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How Much Phosphorus Should We Feed to Transition Dairy Cows?

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Introduction

Phosphorus in dairy cow nutrition is currently receiving much attention from producers, nutritionists, veterinarians and physiologists alike. Reasons for this increased interest are legal incentives implemented in many parts of world aiming at reducing the amounts of phosphorus of animal origin entering the environment, but also the more recently described effect of the dietary phosphorus on the calcium status of fresh cows (Wächter et al., 2022b). A more restrictive use of phosphorus in ruminant nutrition than in previous decades is warranted not only for environmental reasons, but also in view of the scarcity of global phosphorus reserves. On the other hand, the continuously increasing productivity of dairy cows requires an adequate supply of this mineral that is essential to maintain health and productivity in high yielding dairy cows.

Considerable progress was made in understanding the phosphorus metabolism of ruminants in recent years (Grünberg, 2023). The advanced understanding of the regulation of the phosphorus balance in ruminants led a reappraisal of current feeding strategies for this mineral in dairy cows, in particular during the transition period. At the same time important became more relevant and urgent to tackle.

Advances Over the Last Decades

Recognition of phosphorus as environmental pollutant in the last century resulted in a critical reappraisal of feeding recommendations for phosphorus in ruminants (NRC, 2001). Research conducted in the 1970s and 1980s revealed that the absorption efficiency of dietary phosphorus of plant origin in ruminants was grossly underestimated until then. Adjusted absorption coefficients for phosphorus used in official publications led to markedly lowered recommendations for dietary phosphorus supply to cattle (AFRC, 1991, NRC, 2001). These more recent recommendations were evaluated extensively and are generally accepted to be adequate for large parts of the lactation cycle (National Academies of Sciences and Medicine, 2021).

Contentious debate however persists over how much phosphorus to feed to dairy cows during the transition period. For many decades common wisdom stated that inadequate dietary phosphorus is likely to present a risk for health and productivity of the dairy cow. Phosphorus deficiency was and remains of particular concern in early lactation, where it is thought to result in feed intake depression and impaired productivity (Grünberg, 2023). Insufficient phosphorus supply is also empirically linked to medical conditions like the downer cow syndrome or postparturient hemoglobinuria, which further encouraged the common practice of feeding this mineral well in excess of current recommendations in particular during the dry period.

To reliably estimate the phosphorus requirements of the dry cow is challenging. The limited duration of the dry period, the lacking in-depth knowledge of how counter-regulation to phosphorus deficiency works in cattle, and the lack of unambiguous clinical signs of phosphorus deficiency, all precluded meaningful animal response studies in the past. The more recent discovery of novel pathways of phosphorus regulation in ruminants now allow to at least determined at what dietary phosphorus level cows start upregulating counter-regulation to phosphorus deprivation. Recent studies provided compelling evidence that in contrast to earlier concepts the regulation of the phosphorus homeostasis in cattle is not dependent on the regulation of the calcium balance (Cohrs et al., 2018, Cohrs et al., 2022). Although the associated regulatory pathways have not yet been elucidated in every detail, it was shown that bone mobilization that is independent of the secretion of parathyroid hormone (PTH) and the calcium balance is an integral part of the response to phosphorus deficiency (Grünberg, 2023). Furthermore, FGF-23, an endocrine compound recently identified as key regulatory hormone for the phosphorus homeostasis in monogastric species was also found to respond to alterations of the phosphorus balance in ruminants (Köhler et al., 2021). Based on this deeper understanding of the regulatory circuits of the phosphorus homeostasis, it was possible to determine that dry cows do not upregulate counter-regulation during a normal length dry period with rations containing at least 0.20% P in dry matter (DM) (Cohrs et al., 2022).

Exploring the Effect Feeding Different Levels Phosphorus During the Transition Period

Despite of well-established official recommendations in the range of 0,20 to 0,25% P in DM for dairy cows in late pregnancy, dry cows rations with 0.40% P in DM and more are still commonly encountered. Overfeeding this mineral is sometimes intentional with the objective of preventing or at least mitigating a negative phosphorus in early lactation. In Europe dry cow rations often contain 0,40% P in DM and above because of the use of canola as important protein source meeting legal requirements to only use GMO-free feed ingredients in food producing animals.

In the meantime, a continuously increasing body of evidence compellingly shows that feeding phosphorus above requirements during the dry period not only does not mitigate, but rather increases the risk of metabolic disturbances of the fresh cow (Santos et al., 2019). Based on our current knowledge feeding phosphorus in excess of requirements to dry cows should be avoided whenever possible.

Some studies also explored the effect of feeding phosphorus below requirements during the dry period with remarkable results. Deleterious effects of restricted phosphorus supply were observed when cows were phosphorus deprived not only during the dry period but also during early lactation. Clinically apparent signs and symptoms in phosphorus deprived cows consistently only occurred after calving (Valk and Sebek, 1999, Puggaard et al., 2014, Grünberg et al., 2019, van den Brink et al., 2023). Typical symptoms were pronounced feed intake depression, low milk yield and increased incidence of secondary fresh cow diseases such as clinical and subclinical ketosis, abomasal displacement, or postparturient hemoglobinuria. In contrast, studies limiting the phosphorus deprivation to the dry period not only did not identify negative effects during the dry period or the following lactation, but even reported a beneficial effect on the

calcium balance during early lactation (Wächter et al., 2022a, Wächter et al., 2022b). This effect was attributed to marked bone mobilization triggered by a negative phosphorus balance, which results in release of calcium and phosphorus in metabolically relevant amounts into the extracellular space (Cohrs et al., 2018).

In summary, these results indicate that while in the past feeding phosphorus in excess of requirements was considered to be beneficial, and restricting the phosphorus supply during the dry period was presumed to be harmful to the dairy cow the opposite appears to be true.

New Horizon

This novel insight into the regulation of the phosphorus balance in cattle initiated a reappraisal of the current concepts of mineral supply to transition cows aiming at mitigating the risk periparturient hypocalcemia. From a historical perspective, after the dietary calcium-, potassium-, magnesium- and the anion content of dry cow rations, now the dietary phosphorus content is in the focus as a tool to prevent periparturient hypocalcemia. Feeding phosphorus in excess of requirements was unequivocally identified as a risk factor for hypocalcemia, while restricting the dietary phosphorus supply in the last weeks of gestation was found to mitigate calcium balance disturbances in fresh cows. First trials evaluating the concept of restricted phosphorus supply during the last weeks of gestation yielded promising data suggesting that this approach might indeed be innocuous for the dairy cow's health and productivity (Wächter et al., 2022a, Ringseis et al., 2024).

Formulating dry cow rations with a phosphorus content low enough to induce a negative phosphorus balance (i.e. with a phosphorus content well below 0.2% P in DM) is at least as challenging as formulating a dry cow rations with a calcium content sufficiently low to induce a negative calcium balance. In this context feed additives suitable to bind dietary phosphorus are currently receiving increased interest (Frizzarini et al., 2024). Phosphate binders can be expected to be considerably more effective in inducing a negative phosphorus balance in ruminants compared with monogastric species as these compounds would not only bind dietary phosphorus but also remove endogenous phosphorus from the body by making salivary phosphorus less available for reabsorption.

Critical Knowledge Gaps

Restricting the phosphorus supply to dry cows appears to be a promising strategy to address periparturient hypocalcemia, but guardrails have to be established to efficiently and safely use this novel concept in the field. Specifically the maximum intensity and duration of phosphorus deprivation in dry cows that does not impair the metabolism and the productivity of the dairy cow must be determined. As mentioned above, the mechanisms through which phosphorus deficiency causes signs like feed intake depression or hemolysis are not yet understood. This greatly complicates the recognition of phosphorus deficient animals in early stages (ideally at a subclinical stage).

Furthermore an unambiguous parameter to assess the phosphorus status in a cow undergoing phosphorus deprivation is needed. In practice the blood phosphorus

concentration was and is again increasingly used for this purpose. Blood phosphorus reflects the dietary phosphorus intake in the hours prior to sampling with reasonable accuracy, but is poorly suited to assess the phosphorus status of the body (Elizondo Salazar et al., 2013, Grunberg, 2014). Indeed one of the factors affecting the blood phosphorus concentration is the amount of this mineral absorbed from the digestive tract (thus the phosphorus supply). However, in states of phosphorus deprivation where counter regulation in the form of bone mobilization is upregulated, phosphorus enters the extracellular space (and thus blood) not only from the digestive tract but also from bone. Under these conditions blood phosphorus is thus confounded by counter-regulation. Solid data are currently missing, but this author expects that basing the assessment of the level of phosphorus deprivation on blood phosphorus is likely to markedly underestimate the degree of phosphorus deficiency.

Another important knowledge gap concerns the regulation of the phosphorus balance in early lactation. Earlier studies reported that dairy cows fed with rations meeting or exceeding phosphorus requirements during the dry period are in a state of negative phosphorus balance in early lactation (Knowlton and Herbein, 2002, Taylor et al., 2009). It is unclear how phosphorus deprivation before calving affects the phosphorus balance in early lactation, and if and to what extent a cow that was phosphorus deprived during the dry period can adequately replenish phosphate reserves in early lactation. It remains thus uncertain if and to what extent the phosphorus supply in early lactation should be adjusted in a cow that was phosphorus deprived before calving.

Another area of interest is the interaction between different concepts aiming at mitigating periparturient hypocalcemia. This includes the effect of phosphorus deprivation while feeding anionic diets (with or without excessive amounts of calcium) or the inclusion of vitamin D in the dry cow rations.

Based on our current knowledge it seem that the moderate phosphorus deprivation not exceeding 3 to 4 weeks prior to calving is innocuous while phosphorus deficiency in early lactation must be avoided to prevent negative effects on health and productivity. The regulation of the phosphorus balance of the dairy cow in transition however remains unsatisfactorily understood.

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Vitamin D: Support for Immunity and Transition Cow Performance

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Introduction

Cost effective prepartum dietary interventions that support a smooth transition for pregnancy to lactation provide a large return on investment because adverse events in the first week of lactation have substantial negative consequences on lactation and reproductive performance. For example, cows that experience metritis produce about 8 lbs. less milk per day in the first 10 weeks of lactation compared with cows without metritis. Optimal vitamin and mineral nutrition during the transition period is considered important for transition cow performance, but recommendations and practices are often variable. Herein, the focus will be on the influence of vitamin D in immunity of dairy cows and the effects of prepartum vitamin D nutrition on transition cow performance.

Transition Cow Immunity and Inflammation

The transition from pregnancy to lactation presents major challenges to immune processes, such as initiation of parturition, uterine involution, and increased exposure of mammary glands and uterus to opportunistic pathogens. Moreover, metabolic demands of the immune system coupled with metabolic adaptations at the start of lactation may impair immune cell functionality. The resilience and robustness of the cow's immune system is quite remarkable considering the challenges faced upon the onset of lactation.

The mammalian immune system is far more complex than what is generally described. It contains multiple levels of redundancy to compensate for failures of one component. Only a generalized description of the initial innate immune response to an opportunistic pathogen in cases of metritis or mastitis will be provided here because those are two of the most prominent diseases affecting early lactation. A healthy mammary or uterine environment will normally have very little to no microbial load, but the process of parturition and the onset of lactation exposes each respective organ to microbes that cause infections of the mammary gland, leading to mastitis, or uterus, leading to metritis.

The first line of defense against microbes is the epithelial barrier. The epithelial barrier consists of epithelial cells knit together by tight junction proteins. Many epithelial barriers, like those of the intestines, lungs, and uterus produce mucus that includes antimicrobial factors. Epithelial barriers also include intraepithelial immune cells that, along with epithelial cells, have innate pattern recognition receptors (i.e., toll-like receptors) to survey the environment for microbial associated molecular patterns (i.e., lipopolysaccharide). Activation of the pattern recognition receptors triggers a cascade of signaling events that includes production of antimicrobial proteins, chemokines,

cytokines, and eicosanoids. These molecules represent the beginning of an inflammatory response that increases blood flow and recruitment of immune cells to the site of infection.

In the context of intramammary and intrauterine infections, the chemokines attract other immune cells like monocytes and neutrophils. Cytokines, like interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) provide local cell-to-cell signaling that stimulates defense responses of immune cells. Monocytes and neutrophils are phagocytes that engulf microbes and, within intracellular compartments, oxidative enzymes produce reactive oxygen and nitrogen molecules that are lethal to microbes. Monocytes and neutrophils also produce copious amounts of antimicrobial proteins like defensins, cathelicidins, and proteases that are involved in elimination of the microbe. It is important to recognize that functional capacities of immune cells circulating in blood are somewhat diminished in the first few days postpartum. The cause for a change in immune cell functionality postpartum is likely multifactorial and not explicitly the reason for increased risk of postpartum disease.

The immune response involves an intricate system of checks and balances to eliminate infections while also protecting host tissues. A potent proinflammatory response is necessary to defend against infection. The inability of initial defenses to contain infectious microbes will require an increasingly greater pro-inflammatory response to prime the whole body for defense against the infection. Hallmark indicators of a systemic defense response include elevated body temperature and concentrations of haptoglobin, serum amyloid A, and leukocytes in blood. However, the highly oxidative environment created to eliminate an infection can cause tissue damage and necrosis. Therefore, antioxidant and anti-inflammatory mediators are necessary for effective resolution of infection. Nutrients like vitamin E and omega-3 fatty acids that have antioxidant properties, and selenium that is required for enzymatic reduction, play a role in maintaining a balanced immune response.

Vitamin D and Immunity

Vitamin D is best known for its role in skeletal, Ca and P homeostasis, however, it is involved in many biological functions, including immunity. Vitamin D refers to a class of seco-steroid molecules that largely function through an intracellular protein receptor called the vitamin D receptor. An overview of the vitamin D pathway and some outcomes of endocrine and intracrine vitamin D signaling are depicted in Figure 1. Vitamin D_3 is naturally produced from 7-dehydrocholesterol in animals through a process of photoconversion in skin exposed to UVB light. Vitamin D_3 serves as the precursor to the 25-hydroxyvitamin D_3 [25(OH)D_3] metabolite that is the major form of vitamin D circulating in blood. The 25(OH)D has a half-life of approximately two weeks, its concentration in plasma serves as the best indicator of vitamin D status.

The 1,25-dihyroxyvitamin D_3 [1,25$(OH)_2D_3$] metabolite is the biologically active metabolite and its concentration in plasma is typically 20 to 50 pg/mL in lactating and dry cows, and upwards of 100 to 300 pg/mL in 2 to 3 DIM. Plasma 1,25$(OH)_2D_3$ concentrations do not correspond to vitamin D intake (Poindexter et al., 2020). The main

points of control in the vitamin D metabolic pathway center around regulating the concentration of $1,25(OH)_2D_3$ via the activities of 1α-hydroxylase (activation) and 24-hydroxylase (inactivation) enzymes. Most 1α- hydroxylase activity occurs in the kidneys under strict hormonal control, but a small fraction also occurs in adipocytes, immune cells, mammary epithelial cells, and reproductive tissues under control of various signal processes. The $1,25(OH)_2D_3$ induces its own catabolism by upregulation of 24-hydroxylase in a classical feed-back manner. The 24-hydroxylase can be expressed in nearly every cell that has vitamin D receptors, which serves to control vitamin D activity at the cellular level. Collectively, the balance of 1α-hydroxylase and 24-hydroxylase activity serve to regulate vitamin D activity. Most biological activity of vitamin D occurs through intracellular vitamin D receptors (VDR). The DNA-binding domain of the VDR recognizes short, specific sequences of DNA referred to as vitamin D response elements. The elements are located promoter or enhancer segments upstream, downstream, and within vitamin D target genes.

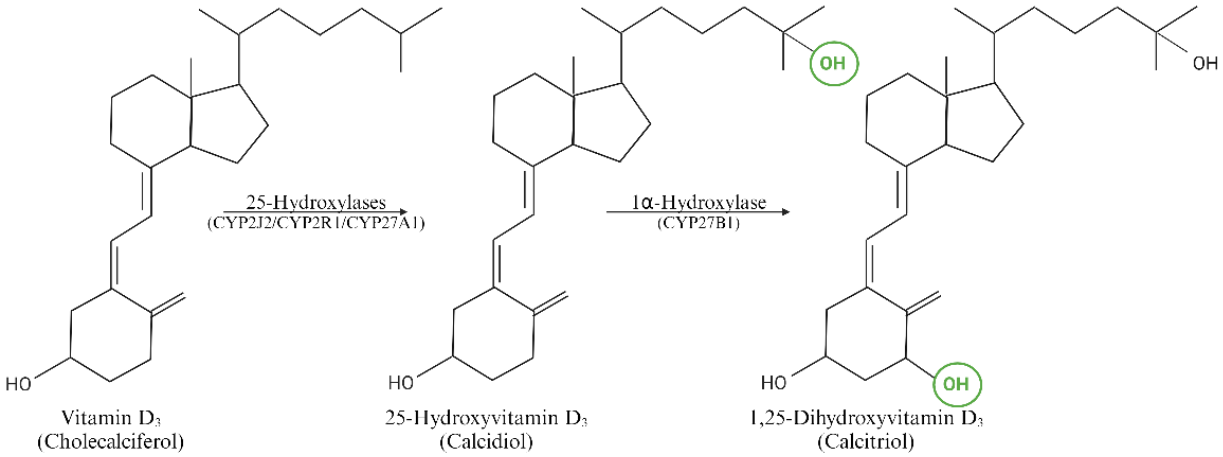

Figure 1. Oxidation of vitamin D_3 to 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3. Oxidation of vitamin D_3 to $25(OH)D_3$ is catalyzed by several hepatic 25-hydroxylases. Subsequent oxidation of $25(OH)D_3$ to 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$, calcitriol], the active metabolite, is catalyzed by the 25-hydroxyvitamin D_3 1α-hydroxylase. Not shown is the oxidation of $25(OH)D_3$ and $1,25(OH)_2D_3$ metabolites to 24,25-dihydroxyvitamin D_3 and 1,24,25-trihydroxyvitamin D_3, respectively, by the 25-hydroxyvitamin D-24-hydroxylase. The 24-hydroxyvitamin D metabolites are reported to have some biological activity, but are generally regarded as degradation products of vitamin D.

Vitamin D activity in immune cells was discovered nearly four decades ago, but it was not until the last two decades that the influence of vitamin D in immunity became appreciated. As with many nutrients that have a role in immunity, there was significant hype regarding vitamin D during the peak of COVID. Many claims regarding vitamin D and immunity may be exaggerated, but substantial evidence indicates it has significant influence in multiple immune processes. As depicted in Figure 2, toll-like receptor recognition of microbial associated molecules like LPS stimulate immune cell 1α-hydroxylase (a.k.a CYP27B1) activity that converts $25(OH)D_3$ to $1,25(OH)_2D_3$ (Nelson et

al., 2010). The upregulation of mRNA for 1α-hydroxylase has been observed in cultures of monocytes and neutrophils cows and in tissues and cells of experimental mammary infections (Merriman et al., 2018). The $1,25(OH)_2D_3$ contributes to antimicrobial, anti-inflammatory, and antioxidant processes of immune cells. For example, vitamin D signaling stimulates nitric oxide production that contributed to the control of *Mycobacterium bovis* growth in monocyte cultures (García-Barragán et al., 2018). Vitamin D signaling also stimulated expression of multiple β-defensins, thioredoxin, and metallothionein genes in monocytes of cows (Merriman et al., 2015 and Kweh et al., 2021). More broadly, vitamin D is known to promote anti-inflammatory processes, such as limit proliferation of pro-inflammatory T cells and promote function of regulatory T cells.

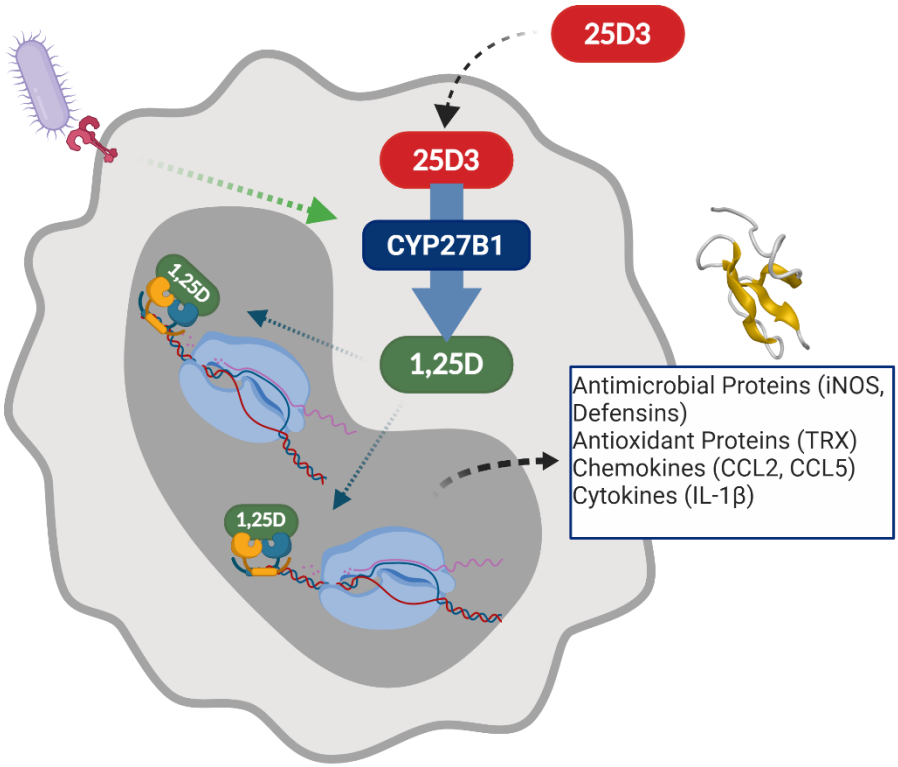

Figure 2. Vitamin D pathway of bovine macrophages. Innate pathogen recognition receptors stimulate expression of 1α-hydroxylase, also known as CYP27B1, and conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3.

Vitamin D Nutrition and Health and Production Outcomes

Recommendations for supplemental vitamin D_3 are largely based on Ca and P-related activity of vitamin D (NASEM, 2021). Most lactating cow diets provide cows 30,000 to 50,000 IU (0.75 to 1.25 mg) supplemental vitamin D_3 per day. Likewise, dry cow and closeup cow diets usually provide at least 20,000 to 30,000 IU supplemental vitamin D_3 per day. Serum 25(OH)D concentrations are typically between 40 and 100 ng/mL regardless of season, housing or geographical location (Nelson et al., 2016). There is scant epidemiological evidence relating vitamin D status to health outcomes, but a study

of 5 dairy herds in Michigan revealed that cows with serum 25(OH)D concentrations below 74 ng/mL were at greater risk of postpartum disease (Wisnieski et al, 2020). Vitamin D deficiency is not common in U.S. dairy cows, so the issue regarding vitamin D is whether increasing vitamin D status provides additional health benefits. At the same time, caution is required for supplemental vitamin D because of risk of vitamin D toxicity (Fraser 2021).

In theory, vitamin D signaling of immune cells should benefit from increased availability of 25(OH)D_3. Unlike the classical endocrine vitamin D pathway that centers on renal control of vitamin D metabolism, immune cell vitamin D activity seems to be rate limited by availability of 25(OH)D_3. There have yet to be reported experiments that provide convincing evidence that a lack of 25(OH)D_3 results in increased risk of infection of cattle. Holstein bull calves that had serum 25(OH)D_3 < 20 ng/mL had more severe responses to an intravenous LPS challenge compared with calves with serum 25(OH)D_3 concentrations between 30 to 40 ng/mL, but it is unknown whether this outcome translates to a difference in risk of infection (Blakely et al., 2023). On the other hand, increasing serum 25(OH)D concentrations above 100 ng/mL compared with the typical concentrations near 60 ng/mL has been shown to deliver better immune protection. Supplementing 25(OH)D_3 in dairy cow diets is an effective means to elevate serum 25(OH)D_3 and, consequently, availability of 25(OH)D_3 for immune cells. Supplemental 25(OH)D_3 is approved for ruminant diets in the U.S. Direct feeding of 25(OH)D_3 bypasses the initial hepatic oxidation (Figure 1) step making it much more effective at increasing serum 25(OH)D_3 compared with feeding vitamin D_3. For example, feeding 3 mg of 25(OH)D_3 to cows increased serum 25(OH)D_3 from 60 ng/mL to 200 ng/mL, whereas feeding 3 mg vitamin D_3 did not cause serum 25(OH)D_3 to increase above 100 ng/mL (Poindexter et al., 2023). In an experiment with lactating dairy cows, Poindexter et al. (2020) showed that feeding 1 or 3 mg of 25(OH)D_3 increased expression of inducible nitric oxide synthase and IL-1β genes in immune cells of milk compared with cows fed 1 or 3 mg of vitamin D_3. Expression of several immune genes also were correlated with serum 25(OH)D_3 concentrations. Feeding 3 mg of 25(OH)D_3 also decrease the severity of intramammary *Streptococcus uberis* infection compared with feeding 1 mg of vitamin D_3 (Poindexter et al., 2020).

The benefits of feeding 25(OH)D_3 are best realized during the transition period. Vieira-Neto et al. (2021) showed that feeding 25(OH)D_3 to closeup cows altered the expression of more than a dozen genes in blood leukocytes that related to immune cell trafficking, cell signaling, and antimicrobial activity. Martinez et al. (2018) also showed that feeding 25(OH)D_3 to closeup cows increased oxidative burst capacity of neutrophils from blood. Whether or not those effects of 25(OH)D_3 truly lead to better immune protection remain to be determined but feeding 25(OH)D_3 to closeup cows has consistently resulted in improved transition cow performance. Martinez et al. (2018), Silva et al. (2021), Poindexter et al. (2023), and Holub et al. (2023) have reported that feeding 3 mg 25(OH)D_3 to closeup cows resulted in 4 to 8 lbs. more milk compared with feeding vitamin D_3. A plausible explanation for increased milk production is the control of inflammation associated with metritis that is common in fresh cows. For example, Martinez et al. (2018) observed that feeding 25(OH)D_3 decreased risk of retained placenta and metritis. Likewise, *post hoc* analysis of data from Poindexter et al. (2023)

revealed the benefits of 25(OH)D_3 were most apparent in cows that were diagnosed with metritis and feeding 25(OH)D_3 decreased concentrations of haptoglobin in serum indicating better containment of inflammation.

Conclusion

Vitamin D signaling has an influential role in shaping the immune response and containing inflammation. Supplemental 25(OH)D_3 provides a more effective alternative to vitamin D_3 for dairy cows and has been reported to influence various immune processes in dairy cows. Important to the bottom line for dairy producers, feeding 25(OH)D_3 to closeup cows is a cost effective approach to increasing serum 25(OH)D of cows during the critical transition period that results in increased milk production.

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Optimal vs. Adequate: Trace Minerals in Transition Cows

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Introduction

Trace mineral nutrition plays a crucial role in the health, productivity, and reproductive performance of dairy cows, particularly during the transition period. This critical time, spanning from about 3 weeks pre-calving to 3 weeks post-calving, is characterized by significant physiological, metabolic, and nutritional changes. The transition period is the most crucial timeframe of the dairy cow lactation cycle, as the performance during this period contributes largely to a cow's ability to remain in the herd. It represents the period of the most dramatic and dynamic daily change in nutrient demands and partitioning to various physiological responses (Overton and Yasui, 2014). The process of calving and the initiation of lactation are associated with a degree of inflammation (Bradford et al., 2015), and oxidative stress. Most cows experience a period of negative energy balance in early lactation as milk production outpaces energy intake. Because of this, transition cows are at risk for various metabolic disorders, including milk fever, ketosis, and fatty liver (Goff and Horst, 1997). The periparturient period is also associated with a degree of immunosuppression, increasing susceptibility to infections.

Adequate trace mineral supplementation is particularly critical in this period to ensure strong performance from the cow, a healthy newborn calf, and success during subsequent lactation and breeding. Feeding more bioavailable sources of trace minerals has been a common solution to increase productivity and health during the transition and fresh periods. Several positive outcomes including increased milk yield, decreased somatic cell count, decreased lameness, and improved reproductive performance have been commonly reported in responses to feeding trace minerals in greater amounts and/or in more bioavailable forms (Overton and Yasui, 2014). This paper examines the current state of micronutrient fortification in transition dairy cows, exploring the optimal timing and levels of supplementation. By critically evaluating our current practices and considering new approaches, we can work towards optimizing trace mineral nutrition for transition dairy cows, balancing the need for adequacy with the risks of excess.

Where Are We Now? Current State of Trace Mineral Fortification in Transition Diets

The NASEM (2021) committee defined adequate intake as that which would meet a general population (say, a Holstein weighing 1500 lbs and producing 80 lbs milk/day) and which supplementing below this amount would decrease performance. But adequate may not mean optimal. The current approach to trace mineral supplementation in transition dairy cows often involves providing concentrations that

exceed the NASEM recommendations. Reasons for this approach include: 1) Nutritionists often include a safety margin above the established requirements to account for variations in feed quality, mineral bioavailability (including effects of water or dietary antagonists like sulfur, molybdenum, or iron), and individual animal needs; 2) Perceived benefits: A belief that higher levels of trace minerals can enhance immune function, reproductive performance, and milk production.; and 3) Lack of precise requirements: The exact requirements for many trace minerals in transition cows are not well-established, leading to a "more is better" approach. While these reasons are understandable, they have led to a situation where many transition dairy cows are receiving trace mineral levels well above their actual requirements. This over-supplementation not only increases feed costs but may also have negative impacts on the environment, and cow and calf health.

The following is a summary of a selection of literature related to trace minerals in transition cows.

Chromium Supplementation:

Supplemental Cr may aid primiparous cows during the transition period as there are heavy stressors of late gestation and early lactation (Mowatl and Subiyatnol, 1995). Smith et al. (2005) found administration of Cr-methionine (0.06 mg Cr/kg $BW^{0.75}$) during the periparturient period (21 d prior to anticipated calving and 28 d postpartum) increased DMI and milk yield during the postpartum period compared to a control group and a group supplemented 0.04 mg Cr/kg $BW^{0.75}$). Increases in milk yield could be accounted for in a variety of processes, such as increased gluconeogenesis, increased activity of the IGF receptor, and amino acid metabolism (McCarty, 1993; Subiyatno et al., 1996; Sano et al., 1997). Supplemental Cr did not have any negative effects on various reproductive parameters (Mowatl and Subiyatnol, 1995). At roughly 9 weeks prior to calving Villalobos et al. (2011) supplemented cows with 3.5 mg d^{-1} CrPic and found that in decreased the incidence of retained placenta. Organic Cr has the ability to reduce serum cortisol concentration in stressed calves (Mowat et al., 1993) and this could partially explain these effects. Lactating dairy cows consume an excess amount of concentrate and commonly experience insulin resistance. Leiva et al. (2015) found that Cr-propionate supplementation was effective in reducing insulin resistance in dairy cows, however it did not have an effect on milk production. Similarly, Cr-methionine supplementation (0.12 mg /kg $BW^{0.75}$) prepartum increased insulin sensitivity and improved glucose tolerance postpartum in a study completed by Hayirli et al. (2001). With recent decreases in Cr costs, this trace mineral may find a place in dry cow diets as well to best set up the cow for transition.

Zinc Supplementation:

Zinc plays a role in metabolic functions such as energy metabolism, nucleic acid metabolism, protein synthesis, epithelial tissue integrity, cell repair and division, and the transport, utilization and absorption of several vitamins. Zinc methionine has been shown to have a multitude of benefits for milk production and somatic cell counts in

dairy cows supplemented various levels of zinc (Kellogg et al., 2004). These authors associated increased milk production with improved udder health. As Zn plays a role in immune function, it is able to activate T-lymphocyte responsiveness, which is also important in the maintenance of health and integrity of epithelial tissues, such as the skin and teats, and mammary tissue. In that cumulation of studies, cows that were supplemented with Zn-methionine produced on average, 4.2% more total milk compared to cows that were supplemented with inorganic zinc sources. They also found that cows fed Zn-methionine produced more total kg in total milk fat. Kellogg also found that there was a 33.3% reduction in somatic cell count (SCC) when cattle were fed Zn-methionine, with the reduction enhanced when Zn-methionine was fed to provide greater than or equal to 360 mg Zn/cow/day.

Zn, Cu, and Mn Together:

Supplementation of Zn, Cu, and Mn in diets may be a way to increase the antioxidant capacity and decreased SCC. Maternal supplementation of Zn, Cu, and Mn lead to their offspring having a better total antioxidant capacity (Roshanzamir et al., 2020). This same study indicated that inorganic and organic sources provide the same opportunity at improving total antioxidant capacities. However, organic sources of Mn, Zn, and Cu have a better advantage at preparing calves with the proper blood immunoglobulins compared to sulfates. Similar to the previous study, Formigoni et al. (2011) found partial supplementation of organic sources of Zn, Cu, and Mn during the dry and lactation periods resulted in higher colostrum immunoglobulin levels. They also noted lower calf mortality at calving. Subcutaneous mineral supplementation (per 100 ml: sodium glycerophosphate 14g, monosodium phosphate 1g, copper chloride 0.4g, potassium chloride 0.6g, magnesium chloride 2.5g, and sodium selenite 0.24g) given approximately 20 days prior to calving, on calving day, and 20 days post-calving reduced SCC in milk in a study completed by Warken et al. (2018). They also noted a reduction in oxidative stress post-calving as well as improved animal health. In that same study, they also saw decreased ketones in the cows given the subcutaneous supplementation.

Selenium Supplementation:

Selenium is crucial for strong reproductive success as it plays a key role in ovarian tissues, follicles, oocytes and embryo development. Selenium readily crosses the placental barrier during gestation and can lead to increased Se concentrations in the calf. Van Emon et al. (2020) concluded additional supplementation may be necessary in the third trimester as the calf needs Se to support growth and development at the expense of the cow. Selenium is also crucial in maintaining a strong antioxidant capacity as it plays a role in eliminating free radicals such as hydrogen peroxide. In a study completed by Jaaf et al. (2020), they found supplementing pregnant primiparous dairy cows with low amounts of Se biofortified alfalfa hay increased Se in the blood and liver, leading to a greater antioxidant activity via glutathione peroxidase.

