between eating and recumbent rumination (adapted from Grant and Cotanch, 2023).

Sieve size, mm	PSPS 2013, % of DM	Miner 2020, % as fed	Comments
19	2-8	2-5	Sortable particles; increases time needed for eating, especially if >10%. Length of 1.3 to 2.5 cm maximum
8	30-50	>50	Physically effective, more so than 4- and 19-mm particles; maximize amount on this sieve, 50 to 60%
4	10-20	10-20	Functions as physical effectiveness factor (pef) sieve; total of the top 3 sieves = pef
---	30-40	25-30	40 to 50% grain diet results in at least 25 to 30% in the pan

To achieve these recommended ration particle size distributions, the dynamic approach in Figure 3 should be used. It is adapted from a similar figure in Grant and Cotanch (2023) and is a concept originally proposed by Woodley (2021).

Figure 3 presents the recommended range in theoretical length of cut (TLC) for forages that are harvested at commonly recommended maturity and moisture content. A sliding scale is proposed for TLC between approximately 12 and 22 mm (~⅜ to ¾ of an inch). Below this range faster passage rates from the rumen may reduce feed efficiency. Above this range, the risk of sorting ramps up. Within the recommended range, the optimal length of chop can be adjusted based on factors such as maturity at harvest, fragility of the crop, and moisture content. As the haycrop becomes more mature (i.e., uNDF240 increases) it should be chopped finer. Likewise, with less mature haycrop, it should be chopped coarser. Drier and more fragile alfalfa should be chopped coarser whereas dry and less fragile grass needs to be chopped more finely. Although not shown in the figure, conventional versus brown midrib corn silage fall into the categories of lesser and greater forage fragility and conventional corn silage generally is chopped more finely than brown midrib.

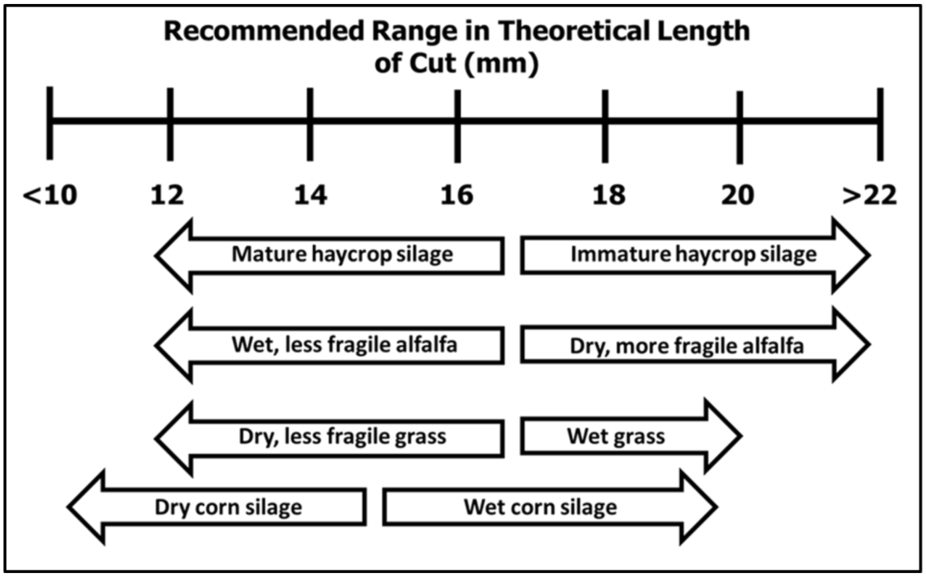


Figure 3. Recommended chop lengths for silages varying in maturity, fragility, and moisture content. Adapted from original concept of Woodley (2021) as described in Grant and Cotanch (2023).

This dynamic approach to forage chop length allows us to match the correct particle size more precisely with a given set of forage-fiber characteristics to produce a forage that optimizes chewing responses of the cow. The combination of pef and uNDF240 can be monitored using peuNDF240 (with some adjustment for fragility if a routine method is developed) and hopefully in the future monitoring of eating and recumbent rumination time will become economically feasible for on-farm use. This system of adjusting chop length means that you would not use one chopper setting throughout a harvest season or always chop dry forages with a standard set of screens. Rather, chop length would be variable with advancing maturity, by growing season, or as the crop becomes drier, especially corn silage. This dynamic approach may become more important over time with the effects of climate change on crop growth and harvesting; something to ruminate on at any rate.

To use this approach, one also needs to consider the potential for further particle size reduction beyond the silo. What silage or dry forage handling and mixing techniques are used on the farm? Think about silage removal and any potential impact of a defacer on reducing particle size. Consider mixer knife sharpness, mixing time, and other factors that all may contribute to a reduction in ration particle size between the silage/hay and the delivered ration. These considerations may necessitate some adjustments to the recommendations in Figure 3 for some farms.

Finally, Table 2 provides some approximate guidelines for recommended particle distributions for common forages that ought to result in total mixed ration particle distributions recommended in Table 1. The information in this table is based on feedback from nutritionists in the field and not research. Remember that considerable variation may exist between TLC and silage particle size distribution. Ultimately, the goal should be a chop length, for any given farm and its unique circumstances, which ensures good silage packing to reduce DM losses and, of course, optimizes chewing and DMI responses of the herd.

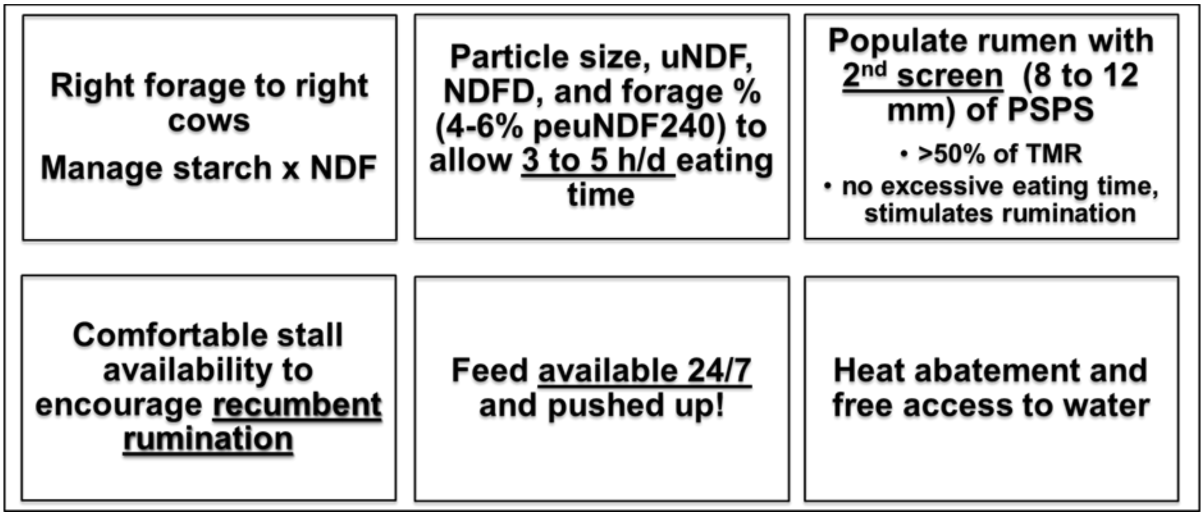
Table 2. Suggested particle distributions using the Penn State Particle Separator.

Screen, mm	Total mixed ration	Corn silage	Alfalfa silage	Grass silage
19-mm	<5	3-8	5-15?	5-15
8-mm	>50	50-65	50-75	50-75
4-mm	10-20	30-40	25-30	20-30
Pan	25-30	<5	<5	<5

The suggestions in Table 2 are definitely a work-in-progress and based on feedback and input from the field. As an example, recent feedback from Mexico and the US (Hugo Ramirez, personal communication, August 2025) suggests the need to revise the recommended particle distributions for corn silage. Ramirez pointed out that, with advancements in kernel processing, he is observing a larger proportion of particles passing through the 4.0-mm sieve into the pan of the Penn State Particle Separator reflecting the fact that many corn kernel fragments now pass through with better corn silage processing. More work is needed, but when Kernel Processing Score is >70% then the proper recommendation may be closer to 15 to 25% for the 4.0-mm sieve and <20% for the pan. Based on his observations, it seems likely that these recommendations may change with time, as equipment evolves, but always with the end goal being dietary particle distributions that optimize chewing and resting behavior.

Precision Chewing Management:
Unlocking Forage Potential from the Cow’s Perspective

Figure 4 depicts six primary components of precision chewing management that should result in optimized chewing and DMI. Fully implementing this approach will require some advancements in monitoring technology for eating and recumbent rumination. However, the core concept of mimicking the cow relative to particle size reduction and managing to ensure the correct balance between eating and recumbent rumination can be achieved now.



Right forage to right cows **Manage starch x NDF**	**Particle size, uNDF, NDFD, and forage % (4-6% peuNDF240) to allow 3 to 5 h/d eating time**	**Populate rumen with 2nd screen (8 to 12 mm) of PSPS** • >50% of TMR • no excessive eating time, stimulates rumination
Comfortable stall availability to encourage recumbent rumination	**Feed available 24/7 and pushed up!**	**Heat abatement and free access to water**

Figure 4. Major components of successful precision chewing management.

In summary, the key elements of precision chewing management are:

- Dynamically selecting forage particle size as a function of dietary forage NDF content, NDF degradability (and fragility?), uNDF240, and moisture content.
  - Chop forage finer or coarser depending on maturity and growing conditions.
- Particle size target should mimic what the cow does naturally as she chews and swallows a feed bolus (i.e., particle size from the cow’s perspective).
  - Make eating as efficient as possible, target 3 to 5 h/d eating time.
  - Enrich ration with particles retained on 8-mm sieve of Penn State Particle Separator; minimize particles on 19-mm sieve as promoting inefficient chewing.
  - Use recommendations for particle size of forages and total mixed rations presented here.
- Ensure ample time for recumbent rumination [11 to 14 h/d resting with 80^{+}% of rumination (8 to 9 h/d) occurring while recumbent].
  - Need technology to monitor eating behavior and recumbent rumination, times per day and when they occur. In the meantime, rumination index will be useful as a practical on-farm tool.
  - Does cow have ability to consume a meal naturally and then lie down comfortably and ruminate undisturbed?

  - Herd management inputs will affect the eating and recumbent rumination balance profoundly. Is feed available? Is resting area comfortable?
- Using peuNDF240 target of ~4 to 6% of ration DM appears to be a good metric for achieving desired eating and recumbent rumination balance; allows dynamic approach to optimize forage fiber characteristics, chewing behavior, and DMI.

Precision chewing management aims to optimize eating and recumbent rumination to boost DMI and healthy production of milk components. The focus is on forage fiber degradability and particle size combined with the feeding environment. Unlocking the nutritional potential of forage demands this overarching focus on cow comfort and forage quality.

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Milk Composition Responses to Changes in Nutrient Supply and Amino Acid Balance

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Introduction

Meeting the amino acids (AA) requirements for lactating dairy cattle are essential to optimizing the synthesis of milk components and likely play a role in different protein-synthetic cascades in the mammary gland (Bionaz and Loor, 2008). Understanding the nutrient demands for milk component synthesis and shifts in milk composition becomes important for the modern dairy cow with high genetic selection and capacity for milk fat and protein. The AA requirements of the modern dairy cow are likely increasing to meet the high demands for milk component synthesis and the efficiency of nutrient use per unit of milk component output as milk composition shifts to higher contents of fat and protein. The objective of the study was to conduct a meta-analysis that evaluated the effect of AA balancing of Histidine (His), Methionine (Met), and Lysine (Lys) on milk composition and milk FA to determine how changes in nutrient supply affect milk component synthesis. The data set for the analysis included ~3,500 individual cow observations from previously conducted pen-level studies evaluating the effect of AA balancing.

