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Is This a Good Microbiome? What About That One? How Does the Microbiome Affect Efficiency and Productivity of My Herd?

W. Huang, J. M. Lourenco, T. D. Pringle, O. Y. Koyun, and T. R. Callaway
Department of Animal and Dairy Science
University of Georgia, Athens, GA

Introduction

Most of what we "know" about the microbial ecosystem of the rumen and the gut of cattle comes from correlative studies based on end-products and animal performance, but largely the function of the microbial ecosystem has remained a "black box" (Krause et al., 2013). There has been a revolution in the past 15 years in terms of information from Next Generation Sequencing (NGS) that has opened the composition and inner workings of the microbial population of the gut in ways that could never be imagined by Hungate or Bryant (Dowd et al., 2008, Callaway et al., 2009, Callaway et al., 2010, Lourenco et al., 2019, Lourenco et al., 2020, Welch et al., 2021). The ability to visualize the microbiome has enabled us to link specific bacteria (or fungi, or protozoa) to specific outcomes in a way that we can finally understand which microbes are most beneficial to the host or are selected for by diet or treatment. However, in many ways, this new power has been wielded like a child with a found handgun; pointed randomly to little purpose but making a loud noise. Instead of adding antibiotics to a diet and expecting "something good" to happen in terms of production response, but not understanding how (Pennycook and Scanlan, 2021); we can now determine which ecological factors impact the composition and degradative activity of the microbial population (Moraïs and Mizrahi, 2019, Grieneisen et al., 2021). As we further our understanding of how the microbiome functions in the real world, we can begin to make directed/targeted changes in the microbial population that can directly impact the animal.

Symbiosis: The Ruminant Gut

The symbiotic relationship between the ruminant animal and the resident microbial ecosystem of the gastrointestinal tract is unique and allows the ruminant animal to thrive on diets that monogastrics cannot (Hungate, 1944, 1947, Bryant and Burkey, 1952, Bryant, 1959, Bryant and Robinson, 1962, Hungate, 1966). The presence of this complex resident microbial consortium of bacteria, protozoa, and fungi gives the ruminant adaptability to utilize a wide variety of feedstuffs; however, this comes at a cost of feed efficiency, and in modern terms, reduced environmental sustainability. The anaerobic environment of the gut means the microbial population must depend upon the process of fermentation which produces the volatile fatty acids which is utilized by the host, but also H_2, CO_2, and CH_4 which are not utilized by the host animal. Methane (CH_4) is a greenhouse gas, but also represents a loss of carbon and energy from the ration that could be used for growth or milk production (McAnally and Phillipson, 1944, Johnson and Johnson, 1995, Boadi et al., 2004, Wright et al., 2004). Thus, the

fermentation characteristics clearly impact the host animal's physiological status, including fetal growth, lactation, and milk composition (Weimer et al., 2017). Moreover, the microbial population will change during the life of the cow from weaning through breeding and will also change dramatically with dietary shifts during the production cycle (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021).

We have long recognized the gastrointestinal microbial population as a great biochemical reservoir of degradative activity (Figure 1), but the relationships between the individual microbes and their substrates, fermentation pathways, affinities, and end-products remain largely unknown except for a few well-studied species (e.g., *Ruminococcus*, *Streptococcus*) who are involved in fiber and starch fermentation (Ransom-Jones et al., 2012, Bandarupalli, 2017, Seshadri et al., 2018, Henderson et al., 2019). Next Generation Sequencing now allows us to "see" the composition of a large microbial population at once, so that instead of measuring what we consider to be "key" or "important" species we can determine the actual keystone organisms in real world conditions (Thomas et al., 2017). For instance, the presence of *Ruminococcus* populations were linked with beef cattle growth efficiency from weaning throughout the backgrounding and feedlot period (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021). This technology allows us to begin asking questions about which specific microbial organisms are important and which are linked with increased milk production or altered body composition.