Vitamin A & β-carotene:

While this paper is focused on trace minerals, fat soluble vitamins support physiological roles that align with mineral function and are an important part of transition cow diets. Based on the amount of retinol needed during colostrum synthesis, NASEM (2021) states that vitamin A supplementation during the prefresh period should in increased to roughly 85,000 IU/day. Michal et al. (1994) concluded cows supplemented with β-carotene and vitamin A had decreased incidence of retained placenta and metritis, supporting the hypothesis that β-carotene enhances host defense mechanisms. Because vitamin A is necessary for maintenance of epithelial integrity, low concentrations of plasma retinol found in unsupplemented cows by Michal et al. (1994) may have been conductive to keratinization of placentomes, increasing the risk of retained placentas. Oldham et al. (1991) did not see any differences in udder health as measured by frequency of clinical mastitis, SCC or intramammary infection when 300 mg/d of supplemental β-carotene or varying ranges in supplemental vitamin A were provided. Cows supplemented with 300 mg/d of β-carotene during the first 10 weeks of lactation had lower SCC than cows fed an equivalent amount as vitamin A (Chew, 1993). Dahlquist et al. (1985) found cows who were fed the same amount of β-carotene (300 mg/d) along with 53,000 IU of vitamin A at dry off had lower rates of new intramammary infection compared to cows who were fed varying amounts of vitamin A (53,000 IU/d or 173,000 IU/d). Collectively, these findings highlight the complex interplay between β-carotene, vitamin A, and immune function, underscoring the importance of appropriate vitamin A supplementation in reducing the risk of postpartum disorders and supporting overall udder health in cows.

Vitamin E:

Supplementation of dietary vitamin E reduced clinical mastitis cases per quarter per lactation by 37%. Smith et al., (1984) concluded that there is evidence that deficiencies of vitamin E and Se can lead to an impaired resistance to disease, specifically in reduced duration of clinical mastitis. In contrast, Erskine et al. (1997) found administration of injectable vitamin E decreased the incidence of retained placenta and metritis but did not affect mastitis. The effects of vitamin E supplementation are largely dependent on the vitamin E tissue status at the time of dietary supplementation or parenteral administration, and interactions with other nutrients, particularly Se.

The Copper Conundrum:

Copper is essential for various physiological functions, including enzyme activities, iron metabolism, and antioxidant defense. The NASEM (2021) recommends ~11 mg Cu/kg DM for lactating dairy cows (using an average weight and milk yield). However, a survey of nutritionist-recommended trace mineral concentrations for lactating dairy cows found that the average Cu concentration in diets was 17 mg/kg DM, with a range of 12 to 20 mg/kg DM (Castillo et al., 2013). This concentration is

significantly higher than the NASEM recommendation. Part of this increase may be explained by fortification of Cu against common antagonists such as sulfur, molybdenum and iron, present in feed or water. Interestingly, while the beef cattle NASEM (2016) suggests one Cu recommendation for all stages of beef production (10 mg/kg DM), it notes in an earlier edition that the likely requirement is 4-6 mg Cu/kg DM, with 10 mg/kg DM addressing the likelihood of Cu antagonists in the diet. However, the 2021 Dairy NASEM committee includes Cu antagonists in consideration of the Cu recommendation more directly, with the user able to make changes based on antagonists present in their situation.

Copper is essential for lipolysis, or the mobilization of fatty acids from adipose stores. Given the negative energy balance of the transition cow, this is a critical metabolic function to contribute to her ability to produce milk, recover from calving and be ready for rebreeding. Lipolysis can occur under stimulation of hormone sensitive lipase, an enzyme activated downstream of cAMP activation. Copper inhibits phosphodiesterase 3B, a molecule which breaks down cAMP, thus preventing decreased cAMP signaling and supporting increased lipolysis (Krishnamoorthy et al., 2016). In cattle or rodents with very low liver Cu concentrations lipolysis is reduced, decreasing downstream effects of lipolysis (Engle, 2011; Messersmith et al., 2022). This, in combination with the roles for Cu in reproduction, neutrophil function, iron metabolism and more all means the transition cow certain needs to have adequate Cu status.

While ensuring adequate Cu status is crucial, excessive Cu supplementation can lead to problems. High dietary Cu can interfere with the absorption and metabolism of other minerals, particularly zinc and iron (Goff, 2018). Moreover, excessive Cu accumulation in the liver can potentially lead to toxicity, especially in breeds that are more susceptible to Cu accumulation, such as Jersey cows (Goff, 2018). The ruminant liver readily stores Cu, and as dietary Cu intake increases as DMI rapidly increases in the transition cow, excess Cu fortification may swiftly lead to high Cu concentrations. Work from Wisconsin suggests liver Cu concentrations over 450 mg/kg DM result in greater oxidative stress in dairy cows (Strickland et al., 2019).

Dry Cow Diet Fortification: Maximizing the Entire Dry Period

The dry period offers a crucial opportunity to optimize a cow's mineral status before the demands of lactation. Traditionally, trace mineral supplementation has focused on the close-up period (the final three weeks pre-calving). However, this short window may be insufficient to fully capture the benefits of trace mineral supplementation for both the cow and the calf. Many trace minerals are transferred from the cow to the fetus during late gestation, supporting fetal growth and the accumulation of critical minerals, such as Cu and Zn, in the fetal liver. These reserves help the calf after birth when trace mineral concentrations in milk are naturally low. Thus, trace mineral supplementation throughout the entire dry period, rather than just the close-up period, is essential to ensure the cow and calf have adequate mineral stores during this critical time (Van Saun, 2023).

Moreover, the concentration of trace minerals in colostrum is largely determined before calving. Supplementing organic trace minerals (Zn, Mn, Cu, and Co) during the dry period may increase the mineral content in colostrum compared to supplementation limited to the close-up period. Since trace minerals, particularly Zn, play a role in mammary gland development and milk production, a broader supplementation strategy throughout the dry period can also support mammary health and optimize milk yield (Nayeri et al., 2014).

Extending trace mineral supplementation beyond the prefresh diet to include the entire dry period is beneficial for several reasons. First, many trace minerals require time for absorption and incorporation into essential proteins and enzymes, making a longer supplementation window more effective. The prefresh period alone may not provide enough time for these processes to occur fully, especially with reduced dry matter intake in the days leading up to calving. Additionally, hormonal and metabolic changes during the close-up period can affect mineral metabolism, further limiting the impact of last-minute supplementation (Goff and Horst, 1997). For example, colostrum formation begins weeks before calving, meaning earlier mineral supplementation is necessary to influence its composition.

The last trimester of pregnancy is a time of rapid fetal growth and mineral accretion, making trace mineral supplementation across the entire dry period essential for supporting fetal development. Earlier supplementation helps ensure adequate mineral levels for the fetus, supporting optimal health at birth and into early life (Hostetler et al., 2003). Additionally, dietary changes in the prefresh period, particularly increases in starch to prepare the rumen for lactation diets, may interfere with mineral absorption due to changes in rumen pH (Goff, 2018). Therefore, focusing solely on the prefresh period for mineral supplementation is insufficient, and a more prolonged approach is necessary.

Finally, while supplementation throughout the dry period is beneficial, excessive mineral intake can be harmful. High maternal intake of Cu can lead to excessive fetal copper accumulation, predisposing calves to oxidative stress (Abuelo et al., 2019). Similarly, over-supplementation of Se can cause toxicity in calves. Excessive intake of one mineral can also interfere with the absorption of others, such as Zn inhibiting Cu absorption, or Fe affecting Zn and Cu metabolism (Goff, 2018). Over-supplementation is not only costly but also inefficient, as excess minerals are excreted in manure, potentially contributing to environmental pollution (Bolan et al., 2004). Therefore, targeted supplementation aimed at achieving optimal trace mineral levels, rather than excessive accumulation, is the most effective approach for maintaining cow and calf health.

In conclusion, focusing trace mineral supplementation across the entire dry period, rather than solely on the close-up diet, provides better support for both the cow's transition into lactation and the calf's early health. By optimizing trace mineral status over a longer timeframe, dairy producers can enhance colostrum quality, support fetal

development, and prevent the negative effects of over-supplementation, ensuring a healthier transition for both cow and calf.

Rebreeding and Health: The Role of Trace Minerals

Proper trace mineral status is crucial for successful rebreeding and overall health of dairy cows, particularly as they move into peak lactation (typically 60-90 days post-calving). Several trace minerals play key roles in reproductive processes. Copper, iodine, iron, manganese, selenium and zinc are all important for early embryonic development and maintenance of pregnancy (Hostetler et al., 2003). Zinc is involved in hormone production, including progesterone, and in the maintenance of uterine and placental tissues. Manganese is necessary for cholesterol synthesis, which is a precursor for steroid hormones (Curran, 1954). Selenium, as a component of selenoproteins, is involved in various aspects of reproduction, including protection against oxidative stress (Mehdi and Dufrasne, 2016).

To support these functions, it's crucial to maintain optimal trace mineral status through the transition period and into early lactation. However, this doesn't necessarily mean increasing supplementation levels during this time. Instead, the focus should be on ensuring consistent, adequate supply and maximizing absorption and utilization. Aim for consistent trace mineral supply throughout the transition period and early lactation, rather than sharp increases or decreases. In some cases, organic (chelated) forms of trace minerals may have higher bioavailability, particularly during times of stress or high production (Rabiee et al., 2010).

Ensure that other aspects of the diet (e.g., energy, protein, macro-minerals) are also optimized, as these can impact the utilization of trace minerals. Regularly monitor reproductive performance, health events, and production, and be prepared to adjust trace mineral strategies if needed. Consider individual cow factors (e.g., parity, production level, health history) when fine-tuning trace mineral strategies. By taking a holistic, balanced approach to trace mineral nutrition, we can support optimal health, production, and reproductive performance as cows move through the transition period and into peak lactation.

Maximizing Calf Transport Success

The health and vigor of newborn calves are crucial for successful calf transport and future productivity. Proper trace mineral nutrition of the dam during gestation plays a significant role in calf health. While factors like dehydration and energy status are likely the major contributors to a calf’s response to transit stress, trace minerals support myriad functions such as glucose metabolism and immune function that may help calves be more resilient to transit stress and shorten recovery time. Trace minerals are known to play a role in the immune system through antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, playing a large part in epithelial integrity, phagocytosis and bacteria killing, leukocyte migration, antibody secretion, cell mediated immunity, and cytokine production (Palomares, 2022).

Heiderscheit and Hansen (2022) noted feeding supplemental Zn to weaned beef steers improved DMI and ADG post trucking, corresponding to decreases in serum lactate (a marker of muscle fatigue) in Zn-fed animals vs. non-Zn supplemented controls. In a retrospective study done in France, Enjalbert et al. (2006) observed that the relationship between zinc status and the risk of infectious diseases was significant for diarrhea in calves. Selenium is another trace mineral known to play an important role in the immune system of cattle. An injection of selenium and vitamin E at birth could improve pre-weaning health by reducing rotavirus infection and diarrhea (Leslie et al., 2019). Supplementation of 3 mg Se and 4,000 IU vitamin E per calf daily improved phagocytic ability by 85% and killing *S. aureus* by 47% by neutrophils (Nunthiya et al., 2007). Selenium and Vitamin E play an important role in innate immune system defense mechanisms. In a recent review exploring various aspects of transportation stress in dairy calves, they concluded that there is a substantial knowledge gap surrounding the mitigation of transportation stress (Goetz et al., 2022). Adequate trace mineral status, particularly Se and vitamin E, can improve calf vigor at birth, which is crucial for successful adaptation to extrauterine life and ability to withstand the stress of transport (Lacetera et al., 1996).

Conclusion

Setting the cow up for a successful transition into lactation begins before the close-up period, expanding thoughtful trace mineral supplementation into the dry period. Strategic supplementation of trace minerals may support rebreeding, overall cow health and calf vigor, but the “more is better” approach requires caution. Copper over supplementation in particular may lead to oxidative stress, environmental concerns, and negative impacts on cow and calf health.

Moving forward, the goal should be to develop more precise, targeted approaches to trace mineral supplementation. This may involve more sensitive diagnostic tools to assess trace mineral status, along with the potential of precision feeding technologies to deliver customized trace mineral supplementation. By shifting our focus from simply providing more to providing better, we can optimize trace mineral nutrition for transition dairy cows, supporting their health and productivity while minimizing waste and potential negative impacts. This approach not only benefits the cows and calves but also contributes to more sustainable and economically viable dairy production systems.

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A Different Perspective on Metabolic Acidosis: Impacts on Glucose and Lipid Metabolism

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Introduction

Use of nutritional strategies to manipulate dietary-cation anion differences (**DCAD**) in dry cow diets has long been used to manage mineral balance and mitigate postpartum health disorders. This broadly used practice was first discovered when Scandinavian researchers fed low ash diets and observed a decrease in postpartum milk fever incidence (Ender et al., 1971; Dishington, 1975) which ultimately led to a cascade of research striving to understand the ions involved and how to formulate diets manipulating these ionic balances (reviewed previously; Erdman and Iwaniuk, 2017; Zimpel et al., 2018). The wealth of knowledge gained and strategies implemented over the decades on how to feed DCAD and the positive impacts on health and production observed has informed management practices. While typically successful, there are cases when DCAD strategies do not have the desired efficacy on farm which may reflect the nuances of cell and tissue physiology and the other peripheral impacts and interactions of manipulating anionic balance and pH.

The biological basis for DCAD diets is that they induce some extent of metabolic acidosis, as detected on farm by measuring urine pH. Within these strategies, urine pH targets can range from 6.0 to 6.8 for mild metabolic acidosis or <6.0 for moderate acidosis (Melendez and Chelikani, 2022). Inducing a mild state of metabolic acidosis has been demonstrated to increase the sensitivity of bone and kidney parathyroid hormone receptors, increase calcium reserve mobilization from bone, increase calcium reabsorption from kidney, increase calcium absorption from small intestine, and overall contribute to improved calcium homeostasis (Goff et al., 2004; Goff, 2018). These physiological changes have been demonstrated to improve calcium metabolism around the time of calving and are successful in reducing the incidence of calcium-related peripartum disorders. As with any intervention, there may be unintended impacts of using a DCAD approach to stimulate metabolic acidosis. In fact, based on cell and tissue physiology, it should be assumed that changing blood pH will have other implications on a broader set of physiological responses. The goal of this review is to explore potential interactions that are less often discussed but may be occurring with DCAD feeding strategies and if these interactions may explain any of the positive or negative indirect outcomes we see on farm.

Peripartum Ruminant Glucose Metabolism

Of the many pathways that metabolic acidosis could impact, one of particular interest and importance in the peripartum dairy cow would be the impacts on glucose metabolism. The ability of the dairy cow to have a coordinated metabolic response to shift from being a dry, pregnant cow to a lactating, non-pregnant cow depends heavily on adaptations within glucose metabolism pathways that allow for glucose sparing for the mammary gland. Although not the primary focus of this review, it is valuable to highlight the key aspects of glucose production in ruminant animals, and in-depth reviews of glucose production can be referenced for more details (Aschenbach et al., 2010). Given that the majority of dietary carbohydrates are fermented within the rumen, glucose is produced predominately through gluconeogenesis in the liver: the ruminant animal is responsible for generating 90% of its total glucose requirement which can equate to 10 pounds of glucose a day to support milk production in high-producing dairy cows. The precursors for gluconeogenesis in the dairy cow are predominately propionate (60 to 74%), lactate (16 to 26%), glycerol (0.5 to 3%), and glucogenic amino acids (alanine: 3 to 5%; other amino acids: 8 to 11%) as reviewed previously (Aschenbach et al., 2010). Given the necessity to generate nearly all glucose de novo by an energetically expensive process (about 10 ATP per glucose molecule), ruminants have a series of adaptations to prioritize glucose utilization. Some of these adaptations include 1) maintaining a blood glucose concentration around half of nonruminants, 2) the ability of several tissues to use other energy sources such as fatty acids and ketone bodies, 3) the absence of liver uptake of blood glucose, 4) less central nervous system glucose need compared with other species, 5) less use of glycogen by muscle, and 6) adipose tissue synthesis primarily from acetate rather than glucose. Altogether, these adaptations allow glucose to be spared for obligatory glucose-utilizing tissues (i.e., mammary gland, fetus, and the activated immune system).

During the postpartum period, the demand for glucose is further taxed by the onset of negative energy balance which often occurs when milk energy output exceeds dietary energy intake. In response to negative energy balance, the cow mobilizes stored body energy (Drackley, 1999; Grummer et al., 2004; Grummer, 2008; White, 2020). Lipolysis of triglycerides stored in adipose tissue results in the release of fatty acids and glycerol which can be used by the liver to generate energy and glucose, respectively (but not interchangeably). Mobilization of adipose stores is well documented during periods of feed restriction and around the time of calving. Central to the adaptations discussed above is that the peripartum cow can develop insulin resistance which prioritizes glucose for the obligatory glucose-utilizing tissues, which can cause atypical metabolic patterns. Typical insulin responsiveness patterns indicate that increased concentrations of insulin suppress adipose tissue lipolysis and hepatic glucose production; however, insulin resistance results in less sensitive or unresponsive tissues even though circulating concentrations of insulin are increasing. Thus, in cows exhibiting insulin resistance, it is possible for them to mobilize adipose tissue to a greater extent and may permit continued or elevated glucose production even in the presence of increased insulin-secretagogues, such as propionate.

Metabolic Acidosis and Insulin Sensitivity

Metabolic acidosis can be induced through dietary interventions directly (i.e., feeding anionic salts) but also as an indirect effect of other conditions such as ketosis which results in increased blood ketone bodies (e.g., ketoacids) that decrease blood pH. In humans, metabolic acidosis has been demonstrated to dampen insulin sensitivity, potentially through interfering with insulin binding to the insulin receptor or the subsequent signaling cascade that occurs after receptor binding (Cuthbert and Alberti, 1978). As an example, diabetic individuals with ketoacidosis are not as insulin responsive as those who are not in an acidotic state (Walker et al., 1963; Schade et al., 1981). This concept has garnered renewed interest with the prevalence of ketogenic diets as a nutritional intervention in people who may already have Type II diabetes, and thus insulin resistance. Recent epidemiological data supports previous findings that mild metabolic acidosis is associated with impaired insulin responsiveness, and additional research supported early indications that this impairment is through interference of cellular insulin translocation and subsequent signaling pathway (Guardia et al., 2018).

Insulin resistance is not a new concept to dairy cattle physiology. As early as 1978 there were reports of clinically ketotic or fasted (48-hr feed restriction) cows having impaired insulin responses to a glucose infusion (Hove, 1978). While urine and blood pH were not reported in that study, other work has indicated that there is a strong negative correlation demonstrated between urine and blood pH (Bateman et al., 2005); thus, it is expected that blood pH would be reduced during clinical ketosis. In 1996, it was identified that metabolic acidosis impaired insulin secretion and thus negatively impacted tissue uptake of glucose (Bigner et al., 1996). Within that study, researchers were trying to determine if neutralizing metabolic acidosis induced by ketosis by using sodium bicarbonate would improve cow responsiveness to intravenous administration of glucose as a ketosis treatment. Interestingly, that research team used DCAD, rather than clinically ketotic or feed restricted animals, to induce metabolic acidosis as a model of ketosis-induced metabolic acidosis and used a glucose tolerance test (**GTT**) to quantify the clearance of a glucose bolus. It was also noted that insulin response to a glucose load was partially recovered by treatment with sodium bicarbonate neutralization of the metabolic acidosis. Partial recovery of insulin sensitivity with bicarbonate neutralization has also been demonstrated in humans (Bellasi et al., 2016).

Impacts of metabolic acidosis on insulin sensitivity are likely dependent on the extent of acidosis induced. Mild acidosis that did not alter blood pH or other acidotic markers did not reduce insulin release or changes in insulin responsiveness as quantified by GTT (Grünberg et al., 2011). In contrast, the impact of two degrees of DCAD diet for two lengths of time prepartum was explored and it was determined that cows with moderate metabolic acidosis had reduced insulin sensitivity, potentially through both blighted insulin release from the pancreas and dampened response to insulin (Vieira-Neto et al., 2021).

While extreme insulin-resistance is negative in dairy cattle, it is innately part of the glucose-sparing mechanisms discussed above (De Koster and Opsomer, 2013; Qiao et al., 2024), and the optimal balance of insulin sensitivity, inflammation, and milk production is nuanced. As an example of this, ketotic cows often exhibit altered insulin sensitivity, increased peripheral tissue utilization of ketone bodies, and presumably acidosis (although blood and urine pH is rarely reported in ketosis research). Across the literature, there are examples of the negative impacts of sub-clinical ketosis timing and severity on milk production (McArt et al., 2012a), recovery of milk production in cows with propylene glycol treatment (McArt et al., 2012b), greater milk production in treated, sub-clinically ketotic cows vs. those without sub-clinical ketosis (Rathbun et al., 2017), and increased milk production with unknown treatment status in a large epidemiological study (Pralle et al., 2021). Within multiparous cows fed a DCAD strategy prepartum, the DCAD fed cows had greater milk production at the first two milk test days and tended to have greater ECM at the first test day (Serrenho et al., 2021). Increased milk production is not always observed with DCAD diets (Glosson et al., 2020; Caixeta and Omontese, 2021), which may reflect the additional nuance of negative impacts on DMI which are sometimes observed with metabolic acidosis (Zimpel et al., 2018).

Metabolic Acidosis and Lipid Metabolism

Another metabolic shift observed with negative DCAD strategies prepartum is a decrease in postpartum blood fatty acid concentrations (Grünberg et al., 2011; Zhang et al., 2022). Mobilization of adipose stores postpartum through lipolysis has been observed in varying extents and reflects energy balance and insulin responsiveness (Drackley, 1999; Grummer et al., 2004; Grummer, 2008; White, 2020). Regulation of lipolysis during periods of negative energy balance is through activity of lipases including hormone-sensitive lipase (**HSL**), adipose triglyceride lipase (**ATGL**), abhydrolase domain containing protein 5 (**ABHD5**), and perilipin (**PLIN**) (Koltes and Spurlock, 2011; Kendall et al., 2024). These lipases have been demonstrated to be dynamic across the periparturient period and in some cases differ by dietary or metabolic states. Their regulation is often through phosphorylation (abbreviated lipase name with a preceding P) and many are responsive to transcription factor regulation. In general, ATGL it is thought to be the rate-limiting lipase for lipolysis in other species; however, it was downregulated postpartum when blood fatty acids are increased (Koltes et al., 2017). Through that research, phosphorylation of HSL and PLIN were identified as potential regulators in dairy cattle (Koltes et al., 2017). Interestingly, those same patterns were not observed in more recent work that either fed a DCAD diet (De Koster et al., 2018) or not (Kendall et al., 2024), and both studies failed to identify a clear lipase pattern that corresponded to the increase blood fatty acid concentrations postpartum.

The regulation of adipose tissue lipases are of interest since they represent a potential point of intervention to modulate how much mobilization occurs. Unfortunately, there has been limited research on the regulation of these lipases with or without nutritional interventions. The ratio of $PHSL_{Ser660}$ to HSL was greater postpartum than prepartum, but there was no difference observed between dietary energy provided in a study examining the impacts of altering energy status in the presence of a DCAD

strategy (no manipulation of DCAD between treatments; Mann et al., 2015). Likewise, energy intake did not influence PHSL (at either Ser563 or Ser660) or HSL; however, they were changed across the periparturient period (Locher et al., 2011), similar to what was observed by Koltes and Spurlock (2011). Gestating Holstein cows were fed oscillating DCAD-level diets, and both HSL and $PHSL_{Ser660}$ were linearly increased when diets alternated, but no effect on the $PHSL_{Ser660}$:HSL ratio was observed (Vieira-Neto et al., 2021). In addition, the lipase abundances were also observed after intravenous GTT, and an interaction of duration and DCAD diet affected HSL abundance and the ratio of $PHSL_{Ser660}$:HSL, but not $PHSL_{Ser660}$ directly (Vieira-Neto et al., 2021).

Although the influence of diet, specifically DCAD strategy, on lipase abundance is unclear, there seems to be potential interactions between diet, insulin responsiveness, and lipolytic activity. The ability to reconcile these differences in conclusive results is further complicated by the differences in DMI across the treatments and the innate variance in DMI and negative energy balance during the peripartum period.

Conclusion

Utilization of DCAD interventions to manage metabolic health peripartum has numerous benefits, but it is very likely that there are impacts on physiology and metabolism that reach beyond mineral metabolism. Evidence exists that inducing metabolic acidosis influences glucose metabolism and insulin resistance which, at least if not over-exacerbated, may support the adaptive mechanisms associated with glucose sparing and permit maintained or increased milk production. Given the decreased circulating blood fatty acids postpartum, and the limited research on lipases with and without DCAD diets, there may also be reason to suspect interactions of dietary DCAD strategies on regulation of lipolysis postpartum. Herds or individual cows may respond differently to dietary DCAD strategies if they have different metabolic baselines (i.e., over-conditioned, insulin-resistant, higher or lower than average DMI, etc.).

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Timing and Extent of Skeletal Muscle Depletion and Accretion

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Introduction

Skeletal muscle is a dynamic tissue, functioning as a pool of amino acids that dairy cattle accrete and deplete throughout their production cycle. To make up for negative energy and protein balances associated with the onset of lactation, dairy cattle extensively mobilize body tissues, including adipose, and muscle, to acquire the necessary nutrients required for maintenance, milk production, and growth (Bauman and Currie, 1980; Bell et al., 2000). Skeletal muscle depletion is a physiological adaptation to the onset of lactation, through increased protein catabolism and reduced protein anabolism (Bauman, 2000). Increased proteolysis contributes amino acids to the developing fetus in late gestation and supports colostrum and milk production in early lactation. The increase in proteolysis is a normal homeorhetic response to animals adjusting to a new normal where their demands have changed without a sufficient increase in intake to support the increased requirements. There is considerable variation in the extent and timing of muscle depletion between cows (van der Drift, 2012; McCabe et al., 2021; Gouveia et al., 2024). Additionally, when and to what extent net muscle accretion occurs is not well documented in dairy cattle.

Skeletal Muscle in Dairy Cattle

Dairy cattle lose an estimated -3 to 17% of their bodyweight from one to five weeks postpartum (Zachut and Moallem, 2017). A dairy cow that weighs 700 kg postpartum may lose an excess of 100 kg through peak milk production. Yet there is considerable variation in bodyweight changes in early lactation, with some cows increasing in bodyweight as they approach peak milk yield. Animals that gained weight postpartum had improved conception rates; however, they had reduced milk yields through the first 30 days of lactation (Zachut and Moallem, 2017). This variation in bodyweight loss exists for animals consuming the same diet due to energy balance differences, as intake and milk yield varies between cows. In individual cows there are marked differences in efficiency of metabolic pathways that allow two cows consuming the same dry matter and producing the same milk output to have differences in bodyweight change. Cows have an estimated 90 – 125 kg of empty body protein with a larger range in adipose tissue, from less than 100 to greater than 200 kg (Komaragiri and Erdman, 1997). Changes in bodyweight are related to changes in both muscle and adipose tissue. Subcutaneous fat is highly correlated to body condition score (Wright and Russel, 1984) and highly correlated to whole body fat (Schröder and Staufenbiel, 2006). Whereas, muscle depth is only moderately correlated to body condition score (Sloniewski et al., 2004; Siachos et al., 2021). Because of this, muscle depth is more difficult to assess with visual observations compared to adipose tissue reserves.

Skeletal muscle makes up the largest internal organ in dairy cattle and plays a critical role in maintaining metabolic homeostasis (Sadri et al., 2023). Skeletal muscle has a role in posture, movement, and protection with a baseline amount of muscle required for an animal to carry out normal bodily functions. Amino acids are stored in skeletal muscle which can be drawn on during times when requirements exceed provided metabolizable protein. Amino acids are used for a variety of purposes that is dependent on stage of lactation. Skeletal muscle serves as both an input and output for the fate of amino acids (Figure 1). In early lactation, it is speculated that specific amino acids, with an emphasis on alanine and glutamine, derived from skeletal muscle are utilized by the liver to produce glucose (Overton, 1998; Drackley et al., 2001). However, in early lactation it appears that amino acids available to the cow, may be preferentially allocated towards milk protein synthesis (Larsen et al., 2014; Larsen et al., 2015). Compared to other tissues, skeletal muscle has the greatest insulin-stimulated glucose uptake and thus helps to maintain insulin sensitivity for the cow (Mohammadabadi et al., 2021). Skeletal muscle plays a key role in glucose metabolism, especially for transition cows, where circulating insulin concentrations are low and cows are insulin resistant.

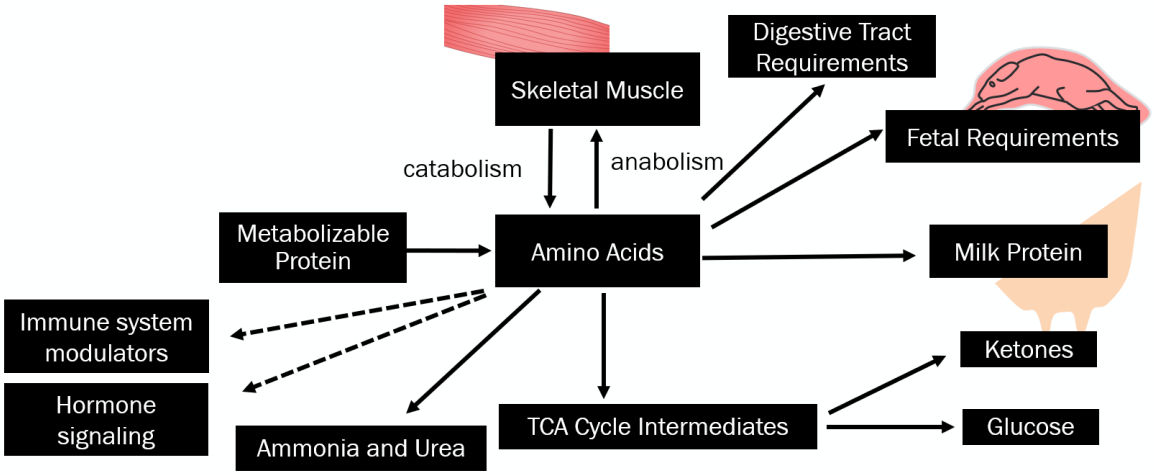

Figure 1. The fates of amino acids in the dairy cow.

Muscle Composition in Dairy Cattle

Skeletal muscle is a heterogeneous tissue comprised of individual multinucleated contractile cells, or myofibers. Bovine skeletal muscle contains three major muscle fiber types (I, IIA, and IIX) that are named according to the specific contractile myosin heavy chain protein present (Hoh, 2023). Type I muscle fibers are fatigue resistant, slow-twitch muscle fibers that have greater oxidative enzymes, myoglobin, and mitochondria present. Type IIX muscle fibers are fast-twitch and rely heavily on glycolytic metabolism. Finally, type IIA is intermediate between type I and type IIX with both an oxidative and glycolytic capacity (Schiaffino and Reggiani, 1994). Muscle fibers exhibit a high degree of plasticity and can undergo phenotypic shifts from type I ↔ IIA ↔ IIX (Cicilot et al., 2013). As a result of this plasticity, in addition to the primary types, hybrid muscle fiber types exist (I/IIA and IIA/IIX), which may be increased in times of stress. The composition of muscle

fibers varies based on the physiological demands of the skeletal muscle groups with the proportion of muscle fiber types changing depending on location. For example, the *semitendinosus* (eye of round) has the greatest frequency of type II muscle fibers and the *psoas major* (tenderloin) has the greatest frequency of type I muscle fibers (Lang et al., 2020). Therefore, the location of a muscle sample has a significant impact on the composition of muscle fibers present. Because of the different characteristics of muscle fibers, there may be preferential degradation of specific muscle fibers and therefore preferential preservation of others. This is not currently understood and is an active research area.

Muscle Depletion

The regulation of muscle catabolism occurs through three interconnected systems: autophagy-lysosomal, calpain calcium-dependent cysteine proteases, and the ubiquitin-proteasomal system (Sadri et al., 2023). These systems are integrated and carefully regulated to maintain protein homeostasis, with each system having specific roles in muscle catabolism. External endocrine inputs like insulin, growth hormone, IGF-1, and glucocorticoids can act to increase muscle catabolism. Changes in skeletal muscle are pronounced in early lactation because muscle has a diminished response to insulin. Additionally, elevated concentrations of glucocorticoids and glucagon may have a catabolic effect on muscle, resulting in increased muscle mobilization (Sadri et al., 2023). Skeletal muscle is undergoing constant turnover as protein catabolism and anabolism occur simultaneously. During the periparturient period, there is an upregulation in proteolytic pathway components that occurs in conjunction with reductions in protein synthesis (Ghaffari et al., 2019).

Muscle Accretion

Skeletal muscle growth occurs through protein accretion, myonuclear accretion, and accumulation of extracellular matrix (Allen et al., 1979). Muscle accretion occurs when the rate of protein synthesis exceeds the rate of protein breakdown (Goll et al., 2008). Skeletal muscle can regenerate itself in response to growth by activation of stem or satellite cells (Brack and Rando, 2012; Relaix et al., 2021). The number of satellite cells, and therefore capacity to regenerate muscle is determined in the late fetal stage (Brack and Rando, 2012). Generally, the number of muscle fibers remain constant from birth with changes in muscle size being the result of changes in muscle fiber area, or hypertrophy of existing muscle fibers, not a change in muscle fiber number. Satellite cells in adult animals remain dormant until acted on by a stimulus, such as exercise or injury. These activated satellite cells can repair or regenerate muscle. The major anabolic pathway that regulates protein synthesis is thought to be the mTOR signaling pathway (McCarthy and Esser, 2010). Protein synthesis is responsive to several signals including growth factors, hormones, cytokines, oxidative and metabolic stress, and nutrient availability (McCarthy and Esser, 2010). Interplay between these signals exists, as nutrient availability of the animal alters growth factors, thus impacting skeletal muscle growth (Thornton, 2019). Therefore, there may be stages of lactation when skeletal

muscle growth can be greater impacted by nutrition because of what other signals are occurring simultaneously.