Methodology

Individual cow observations from two studies (Benoit et al., 2021; Danese et al., 2024) conducted at the Cornell University Ruminant Center (CURC) were used in the meta-analysis to determine the effect of AA treatments on milk yield and composition. Descriptive statistics for individual cows and diet composition are in Table 1. Study experimental design, diet formulation, and data collection were similar among studies. The treatments were: diets that supplied, on a metabolizable basis, His, Met, or Lys below the recommended requirements (LaPierre et al., 2020; Higgs et al., 2023) of 1.19g His/Mcal ME, 1.19g Met/Mcal ME, or 3.20g Lys/Mcal ME (-AA), or diets that supplied His, Met and Lys at or above the recommended requirements (+AA). The meta-analysis included data from 240 and 96 individual cows, for +AA and -AA, respectively, collected over an average 10-week period, providing a total of 2,400 and 1,104 individual observations for +AA and -AA, respectively. Pen-level AA supplies and ratios were predicted using the Cornell Net Carbohydrate and Protein System (v6.5.5; Van Amburgh et al., 2015) with updated feed chemical composition and intakes. The statistical model included the fixed effect of treatment (-AA or +AA), the random effect of study, cow within study, and experimental week within study.

Table 1. Descriptive statistics of cow-level variables and pen-level dietary composition.

	Treatments[1]							
	-AA				**+AA**			
Item	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Study DIM	135	31.0	84.0	210	157	48.0	78.0	254
Lactations	2.87	1.60	1.00	7.00	2.87	1.60	1.00	7.00
BW, kg	698	12.0	656	741	692	8.20	631	735
DM, % as fed	42.5	2.12	38.2	46.4	44.3	1.98	38.4	47.4
Nitrogen, g/d	713	22.6	666	777	698	27.6	625	775
CP, % DM	15.8	0.25	15.2	16.3	15.9	0.25	15.4	16.4
SP, % CP	39.8	2.53	36.4	43.9	35.5	3.64	30.4	43.2
ADICP, % CP	5.44	0.28	4.89	5.93	4.80	0.54	4.09	5.88
aNDFom, %DM	29.9	1.07	28.1	32.1	29.6	1.70	24.5	32.0
ADF, % DM	18.7	2.27	15.6	23.2	18.2	1.52	15.8	23.2
Sugar, % DM	6.40	0.91	5.14	7.53	5.89	0.68	5.14	7.46
Starch, % DM	26.0	2.22	22.7	30.2	26.4	1.82	22.4	30.0
Ether extract, % DM	4.27	0.33	3.75	4.67	4.31	0.26	3.75	4.75
Total FA, % DM	3.29	0.33	2.82	3.62	3.39	0.26	2.85	3.80
ME supply, Mcal/kg[2]	2.75	0.03	2.70	2.81	2.74	0.04	2.67	2.83
MP supply, g/d[2]	3332	110	3082	3692	3338	130	3028	3803
His, g/Mcal ME[2]	1.21	0.06	1.14	1.19	1.24	0.05	1.19	1.31
Met, g/Mcal ME[2]	1.11	0.14	0.88	1.19	1.23	0.14	1.19	1.27
Lys, g/Mcal ME[2]	3.15	0.13	2.92	3.19	3.21	0.04	3.20	3.35

[1] Diets formulated to supply <1.19g His/Mcal ME, <1.19g Met/Mcal ME, or <3.20g Lys/Mcal ME = -AA. Diets formulated to supply ≥ 1.19g His/Mcal ME, ≥ 1.19g Met/Mcal ME, and ≥ 3.20g Lys/Mcal ME = +AA

[2] Predicted using CNCPS v6.5.5 with updated feed chemistry and DMI.

SP = soluble protein, ADICP = acid detergent insoluble crude protein, aNDFom = neutral detergent fiber treated with amylase and ash-corrected, ME = metabolizable energy, MP = metabolizable protein

Preliminary Results

Table 2. Preliminary meta-analysis: milk yield, components, and FA for individual cows fed AA treatment diets.

	Treatments[1]			
Item	**-AA**	**+AA**	**SEM**	**P-value**
DMI, kg[2]	28.3	27.8	0.51	0.32
Milk, kg	43.2	44.0	2.36	0.38
ECM, kg	48.0	49.4	1.51	0.14
Fat, kg[3]	1.84	1.88	0.04	0.27
Denovo, g	486	506	29.4	0.08
Mixed, g	722	741	12.9	0.20
Preformed, g	519	527	8.03	0.34
C18:0, g	149	150	6.75	0.89
cis-9 C18:1, g	290	303	6.28	0.02
Protein, kg	1.37	1.44	0.06	<0.01
Lactose, kg	2.05	2.06	0.12	0.77
Fat, g/100g milk	4.25	4.39	0.15	0.03
Denovo, g/100g milk	1.13	1.17	0.02	0.05
Mixed, g/100g milk	1.68	1.71	0.10	0.26
Preformed, g/100g milk	1.20	1.23	0.06	0.10
C18:0, g/100g milk	0.34	0.35	0.01	0.30
cis-9 C18:1, g/100g milk	0.68	0.70	0.04	0.03
Protein, g/100g milk	3.17	3.34	0.03	<0.01
Lactose, g/100g milk	4.74	4.68	0.03	<0.01
MUN, mg/dL	9.85	9.63	0.86	0.24

[1] Diets formulated to supply <1.19g His/Mcal ME, <1.19g Met/Mcal ME, or <3.20g Lys/Mcal ME = -AA. Diets formulated to supply ≥ 1.19g His/Mcal ME, ≥ 1.19g Met/Mcal ME, and ≥ 3.20g Lys/Mcal ME = +AA.

[2] Analysis conducted on pen-level

[3] Milk FA analyzed according to Woolpert et al. (2016).

Conclusions

Balancing AA to meet 100% of the requirements shifted milk composition in favor of increased milk fat and protein content with a decrease in lactose content likely to due to allocation of carbons for milk component synthesis or dilution. The +AA diets increased the yields of de novo FA and *cis*-9 C18:1, suggesting a role for AA in the synthesis of FA and triglyceride formation in the mammary gland. The +AA diet did not affect the yields of mixed or preformed FA, thus balancing the supply of AA and dietary FA may be important to increase milk fat synthesis further. Additionally, as the industry continues to select for higher milk component yields genetically, it is important to understand how this changes milk composition and the nutrient demands of the mammary gland to meet this high genetic capacity.

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Hemp Seed Meal in Equine Nutrition: Current Knowledge and Ongoing Research

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Introduction

Hemp seed meal (HSM) is composed of high-quality protein, beneficial fatty acids, low starch, and high fiber, giving it a unique nutritional profile. Despite its potential, little research has evaluated the nutritional application of HSM in equine diets. To date, equine studies have focused primarily on hemp seed oil and cannabinoids, with only limited work on HSM as a broader feed component (Springer et al., 2023). Preliminary findings suggest that HSM is readily consumed by horses and may provide a valuable alternative protein and fiber source. However, the nutrient digestibility and safety of HSM for horses remain largely uncharacterized (House et al., 2010; Presto et al., 2011).

Materials and Methods

A two-phase project is underway at Cornell University to evaluate the use of HSM as an ingredient in horse diets. The first phase will assess nutrient digestibility using eight mature geldings in a repeated 4 × 4 Latin square design. Horses will receive grass hay and feed concentrates formulated to contain graded concentrations of HSM. Following dietary acclimation, fecal collections will be conducted to determine whole-tract digestibility of protein, fiber, and energy using established marker and regression approaches.

The second phase will examine the long-term safety of feeding HSM. Eighteen horses will be fed diets with graded concentrations of HSM over a 12-week period. Body weight, condition score, and standard health parameters, including blood chemistry, will be monitored to ensure tolerance and identify potential health risks. In addition, the blood cannabinoid profile will be measured at the beginning and end of the study to assess the potential contribution of cannabinoids from HSM.

Expected Outcomes

This project will provide the first controlled evaluation of HSM digestibility and safety in horses. Given its nutritional characteristics, HSM may serve as a suitable feed ingredient for horses, particularly those requiring reduced starch diets. Results will contribute to filling a critical knowledge gap and inform future application of HSM in equine nutrition. Importantly, these studies may provide the foundational data needed to support future approval of HSM as an accepted feed ingredient for horses.

Summary

Hemp seed meal shows promise as a novel feed ingredient for horses due to its protein quality, fatty acid profile, and low non-structural carbohydrate content. The Cornell studies will provide much-needed data on nutrient utilization and safety, helping to inform decisions regarding the potential role of HSM in equine diets.

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MyCow$©: A Real Time Profitability Tool for Smarter Dairy Management

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Introduction

Traditionally, on-farm decisions that affect farm profitability are made using herd-level or group averages for cow profitability. Often, this masks individual variation and can lead to suboptimal outcomes when applied to cow-level management. As a result, there is a growing need for tools that allow for individualized decision-making or for group level decisions based on actual individual cow performance and economic data. To meet this need, we developed MyCow$$^©$, a novel tool that automatically calculates the real-time net value of individual cows. By leveraging existing precision technologies and incorporating up-to-date market data, MyCow$$^©$ provides an individualized economic profile for each animal.

Materials and Methods

The MyCow$$^©$ tool uses data from several on-farm data sources, including, cow features, performance, and events. Farm specific prices are entered during the initial tool set up to include values for inputs and outputs. Data reports from each data source are created daily and ingested into the MyCow$$^©$ tool. Once ingested, data is cleaned and processed before it is used by a series of algorithms to calculate the net value of each individual cow by subtracting expenses from revenues. Values are reported to the user in various formats via the MyCow$$^©$ website.

Results

A case study was conducted using the MyCow$$^©$ tool to compare the difference in daily and accumulated cash flow up to 60 DIM for a selected group of cows diagnosed or not diagnosed with metritis. Nine cows diagnosed with metritis were matched with nine healthy cows controlling for parity, calving date, and genomic net merit. The comparison found that on average, the cows diagnosed with metritis incurred $168 less profit in the first 60 DIM than their healthy counterparts (Figure 1).

Summary

The MyCow$$^©$ tool provides a novel decision-support system that calculates real-time net value at the individual cow level. By combining precision technologies with economic modeling, the tool allows for more nuanced and efficient decision-making than traditional herd-average approaches. Ongoing validation across diverse herd sizes and management systems will further refine the model and expand its applicability.

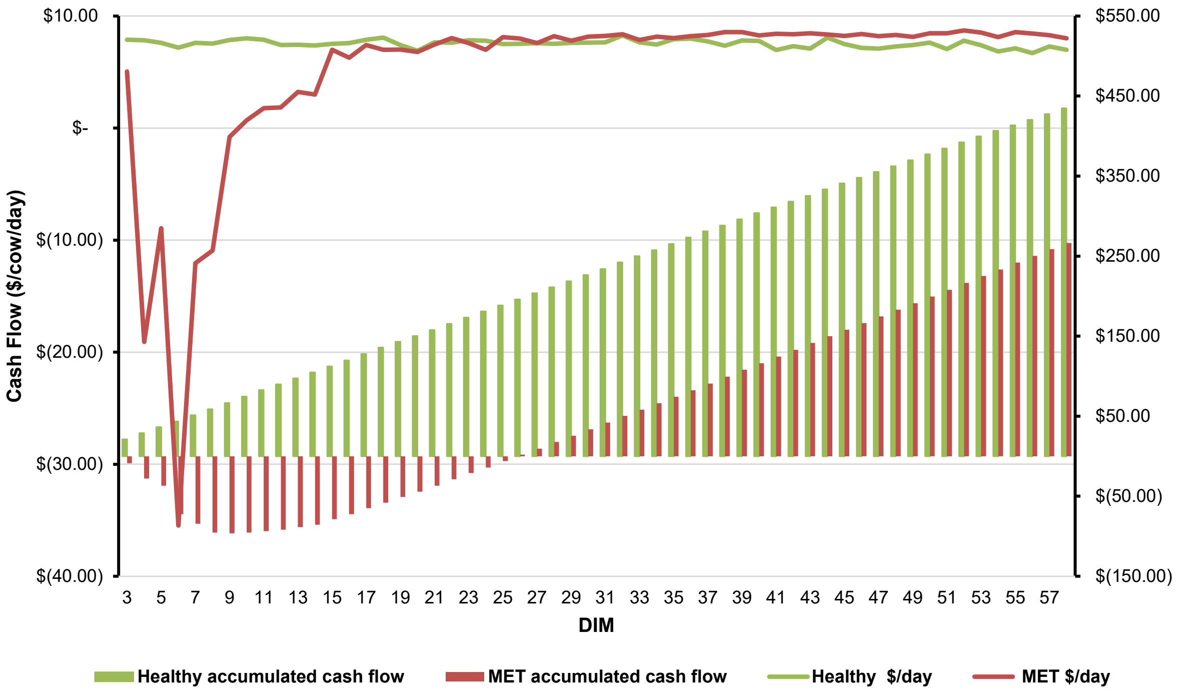

Figure 1. Difference in daily and accumulated cash flow from 3 to 60 DIM for cows affected and not affected by metritis calculated using the MyCow$$^{©}$ tool.