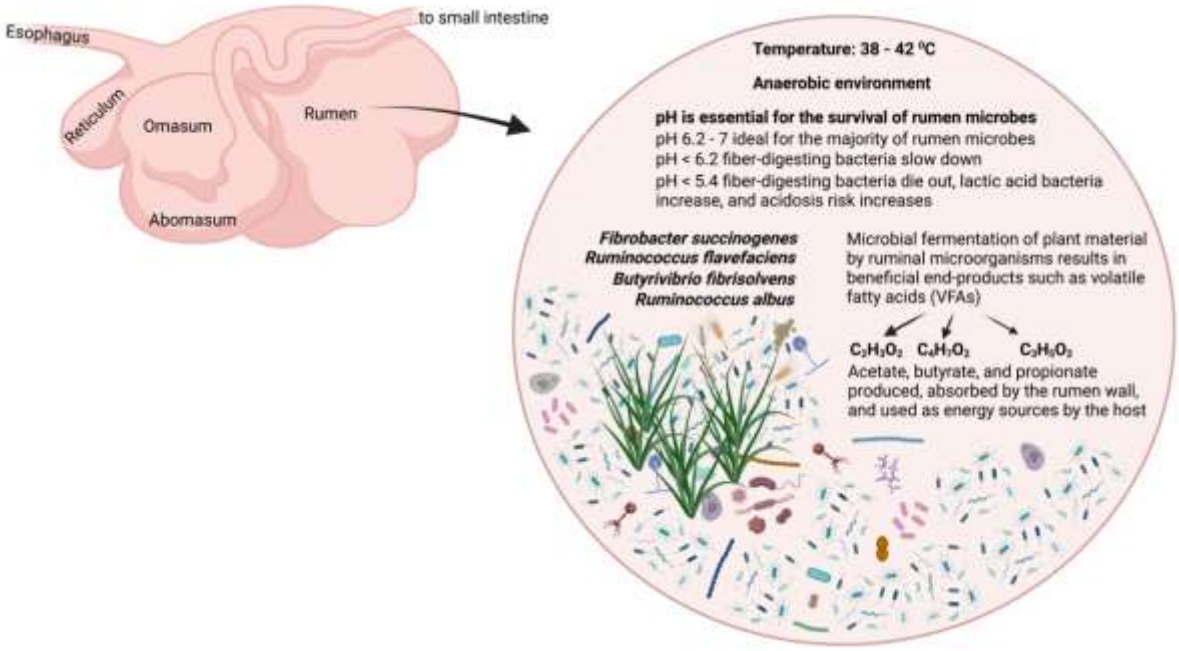

Figure 1. Role of the ruminal microbial population fermentation on ruminant nutrition.

Changes in the Gastrointestinal Microbial Ecosystem

The specific end-products of the gastrointestinal microbial fermentation vary based upon diet, and most especially upon energy density of the ration. We understand the impact of starch feeding on lactate production leading to acidosis (or subacute

acidosis) in our highest producing cows; but we have not demonstrated how starch impacts the microbial population of the gut in relation to the diet. While we do know that propionate is a primary end-product of ruminal starch fermentation, it results in a certain degree of milkfat depression (Hook et al., 2011). However, increasing acetate production from forage feeding to enhance milkfat production, results in increased methane production, which is a potent greenhouse gas and represents a significant loss of carbon and energy to the host cow (Hornung et al., 2018, Wallace et al., 2019, Bowen et al., 2020). Thus, we need to understand which organisms in the microbial population are linked with production of each of these important short chain fatty acids to manipulate the ruminal fermentation to meet the goals of your specific producers.

As calves mature, the microbiome changes throughout the gut and include the calf becoming a functional ruminant animal (Welch et al., 2021). Studies following beef calves from weaning through backgrounding and into the feedlot have found increasing microbial diversity in the rumen and hindgut as calves age (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021). Specific members of the microbial population have been identified that are linked with increased production efficiency (Feed:Gain), and with carcass quality (marbling) (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021). The fecal populations of methane producing organisms (archaea; methanogens) were increased in feedlot steers that were less efficient, compared to the more efficient steers (Carmichael et al., 2022). These studies indicate that the specific composition of the microbial population inhabiting the gut can have profound impacts on cattle physiology and energetic status.

While we have used antibiotics for years to alter the end-products of the ruminal (and gastrointestinal) fermentation in animals, we have not understood how antibiotics work to improve food production efficiency. As we increasingly regulate the use of antibiotics in animal agriculture, we have got to replace their benefits with some alternatives. To do so, we must understand HOW these compounds impact (i) animal performance, (ii) fermentation characteristics, and (iii) the native microbial population. The use of NGS allows us to finally begin to understand these impacts, and how alternatives to antimicrobials (ATA) genuinely work. Afterwards, we can begin to understand how each ATA impacts the microbial population and resultant milk production. Once we understand how those linkages actually function (as opposed to theoretically), then we can develop specific strategies tailored for specific production stages, specific health challenges (e.g., hemorrhagic bowel syndrome), production goals (e.g., fluid milk or milk fat), or even down to individual farms.

The improved understanding of antibiotic action will allow us to fully understand what probiotic approaches (including eubiotics, prebiotics, organic acids, and postbiotics) accomplish in the gut in terms of microbial ecological impacts. Figure 2 demonstrates the hypothesized impacts of probiotic approaches. Most of the effects are derived from stimulating a native (or introduced in case of eubiotics) microbial population to produce antimicrobial proteins (AMPs) or short chain fatty acids (SCFAs). These end-products can inhibit opportunistic (or obligate) pathogens from inhabiting the mucus layer near the epithelium of the gut. Excluding pathogens from this proximate

layer can prevent pathogen entry to epithelial cells and prevent them instigating inflammation. When epithelial cells undergo inflammation, the proteins holding epithelial cells in close proximity become weaker, and this loosens up the tight junctions between cells, which can allow the passage of toxins and pathogens into the bloodstream which can have very deleterious impacts throughout the animal. Probiotic action is thought to prevent this pathogen proximity but can also modulate the immune system by stimulating dendritic cell "sampling" of the gut microbiota, altering T- and B-cell proliferation which affects downstream cytokine regulation. Interestingly, a recent study has shown that when probiotics were fed to high producing lactating Holstein cows, the probiotic altered the expression of more than 11,000 genes of these cows (Adjei-Fremah et al., 2017). The genes that were both up- and down-regulated were scattered across 87 different bovine metabolic pathways, many of which involved suppression of inflammation and growth hormone production (Adjei-Fremah et al., 2017). Further studies have demonstrated that probiotic feeding in cattle affects changes in the production of many of the B-vitamins that meet the cow's requirements (Vandana et al., 2013).