Assessment of Tissue Reserves and Mobilization

The main tissues depleted in early lactation are adipose and protein, with protein coming primarily from skeletal muscle tissue. In a protein restricted diet that was fed to achieve an N balance of 0, Botts et al. (1979) estimated that the labile supply of protein that is able to be mobilized is approximately 27% of empty body protein. In diets that are supplying more dietary protein, lower amounts of protein mobilized from calving through early lactation were reported, with approximately 20% of empty body protein mobilized (Komaragiri and Erdman, 1997; van der Drift et al., 2012). For the transition dairy cow, depending on body condition score, adipose tissue may contribute to over 25% of the empty bodyweight of the cow, with protein representing approximately 13% of empty bodyweight (Komaragiri and Erdman, 1997). Metabolic adaptation to lactation may result in mobilization of an excess of 80 kg of adipose tissue and 20 kg of protein. Compared to most other livestock species, dairy cattle skeletal muscle estimates, especially for healthy, productive, lactating cows, are not well documented. Accurate values of whole-body skeletal muscle can be determined through slaughter studies of animals across stages of lactation to determine body composition and specifically muscle changes across lactation. This is an exceptional resource commitment, and therefore alternative approaches to approximating skeletal muscle have been established.

Metabolites related to skeletal muscle metabolism are measured in dairy cows to estimate the amount of muscle mass and proteolysis that is occurring. Muscle mass can be estimated from creatinine, the waste product produced at a constant rate by muscle from the breakdown of creatine and phosphocreatine (Wyss and Kaddurah-Daouk, 2000). Creatinine is increased during periods of time when an animal has more muscle mass (i.e. prepartum) and reduced after skeletal muscle is extensively mobilized (i.e. post-peak milk production). Megahed et al. (2019), indicated that plasma concentrations of creatinine were decreased 28 days postpartum compared to 3 days prepartum, indicating muscle loss in the first month of lactation. Plasma 3-methylhistidine (3-MH) concentrations are also used to assess muscle degradation, as 3-MH is an amino acid present in actin and myosin that is primarily located in skeletal muscle. When muscle is degraded, 3-MH is released and not able to be reincorporated into muscle and therefore can be used as a marker for muscle degradation. As muscle is constantly degraded and accreted, there will always been 3-MH present; however, relative differences across time or between treatments can be used to assess protein degradation. To standardize across animals, 3-MH to creatinine ratio indicates relative differences in protein mobilization per unit of muscle mass; the higher the ratio the more protein being degraded.

Extent of Muscle Mobilization Using Ultrasounds

In beef cattle, ultrasound measurement of the *longissimus dorsi* muscle depth is highly correlated to whole body protein (Greiner et al., 2003). Ultrasounds in dairy cattle of the *longissimus dorsi* and *gluteus medius* have been used to assess the amount of muscle

depth at specific locations with extrapolation to whole body protein (van der Drift et al., 2012; Megahed et al., 2019). Through ultrasonography, individual animals during the transition period have mobilized over 40% of their *longissimus dorsi* muscle depth (McCabe et al., 2021). Representative images of longissimus dorsi depth from 6 weeks prepartum to 4 weeks postpartum are shown in Figure 2, showing a 33% reduction in *longissimus dorsi* depth. Using these ultrasonography techniques, it is observed that muscle mobilization begins prior to calving, to provide amino acids to the fetus and for colostrogenesis, with muscle depth stabilizing by approximately one month postpartum (van der Drift et al., 2012; McCabe et al., 2021). It is important to note that skeletal muscle depth estimates also contain intramuscular fat and reductions of muscle depth are a combination of reduced muscle fiber size as well as intramuscular fat loss.

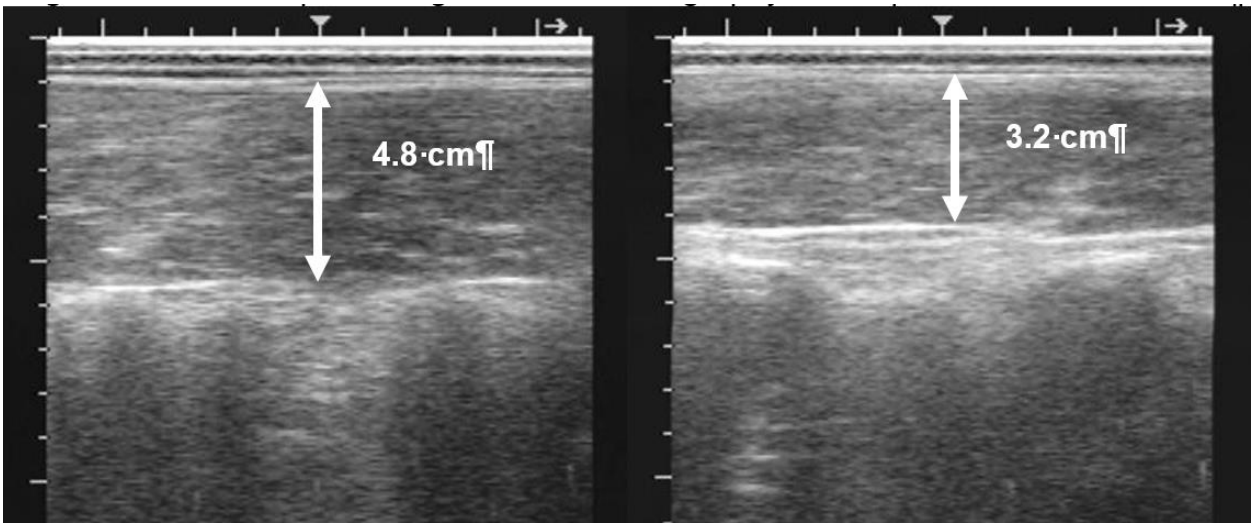

Figure 2. Left image is captured from a cow six weeks before parturition, right image is captured from a cow four weeks postpartum. White arrows indicate longissimus dorsi muscle depth and the values are listed to the right of the arrow in cm.

In a series of research trials conducted at Purdue University, ultrasound images of the *longissimus dorsi* were collected starting 5-6 weeks before expected calving until 8 weeks in lactation (McCabe et al., 2021; Gouveia, et al., 2024; Hanno, 2024; Figure 3). Dairy cattle had considerable variation in muscle depth when measured during the dry period, ranging from 2 cm to 6.5 cm. Dairy cows with greater muscle reserves prepartum, mobilized a greater amount of their *longissimus dorsi* depth by 60 days in milk and subsequently produced greater milk yields in early and mid-lactation (Gouveia et al., 2024; Hanno, 2024). Alternatively, cows with less muscle reserves, can gain muscle from approximately one-month prepartum to two months postpartum. This is similar to what has been observed with adipose tissue, as cows with greater adipose reserves mobilize more of their adipose tissue in early lactation. van der Drift et al. (2012) indicated that *longissimus dorsi* depth was reduced prepartum and continued to be reduced into the first weeks of lactation, whereas adipose tissue reserves are drawn upon following parturition. Recently, we reported that cows with greater muscle reserves, during the far-off dry period, lose muscle prepartum and cows with less muscle reserves can gain muscle prior

to calving (McCabe et al., 2021; Gouveia et al., 2024). These cows with greater muscle reserves also give birth to heavier calves, likely due to increased amino acids available for fetal growth.

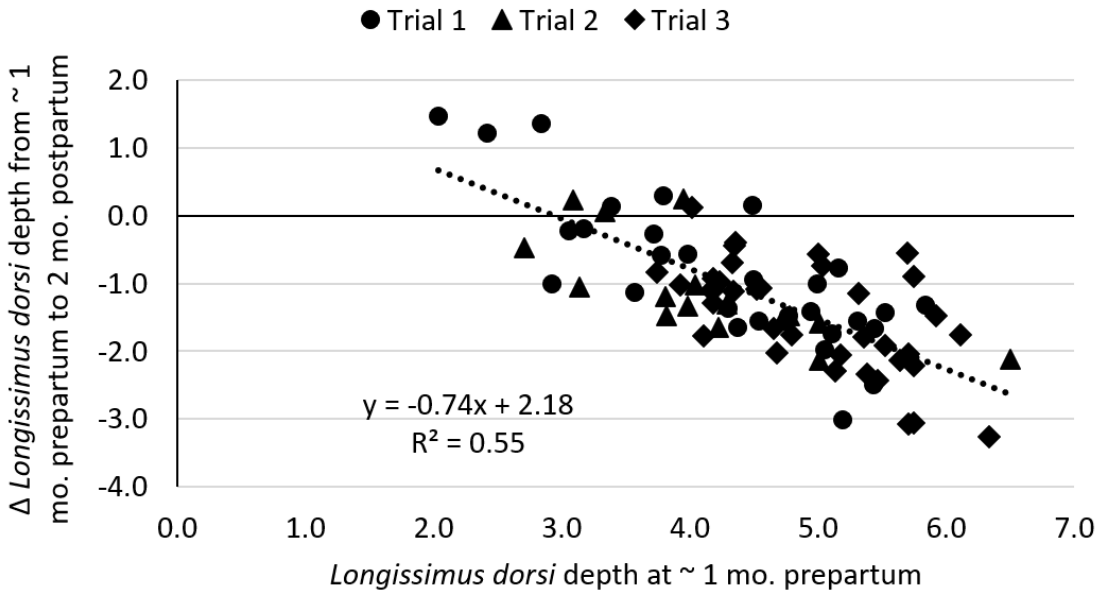

Figure 3. Longissimus dorsi muscle depth collected approximately one-month prepartum compared to change in muscle depth from approximately one-month prepartum to two months postpartum. Negative values on the y-axis indicate that the cows lost muscle depth assessed through ultrasound images, positive values on the y-axis indicate that cows gained muscle depth. Trial 1 and 2 are adapted from (McCabe et al., 2021) and Trial 3 is adapted from (Gouveia et al., 2024 and Hanno, 2024).

Ultrasound images from 40 multiparous cows were collected monthly from parturition to 300 days in milk (DIM) and of the timepoints measured, the greatest *longissimus dorsi* depth was observed at 0 DIM (Figure 4; adapted from Hanno, 2024). Cows then lost muscle depth until 60 DIM and did not appreciably gain muscle until 270 DIM. Cows on average mobilized 30-35% of their muscle reserves between parturition and 60 DIM. At 300 DIM, cows were not back to their muscle depth that they had at parturition. Interestingly, even when cows were post-peak and gaining body weight and adipose tissue (after 90 DIM) they did not appreciably gain muscle until much later in lactation. These findings indicate that as cows approach the dry period, they may have less muscle than they did at their last parturition.

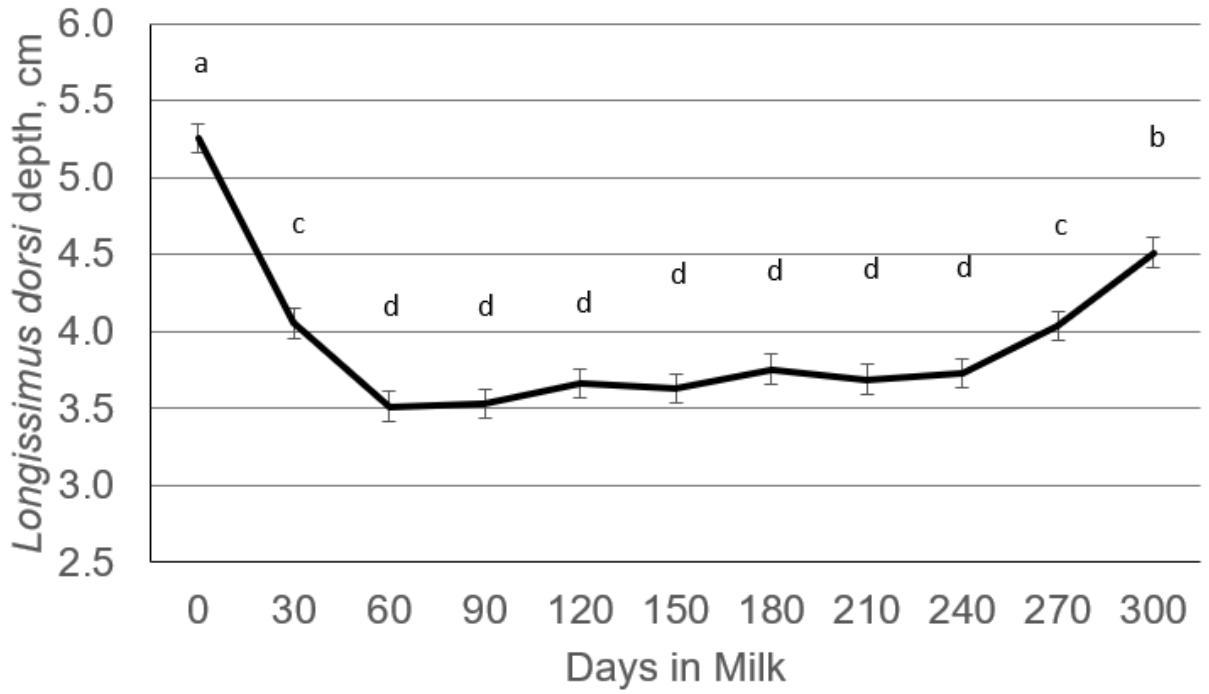

Figure 4. Longissimus dorsi depth from parturition to 300 days in milk. Adapted from Hanno, 2024.

Summary

There is considerable variation that exists between cows regarding skeletal muscle depletion. The extent of skeletal muscle depletion in early lactation is dependent on the amount of muscle present, as cows with greater reserves deplete more of their skeletal muscle reserves. The amino acids that are mobilized from skeletal muscle in late gestation are likely allocated to the fetus, resulting in increased calf birthweight. From recent work done in our laboratory, muscle depletion occurs in early lactation with appreciable accretion not occurring until late lactation. Further work is needed to understand how nutrition can impact skeletal muscle depletion and accretion across stages of lactation.

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Simulating Diverse Dairy Management Systems with the RuFaS Model

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Introduction

Dairy industry progress towards sustainable production practices requires methods for whole farm environmental footprinting to support both on-farm decision-making and inventory analyses for emissions tracking to inform corporate and governmental GHG reduction targets. One challenge in the effort to build methods and models that estimate farm footprints is the ability to represent the diverse management practices found in agricultural systems with enough specificity to capture the impact of changes in management or differences between farms. Quantification of GHG emissions from US dairy production epitomizes this challenge with a huge range in both animal and manure management practices that affect production, nutrient use efficiency, and GHG emissions. The Ruminant Farm Systems (RuFaS) model is a next-generation simulation model of dairy production being developed by a trans-disciplinary team of scientists and engineers to meet the needs for environmental footprinting of diverse dairy production systems. We have built the Animal and Manure Modules to flexibly represent the most common management options seen in US dairy. To evaluate the capacity of the RuFaS model to represent diverse dairy animal and manure management systems, we collected information on management practices to inform model inputs from 32 farms across the US and applied the RuFaS model to estimate the GHG emissions from animals (enteric CH_4), manure (CH_4, direct N_2O, and indirect N_2O), and feed production (CO_2-eq). Our sampled farms vary in geographic location, herd size, husbandry practices, and manure management strategies. We summarize the availability of input data and the translation of farm management practices into inputs that achieve the closest representation by the RuFaS modeling framework and the ways in which RuFaS v. 0.9 does and does not represent the management systems found in our sampled farms. We then explore the variability in RuFaS emissions estimates and illustrate alignment of model greenhouse gas (GHG) footprints with existing estimates of dairy farm GHG emissions.

Input Data Collection

RuFaS v. 0.9 requires over 280 user inputs, a library of feed compositions, a weather dataset that includes daily temperature, precipitation, and radiation, and a dataset that includes the embedded emissions associated with feeds. The RuFaS model receives the user input data via a series of JavaScript Object Notation (JSON) files and receives the feed composition, weather, and embedded feed emission data via a series of CSV files. To reduce the burden of collecting the user input data, we identified a subset of 43 minimum required inputs and established default values for the remaining inputs based on published literature and expert opinion. Further, because JSON files are not a

commonly used file type for most people collecting farm management data, we created a more user-friendly Data Collection Sheet in an excel worksheet that includes variable names and definitions, indicates which variables were minimum required inputs, provides the default values used for all optional inputs, and explains how to navigate the Data Collection Sheet. The structure and level of detail of the RuFaS model inputs reflect the fact that the model is being developed with multiple categories of model users and applications in mind. As such, there are instances where the direct model inputs are not reflective of data or management descriptions commonly used in commercial dairies. In preparation for application of the RuFaS model for commercial farm footprinting, the data requested of the farm was modified or translated from the RuFaS input variables to increase the likelihood that the request would be interpreted correctly, and that the information would be available.

Farm Enrollment and Data Collection Process

Our objective for farm enrollment in the model evaluation dataset was to include farms that represent the diversity of management practices and environments in the US dairy industry. Our goal was not to conduct an inventory of US dairy farms or dairy emissions and thus the farms are not a representative sample of management practices but rather the widest range of practices that would challenge the ability of the RuFaS model to represent each unique set of management and environmental conditions. To ensure fidelity and consistency in the data collection process, in collaboration with the FARM Environmental Stewardship program, we trained evaluators on the data requirements of the RuFaS model, provided each with a Data Collection Sheet, and provided technical support during the data collection process when needed. Evaluators collected farm management data reflecting management practices for 2022 over 4 months in 2023. In the following sections, we will summarize the methods we used to adapt RuFaS input requirements for collection of commercial farm data and instances where default values were provided to overcome data disparities.

Animal Feeding Data Collection and Translation

The diet recipes in RuFaS are either formulated through least-cost formulation (Li et al., 2021) or provided by the user for each of 4 animal categories (calves, growing heifers, dry and close-up cows, and lactating cows). The composition of the available feeds are provided by a feed library that was adapted from the NASEM Dairy Cattle Nutrient Requirements model (2021). The amount of feed required by the farm is tracked by pen and multiple pens of each animal category can be simulated; however, at this time, the RuFaS model can only accept 1 user-defined diet per animal category even if there are multiple simulated pens for that animal category.

In practice, animal feeding on dairy farms is a constantly evolving process that responds to fluctuations in feed availability, animal requirements and responses, and management goals. Although the feed delivery algorithms will make small modifications to the diet recipe in response to animal nutrient requirements and adjust the total amount of feed delivered based on the number of animals and their requirements, the diversity

and degree of fluctuation in the diets is less than what is expected on a commercial dairy. To account for this discrepancy, if a farm provided more than one diet for animals that RuFaS represents as a single category of animals (e.g. a high- and low-lactating cow diet), a weighted average of the diets was used to define the RuFaS diet inputs.

Another element of the feed data inputs that required some translation between farm data and RuFaS inputs are the feed types themselves. Dairy farmers feed numerous feeds that are not represented in the RuFaS feed library. Although the RuFaS feed library can be modified by changing the compositions of the existing feeds or by adding new feeds we did not provide that option during this evaluation study. If a feed was reported by a farm that was not available in the existing library, we selected the feed that most closely represented the reported feed.

Finally, while the case of averaging multiple diets illustrates an example where farms provided more feeding data that RuFaS could accept, lack of availability of feeding data was another common problem. To reduce the burden of collecting and reporting feeding data, we provided the option to use average regional diets for growing animals and dry cows (lactating cow diet information was required) and added 4 by-product mixes to the RuFaS feed library with compositions that represented regional average by-product inclusion rates. Thus, the resulting minimum required data inputs for feeding data was a diet for lactating cows that had the option to include a byproduct mix of feeds common for that region.

Herd Management Data Collection and Translation

The RuFaS Animal Module is a dynamic, process-based model that simulates individual animals on a daily time-step from birth or purchase through their exit from the herd (death or sale) (Hansen et al., 2021; Li et al., 2023). To facilitate flexibility and granularity in simulation of different animal classes, the Animal Module requires detailed inputs of the reproductive management and culling practices that are either not readily available or do not easily translate into farm management information. For commercial farm data collection purposes we translated and simplified 4 groups of RuFaS user inputs within the Animal Module to achieve the desired simulated outcomes. The 4 categories of user inputs are 1) milk production, 2) breeding programs, 3) heifer replacements, and 4) cow exits and are described in more detail below and summarized in Table 1.

Milk Production

The RuFaS model uses a simple model of milk production over time, the Wood's lactation curve, that uses 3 parameters to determine the shape and total milk production over time. These parameters are not information that most scientists have, let alone a commercial dairy farm. To overcome this barrier to application of the RuFaS model in commercial farm settings, we developed a method to estimate farm-specific lactation curve parameters using previously published parameter estimates (Li et al., 2022) and the farm-provided total annual milk production. Heuristically, we select the 2 parameters that define the shape of the curve (the rate of increase and rate of decrease) based on

the location and management practices from the published set of parameter estimates and then fit the scale parameter to farm-specific total milk production.

Table 1. Groups of user inputs required by the RuFaS Animal Module that were modified during data collection to align with farm data availability and granularity

Category of input	RuFaS Animal Module Inputs	Data and Information Collected from Farm
Milk Production	Estimates of the mean and standard deviation of the 3 parameters that define the Wood's lactation curve for parities 1, 2, and 3+	Annual fluid milk production
Breeding Program	Heifer and cow breeding start and end dates, general reproduction strategy, specific reproduction protocols, conception rates	Age or DIM at first service; average conception rates for each service for heifers and cows
Heifer Replacements	Male calf rate, keep female calf rate, still birth rate	Number of replacements raised on farm
Cow Exits	Cow do not breed day in lactation, milk production threshold for culling, non-production cull rates for each parity	Number of cows sold and died

Breeding Programs

In practice, dairy cow breeding programs are made up of a complex series of decisions that determine when the heifer or cow is eligible for breeding, how to know the best time to breed a cow including whether or not to manipulate her estrus cycle, and when to stop trying to breed an animal and sell her. To provide flexibility in representation of reproductive management practices, the RuFaS model requires inputs for the days in an animal's life cycle when she becomes eligible for breeding (age at first breeding and the voluntary waiting period), the general breeding strategy (estrus detection and timed artificial insemination), the specific synchronization and re-synchronization protocols (e.g. Ovsynch-56 and Ovsynch at diagnosis of an open cow), the day in the animal's life when breeding attempts are stopped (for both heifers and cows), and the insemination and conception rates for each breeding protocol. To reduce the complexity and number of inputs required to define the reproduction program for commercial farm footprinting, the FARM-ES evaluators were trained to ask for a description of the practices and average timing of the average timing for first service and protocol for open cows at pregnancy check for cows and heifers separately. From this information, we specified the breeding start dates, success rates, and protocols that aligned with the breeding schedules provided.

Heifer Replacements

RuFaS V0.9 has the option to specify different semen types (conventional, sexed, and beef) as well as expected rate of male calves produced from each type of semen. However, at this time, the model does not support use of multiple semen types in the same simulation which is not representative of what happens on a farm. To account for this gap in desired functionality, we modified the application of how the semen type and male calf rate of the semen type are used in coordination with the input that determines the percent of female calves that are kept and raised on farm to achieve the desired number of replacement heifers. To collect the information needed for defining these inputs from the study farms, we asked for the total number of replacements needed by the farm and the number or percent of those that were raised on the farm. If this information was not available, we assumed all replacements were raised on the farm and calculated the number required to achieve the herd turnover rate. Future versions of RuFaS will improve the representation of semen types so that more than one type of semen can be used in the same scenario/farm.

Cow Exits

RuFaS v0.9 has several mechanisms that determine when cows leave the herd that can be grouped into two categories. The first category of simulated cow exits are when cows do not conceive by the user-defined breeding cutoff date. When the simulated cow exceeds the breeding window cutoff without conceiving, she is tagged for removal from the herd but will proceed with her lactation until her production drops below a milk production threshold (also a user input). The second group of simulated cow exits are when cows leave the herd due to death or other health and disease incidents. These cow exits are determined by designated parity-specific probabilities for death and other diseases. We have found that these two separate mechanisms for cow removals present a challenge for translating the farm management practices to achieve the herd dynamics and demographics based on commonly available management data and strategies in part because the numbers of cows exiting the herd are not grouped into these two categories in practice and because the reasons cows do leave the herd are often complex and no standard reporting/recording metrics exist.

The default inputs for cow death and non-production removal rates are based on a literature report and can be used as a starting place for these values that are not typically available on farm. However, these rates should be adjusted if the combination of the number of cows leaving for production (failure to conceive) and non-production reasons do not match the farm's herd turnover rate calculated as the ratio of the number of cows sold to the adult herd size. Thus, the number of adult cows sold is the minimum required user input which can be used to adjust the default inputs for the parity specific rates of cow death and sale by parity, after taking into account the expected number of unsuccessful breedings.

Manure Management Data Collection and Translation

The Manure Module provides the option to define as many manure management systems as desired and includes a range of liquid and solid manure management options. The systems follow manure from collection through storage and are assigned to the animal pens with 1:N relationships – meaning each manure system can be assigned to many pens. While this provides a reasonable amount of flexibility, it does not account for settings where animals spend part of their time within a day or across the year in a different location and thus deposit their manure in more than one location. Thus, allocation of manure management systems to pens of animals in RuFaS also required some translation to most closely represent the amount of manure managed in each system. To do this, in addition to collecting information about the number and types of animals contributing to each manure management system, in cases where manure from the same animals contributes to multiple manure management systems, we also gathered information about those animals' time budgets and defined separate animal pens with animal numbers that reflected the percent of time or manure that should be allocated to each system. For example, if a farm had 100 cows in a compost-bedded pack barn for 4 months and on pasture for 8 months, then we would create two pens, one that is assigned a compost bedded pack manure management system and contains 33 cows and the other that would be assigned to a daily spread management system with 67 cows. Farms were required to specify at least one manure management system for their lactating herd.

Farm Characteristics and Input Data Availability

The resulting set of 32 farms included representation of farms from 16 states, 26 of which raised predominantly Holstein cows and 7 of which raised predominantly Jersey cows. The median adult herd size of the farms was 1,014 and ranged from 51 to 9,300 with a median Fat and Protein Corrected Milk (FPCM) production of 33.3 kg ranging from 19.8 kg to 47.7 kg. The herd management strategies included examples of low input practices like estrus detection, long breeding windows, low thresholds for dry-off, and minimal selection pressure that resulted in low herd turnover rates as well as herds with higher input systems that resulted in high turnover rates. Housing and manure management practices also varied widely and included farms in which all cows were housed in confined pens with liquid manure management and those using open-lot or compost-bedded back barns and relying almost exclusively on dry manure management systems.

As the information provided in the Data Collection Sheets was translated into RuFaS JSON input files, we recorded notes about instances where the provided data and information did not directly match or required interpretation to achieve inputs that matched the RuFaS required input. These notes were coded and summarized into 13 different types of input translations, modification, and data availability (Table 2). We found that the 3 most common needs to adapt the farm-provided information for input into RuFaS were in the selection of the appropriate feeds from the feed library, calculation of the non-production cull rates by parity from the total number of cow sales, and calculation of the male calf rate/ keep female calf rate to achieve the desired number of youngstock.

Another common gap between the available data and the model inputs were instances where the combination of the herd management and demographic data did not result in a viable herd (i.e. the reported number of youngstock or conception rates were too small to maintain the reported lactating herd). In addition, there were several farms that required application of pre-planned adaptations of the model to most closely represent their management system. These included 7 cases where manure management systems and animal pens were allocated according to the amount of the animal's time budget contributing to each manure system; 9 cases where the average regional diet for youngstock was used; 6 cases where the average regional diet for dry cows was used; and 5 cases where the farm raised heifers offsite so an average heifer raising management system for that region was assumed.

Table 2. Summary of farm input data availability and instances where management practices were not directly represented by the RuFaS model

Description of missing inputs or lack of representation within RuFaS	**Number of Farms that Required this input translation**
No direct match for feed in feed library	14
Non-production cull rates modified to match farm provided inputs	14
Calculated male calf rate/ keep female calf rate to achieve desired youngstock population	13
Updated death rates by lactation to match farm provided input	11
No youngstock diets provided; default regional diets used	9
Herd demographic inputs not provided or provided demographics are not representative of a realistic herd	8
Pens created to support allocation of manure management by time budget	7
Modified production cull management inputs to align with herd demographics	6
No dry cow diets provided; default regional diets used	6
Heifers raised offsite	5
No heifer reproduction management inputs provided	5
Translated farm manure separator(s) description to a single RuFaS separator option by updating separation efficiency	5
Did not provide manure management info for youngstock; used regional default or same as lactating cow	9

Emissions Estimates

The RuFaS model reports a wide range of results related to production and nutrient cycling in addition to GHG emissions. Here we focus on the GHG emissions outcomes as one of the most important use cases for the dairy industry. As a process-based model, RuFaS directly reports total emission losses from each pen, animal group, and manure management system on a daily basis. Records of animal bodyweights, sales, and

purchases enable allocation of emissions milk and meat. To summarize the daily, granular data reported by the RuFaS simulation model, we applied the newly developed data processing features in the larger RuFaS model to calculate more commonly used metrics in alignment with emissions reporting guidance and are reporting annual emission intensity using the biophysical allocation method recommended by the International Dairy Federation (2022).

In our testing dataset, estimates for GHG emissions intensity ranged from 0.831 to 1.91 kg CO_2-eq / kg FPCM with a median value of 1.18 kg CO_2-eq / kg FPCM. These estimates are in line with previously reported values for whole farm carbon footprints of US Dairy Farms (i.e. Capper and Cady, 2020; Rotz et al., 2021; Uddin et al., 2021; Olivo et al., 2024). In their 2021 analysis, Rotz et al. applied a process-based model to a set of archetypal farms designed to create a representative sample of US production in 2019 that resulted in estimates of GHG emissions intensity that ranged from 0.69 to 1.45 kg CO_2-eq / kg FPCM with a weighted average of 1.01 kg CO_2-eq / kg FPCM. Results are not directly comparable due to differences in model structure and assumptions, but it is worth noting that the intensity estimates produced by the RuFaS model encompass a wider range and a higher maximum estimate than those from the IFSM model reported by Rotz et al. (2021).

To further our understanding of the factors contributing to dairy emissions, boxplots of the sources of emissions are provided in Figures 1 and 2. As expected, on average, enteric emissions are the largest contribution to dairy production emissions intensity with an average contribution of 0.46 kg CO_2-eq/kg FPCM which represents 37.7% of the carbon footprint. The second largest source of emissions is feed production which contributed an average of 0.41 CO_2-eq/kg FPCM to the emissions intensity, or 33.4% of the footprint. Manure methane and nitrous oxide emissions combined produced an average of 0.35 kg CO_2-eq/kg FPCM and represented 29.0% of the footprint. The relative contribution of each emission source is similar to the 43% for enteric emissions, 26% for feed production, and 25% from manure emissions reported by Rotz et al. (2021).

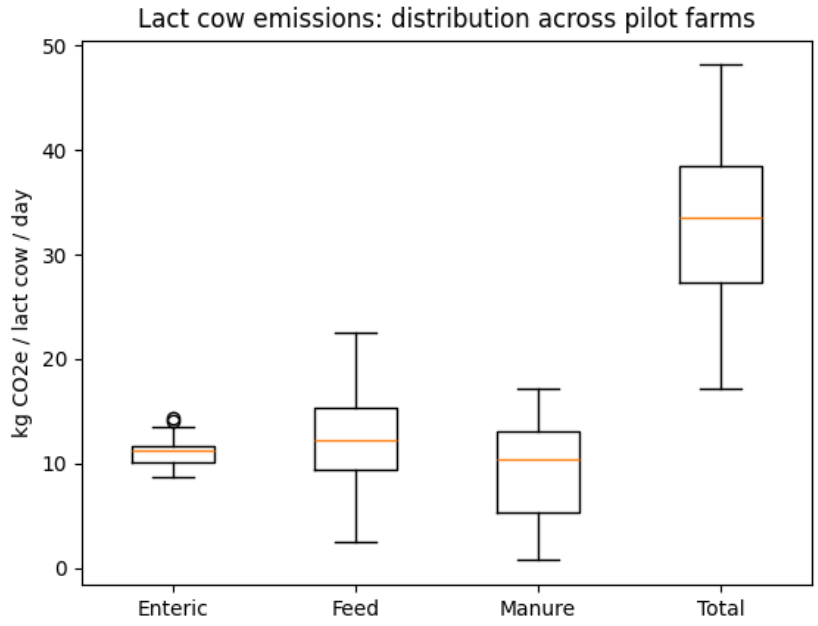

Figure 1. Distribution of whole farm greenhouse gas emissions estimates in units of CO_2-eq per lactating cow per day by emissions category (Enteric, Feed, and Manure), and the total emissions.

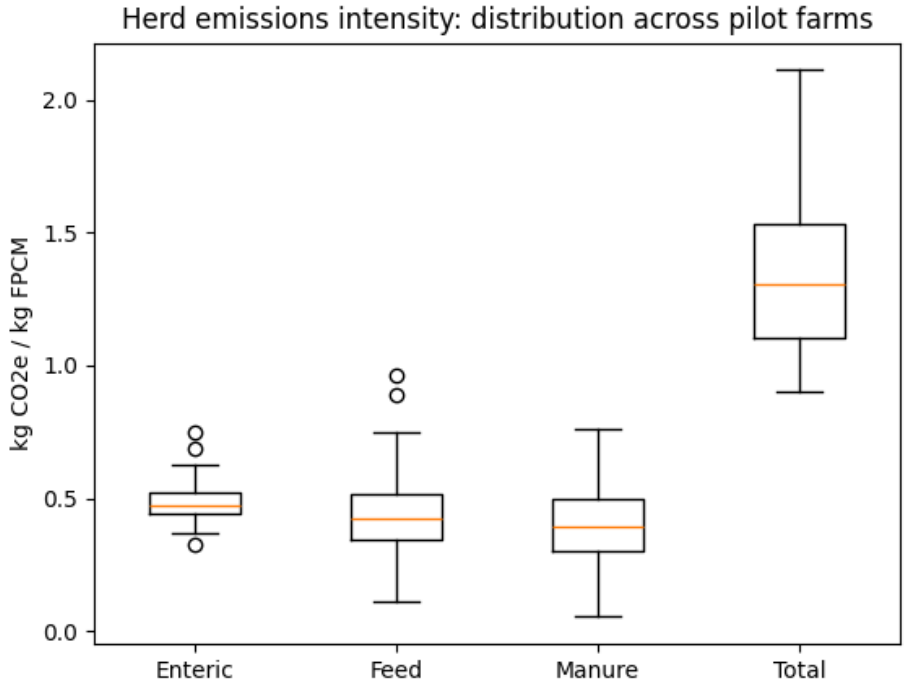

Figure 2. Distribution of whole farm greenhouse gas emissions intensity estimates in units of CO_2-eq per kg of FPCM by emissions category (Enteric, Feed, and Manure), and the total emissions.