Effects of Dietary Fat and Cashew Nutshell Liquid with Castor Oil Supplementation on Milk Production and Ruminal Methane Emissions in Dairy Cows: Current Insights and Future Perspectives

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Introduction

Accelerated global warming is driven not only by long-lived carbon dioxide (CO_2) as well as short-lived climate pollutants, particularly methane (CH_4). Cutting CH_4 emissions in the near-term is among the fastest strategy to slow warming within this decade, while measures targeting long-lived CO_2 gradually take effect (IPCC, 2023). The livestock sector alone contributes about 14.5% of anthropogenic greenhouse gas (GHG) emissions, and mitigating enteric CH_4 from ruminant production is a critical component of global efforts to limit temperature rise to 1.5 °C (FAO, 2023).

In dairy production systems, nutritional interventions represent some of the most scalable and practical approaches for mitigating enteric CH_4 emissions. Feeding cows properly formulated diets along with feed additive supplementation has been shown to lower CH_4 output without negatively affecting animal productivity, underscoring the need for strategies that balance efficacy, persistence, and feasibility for on-farm implementation. Among the available options, lipid supplementation and plant-derived functional oils stand out as particularly relevant for incorporation into commercial feeding practices.

Dietary Fats and Fatty Acids

Supplementation of dietary lipids including fatty acids (FA) is one of the most promising nutritional strategies for mitigating enteric CH_4 emissions in ruminants (Hristov et al., 2013; Grainger & Beauchemin, 2011). Lipid inclusion reduces CH_4 primarily through three mechanisms: (i) lowering organic matter (OM) and fiber degradation, which decreases hydrogen availability for methanogenesis; (ii) directly inhibiting ruminal methanogens and protozoa; and (iii) providing alternative hydrogen sinks through biohydrogenation of unsaturated fatty acids (Martin et al., 2008; Knapp et al., 2014; Beauchemin et al., 2022).

Meta-analyses consistently demonstrate that lipid supplementation decreases CH_4 yield. Beauchemin et al. (2007) reported a 5.6% decline in CH_4 per 1% increase in dietary fat (% DM), while Knapp et al. (2014) estimated a 5% reduction in CH_4 intensity per 1% ether extract (EE). Patra (2013) similarly found linear reductions in daily CH_4 production, yield (g/kg DMI), and intensity (g/kg milk) with fat inclusion across a broad range of dietary fat concentrations (1.2–11.4% DM). A more recent study revealed that supplemental rumen-available fatty acids were able to mitigate enteric CH_4 (g/d) emissions in dairy cows, with reductions of approximately 6.9% per unit increase in polyunsaturated FA

(PUFA; C18:2, C18:3) and 4.7% per unit increase in unsaturated FA (UFA; C18:1, C18:2, C18:3), while overall decreases averaged 3.8% per 1% supplemental rumen-available EE (De Ondarza et al., 2024).

The effectiveness of CH_4 mitigation is influenced by FA type where, UFA (e.g., linoleic, linolenic, and docosahexaenoic acid) exhibit stronger antimethanogenic activity compared to saturated fatty acids (SFA), largely through direct inhibition of methanogenic archaea (Boeckaert et al., 2008; Zhang et al., 2008; Doreau et al., 2011). Medium-chain fatty acids (MCFA; C6–C12) are also potent inhibitors of protozoa and methanogens yet may negatively impact dry matter intake (DMI), fiber digestibility, and milk yield when included above 1–2.5% of dietary DM (Machmuller, 2006; Dohme et al., 2008; Faciola & Broderick, 2013).

Despite consistent reductions in CH_4, lipid supplementation presents trade-offs for animal performance. While moderate inclusion levels can increase dietary energy density and support milk yield (Larsen et al., 2012), higher levels may reduce fiber digestibility, DMI, and milk fat synthesis due to biohydrogenation intermediates (Alvarez-Hess et al., 2019; Dewanckele et al., 2020). For instance, adding 0.8 kg canola oil reduced CH_4 yield, also decreased milk fat percentage from 4.8% to 3.0% (Alvarez-Hess et al., 2019). Thus, a dietary lipid concentration of ~6% DM is generally recommended to balance CH_4 mitigation and productivity (Patra, 2013).

In addition to CH_4 reduction, milk fatty acid profiles offer potential as biomarkers of rumen fermentation and CH_4 output. Saturated and branched-chain fatty acids (BCFA) are positively correlated with CH_4 emissions, while UFA, including cis- and trans-isomers of C18:1, C18:2, and C18:3 show strong negative associations (Chilliard et al., 2009; Engelke et al., 2019; Youngmark & Kraft, 2025). Odd- and branched-chain fatty acids (OBCFA), derived from rumen microbes, further reflect fermentation patterns and CH_4 production, although associations vary across studies (Vlaeminck et al., 2006; Castro-Montoya et al., 2016; Engelke et al., 2019).

Overall, dietary lipid supplementation represents a reliable methane mitigation strategy in dairy systems. Strategic use of fat supplementation, combined with careful balancing of diet composition, offers a pathway to achieve CH_4 reductions without sacrificing milk yield and composition.

Cashew Nutshell Liquid and Castor Oil as Functional Feed Additives

Functional oils derived from cashew nutshell liquid (CNSL) and castor oil (CO) have emerged as promising natural modulators of rumen fermentation due to their antimicrobial, antioxidant, and anti-inflammatory properties (Vieira et al., 2000; Trevisan et al., 2006). CNSL is a by-product of cashew nut processing and contains phenolic lipids such as anacardic acid, cardanol, and cardol, whereas castor oil provides ricinoleic acid, a hydroxylated fatty acid structurally similar to oleic acid (Lubi & Thachil, 2000; Alves et al., 2017). Both compounds exhibit strong activity against Gram-positive bacteria and protozoa, which are key players in methanogenesis (Kubo et al., 1993).

Several in vitro and in vivo studies have demonstrated the CH_4 mitigation potential of CNSL. Shinkai et al. (2012) reported reductions of up to 38% in non-lactating cows, accompanied by a shift in volatile fatty acid (VFA) profiles toward propionate and decreased abundance of methanogenic archaea. Similarly, Watanabe et al. (2010) observed CH_4 reductions of 36–70% in Rusitec fermenters with CNSL supplementation, while Mitsumori et al. (2014) showed that CNSL decreased acetate and CH_4 production while increasing propionate and hydrogen gas in vivo. These effects are attributed to the phenolic constituents disrupting microbial membranes and selectively inhibiting archaea and hydrogen-producing bacteria (Oh et al., 2017; Danielsson et al., 2014). However, technical grade CNSL (TCNSL), which contains mainly cardanol and cardol yet a little anacardic acid, has shown more variable effects, with Branco et al. (2015) reporting only modest CH_4 reductions (~8% per kg DMI) and no changes in milk yield.

Ricinoleic acid (RA) has also shown CH_4 reducing effects, primarily through altering ruminal fermentation. Morales et al. (2012) demonstrated that RA supplementation reduced CH_4 production by 28% in vitro, while decreasing acetate and butyrate proportions and increasing propionate. In dairy cows, RA improved fat-corrected milk yield and milk fat content, though at the expense of DMI (Gandra et al., 2014). Mechanistically, RA appears to inhibit biohydrogenation by ruminal bacteria such as *Butyrivibrio proteoclasticus*, leading to the accumulation of rumenic and vaccenic acids, which also have potential health benefits in ruminant products (Wallace et al., 2007; Morales et al., 2012).

Blends of CNSL and CO have been developed to enhance efficacy and reduce the laxative effect of CO. Ferreira de Jesus et al. (2016) reported that a CNSL+CO mixture increased milk and protein yields while decreasing blood urea nitrogen, with positive shifts in ruminal fermentation comparable to ionophore supplementation. Other studies further support improvements in fiber digestibility, ruminal short chain FA (SCFA) concentrations, and nitrogen balance when these oils are fed together (Gandra et al., 2022).

Collectively, CNSL and CO represent functional oils with dual benefits: (i) consistent reductions in enteric CH_4 emissions via inhibition of methanogens and redirection of hydrogen toward propionate, and (ii) improvements in energy metabolism, milk production, and nitrogen utilization under certain conditions. Nevertheless, their effectiveness is influenced by the form of oil (natural vs. technical CNSL), dietary inclusion rate, and animal physiological state, highlighting the need for further targeted studies in lactating dairy cows.

Rationale and Objectives

In response to the urgent need to mitigate ruminant CH_4 emissions and given the potential of dietary fats and functional oils to reduce methane while sustaining animal performance, the combined effects of dietary fat and a cashew nutshell liquid (CNSL) plus castor oil (CO) supplement were investigated in relation to performance and enteric CH_4 emissions in lactating dairy cows. It was hypothesized that (i) moderate dietary fat inclusion would reduce CH_4 yield without impairing milk production, and (ii) co-supplementation with CNSL+CO would further suppress methanogenesis and promote propionogenesis, thereby enhancing CH_4 mitigation while maintaining feed intake and milk composition.

Materials and Methods

The study was conducted at the Cornell University Dairy Research Center (Harford, NY), and all experimental procedures were approved by the Institutional Animal Care and Use Committee at Cornell University (Ithaca, NY). Thirty-six multiparous Holstein cows (2.7 ± 0.9 parity; 87 ± 29 DIM; 40 ± 5.3 kg milk/d) were used in a 4 × 4 Latin square design with four 21-d periods, consisting of 16 d of adaptation and 5 d of sampling and data collection. Cows were fed a corn silage- and haylage-based diet (DM basis: 33% aNDFom and 23% starch) for a 21-d covariate period and then assigned to one of four treatment groups (n = 9): (1) basal TMR (CON); (2) CON with 12 g/d of a cashew nutshell liquid and castor oil blend (CN); (3) CON with 2.0% (DM basis) fatty acids (C16:0, 51.47%; C18:0, 30.66%; C18:1, 8.78%; C18:2, 1.88%) (FA); and (4) CN plus FA (CNFA).

Cows were fed treatment diets daily between 0700 and 0900 h and milked three times daily at 0600, 1400, and 2200 h. Daily orts were maintained at 5–10%. Samples of individual feed ingredients, TMR, and orts were collected weekly and composited by period for chemical analysis using wet chemistry procedures. Feed intake and milk yield were recorded daily, while body weight (BW) and body condition score (BCS) were measured periodically. Cows were equipped with SCR collars to continuously monitor rumination and physical activity.

Milk samples were analyzed for components using Fourier-transform infrared spectroscopy. Yields of fat-corrected milk (FCM) and energy-corrected milk (ECM) were calculated, and efficiencies of milk, FCM, and ECM production were expressed as the ratio of respective yields to DMI. Milk fatty acid profiles were determined using a two-step methylation procedure followed by gas chromatography. Milk somatic cell count (SCC) was measured by flow cytometry, and somatic cell score (SCS) was calculated using the logarithmic transformation described by Ali and Shook (1980).

Rumen fluid was collected by transesophageal sampling 5 h post-feeding to coincide with peak ruminal VFA production. VFA were quantified using high-performance liquid chromatography. Blood samples were collected via caudal venipuncture, and plasma glucose concentrations were measured using an enzymatic colorimetric method.