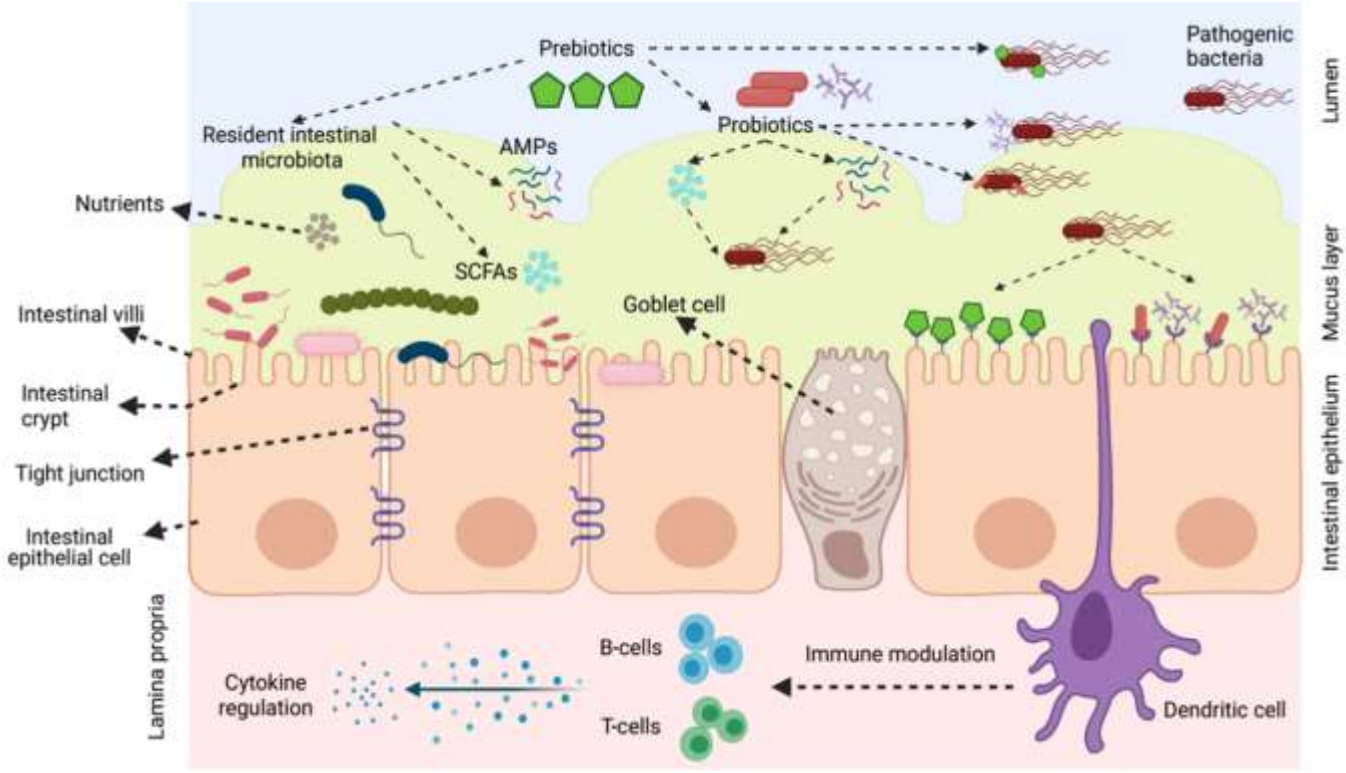

Figure 2. Modes of action of probiotic-type approaches to harness the power of the microbiome. An area, where we can now fully determine the impacts of both pro- and anti-biotic treatments, that offers opportunities to improve production efficiency.

Words of Caution

Being able to identify the microbial populations in detail has been intoxicating to microbial ecologists, and they have been tempted to use the microbiome analytic tools to answer all questions similarly to the axiom- "all the world looks like a nail when you

have a hammer". The temptation has been to perform microbiome analyses on all cattle in an undirected fashion, but without understanding the microbial activity or the nutrition and animal production factors this knowledge carries limited practical impact. We can look at a microbiome analysis like we would someone using a class photograph of elementary students to predict the impact of a group of children on society as adults. A class photo simply demonstrates the presence of absence of a member of the "class" on a specific day. This information in isolation has little value in our pursuit of societal-level impact, but when compiled over time/locations and other metrics aggregates value. Combining several metrics such as: continued daily attendance (which is often correlated with grades), discipline issues, grades, activities, clubs, internships, and goals; along with collegiate selection, work ethic, majors, personal ethics, activities, and jobs after college; begins to accumulate predictive value. Compiling this spectrum of "production" metrics can allow us to build models that are predictive of outcomes, both at an individual and societal level. So, by looking at the microbiome composition in cattle can tell us what is happening at that moment, but not much more. As we accumulate more information from multiple herds and conditions, including beef and dairy, we will be able to overlay a number of cattle over time, fed different diets, producing different milk fat and/or protein percentages, and different milk yields along with end-products (e.g., methane, VFA, ammonia, microbial crude protein). As this data accumulates, we can begin to construct a predictive model to harness the power of the microbiome.

Summary

Much like the new James Webb Telescope that allows us to see deeper into space, Next Generation Sequencing allows us to see deeper into the microbial world, including that within the gut of humans and food animals. The ability to understand which microbes are present in specific diets and production conditions, and which end-products they are linked to provides potential power to be able to predict outcomes. Clearly, the microbial population of the gastrointestinal tract impacts animal performance, efficiency, sustainability, animal health, and food safety. NGS allows us to be able to finally understand what probiotic and pathogen controlling approaches do to replace antibiotics and improve fermentation efficiency, production sustainability, production quality/quantity, animal health, and food safety. These new techniques offer the possibility of truly understanding how the ruminant microbial population works with the host animal to degrade feed and can allow us to simultaneously control production efficiency, end-products, and wasteful fermentations to improve sustainability and (more directly important to us) profitability of dairy production.

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Nutritional Opportunities and Challenges with Robot Milked Cows

T. J. DeVries[1*] and G. B. Penner[2]
[1]Department of Animal Biosciences, University of Guelph, Guelph, ON; [2]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK

Introduction

With continued increases in the adoption of automated (robotic) milking systems (AMS), we have experienced a fundamental shift in nutritional management, with the division of the ration into a partial mixed ration (PMR) and the AMS pellet. In addition, the composition of the PMR, allocation of the PMR, type of pellet, and feeding strategy of the pellet delivered in the AMS differ. The large diversity coupled limited controlled research regarding feeding management have led to many recommendations being largely based on survey studies or based on anecdotal data from single-farm case studies. However, research on feeding management strategies for cows managed in AMS has increased, and this paper will describe the current state of knowledge along with areas where research is needed.