Although, Rotz et al. (2021) did not report the distribution of emissions by source, the boxplots in Figure 1 illustrate that predicted emissions per unit of milk and per animal from both feed production and manure management have a larger range than those of enteric emissions. The contribution of emissions from feed production is also likely the most different between the two studies and can be attributed to the different values used to estimate the emissions associated with feed production. In our study, we used a spatially explicit library of emissions associated with feed production developed for the RuFaS model. This library is based on county-specific estimates for 7 of the most common dairy feeds provided by the FoodS^3 group (Pelton et al. 2024, Pelton et al. 2021, http://www.foodscubed.umn.edu/); regionally specific estimates for 17 common by-product feeds produced by an LCA conducted by LEIF, LLC and commissioned by NMPF; and national estimates from the IPCC 2021 report for all remaining feeds. The feed emissions intensities used by Rotz et al. (2021) reported in their Table 2 are often ½ to 1/3 of the median estimates in the database compiled for the RuFaS model. For example, the emissions intensities of corn grain and alfalfa hay used in the IFSM study are 0.37 and 0.18 kg CO_2-eq/kg DM while the median estimates from the FoodS^3 group for these important dairy feeds are 0.63 and 0.50 kg CO_2-eq/kg DM. Similarly, the estimates for byproduct feed emissions intensities produced by the recent LCA resulted in higher estimates than those reported by Rotz et al. (2021). The average estimates for emissions associated with corn gluten feed and dried distiller's grain production in our database were 0.66 and 1.21 kg CO_2-eq/kg DM, respectively which is substantially greater than the values of 0.30 and 0.60 kg CO_2-eq/kg DM used by Rotz et al. (2021) for the same feeds. The higher average values of emissions associated with feeds and the spatially explicit estimates for this source of emissions likely explain much of the higher, wider range of estimates seen in this study compared to that of Rotz et al. (2021). The aggregate CO_2-eq estimates from both the regional LCA and the county level estimates from the FoodS^3 group include both carbon sequestration and the emissions associated with land use change; the latter of which is likely a key factor contributing to the higher estimates of feed emissions. Future work in the RuFaS model will add methods to estimate the carbon sequestration and land use change emissions from purchased feeds separately.

Summary

The RuFaS model is developed for application in both research and industry decision support. To meet these dual objectives, some adaptation of model inputs is required. We found that methods to simplify input requirements for animal diets and available feeds, interpret breeding and herd demographics within the RuFaS dynamic animal life cycle module, and translation of manure management systems based on animal time budgets were required to represent the diverse set of management practices captured in the set of farms enrolled in this study. Simulation of these farms produced estimates for GHG emissions intensity that have a wider range of values than previously reported estimates for emissions associated with feed production and manure management in particular.

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Impacts of Yeast Culture (Yeasture Elite-4 Plex) on the Growth Performance and Intestinal Health of the Broiler Chickens

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Introduction

The United States is the world's largest poultry producer, with the combined value of broilers, eggs, and turkeys reaching $67.1 billion (USDA, 2024). The U.S. broiler industry is highly efficient and vertically integrated, increasingly providing customers nationwide and globally. In 2023, the U.S. broiler industry remained strong, producing approximately 9 million broilers valued at $42.5 billion (USDA, 2024). The high production of chicken is due to several advantages of broiler farming. Chicken meat is a nutritious and healthy source of animal protein with all nine dietary essential amino acids and is low in calories, sodium, and fat (Nyam et al., 2023). Additionally, broiler chickens are highly efficient at converting feed into meat under a relatively short growing period (Clune et al., 2017). This efficiency allows broiler production to meet the growing global demand for poultry more efficiently, which is essential for feeding the increasing population while maintaining the overall sustainability of food production. As demand rises and technology advances, the poultry industry is expected to continue its growth in the future.

Despite advancements in poultry production, intestinal health remains a significant challenge due to its complex role in nutrient digestion, absorption, and overall growth performance. A healthy gastrointestinal system is essential for optimizing nutrient utilization, maintaining immune function, and supporting the growth of poultry (Sun et al., 2021). However, research indicates that intestinal health issues are prevalent in broiler chickens, largely due to the high feed intake placing considerable stress on the gastrointestinal tract (Ahmad et al., 2022). Ensuring a well-functioning intestinal system involves balancing immune status, oxidative systems, intestinal morphology, and microbiota composition (Moita and Kim, 2022). One major health concern related to intestinal health is leaky gut syndrome, particularly in broiler chickens. This condition occurs when the connections between intestinal epithelial cells weaken, increasing intestinal permeability and allowing bacteria, pathogens, and toxins to pass through the intestinal lining into the bloodstream (De Meyer et al., 2019). Leaky gut syndrome in broilers is partly caused by the rapid turnover of intestinal epithelial cells, a process heavily influenced by nutrient absorption and environmental factors (Stewart et al., 2017). Under conditions such as heat stress or malnutrition, a slower turnover rate can lead to damage in the intestinal lining, exacerbating the severity of the condition (Ahmad et al., 2022). Intestinal inflammation further impairs the structure and tightness of the connection, reducing its ability to absorb nutrients and potentially leading to clinical conditions such as necrotic enteritis and diarrhea, or even death (Dal Pont et al., 2020). Addressing these intestinal challenges, including preventing and mitigating leaky gut syndrome, is critical

for maintaining production efficiency and ensuring the overall sustainability of poultry farming.

Yeast culture, especially *Saccharomyces cerevisiae* (SC), has been known as a probiotic that enhances growth performance and intestinal health in animals (Bidura et al., 2019). Multiple bioactive compounds can be found in SC, such as nucleotides, B vitamins, amino acids, and yeast cell walls like beta-glucan and mannan (Lin et al., 2023). Yeast nucleotides could boost immune function and repair gastrointestinal tract (Superchi et al., 2012), while oligosaccharides act as prebiotics to regulate immune responses (Fadl et al., 2020). The B vitamins and amino acids also contribute to the poultry intestine development (Gil-Rodríguez and Garcia-Gutierrez, 2021). Thus, SC could benefit chickens by improving tight junction protein expressions and gut morphology (Lin et al., 2023), enhancing immune function and antioxidant properties (Nelson et al., 2020), modulating gut microbial community (Wang et al., 2016), and increasing intestinal nutrient digestibility (Nari and Ghasemi, 2020).

Trace minerals such as zinc (Zn), manganese (Mn), copper (Cu), and cobalt (Co) play essential roles in energy metabolism, tissue growth, and nucleic acid synthesis (National Research Council, 1994; DeFrain et al., 2009; Star et al., 2012). Chelated forms of these minerals offer greater bioavailability than inorganic forms, enhancing embryonic and bone development, immune systems, and antioxidative systems (Wedekind et al., 1992; Salim et al., 2010; van den Brand et al., 2023). They have also been found to improve intestinal health by increasing villus height/crypt depth ratio (Seyfori et al., 2019) and enhancing intestinal digestive enzymes and barrier functions in poultry (Xie et al., 2021).

We hypothesized the supplementations of yeast culture Yeasture Elite-4 Plex could improve intestinal health and overall growth in broiler chickens. Besides, chelated minerals with amino acids are also widely used to enhance growth with better intestinal health. Comparing these two products in promoting intestinal health and investigating whether they modulate the same pathways would be intriguing. Therefore, the objectives were to explore the effects of Yeasture Elite 4-Plex and Zinpro Availa Sow on broiler growth, intestinal health, and gas emission and to investigate what metabolic pathways are involved and modified.

Yeasture and Zinpro did not show differences on growth performance

A total of 144 Cobb500 chicks from Cobb hatchery (Athens, GA, USA) were divided into four dietary treatments (6 cages/treatment, 6 birds/cage) and housed in a temperature-controlled facility at Cornell Poultry Research Building. The experimental diets were supplemented with Yeasture Elite 4-Plex (Cenzone Tech Inc., San Marcos, CA, USA) and Zinpro Availa Sow (Zinpro Corporation, Eden Prairie, MN, USA). The dietary treatments were: 1) a control corn-soybean meal diet (**Control**); 2) Control + 0.1% Yeasture Elite 4-Plex (**0.1% Yeasture**); 3) Control + 0.2% Yeasture (**0.2% Yeasture**); and 4) Control + 0.2% Zinpro Availa Sow (**0.2%**

Zinpro). The experimental diet design is provided in **Figure 1**. The starter (0-3 weeks) and grower (3-6 weeks) diets were formulated to meet the broiler's nutritional requirements (National Research Council, 1994). Birds were fed with the control diet for acclimation from day 0 to 6, and then switched to the experimental diets from day 6 to 42. Unrestricted access to feed and water was provided for birds over a 6-week period under a lighting schedule of 22 h light: 2 h dark.

2 nutrient supplements	Control	Yeasture	Yeasture	Zinpro
Yeasture Elite 4-Plex	Corn, SBM basal diet	0.1%	0.2%	
Zinpro Availa sow				0.2%

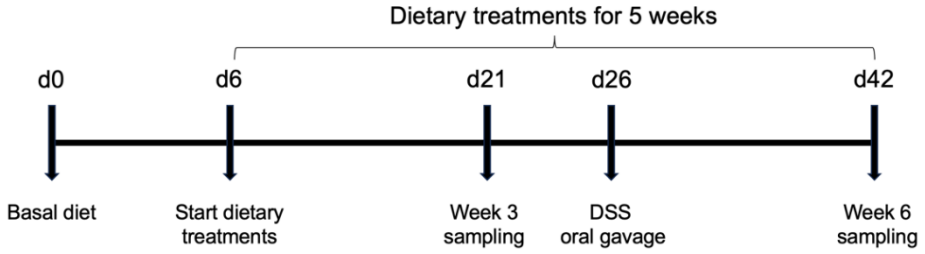

Figure 1. The schematics of dietary treatments and experiment timeline.

During the acclimation period from week 0 to 1, no differences in growth performance were observed across all treatments (**Table 1**). After transitioning to the experimental diets, there were no differences in body weight gain (BWG) and average daily feed intake (ADFI) from week 1 to 3. However, the 0.1% and 0.2% Yeasture treatment improved (P = 0.077) the feed conversion ratio (FCR) by 12.8% and 14.8%, respectively, compared with the control. From weeks 3 to 6 and overall from weeks 1 to 6, no treatments showed any significant differences in growth performance from the Control, though the 0.2% Zinpro showed lower (P = 0.069) BWG than the 0.1% Yeasture group. Overall, no significant differences in growth performance were found among Yeasture or Zinpro groups. The only exception was that the 0.1% and 0.2%Yeasture treatments improved FCR during Week 1 to 3, but this effect was not sustained over the 6-week period.

Yeasture and Zinpro improved the intestinal integrity and morphology

Intestinal tightness and morphology were also assessed by inducing stress in the broilers through oral gavage of dextran sulfate sodium (DSS). On day 25, two chicks/cage were administered 0.45 g DSS/100 g of body weight (Alfa Aesar, Ward Hill, MA, USA; MA: 40 kDa), followed by a second DSS dose 24 hours later (Menconi et al., 2015; **Figure 2A**) To evaluate the intestinal damage, fluorescein isothiocyanate dextran (FITC-d; Sigma Aldrich, St. Louis, MO, USA; MW: 3,000-5,000) was given orally at 8.32 mg/kg of body weight 2.5 hours after the second DSS dose. The chicks were then euthanized for blood and tissue collection 2.5 hours after the FITC-d administration (Sun et al., 2024). Following DSS-induced stress, the 0.1% and 0.2% Yeasture, as well as the 0.2% Zinpro

treatments, significantly decreased serum FITC-d levels compared with the control, indicating improved intestinal integrity under DSS-induced stress (**Figure 2B**).

Table 1. Effects of dietary treatments on growth performance of broiler chicks[1].

Treatment	Control	0.1% Yeasture	0.2% Yeasture	0.2% Zinpro	SEM	*P*-value
BWG[2], gram						
Week 0-1	15.8	15.4	16.6	16.5	0.33	0.542
Week 1-3	46.4	47.6	47.2	46.2	0.62	0.864
Week 3-6	111	114	111	105	1.41	0.115
Week 1-6	92.5^{ab}	95.3^{a}	93.0^{ab}	88.9^{b}	0.88	0.069
ADFI[2], gram						
Week 0-1	20.0	20.8	21.0	22.1	0.45	0.458
Week 1-3	69.2	62.1	59.8	63.5	1.68	0.235
Week 3-6	225	225	215	216	2.45	0.233
Week 1-6	168	165	158	160	1.76	0.173
FCR[2]						
Week 0-1	1.27	1.35	1.26	1.34	0.02	0.273
Week 1-3	1.49^{a}	1.30^{b}	1.27^{b}	1.37^{ab}	0.03	0.077
Week 3-6	2.04	1.98	1.94	2.06	0.03	0.387
Week 1-6	1.81	1.74	1.70	1.80	0.02	0.112

[1]Data are expressed as means (n = 6). Main effects were analyzed by one-way ANOVA and Duncan's multiple-range test. Means bearing the difference superscripts in a row differ significantly ($P < 0.05$).
[2]BWG: body weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio, as ADFI divided by BWG.

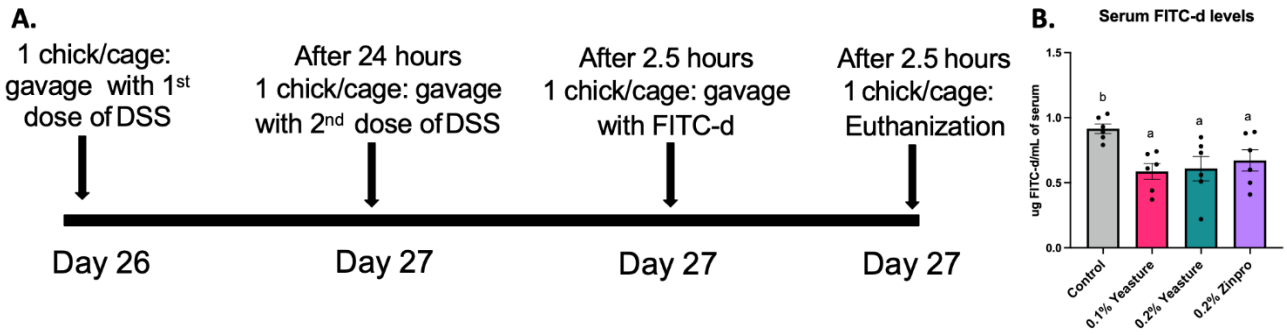

Figure 2. Schematics of oral gavage of dextran sulfate sodium and FITC-d (A) and serum FITC-d levels at week 4 (B).

Additionally, three intestinal segments were collected representatively and processed for morphometric analysis of intestinal structure (**Figure 3**). After DSS-induced stress, small intestinal segments were collected, processed, embedded, and stained with hematoxylin and eosin for morphometric analysis using a microscope at the Cornell Imaging Facility. Villus height (VH) was measured from the tip of villus to the top of lamina propria, while crypt depth (CD) was measured

from the base upwards to the region of transitions between the crypt and villi (Biloni et al., 2013). In the duodenum, both the 0.2% Yeasture and 0.2% Zinpro groups showed an increase ($P < 0.05$) in VH than the control group. Meanwhile, the 0.2% Yeasture but not 0.2% Zinpro group, exhibited higher VH ($P < 0.05$) in the jejunum compared with the control. No differences were found in other intestinal morphology parameters.

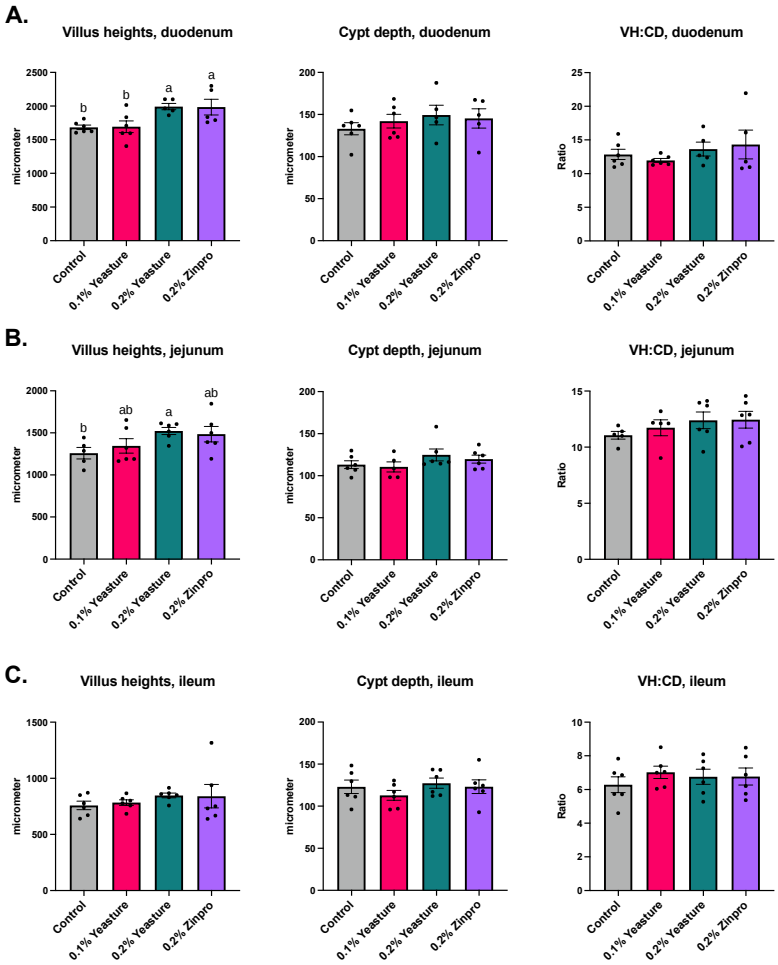

Figure 3. The morphologic analysis of intestinal segments including duodenum (A), jejunum (B), and ileum (C) after DSS-induced stress.

In addition to changes in intestinal morphology and serum FITC-d concentration, quantitative PCR and western blot analysis were performed to investigate the mRNA abundances or protein expression in the duodenum, jejunum, and ileum samples shown in **Figure 4** by following the previous protocols (Ou et al., 2023). Supporting previous findings, mRNA expression of *Claudin-1* was increased ($P < 0.05$) by both 0.2% Yeasture and Zinpro. However, there were no significant differences in the mRNA of Occludin and ZO-1 compared with the control. Notably, the gene expression results were not fully consistent with the western blot analysis, as only jejunal Claudin-1 protein expression was increased by the 0.2% Yeasture treatment. Overall, supplementation with Yeasture and Zinpro at either 0.1% or 0.2% improved intestinal epithelial tightness and structure, as further confirmed by related mRNA and protein expressions.

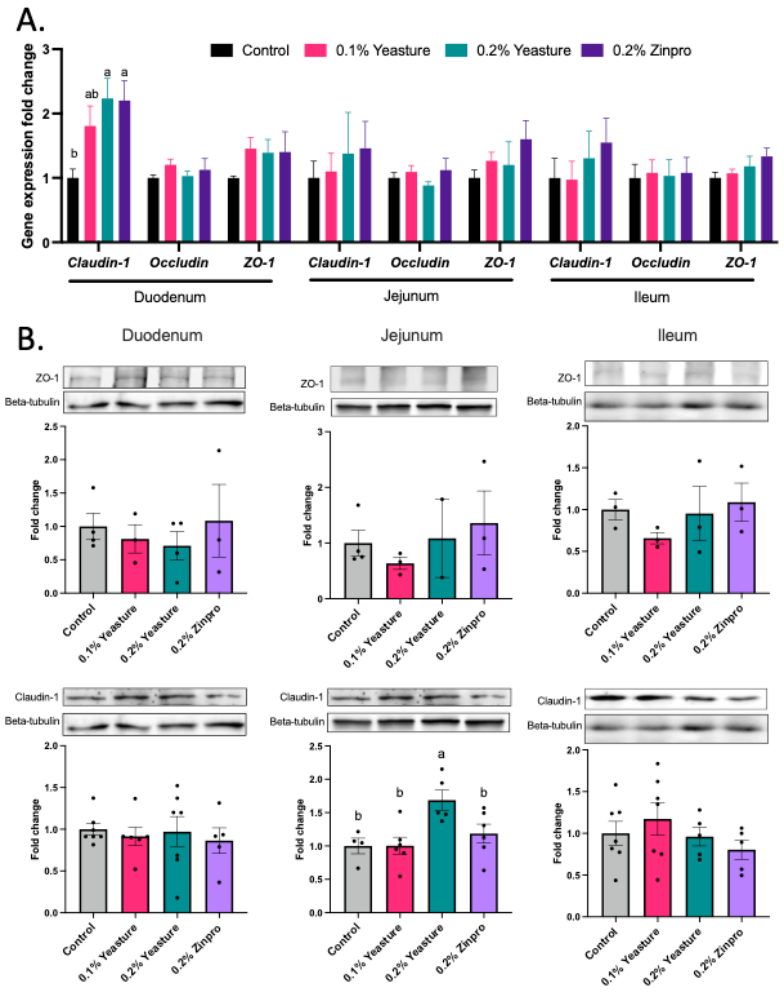

Figure 4. The mRNA (A) and protein (B) expression of the tight junction protein in the small intestine after DSS-induced stress.

Summary and Implication

In summary, yeast culture Yeasture Elite-4 Plex led to minor improvements in feed efficiency during the early stage of growth, but no significant differences were observed over the entire production period compared with other treatments. However, both Yeasture and Zinpro supplementations demonstrated considerable improvements to intestinal health, particularly in intestinal integrity and morphology, with no notable difference between the two products. In conclusion, both Yeasture and Zinpro products were beneficial for improving intestinal health in broiler chickens.

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The Next Frontier: Does Dam Nutrition Influence Offspring Performance?

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Introduction

Developmental programming is the alteration of an individual's phenotype resulting from the gestational or neonatal environment (Reynolds and Vonnahme, 2017). Classically, these programming influences are considered to be negative, such as reduced nutrient supply, exposure to heat, or intra-uterine growth retardation from pregnancies with multiple fetuses – all insults that regularly affect livestock around the world.

One of the most extensive examples of developmental programming is through the atrocities experienced during World War II, where starvation and malnutrition occurred through swaths of Europe over time periods of a few months to several years. Remarkably, children born to women who experienced malnutrition during this period experienced greater incidence of metabolic disease during adulthood. The British researcher David Barker coined this phenomenon as the 'Thrifty Phenotype Hypothesis' (Hales and Barker, 2001). This phenomenon has been replicated in other species (Yura et al., 2005; Tipton et al., 2018), and the thrifty phenotype can also be induced by maternal overnutrition (Long et al., 2010).

The ability to induce long-term developmental programming on offspring appears to be possible at many points throughout gestation (Du et al., 2017), and even occurs as early as the periconception period (Fleming et al., 2012; Wu and Sirard, 2020). The mechanisms of developmental programming are likely multifactorial. For example, insults may alter placental mass, blood flow, and nutrient transport to the fetus (Reynolds et al., 2023). Ultimately, the long-term implications of developmental programming are likely realized at the fetal cellular level through epigenetic changes. Epigenetics refers to the alteration of DNA through the addition of methyl groups to primarily cytosine residues of CpG dinucleotides, and of post-translational modifications of histone tails – collectively they influence the genomic material that is transcribed and ultimately activates cellular functions (Gabory et al., 2011; Chavatte-Palmer et al., 2018). Identification of mechanisms by which epigenetic programming can influence offspring performance is a valuable goal for future research and holds promise for continued improvement of our understanding of biology.

Developmental Programming in Meat vs. Dairy Animals

Developmental programming research in the animal sciences has largely focused on meat animals. The swine and sheep industries have focused extensively on intra-uterine growth retardation as large litter size creates restraints for fetal nutrient

availability (Wu et al., 2006). Offspring suffering from intra-uterine growth retardation have decrease skeletal muscle mass, growth rates, feed efficiency, and increased whole-body fat mass and metabolic disorders (Wu et al., 2006).

In the beef industry, work has focused on evaluating dam plane of nutrition and dietary protein supply on offspring performance. Beef cattle often experience a nutritional deficit during gestation while grazing rangeland or poor-quality stored feeds in the winter and may not have sufficient energy to dedicate to reproductive purposes. In these studies, cows that were supplemented with either energy or protein prepartum had offspring with greater body weight from calving through slaughter (Corah et al., 1975; Stalker et al., 2007; Larson et al., 2009; Noya et al., 2019; Long et al., 2021), demonstrating financially important outcomes for the beef producer. Additionally, heifers from supplemented dams reached puberty earlier (Noya et al., 2019). Fewer studies followed offspring through to harvest, for which it is often difficult to achieve sufficient statistical power to detect carcass differences. Those studies that did detect statistical differences when the dam was supplemented with dietary energy or protein observed greater marbling, quality grades, and ribeye area (Larson et al., 2009; Long et al., 2021), which are important value-added revenue streams in finished beef animals.

It is difficult to extrapolate these data to the dairy industry. The work conducted in swine and beef systems is inherently confounded with postpartum variation in milk production and component composition. These milk supply variables are underreported in beef and swine projects due to the difficulty in testing milk in those species, and the intensive nature of suckle and weigh experimental designs. Altering dairy cow prepartum dietary energy density affects the postpartum milk and fat-corrected milk yields (Janovick and Drackley, 2010), so it is reasonable to expect that similar differences would be observed for the energy available to piglets and beef calves. The dairy industry has an ideal model to isolate and assess developmental programming effects since calves are separated from the dam at birth and reared in a consistent environment. The feeding of milk replacer to calves with similar housing allows for remarkable consistency of experimental design that is difficult to replicate in other species. Therefore, dairy calves are primed as the premier model for evaluating gestational developmental programming effects. There has been a considerable amount of research conducted on dairy cow prepartum nutrition and management strategies to maximize production and health of the postpartum cow. The intensive nature and expense of these studies has precluded researchers from having sufficient time, labor, and financial resources to pursue additional measurements on the offspring. There has been a flurry of recent and convincing research evaluating the negative impact of gestational heat stress on calf performance, but comparatively fewer investigations have focused on effects of dam nutritional interventions. The remainder of this paper will characterize what we know about dairy cattle gestational nutrition on offspring outcomes.

Dam Energy Balance

Dairy cows experience a range of energy balance throughout lactation. After encountering negative energy balance postpartum (Albornoz et al., 2019), cows gradually return to neutral or positive energy balance around the time of insemination. From that point forward, cows ideally remain in neutral or a slightly positive energy balance through the cessation of lactation. Cows may be in a slightly positive energy balance during the dry period when their body condition is at its greatest during the lactation cycle.

The degree of positive or negative energy balance of a dairy cow during gestation likely has long-term implications for the developing fetus. Cows that conceive during peak milk production and have strong persistency for milk production may be challenging their growing fetus to compete for nutrients with the mammary gland. Several studies utilizing large datasets have observed that greater dam milk production during gestation, including during embryogenesis, results in progeny with reduced milk yield (Banos et al., 2007; Berry et al., 2008; González-Recio et al., 2012). The reduced milk yield in the daughters was particularly evident in the first and third lactations (Berry et al., 2008), and these offspring had decreased survival to second parity or overall reduced functional lifespan in the herd (Berry et al., 2008; González-Recio et al., 2012). Intuitively, the dams with greater milk production might have greater genetic merit for milk production which could be passed along to their offspring; however, if epigenetic changes are occurring from reduced nutrient supply to the fetus while competing with the mammary gland, then this could limit the progeny milk production potential.

More recent work has evaluated dam body condition score (BCS) at calving as a proxy for gestational energy balance and its effect on calf developmental programming. Within these studies, the underlying assumption is that a cow with greater body condition at calving experienced greater energy balance throughout gestation, or at least during the last trimester. While these may not represent a perfectly controlled experiment, they can provide initial insight into the effect of long-term dam energy balance during gestation. Dairy calf birthweight increases about 2 kg with each 0.5 unit increase in dam BCS at calving (5-point scale; Poczynek et al., 2023). This is not altogether unexpected and appears to be a positive outcome for the offspring; however, data from other species indicate dams with excessive energy intake during gestation have deleterious effects for the neonate's long-term ability to regulate feed intake and body composition. Overfeeding ewes during gestation imparts an obese phenotype, greater feed intake, and reduced insulin sensitivity in the adult offspring (Long et al., 2010, 2015; Shasa et al., 2015). Interestingly, the body composition of the F2 progeny was greater for lambs whose granddams were overfed during gestation, implicating multigenerational effects (Shasa et al., 2015). If the same holds true for dairy cows, those with excessive energy balance during gestation (calving BCS >4), could be predisposing their offspring to poor feed intake regulation capabilities, excessive adulthood BCS, and a propensity for common transition cow metabolic disorders. Indeed, dam gestational BCS is associated with greater offspring first lactation BCS and

reduced milk yield, and these negative associations between dam BCS and offspring milk yield were more pronounced in the last 2 months of gestation (Banos et al., 2007).

Data from mice show that the obese phenotype in offspring from overfed dams is mediated by impaired hypothalamic development of feeding control centers during the neonatal period, which is normally facilitated by a neonatal leptin surge (Bouret and Simerly, 2004). It is unclear if the neonatal leptin surge and subsequent hypothalamic development of feed intake control exists in dairy cattle, but the leptin surge can be altered by dam gestational nutrition in beef calves (LeMaster et al., 2017). Calves from Holstein cows that were overfed during the prepartum period compared with being fed a controlled-energy diet did not exhibit an altered leptin surge (Osorio et al., 2013). From a more observational perspective, Brown et al. (2023b) attempted to determine if the aforementioned leptin signaling pathways critical to neonatal hypothalamic development were disrupted in calves born from dams with greater BCS. While the researchers did not observe differences in the offspring neonatal leptin surge, calves from dams with greater BCS had a characteristic spike in cortisol at birth (Brown et al., 2023b). This spike in cortisol is observed in both lambs and beef calves from dams on extreme planes of nutrition during gestation and coincides with an ablated leptin surge (Long et al., 2011; LeMaster et al., 2017; Smith et al., 2018). Overall, there appears to be alterations of endocrine function in neonatal ruminant offspring when the dam experiences extreme energy intake during gestation, but it is not clear if controlled energy diets utilized in modern prepartum dairy cow diets allow sufficient deviation from energy demand to affect the offspring. Identifying mechanisms by which these endocrine changes may be related to or influence long-term production and metabolic characteristics in the offspring may facilitate more strategic management of transition dairy cows in the future.

Dam Dietary Additives

Many feed additives are administered to the prepartum cow to boost postpartum milk production and ameliorate common maladies encountered by cows during this period. Developing research demonstrates that these nutritional additives may also have positive effects on the calf.

Rumen-Protected Choline

Choline is a quasi-vitamin that has received substantial interest for transition dairy cows in the last two decades, and there has been recent discussion of classifying choline as a required nutrient for transition dairy cows (Santos et al., 2024). Feeding rumen-protected choline (RPC) to gestating ewes starting at conception increases long bone growth in sheep fetuses (Sawant et al., 2019). In transition dairy cows, supplementation with RPC for 21 days prepartum increased birthweight in their Holstein heifer offspring (Zenobi et al., 2018), and increased preweaning gain and feed efficiency in a sex- and breed-specific manner for Holstein and Holstein x Angus calves (Holdorf et al., 2023). In another study, there was no evidence of body weight advantage over the

first 3 weeks of life for Holstein calves whose dams were fed increasing rates of dietary RPC, but it did reduce measures of oxidative stress (Swartz et al., 2022).

The growth advantages imparted by maternal choline supplementation were noted in offspring up to 9 to 12 months of age (Zenobi et al., 2018; Brown et al., 2023a), but this was not observed in pure beef calves at weaning when their dams were supplemented a low dose of 4.5 g choline/day for at least 60 days prepartum (Pacheco et al., 2010). Beef x dairy calves were followed through to harvest to evaluate carcass quality; those animals exposed to greater prenatal doses of choline had greater marbling, and kidney, pelvic, and heart (KPH) fat stores at slaughter (~15 months of age; Brown et al., 2023a). Furthermore, the beef x dairy cattle exposed to choline during the prenatal period also had markers of greater insulin sensitivity when fed a finishing diet (lower plasma glucose and insulin concentrations; Brown et al., 2023a), which may point to a potential mechanism of action for the greater fat deposition. There are currently no nutritional mediators to improve marbling other than feeding diets with greater energy density, so the possibility of improving beef marbling through calf developmental programming is intriguing. Admittedly, there may be a tradeoff as carcasses that possess greater internal fat result in reduced carcass value, so further research is warranted.

Of particular interest from these studies is the feeding rate of RPC that should be fed to cows to impart offspring growth effects, as there has been a range of RPC inclusion rates for prepartum dairy cow diets (7 to 25 g of choline ion/day; Arshad et al., 2020). Holdorf et al. (2023) evaluated calf performance based on linear amounts of dam choline intake and noted linear and quadratic increases in preweaning average daily gain and feed efficiency, especially in male beef x dairy calves. These linear effects carried over to the growing period and carcass fat deposition characteristics previously discussed (Brown et al., 2023a). While current recommendations for RPC inclusion in prepartum diets are around 13 g of ion/cow/day, higher inclusion rates may be beneficial for those seeking greater growth and performance benefits for the resulting calves.

Rumen-Protected Amino Acids

Supplementation with rumen-protected amino acids is a common strategy in dairy cattle to meet metabolizable protein requirements and reduce overall nitrogen concentration in the diet, and supplementation during the peripartum period has proven to be beneficial in the performance of lactating dairy cows (Zanton and Toledo, 2024). A body of work at the University of Illinois has evaluated the effects of rumen-protected methionine (RPM) supplementation to prepartum cows on the performance of their calves. Overwhelmingly, RPM supplementation for 21 to 28 d prepartum increases offspring birthweight (up to 2 kg; Alharthi et al., 2018), body weight through 9 weeks of age (up to 4.2 kg advantage; Alharthi et al., 2018, 2019; Batistel et al., 2019; Elolimy et al., 2019), wither and hip height (Alharthi et al., 2018; Batistel et al., 2019; Elolimy et al., 2019), and average daily gain (Alharthi et al., 2018). Combining supplementation of RPM with rumen-protected lysine additively improved dairy calf average daily gain and weaning weight, while reducing the number of days to weaning (Wang et al., 2021).