Enteric CH_4, CO_2, and H_2 emissions were measured for 3 days within each 5-d sampling period using the GreenFeed system (C-Lock Inc., Rapid City, SD). Spot measurements were obtained at 0200, 1000, and 1800 h. Each sampling period lasted 5 to 8 min, with an additional 2 min allocated for background gas collection. Enteric CH_4 production (g/d), CH_4 yield (g/kg DMI), and CH_4 intensity (g/kg milk yield and g/kg ECM) were calculated.

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The statistical model included the fixed effects of treatment, period, square, and the treatment × period interaction, with cow nested within square as a random effect. Outliers were excluded when studentized residuals were >3.0 or < −3.0. Treatment effects were tested using ANOVA, and least squares means were separated using Tukey's adjustment when a significant F-test ($P \leq 0.05$) was detected.

Results

DMI was comparable between CON (27.5 kg/d) and CN (27.2 kg/d) yet was reduced by FA (26.6 kg/d; $P < 0.01$) and further decreased by CNFA (25.9 kg/d; $P < 0.01$). Organic matter intake (OMI) was also differed among diets ($P = 0.02$), with CNFA (22.9 kg/d; $P = 0.02$) and CN (23.8 kg/d; $P = 0.05$) were lower than CON (24.0 kg/d), whereas FA (23.5 kg/d) did not differ from CON ($P = 0.57$). In contrast, crude protein intake (CPI) was unaffected ($P = 0.53$) and averaged ~4.2 kg/d across treatments. FA and CNFA reduced NDF intake (8.36 and 8.23 kg/d, respectively) compared with both CON (9.18 kg/d) and CN (8.92 kg/d; $P < 0.01$) and did not differ between CON and CN ($P = 0.14$). Starch intake differed among diets ($P < 0.01$), where FA (5.83 kg/d; $P < 0.01$) was lower than CON (6.32 kg/d), CN (6.26 kg/d), and CNFA (6.12 kg/d), whereas CON, CN, and CNFA did not differ from each other ($P > 0.05$). Total FA intake increased substantially with FA (1123 g/d) and CNFA (1142 g/d), relative to CON (632 g/d) and CN (612 g/d; $P < 0.01$). Intake of SFA (C12:0, C14:0, C16:0, C18:0) was higher in FA and CNFA compared to CON and CN (g/d; $P < 0.01$), with CNFA showing greater intake of C14:0 and C18:0 than FA ($P < 0.01$). Similarly, monounsaturated FA (MUFA; C16:1, C18:1n-9c, C18:1n-7) intake was higher in FA and CNFA ($P < 0.01$). Intake of linoleic acid (cis-9, cis-12 C18:2) was also higher in FA and CNFA, relative to CON and CN ($P < 0.01$), with no difference between FA and CNFA ($P = 0.99$).

Despite reduced DMI, milk yield was higher in FA (38.5 kg/d) and CNFA (37.9 kg/d), relative to CON (36.6 kg/d) and CN (36.1 kg/d; $P < 0.01$). Consequently, feed efficiency (milk yield/DMI) improved in FA (1.45 kg/kg) and CNFA (1.47 kg/kg) compared with CON and CN (1.35 kg/kg; $P < 0.01$). ECM and 3.5% FCM yields followed the same pattern. FA and CNFA increased ECM (46.0 and 45.2 kg/d, respectively) and FCM (45.8 and 45.0 kg/d) compared with CON (43.3 and 42.7 kg/d) and CN (42.7 and 42.5 kg/d; $P < 0.01$). Feed efficiency ratios (ECM/DMI and FCM/DMI) were also higher in FA (1.73 and 1.72 kg/kg) and CNFA (1.75 and 1.75 kg/kg) relative to CON and CN (1.59 and 1.57 kg/kg; $P < 0.01$).

BCS did not differ among treatments (~3.06–3.07; $P = 0.98$). Body weight (BW) was slightly lower in CNFA (706 kg) compared with CON (712 kg; $P = 0.04$), whereas CN (709 kg) and FA (710 kg) were intermediate. No differences were observed in ruminal pH (6.82–6.89; $P = 0.88$), daily rumination time (499–509 min/d; $P = 0.53$), or physical activity (424–431 min/d; $P = 0.09$). Plasma glucose was unaffected by diet (67.2–68.8 mg/dL; $P = 0.74$).

Milk fat concentration was greater in FA (4.67%) and CNFA (4.72%) than in CON (4.57%) and CN (4.53%; $P < 0.01$). Consistently, milk fat yield was increased in FA (1.80 kg/d) and CNFA (1.77 kg/d) compared with CON (1.66 kg/d) and CN (1.64 kg/d; $P < 0.01$). In contrast, milk protein concentration declined in FA (3.47%) and CNFA (3.46%) relative to CON (3.57%) and CN (3.54%; $P < 0.01$). However, protein yield was not significantly different among treatments ($P = 0.08$), averaging 1.27–1.31 kg/d. Milk lactose percentage was slightly elevated in FA (4.91%) and CNFA (4.92%) compared with CON and CN (both 4.88%; $P < 0.01$). This translated into higher lactose yields in FA (1.89 kg/d) and CNFA (1.87 kg/d) relative to CON (1.79 kg/d) and CN (1.76 kg/d; $P < 0.01$). Total solids (TS) content was marginally affected by diet ($P = 0.02$), with FA and CNFA showing a slightly higher TS percentage (both 14.1%) than CON and CN (14.0%). However, TS yield was greater in FA (5.44 kg/d) and CNFA (5.32 kg/d) than in CON (5.14 kg/d) and CN (5.08 kg/d; $P < 0.01$). SCC was reduced in FA (30.9×10^3 cells/mL) compared with CON (38.2×10^3 cells/mL) and CN (37.8×10^3 cells/mL), while CNFA (34.6×10^3 cells/mL) was intermediate ($P < 0.01$). Similarly, SCS was lowest in FA (0.80) and CNFA (0.86) compared with CON (0.97) and CN (1.12; $P < 0.01$).

Dietary treatments significantly affected milk fatty acid composition and yield. De novo FA (DFA) % was lower ($P < 0.01$) in FA (21.50%) and CNFA (21.28%) compared with CON (23.32%) and CN (23.24%), with no difference between CON and CN ($P = 0.98$) or between FA and CNFA ($P = 0.69$). Mixed FA (MFA) % was not affected by diet ($P = 0.22$), averaging 35.39%, 35.38%, 35.29%, and 35.77% for CON, CN, FA, and CNFA, respectively. Preformed FA (PFA) % was greater in FA (29.22%) and CNFA (28.92%) compared with CON (27.26%) and CN (27.34%; $P < 0.01$), with no difference between CON and CN ($P = 0.99$) or between FA and CNFA ($P = 0.78$). SFA% was lower in FA (67.28%) compared with CON (68.49%; $P > 0.01$) and CN (68.37%; $P = 0.01$), while CNFA (67.67%) did not differ from the other diets ($P > 0.05$). No difference was observed between CON and CN ($P = 0.99$) or between FA and CNFA ($P = 0.67$). MUFA% was higher in FA (18.96%) than in CON (17.51%; $P < 0.01$) and CN (17.63%; $P < 0.01$), yet similar to CNFA (18.57%; $P = 0.67$). Additionally, MUFA% in CNFA was higher than in CON ($P = 0.02$) and CN ($P = 0.04$), with no difference between CON and CN ($P = 0.99$). PUFA% was lower in FA (2.16%) and CNFA (2.16%) compared with CON (2.33%) and CN (2.33%; all $P < 0.01$), with no difference between CON and CN ($P = 1.00$) or between FA and CNFA ($P = 1.00$).

Yield of DFA (g/d) was not affected by diet ($P = 0.87$). However, MFA yield was higher ($P < 0.01$) in FA (629.8 g/d) and CNFA (638.8 g/d) compared with CON (589.5 g/d) and CN (582.7 g/d), whereas there were no differences between CON and CN ($P = 0.88$) or FA and CNFA ($P = 0.77$). Similarly, PFA yield was greater ($P < 0.01$) in FA (511.4 g/d) and CNFA (511.8 g/d) than in CON (450.8 g/d) and CN (447.1 g/d). Total SFA yield increased in FA (1201.1 g/d) and CNFA (1212.2 g/d) compared with CON (1139.6 g/d; both $P < 0.01$) and CN (1124.9 g/d; both $P < 0.01$). Similarly, MUFA yield was elevated in FA (328.5 g/d) and CNFA (326.4 g/d) compared with CON (289.2 g/d; both $P < 0.01$) and CN (288.4 g/d; both $P < 0.01$). In contrast, PUFA yield was not different among diets ($P = 0.85$).

Total VFA concentration (119–126 m*M*) was not affected by diet ($P = 0.68$). Also, molar proportions of acetate (A), propionate (P), and butyrate (B) were statistically similar across treatments ($P > 0.30$). Numerical trends suggested that CN increased propionate (44.0 vs. 41.9 m*M* in CON), while FA and CNFA had slightly lower values (39.1 and 39.0 m*M*), consistent with a modest dilution of propionate with fat supplementation. Acetate tended to be slightly higher in CNFA (53.1 m*M*), and CN (53.7 m*M*) compared with CON (52.5 m*M*) and FA (51.6 m*M*), although not significant. Butyrate was relatively stable (22.2–23.5 m*M*) across diets. VFA ratios did not differ ($P > 0.29$), yet FA and CNFA had numerically higher A:P ratio (both 1.37 vs. 1.28 in CON) and (A+B):P ratios (1.95 and 1.96 respectively vs. 1.79 in CN), consistent with a shift toward more acetate-type fermentation when fat was included.

Cows fed CNFA emitted 424 g/d CH_4, which represented a 4.7% reduction ($P < 0.01$) compared with CON (445 g/d). Neither CN (442 g/d) nor FA (446 g/d) differed significantly from CON ($P > 0.05$). Carbon dioxide output followed a similar trend ($P < 0.01$). CNFA reduced CO_2 to 14,989 g/d, corresponding to a 3.3% decrease compared with CON (15,501 g/d). CN and FA remained comparable to control. Hydrogen emissions were unaffected ($P = 0.24$), although CNFA showed a numerical decline (1.25 vs. 1.35 g/d in CON; ~7% lower).

When expressed relative to productivity, CH_4 yield per unit of milk was significantly lower in fat-supplemented diets. Compared with CON and CN (both 12.5 g/kg), CNFA reduced CH_4 intensity by 8.8% (11.4 g/kg; $P < 0.01$), while FA reduced it by 4.8% (11.9 g/kg; $P < 0.01$). Similarly, CH_4 per unit of ECM was reduced by 9.5% in CNFA compared with CON (9.50 vs. 10.5 g/kg; $P < 0.01$) and by 5.9% in FA (9.88 g/kg; $P < 0.01$). Expressed per unit of 3.5% FCM, CNFA showed the greatest reduction compared with CON (9.54 vs. 10.7 g/kg; 10.8% decrease; $P < 0.01$), while FA showed a 6.9% reduction (9.96 g/kg; $P < 0.01$). CN did not affect CH_4 intensity, whether expressed per ECM (10.6 g/kg) or FCM (10.8 g/kg; $P > 0.05$). Methane yield per unit of DMI was unaffected (16.4–17.0 g/kg; $P = 0.27$), indicating that reductions in CH_4 emissions were largely efficiency-driven through improved milk output rather than lower feed intake.

Summary

Supplementation with dietary FA and the combination of cashew nutshell liquid plus castor oil with fat (CNFA) significantly influenced intake, performance, and methane emissions in lactating cows. Both FA and CNFA reduced dry matter and fiber intake ($P < 0.05$), while markedly increasing fatty acid intake ($P < 0.01$). Despite lower intakes, FA and CNFA improved milk yield, feed efficiency, and energy-corrected milk production compared with the CON and CN diets ($P < 0.01$). Furthermore, FA and CNFA increased milk fat concentration and yield, whereas milk protein concentration declined slightly; however, protein yield was unchanged. FA and CNFA also altered the milk fatty acid profile, reducing de novo and microbial-derived fatty acids while increasing preformed and long-chain fatty acids ($P < 0.01$). Ruminal VFA concentrations were not significantly affected, yet enteric CH_4 emissions decreased with CNFA (–4.7% vs. CON; $P < 0.01$), and CH_4 intensity per unit of milk, ECM, and FCM was reduced by 5–11% in FA and

CNFA. Collectively, these results indicate that dietary fat, particularly when combined with CNSL+CO, can enhance milk production efficiency and reduce CH_4 intensity in dairy cows.