Varied Concepts in Feeding Management in AMS Herds

There are two main goals when considering the nutritional program for cows milked with AMS. The first, as with all planned nutritional programs, is to provide a diet that meets nutrient requirements for maintenance and production. However, with AMS, there is a perception, and potentially some opportunity, that this goal can be shifted from the pen level to the cow level. Thus, producers could be providing a different diet for each cow within the same pen by adjusting the amount of pellet provided in the AMS. The second goal, which is unique to AMS, is to stimulate cows to voluntarily enter the AMS by dispensing pellet in the AMS. A disproportionately large focus has been placed on the AMS pellet, considering that the PMR provides the majority of the dry matter and nutrients consumed. For example, assuming a static dry matter intake (DMI) of 28 kg, the PMR could be estimated to contribute between 89 and 71% of the total dietary dry matter for cows offered three and eight kg of pellet in the AMS (dry matter basis), respectively.

Some survey data suggest that producers with free-flow traffic barns program greater AMS pellet allocations than those with guided-flow traffic barns (Salfer and Endres, 2018). Feeding greater quantities of pellet in the AMS, by default, also indicates the PMR will be less nutrient dense. While this may not be considered to be a problem, recent research has demonstrated that feeding a PMR with a greater proportion of forage increases the ability of cattle to sort that PMR (Menajovsky et al., 2018; Paddick et al., 2019). Providing more pellet in the AMS with free-flow barns is typically done because cows can choose when, and if, they voluntarily enter the AMS, whereas with guided flow barns, cows are ultimately directed to the commitment pen and the AMS using automated sorting gates. While the survey data indicate that producers with free-

flow barns provide more pellet in the AMS, it is not known whether those cows consume more AMS pellet because the amount actually delivered and the amount consumed are not necessarily known nor reported. The difference between the computer programmed value, amount delivered, and amount consumed for the AMS pellet is of major importance. Moreover, survey-based studies have neglected to evaluate PMR composition and do not have the ability to evaluate PMR intake at a cow level (Bently et al., 2013; Tremblay et al., 2016; Salfer and Endres, 2018). Thus, caution should be applied when considering survey-based data as a means to evaluate potential recommended feeding strategies.

Salfer and Endres (2018) reported that the upper limit for pellet allocation in AMS (computer programmed value) in their survey was 11.3 kg /cow/d. Assuming cows could consume 11.3 kg/d, each cow would need to consume over 2.8 kg/milking (assuming 4 milkings/day) equal to 350 to 400 g/min if milking duration was between seven and eight minutes. This high rate of pellet feeding may outpace the ability of cows to consume pellet while milking, and likely would result in a significant quantity of pellet that is either not delivered to the cow (Penner et al., 2017) or delivered in the AMS, but not consumed by the cow (Bach and Cabrera, 2017).

Unfortunately, there is a lack of data evaluating whether traffic flow truly affects the amount of pellet required to be offered in the AMS. A study conducted in a feed-first, guided-flow barn reported no effect on voluntary attendance or milk yield when the amount of pellet delivered varied from 0.5 to 5.0 kg of DM/d (Paddick et al., 2019), whereas similar treatments in a free-flow barn resulted in more frequent voluntary milkings (Schwanke et al., 2019). One might conclude that these data provide support for allocating greater quantities of AMS pellet under free-flow systems; however, the AMS pellet composition, PMR composition, total DMI, and days in milk also differed between the two studies thereby preventing a direct comparison. Moreover, Bach et al. (2007) reported that the amount of pellet provided in a free-flow system did not affect voluntary attendance or milk yield. As a result, studies should not be interpreted to indicate the absolute amount of pellet provided because the amount likely differs on a farm-to-farm basis.

Effect of AMS Pellet Allocation on DMI, Voluntary Milking, and Milk Yield

One of the most common claims with AMS feeding strategies is that increasing the amount of pellet delivered in the AMS will stimulate voluntary attendance and milk yield. The approaches used to increase the AMS pellet allocation should be considered because there are two very different nutritional strategies. First, producers need to decide how much pellet is required from a basal level and this basal amount must consider the formulation of the PMR. Studies have been conducted in the past to evaluate how the amount of pellet offered in AMS affects production responses when the total dietary nutrient supply is equivalent. In other words, with this strategy, increasing the amount of pellet provided in the AMS requires an equal reduction in the amount of pellet in the PMR, such that the total diet (PMR + AMS) does not differ. The first study published using this nutritional strategy compared treatments with computer

programmed values of three or eight kg of pellet in the AMS in a free-flow barn design (Bach et al., 2007). In that study, despite having programmed values of 3 and 8 kg/d, pellet delivery was 2.6 and 6.8 kg/d (dry matter basis) and the amount of pellet delivered did not affect milk production or milk component production. In two recent studies conducted in a feed first guided-flow barn at the University of Saskatchewan, AMS pellet delivery ranged between 0.5 and 5.0 kg of dry matter/cow/d (Hare et al., 2018; Paddick et al., 2019). Altering the amount of AMS pellet while maintaining equal dietary nutrient composition did not affect voluntary visits, milk yield or milk component yield. In contrast, in a recent study conducted at the University of Guelph in a free-flow barn, it was reported that with total diets (PMR + AMS pellet) that were the same in nutrient composition, increasing the AMS pellet from 3 to 6 kg/d (and correspondingly reducing the same pellet in the PMR), stimulated greater DMI (+1.3 kg/d), increased voluntary visits by 0.5 milkings/d, and numerically increased milk yield by 1.5 kg/d (Schwanke et al., 2019). In a similar study at the same facility, Schwanke et al. (2022) demonstrated that by increasing AMS pellet (6 vs 3 kg/d) when cows were fed the same PMR, cows again demonstrated greater total DMI (+1.3 kg/d) and numeric increase in milk yield (+1.6 kg/d)