Supplementation of rumen-protected lysine alone to dairy cows 26 days before parturition increased body weight and average daily gain in progeny during weeks 6 to 8 of life, but there were no other key production benefits to the offspring in the first 8 weeks of life (Thomas et al., 2022). Despite the positive effects observed in neonatal calves exposed to greater methionine in utero, longer-term data on the effects of RPM or lysine supplementation on the offspring is not yet available.

The mechanisms for the performance advantages from RPM appear to be mediated by several factors. First, the prepartum dry matter intake was greater for dams supplemented with RPM (Batistel et al., 2017), which indicates potentially greater nutrient supply to the calf. Secondly, RPM alters placentome gene expression for enzymes related to transport of amino acids, glucose, and the mTOR pathway (Batistel et al., 2017) and may be indicative of greater nutrient uptake. Third, DNA methylation plays a key role in developmental programming changes, and although dam RPM supplementation did not alter global DNA methylation in heifer calves, it did increase placentome DNA methyltransferase gene expression (Batistel et al., 2019). Fourth, in the calf itself, there are further changes in hepatic gene expression for genes associated with methionine metabolism, DNA methylation, and transsulfuration pathways in calves through at least 50 days of age when the dam is supplemented with RPM prepartum (Jacometo et al., 2017). These calves also experienced improved metrics for maturation of gluconeogenesis and insulin sensitivity (decreased glucose:insulin ratio; Jacometo et al., 2016). The provision of additional methionine during the prenatal period could function through multiple pathways to promote a more positive growth environment for dairy calves.

Omega-3 Fatty Acids

Fatty acid supplementation is not a common strategy for prepartum cows, but some limited work has explored the concept as a potential opportunity to influence calf performance. Of particular interest is the use of omega-3 fatty acids; Moallem and Zachut demonstrated that supplementation of DHA to gestating cows increased DHA composition in plasma of the calves at birth, but supplementation of dams with alpha-linoleic acid did not alter calf plasma fatty acid profile compared with controls (Moallem and Zachut, 2012). Similarly, calf plasma fatty acid profile is correlated with both the dam plasma and colostrum fatty acid profile (Uken et al., 2021). Compared with a saturated fatty acid supplement, gestational essential fatty acid (C18:2 and C18:3) supplementation for 8 weeks prepartum in dairy cows increased average daily gain through weaning for the offspring (Garcia et al., 2014). Dam gestational supplementation with calcium salts of soy oil and fish oil both increased weight and growth of calves through weaning (Jolazadeh et al., 2019).

There has also been some interest in determining whether dam supplemental fat enhances offspring health and immune function given the importance of omega-3 fatty acids in health outcomes in other stages of life. Dam fatty acid supplementation with essential fatty acids did not alter calf leukocyte counts or neutrophil function (Garcia et al., 2014), but it did decrease offspring hepatic gene expression related to immune

activation (Garcia et al., 2016). Others have noted that dam supplementation with various fatty acid types for 2 months prepartum variably altered plasma IL-1β and reactive oxygen metabolites in neonatal calves less than 5 days of age (Liermann et al., 2021), but additional studies are required to validate these effects more broadly and to assess the health implications for these calves through the milk feeding period and beyond.

It is possible that these limited influences on performance may be mediated primarily through changes in colostrum versus in utero programming effects. For example, altering the dam's supplemental fat source prepartum changes the colostrum dry matter and fat composition through the first 5 milkings (Uken et al., 2021), and increases immunoglobulin intake (Jolazadeh et al., 2019). However, the effects on IgG passive transfer are variable. Supplementation in prepartum cows using calcium salts of fatty acids compared with a basal diet without supplemental fat markedly increased the passive transfer of immunoglobulins in their Holstein calves (Jolazadeh et al., 2019), whereas essential and saturated fatty acid supplementation during gestation failed to alter serum IgG in another study (Garcia et al., 2014). Liermann et al. (2021) showed that conjugated linoleic acid supplementation to dams prepartum decreased plasma total protein concentration compared with essential fatty acid supplementation. The variability in these results likely reflect differences in supplemental fat inclusion rates and the profile of fatty acids in those supplements. Despite these variable results, systematic research to determine effects of fatty acid supplementation on offspring immune function and longer-term performance past the weaning period is warranted.

Summary and Implications

The use of RPC and RPM in prepartum dairy cows have been established as useful nutritional management tools to prepare the cow for successful transition period, and a growing body of evidence indicates these tools can also be successfully used to improve performance in the offspring. Epigenetic programming from additional methyl donors supplied by these supplements is the most likely contributing factor for these improvements, but much more research is needed to validate the exact mechanisms imparting the changes observed. Fat supplementation may have some beneficial characteristics on the offspring, but more research is needed to elucidate the situations where it is most beneficial to production responses. Given the plethora of prepartum nutritional supplements available combined with an appearance of potential challenges from excessive dam body condition during gestation on offspring performance, there are many factors that should be evaluated as mediators of offspring performance. As this body of research grows, the future of the dairy industry may involve more deliberately setting up calves for success through the use of dam nutritional management during gestation.

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From Moo to Measurement: Investigating Enteric Methane with Cutting-Edge Technology

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Introduction

Methane (CH_4) is a potent greenhouse gas (GHG) contributing significantly to global warming and climate change. Ruminant livestock, particularly beef and dairy cattle, are major contributors to GHG emissions due to enteric CH_4 fermentation. In this context, methods to estimate enteric CH_4 emissions are extremely important. While respiration chambers (RC) are the gold standard for measuring CH_4, they are costly and may affect cattle behavior. A commercially available head-box system (GF) offers portability but has higher variability and lacks standardization. The tracer gas technique (SF_6) is useful for grazing systems but has limitations in measuring whole animal emissions and potential interferences. Sniffer systems (SNF) offer repeated measurements during milking but exhibit within-animal and between-animal variability. This conference proceeding aims to review the fundamental concepts related to enteric CH_4 emissions in dairy cattle considering methodologies and interventions aimed at enteric CH_4 monitoring and reduction. The most common methodologies to measure enteric CH_4 emissions in dairy cattle are summarized. An initial assessment on methodologies agreement to the RC is presented and discussed. Preliminary results from a recent study that investigated the agreement between the GF and RC systems in pregnant Holstein heifers are presented here.

Measuring Current Enteric Methane Emissions and Impact of Interventions

The current measurement of enteric CH_4 emissions and the impact of interventions are subjects of active scientific investigation aimed at understanding and mitigating GHG emissions from livestock. Researchers employ various methodologies to quantify enteric CH_4 emissions (Tedeschi et al., 2022), while interventions seek to reduce these emissions through targeted strategies (Fouts et al., 2022). Measurement of enteric CH_4 emissions encompasses a range of sophisticated techniques that have been the focus of several review papers in the past couple of years (Storm et al., 2012; Hammond et al., 2016; Zhao et al., 2020; Tedeschi et al., 2022). Our purpose is not to review these, but to briefly highlight the different methodologies and techniques that can be used in the current context of CH_4 mitigation space.

Respiration chambers, considered the gold standard, involve housing animals within controlled environments to capture exhaled gases, enabling direct measurement of gaseous exchange and emissions, including but not limited to CH_4, alongside other metabolic parameters in energy metabolism (Kleiber, 1961; Pullar, J.D., Brockway, J. M.,

1967). Automated headbox systems, such as the commercially available GreenFeed system (GF; C-Lock Inc., Rapid City, SD), employ similar principles of indirect calorimetry relative to the respiration chamber system, but it does so during regular feeding events, measuring the concentration of CH_4, carbon dioxide (CO_2) and hydrogen (H_2) in an animal's breath during a feeding bout (Huhtanen et al., 2019).

Respiration masks, sniffers, and eructation peaks (SNF) are all similar methodologies that utilize eructated breaths to measure CH_4 in a feed bin located near the milking station (Garnsworthy et al., 2012). The breathed air is drawn continuously from the feed bin through the instrument by an integral pump between the gas inlet port and analyzer. Because the CH_4 concentration is usually logged at very short intervals, the data follows a very distinctive pattern of peaks over each breathing movement. And thus, like the GF system, the overall assumption is the close relationship between daily CH_4 production and the concentration in animal's breath.

Tracer gas techniques, such as sulfur hexafluoride (SF_6), have also been used and especially in grazing conditions. The SF_6 technique consists of a permeation tube or bolus that releases SF_6 gas into the reticulo-rumen at a known and predetermined constant. The sampled breath is evacuated with a cylinder by placing a tube near the nostril of the animal, that is usually positioned on a halter. The CH_4 emission rate is computed by the known release rate of the tracer gas and the ratio of expired CH_4 and tracer gas concentrations in the canister while accounting for background concentrations of CH_4 and SF_6 in ambient air (Johnson et al., 1994; Beauchemin et al., 2012).

The development of advanced monitoring systems, including open-path lasers, can also be highlighted to assess CH_4 emissions from cattle. This technique assesses gas dispersion from a source and subsequent downwind concentrations to calculate emission rates using an "inverse dispersion" approach. This method has been applied to quantify CH_4 and ammonia (NH_3) emissions from animals housed in feedlots and pastures (McGinn et al., 2006, 2008). Advancements have extended the technique's utility to aircraft and drones, demonstrating reliability for CH_4 and NH_3 detection over significant distances (Hacker et al., 2016). However, studies have compared open-path laser estimates of daily CH_4 emissions on pasture with respiration chamber results and showed variations based on forage types and herds (Tomkins et al., 2011; Tomkins and Charmley, 2015). In addition, validation has highlighted limitations related to data collection time, as spot measurements during daylight hours may not fully represent 24-hour emission patterns (McGinn et al., 2006, 2008). Nonetheless, the open-path laser technique proves valuable for measuring CH_4 emissions from herds in both grazing scenarios and intensive livestock operations, offering insights at the herd scale.

Various interventions are being explored to mitigate enteric CH_4 emissions from dairy cattle. Dietary modifications are highlighted as one of the most immediate solutions, though effects can be reversible (Hristov et al., 2022). These modifications encompass changes in diet composition such as forage-to-concentrate ratio, nutrient balance, and the inclusion of additives like fats, overall targeting the rumen's microbial ecosystem and thus consequently impacting CH_4 production while still upholding animal productivity and

health. The addition of feed additives like 3-nitrooxypropanol (3-NOP) and essential oils are investigated for their potential to inhibit CH_4 -producing microbes (Garcia et al., 2020; Alemu et al., 2021; Pitta et al., 2022). Incorporation of specific forage components into the diet like tannins and saponins have also been described to hinder CH_4 production by altering rumen fermentation processes (Ku-Vera et al., 2020). Microbial manipulation exploring the use of probiotics, prebiotics, and direct fed microbials to alter the rumen's microbial community have also been alluded to as a possibility to decrease CH_4 production (Ban and Guan, 2021). Early-life interventions during critical developmental stages like in utero or post-natal periods have been considered to program the gut microbiome for reduced CH_4 emissions later in life (Cristobal-Carballo et al., 2021; Meale et al., 2021). Lastly, genetic selection strategies have been considered to breed animals with genetic traits linked to reduced CH_4 emissions and incorporating CH_4 -related genes into breeding programs (Lassen and Difford, 2020). Rigorous scientific experimentation through controlled studies, randomized trials, and field trials assesses these interventions, considering factors such as CH_4 emissions, animal performance, nutrient utilization, and environmental impacts. Ongoing research endeavors strive to enhance measurement techniques and establish a standardized framework for measuring enteric CH_4 emissions, optimize intervention approaches, and attain a comprehensive understanding of enteric CH_4 emissions' role in global GHG dynamics.

Standardization of Methodologies: Why Should We Care?

Collaborative efforts between Cornell University, Environmental Defense Fund, University of California, Davis, Global Research Alliance, and others are currently in progress to establish comprehensive standards for assessing the efficacy of feed additives designed to diminish enteric CH_4 production. Traditionally, researchers have taken an atomistic approach to gauge the effectiveness of these dietary interventions, largely due to constraints in resources and expertise. However, a more holistic perspective is essential, considering aspects like energetic efficiency, nutrient digestibility, manure GHG emissions, animal well-being, and residues in tissues and milk, all within the context of CH_4 reduction strategies. Additionally, methodologies are being developed to quantify actual reductions in CH_4 emissions on farms, which can support the creation of carbon offsetting or insetting initiatives. Nevertheless, the complex nature of CH_4 reduction assessment is compounded by the absence of standardized methods for quantifying changes in CH_4 emissions from individual cows or CH_4 inventories on specific farms.

To attain higher precision and accuracy in determining both baseline emissions from the livestock sector and the effectiveness of mitigation strategies, such as enteric CH_4 -inhibiting feed additives, the establishment of standardized, robust, science-based quantification methodologies for measuring enteric CH_4 emissions from cattle is imperative. While alternative methodologies for measuring gas exchange in ruminants hold promise, they must undergo rigorous validation before being accepted. This validation should involve direct comparison with the gold-standard respiration chamber system, which captures total gas exchange over a 24-hour period within a controlled environment. For any validation of methodologies, comprehensive assessments should

be conducted, including repeated measurements of gas emissions from the same animal(s) using alternative CH_4 measurement techniques over various days and within a day, all under controlled experimental conditions.

The results of such validation should be openly reported in the public domain, including concordance correlation coefficients and confidence intervals. Given that some methods may exhibit notable within-day and within-animal variance relative to a pre-established standard, a thorough understanding of experimental parameters, such as the number of animals measured, breed, diet, and performance indicators, is crucial. Equally important is validating emissions based on daily CH_4 production within a 24-hour interval, considering the temporal variability in CH_4 emissions and the variation in measurement frequency across different methods. Documentation of the frequency and type of calibration and gas recovery tests is essential and should be made available for public scrutiny.

To consolidate this effort, a comprehensive database should be constructed. This database should encompass standardized methodologies and their applications under various management scenarios, while also accounting for variables like breed, life stage, feed composition, and management systems. The inclusion of data demonstrating the effectiveness of mitigation strategies using these standardized methodologies would further enhance the database's utility.

An Initial Assessment of the Validation and Reliability of Methods to Measure Enteric Methane Emissions in Dairy Cattle

With this initial assessment, our objectives were to evaluate the predictions of GF, SF_6, and SNF, relative to the RC enteric CH_4 emissions. This evaluation was performed using a meta-analytic approach using original research articles and comparative studies. Statistical analysis was performed using R (v. 4.2.1); the comparison between GF, SF_6, and SNF with RC data was assessed with Forrest plots, and the random effect model correlation coefficients using the metafor package. A total of 14 studies met the criteria for inclusion in the meta-analysis. Moderate agreement was observed for total CH_4 production between GF and RC (r = 0.61 [95% confidence interval [CI]: 0.40, 0.76]). The SF_6 and SNF methods showed stronger agreement with RC (r = 0.82 [95% CI: 0.67, 0.90] and r = 0.81 [95% CI: 0.67, 0.90], respectively). Overall, this meta-analysis uncovered inconsistencies in CH_4 production metrics across different measurement approaches, highlighting both the advantages and limitations of each methodology. These findings underscored the need for standardized procedures and careful calibration to ensure accurate and reliable data. Recognizing the importance of addressing these issues, we recently completed a trial to test and validate certain methodologies (namely GF relative to the RC). Our goal was to assess measurement accuracy and support transparent comparisons in enteric CH_4 emissions.

Eight pregnant Holstein dairy heifers (18 ± 0.49 months of age [mean ± SD], 164 ± 4.42 days carrying calf, and 556 ± 39.4 kg of body weight [BW]) were enrolled in a study at the Large Animal Research and Teaching Unit at Cornell University (LARTU; Ithaca,

NY). The heifers were provided by the Cornell University Ruminant Center (CURC; Harford, NY) and transported to LARTU. Heifers were acclimated to the LARTU facility, trained to use GF units, and the Cornell University Animal Respiration Chambers for up to 2 weeks prior to the start of the experiment. Following a ~2-week training period, heifers were equally divided into two groups and underwent 5 repetitions in a switchback design. During each repetition, measurements were made using GF and RC for 3 days in each system, totaling 6 days in each repetition. For the GF system, each heifer was brought to the unit every 9 hours during the 3-day period. The unit was maintained stationary in the hallway. This approach allowed for a representative sample of a 24-hour period when assessments from the GF were averaged and ensured all animals had the same number of measurements. For the RC system, the interval between measurements was 10 minutes during the 3-day period. Both RC and GF were properly calibrated prior to beginning of the experiment. Recoveries of CH_4 and CO_2 were 100 and 104% in the RC system. Recovery of CO_2 was 99.2% for the GF system. Environmental conditions at the research facility were set to a temperature of 16 to 20°C and 30 to 40% relative humidity. Body weight was recorded at the beginning and end of the experiment. Heifers were fed a conventional total mixed ration composed mainly of corn and grass silages, grass hay with a hand-add of mineral premix. Feed and water were provided ad libitum.

Preliminary results (Table 1) indicated that the RC system had improved precision, as evidenced by its generally smaller standard deviation (SD) and coefficient of variation (CV). The CH_4 emissions obtained with RC had less variability and greater consistency compared to GF, suggesting better gas recovery. When we compare the percent difference between CH_4 emissions generated by the two methods, while it is true that for most observations (i.e., 38 out of 40), the GF system estimated CH_4 to be lower than the emissions from RC. The range of this difference was quite large. Overall, the GF system estimated CH_4 emissions to be 14% less than RC, but with a range that went from -27.7 to +7.6%. This is particularly important when evaluating dietary treatments that result in a reduction in CH_4 emissions, especially supplements or compounds that offer smaller reductions (i.e., 5 to 20%, for example; Hristov et al., 2022). Overall, the CO_2 emissions generated by GF were generally +3.3% higher than RC, but again, these emissions spanned from a minimum of -8.7 to +18.5%.

To investigate both correlation and agreement between CH_4 emissions measured by RC and GF, we initially calculated Lin's concordance correlation coefficient (CCC). This metric allows us to evaluate whether GF measurements are consistently lower or higher than those of the RC and to assess how well the two methods match in terms of actual values. This analysis revealed poor agreement between the two methods (CCC = 0.06 [-0.06, 0.17 95% CI]). Similar results were found by Hammond et al. (2015), who evaluated growing Holstein heifers in two different experiments. The authors observed poor agreement in CH_4 emissions measured by RC and GF, with CCC values ranging from 0.05 to 0.10 in their experiments. Interestingly, Doreau et al. (2018), when evaluating the agreement between RC, GF, and SF_6, found no agreement between RC and GF, and a 78% agreement between SF_6 and RC. However, it is important to highlight that the agreement between RC and GF varies across the literature. For example, Velazco et al. (2016) observed an 85% agreement in CH_4 emissions measured by RC and GF in Angus

steers. This high agreement in their study is likely a reflection of the comparison metric chosen (i.e., Pearson correlation). Pearson's correlation coefficient should not be used to assess method agreement, as this metric does not account for how well the two variables agree in terms of their actual values. Two methods could have a high Pearson correlation but still produce results that are systematically different.

Table 1. Descriptive statistics.

Item	Replicate 1 (n = 8)	Replicate 2 (n = 8)	Replicate 3 (n = 8)	Replicate 4 (n = 8)	Replicate 5 (n = 8)
Respiration chamber					
CH_4, g/d	224	223	220	222	211
SD	9.38	10.9	8.78	22.4	10.5
CV	4.19	4.89	4.00	10.1	4.96
CH_4 yield, g/kg DM	23.8	26.4	25.3	25.6	24.4
SD	1.53	1.67	1.76	1.05	1.22
CV	6.43	6.33	6.96	4.10	5.01
CO_2, g/d	8249	8388	8363	8552	8338
SD	199	449	392	424	255
CV	2.42	5.36	4.69	4.96	3.06
CO_2 yield, g/kg DM	877	995	964	990	964
SD	58.7	63.6	71.7	82.3	34.4
CV	6.70	6.39	7.44	8.31	3.57
GreenFeed					
CH_4, g/d	190	184	196	195	181
SD	18.2	11.8	17.3	20.1	19.6
CV	9.58	6.42	8.82	10.3	10.8
CH_4 yield, g/kg DM	19.5	20.6	24.0	24.5	22.1
SD	2.17	1.91	3.94	3.42	2.48
CV	11.1	9.25	16.5	14.0	11.2
CO_2, g/d	8276	8460	8771	8989	8699
SD	475	231	410	432	349
CV	5.74	2.73	4.67	4.81	4.01
CO_2 yield, g/kg DM	850	953	1067	1133	1065
SD	78.2	114	107	144	79.4
CV	9.20	11.9	10.1	12.7	7.46

CH_4: methane; CO_2: carbon dioxide; CV: coefficient of variation; SD: standard deviation.

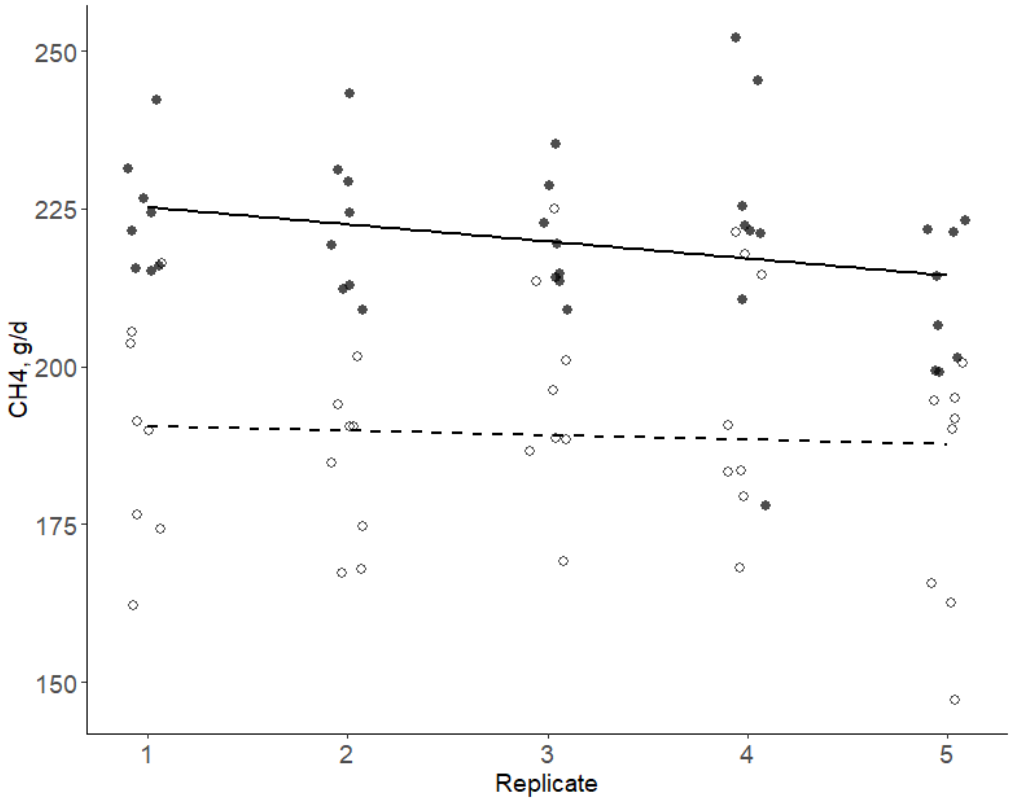

Figure 1. Total methane (CH_4) emissions measured by respiration chambers (RC) and GreenFeed system (GF). $CH_{4\text{-RC}}$ = 228 – (2.71 × Replicate); $CH_{4\text{-GF}}$ = 191 – (0.71 × Replicate); Intercept $P < 0.001$, Replicate $P = 0.71$, Method $P < 0.001$; Replicate × Method $P < 0.001$. RC measurements represented by ● and solid line; GF measurements represented by ○ and dashed line.

By employing a switchback design, we were able to assess the slope of CH_4 (g/d and g/kg DM) emissions measured using RC and GF over the 5 weeks of the experiment (Figures 1 and 2). We observed a similar, slightly negative slope for CH_4 emissions in both RC and GF. The differing intercepts reflect the expected difference: RC typically shows higher values because it accounts for total CH_4 produced, while GF measures only eructated CH_4. It is important to note that Figure 1 also illustrates greater variation in the data points from GF measurements compared to RC. This aligns with our previous observations of larger standard deviations and coefficients of variation for the GF system. When evaluating trends over time for CH_4 yield, we noticed a discrepancy in the slopes for RC and GF (Figure 2). While the slope for RC appears to remain constant throughout the weeks, the GF slope shows an upward trend. A potential source of variation could be differences in dry matter intake (DMI). However, we did not find any statistically significant differences in DMI at either the method level or the interaction of method by week. Overall, based on the preliminary findings of our experiment, it is suggestive that the CH_4 emissions measured by RC and GF are not agreeing closely enough to be considered interchangeable. Importantly, caution should be taken when evaluating CH_4 intensity measures, especially when testing different nutritional strategies aimed at enteric CH_4 reduction using the GF system.

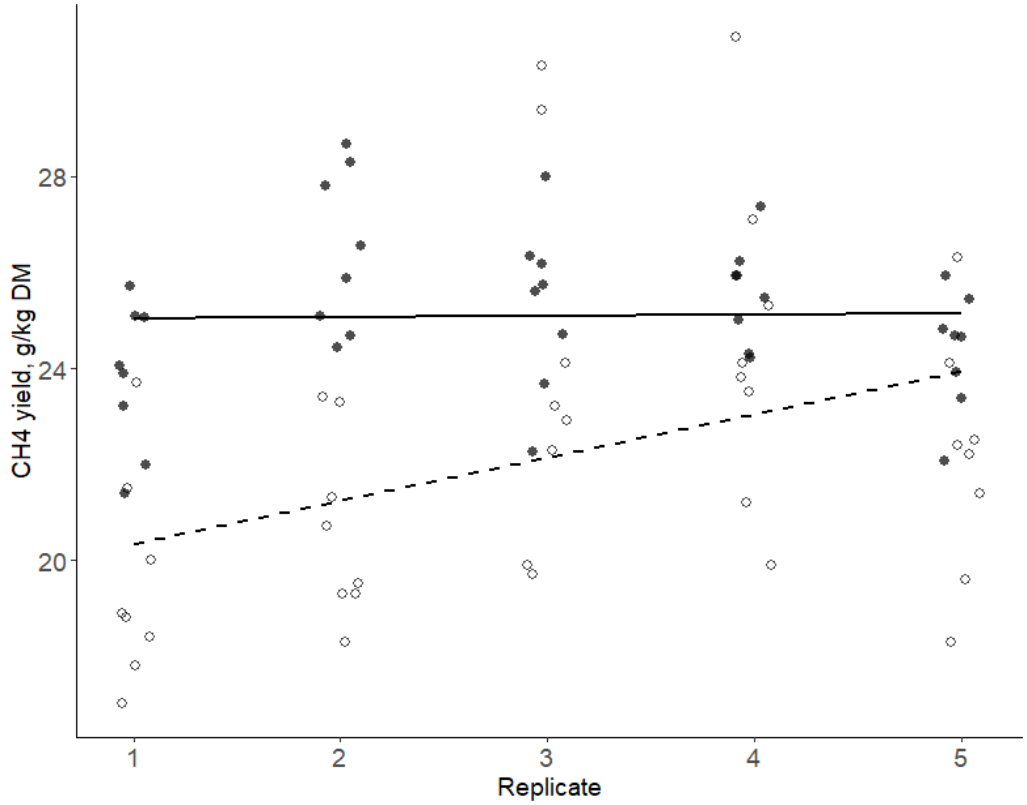

Figure 2. Methane (CH4) yield emissions measured by respiration chambers (RC) and GreenFeed system (GF). CH4 yield$_{RC}$ = 25.0 + (0.02 × Replicate); CH4 yield$_{GF}$ = 19.43 + (0.90 × Replicate); Intercept $P < 0.001$, Replicate $P < 0.001$, Method $P < 0.001$; Replicate × Method $P = 0.08$. RC measurements represented by ● and solid line; GF measurements represented by ○ and dashed line.

Summary

Our work highlights the complexities and challenges of accurately measuring enteric CH_4 emissions from dairy cattle using various methodologies. Our meta-analysis and preliminary results highlight that the GF system, despite its practicality and portability, shows notable discrepancies compared to the RC, with a significant tendency to overestimate CH_4 yield and a large range in variation animal-to-animal. This discrepancy emphasizes the need for improved standardization and validation of measurement techniques. The observed variability between methods stresses the importance of rigorous calibration and methodological consistency to ensure reliable data. Moreover, we advocate for a more integrated approach to developing and validating these technologies, including the establishment of comprehensive standards and databases. Such efforts are crucial for enhancing the accuracy of CH_4 emission assessments and the efficacy of mitigation strategies. Moving forward, a unified framework for measurement and validation will be essential for advancing our understanding of enteric CH_4 emissions and effectively addressing their environmental impact.

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Cows, Climate and Carbon:
Challenges and Opportunities for the Northeast Dairy Industry

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Agriculture globally is under a great deal of pressure to reduce resource use and impact on the environment. While farming has long been about “doing more with less”, and US and Northeast farmers are very good at this, new pressures are piling up and we will need to do even more with even less going forward.

First, the pressure points. With so many wonderful lakes, streams and rivers in the Northeast, we have been working on water quality for decades now and this is familiar territory for most dairy farms. Many dairy farms have implemented nutrient management plans that address potential pollution from the farmstead area including cattle housing, manure and feed storage and barnyards as well as manure and fertilizer management plans for crop fields and pastures.

A developing pressure point that some dairy farms may be starting to feel relates to climate change and reducing greenhouse gas emissions. For many dairies that have not felt it, the time will come, soon. There are several reasons for this. First, the Innovation Center for US Dairy, working with member organizations such as dairy cooperatives and processors, has developed a stewardship commitment for US Dairy. Among other things, this commits US Dairy to be greenhouse gas neutral by 2050. Second, thousands of companies from the US and around the world have made commitments to reduce greenhouse gas emissions within their operations AND throughout their corporate supply chain through Science Based Targets. A number of the organizations include well-recognized food companies with supply chains that include milk or milk products. The commitments often include 30, 40 and even 50% reductions in greenhouse gas emissions across the supply chain by 2030, and, just like the US Dairy stewardship commitment, to greenhouse gas neutrality by 2050. A third reason includes legislation: the European Union has a statute on the books requiring greenhouse gas neutrality by 2050. Many international dairy food companies are headquartered in the EU and are working across supply chains around the world to reduce greenhouse gas emissions from dairy farm production in order to comply. While the US has not seen national legislation setting a greenhouse gas neutrality goal for 2050, state legislation is starting to take shape. The NYS Climate Leadership and Community Protection Act, or CLCPA is a prime example. The CLCPA targets overall greenhouse gas reductions of 40% by 2030 and 85% by 2050, with 1990 emission levels as the baseline. While agriculture is less than 10% of NY emissions, dairy is a large player in the NY ag emission inventory. The CLCPA called for a group called the Climate Action Council to develop a scoping plan by Jan 1, 2023.

The scoping plan identifies reduction of methane emissions from dairy production as an important contributor toward meeting goals of the CLCPA:

> "Advance Livestock Management Strategies: Livestock management strategies could contribute to the deepest reductions in agricultural emissions by mitigating methane through manure management practices and precision animal feeding. Alternative manure management strategies rely heavily on the advancement and expansion of current programs. Precision feed, forage, and herd management strategies rely mainly on increased training and support to the farm community, expanded use of monitoring and decision tools, and continued and enhanced research and development of feed supplements and additives for further methane reductions."

Here, dairy can be viewed as an important part of the solution to limit climate change. The state is deploying both state and federal resources toward grants for emission reductions from dairy, especially methane. For example, cover and flare projects for manure storage structures are being rolled out through Soil and Water Conservation Districts. USDA-NRCS also has grant programs supporting greenhouse gas mitigating practices. Projects take time to develop and farmers are advised to speak to local soil and water conservation district staff as a way to get started.

There are additional pressures that are less developed but will emerge more firmly in the coming months and years. Water use is getting significant attention in agriculture, including dairy. Since a very large portion of dairy-related water use comes from the water used to produce feed in an irrigated system, Northeast dairy is under much less scrutiny than arid areas. However, we will need to pay attention to using water efficiently and there may be more irrigation needed to maintain high forage yields in our future depending on changes in weather patterns. Loss of ammonia-nitrogen from housing, manure storage and field application of manure is also getting more attention. Ammonia is a precursor to PM-2.5, a human-health concern and ammonia deposition across the landscape and to waterbodies is a water quality concern and also results in production of nitrous oxide, a very potent greenhouse gas. Lastly, biodiversity is going to be on our future dairy farm management list. This topic is tough because defining and measuring biodiversity is a major challenge. However, as pressure builds toward ensuring that the regenerative attributes of agriculture are strengthened, we will all be hearing more about biodiversity standards and measures.

The future of northeast dairy is exciting. The industry has made tremendous strides in productivity. An excellent paper on this was recently published in the Journal of Dairy Science (JDS): Fifty Years of Environmental Progress for United States Dairy Farms. This paper divides US dairy into regions, one covering 10 states from Maryland to Maine. In the 50 years from 1971-2020, this region has increased milk per cow by 150% and overall milk by 27% while decreasing both carbon intensity and absolute greenhouse gas emissions among several other notable improvements.

The JDS article shows us the amazing capabilities and ingenuity that the dairy industry here has deployed to support both economic and environmental well-being of our farms and surrounding communities. In this region, we have some of the best farmers in the world, highly capable and engaged allied industry partners, supportive governmental agencies and researchers and extension teams that know the industry well and who focus on developing practices that the industry can implement. With the strength of these partnerships, we can continue to meet the changing needs of Northeast dairy.

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Effects of Dietary Lipid Supplements and Feeding Level on Milk Production and Methane Emissions in Holstein Dairy Cows

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Introduction

Methane is a potent greenhouse gas (GHG) and climate pollutant (Jones et al., 2023). Emissions from enteric fermentation and manure are major sources of anthropogenic methane (CH_4) (Smith et al., 2021). However, we must contend with the reality that the global demand for dairy products is projected to increase due to population growth and rising incomes (Bojovic and McGregor, 2023). This growing demand for dairy is expected to increase methane emissions if measures are not taken (Li et al., 2023). Yet, the short atmospheric lifetime for methane (7 to 12 years) presents a unique opportunity to reduce its impact on climate change in a short period (Mar et al., 2022). Lowering enteric methane emissions without compromising productivity, efficiency, animal health, and human food safety is a valuable strategy for decarbonizing the dairy industry while achieving food security.