Future Perspectives

Building on the present findings with dietary fat and CNSL+CO blend supplementation, the next wave of CH_4 mitigation and productivity research in dairy cattle should be integrated with systems-level microbiome and host-omics analyses. Below, we outline high-priority research directions that are both feasible and impactful.

Microbiome Mechanics: Identifying Microbial Responders and Why It Matters

Re-examining lipid supplementation with a microbiome-focused perspective suggests that fats do more than create hydrogen “back-pressure.” Specific FA; palmitic, stearic, and oleic can reshape rumen bacterial communities, driving shifts in genera such as *Ruminococcus*, *Prevotella*, and *Butyrivibrio* (Sears et al., 2024). Purpose designed combinations, for instance FA-enriched blends with CNSL+CO, can be evaluated for microbiome signatures linked to increased milk-fat yield and reduced methane intensity.

Furthermore, rumen community analysis should extend beyond 16S rRNA amplicon sequencing, employing shotgun metagenomics and metatranscriptomics to quantify methanogenesis and alternative hydrogen-utilizing pathways, including formate dehydrogenases, fumarate reductases, and biohydrogenation routes. Specifically for fatty acids and CNSL+CO, investigations should focus on: (i) downregulation of the archaeal MCR complex, (ii) suppression of protozoal networks, and (iii) diversion of reducing equivalents toward propionate (via the mmd operon or acrylate pathway) or microbial lipid synthesis. Integrating quantitative PCR for methyl-coenzyme M reductase subunit A (*mcrA*), archaeal and bacterial metagenome-assembled genomes (MAGs), and network analysis will strengthen causal links between feed additives, microbial guild shifts, and CH_4 production. Insights into rumen modifiers and microbiome functionality can inform practical assay frameworks adaptable to these trials (Denman et al., 2015).

Nutritional Epigenomics: WGBS and RNA-Seq to Connect Diet to Function

Dietary inputs can establish stable, tissue-specific epigenetic marks that fine-tune gene networks governing metabolism and immunity. In dairy cattle, nutritional components, particularly methyl donors such as methionine and choline have been shown to modulate DNA methylation and gene expression in the liver, mammary gland, and embryos. These effects link one-carbon metabolism to animal performance and health outcomes. Building on this, future studies should explicitly evaluate whether fatty acids and functional feed additives such as CNSL and CO, alone or in combination with other agents (e.g., 3-NOP or nitrate), induce DNA methylation changes (via whole-genome bisulfite sequencing, WGBS) and transcriptional reprogramming (via RNA-seq) in the mammary gland, liver, and rumen epithelium. Existing literature already documents diet-associated methylation shifts and transcriptional remodeling in these tissues, supporting the feasibility and biological relevance of such approaches (Murdoch et al., 2016; Wang and Ibeagha-Awemu, 2021; Zhang et al., 2025).

Whole-genome bisulfite sequencing can be employed in target tissues to map differentially methylated regions (DMRs) in proximity to genes involved in lipid metabolism, SCFA signaling, and one-carbon pathways. These genome-wide maps can be complemented with targeted bisulfite amplicon assays to validate specific DMRs. Recent studies on bovine methylomes provide essential baseline data and detailed CpG landscape information, facilitating contextual interpretation of diet-induced epigenetic remodeling (Zhou et al., 2020). RNA sequencing can further quantify pathway-level responses to FA and CNSL+CO supplementation, including SREBF1/PPAR signaling, fatty-acid transport, elongation, and desaturation. This approach also enables the detection of CLA- or trans-10 isomer shifts, which are known to modulate the expression of mammary lipogenic transcripts (Baumgard et al., 2002). Repeated sampling through mammary biopsies or milk somatic cells provides the opportunity to track dynamic transcriptional changes across experimental periods (Toral et al., 2016; Ibeagha-Awemu et al., 2016). Additionally, monitoring one-carbon metabolism, including the SAM:SAH ratio, folate and B12 levels, and methyl-donor availability, can provide mechanistic insight into how nutritional interventions influence the epigenome.

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Re-evaluating Nutrition Models to Predict Calf Growth

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Introduction: Why nutrition models for calf growth matter

The rearing of calves and heifers represents one of the largest investments in dairy operations, accounting for approximately 15–20% of the total cost of producing milk (Heinrichs, 1993; Van Amburgh et al., 1998). These animals do not generate revenue until first calving, which makes the efficiency of their growth programs essential for farm profitability. Inaccuracies in predicting growth may translate into not optimized feeding programs that either oversupply or undersupply nutrients, both of which can be costly. Overfeeding leads to excess rearing expenses and metabolic problems, while underfeeding delays age at first calving (AFC), prolonging the non-productive period.

Nutrition models have been developed aiming to match supply with requirements by predicting energy and protein needs for target growth rates. However, these predictions rely heavily on the accuracy of the underlying growth equations. If models underestimate or overestimate growth potential, heifers may reach breeding size either too late or in suboptimal condition, which affects reproduction, longevity, and lifetime productivity (Kusaka et al., 2023). Importantly, growth in early life has programming effects that extend into lactation. For example, calves that achieve higher preweaning average daily gain (ADG) consistently produce more milk in their first lactation (Soberon et al., 2012; Van De Stroet et al., 2016). This demonstrates that growth is not simply a cost to be minimized, but a process with long-term biological and economic implications, and highlights the need for accurate and adaptable prediction models.

Biological basis of calf and heifer growth

Growth in dairy calves and heifers occurs in distinct developmental phases, each with unique nutritional priorities. During the preweaning phase, rapid skeletal and organ development predominates. Increased nutrient supply at this stage, particularly through greater liquid feed allowances, has been linked to earlier puberty, increased milk yield, and higher economic returns (Davis Rincker et al., 2011; Soberon et al., 2012).

In the post-weaning to puberty phase, skeletal growth accelerates, but the mammary gland is particularly sensitive to nutritional balance. Studies have shown that excessive energy intake during this window can increase fat deposition within the gland and impair parenchymal tissue development, ultimately reducing future milk yield (Sejrsen et al., 1982; Sejrsen & Purup, 1997).

After puberty, growth increasingly reflects fat deposition relative to frame size. Proper ration formulation is required to support breeding readiness—commonly targeted at 55% of mature BW—without creating over-conditioned heifers that are prone to dystocia or metabolic issues (Heinrichs, 1993; Hoffman, 1997). Finally, during gestation through calving, nutrients are partitioned to support the fetus growth. Inadequate supply compromises calf development, while excessive energy predisposes heifers to calving difficulties and metabolic diseases.

These biological realities are captured imperfectly by models such as NASEM (2021), which predict nutrient requirements based on BW and target ADG. While such models have provided a strong foundation, they often fail to account for structural growth (e.g., height, body proportions) or the long-term programming effects of early nutrition, which limits their ability to guide precision feeding strategies across diverse environments.

Protein requirements considerations

Microbial protein for calves

For decades, nutrition models have assumed that preweaned calves derive virtually all of their metabolizable protein from dietary and endogenous sources, largely ignoring microbial crude protein (MCP) as a contributor. This assumption stems from the fact that the esophageal groove shunts most of the liquid diet directly to the abomasum, bypassing the rumen. However, recent research challenges this view, demonstrating that microbial protein production begins much earlier in life than previously recognized.

Pinheiro et al. (2025) quantified the extent of milk replacer leakage into the rumen and measured MCP synthesis in preweaned dairy kids. On average, 56% of the milk replacer was absorbed into the rumen, providing a substrate for microbial fermentation. This process generated volatile fatty acids, ammonia, and microbial biomass, with MCP synthesis averaging 1.95 g/d. Although the average MCP synthesis was modest (~1.95 g/d), its nutritional significance becomes clearer when expressed relative to crude protein (CP) intake. The kids in the study consumed between 28.3 and 56.7 g CP/d, meaning that MCP represented on average a small fraction of their protein supply (~4.5%). However, the variability was substantial: MCP ranged from as low as 0.625 g/d to as high as 4.0 g/d. Consequently, the proportion of dietary CP (from milk replacer) converted into MCP spanned from 1.1% up to 14%. At the lower end, 1.1% supports the traditional assumption that MCP contribution in preweaning ruminants is negligible. Yet at the upper end, 14% challenges this notion and suggests that microbial activity may represent a meaningful and previously underestimated source of protein during early life. This degree of variation highlights both the potential importance of MCP and the limitations of current requirement models that ignore it. Unfortunately, the factors driving such variability in MCP production remain unclear, and future research is warranted to identify dietary, physiological, or microbial drivers that could allow nutritionists to harness this early microbial contribution to enhance preweaning nutrition.

Complementary evidence from Pinheiro et al. (2025) shows that calves fed higher milk replacer allowances exhibited improved nitrogen retention, increased nutrient flow to the gastrointestinal tract, and enhanced tissue hypertrophy, including skeletal muscle development. This suggests that although MCP yield may be modest in absolute terms, early microbial activity interacts with systemic metabolism, influencing nitrogen utilization efficiency and muscle protein synthesis.

These insights have important implications for re-evaluating protein requirements in calves. Current requirement models (NRC, 2001; NASEM, 2021) do not account for MCP contribution in preweaning diets, essentially treating the calf as a monogastric animal. Yet the demonstrated leakage of milk replacer into the rumen and measurable MCP synthesis call for adjustments in how we model metabolizable protein supply during early life. Even small contributions of MCP may reduce the reliance on dietary protein or interact with growth signals such as insulin-like growth factor-1 (IGF-1), which has been linked to muscle development in calves and kids fed higher liquid allowances (Pinheiro et al., 2025).

Overall, the emerging picture is that microbial protein synthesis starts earlier than traditionally recognized, albeit at a lower magnitude than in post-weaned animals. Integrating this contribution into calf protein requirement models could improve the accuracy of predictions and better align nutritional strategies with the biological reality of early rumen development.

Starter crude protein for calves

As the milk allowance is reduced during the preweaning period, calves become increasingly dependent on starter to supply the necessary nutrients for growth. Importantly, the rise in nutrient demand is not uniform: requirements for energy increase in a greater proportion than requirements for protein. This has led to the hypothesis that starter crude protein (CP) concentration could be gradually reduced over time without compromising performance, provided that intake is sufficient. Results from requirement models support this idea, showing that the efficiency of utilizing metabolizable protein (MP) and metabolizable energy (ME) from starter is markedly lower than from milk, with estimates of 44.4% for MP and 41.2% for ME from starter compared with 71.9% and 57.6%, respectively, from milk (Marcondes and Silva, 2021). Thus, energy capture tends to become the primary limiting factor once milk is stepped down, and excess protein supplied in this context may be used inefficiently.

In an attempt to align dietary supply with these changing requirements, we recently evaluated a decreasing-CP starter strategy, which offers calves a higher CP concentration early in life (when starter intake is minimal) and progressively reduces CP content as milk is withdrawn and starter intake increases (Silva et al., 2025). Contrary to our expectations, the decreasing CP approach did not enhance preweaning growth. Instead, calves on the fixed 18% CP starter exhibited greater weight gains and feed intake, while those on the decreasing CP program had lower starter intake and reduced fecal output near weaning. Nitrogen-use efficiency improved with the decreasing CP program, and fecal N excretion was reduced, suggesting potential environmental benefits; however, these came at the expense of calf growth and robustness of performance.

Given these findings, why have some nutritionists moved in the opposite direction, formulating calf starters with 24–25% CP? The rationale stems from two factors. First, the 2021 NASEM model shifted calf protein requirements from a crude protein to a metabolizable protein framework. In this system, MP needs are driven by target daily gain, and the efficiency of using MP varies by source: ~0.95 for milk proteins, ~0.75 when liquid and starter are fed together, and ~0.70 once the rumen is more developed (NASEM, 2021). Second, microbial protein supply only becomes a major contributor once starter intake exceeds ~1.3 kg/d, which often occurs after weaning. At typical preweaning starter intakes, microbial N is still limited, so nutritionists usually hedge by increasing CP density to ensure that essential amino acid requirements (particularly lysine and methionine) are met from plant proteins.