It might seem counter-intuitive that increasing the AMS pellet allocation does not necessarily stimulate voluntary visits or milk yield in all situations. However, simply providing more pellet in the AMS does not necessarily translate to greater DMI, as cows will generally eat to a set level of intake based on BW and requirements (including production and DIM).. For example, Hare et al. (2018) reported that for every 1 kg increase in AMS pellet delivered, there was a corresponding decrease in PMR DMI of 1.58 kg. Bach et al. (2007) reported a 1.14 kg reduction in PMR DMI and Paddick et al. (2019) reported that PMR DMI decreased by 0.97 kg for every one kg increase in AMS pellet delivered. The large or at least equal reduction in PMR DMI with increasing AMS pellet intake demonstrates that nutrient intake may not be positively affected. These effects of greater concentrate consumption in the AMS and subsequent PMR substitution rate may also vary due to the energy density of the PMR; Menajovsky et al. (2018) reported a 0.78 and 0.89 kg/d reduction of PMR for every 1 kg of concentrate, depending on PMR energy density (low or high). In contrast, in Schwanke et al. (2019) and (2022) it was reported that for every 1 kg increase in AMS pellet intake there was only a 0.63 kg and 0.54 kg, respective, reduction in PMR DMI (Table 1).

In those two later cases, providing more pellet in the AMS resulted in greater total DMI and likely explains their numerical improvement in milk yield. Across studies, the variable and currently unpredictable substitution rate may challenge the ability to formulate diets for individual cows in the same pen given that only the amount or types of pellet in the AMS can differ.

Table 1. Effect of increasing pellet in the automated milking system (AMS) on the reduction in PMR intake (DM basis).

Study	DIM (mean ± SD)	Cows, parity, and study design	Traffic and diet, dietary scenario	Substitution ratio, kg PMR/kg AMS concentrate
Bach et al., 2007	191 ± 2.13	69 primiparous Holstein, 46 multiparous Holstein Completely randomized design	Free Isocaloric	1.14
Hare et al., 2018	227 ± 25 123 ± 71	5 multiparous Holstein 3 primiparous Holstein	Guided Isocaloric	1.58
Henriksen et al., 2018	32-320 14-330	22 primiparous Holstein, 19 multiparous Holstein 11-week study	Free Static PMR with 2 concentrate	0.58 – 0.92
Henriksen et al., 2018	29-218 17-267	14 primiparous Jersey 28 multiparous Jersey 11-week study	Free Static PMR with 2 concentrate allocations	0.69-0.50
Menajovsky et al., 2018	141 ± 13.6	8 multiparous Holstein Replicated 4x4 Latin square	Guided Low energy PMR High energy PMR	0.89 0.78
Henriksen et al., 2019	Early (5 to 14) Mid (15 to 240) Late (241 to 305)	128 cows (68 Holstein + 60 Jersey) Continuous lactation study	Free Static PMR with 2 differing concentrate allocations	5 1.1 2.9
Paddick et al., 2019	90.6 ± 9.8	8 primiparous Holstein Replicated 4x4 Latin square	Guided Isocaloric	0.97
Schwanke et al., 2019	47.1 ± 15.0	15 primiparous Holstein cows, crossover design	Free, Isocaloric	0.63
Schwanke et al., 2022	123.9 ± 53.2	14 muliparous, 1 primiparous Holstein cows, crossover design	Free, static PMR	0.54

As a second strategy, the energy density of the diet for an individual cow can be changed by increasing or decreasing the AMS pellet allocation without changing the composition of the PMR. This approach is one strategy to apply precision feeding management. There has been limited research with this strategy; however, in a recent study where cows received 2 or 6 kg of AMS pellet (dry matter basis), there were only subtle differences in milking frequency and only numerical improvements for milk and milk protein yield (Menajovsky et al., 2018). At a farm level, Tremblay et al. (2016) reported a negative relationship between the amount of pellet offered in the AMS and milk yield. Their rationale was that poor forage quality requires more pellet; however, there was no information provided on PMR characteristics. To our knowledge, there is still a lack of research focusing on the use of precision feeding strategies, particularly with high-yielding and early lactation cows.

A challenge with adopting precision feeding strategies is that predictions are needed for the amount of PMR and AMS pellet that the cow will consume on a daily basis. The data are clear that increasing the quantity of AMS pellet offered in the AMS increases the day-to-day variability in the consumption of the AMS pellet and hence

creates more dietary variability (Hare et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019; Schwanke et al., 2019). Based on the available data, the coefficient of variation (CV) in AMS pellet delivered averages 13.5%.

In most studies, a fundamental assumption is that as AMS pellet delivered, and presumably consumed, increased, PMR intake would decrease with an equal magnitude. We know this assumption is not true as substitution rates (amount of decrease in PMR intake for every 1 kg increase in AMS pellet intake) range from 0.54 to 1.58 kg (Table 1). Obviously, the reduction in PMR intake with increasing AMS pellet allocation will change the nature of the total diet and depending on the direction and magnitude of the PMR substitution, the proportions of forage neutral detergent fibre (NDF) or physically effective NDF may become marginal coupled with increases in ruminally degradable starch.