Developing strategies to reduce methane emissions can be grouped into three categories: dietary interventions, breeding for low-methane emission animals, and advanced manure management. Breeding can take several generations to cause a significant reduction in methane emissions (Lassen and Difford, 2020). Manure management to reduce methane production is feasible under intensive operations (Sefeedpari et al., 2019). Inhibiting ruminal methanogenesis using dietary approaches offer the potential for positive immediate environmental impact, are adaptable across various farming systems, and can be designed to meet the needs of different production systems.

Dietary ingredients and technologies being evaluated to reduce methane production include bromoform technologies (e.g. red seaweed), fatty acids (FA), 3-nitrooxypropanol (3-NOP), essential oils, tannins, saponins, ionophores, and nitrates. Dietary supplementation of *Asparagopsis taxiformis* has been proven effective at reducing methane production because it contains bromoform, a halogenated compound that inhibits ruminal methanogenesis (Sofyan et al., 2022). However, bromoform and iodine toxicity in milk are a potential safety concern (Muizelaar et al., 2021). Additionally, the availability and cost of seaweed represent key challenges for the industry (De Bhowmick and Hayes, 2023). Alternatively, dietary 3-NOP supplementation reduces methane yield up to 30.9% (Kebreab et al., 2023) but has demonstrated minor or no improvements in milk production (Melgar et al., 2021; van Gastelen et al., 2024). Products like essential oils, tannins, saponins, nitrates, and ionophores present variability in study outcomes when fed to cattle (Almeida et al., 2021). Feedings lipids is potentially

advantageous because they are available in the market, are safe to use, and a well-established supply chain is available.

Fatty acids reduce methanogenesis via several mechanisms. First, replacing fermentable organic matter (OM) with FA dilutes the substrate available for methanogenesis (Alstrup et al., 2015). Second, specific FAs directly inhibit fibrinolytic bacteria and methanogens, decreasing methane production (Amanullah et al., 2021). Lastly, biohydrogenation of unsaturated FAs to saturated FAs acts as a minor hydrogen sink, diverting hydrogen from methanogenesis (Zhang et al., 2019). The dilution effects and microbial inhibition contribute significantly to methane reduction. In contrast, the hydrogen sink effect from biohydrogenation is relatively modest. Patra (2013) reported a 3.77% reduction in methane emissions for each percentage unit increase of lipids within dairy cattle diets. More recently, de Ondarza et al. (2024) found a 3.91% reduction in methane yield of 3.91% per unit of rumen-available lipids. Despite their benefits, lipid feeding should be limited to 6 to 7% of total dry matter of the diet due to adverse effects on intake and fiber digestibility at higher feeding levels (Beauchemin et al., 2008).

Fatty acids have unique effects on metabolism, opening new frontiers to enhance productivity. For example, palmitic acid (C16:0), a saturated FA, has improved energy partitioning toward milk production, increasing yields of milk and energy-corrected milk (de Souza and Lock, 2018). On the other hand, unsaturated FA (UFA), such as oleic acid (C18:1), promote energy partitioning toward tissue gain, supporting body condition and recovery during lactation (Abou-Rjeileh et al., 2023). So, we must consider how dietary FAs uniquely modulate nutrient partitioning and milk synthesis while also evaluating changes in methane production to define the effects of individual dietary FAs on enteric GHG emissions. Our objective was to assess the impact of two dietary lipid supplements on milk production and composition and ruminal GHG emissions.

Materials and Methods

Thirty-six multiparous Holstein cows [147.1 ± 5.9 DIM (mean ± SD), 2.6 ± 0.8 parity; 158 ± 8 DIM, 2.6 ± 0.8 parity] were enrolled in a study with a split-plot Latin square design. The study was conducted to test the interaction between feeding a diet with two types of lipid supplements with different FAs compositions and three fat-feeding levels. Cows were assigned to one of 2 main plots (FA type): a blend of palmitic and oleic acids (PO; Megalac®) or high palmitic acid (HP; Wilfarin®). Within each plot, cows were randomly assigned to a sequence of 3 supplementation levels of dietary FA: 0, 1.5, or 3.0% in a 3 × 3 Latin square design with three 21-d periods. At the end of each period, during a 3-d collection, dry matter intake (DMI), milk yield (MY), milk composition, body weight (BW), body condition score (BCS), and enteric methane (CH_4), carbon dioxide (CO_2), and hydrogen (H_2) emissions were evaluated. Data were analyzed using a mixed model with fixed effects of plot, FA level, period, square, plot × FA level, and the random effect of cow nested in the square. Preplanned contrasts compared HP 1.5% vs. PO 1.5% and HP 3.0% vs. PO 3.0%.

Results

Dry matter intake did not show significant differences between FA type; however, as the level of FA supplementation increased, there was a reduction in DMI ($P < 0.05$). Milk yield positively responded to FA supplementation, specifically at higher dose levels. At the 3% supplementation level, cows receiving PO had significantly greater milk yields than those receiving PO (42.9 vs. 40.6 kg/d; $P < 0.05$). Cows fed HP at 1.5% or 3.0% FA had higher milk fat content than PO (4.56% vs. 4.39% and 4.63% vs. 4.30%; $P < 0.05$). Cows fed HP at 1.5% or 3.0% FA had higher milk protein content than PO (3.54% vs. 3.43% and 3.54% vs. 3.34%; $P < 0.05$). Fat, lactose, and total solids yields increased with higher FA supplementation for both products ($P < 0.05$). As the dietary FA levels increased for both HP and PO, it resulted in a reduction of methane production (g/d), methane intensities (g/kg of milk or ECM), and methane yields (g/kg of DMI), with no significant differences between FA type ($P < 0.05$). Feed efficiencies (milk/DMI or ECM/DMI) improved as FA levels increased for both supplements ($P < 0.05$), with cows fed PO at 1.5% or 3.0% FA showing greater feed efficiency (MY/DMI) compared to those on HP ($P < 0.05$). Carbon dioxide emissions decreased as the FA dose increased ($P = 0.05$), though no differences were observed between FA type. Interestingly, hydrogen emissions showed a slight reduction in cows fed HP as FA levels increased ($P < 0.05$), a trend not observed in cows receiving PO. Furthermore, as FA levels increased, there was a reduction in body weight, mainly in cows supplemented with the PO product ($P < 0.05$). Fatty acid treatment (i.e., plot) did not affect body condition score (BCS) at any supplementation level.

Conclusion

We conclude that despite a reduction in dry matter intake at higher FA levels, milk yield was positively affected, particularly in cows receiving PO. Cows supplemented with HP had increased milk fat and protein contents compared to those receiving PO. Both FA sources, HP and PO, effectively reduced methane production, intensities, and yields alongside carbon dioxide emissions, with a slight reduction in hydrogen emissions observed in cows supplemented with HP. Feeding high palmitic acid led to a 4.6% reduction in methane intensity for each 1% increase in dietary fatty acids, while PO resulted in a 3.75% reduction per 1% fatty acid increase. These findings confirm that FA supplementation can serve as a viable strategy to enhance milk production efficiency and reduce greenhouse gas emissions in lactating dairy cows.

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The Effect of Increasing Lysine Supplementation on Milk Production Performance

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Introduction

Amino acids (AA) are essential for maintenance, growth, reproduction, and component synthesis in dairy cows with the most limiting AA being lysine (Lys), methionine (Met), and histidine (His). We have known that AA are required for milk protein synthesis, but there is emerging data that suggests AA are also required for milk fat synthesis. Li et al. (2019) observed that Lys enhanced fatty acid binding protein (FABP) and sterol regulatory element binding protein (SREBP) in bovine mammary epithelial cells which are key regulators of milk fat synthesis. Additionally, Danese et al. (2024) observed that increasing the supply of metabolizable Met increased the contents of milk fat, de novo fatty acids (FA; <16 carbons), and mixed FA (16-carbon). The objective of this study was to evaluate the effect of increasing metabolizable Lys supply on DMI, milk component yield, and milk FA synthesis.

Methodology

One hundred forty-four lactating dairy cows (88 ± 28 d in milk; DIM) were randomly assigned to 9 free stall pens containing 16 cows (12 multiparous and 4 primiparous) per pen stratified by DIM, milk yield, and parity. The formulated treatment diets were 1.) a diet that supplied 2.81g of Lys per Mcal ME, 2.) a diet that supplied 2.98g of Lys per Mcal ME, and 3.) a diet that supplied 3.18g Lys per Mcal ME. A rumen protected Lys product was fed at 0.0%, 0.16%, and 0.27% of DM in treatment diets 1, 2, and 3, respectively, to increase the supply of Lys (LysiGEM™ Extend, Kemin Industries, Inc, Des Moines, IA). Diets were formulated with the Cornell Net Carbohydrate Protein System (CNCPS v6.5, Van Amburgh et al., 2015), and all treatment diets provided 1.18 g metabolizable Met/Mcal ME and 1.18 g metabolizable His/Mcal ME to meet 100% of the requirements (LaPierre et al., 2020; Higgs et al., 2023).

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

Table 1. Ingredient composition of treatment diets.

	Treatment[1]		
Ingredient, % DM	2.81	2.98	3.18
Corn silage	44.2	44.1	44.2
Grass silage	14.7	14.8	14.8
Corn grain	9.65	9.65	9.57
Soybean meal	5.98	6.03	5.94
Concentrate mix	25.4	25.4	25.6

[1]Grams of metabolizable Lysine (Lys) per Mcal of metabolizable energy (ME)

Table 2. Formulated nutrient composition of treatment diets.

	Treatment[1]		
Item, % DM	2.81	2.98	3.18
DM	44.0	44.0	44.1
CP	15.7	15.7	15.8
aNDFom	28.9	28.9	28.8
Starch	25.7	25.7	25.6
Sugar	7.23	7.21	7.20
Ether extract	4.50	4.54	4.59
Ash	5.75	5.77	5.79
NFC	45.6	45.6	45.5
ME, Mcal/kg	2.80	2.80	2.82
MP supply, g/d	3,120	3,130	3,150
FA, % DM			
Total	3.66	3.70	3.75
C16:0	1.07	1.10	1.13
C18:0	0.25	0.27	0.29
c*is*-9 C18:1	0.69	0.69	0.69
c*is*-9, 12 C18:2	1.20	1.20	1.19
c*is*-9, 12, 15 C18:3	0.27	0.27	0.27
AA, g/Mcal ME			
Lysine	2.81	2.98	3.18
Methionine	1.18	1.18	1.18
Histidine	1.18	1.18	1.18

[1]Grams of metabolizable Lysine (Lys) per Mcal of metabolizable energy (ME)

Preliminary Results

Table 3. Preliminary dry matter intake and milk performance of cows fed treatment diets.

	Treatment[1]				*P*-value[2]			
Item	2.81	2.98	3.18	SEM	Linear	Quad	Trt	Trt × Week
DMI, kg/d	28.2	28.1	27.5	0.26	0.11	0.53	0.20	<0.01
Lys, g/d	222	235	247	-	-	-	-	-
Yield, kg/d								
Milk	46.4	46.6	46.7	0.28	0.30	0.99	0.69	0.65
ECM	49.5	49.8	49.8	0.36	0.50	0.74	0.75	0.22
FCM	50.0	50.2	50.4	0.39	0.51	0.90	0.80	0.39
Fat[3]	1.85	1.86	1.85	0.02	0.90	0.67	0.91	0.77
Denovo, g/d	517	524	519	5.5	0.75	0.41	0.68	0.48
Mixed, g/d	696	705	697	7.2	0.98	0.36	0.65	0.74
Preformed, g/d	532	525	533	5.4	0.82	0.28	0.55	0.97
Protein	1.45	1.47	1.46	0.01	0.42	0.34	0.45	0.12
Lactose	2.19	2.20	2.23	0.02	0.14	0.72	0.32	0.04
Content, g/100g milk								
Fat	4.05	4.03	3.98	0.03	0.14	0.71	0.31	0.50
Denovo	1.13	1.14	1.11	0.01	0.26	0.21	0.24	0.60
Mixed	1.52	1.53	1.50	0.01	0.13	0.24	0.16	0.54
Preformed	1.17	1.14	1.15	0.01	0.35	0.28	0.36	0.89
Protein	3.14	3.18	3.14	0.01	0.92	0.03	0.08	<0.01
Lactose	4.75	4.75	4.76	0.01	0.32	0.73	0.67	<0.01
MUN, mg/dL	8.48[ab]	8.29[b]	8.86[a]	0.12	0.02	0.01	<0.01	0.10
ECM/DMI, kg/kg	1.77	1.77	1.80	0.01	0.10	0.53	0.18	<0.01
FCM/DMI, kg/kg	1.79	1.80	1.80	0.01	0.39	0.89	0.62	<0.01
BCS	2.95	2.90	2.96	0.01	0.39	<0.01	<0.01	0.04
BW, kg	698[a]	692[ab]	691[b]	2.19	0.01	0.20	0.02	0.19
BW change, kg/d	0.49[a]	0.33[ab]	0.26[b]	0.06	<0.01	0.41	0.01	-

[a-d]Means within a row differ with different superscripts ($P < 0.05$).
[1]Grams of metabolizable Lysine (Lys) per Mcal of metabolizable energy (ME)
[2]Week *P*-value < 0.01 for all variables
[3] FA sources classified as de novo < 16 carbon FA, mixed = 16-carbon and 17-carbon FA, and preformed ≥ 18 carbon FA

Conclusion

Increasing Lys supply tended to linearly increase ECM feed efficiency. Increasing Lys quadratically affected milk protein content where 3.05 g/Mcal ME increased milk protein content compared to 2.80 and 3.20 g/Mcal ME. Understanding the integration between nutrient supply though energy, AA, and FA, cell signaling, and gene expression is important for increasing milk component synthesis. Continued research is warranted to understand the effect of AA on gene expression, protein synthesis, and cell signaling related to milk component synthesis.

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Nutritional Benefits of Feeding Hempseed Meal to Laying Hens as a Sustainable Feed Protein Alternative

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Introduction

Industrial hemp (*Cannabis sativa* L.) is a versatile commercial crop with its materials being used in construction, clothing, and human consumption. Hempseed is produced after the oil extraction and hemp hearts processing from industrial hemp production (Kaur and Kander, 2023). With the recent passage of the 2018 Farm Bill, there has been increasing interest in incorporating industrial hemp production byproducts in animal agriculture, particularly in the New York State (NYS) for its sustainable agriculture initiative (Congressional Research Service, 2019; AAFCO, 2021). Hempseed is a nutritious ingredient with high levels of protein, dietary essential amino acids, and essential fatty acids, which can be a great substitute to soybean mean that is commonly used in the poultry industry (Bailoni et al., 2021; Montero et al., 2022; Rodriguez-Leyva and Pierce, 2010). Repurposing hempseed from landfill to animal agriculture as a valuable feed ingredient can reduce agricultural waste, decrease the food-feed competition, and ultimately promote environmental sustainability. However, despite the potential broad applications of hempseed in animal agriculture, its use in animal feed has not been approved by the Food and Drug Administration due to the lack of scientific evidence for the safety of animals and the food (Congressional Research Service, 2019). More recently in December 2023, NYS Senate Bill S6326 on authorizing the use of hempseed in commercial feed for pets, horses, and camelids was vetoed by Governor Hochul due to similar concerns on the safety of such uses (The New York State Senate, 2023). Trace amounts of total cannabidiol (CBD) and Δ-9 tetrahydrocannabinol (Δ-9 THC) were detected in the adipose tissue of beef heifers that were fed 20% hempseed cake for 111 days as well as in the plasma and urine of the animals (Smith et al., 2023). Therefore, further research is warranted to investigate the implications of feeding hempseed as an animal feed to animal health.

Materials and Methods

The objective of this study was to assess the impacts of supplementing hempseed meal (HSM) in laying hens' diets on animal health as well as egg production and egg nutrient profiles. The animal experiment protocol was approved by the Institutional Animal Care and Use Committee of Cornell University (Ithaca, NY). A total of 40 Dekalb White laying hens was fed a corn-soybean meal basal diet supplemented with HSM (IND HEMP, LLC, Fort Benton, MT) in a stepwise addition fashion at 5% increment (i.e., 0%, 5%, 10%, and 15%) for 6 weeks (n = 10 each for 4 groups). During the experiment, eggs were recorded and collected daily, feed was recorded and replenished weekly, and body weights of animals were measured biweekly. At weeks 0,

3, and 6, blood samples were drawn from the brachial wing veins from each animal. Egg yolk samples were freeze-dried, and the fatty acids were extracted according to the Folch method and quantified using a gas chromatography system (Agilent 6890N) with a flame ionization detector and a fused-silica capillary column (CP-Sil 88) as described previously (Ou et al., 2023). Data were analyzed by one-way ANOVA and followed by Duncan's multiple-ranged method as well as linear and quadratic regression models using R (version 4.1.3). Data were presented as means with standard error of the mean (SEM), and statistical significance was declared at $P < 0.05$.

Results

Supplementing HSM at up to 15% did not affect body weights or feed intake of the animals. Egg production and characteristics were also not impacted by the HSM supplementations. The HSM supplementations significantly enriched the eggs with the omega-3 polyunsaturated fatty acid (n-3 PUFA), docosahexaenoic acid (DHA; C22:6n-3), dose-dependently ($P < 0.001$, $R^2 = 0.93$) at week 6 (**Table 1**). Egg yolks from the 15% HSM treatment contained approximately 40 mg DHA per egg, which was 2-fold higher ($P < 0.001$) than the DHA content in the control egg yolks (18 mg DHA per egg). The other n-3 PUFA, α-linolenic acid (ALA; C18:3n-3), was also significantly increased ($P < 0.001$) in the egg yolks from the HSM treatments. The increases of n-3 PUFAs were associated with the decreases of some monounsaturated fatty acids, including the palmitoleic acid (C16:1n-7; $P < 0.001$). The n-6 PUFAs were not impacted by the HSM supplementations, including linoleic acid (LA; C18:2n-6) and arachidonic acid (ARA; C20:4n-6). Therefore, the overall n-6 to n-3 ratios of the eggs from the HSM treatments were improved ($P < 0.001$) compared with the control eggs. The supplementations of HSM also impacted the animals' metabolism and the greenhouse gases production from their manure. Hens from the 10% HSM group produced 14%-17% less ($P < 0.05$) respiratory carbon dioxide (CO_2) than the hens in the control and the 15% HSM treatment when placed in a respiratory chamber, but the difference was not significant ($P = 0.11$) after correcting for their metabolic body weights ($BW^{0.75}$). In the fecal samples, the HSM treatments decreased the methane (CH_4) production ($P < 0.01$, $R^2 = 0.35$), where fecal samples in the 15% HSM treatment had significantly lower ($P < 0.05$) methane production than those of the control.

Table 1. Effects of hempseed meal supplementations at incremental doses on selected fatty acid concentrations of egg yolk of hens at week 6.*

mg/g dried yolk	0%	5%	10%	15%	SEM	*P* value
Linoleic acid	28.2	28.8	29.6	27.6	0.56	0.67
α-linolenic acid	0.685^{c}	0.900^{b}	1.14^{a}	1.07^{ab}	0.046	< 0.001
Arachidonic acid	3.67	3.47	3.72	3.62	0.065	0.59
Docosahexaenoic acid	1.83^{d}	2.51^{c}	3.45^{b}	3.97^{a}	0.18	< 0.001
n-6/n-3 ratio	12.9^{a}	9.56^{b}	7.34^{c}	6.28^{d}	0.54	< 0.001

*Data are reported as means with standard error of the mean (SEM), $n = 6$. Values within the same row without a common letter are different, $P < 0.05$.

Summary and Conclusions

In conclusion, HSM supplementations at up to 15% in laying hens were safe and could enrich eggs with considerable amounts of health-promoting n-3 PUFAs. Further studies are warranted to explore the use of HSM in other livestock species as well as to confirm the potential CBD and Δ-9 THC residues in the animal products for both human and animal health. Additionally, HSM may be supplemented with other beneficial feed additives concurrently (such as microalgae) in diets to explore the full potentials of n-3 PUFAs and DHA enrichments.

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Effect of Feeding a Cold Extracted Cashew Nutshell Extract Product With Varying Concentrations of Starch and Sugar to Post-Peak Lactating Holstein Cows

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Introduction

The dairy industry globally is presently faced with the task of improving the productivity of dairy animals, especially cows, to meet the growing demand for dairy products. This must be done without the negative impact on the environmental footprint of the dairy industry via greenhouse gas emissions. Improving feed efficiency provides an avenue to tackle these problems, as increasing the production per cow can reduce emissions and improve profitability in dairy cattle production systems (Connor, 2015). Methane (CH_4) is a potent greenhouse gas which is a byproduct of enteric fermentation and manure storage in ruminant livestock production systems, and in a bid to reduce enteric CH_4 emissions from livestock agriculture, several strategies have been employed, such as diet modification and feed additive inclusion (Hristov et al., 2013). Feed additives have been researched as a promising solution, and there is a need for more research to ascertain feed additives that are economical, sustainable, and safe, without adverse effects on animal health and productivity.

Cashew nutshell extract (CNSE) is a by-product of cashew nut processing that contains anacardic acid which has antibacterial activity against gram-positive bacteria (Watanabe et al., 2010). In previous in-vitro studies, raw cashew nutshell liquid (CNSL) has been shown to enhance propionate production by up to 44% and exhibit anti-methanogenic effect by up to 70.1% in a dose-dependent manner (Watanabe et al., 2010), while another study reported up to 18% reduction in CH_4 production (Danielsson et al., 2014). However, an in-vitro study by Compton et al., (2023) did not observe any effect of CNSE on propionate production. The inclusion of CNSE in in-vivo animal studies showed up to 38% reduction in enteric CH_4 and an increase in propionate production when non-lactating Holstein cows were fed 4 g/100 kg of body weight per day of CNSL (Shinkai et al., 2012). When Branco et al., (2015) included technical-grade cashew nutshell liquid (TCNSL) in the diet of multiparous Holstein cows at 30 g/cow per day, they observed no effect of the treatment on CH_4 emissions, but there was a tendency for decreased CH_4 yield (g CH_4/kg dry matter intake) by up to 8%. Cold-extracted CNSE contains anacardic acid, while TCNSL, which is obtained after roasting the nut, contains little to no anacardic acid because it is lost in the heating process (Gandhi et al. 2012).

Higher sugar diets have been shown to increase dry matter intake (DMI) and milk fat content (Broderick et al., 2008; Firkins et al., 2008; Penner and Oba, 2009). A review of several animal studies by De Ondarza et al. (2017) showed that the inclusion of 6.8 – 8.0% dry matter (DM) dietary sugar in the diets of lactating dairy cows had the highest effect on fat-corrected milk yield and milk protein yield. A higher sugar inclusion (~15.9%

of diet DM) in cattle diets showed a decrease in the rate of ruminal neutral detergent fiber (NDF) digestion (Huhtanen and Khalili, 1991), and Heldt et al., (1999) reported a similar decrease in NDF digestion when sugar was supplemented in the diet at 0.3% per body weight per day. Penner et al. (2009) observed no effect of dietary sugar inclusion on total tract digestibility (TTD) of nutrients when they fed a low sugar diet (2.8 %) and a high sugar diet (5.7 %) to lactating Holstein cows. The review by De Ondarza et al., (2017) showed that the inclusion of dietary sugar did not have an effect on feed efficiency (kg of 3.5% FCM/kg of DMI). Studies that used 3-5 % of added dietary sugar had the highest mean efficiency (1.52) while the lowest mean efficiency value was obtained from studies that used 5-7 % of added dietary sugar. Hindrichsen et al. (2005) noted that there was no statistical difference in enteric CH_4 emissions when they fed six diets with dietary sugar ranging from 6.2 – 17.7 % to Brown Swiss dairy cows. This was supported by Staerfl et al. (2012) who observed no effect of feeding a high-sugar ryegrass containing 193 g/kg water-soluble carbohydrate (DM) on enteric CH_4 emissions in primiparous Holstein cows.

The objective of this study was to determine the effect of CNSE inclusion in diets with different starch to sugar (SS) ratios on feed intake, milk yield and composition, rumen fermentation, CH_4 emission, and TTD in post-peak lactating Holstein cows.

Methodology

A total of 88 lactating Holstein cows (28 primiparous and 60 multiparous) were enrolled across two enrollments. A randomized block design was used with a 2 by 2 factorial arrangement of treatments. Cows were enrolled at 114 ± 16 (standard deviation; SD) days in milk (DIM) and fed a common diet for the 3-wk covariate period. At the end of the covariate period cows were blocked by parity, DIM, and milk yield, and were assigned randomly to one of four treatment diets for 8 wk. The diets were formulated to evaluate the effect of two ratios of starch to sugar in the diet (SS) [i.e. low sugar (LSu) vs. high sugar (HSu)] and cold extracted nutshell extract (CON vs. CNSE). The treatment diets were:1) a high starch (26.8% of DM), low sugar (4.5% of DM) diet with no CNSE (LSu-CON), 2) a high starch (26.8% of DM), low sugar (4.5% of DM) diet with CNSE (LSu-CNSE), 3) a low starch (23.5% of DM), high sugar (7.8% of DM) diet with no CNSE (HSu-CON), and 4) a low starch (23.5% of DM), high sugar (7.8% of DM) diet with CNSE (HSu-CNSE).

The cold extracted CNSE product (SDS Biotech K.K., Tokyo, Japan) was incorporated into a corn meal carrier (0.45 kg) on a DM basis so that 5 g of cashew nutshell extract product was fed for a cow consuming 28.6 kg of dry matter intake (DMI) and was mixed using a stationary mixer (model V-1401; Hobart Manufacturing Company, Troy, OH). The mixed product was included into the diets at 1.59% of DM (Table 1). As a result, the actual amount of treatment provided was dependent on individual cow intake. The diets were formulated using a commercial ration formulation platform (AMTS.Cattle.Professional, Agricultural Modeling & Training systems, LLC, Groton, NY; version 4.18 with CNCPS biology (Cornell University, Ithaca, NY). The diets were devoid of monensin and the covariate period served as a washout for all cows.

		Starch/Sugar content	
		High Sugar (7.8%) Low Starch (23.5%)	Low Sugar (4.5%) High Starch (26.8%)
Cashew Nutshell Extract (CNSE)	None (control)	HSu-CON	LSu-CON
	Yes (CNSE)	HSu-CNSE	LSu-CNSE

Figure 1: Schematic of treatment assignment.

Feed ingredients were collected, composited, and analyzed for chemical composition (CNCPS Package; Cumberland Valley Analytical Services, Inc., Waynesboro, PA). Individual DMI was determined by recording feed offered and refused daily for each cow during the study. Milk yield was recorded electronically (ProVantage Information Management System; Bou-Matic, Madison, WI) at each milking (0430, 1230, and 2030 h) of the covariate and treatment periods. Milk samples from 6 consecutive milkings for each cow were collected weekly and were analyzed for fat, true protein, lactose (anhydrous), solids-not-fat, urea nitrogen, fatty acid groups, and chain length by mid-infrared procedures (LactoScope FTIR Advanced milk analyzer, Combi 300, Delta Instruments, Drachten, The Netherlands; Wojciechowski and Barbano, 2016; Wojciechowski et al., 2016; Woolpert et al., 2016). Body weight, expressed in kg, was measured (Allweigh computerized scale; Allweigh Scale System Inc., Red Deer, AB, Canada), and BCS was assigned in 0.25-unit increments on a 1 to 5 scale (Ferguson et al., 1994) prior to enrollment on the study, and weekly throughout the covariate and treatment periods. Fat corrected (4.0%; FCM) and energy corrected milk (ECM) were calculated by week. Feed efficiency (kg/kg) was calculated and expressed as milk/DMI, 4.0% FCM/DMI, SCM/DMI, and ECM/DMI.

Rumen fluid was sampled by esophageal tube from a subset of cows (12 cows per treatment) during wk 3 of the covariate period, and wk 4 and 8 of treatment period. The rumen fluid was analyzed for volatile fatty acids (VFA) concentration. Total tract digestibility was determined using samples of diets, orts, and feces during wk 3 of the covariate period and wk 8 of the treatment period. Enteric CH_4 emissions were measured continuously throughout the study. The cows had access to two GreenFeed systems (C-Lock Inc., Rapid City, SD) within the pen, and they were attracted to the machine with pelleted grain (Ultra 20% Dairy/Beef Pellet; Poulin Grain, Newport, VT).

Statistical analyses were performed using the Statistical Analysis System (version 9.4; SAS Institute Inc., Cary, NC). Data from the analysis of feed ingredients and diets were analyzed using the MEANS procedure of SAS and were reported as descriptive statistics (mean ± SD). Data from the cows were analyzed as a randomized block design with a 2 by 2 factorial arrangement of treatments. Cow was defined as the experimental

unit since the treatment was applied to each cow with an individual feeding bin. Data collected over time (e.g., intake, milk yield, milk composition, feed efficiency, and CH_4 gas emission) were reduced to a covariate period mean or a weekly mean during the treatment period. Data were checked for homogeneity of variance and normality assumptions using Levene and Shapiro-Wilk's test and subjected to analysis of covariance using the MIXED procedure of SAS (Littell et al., 1996). The model included a covariate and the fixed effects of SS, CNSE, time, and their interaction, and block within enrollment. Cow within block and enrollment were random effects in the model. Least squares means were reported, significance declared at $P \leq 0.05$, and trends discussed at $0.05 < P \leq 0.10$. The model for TTD and rumen fluid included a covariate and the fixed effects of SS, CNSE, and their interaction and block within enrollment.

Results

Diets and Feed Ingredients

The ingredient composition of the covariate and treatment diets fed during the first and second enrollments of the study are presented in Table 1. These diets contained ~60% forage from corn silage and alfalfa-grass silage. The corn silage source was changed from 2022 corn to 2023 corn during wk 3 of the treatment period for enrollment 2, and the ingredient formulation was adjusted to reflect this change based on the nutrient analysis of the new corn silage that was sampled before incorporation into the study diet. The average calculated chemical composition of the covariate and treatment diets is shown in Table 2 for both enrollments. The calculated chemical composition for the LSu diets were 26.3% starch and 3.6% sugar while the HSu diets were 23.6% starch and 5.9% sugar. These values were lower relative to the desired formulated composition for both diets; however, the overall relative difference was maintained as the goal of the formulation was to maintain the total amount of starch and sugar at 31.3% DM. The lower sugar values for the Hsu and LSu diets could have been due to the sugar content of the different ingredients used for the formulation. The NDF and the crude protein (CP) were similar among diets at 29.2 and 16.4% of DM, respectively.

Dry Matter and Nutrient Intake

Dry matter intake values are shown in Table 3. There was no effect ($P = 0.62$) of CNSE on DMI (kg/d), but a trend was observed ($P = 0.10$) for the effect of SS, with cows fed the LSu diets (27.3 kg/d) tending to have decreased DMI compared to cows fed the HSu diets (27.7 kg/d). There was an effect of SS ($P = 0.04$) on DMI as a percentage of body weight (% of BW), and a trend ($P = 0.06$) was observed for the effect of CNSE, with the LSu diets having a lower DMI % BW (average of 3.80% of BW) compared to the HSu diets (average of 3.86% of BW), and cows fed the CNSE diets tending to have lower DMI % BW (3.80% of BW) compared to cows fed the CON diets (3.86% of BW). Based on the inclusion of premix (1.59% of DM) and the average intake of 27.4 kg/d, cows consumed 4.79 g DM of CNSE/cow/d. The target was 5 g DM for an average intake of 28.6 kg/d.

Table 1. Ingredient composition (% of dry matter) of the covariate and treatment diets fed to lactating Holstein dairy cows during enrollments 1 and 2.

	Enrollment 1					Enrollment 2								
		Diets[1]					Diets[1,2]				Diets[2,3]			
Item	Covariate	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE	Covariate	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE
Conventional corn silage	44.44	41.27	41.27	41.27	41.27	41.34	41.28	41.28	41.27	41.27	41.23	41.23	41.27	41.27
Alfalfa-grass silage	15.87	19.05	19.05	19.05	19.05	19.08	19.05	19.05	19.05	19.05	19.03	19.03	19.05	19.05
Beet pulp	7.22	7.21	7.21	7.12	7.12	7.22	7.21	7.21	7.12	7.12	7.19	7.19	7.20	7.20
Steam flaked corn	5.49	5.87	5.87	3.28	3.28	5.88	6.83	6.83	4.57	4.57	5.80	5.80	4.08	4.08
CON premix[4]	1.59	1.59	-	1.59	-	1.59	1.59	-	1.59	-	1.58	-	1.59	-
CNSE premix[4]	-	-	1.59	-	1.59	-	-	1.59	-	1.59	-	1.58	-	1.59
Grain mix														
Soybean meal	6.90	6.90	6.90	5.52	5.52	6.91	7.18	7.18	8.29	8.29	6.90	6.90	7.63	7.63
Amino Max PGI[5,6]	3.09	3.09	3.09	3.05	3.05	3.09	1.79	1.79	1.37	1.37	1.10	1.10	0.96	0.96
Amino Enhancer PGI[6]	0.71	0.71	0.71	0.70	0.70	0.71	0.71	0.71	0.85	0.85	0.21	0.21	0.21	0.21
Soy hulls	1.44	1.44	1.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wheat middlings	0.00	0.00	0.00	2.10	2.10	0.00	0.00	0.00	0.00	0.00	3.53	3.53	1.99	1.99
Urea	0.14	0.34	0.34	0.40	0.40	0.29	0.39	0.39	0.42	0.42	0.39	0.39	0.52	0.52
Fine corn meal	3.89	4.14	4.14	1.82	1.82	6.22	5.39	5.39	3.04	3.04	4.14	4.14	2.67	2.67
Canola meal	2.82	2.75	2.75	4.94	4.94	2.76	3.53	3.53	3.77	3.77	4.33	4.33	5.08	5.08
Calcium Salt Fat[7]	1.00	1.00	1.00	0.99	0.99	1.00	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
Sugar	0.88	0.88	0.88	3.59	3.59	0.22	0.80	0.80	3.68	3.68	0.32	0.32	2.91	2.91
Cane molasses	0.76	0.00	0.00	0.87	0.87	0.00	0.00	0.00	0.77	0.77	0.00	0.00	0.63	0.63
C16 fat[8]	1.03	1.03	1.03	1.01	1.01	1.03	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Sodium sesquicarbonate	0.78	0.78	0.78	0.77	0.77	0.78	0.78	0.78	0.77	0.77	0.78	0.78	0.77	0.77

Calcium carbonate	0.89	0.89	0.89	0.88	0.88	0.89	0.89	0.89	0.88	0.88	0.89	0.89	0.88	0.88
Magnesium oxide	0.33	0.33	0.33	0.32	0.32	0.33	0.33	0.33	0.32	0.32	0.33	0.33	0.32	0.32
Salt	0.43	0.43	0.43	0.42	0.42	0.43	0.43	0.43	0.42	0.42	0.43	0.43	0.42	0.42
Trace min/vit mix[5,9]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Smartamine M[10]	0.08	0.08	0.08	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Smartamine ML[10]	0.05	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.04	0.04	0.05	0.05	0.04	0.04
Clarifly larvacide[11]	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
XPC Yeast Culture[12]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[1]High starch (26.8% DM)-low sugar (4.5% DM) and no cashew nutshell extract product (LSu-CON), high starch (26.8% DM)-low sugar (4.5% DM) with cashew nutshell extract product (LSu-CNSE), low starch (23.5% DM)-high sugar (7.8% DM) and no cashew nutshell extract product (HSu-CON), and low starch (23.5% DM)-high sugar (7.8% DM) with cashew nutshell extract product (HSu-CNSE).