Several trials illustrate the outcomes of this high-CP approach. Stamey et al. (2012) compared a 19.6% CP starter with a 25.5% CP starter in calves receiving either conventional or enhanced milk replacer. Within the enhanced program, calves fed the higher-CP starter showed greater starter intake around weaning, a tendency for higher average daily gain, and heavier body weights at 8–10 wk. In a follow-up study, Lanier et al. (2021) found that increasing starter CP from 21.5 to 26% altered tissue composition (more lean, larger visceral organs) under a high milk replacer program, but did not increase body weight gain per se. More recently, Yousefinejad et al. (2021) reported that increasing starter CP from 18 to 22% improved average daily gain, weaning weight, and feed efficiency, regardless of the proportion of rumen-undegradable protein. Collectively, these studies explain why 24–25% CP starters are attractive in accelerated growth programs: they can support lean gain and smooth the transition at weaning, especially when milk supply is reduced early and starter intakes remain modest. It is important to emphasize that energy is usually the most limiting nutrient for calves. Therefore, the optimal crude protein concentration in the starter should be considered in relation to both milk allowance and starter intake. In practice, higher CP levels tend to elicit stronger responses primarily when calves are receiving greater amounts of milk.

Nevertheless, evidence is inconsistent, and our own work suggests caution. Our trial demonstrated that although decreasing CP increased nitrogen efficiency, reducing CP during the step-down phase compromised growth—likely due to amino acid imbalances, lower efficiency of using starter-derived MP, and intake dynamics near weaning. NASEM (2021) does not prescribe a universal CP level; instead, it emphasizes formulating to meet MP and essential amino acid requirements according to growth targets and intake predictions. From a practical standpoint, a CP level of 20–22% is sufficient in most conventional milk programs. Higher CP (22–24%, occasionally up to 25%) may be justified in aggressive weaning systems with high growth targets and limited starter intake, but only if amino acid balance is ensured. Importantly, as starter intake rises and microbial protein supply stabilizes, phasing CP downward post-weaning is biologically sound and environmentally responsible.

In summary, the rationale for 24–25% CP starters is strongest in aggressive programs where calves consume little starter preweaning and amino acid supply from solid feed is at risk of being limiting. However, responses are variable, and excessive reliance on CP percentage rather than amino acid balance may lead to inefficiencies. Current evidence does not support blanket recommendations for very high CP starters. Instead, formulation should focus on supplying metabolizable protein and essential amino acids in synchrony with energy, aligning with NASEM (2021) principles. Further research is needed to optimize amino acid-balanced strategies that separate "more protein" from "the right protein at the right time."

Metabolizable protein and energy for postweaned heifers

In postweaned dairy calves, the balance between metabolizable protein (MP) and metabolizable energy (ME) intake is critical for guiding tissue partitioning and mammary development. A higher MP:ME ratio has been associated with improved deposition of mammary parenchymal tissue while limiting undesirable fat accumulation in the mammary gland, particularly when heifers are allowed higher rates of gain. Albino et al. (2015) demonstrated that Holstein heifers gaining 1.0 kg/d maintained adequate mammary development only when diets supplied more than 43 g of MP/Mcal of ME, whereas lower MP:ME ratios led to greater fat deposition in the gland. This aligns with recent NASEM (2021) recommendations for heifers with BW > 125 kg, which propose estimating the minimum MP requirement relative to energy as: MP (g/Mcal ME) = 53 − 25 × (BW/Mature BW). For a 200-kg heifer with a mature BW of 700 kg, the equation predicts ~46 g MP/Mcal, which is consistent with Albino's threshold. However, by 400 kg BW the equation allows the ratio to decline to ~39 g/Mcal. Our results and those of Albino et al. (2015, 2017) suggest that such low values are not adequate when heifers are gaining around 1 kg/d, and a practical minimum of ≥43 g/Mcal ME should be maintained to avoid excessive fat deposition in the mammary gland.

Similarly, Weller et al. (2016) and Albino et al. (2017) confirmed that high nutrient intake levels stimulated systemic IGF-1 but also promoted lipogenesis in the mammary gland, indicating that energy supply in excess of available MP can shift nutrient partitioning toward adipose tissue rather than functional parenchyma. These findings support the idea that mammary gland development is more sensitive to the protein-to-energy balance than to overall growth rate alone (Silva et al., 2002; Albino et al., 2015). In fact, Silva et al. (2002) showed that body fatness, rather than body weight gain per se, was the stronger predictor of impaired parenchymal development—further reinforcing that maintaining an adequate MP:ME ratio is crucial.

Beyond parenchymal development, long-term performance implications are also linked to the MP:ME ratio. Excessive fat deposition in the mammary gland during the allometric growth phase (3–10 mo) is consistently associated with lower milk yield in the first lactation (Capuco et al., 1995; Sejrsen and Purup, 1997; Albino et al., 2017). Conversely, maintaining growth with an adequate MP supply allows heifers to reach breeding size earlier without impairing mammary development, potentially reducing age at first calving without compromising future milk yield (Silva et al., 2002; Albino et al., 2015; Weller et al., 2016).

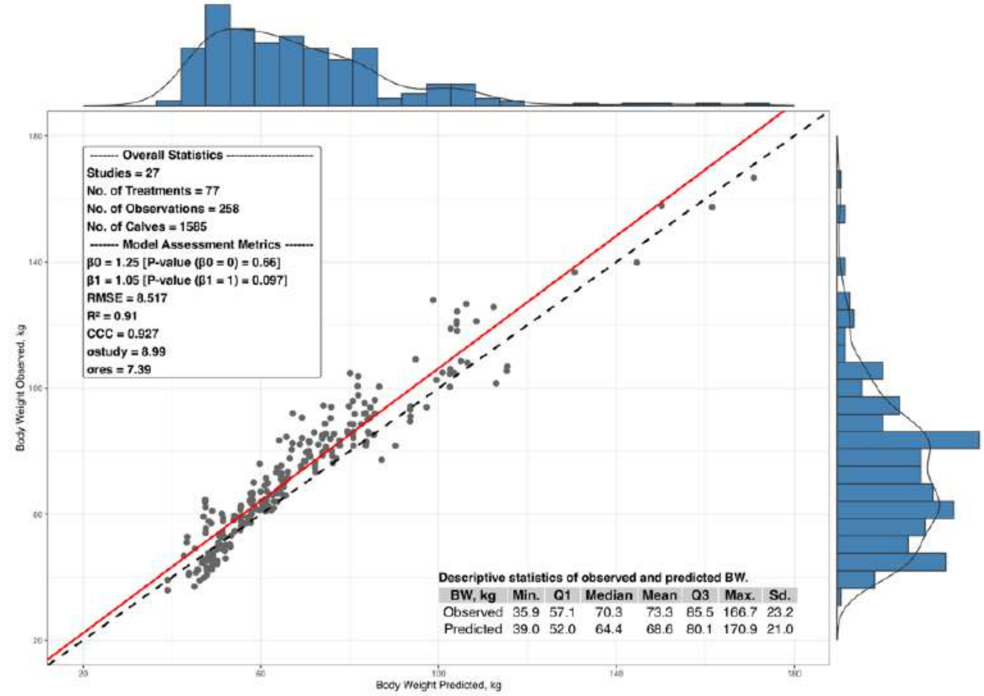
One nutritional strategy to improve the MP:ME ratio without excessively raising dietary CP is to increase the proportion of rumen-undegradable protein (RUP). Diets with higher RUP enhance MP supply because a larger fraction of protein escapes rumen degradation and contributes directly to intestinal amino acid absorption. Silva et al. (2018) showed that Holstein heifers fed diets with approximately 51% of CP as RUP achieved greater average daily gain, feed efficiency, and N retention compared with lower RUP levels, without compromising mammary ultrasonography traits. This aligns with earlier evidence that additional dietary protein supplied as RUP (e.g., from fish meal or heat-treated soybean meal) sustains growth while reducing the risk of excessive mammary fat deposition (Moallem et al., 2004). Thus, increasing RUP is a practical approach to elevate MP intake while keeping dietary CP moderate, improving efficiency and mitigating environmental N excretion.

In summary, the MP:ME ratio is a key nutritional lever in postweaned heifer diets. Higher ratios favor mammary parenchymal development and reduce the likelihood of excess fat deposition, supporting both heifer growth efficiency and long-term milk production potential. Strategic use of high-RUP feeds can help meet MP requirements without resorting to overly high dietary CP, aligning with both performance and sustainability goals.

Traditional nutrition models: Foundations and limitations

Nutrition models for calf and heifer growth have historically relied on empirical and mechanistic functions that link BW to age and nutrient intake. The NRC (2001) and NASEM (2021) frameworks, for example, calculate nutrient requirements for energy and protein deposition based on BW, target ADG, and mature size. Growth itself is often represented using simple functions such as the Brody, Gompertz, or Richards equations (Richards, 1959; Owens et al., 1993).

While useful, these models present clear limitations. First, they often treat growth as a unidimensional process, relying on BW as the sole indicator. This approach ignores the importance of skeletal growth and body composition, both of which are crucial for predicting developmental milestones like puberty or breeding readiness (Heinrichs & Losinger, 1998). Second, most validation datasets are derived from North American or European populations, which may not proper reflect genetic selection or management systems in tropical or subtropical environments (Silva et al., 2021). Finally, traditional models rarely incorporate variability across individuals, even though differences in tissue accretion rates or feed efficiency strongly influence outcomes. The result is that models provide general targets but often fall short when applied to specific herds, breeds, or climates. Finally, most frameworks do not account for the incidence of disease or its effects on nutrient requirements and tissue accretion, despite the profound impact of morbidity on calf growth trajectories.



------- Overall Statistics -------------------
Studies = 27
No. of Treatments = 77
No. of Observations = 258
No. of Calves = 1585
------- Model Assessment Metrics -------
β0 = 1.25 [P-value (β0 = 0) = 0.66]
β1 = 1.05 [P-value (β1 = 1) = 0.097]
RMSE = 8.517
R² = 0.91
CCC = 0.927
σstudy = 8.99
σres = 7.39

Descriptive statistics of observed and predicted BW.

BW, kg	Min.	Q1	Median	Mean	Q3	Max.	Sd.
Observed	35.9	57.1	70.3	73.3	85.5	166.7	23.2
Predicted	39.0	52.0	64.4	68.6	80.1	170.9	21.0

Figure 1. Relationship between predicted BW (kg; x-axis) from the CalfSim tool and observed BW (kg; y-axis) from 27 dairy calf nutrition studies extracted during the literature review. The dashed black line represents the line of equality (X = Y), and the solid red line represents the fitted regression line. Main model assessment statistics are displayed in the inset box within the scatter plot (upper left). The descriptive statistics of observed and predicted BW, including the minimum (Min.), first quartile (Q1), median, mean, third quartile (Q3), maximum (Max.), and SD, are shown in the bottom right. The histograms above and to the right of the graph show the distribution of predicted and observed values, respectively. RMSE = root mean square error; CCC = concordance correlation coefficient; σstudy = study-level standard deviation; σres = residual standard deviation.

In fact, when comparing the chapter dedicated to dairy calves in NRC (2001) with NASEM (2021), a notable evolution can be observed. The more recent framework not only details protein and energy requirements but also addresses calf starter intake more explicitly, with equations developed for both temperate and subtropical climates. Moreover, NASEM (2021) incorporated extensive model testing, validating its predictions against 397 literature treatment means.

Building on this foundation, Da Silva and Costa (2025) developed the CalfSim tool (go.uvm.edu/calfsim), a web-based decision-support platform that uses NASEM (2021) equations as its backbone. Their evaluation of predicted versus observed BW, based on 27 studies from the literature, demonstrated both accuracy and precision of the NASEM (2021) predictions (Figure 1). However, it is important to highlight that in their regression analysis of predicted versus observed BW, the effect of study was modeled as a random effect (σstudy = 8.99 kg), with an intraclass correlation coefficient (ICC) of 0.59. This indicates a strong study effect, which in practical terms may be viewed as a proxy for "farm effect," encompassing unmeasured factors such as housing, environmental conditions, health management, disease incidence, and genetics, as pointed out earlier. These contextual differences suggest that although the model performs well overall, prediction errors can emerge depending on the specific production conditions.