In AMS systems, there are three values that are relevant when considering AMS pellet delivery. The first value is the computer programmed target value. This value is the maximum amount that can be offered to cows in the AMS, assuming that carry-over of pellet is not included in the equation. The second value is the amount that is delivered to the cows in the AMS. The third value is the amount consumed in the AMS. The amount of pellet programmed in the computer does not correspond with the amount delivered. For example, Bach et al. (2007) allocated either 3 or 8 kg/d in the AMS but only 2.6 and 6.8 kg/d were delivered, respectively. Halachmi et al. (2005) offered either 7 kg/d or 1.2 kg/visit to cows and reported that cows offered 7 kg/d were only delivered 5.2 kg/d while those offered 1.2 kg/visit received 3.85 kg/d. Pellet delivery and pellet consumption below that of the formulated diet are major concerns. Evaluating the deviation between the amount programmed and the amount offered is an important management tool because it demonstrates the ability to deliver the formulated diet to the cows. The deviation between the amount programmed and the amount delivered increases as the amount programmed increases. While it cannot be evaluated on farm easily, residual pellet left in the AMS feeder also increases with increasing pellet allocation in the AMS (Bach and Cabrera, 2017). Differences among the amount of pellet programmed, amount delivered in the AMS, and amount consumed by cows in the AMS can pose a challenge to dairy producers and their nutritionists, and diminish the ability to formulate diets that reasonably predict production outcomes.

Type of Supplement Provided in the AMS

Another factor which influences the amount of feed provided and consumed in the AMS is it composition, palatability and physical form. The rate of consumption of various feeds may limit the amount which may be consumed in the AMS. It is well established that eating rates vary with physical form of concentrate. For example, Kertz et al. (1981) demonstrated that a 4mm pellet was consumed by cows quicker than a pellet with cracked corn, a crumbled pellet, and a meal (in that order), with a maximal rate of consumption of ~430 g/min of the pellet. Pellet consumption rate in other studies has averaged 265 g/min (Beauchemin et al., 2002) and 199 g/min (Maekawa et al., 2002). Sporndly and Asberg (2006) recording concentrate intake rates of up to 200

g/min, with preferences of pellets to ground grain. Additionally, Harper et al. (2016) recorded eating rates varying from 223 - 312 g/min of non-pelletized concentrates with various flavors. Across the literature, it appears that the 'average' cow consumes concentrate at ~250 g/min. In a typical 7 min milking, this would equate to 1.75 kg/milking that the average cow can consume in concentrate. Thus, with a target of ~3 milkings per day, the 'average' cow would be expected to be able to consume ~5 to 5.5 kg/d of feed in the AMS.

The palatability of the pellet provided in the AMS may also be important. Madsen et al. (2010) evaluated pellets containing barley, wheat, a barley-oat mix, maize, artificially dried grass, or pellets with added fat, with all cows fed a common PMR. Those researchers observed that AMS pellet intake and voluntary visits were greatest when the pellets contained the wheat or the barley-oat mix. However, pelleted barley and wheat are expected to have a rapid rate of fermentation in the rumen and feeding substantial quantities would be expected to increase the risk for low ruminal pH. To reduce fermentability, pellets could be prepared with low-starch alternatives (Miron et al., 2004; Halamachi et al., 2006; 2009). Substituting starch sources with soyhulls did not negatively affect voluntary attendance at the AMS or milk yield (Halamachi et al., 2006, 2009), and may slightly improve milk fat and reduce milk protein concentrations (Miron et al., 2004).

Producers may also choose to use home-grown feeds in the AMS. In a more recent study at the University of Saskatchewan, it was tested whether feeding a pellet was required or if they could deliver steam-flaked barley as an alternative (Johnson et al., 2022) in a feed-first guided-traffic flow barn. In that study, the pellet comprised only barley grain and the same source of barley grain was used for the steam-flaked treatment. In all cases, cows were programmed to have 2.0 kg of the concentrate in the AMS delivered. While PMR (27.0 kg/d DM basis) and AMS concentrate intake (1.99 kg/d DM basis) did not differ among treatments, cows fed the steam-flaked barley had fewer visits (2.71 vs 2.90 visits/d) to the AMS, tended to have a longer interval between milking events (541.7 vs. 505.8 min), and spent more time in the commitment pen prior to entering the AMS (139.9 vs. 81.2 min/d) than those fed pelleted barley. While this did not translate into differences in milk yield (average of 44 L/d), it may be expected that with a longer-term study, production impacts would be observed. In contrast, Henriksen et al. (2018) reported greater voluntary visits when a texturized feed (combination of pellet and steam-rolled barley) was provided in comparison to a pellet alone. Regardless, utilization of a pellet as the sole ingredient or part of the mix may limit the ability of producers to use home-grown feeds in the AMS.

Management of the Partial Mixed Ration

As mentioned above, all surveys that have been published to date focus on AMS feeding with little or no information collected to describe PMR composition or intake. The lack of focus on the PMR is likely because only group intakes can be determined and many of the studies have been conducted using retrospective analysis. However, drawing conclusions or making recommendations for feeding management without

considering the PMR may lead to erroneous decisions. We completed a study where we varied the formulation of the PMR such that we increased the energy density of the PMR by a similar magnitude to that commonly used when increasing the amount of pellet in the AMS (Menajovsky et al., 2018). Feeding the PMR with a greater energy density tended to increase milk yield (39.2 vs. 37.9 kg/d) likely because of greater energy supply.