[2]Conventional corn silage 2022 crop year, the formulation was fed from wk 1 to 2 of the treatment period.

[3]Conventional corn silage 2023 crop year, the formulation was fed from wk 3 to 8 of the treatment period. Water was added to each diet from wk 4 to 8 to maintain a target DM of 46%.

[4]CON premix was corn meal only, CNSE premix was cold extracted cashew nutshell extract product (SDS Biotech K.K., Tokyo, Japan) incorporated into a corn meal carrier (0.45 kg) on a DM basis so that 5 g of cashew nutshell extract product was fed for a cow consuming 28.6 kg of dry matter intake (DMI).

[5]Poulin Grain Inc., Newport, VT.

[6]Afgritech, LLC, Watertown, NY.

[7] Darby Trading Inc., Purchase, NY.

[8]Harbor Point Minerals, Utica, NY.

[9]Micronutrients, Indianapolis, IN

[10]Adisseo USA Inc., Alpharetta, GA.

[11]Central Garden and Pet Company, Schaumburg, IL

[12]Diamond V, Cedar Rapids, IA.

Table 2. Calculated chemical composition of the diets fed to lactating Holstein dairy cows during the 3-wk covariate and 8-wk treatment periods (dry matter basis, unless otherwise noted; mean ± standard deviation).

	Diets[1]				
Item	Covariate	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE
n	2	9	9	9	9
Dry matter, %[2]	46.1±4.4	47.4±3.5	47.6±3.6	47.4±3.2	47.4±3.8
Crude protein (CP), %	15.6±0.1	16.4±0.2	16.2±0.3	16.4±0.5	16.4±0.5
Soluble protein, % CP	5.9±0.3	6.4±0.3	6.4±0.3	6.3±0.5	6.3±0.5
Neutral detergent CP, %	1.4±0.1	1.6±0.1	1.6±0.2	1.7±0.2	1.7±0.2
Acid detergent fiber, %	18.2±1.1	18.5±0.3	18.8±1.0	18.4±0.5	18.4±0.5
Neutral detergent fiber (NDF), %	29.0±0.9	29.3±1.0	29.8±1.5	29.2±0.8	29.2±0.8
Acid detergent lignin, %	3.3±0.5	3.0±0.2	3.1±0.3	3.2±0.2	3.2±0.2
Nonfiber carbohydrates, %	43.0±0.3	43.2±0.7	43.1±0.8	43.4±1.0	43.4±1.0
Nonstructural carbohydrates, %	30.2±0.0	30.0±0.9	29.7±0.9	29.5±1.0	29.5±1.0
Starch, %	25.8±0.4	26.4±0.9	26.2±0.9	23.6±1.0	23.6±1.0
Sugar (ESC[3]), %	4.4±0.4	3.6±0.3	3.5±0.3	5.9±0.5	5.9±0.5
Ether extract, %	4.7±0.5	4.7±0.6	4.7±0.6	4.6±0.6	4.6±0.6
Ash, %	8.1±0.5	7.9±0.4	7.9±0.4	8.1±0.4	8.1±0.4
Calcium, %	0.97±0.05	0.94±0.05	0.92±0.08	0.94±0.06	0.94±0.06
Phosphorus, %	0.32±0.00	0.33±0.02	0.33±0.02	0.34±0.02	0.34±0.01
Magnesium, %	0.42±0.01	0.40±0.02	0.39±0.02	0.44±0.03	0.44±0.03
Potassium, %	1.10±0.04	1.17±0.05	1.19±0.07	1.19±0.05	1.19±0.05
Sulfur, %	0.21±0.02	0.22±0.02	0.22±0.01	0.23±0.01	0.23±0.01
Sodium, %	0.45±0.02	0.46±0.04	0.44±0.07	0.47±0.02	0.47±0.02
Chloride ion, %	0.51±0.00	0.51±0.09	0.51±0.08	0.53±0.06	0.53±0.06
Iron, mg/kg	201±9	197±23	198±23	191±10	192±10
Copper, mg/kg	15±0	17±5	17±5	16±2	16±2
Manganese, mg/kg	48±1	50±4	50±3	54±4	54±4
Zinc, mg/kg	58±5	57±3	56±5	62±5	62±5
Net energy for lactation, Mcal/kg	1.64±0.00	1.67±0.02	1.67±0.03	1.66±0.02	1.66±0.02

[1]High starch (26.8% DM)-low sugar (4.5% DM) and no cashew nutshell extract product (LSu-CON), high starch (26.8% DM)-low sugar (4.5% DM) with cashew nutshell extract product (LSu-CNSE), low starch (23.5% DM)-high sugar (7.8% DM) and no cashew nutshell extract product (HSu-CON), and low starch (23.5% DM)-high sugar (7.8% DM) with cashew nutshell extract product (HSu-CNSE).

[2]Dry matter (105°C) for diets collected during the collection period for each period, n=16 for covariate and 48/treatment diet.

[3]Ethanol soluble carbohydrates.

Milk Yield, Composition, and Feed Efficiency

Milk yield and milk composition data are shown in Table 3. There was no effect of SS or CNSE on milk yield (kg/d; $P > 0.40$). There was an effect of SS ($P = 0.04$) on milk fat concentration with the HSu diets having a higher milk fat concentration compared to the LSu diets (4.05 vs 3.97%, respectively). There was a tendency for a time and CNSE interaction on milk fat concentration ($P = 0.06$). There was no effect of SS on milk true protein ($P = 0.68$), lactose ($P = 0.12$), or SNF ($P = 0.22$). Cows fed CNSE had a lower lactose concentration compared to cows fed CON (4.63 vs 4.68%, respectively; $P = 0.01$). Milk urea nitrogen (MUN; mg/dL) was not affected by CNSE (P = 0.82) but there was an effect of SS ($P = 0.02$) with cows fed the LSu diets having a lower MUN (11.29 mg/dL) compared to cows fed the HSu diets (11.72 mg/dL). De novo fatty acid (FA) and preformed FA were not affected ($P > 0.10$) by SS. There was an interaction effect between SS and CNSE on FCM/DMI ($P = 0.05$), SCM/DMI ($P = 0.02$), ECM/DMI ($P = 0.02$).

Rumen Fermentation

Rumen fermentation data are shown in Table 4. There was a tendency for an effect of SS during wk 4 for total VFA concentration with cows fed the HSu diets having a lower total VFA concentration (143 mM) compared to cows fed the LSu diets (151 mM). Total VFA concentration was not affected by CNSE (P > 0.51), or SS on wk 8 ($P = 0.76$). There was a tendency ($P = 0.10$) for an interaction of SS and CNSE during wk 8 with cows fed the HSu-CNSE diet having the highest butyrate concentration, whereas the cows fed the CON diets were intermediate to cows fed the LSu-CNSE diet. There were no other interaction effects of SS and CNSE on any of the other rumen fermentation variables ($P > 0.10$). There was no effect main effect of SS or CNSE on acetate on wk 4 ($P >0.39$) and wk 8 ($P >0.16$). The was an effect of CNSE during wk 8 on propionate ($P = 0.02$). Cows fed the CNSE diets had a lower average propionate (24.9% of total VFA) compared to cows fed the CON diets with an average propionate of 26.6% of total VFA. There was a tendency for the effect of SS on butyrate during wk 8 ($P = 0.10$), with cows fed the HSu diets having a higher average butyrate (15.2% of total VFA) compared to cows fed the LSu diets (14.4% of total VFA). There was an effect of SS on isovalerate during wk 4 ($P = 0.05$), where cows fed the HSu diets had a lower value (1.92% of total VFA) compared to cows fed the LSu diets (2.28% of total VFA).

Total tract digestibility data are presented in Table 5. There was no interaction effect of SS and CNSE on any of the total tract nutrient digestibility variables ($P > 0.10$). There was an effect of SS on DM digestibility ($P = 0.01$), organic matter digestibility ($P = 0.01$), and aNDFom digestibility ($P < 0.001$), but starch digestibility was not affected by SS ($P = 0.23$). The average digestibility values for cows fed the LSu diets were higher compared to cows fed the HSu diets for DM digestibility (79.5 vs 77.1% of DM), organic matter digestibility (80.8 vs 78.7 % of DM), and aNDFom digestibility (63.2 vs 56.7% of DM). The starch digestibility for all diets was high and not different across all diets (99.5% of DM). There was no effect of CNSE on total tract digestibility ($P > 0.30$).

Table 3. Dry matter intake (DMI), milk yield and composition, body weight (BW), and body condition score (BCS) in lactating Holstein dairy cows (n = 86) fed the treatment diets during the treatment period.

	Treatment[1]					*P*-value			
Item	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE	SEM	Block (Enrollment)	SS	CNSE	SS × CNSE
TMR DMI, kg/d[2]	26.6	26.7	27.3	27.0	0.2	<0.01	0.03	0.68	0.40
GF DMI, kg/d[3]	0.7	0.6	0.5	0.5	0.0	0.43	<0.01	0.73	0.86
DMI, kg/d[4]	27.3	27.3	27.9	27.5	0.2	<0.01	0.10	0.62	0.38
DMI, % of BW	3.81	3.78	3.90	3.81	0.03	<0.01	0.04	0.06	0.28
Milk, kg/d	45.3	44.6	45.0	44.7	0.6	<0.01	0.79	0.40	0.71
Milk/DMI, kg/kg	1.66	1.64	1.62	1.62	0.01	<0.01	0.03	0.52	0.22
ECM[5], kg/d	48.9	48.0	48.8	48.8	0.6	<0.01	0.56	0.44	0.43
ECM/DMI, kg/kg	1.80	1.76	1.75	1.77	0.01	<0.01	0.27	0.46	0.02
FCM[6], kg/d	44.8	44.0	44.9	44.9	0.5	<0.01	0.39	0.44	0.48
FCM/DMI, kg/kg	1.65	1.61	1.61	1.63	0.01	<0.01	0.48	0.47	0.05
SCM[7], kg/d	45.3	44.2	45.0	44.9	0.6	<0.01	0.70	0.32	0.39
SCM/DMI, kg/kg	1.67	1.62	1.62	1.63	0.01	<0.01	0.19	0.25	0.02
Milk									
Fat, %	3.98	3.95	4.03	4.07	0.04	0.01	0.04	0.95	0.35
Fat, kg/d	1.78	1.74	1.79	1.80	0.02	<0.01	0.13	0.55	0.38
True protein, %	3.20	3.16	3.16	3.18	0.02	<0.01	0.68	0.73	0.09
True protein, kg/d	1.44	1.41	1.41	1.42	0.02	<0.01	0.72	0.56	0.34
Lactose, %	4.71	4.63	4.65	4.62	0.02	<0.01	0.12	0.01	0.21
Lactose, kg/d	2.14	2.07	2.09	2.07	0.03	<0.01	0.48	0.12	0.44
SNF, %	9.02	8.91	8.92	8.93	0.03	<0.01	0.22	0.13	0.09
SNF, kg/d	4.09	3.97	4.01	3.99	0.05	<0.01	0.54	0.23	0.37
MUN, mg/dL	11.40	11.17	11.56	11.88	0.20	0.62	0.02	0.82	0.16
De novo FA[8], g/100 g milk	1.04	1.02	1.06	1.07	0.01	<0.01	<0.01	0.72	0.28
De novo FA, g/100 g FA	27.6	27.2	27.7	27.6	0.2	0.01	0.18	0.13	0.36
Mixed origin FA, g/100 g milk	1.50	1.49	1.52	1.53	0.01	<0.01	0.01	0.91	0.39
Mixed origin FA, g/100 g FA	39.8	39.6	39.5	39.5	0.2	<0.01	0.35	0.66	0.74
Preformed FA, g/100 g milk	1.23	1.22	1.23	1.24	0.02	<0.01	0.57	0.99	0.63
Preformed FA, g. 100 g FA	33.0	32.6	32.2	32.0	0.3	<0.01	0.02	0.31	0.67
Chain length, carbons/FA	14.35	14.24	14.17	14.21	0.04	0.03	0.01	0.40	0.06
BW, kg	717	722	718	723	3	0.23	0.77	0.06	0.93
BW change, kg (wk -1 to 8)	11	18	17	13	4.04	0.08	0.93	0.73	0.12
BCS	2.93	2.98	2.93	2.97	0.01	0.03	0.89	0.01	0.86
BCS change (wk -1 to 8)	-0.01	0.10	0.07	0.08	0.03	0.76	0.46	0.14	0.22

[1]High starch (26.8% DM)-low sugar (4.5% DM) and no cashew nutshell extract product (LSu-CON), high starch (26.8% DM)-low sugar (4.5% DM) with cashew nutshell extract product (LSu-CNSE), low starch (23.5% DM)-high sugar (7.8%

DM) and no cashew nutshell extract product (HSu-CON), and low starch (23.5% DM)-high sugar (7.8% DM) with cashew nutshell extract product (HSu-CNSE).
[2]DMI of the total mixed ration (TMR).
[3]DMI of the GreenFeed pellet
[4]Total DMI of the TMR and GreenFeed pellet
[5]Energy-corrected milk.
[6]4.0 % Fat-corrected milk.
[7]Solids-corrected milk.
[8]Fatty acids.

Table 4. Least squares means of rumen fermentation data in lactating Holstein dairy cows (n = 47) fed the treatment diets during the treatment period.

	Treatment[1]					*P*-value			
Item	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE	SEM	Block (Enrollment)	SS	CNSE	SS × CNSE
Week 4									
n	12	12	11	12					
Total volatile fatty acids (VFA), m*M*	152.9	149.5	138.1	147.9	4.7	0.12	0.09	0.51	0.17
VFA, % of total VFA									
Acetate (A)	53.8	55.6	54.8	54.4	0.9	0.08	0.90	0.39	0.24
Propionate (P)	26.7	25.4	25.7	26.3	0.8	0.03	0.97	0.66	0.30
Butyrate (B)	14.4	13.7	14.7	14.3	0.5	0.43	0.35	0.26	0.71
Isobutyrate	0.67	0.70	0.64	0.65	0.02	0.06	0.09	0.32	0.63
Valerate	2.34	2.19	2.32	2.32	0.07	0.11	0.42	0.27	0.31
Isovalerate	2.23	2.33	1.75	2.08	0.19	0.25	0.05	0.31	0.51
A:P	2.07	2.22	2.16	2.12	0.10	0.04	0.98	0.56	0.38
A+B:P	2.62	2.75	2.74	2.68	0.11	0.02	0.85	0.75	0.40
Week 8									
n	12	11	11	12					
Total VFA, m*M*	154.0	147.7	145.7	152.6	5.4	0.65	0.76	0.95	0.24
VFA, % of total VFA									
A	53.2	55.4	54.1	54.1	0.7	0.12	0.81	0.16	0.16
P	27.1	24.8	26.0	24.9	0.7	0.02	0.47	0.02	0.39
B	14.6	14.2	14.6	15.7	0.4	0.28	0.10	0.43	0.10
Isobutyrate	0.69	0.72	0.68	0.69	0.03	0.70	0.46	0.50	0.80
Valerate	2.37	2.27	2.34	2.47	0.07	0.16	0.26	0.87	0.16
Isovalerate	2.23	2.54	2.06	2.20	0.19	0.09	0.21	0.25	0.67
A:P	2.00	2.28	2.12	2.18	0.08	0.02	0.88	0.04	0.20
A+B:P	2.55	2.85	2.69	2.81	0.09	<0.01	0.54	0.02	0.31

[1]High starch (26.8% DM)-low sugar (4.5% DM) and no cashew nutshell extract product (LSu-CON), high starch (26.8% DM)-low sugar (4.5% DM) with cashew nutshell extract product (LSu-CNSE), low starch (23.5% DM)-high sugar (7.8% DM) and no cashew nutshell extract product (HSu-CON), and low starch (23.5% DM)-high sugar (7.8% DM) with cashew nutshell extract product (HSu-CNSE).
Total Tract Nutrient Digestibility

Table 5. Total tract digestibility data of lactating Holstein dairy cows (n = 46) fed the treatment diets during the treatment period.

	Diets[1]					*P*-value			
Variable	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE	SEM	Block (Enrollment)	SS	CNSE	SS × CNSE
Dry matter (DM), %	79.0	80.0	77.4	76.8	0.85	<0.01	0.01	0.83	0.35
Organic matter, % of DM	80.4	81.1	78.9	78.4	0.78	<0.01	0.01	0.92	0.41
aNDFom[2], % of DM	62.1	64.3	56.2	57.1	1.58	<0.01	<0.01	0.34	0.69
Starch, % of DM	99.5	99.4	99.5	99.5	0.08	<0.01	0.23	0.70	0.45

[1]High starch (26.8% DM)-low sugar (4.5% DM) and no cashew nutshell extract product (LSu-CON), high starch (26.8% DM)-low sugar (4.5% DM) with cashew nutshell extract product (LSu-CNSE), low starch (23.5% DM)-high sugar (7.8% DM) and no cashew nutshell extract product (HSu-CON), and low starch (23.5% DM)-high sugar (7.8% DM) with cashew nutshell extract product (HSu-CNSE).
[2]Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.

Methane Emissions

The CH_4 emissions data are shown in Table 6. There was no effect of SS, CNSE, or an interaction between SS and CNSE on CH_4 emissions (g/d), CH_4 yield (g/DMI), and CH_4 intensity (g/ECM; $P > 0.10$). There was an effect of time on CH_4 yield ($P = 0.03$), CH_4 intensity ($P = 0.01$), and a tendency for time on CH_4 emissions ($P = 0.10$).

Summary

This study evaluated the effect of the inclusion of cold extracted cashew nut extract (CNSE) in diets with different starch to sugar ratios (SS) on lactation performance, rumen fermentation, total tract digestibility (TTD), and enteric CH_4 emission in post-peak lactating Holstein cows. The results showed that the cows fed the high sugar (HSu) diets had a higher dry matter intake (DMI; 27.3 kg/d) compared to cows fed the low sugar (LSu) diets (27.7 kg/d). The inclusion of CNSE did not have any effect on DMI. Cows fed the LSu diets had a higher DMI % BW (average of 3.80%) compared to those fed the HSu diets (average of 3.86%), and cows fed the CNSE diets tended to have lower intake as a percent of BW (average of 3.80%) compared to cows fed the CON diets (average of 3.86%). There was no effect of SS (LSu and HSu) or the feed additive (CNSE and CON) on milk yield, with cows producing an average of 44.9 kg/d. The HSu cows had a higher fat concentration (4.05%) compared to the LSu cows (3.97%). There was no effect of CNSE on TTD, but there was an effect of SS on dry matter (DM) digestibility, organic matter digestibility (OM), and neutral detergent fiber (aNDFom) digestibility, with cows fed the LSu diets having higher DM, OM, and aNDFom digestibility compared to cows fed the HSu diets (79.9 %, 81.1 %, and 63.1 % vs 77.4 %, 78.9 %, and 56.5 % for DM, OM, and aNDFom digestibility, respectively) There was no effect of SS or CNSE on CH_4 emissions. The high sugar diets (5.9% of DM) improved DMI and milk fat concentration, while the inclusion of CNSE tended to enhance body weight gain (722.5 vs 717.5 kg for CNSE and

CON diets, respectively) and body condition score (2.98 and 2.93 for CNSE and CON diets, respectively).

Table 6. Methane (CH_4) emission data of lactating Holstein dairy cows (n = 84) fed with the treatment diets during the treatment period.

	Treatment					*P*-value			
Item	LSu-CON	LSu-CNSE	HSu-CON	HSu-CNSE	SEM	Block (Enrollment)	SS	CNSE	SS × CNSE
n	20	22	20	22					
CH_4 g/d	426	442	435	438	7	0.04	0.73	0.18	0.32
CH_4 yield (g/kg DMI[1])	15.8	16.3	16.0	16.1	0.3	<0.01	0.98	0.26	0.46
CH_4 intensity (g/kg ECM[2])	8.9	9.4	9.2	9.2	0.2	<0.01	0.76	0.19	0.13

[1]High starch (26.8% DM)-low sugar (4.5% DM) and no cashew nutshell extract product (LSu-CON), high starch (26.8% DM)-low sugar (4.5% DM) with cashew nutshell extract product (LSu-CNSE), low starch (23.5% DM)-high sugar (7.8% DM) and no cashew nutshell extract product (HSu-CON), and low starch (23.5% DM)-high sugar (7.8% DM) with cashew nutshell extract product (HSu-CNSE).
[2]Dry matter intake.
[3]Energy-corrected milk.

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Transition Cow: Immune Suppression or a State of Immune Robustness?

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Introduction

Immunosuppression is traditionally considered a hallmark of transition cow biology and the ascribed liability for subsequent infectious and inflammatory conditions disproportionately affecting periparturient cows. The metabolic profile dominating the peripartum period (i.e., hypocalcemia, increased non-esterified fatty acids [NEFA] and ketones) is thought causal to immune dysfunction that inherently leads to increased morbidity and susceptibility to infectious diseases. However, there is now a better understanding of inflammation's role in circulating leukocyte dynamics, and new evidence stemming from controlled experimentation lead us to suggest transition cows are not immune-suppressed or dysregulated, but rather are capable of engaging a robust immune response.

Immunosuppression

Traditional Paradigm

Lloyd (1983) originally defined immune suppression as "immunological unresponsiveness manifest as an increased susceptibility to infection and/or a recrudescence of infection around pregnancy and lactation". The immunosuppression posit is often supported by experiments isolating circulating leukocytes from peripartum cows and identifying reduced functionality *ex vivo* (i.e., decreased oxidative burst, adhesion and migration, cytotoxicity, antibody secretion, etc.; Kehrli et al., 1989; Lee and Kehrli, 1998; Lacetera et al., 2005). Further, postpartum animals affected by mastitis or metritis display a more severe peripheral leukocyte functional deficit (Loftstedt et al., 1983; Cai et al., 1994; Kim et al., 2005). Overall, reports suggest impaired immune functions (typically corresponding with microbial killing) lead to increased morbidity risk and susceptibility to infections during the transition period.

Limitations of the Dogma

Collecting circulating leukocytes from transition cows and evaluating their *ex vivo* functionality is an incredibly powerful technique that has provided an enormous amount of valuable information. This procedure has laid the foundation for our understanding of periparturient immunobiology and scholars like Sheelagh Lloyd (University of Cambridge), Mark Kehrli (NADC in Ames Iowa), and James Roth (Iowa State University) have thoroughly and eloquently established a biological rationale for why transition cows are more likely to have an adverse health event. However, our understanding of how leukocytes traffic in and out of the circulating pool has recently (within the last decade) become more advanced. We now know that this *ex vivo* leukocyte analysis approach has

limitations, especially post-calving where a kinetic shift in neutrophil trafficking occurs. Peripheral mature leukocytes extravasate to tissues in response to chemical recruiting signals at infection sites or tissue remodeling (i.e., uterus and mammary gland). In response to inflammation, the bone marrow replenishes the pool with immature neutrophils that are not yet fully developed and thus have altered functionality (Shynlova et al., 2013; Abuelo et al., 2023). This has profound implications for our understanding of periparturient "immune suppression". All transition cows are inflamed, which stems from sterile inflammation associated with parturition, placenta expulsion and uterine involution AND pathogenic inflammation arising from subclinical and clinical metritis, mastitis, and gastroenteritis (Horst et al., 2021). This temporary inflammatory state then triggers the bone marrow to release immature leukocytes and this is simultaneously coupled with circulating mature neutrophils infiltrating into inflamed tissues; a collective scenario that markedly alters the "functionality" of circulating neutrophils obtained during the peripartum period. An explanation for how even healthy periparturient animals express reduced peripheral leukocyte functions relative to other stages of lactation and how postpartum cows encountering increased inflammation such as mastitis or metritis have a more severe "left shift" (higher immature neutrophil populations as a consequence of immune activation; Stockham and Scott, 2008) in neutrophil composition and functionality than their healthy counterparts is described in Figure 1.

Metabolites as Suspected Immunosuppressants

Several causal factors of peripartal immune dysfunction have been proposed and negative energy balance (NEBAL) and its ensuing metabolic consequences including increased NEFA, hyperketonemia, and the associated mineral adjustments of lactation onset (hypocalcemia) are primarily speculated (Kehrli et al., 2006; Ingvartsen and Moyes, 2013; Abuelo et al., 2023). We have argued that these metabolic footprints are either normal and homeorhetic adaptations healthy cows enlist to produce milk or consequences of immune activation (Horst et al., 2021). *Ex vivo* functional assays including oxidative burst, antibody and cytokine secretion, activation, or phagocytosis decrease when incubating leukocytes with elevated ketones, NEFA, or reduced Ca concentrations (Hoeben et al., 1997; Suriyasathaporn et al., 1999; Ducusin et al., 2003; Lacetera et al., 2004; Ster et al., 2012). However, others report little to no consequences to proliferation or phagocytosis (Franklin et al., 1991; Scalia et al., 2006), and some even report elevated leukocyte adhesion, cytokine production, or oxidative burst (Scalia et al., 2006; Zhang et al., 2006; Zhang et al., 2018) with similar *ex vivo* conditions. The inconsistencies in the periparturient leukocyte function literature (reviewed by LeBlanc, 2020) shed light on the dogma's limitations, as a solid foundation of any tenet should be a consistent scientific pattern.

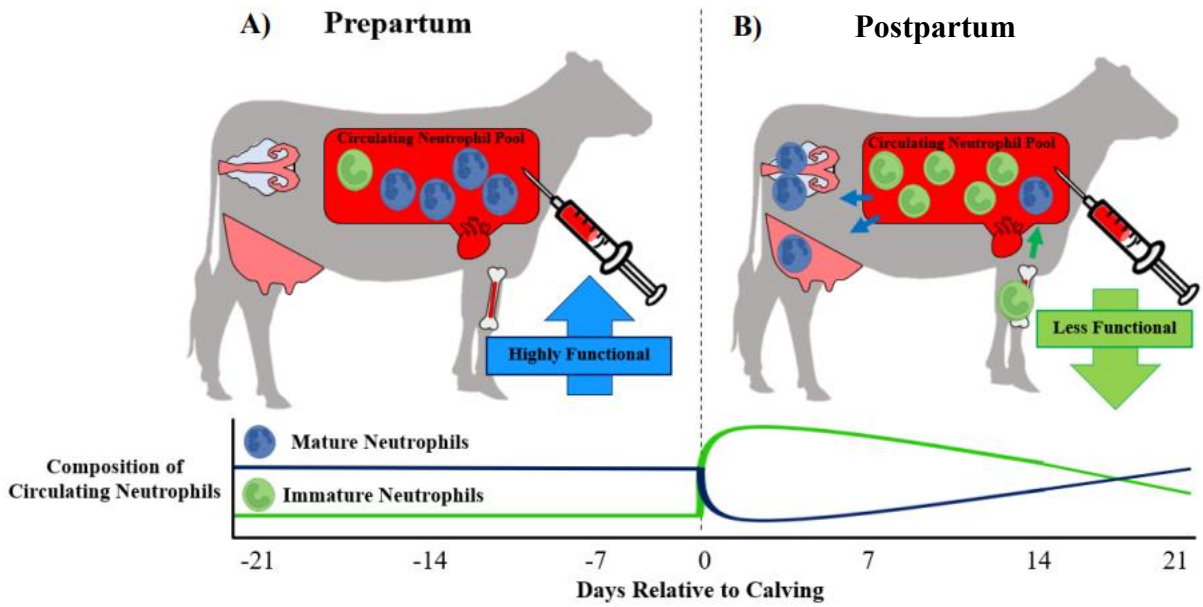

Figure 1. Parturient alterations in neutrophil maturity and function. Before parturition (A), circulating neutrophil composition is primarily comprised of mature populations and are highly functional when evaluated under ex vivo conditions. Postpartum (B), circulating neutrophils migrate to extravascular tissues to facilitate uterine involution and lactogenesis (Shynlova et al., 2013). Immature neutrophils replenish the circulatory pool and their proportion in peripheral neutrophil composition increases (Stockham and Scott, 2008); however, they are still developing and not yet fully functional (McKenna et al., 2021). As a result, postpartum peripheral leukocytes may appear immunosuppressed when functionality of peripheral leukocytes is assessed under ex vivo conditions.

Further, *in vitro* assays typically maintain tightly controlled conditions, a technical environment unable to replicate dynamic *in vivo* adjustments in hormonal signaling and metabolism. For instance, *in vivo* immune activation increases hepatic glucose output (Lang et al., 1985), which provides fuel for the metabolic switch of resting immune cells that primarily rely on oxidative phosphorylation, to activated immune cells that preferentially undergo aerobic glycolysis (the Warburg effect; Srivastava and Mannam, 2015). This altered method of generating ATP is accompanied with a very large insulin surge (Kvidera et al., 2017). Under *in vitro* conditions with increasing NEFA concentrations and limited glucose, energetically expensive effector functions evolutionarily designed to be fueled by aerobic glycolysis may be restrained; as metabolic pathways dictate immune programming (Ganeshan and Chawla, 2014; Olenchock et al., 2017). In support, some reports of decreased functions of leukocytes *in vitro* with media containing high BHB concentrations disagree with results indicating no impairments to leukocytes isolated from hyperketonemic transition cows or ruminants in NEBAL (Perkins et al., 2001; Schulz et al., 2014). In other words, *in vitro* assays lack the hormonal and metabolic temporal fluctuations that occur *in vivo* and thus force the activated leukocyte to burn a fuel it would not naturally. Further, *in vitro/ex vivo* experiments can widely vary by methods (e.g., metabolite concentrations or media components) and often only isolate one leukocyte type (neutrophils vs. monocytes vs. lymphocytes) or circulating peripheral

cells; specific results may precariously lead to oversimplifying the status of the entire immune system – which is especially dynamic and purposeful during the transition period.

The Role of Immune Activation

While some peripheral immune cell functions appear to be reduced postpartum, increasing evidence points to a distinctly active inflammatory profile (enhanced circulating pro-inflammatory cytokines and leukocyte activation from stimuli; Jahan et al., 2015; Trevisi and Minuti, 2018). We now know all transitioning dairy cows experience some degree of inflammation around calving, and the extent and length of inflammation appears to be indicative of transition cow performance (Trevisi et al., 2002; Bertoni et al., 2008; Trevisi and Minuti, 2018). During the weeks surrounding calving, cows are exposed to a myriad of stressors which may permit pathogen associated molecular patterns (PAMPs) entry into local and systemic circulation and thereby initiate an inflammatory response (Khafipour et al., 2009; Kvidera et al., 2017; Proudfoot et al., 2018; Koch et al., 2019). Further, other investigators suggest sterile inflammation contributes to the altered immune state of transition cows. Hypothesized sources include adipocyte lipolysis (Contreras et al., 2017; Pascottini et al., 2020), tissue trauma from dystocia (Benzaquen et al., 2015), or parturition (Negishi et al., 2020). Unlike pathogenic inflammation, the consequences of sterile inflammation on productive performance in the ruminant (i.e., through inducing hypophagia) are less clear, so its relevance to transition period management remains uncertain. Sources of sterile inflammation excluding normal pregnancy and parturition remain questionable, however, because even the healthy reproductive tract has a microbiome (i.e., is not sterile; Santos et al., 2011) – particularly after parturition during which uterine bacterial contamination occurs in most cows (Paisley et al., 1986). Further, microbe-derived inflammation increases tissue damage and gives rise to damage associated molecular pattern (DAMPs) sterile inflammatory (Ekaney et al., 2014), so it is difficult to disentangle sterile and pathogenic inflammatory sources. Collectively, transition cows are inflamed, and depending on the source, severity, and promptness of resolution (Bertoni et al., 2015), it may alter circulating populations of mature and immature leukocyte that contribute to the distinct *ex vivo* immune status of periparturient cows.

We speculate that transition cows are more susceptible to disease not due to a disadvantaged immune system by the metabolic profile of lactation onset and NEBAL, but because they are at an increased risk of exposure to lipopolysaccharide (LPS) and other PAMPs via uterine, mammary, and intestinal epithelial hyperpermeability from parturition, lactogenesis, and dietary and social changes (Eckel and Ametaj, 2016; Horst et al., 2021). Interestingly, the literature often implies that postpartum cows have reduced toll-like receptor (TLR)-4 expression (indicating LPS tolerance; Nomura et al., 2000) in rumen epithelial cells, milk somatic cells, and peripheral blood mononuclear cells (Catalini et al., 2010; Cui et al., 2013; Mukherjee et al., 2013; Minuti et al., 2015). Some have considered LPS tolerance – developed after repeated exposure to LPS over time - protects against infections or resulting immune activation (Wheeler et al., 2008; Petzl et al., 2012), whereas others speculate it slows the immune response (López-Collazo and del Fresno, 2013; Pena et al., 2014). Further, several dairy investigators have suggested LPS tolerance may occur in periparturient cows (Zebeli et al., 2011; Eckel and Ametaj,

2016; Filipe et al., 2021). We hypothesized that transition cows could develop LPS tolerance from heightened exposure, which could explain, at least in part, some of their ostensibly reduced immune responses relative to cows later in lactation and contribute to their increased vulnerability to infections; a rationale that starkly contrasts from the traditional metabolic stress paradigm.

Immune Activation in Response to LPS in Early Lactation

Properly conducting periparturient *in vivo* experiments targeting the immune system is difficult because the physiology, metabolism, endocrinology, and health status (and ensuing inflammation) are all highly variable during this unique stage of lactation. Despite the challenges, we have recently conducted two controlled and intervening experiments that directly compare the immune response in transition vs. mid-lactation dairy cows.