A key aspect, and often a bottleneck when comparing studies or farms, is starter intake. Although new equations were proposed in NASEM (2021), and other formulations are available in the literature (e.g., Quigley et al., 2021; Silva et al., 2019), starter consumption is highly sensitive to factors such as management, environment, and health status. Because starter is a critical nutrient supply (particularly energy), this variable becomes especially important in systems with lower milk allowances and after the first month of life. Accurate equations for predicting starter intake are therefore fundamental to obtaining growth predictions that more closely reflect on-farm reality.

Looking ahead, one promising avenue lies in the development of hybrid models, which is a mathematical modeling paradigm that can aggregate empirical or semi-mechanistic nutrition models with machine learning approaches (Tedeschi, 2023). Such hybrid models could refine predictions by incorporating additional variables not currently considered in nutrition models, such as disease occurrence, behavioral indicators, or environmental stressors. However, these approaches require access to larger and richer datasets, which may be increasingly feasible through Precision Livestock Farming Technologies (PLF), such as wearable sensors, automated feeders, and continuous behavioral monitoring systems (Costa et al., 2021). The integration of nutritional models with data-driven corrections represents a promising strategy to enhance predictive capacity and support on-farm decision making in diverse production environments

Considerations about age at first calving

AFC is a pivotal outcome shaped by nutrition and growth, with profound implications for lifetime productivity, health, and culling risk. Traditional recommendations in the United States encourage calving between 22 and 24 months to minimize rearing costs and accelerate returns (Hoffman, 1997; Ettema & Santos, 2004). However, recent evidence highlights that the relationship between AFC, calving difficulty, and long-term milk production is more complex than previously assumed.

In a large dataset covering over 687 herds and 1 million calving observations across the United States, Marcondes et al. (2025) show that heifers calving later, at around 27–28 months, produced more milk across their lifetime and likely benefited from additional time for mammary development. However, these older heifers also experienced higher levels of dystocia, which depressed first-lactation yield and increased health risks. This highlights the trade-off: while delayed AFC may enhance mammary development and lifetime output, it also increases the likelihood of calving complications.

For this reason, the overall recommendation continues to favor targeting 23–24 months as the ideal AFC. At this age, heifers have reached sufficient skeletal maturity to minimize calving difficulty while still entering the herd early enough to balance rearing costs with productive life. The challenge today is that many farms are pushing AFC lower, to around 21–22 months, in an effort to reduce the high costs of heifer rearing, which often exceed $3,000 per animal. While this strategy reduces upfront expenses, the assumption that early calving has little to no effect on first-lactation performance is not supported by the evidence. The findings of Marcondes et al. (2025) demonstrate that heifers calving at 21–22 months not only produced less milk at first parturition but also faced a greater likelihood of dystocia, compounding the negative impact on both performance and welfare.

Thus, the re-evaluation of nutrition models must account not just for growth rate and cost efficiency, but also for how growth trajectories interact with biological maturity, calving ease, and long-term productivity. The ultimate goal should be to support breeding strategies that consistently achieve 23–24 months AFC while avoiding the risks of both too-early and too-late calving.

New tools and directions

The advent of precision livestock farming (PLF) technologies offers new opportunities to improve growth monitoring and prediction in dairy cattle. Advances in automated imaging, computer vision, and 3-dimensional systems enable objective assessment of body weight, body condition, and conformation, reducing reliance on manual scoring and generating high-frequency data for longitudinal analysis (Azzaro et al., 2011; Salau et al., 2017; Van Hertem et al., 2020; Xavier et al., 2022). Walk-over weighing systems also provide continuous and labor-efficient body weight monitoring with strong agreement to conventional scales (Dickinson et al., 2013). Beyond measurement, predictive analytics are being applied to these data streams: machine-learning algorithms trained on image features can accurately predict body weight and track composition of body weight changes throughout lactation (Song et al., 2018; Xavier et al., 2022). Importantly, because most farmers do not routinely weigh their growing heifers or evaluate body condition score (BCS), camera-based systems offer a practical and impactful improvement in assessing heifer development. Looking ahead, these tools will not only enable body weight monitoring but also withers height measurement and analysis of the BW:height relationship. Such metrics can help determine whether accelerated growth programs (higher ADG targets) are driving true structural development (muscle and bone) rather than undesirable fattening. Combining these PLF tools with traditional nutrition models provides a path to refining growth predictions in a way that is both biologically meaningful and practically useful.

Summary

Despite advances, significant challenges remain. Growth trajectories vary across breeds, regions, and management systems, limiting the transferability of models. Longitudinal datasets spanning multiple lactations remain rare, making it difficult to validate how early-life growth influences lifetime productivity. Additionally, mechanistic models such as NASEM (2021) require extensive input data that may not be readily available on commercial farms, while simpler empirical curves may lack biological depth.

The way forward lies in integrating biological understanding with modern data streams. Nutrition models must account for both weight and structural growth, incorporate early-life programming effects, and leverage precision technologies to reflect real-world variability. Re-evaluating models in this way will allow for more accurate feeding strategies, optimized AFC, and improved lifetime productivity.

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Implications of High Milk Component Yield in Dairy Cattle – Do We Really Understand the Nutrient Requirements

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The objective of this paper is to discuss observations on the yields of milk and milk components in dairy cattle in the Northeast US and integrate these observations with the potential implications for updating nutrient requirements to meet the synthesizing capability of fat and protein as selection pressure continues to drive genetic changes in phenotypic expression of these components.

With the development of genomic evaluation and selection, there has been greater selection pressure on traits like milk fat and protein content and yield (Guinan et al., 2023). Accordingly, in the Northeast Federal Order, over the last fourteen years, milk fat has risen from 3.78% to approximately 4.32% and milk protein from 3.11% to 3.27% resulting in a 14.3% increase in fat content and a 5.1% increase in protein content (https://www.fmmone.com/Statistical_Report.htm). The increase in milk fat is more than double the increase in protein, which calls into question both the energetics of the differences in expression and the nutrient supplies required for the synthesis of both components and will be discussed later in the paper. Over that period, one of the factors leading to increased milk fat was the recognition that the industry was overfeeding unsaturated fatty acids (FA) leading to milk fat depression caused by conjugated linoleic acid isomer production, specifically cis10-trans12 (CLA; Jenkins and McGuire, 2006). Reducing the rumen unsaturated FA load (RUFAL) led to increases in milk fat content and yield (Bauman and Griinari, 2003; Palmquist and Jenkins, 2017). Then, with the introduction of genomic selection and high-palmitic acid (C16:0) FA products, the industry observed more consistent increases in milk fat content. In the early stages, the addition of C16:0 products to the diet looked like magic, as the mammary gland had a ready source of C16:0 to use for triglyceride synthesis that was not affected by the downregulation of de novo FA synthesis and Stearoyl Co-A Desaturase (SCD) activity by 1 to 3 grams of CLA. By about 2013, the industry had fully implemented genomic selection on the male side, resulting in profound effects on milk fat and protein yield capacity. As reviewed by Guinan et al. (2023), the generation interval on the male side has decreased by about 50%, which accelerates the selection intensity. Additionally, more knowledge about which genes and alleles to target for milk fat increases the selection pressure for that trait as well (Bionaz et al., 2015; Carvajal et al., 2016).

With the rederivation of amino acid (AA) requirements for lactating dairy cattle by Higgs, (2014), Higgs and Van Amburgh (2016), and Higgs et al., (2023) and the evaluation of those values by LaPierre et al., (2019), more precise and higher requirements were established. Further, the evaluation of CNCPS v7 essential AA (EAA) requirements integrated into CNCPS v6.5 enabled the implementation of these requirements for Methionine (Met), Lysine (Lys), and Histidine (His) in commercial diet formulation much faster than the release of v7. The first implementation of these data, in a prospective research study unrelated to AA, was the study of Benoit et al. (submitted), that evaluated monensin supplementation. In that study, balancing for Met, Lys, and His led to a 14% increase in milk fat content and yield and a 9.4% increase in milk protein content and yield in established lactation compared to pre-implementation of the treatment diets. This demonstrates that overcoming a deficiency in EAA supplies could have a profound effect not only on the synthesis of milk protein but also on fat, leading to the idea that we should not think narrowly about the longstanding linear approach of AA and milk protein. A surprising observation was that milk fat was increased through increased synthesis of de novo and mixed FA in agreement with previous data showing the integration of AA and FA synthesis residing in the mammary gland (Li et al., 2019; Bionaz and Loor, 2008; Ding et al., 2020). Reviewing the literature, there is good evidence that suggests several AA are intimately linked to FA synthesis, such as His, Serine, Cysteine, Lys, Met, and Arginine, and also some non-essential AA (NEAA; Smith et al., 2003; von Wettstein-Knowles et al. 2005; Dudek and Semenkovich, 1995; Fukuda and Iritani, 1985; Li et al., 2014; Ding et al., 2019). Thus, as we consider the yield capacity of modern dairy cattle, specifically Holsteins, we need to integrate AA requirements into our formulation strategies to meet the large protein-synthetic demand for milk FA synthesis, even though the idea is not historically intuitive. The approach to AA requirements by Higgs et al. integrated energy and AA requirements with these concepts in mind. Similar to the energy demands for milk component synthesis, AA requirements are wide-spread and not specific to any one protein synthetic pathway, thus a global efficiency of use of AA incorporates the non-milk protein uses of AA in metabolism (Lobley, 2007; Beckett et al., 2006).

Evaluating the studies conducted internally at Cornell University over the last 7 years that fed diets formulated to meet the Met, Lys, and His requirements (1.19 g/Mcal, 3.20 g/Mcal and 1.19 g/Mcal ME, respectively), we have observed higher de novo and mixed FA content and yield, which accounts for the majority of the increase in overall milk fat (Benoit et al., submitted, Danese et al., 2023; Benoit et al., 2024). Using a meta-regression approach with pen-level individual production data and AA supply predictions using CNCPS v6.55 from treatment diets formulated to be below or above the AA requirements above, we evaluated the relationship between increasing the supply of Lys with ECM yield and milk components. Increasing metabolizable Lys increased ECM yield (Figure 1), milk fat yield (Figure 2A), milk protein yield (data not shown), and de novo FA yield (Figure 2B). Part of this relationship was due to the overall increase in DMI leading to an increase in AA and energy supply, although this data suggests an integrated response to increases in these nutrients, and the positive effect of increasing AA supply on milk component synthesis. Implementation of these AA requirements and supply on regional dairy farms has also demonstrated increases in milk fat content and yield, most likely related to increased production of de novo and mixed FA and, in some cases, the availability of oleic acid from high-oleic soybeans that appears to enhance component yield when the soybeans have been processed appropriately (heat and particle size). Amino acid balancing in NY Farms has led to bulk tank milk fat contents of 4.5% to 4.6% and further increased to 4.8% to 5.0% with the addition of high-oleic soybeans. This suggests there are synergies between AA and FA, and likely FA requirements for energy metabolism and lactation that are not well described and require further investigation. Additionally, meeting the AA requirements to increase the synthesis of de novo FA and an adequate level and ratio of dietary FA to increase the supply of preformed FA may be optimal to increase overall milk fat production.