Management of the PMR may be a key factor in success of AMS, largely due to the fact that milking activity in AMS is largely tied to PMR feeding activity (DeVries et al., 2011; Deming et al., 2013). Stimulation of PMR eating behavior, through frequent feed delivery and push up across the day may, thus, be important for optimizing AMS usage. Interestingly, in recent observational study of AMS herds, Siewert et al. (2018) reported that farms with automatic feed push-up produced 352 kg more milk/robotic unit and 4.9 kg more milk/cow per day than farms that manually pushed up feed. In an even more recent study by our group (Matson et al., 2021), we demonstrated in an observational study of 197 Canadian robot milking farms, that each additional 5 feed push-ups per day was associated with 0.35 kg/d/cow greater milk yield. Interestingly, given the mean push up frequency between those that pushed up feed manually (4.4 times per day; 19% of farms) and those that used a robotic feed pusher (16.8 times per day; 71% of farms) in our study, it is likely that our findings and that of Siewert et al. (2018) were driven by the frequency feed was pushed up within each system, rather than by the method itself. More specifically, these effects may not be directly attributable to the use of an automated feed pusher, but rather that those farms using such automated equipment had more consistent feed push-up, and thus continuous feed access, than those pushing up feed manually.

Early lactation challenges?

Automated milking systems provide the ability to milk and feed cows individually based on production potential and stage of lactation. However, individualized milking may not only lead to more frequent milking and greater milk yield in early lactation, but may lead to issues with negative energy balance and metabolic disorders. Tatone et al. (2017) reported that AMS herds in Ontario, Canada had higher within-herd prevalence of SCK (26%; as measured through milk ketone levels) than did conventional herds (21%). Those researchers also reported that multiparous cows in AMS herds were more likely to have SCK than in conventional herds (Tatone et al., 2017). Higher SCK prevalence may be the result of increased frequency of milking during early lactation or inadequate supplemental feeding of concentrates in the robot. In a field study King et al. (2018) reported that development of SCK in AMS cows was associated with greater production of milk relative to the amount of feed consumed in the AMS, suggesting that inadequate supplementation was potentially occurring at that time. This provides evidence that robot feed supplementation must be based on stage of lactation and production level. Alternative and additional energy sources may also be beneficial in early lactation. Specifically, alternatives to starch (to improve rumen conditions) including sugars and other gluconeogenic precursors may have benefits. As one example, we demonstrated that we could improve energy balance and minimize body

condition loss in early lactation by supplementing cows milked in AMS with a molasses-based liquid feed supplement in addition to their regular AMS concentrate (Moore et al., 2020).

Conclusions

The adoption of AMS systems continues to rise and sound feeding management practices are needed to support efficient and cost-effective milk production. Feeding strategy in AMS herds must take into account the stage of lactation and production level, as well as the behavioral capabilities of dairy cows. It is well established that the feeding strategy at the AMS will impact PMR consumption levels, thus this needs to be accounted for when formulating dietary plans. Finally, encouraging PMR feeding will help drive total intake and milking activity.

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Effects of Seaweeds on Dairy Production

A.F. Brito
Department of Agriculture, Nutrition, and Food Systems
University of New Hampshire

Introduction

There is a growing interest in incorporating seaweeds in ruminant diets motivated by the effects of algal feeds on reducing enteric methane (CH_4) emissions while improving animal health (Allen et al., 2001; Makkar et al., 2016; Stefenoni et a., 2021). Seaweeds are macroalgae species growing primarily in littoral zones, with varying shapes, sizes, and pigmentation (Makkar et al., 2016), and typically classified as brown (Phaeophyceae), red (Rhodophyceae), and green (Chlorophyceae) algae. Historically, seaweeds have been used as feed supplements, soil conditioners, and as sources of minerals for plants and animals (Allen et al., 2001; Makkar et al., 2016). Occasional or systematic feeding of seaweeds to ruminants during the 19th and early 20th centuries have been reported in Europe (Makkar et al., 2016). In addition, the feral sheep from the North Ronaldsay island (Orkney, Scotland) rely almost exclusively on grazing a variety of beach cast seaweeds (mostly *Laminaria* species) as their sole nutritional source (Hansen et al., 2003). Currently, algal-based feed supplements available in the market across Europe, Asia, and North America are made from dried, milled seaweeds such as *Ascophyllum nodosum* (ASCO) and *Laminaria* and commercialized as seaweed meals (Makkar et al., 2016).

Seaweeds contain high concentrations of minerals, particularly iodine, and a wide spectrum of nutritional compounds including polysaccharides, polyunsaturated fatty acids, polyphenols, bioactive peptides, and vitamins (Kumari et al., 2010; Tierney et al., 2010; Fitzgerald et al., 2011). There are approximately 11,000 species of seaweeds (7,500 red, 2,000 brown, and 1,500 green; https://www.seaweed.ie/algae/seaweeds.php), but few of them have been used for livestock diets (Makkar et al., 2016; Morais et al., 2020). Among the seaweed used for livestock, the brown seaweed ASCO is one of the most researched (Allen et al., 2001), and it has been fed to confined (Antaya et al., 2015; Silva et al., 2022) and grazing dairy cows (Antaya et al., 2019). *Ascophyllum nodosum* is rich phlorotannins, which are polyphenolic compounds that resemble terrestrial tannins in their ability to bind proteins and carbohydrates (Ragan and Glombitza, 1986) and to inhibit growth of pathogenic bacteria (Wang et al., 2009). Moreover, ASCO contains compounds with antioxidant activity such as β-carotene and fucoxanthin (Haugan and Liaaen-Jensen, 1994), which may improve animal health (Allen et al., 2001). Table 1 shows various seaweeds species used as feed supplements to livestock based on the literature reviews of Evans and Critchley (2014), Makkar et al. (2016), Morais et al. (2020), and Fouts et al. (2022).