We administered an acute i.v. LPS dose (0.09 μg/kg body weight) in both early (EL; 20 ± 2 DIM) and mid-lactation (ML; 131 ± 31 DIM) multiparous dairy cows to compare their immune, metabolic, and production response in the 3 d following LPS administration, hypothesizing EL cows would respond to LPS in a manner consistent with LPS tolerance: reduced immune responsiveness and attenuated consequences on metabolism and production (Opgenorth et al., 2024a,b; Figure 2). As anticipated, LPS caused an inflammatory response in both lactation stages. However, the vast majority of immune and inflammatory parameters measured were actually more robust in EL cows, including the febrile response, haptoglobin and LPS-binding protein, IL-6, tumor necrosis factor-α, and several chemokines, as well as the extent of neutrophilia and monocytosis. The data indicate that EL cows were not more LPS tolerant than ML cows, but were fully capable of engaging a robust immune response. Clearly, our tenet could not have been more wrong, and reasons why EL cows had such a heightened immune activation toward LPS are not obvious. Irrespective of the mechanism(s), many aspects of the immune system are highly sensitive to endotoxin in early lactation and are actually incredibly robust; having a better appreciation for these lactation stage differences will likely have important implications for transition cow management, nutrition, and veterinary care.

Immune activation in response to LPS caused substantial losses in production metrics. Dry matter intake was reduced and the hypophagia was more severe in EL cows. Similar to DMI, milk yield also decreased in both lactation stages. Importantly though, milk synthesis did not differ overall by lactation stage. Interestingly, metabolic data suggest a more engaged glucose sparing (blunted insulin and increased glucagon) and catabolic (increased change in circulating urea nitrogen) response in EL cows that likely enabled them to recover milk yield when DMI was limited.

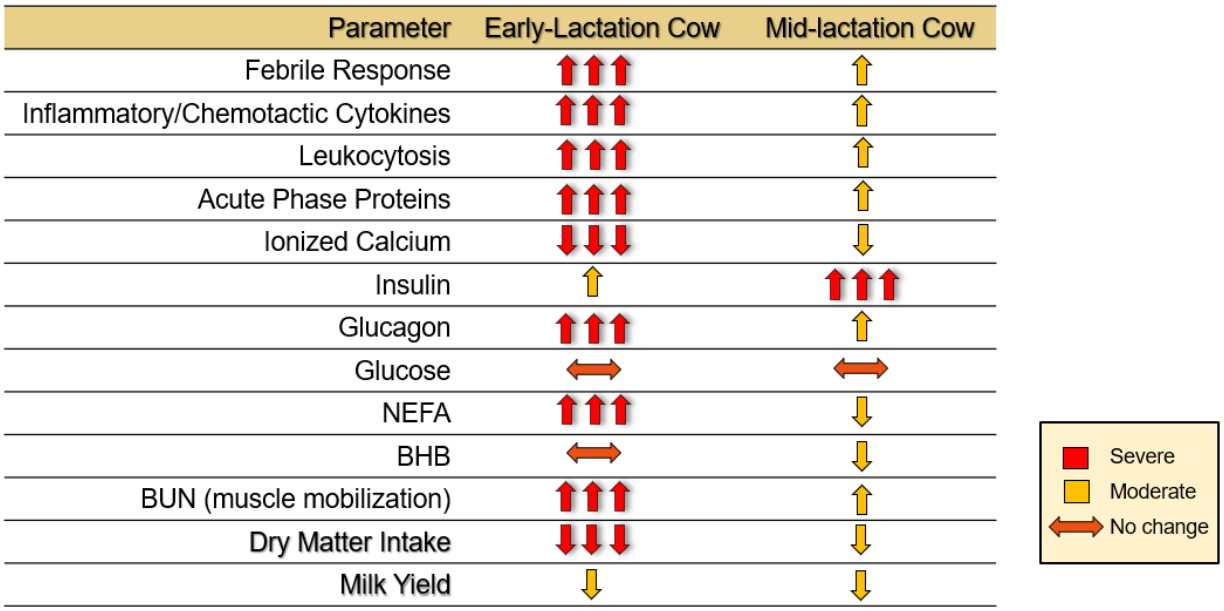

Figure 2. Summary of the effects of i.v. LPS on the immune and inflammatory response, metabolism, and production of early relative to mid-lactation dairy cows (Opgenorth et al., 2024a,b). The immune system in early lactation cows was more sensitive to LPS and mounted a heightened response; their metabolic profile indicated enhanced catabolism and glucose-sparing which likely supported their efforts to recover milk synthesis alongside mid-lactation cows, despite more severe reductions in dry matter intake.

We conducted a similar experiment with intramammary LPS (100 µg) to evaluate the repeatability of our i.v. results and recreate a more natural route of LPS infiltration (Opgenorth et al., 2024c). In agreement with the i.v. LPS challenge, many parameters were similarly altered in EL (20 ± 4 DIM) compared to ML cows (155 ± 40 DIM) that suggested an increased immune and inflammatory response (augmented febrile response, neutrophilia, and circulating cytokines) and subsequent metabolic alterations (blunted insulin and increased change in circulating urea nitrogen). Intramammary LPS reduced DMI to a similar magnitude in all cows, and the milk production response did not differ by lactation stage.

The use of homeorhetic mechanisms to quickly recover milk synthesis in EL cows despite the staggering nutrient expense of reduced feed intake and heightened immune activation implies EL physiology continues to prioritize milk production (likely an evolutionary adaptation to ensure neonatal calf survival accomplished through an intensely catabolic, but coordinated, metabolic response).

Practical On-Farm Examples Supportive of Our Interpretation

The pattern of early lactation cows mounting a heightened immune response to a challenge but maintaining a similar milk production response with later lactation cows deserves special attention and corroborates other reports indicating transition cows have

a similar or less severe of a reduction in milk synthesis following various stressors than cows in later lactation. In response to an intramammary LPS challenge, cows in early lactation have more severe clinical signs but recover milk yield a day earlier than in late lactation (Lehtolainen et al., 2003). During summer heat stress (an immune activating event), milk production in early lactation is less affected (Maust et al., 1972; Perera et al., 1986). Additionally, the recent avian influenza outbreak in dairy cattle can severely decrease milk production in established or late lactation cows whereas early lactation animals appear to be less susceptible to production loss (Burrough et al., 2024). That transition cows are less sensitive to both heat stress and High Pathogenic Avian Influenza is seemingly incompatible with the dogma of periparturient immune suppression.

Efforts to modulate the immune system around calving as a strategy to “improve” peripartal immunity (i.e., use of non-steroidal anti-inflammatory drugs [NSAID] or bovine granulocyte colony stimulating factor) do not produce consistent results and sometimes even have detrimental side-effects (Horst et al., 2021; Yáñez et al., 2024). That pharmaceutically increasing circulating neutrophils and targeting systemic inflammation do not consistently ameliorate transition period health is seemingly incompatible with the dogma of periparturient immune suppression.

Collectively, there are multiple pragmatic lines of evidence that does not harmonize with the tenant of early lactation immune dysregulation. This is probably because the peripartal immune system is not inherently dysfunctional. Thus, while the concept of modulating the transition cow immune system likely holds promise, our current suboptimal understanding of this incredibly complex system needs a gigantic leap forward in order to provide meaningful solutions for dairy farmers.

Summary

It is becoming increasingly clear that transition cows are not simply immunosuppressed, but rather engage robust aspects of the immune response. This perplexing immune status paradigm of reduced leukocyte functions in some experiments but increased inflammation in others is often described as dysfunctional or dysregulated (Sordillo and Raphael, 2013; Trevisi and Minuti, 2018; Minuti et al., 2020). Regardless of nomenclature, the descriptors “immunosuppression”, “dysregulation”, and/or “dysfunction” all inherently imply a problem with postpartum immunity. Human literature has already recognized the limitations of defining the immune status during natural biological events as immune-suppressed or unfavorable, such as pregnancy and the neonatal period (Mor and Cardenas, 2010; Harbeson et al., 2018). Alterations in immunity are intentional to address the homeorhetic requirements of each physiological state while maintaining host defense, and the term immune suppression oversimplifies a dynamic immune system where some functions are downregulated, others are upregulated (observed in peripartum leukocytes by Mann et al., 2019 and Minuti et al., 2020), and some remain unchanged (i.e., mucosal immunity; Cortese et al., 2024). While there are reports describing extreme immune impairments in some transition cows (Hill et al., 1979; Frost and Brooker, 1986), this likely signals other issues (i.e., recrudescence of or chronic infection) and does not define the typical immune status of the majority around calving.

We find it difficult to understand how evolution would favor an immune suppressive or dysregulated state in early lactation; a physiological scenario that would clearly not benefit survival or fitness of both the mother and offspring (Horst et al., 2021). Notwithstanding the natural selection speculation, our data further propounds the notion that the term “immune-suppressed” does not comprehensively or accurately describe many aspects of the transition cow's immune system.

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Using High Oleic Soybeans in Dairy Diets

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Introduction

Monitoring Income Over Feed Cost (IOFC) for dairy herds has been a longstanding metric used to evaluate the economics of feeding programs. One of the reasons for its popularity is that it is relatively straightforward to calculate in most situations and data from the Cornell Dairy Farm Business Summary and Analysis program indicates that it consistently accounts for about 50% of the variability in overall farm profitability (Karszes, personal communication). Work that we have done within the PRO-DAIRY discussion group program to identify key factors contributing to variance in IOFC suggests that milk and milk component yields account for about 50% of the variance, feed efficiency (ECM/DMI) accounts for an additional 25% of the variance, and cost per pound of TMR dry matter accounts for only 10% of the variance. In total, these three factors account for 85% of the variance in IOFC in herds that we have studied, and milk component yields are by far the biggest influence on IOFC.

Most of the dairy industry in the U.S. has been paid using multiple component pricing for more than 20 years, with the value of fat, protein, and other solids determined on a monthly basis. During most of the early part of that time and until about 2015, milk protein was more valuable than milk fat on a per pound basis. Starting in about 2016 and continuing until today with few exceptions, the value of milk fat has been greater than milk protein. Data from the Producer Price Forecast prepared by Upstate Niagara Cooperative https://www.membership.upstateniagara.com/_membership_payprices.asp accessed 9/12/24 indicates that the value of fat and protein averaged $2.96/lb and $1.90/lb, respectively for 2023. Including projections, the values for fat and protein are expected to be $3.41/lb and $1.66/lb, respectively for 2024. Thus, while both fat and protein are very meaningful contributors to the milk check, there is clearly reward for strategies that boost milk fat and penalty for things that decrease milk fat percentage and yield.

Factors That Affect Milk Fat Production

Research conducted over the past 25 years has dramatically enhanced our understanding of factors that affect milk fat production. Primary non-nutritional factors include genetics and seasonality (Salfer et al., 2019), but nutritional factors play a major role in modulating milk fat production (Bauman et al., 2011). Among these are unsaturated fatty acid load and biohydrogenation in the rumen along with factors that contribute to altered biohydrogenation resulting in the production of unique bioactive fatty acids that directly downregulate genes related to lipogenesis in the mammary gland.

Biohydrogenation pathways under “normal” and “altered” ruminal metabolism are described in Figure 1. Key risk factors for milk fat issues include the amount and

availability of linoleic acid and factors that contribute to the “isomerase shift” causing the bacteria to convert linoleic acid to the milk fat depressing *trans*-10, *cis*-12 conjugated linoleic acid (CLA) rather than the *cis*-9, *trans*-11 CLA. These risk factors include decreased rumen pH either because of diet formulation or feeding behavior factors such as slug feeding in overcrowded group situations, mycotoxins, and other undetermined factors.

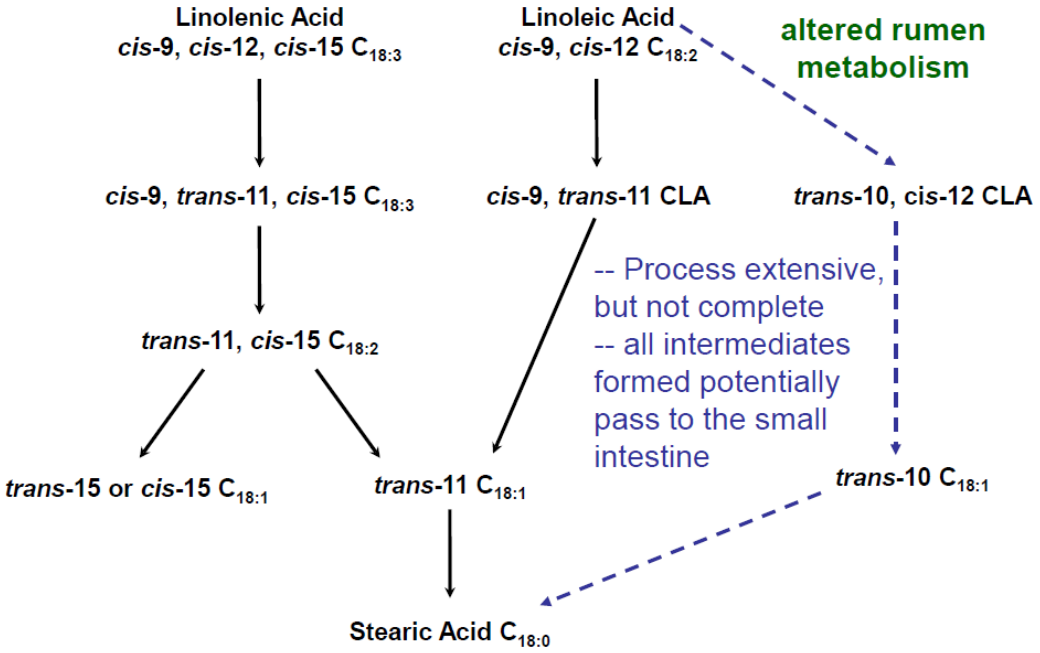

Figure 1. Biohydrogenation pathways during normal and altered ruminal fermentation. Adapted from Bauman and Griinari (2003).

Corn and conventional soybeans typically contain linoleic acid at 50 to 60% of their total fatty acid content (Cornell Net Carbohydrate and Protein System v. 6.5.5; CNCPS) and as such lactating cow diets based heavily on corn silage, other corn products, and soy products can approach or exceed 400 g/d and it only takes 2 to 3 grams of the *trans*-10, *cis*-12 CLA escaping the rumen to meaningfully decrease milk fat. Because of the concern over too much linoleic acid in the rumen affecting milk fat, nutritionists have commonly limited inclusion rates of conventional full-fat soybeans to no more than 3 to 5 lbs per cow per day.

High oleic soybeans have been produced by genetic modification (Wilson, 2012), resulting in much higher oleic acid and much lower linoleic acid content than conventional soybeans. Feed library values for conventional soybeans would be ~23% of total fatty acids as oleic acid and ~54% of total fatty acids as linoleic acid; high oleic soybeans would have ~76% of total fatty acids as oleic acid and ~7% as linoleic acid. This would mean that there is inherently less risk for negative impacts on milk fat by feeding high oleic acid soybeans compared to conventional, and I am aware of nutritionists feeding upwards of 7 to 8 lbs per cow per day of high oleic soybeans.

However, inherently less risk doesn't mean no risk. Interestingly, there are negative relationships between oleic acid intake and milk fat. McCarthy et al. (2018) reported, from data collected in a cross-sectional observational study involving 79 Holstein herds in the Northeast and Upper Midwest, that increasing oleic acid intake was associated with lower milk fat percentage whereas, also interestingly, linoleic acid intake was not. In a controlled feeding study evaluating the independent and combined effects of oleic acid and linoleic acid on milk fat, He et al. (2012) determined that linoleic acid was more potent than oleic acid at decreasing milk fat; however, there was a negative relationship between oleic acid intake and milk fat.

Studies Feeding High Oleic Soybeans to Lactating Dairy Cows

Lopes et al. (2017) fed cows one of three soybean sources varying in FA profile and processing method in diets with forage base of mostly corn silage. Treatments were "Extruded conventional soybean meal; ether extract 8.7% with 15% of FA as oleic and 54% as linoleic"; Extruded high oleic soybean meal; ether extract 8.4% with 73% of FA as oleic"; and "whole roasted high oleic soybeans with 20% ether extract". Total dietary ether extract averaged ~4.0% and were very similar across treatments. They reported similar DMI and milk yield across treatments, but milk fat percentage was increased for cows fed the two high oleic soy treatments compared to the conventional soybean meal control (~ 3.75 vs. 3.55%).

Weld and Armentano (2018) conducted two experiments to evaluate the role of high oleic soybeans in diets for dairy cattle. In their first experiment, they fed whole conventional vs. whole high oleic soybeans, both raw (unroasted). Total dietary ether extract was ~ 5.0%. In this study there were no overall effects of treatment on outcomes, but there were some relatively minor parity interactions between primiparous and multiparous cows on milk fat percentage and yield. In a second experiment, they assigned cows to either a low-fat control diet or one of four treatments: ground raw conventional soybeans, ground raw high oleic soybeans, whole raw conventional soybeans, and whole raw high oleic soybeans. Of note is that the ether extract content of the low-fat control was 3.2% whereas the four other treatments were between 6.3 and 7.1% ether extract. Further, the starch content was relatively high (~30%) and particle size of the TMR was very small, particularly for the diets containing ground soybeans (~70% of the TMR was on the 4-mm screen and pan). Not surprisingly, milk fat percentage was low for the control (3.25%) and higher for ground high oleic vs. ground conventional (3.50 vs. 3.09%). Milk fat percentage was similar among the two whole soybean treatments.

Most recently, the group at Michigan State has conducted studies evaluating high oleic acid soybeans in diets for lactating dairy cattle. In the first study, Bales and Lock (2024a) fed roasted and ground high oleic soybeans at 0, 8, 16, and 24% of diet dry matter, replacing soybean meal and soyhulls as the high oleic soybeans increased. Diets were isonitrogenous, but as inclusion rate of the high oleic soybeans increased, total fatty acids increased linearly from ~2.6 to 5.7% of diet DM and NDF decreased from about 29 to 27% of diet DM. Diets contained about 45% forage, predominantly from corn silage.

As high oleic soybean inclusion rate increased, DMI decreased linearly, milk yield increased quadratically, yields of ECM increased linearly, yields of milk fat increased linearly, and yields of protein were quadratically affected such that it increased when high oleic soybeans were fed at the 8% level but plateaued thereafter. Feed efficiency (ECM/DMI) was increased linearly with greater inclusion rates of high oleic soybeans.

In a second experiment, Bales and Lock (2024b) sought to compare the effects of feeding raw versus roasted high oleic soybeans and also to determine whether adding additional ruminally undegradable protein to a diet containing raw high oleic soybeans would improve performance. They had a control without added soybeans with a total dietary fatty acid content of 2.8%. They had two treatments with high oleic soybeans added at the 16% of dietary DM level – one raw and one roasted. They had a fourth treatment in which raw high oleic soybeans were fed and in which soyhulls were replaced by expeller soybean meal. Diets were isonitrogenous but the total fatty acid content of the three treatments with high oleic soybeans added ranged from 4.9 to 5.1%. Dry matter intakes were increased in all three soybean treatments compared to the control. Yields of milk and milk fat were increased in the soybean treatments compared to the control, but the greatest increases occurred in the cows fed roasted high oleic soybeans. Feed efficiency was increased in cows fed the soybean treatments. From my perspective, this study clearly reinforces the need to roast high oleic soybeans for maximum feeding value.

Nicholson et al. (2024) conducted an economic analysis for inclusion of high oleic soybeans in diets for dairy cattle. They used results from the Lopes et al. (2017) study and the two studies described in Weld and Armentano (2018) and results from two unpublished studies. In general, they demonstrated positive economic responses from inclusion of high oleic soybeans, with most responses in the $0.12 to $0.20 per cow per day range. I believe that the methodology that these authors used was sound; however, in two of the five studies there was clearly milk fat depression that was being at least partially rescued by the inclusion of the high oleic soybeans. In surveying the available literature, I think that the only time one might expect a milk fat response is in a case where there is some milk fat depression with elevated linoleic acid supply and availability in the rumen as a risk factor.

Summary

The availability of high oleic soybeans offers the opportunity to feed more whole soybeans than conventional with inherently (but not zero) risk for milk fat issues. One should not routinely expect increased milk fat percentage if there is not evidence of milk fat depression, so the opportunity may be to examine whether or not there are ways to decrease feed cost. Roasting high oleic soybeans greatly increases their feeding value by increasing the ruminally undegradable fraction. Until these become widely available as feed ingredients in feed mills, this is largely an opportunity for a farm to utilize “extra” acres or partner with a crop farm or purchase as a commodity.

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Does the Concept of Nitrogen Efficiency Make Sense for Dairy Cattle?

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Introduction

In many countries with a significant dairy industry, there is pressure to mitigate carbon and methane and to reduce fugitive nitrogen (N) from dairy production. Fugitive N consists of urinary and fecal N that was not utilized by the dairy cow for productive functions such as milk or tissues and fertilizer N that migrates to soil and plants roots or volatilizes away. Inherently, cattle will always excrete N through fecal and urinary losses; however, the potential to minimize and mitigate these losses through more precise estimations of both ruminal N requirements and post-ruminal amino acid (AA) requirements and supply is significant. Additionally, this precision can help reduce the societal pressure on the dairy industry. Over one-half of all ammonia emissions in the US are sourced from domesticated farm animals due to the presence of ureases in feces that mix with urine to convert urinary urea into volatilized products (Hristov et al., 2011). There is a direct effect of ammonia production and fine particulate matter in the air that can negatively impact human health (Wyer et al., 2022). Further, N has often been considered the primary cause of eutrophication in marine ecosystems (Liu et al., 2024) and reducing excretion would lead to increased water quality. Although still not fully quantified among all conditions and regions, nitrous oxide emissions from cattle manure range from 0.5 to 1.5 percent of total excretion. This means, for every 100 g of N excretion, nitrous oxide emissions are approximately 1 g, which is 273 times more potent as a greenhouse gas compared to carbon dioxide and about 10 times more potent than methane (Nichols et al., 2016; Rivera and Chara, 2021)

The concept of nitrogen efficiency in lactating dairy cattle has been proposed, published, and discussed for many years (Van Vuuren and Meijs, 1987; Dijkstra et al., 2013; Reed et al., 2015). Many concepts previously published on N efficiency focused on the amount of intake N transferred into milk N or productive N (maintenance, tissue, fetus, and milk). In most cases, improving N efficiency can be achieved by partitioning more nutrients to the mammary gland, which increases energy-corrected milk (ECM) yield following the same concept as feed efficiency and dilution of maintenance. As most practicing nutritionists know, it is difficult to change metabolizable energy (ME) supply and milk volume during established lactation or post-peak milk; however, modifications to the form and amount of N being fed are possible and can reduce the amount of excreted N in cattle diets. This approach does not significantly change N efficiency but reduces environmental impact.

Adhering to the classical definition of efficiency, N efficiency can be improved by creating an N deficiency from the diet, as the available N is a scarcer resource and is used where the demand is greatest. The problem with this approach is that high-

producing cattle cannot perform in a deficient state, which would result in the highest efficiencies, so we must work with optimum efficiencies, not maximum efficiencies. This has been discussed previously (Higgs and Van Amburgh, 2016; Van Amburgh and LaPierre, 2022), where the AA requirements were described based on ME supply anchoring the requirements to the energetic state of the animal, whether at fresh, peak, or post-peak milk yield. Most N efficiency calculations for cattle include the non-protein nitrogen (NPN) intake, which confuses the comparisons between ruminant and monogastric species. When evaluating the N efficiencies for pigs and chickens, only the efficiency of use of AA is considered, which makes the comparison biased by the NPN content of ruminant diets. This also suggests that N efficiency might be more variable for a ruminant on a total N basis due to the inability to account for NPN utilization and loss, as well as urea production and recycling.

An efficiency of use of less than one demonstrates that not all N is allocated to a productive use, thus, the difference is what we cannot recover as milk or tissue. Higgs (2014) discussed this during CNCPS v7 development, where it was recognized that after accounting for rumen N requirements and all supplies of AA, the overall efficiency of use of the essential AA (EAA) on a metabolizable protein (MP) basis was 0.73 or 73%. This is reasonably high compared with pigs and chickens and suggests that the difference in efficiency in N utilization by lactating dairy cattle is the metabolism and fate of the NPN. The overall N efficiency when NPN is included is approximately 48% lower or essentially one-half of what we observe on farms or in research studies. In addition, even if we knew the non-essential AA (NEAA) requirements, it is unlikely that the efficiency of the use of EAA could be increased in high-producing lactating dairy cattle because we need to operate at an optimum efficiency, not a maximum. Additionally, we need to recognize that the NEAA is required for optimum energy metabolism and is used for many functions unrelated to N output, including energy substrates not captured in net protein yield. Thus, the opportunity to improve overall N efficiency is to reduce the amount of N excreted in the urine because that demonstrates overfeeding of nutrients to the cow that cannot be used for productive functions, ultimately contributing to excess excretion into the environment.

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.

Further, the recognition that some EAA can be utilized as signaling mechanisms and are required for the synthesis of most end-products should be prioritized in future models to optimize our predictions for metabolic demand. For example, Li et al. (2019) observed that lysine (Lys) enhanced fatty acid binding protein (FABP) and sterol regulatory element binding protein (SREBP) in bovine mammary epithelial cells, which are vital regulators of milk fat synthesis. Lysine is also part of many carrier proteins that support the synthesis of milk fatty acids (FA). Thus, some Lys will never be available for milk protein synthesis due to these other metabolic demands. This does not mean the mammary gland inefficiently uses Lys or other AA, rather the mammary gland has a requirement for these AA for other metabolic pathways such as lactose and fat synthesis. Ultimately, this leads to efficiencies that are optimums as the cattle cannot operate at a deficiency of EAA or NEAA while partitioning all nutrients to ECM yield. Additionally, the efficiency of use of energy for milk synthesis is improved when balancing for AA. For example, Table 1 shows actual treatment outputs from cows fed a basal diet not balanced for AA and actual treatment outputs from cows fed a diet balanced for Methionine (Met), Lys and Histidine (His). The basal and AA-balanced diets were formulated iso-calorically, but the cows fed the AA-balanced diet had 1.7 kg/d greater ECM yield, suggesting that balancing for AA improved the efficiency of energy use by partitioning more nutrients to the mammary gland for increased milk component synthesis. If calculated correctly, the supply of EAA from escape feed, bacteria, protozoa, and endogenous protein is accounted for to meet the needs for productive functions and can be incorporated into the concept of productive N. This condenses the N for productive purposes into one metric, which can be used to evaluate the diet formulation for N intake relative to the productive N requirements. Consideration of ruminal N requirements is paramount in leveraging a ruminant's ability to optimize productivity. It is likely that we will not make significant improvements to post-ruminal N efficiency without considering the N demand for efficient microbial growth, as the fermentation of carbohydrates and proliferation of microbial supply led to a larger source of AA and energy for cattle.

The ruminal requirements for N have been discussed extensively over the last 30 years (Russell et. al., 1992; Chen et al., 1987; Wallace, 1996; Firkins et al., 2007). There are other considerations around the idea of N efficiency, as the cow has two separate metabolic systems operating in synchrony. Rumen microbes require N, in the form of amino or ammonia N sourced from either the diet, protein degradation, or hepatic urea recycling secreted by the salivary glands (Van Kessel and Russel, 1996; Firkins et al., 2007; Hackman and Firkins, 2015). Generally, ruminal N requirements have been

reasonably well described in the CNCPS and applied in a manner that is consistent with good diet formulation practice. However, as the industry looks to reduce protein feeding in cattle, more discrete metabolic demands tend to elucidate themselves as sources of N become scarcer in the rumen. One such demand is the obligate requirement of fibrolytic bacteria for branch chain volatile fatty acids (BCVFA).

Nearly all diet formulation systems that utilize CNCPS provide a rumen ammonia value expressed as a percent of requirement, which is calculated based on the ammonia N requirement of rumen bacteria to digest the amount of fermentable carbohydrates (fCHO). Bacteria have an N requirement to ferment the feed and grow. In a simple approach, bacteria range from 9 to 10% N; thus, to digest 100 grams of fCHO, the model would calculate 0.5 grams bacteria/gram fCHO/h, resulting in 50 grams of bacteria/h. If the bacteria are 10% N, the requirement for growth would be 5 grams of N. If this is scaled up to the total fCHO intake, considering the rate of passage and digestion, the result is calculated by the pool size of fCHO, the grams of bacteria produced, and the N required to allow it to happen. Most of the bacteria in the rumen require either ammonia or amino N to synthesize AA for growth and metabolism (Russell et al., 1992). As characterized in the CNCPS (Russell et al., 1992; Chen et al., 1987), the amylolytic bacteria (starch and sugar utilizers) have an enhancement in growth dependent on the concentration of dietary peptides in the rumen. One of the factors that have impeded the ability to formulate at the lower rumen degradable protein (RDP) is the consideration for more discrete nutrient needs, including BCVFA needs for fibrolytic bacteria (Allison, 1969; Firkins, 2023; LaPierre and Van Amburgh, 2023). As such, a sub-model has been developed that will allow users of the CNCPS to predict any ruminal shortcomings of BCVFA, which, when implemented, should allow for the formulation of diets with lower and more targeted N intake. There has been little focus on the precision of predicting the ruminal N requirements and the minimum N supply in the diet that should be fed and still meet the requirements of the rumen microbes for maximum fCHO digestion and growth. Further refinements of these BCVFA predictions will look to predict what dietary strategies promote the most efficient production of microbial protein in the rumen.

Another consideration when estimating and managing the efficiency of N use in dairy cattle is the amount of ammonia produced, diffused to the mesentery and portal blood, and converted to urea in the liver. This is a continuous process and is a function of the total intake of N in the diet and the pool size of urea N in the blood. On average, dairy cattle intake from about 600 to 870 grams of N per day in a modern dairy diet. Of this, 50% to 70% will be converted to ammonia in the rumen, of which a significant portion will diffuse out of the rumen and be converted to urea in the liver (Reynolds and Kristensen, 2008; Recktenwald et al., 2014). Of the urea production, the amount recycled back to the gastrointestinal tract will range from 40% to 60% in high producing cattle in the range of most N intakes, with the balance being excreted in the milk and urine (Reynolds and Kristensen, 2008). The efficiency of use of the recycled N is a probability function based on the amount of consumed N relative to the demand from fCHO digestion. Given that urea recycling is essentially a function of the relatively constant plasma pool size, the greater the intake of N, the lower the probability of recycled N being captured by bacteria and moving into the AA pool; thus, the efficiency is reduced, and more urea is excreted in

the urine. This is where there is an opportunity to alter this formulation and N supply condition to increase the efficiency of the use of the recycled N. This requires having accurate DMI, appropriate and comprehensive feed chemistry, aNDFom and other fCHO digestibility, and appropriate “safety factors” to account for on-farm management like feed pushup, time budgets, and overcrowding at the feed bunk. Improved feed and feeding management reduce the need for “N safety factors” in the diet and this will increase the efficiency of use of recycled N for microbial protein yield.

One additional factor necessary to monitor the efficiency of N use is urinary N excretion predictions. Any N not utilized in the rumen or in post-ruminal metabolism is excreted in the urine, and although this number cannot be zero, it can be lower than what is observed in most dairy cattle. There are many diets being fed where urinary N excretion is over 200 grams and, in some situations, up to 280 grams per day. On a CP basis, that is 1.25 to 1.74 kg of protein being excreted. The opportunity is to lower urinary N so that it equals the amount of productive N (total protein used for all functions, converted to N basis) – 1:1 productive N to urinary N or achieve a ratio greater than 1 for productive N to urinary N (Table 1). In Table 1, Diets 1 and 2 were diets from Benoit et al (2021) and were the diets fed. Diet 3 is a formulated diet to show the potential to reduce N from the diet, especially rumen available N.

Achieving a 1:1 ratio in most herds results in at least a 40-gram reduction in urinary N excretion with a potential of up to 80 grams based on the starting point, the available alternative feeds, and our knowledge of how low we can formulate rumen N balance. Reducing the urinary N will reduce the potential for ammonia and nitrous oxide production, which are two targets for reduction in many countries around the world to improve air quality and global warming.

Table 1. Intake, production, and excretion parameters among three diets. A base diet and two diets modified from the base – one balanced for amino acids (AA) and increased nitrogen (N) efficiency through AA balancing. The final diet uses the AA balanced diet and reduces N intake by refining the formulation of the ruminal N supplies to reduce excretion. The days in milk are not equivalent among the diet comparisons between the base and modified diets, which explains part of the milk volume difference. For Diet 1, days in milk average 120, whereas for Diet 2 and 3, days in milk are 190.

Input/Output variables	Diet 1 Base Diet	Diet 2 Balanced for Amino Acids and N efficiency	Diet 3 Balanced for reduced urinary N excretion
DMI, kg/d	26.4	26.7	26.6
Milk, kg/d	41.7	39.6	39.6
ECM, kg/d	44.4	46.1	45.9
Milk fat, %	4.10	4.72	4.70
Milk protein, %	3.10	3.37	3.35
ME Allow milk, kg	43.0	43.6	43.6
MP Allow. Milk, kg	43.4	47.3	45.4
Carbohydrate allowable bacteria, g	4,021	4,184	4,210
N allowable bacteria, g	5,019	4,646	4,668
N intake, g/d	684.9	683.5	668.9
Rumen NH_3 balance, %	146	121	125
Rumen Degradable Protein, % DM	10.5	9.0	9.0
True RDP/Fermentable CHO, g/kg	199	188	178
Productive N, g/d	219	223	223.1
Urine N, g/d	221	215	192.6
Fecal N, g/d	257	258	256.1
Manure N, g/d	478	473	448.7
Productive N/Total N, %	32.0	32.6	33.5
Productive N/Urinary N, %	99.1	103.7	116.1
MP Supply, g/d	2888	3214	3131
MP Required, g/d	2820	2825	2840
MP Met, g/d	78.9	85.0	84.9
MP Met, g/Mcal	1.13	1.18	1.17
MP Lys, g/d	191.0	222.4	221.1
MP Lys, g/Mcal	2.74	3.20	3.05
MP His, g/d	76.0	88.9	86.7
MP His, g/Mcal	1.08	1.19	1.20

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