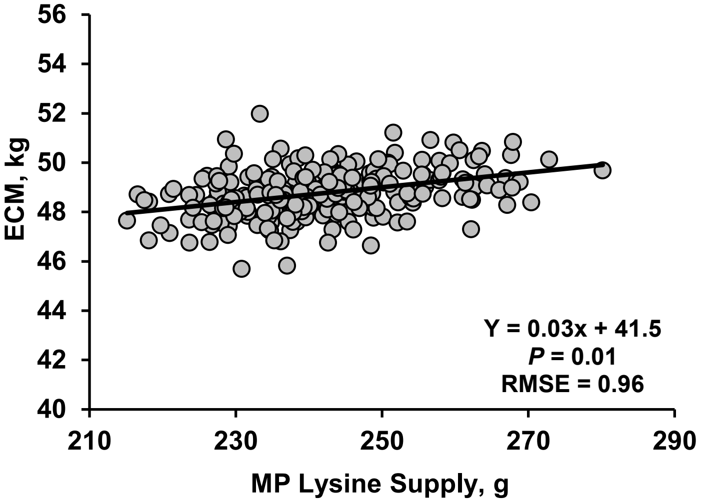

Figure 1. The effect of increasing metabolizable (MP) Lys supply on ECM yield using pen-level individual observations from three studies conducted at Cornell University. The predicted equation is: ECM kg/d = 0.03 × MP Lys supply + 41.5; P = 0.01; RMSE = 0.96.

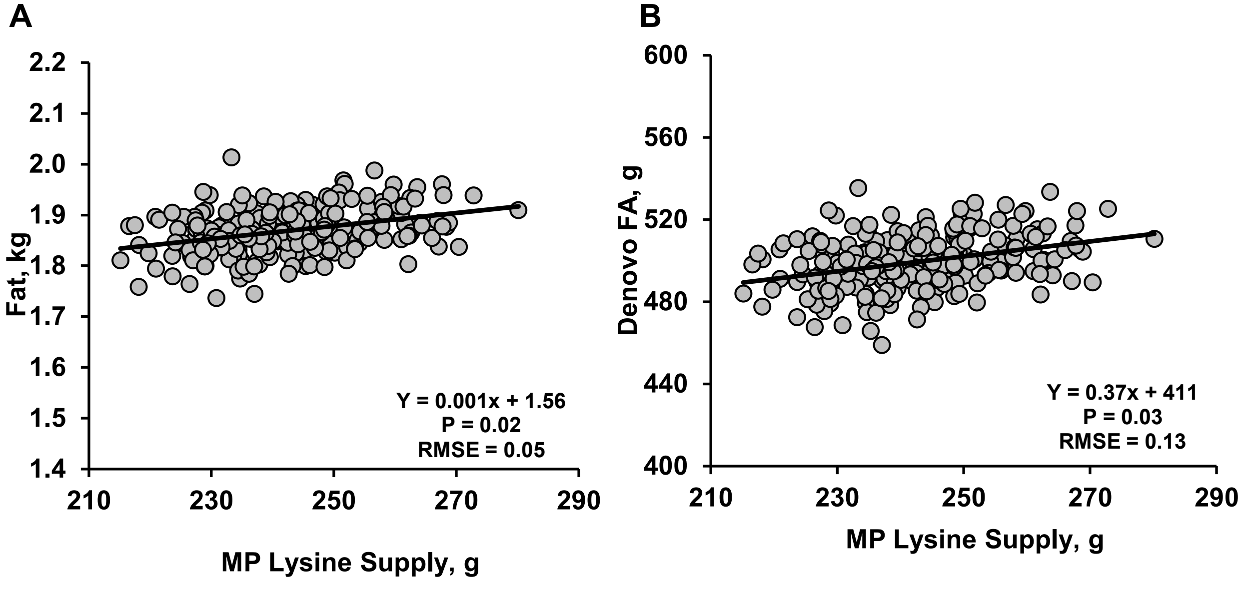

Figure 2. The effect of increasing metabolizable (MP) Lys supply on milk fat yield (A) and de novo FA yield (B) using pen-level individual observations from three studies conducted at Cornell University. The predicted equation is: (A) milk fat kg/d = 0.001 × MP Lys supply + 1.56; P = 0.02; RMSE = 0.05, and (B) de novo FA g/d = 0.37 × MP Lys supply + 411; P = 0.03; RMSE = 0.13.

Over time, with greater utilization of genomic selection and genotyping of both bulls and cows, there is more efficient selection pressure on specific traits such as milk fat and protein. The federal order composition data clearly demonstrates the effectiveness of this new set of tools in advancing the genetic capacity of dairy cattle to synthesize milk components. If dairy cows were pigs, we would suggest that new genotypes with a greater capacity for protein synthesis would require increased nutrient requirements for substrates like AA; thus, the implication is that we might not know the current requirements for nutrients beyond energy for high genomic, modern Holsteins. We hypothesized that linking AA requirements to energy supply would enable a process that maintains continuous AA supply with increasing dry matter and metabolizable energy intake; however, this hypothesis may not be entirely accurate. On commercial dairy farms, first-lactation highly selected Holstein dairy cows partition nutrients to milk components in a manner that suggests their capacity to synthesize milk fat and protein at a given energy intake is greater per unit of lactose synthesis than previously observed. Thus, the nutrient demand per unit of ME intake is likely greater to allow the cow to partition nutrients and synthesize fat and protein closer to her capacity. As stated above, increases in milk fat synthesis in these high genetic-potential cattle are largely achieved by increases in de novo and mixed FA synthesized within the mammary gland. Previous data would suggest genetic variation in milk fat was largely due to DGAT-1 (Schennink et al., 2007), a known regulatory enzyme in triglyceride synthesis, although increases in the synthesis of de novo FA and formation of milk fat in high-genetic cattle would suggest the activity of many different enzymes involved in the entire synthesis pathway are being upregulated to achieve greater milk fat production. Additionally, AA requirements for the increased protein-synthetic capacity needed to optimize milk fat synthesis should be considered when formulating diets for highly selected dairy cows.

In previous internal studies, we observed differences in the ratios of protein to lactose yield and fat to lactose yield, suggesting that the partitioning of nutrients for component synthesis may be modified in unexpected ways. For example, in our studies with adequate AA supply and modest dietary FA supply (3.7-4.0% total FA), the ratio between protein and lactose yield on a fat-free basis is 0.81:1. At the low end of the relationship, the ratio is 0.63:1, suggesting that the relationship between protein and lactose is not as constant as previously considered (Jenness, 1985). For ratios of fat to lactose, the high end of the relationship is 1.13:1 and the low end is 0.72:1. When fat and protein are combined the ratios are 1.93:1 and 1.35:1. Some of these differences in milk component ratios are due to AA balancing, subtle differences in stage of lactation, or likely genotype differences that we don't fully understand yet. To make an appropriate comparison of protein and lactose yield, it should be conducted on a fat-free milk basis, as the butterfat is not integral to the fluid compartment of milk, and when compared on that basis, this leads to similar relationships between lactose and protein. The implication that there is potential to improve the solids content of milk is not new. Still, the difference in component yield per unit of lactose implies the need to formulate diets that are more nutrient-dense per unit of energy to meet the requirements for high component synthesis. Dry matter intake needs to be considered; however, conversations at the farm level with cows yielding higher component levels suggest feed intake is not increasing consistently with expectations, in situations where milk volume remains somewhat constant while fat and protein content and yield increase. This implies a change in energetic efficiency as was observed in the study of Benoit et al. (submitted) as the change in component yield did not appear to have an impact on DMI. Another observation is the high-genetic cows are responding positively to greater increases in AA supply, beyond our current estimates, suggesting their AA requirements are higher per unit of energy intake and they are more efficient with how they partition nutrients, consistent with a genotype response.

On some dairy farms in NY, there are opportunities to observe Holstein cattle with more intensive genomic selection, and an evaluation of their milk, fat, and protein yields demonstrates significant increases in fat and protein content and yield, and more moderate increases in milk volume. For example, in one herd, high genomic first lactation Holstein cattle are averaging about 38 kg/d milk yield, with 5.48% fat and 3.53% true protein, and the range on milk fat is 4.40% to 7.08% and protein ranging from 2.88% to 4.20%. We are observing more cattle like this in the first lactation, which begs a couple of questions: Do we understand the nutrient requirements for these cattle, and are these cattle a new genotype? Most of these cattle receive diets that are balanced for AA, have moderate FA supplies, and have good cow comfort and management. Dr. Curt Van Tassell at USDA Beltsville was one of the three people who developed the genomic selection process we currently use. In a recent conversation with him about this, we asked him, compared to swine and poultry, when do we know if we have a new genotype in dairy cattle? His response was “constantly” because our selection process, compared to swine and poultry, is continuous, as we don’t line breed. Thus, according to Curt, under the current systems for genetic selection and the technology being used to reduce the generation interval, each calf born has the potential to be a new genotype. This means that the nutrient requirements for milk and milk component synthesis are increasing at a steady rate, and that we need to conduct frequent studies to challenge the requirements for nutrients like AA or FA, similar to what was done by Higgs (2014) and Higgs et al. (2023). Considering these cattle produce more than 5.5% milk fat under the current dietary conditions, they likely have a greater capacity for fat and protein synthesis. And they are unable to demonstrate it because of a lack of nutrient resources. So, what might this mean for nutrient requirements? In the conversation with Dr. Van Tassell, he suggests we should re-evaluate these requirements every 5 to 10 years as the capacity for component yield increases. This already presents a conundrum for our modeling efforts, as Higgs (2014) utilized literature data to develop the current values we are starting to use, and Dr. Van Tassell’s comments suggest those values are already outdated for modern Holsteins. The practical situation that makes this difficult is that the cattle we need to re-evaluate the EAA requirements for don’t exist in literature or on most research farms; they are on commercial dairies. As we contemplate re-evaluating these values, determining how to gain access to high genomic Holsteins to test these concepts is one of our most significant considerations.

This highlights the current observation and challenge that the phenotypic expression of milk fat content and yield is outpacing the same expression of milk protein. When we evaluate the rate of genetic change for milk protein yield, we observe that the rate of change is slightly less than milk fat but not significantly less, and that the rate of change in both males and females is similar (Guinan et al., 2023; Counsel on Dairy Cattle

Breeding, https://uscdcb.com/); thus, the potential to observe higher average milk protein yields is similar to that of fat. So why are we not making the same progress with milk protein? Many times, the answer to that question is that the market is not paying for it, so why produce it? But we believe the answer is more nuanced than that. The amount of fat currently being produced has more to do with genetics and nutrition than it does with the market price; however, we are happy the market price has helped support the productivity of the cattle. For the protein yield, we would hypothesize that the limiting factors are more related to limiting AA, such as leucine (Leu), isoleucine (Ile), and possibly arginine and proline (Pro). In our re-evaluations of research studies through CNCPS v7, the next limiting AA after Met, Lys, and His tends to be Leu and Ile. The limitation varies from approximately 10 grams per day to up to 22 grams per day on a metabolizable basis of each AA. If the objective is to produce more casein, the three most required EAA for synthesis are Leu, Ile, and Valine, and for the non-essentials, Pro and glycine. The most limiting AA observed using CNCPS v7 are consistent with the prioritization from the NASEM document in 2021 (NASEM, 2021); thus, we are consistent in our modeling efforts to identify the AA we should focus on for improving productivity. Proline is essential for casein synthesis because of its structure and binding sites, which provide the three-dimensional structure of casein. In high-producing cows with limited resources, synthesizing Pro might be too resource-intensive; thus, it becomes a limitation.

One other observation concerning formulating diets with AA is that the dietary addition and increased supply of AA must be greater than what is required to overcome the AA deficiency in the diet completely. In other words, adding 10 grams of a rumen-protected AA product to the diet of cattle that are deficient in 20 grams of metabolizable AA will likely not produce any observable positive effects. In the Northeastern US, there has been greater adoption of AA balancing by producers and nutritionists, as we have been taught that they are nutrients, not ‘feed additives”. As an industry, we must recognize AA as nutrient requirements needed to optimize productive functions such as lactation, specifically when considering the increased protein-synthetic capacity and milk component synthesis of high genomic cattle. In most cases, this means feeding AA at much higher levels than previously considered. For example, in dairy cattle yielding about 52 kg/d milk at 4.35% fat and 3.29% true protein with a metabolizable energy intake of ~87 Mcals/d, the supply of His and Met should be 103 grams per day, and the supply of Lys should be 278 grams per day. The supply adheres to the 1.19 gram/Mcal for Met and His and 3.2 grams/Mcal guidelines, achieved through forage digestibility and management that enables dry matter intakes sufficient for the level of productivity.

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