There is limited research investigating the impact of seaweeds on production performance and enteric CH_4 emissions in lactating dairy cows. Studies done with dairy

cows fed varying amounts (0, 57, 113, and 170 g/d) of the brown seaweed ASCO did not affect dry matter intake (DMI) and milk yield (Antaya et al., 2015, 2019; Silva et al., 2022). In contrast, DMI and milk yield decreased by up to 38 and 11.6%, respectively, in dairy cows fed incremental amounts [0, 0.5, and 1%; diet organic matter (OM) basis] of the red seaweed *Asparagopsis armata* (Roque et al., 2019). Both DMI and milk yield decreased by up to 7.1 and 6.5%, respectively, with feeding increasing levels [0, 0.25, and 0.5%; diet dry matter (DM) basis] of the red seaweed *A. taxiformis* to dairy cows (Stefenoni et al., 2021). Enteric CH_4 production decreased by 10.3% during the first period of the study (diet x period interaction) in grazing dairy cows supplemented with 113 g/d of ASCO meal but not thereafter (Antaya et al., 2019). Feeding the greatest level of *A. armata* (Roque et al., 2019) or *A. taxiformis* (Stefenoni et al., 2021) were much more effectively to reduce enteric CH_4 production (-67.2 and -34.4%, respectively) than ASCO meal likely because the presence of halogenated compounds (e.g., bromoform) in both red seaweeds.

Table 1. Predominant seaweed genera and species used as feed supplements to ruminants, chickens, swine, equine, fish, oyster, and shrimp.

Seaweed genera or species	**Seaweed type**	**Animal species fed**
Ascophyllum nodosum	Brown	Beef cattle, broiler chicken, dairy cattle, horse, fish, sheep swine
Laminaria	Brown	Dairy cattle, fish, sheep, swine
Lithothamnion	Red	Beef cattle, rabbit
Macrocystis pyrifera	Brown	Beef cattle, dairy cattle, goat, shrimp
Sargassum	Brown	Broiler chicken, dairy cattle, fish, goat, laying hen, sheep
Palmaria palmata	Red	Sheep
Ulva	Green	Broiler chicken, laying hen, fish, oyster, rabbit, sheep, shrimp
Asparagopsis taxiformis	Red	Beef cattle, dairy cattle, sheep
Asparagopsis armata	Red	Dairy cattle
Chondrus crispus	Red	Dairy cattle, laying hen

Another topic that attracts public attention regarding the use of seaweeds in dairy diets is the transfer of iodine and brominated metabolites to milk due to potential risks to human health (Brito, 2020; Fouts et al., 2022). Consequently, development of commercial algal-based feeds must consider tradeoffs between mitigation of enteric CH_4 emissions, and any human food safety, or environmental hazards linked to seaweeds (Vijn et al., 2020). Additional barriers to seaweed commercialization include production scalability and regulatory approval (Makkar et al., 2016; Vijn et al., 2020; Honan et al., 2021). One of the objectives of the present paper is to report the effect of the brown seaweed species ASCO and the red seaweed species *Chondrus crispus* on production performance,

enteric CH_4 emissions, milk iodine concentration, and cow health. These seaweeds grow in the Atlantic coast of North America, and ASCO meal is commercially available in the US and popular among organic dairy producers in the country (Hardie et al., 2014; Antaya et al., 2015; Sorge et al., 2016; Snider et al., 2021), thus justifying research and review of both ASCO and *C. crispus*. A second objective of this paper is to review the effect of seaweeds with high CH_4 mitigation potential (i.e., *Asparagopsis* species) on production performance, enteric CH_4 emissions, milk iodine concentration, and cow health. It is beyond the scope of this paper to provide a systematic review of all seaweeds or seaweed mixtures that have been fed to ruminants including lactating dairy cows. The nutrient composition of 4 selected seaweed species (ASCO, *A. armata*, *A. taxiformis*, and *C. crispus*) is also reviewed.

Nutrient Composition of Selected Seaweeds

Table 2 shows the nutrient composition of selected seaweeds used in diets of lactating dairy cows. It was detected some variation in the nutrient composition of ASCO meal, particularly in the fibrous fractions (neutral detergent fiber, acid detergent fiber, and lignin) and minerals such as iron, zinc, and iodine. These discrepancies in nutrient composition may be associated with various sources of ASCO meal used in the studies, as well as different harvesting and processing procedures adopted by seaweed companies and seasonality (Evans and Critchley, 2014). It is important to note that ASCO is wild harvested in the US, which can lead to inconsistencies in nutrient composition due to less controlled conditions.

Concentration of crude protein was greater in the red seaweeds *A. armata*, *A. taxiformis*, and *C. crispus* than in the brown seaweed ASCO, with *A. armata* showing the greatest content (18.3%). Similarly, the red seaweeds had greater ash concentration than ASCO meal, particularly A*. taxiformis* that averaged 55.5% ash. *Asparagopsis taxiformis* also had the greatest concentrations of iron (4,964 mg/kg of DM) and bromoform (~10 mg/g of DM). Compared with *C. crispus*, ASCO meal had a greater proportion of the total crude protein constituted by soluble crude protein (Table 2). In general, the concentration of neutral detergent fiber was greatest in ASCO meal, intermediate in *C. crispus*, and lowest in both *Asparagopsis* species. As discussed above, variation in nutrient composition between brown and red seaweeds possibly reflect differences in methods used for harvesting, processing, and storage, as well as seasonality and geographical location.

The dietary inclusion of seaweeds ranged from about 0.27 to 1% in studies done with ASCO meal (Antaya et al., 2015, 2019; Silva et al., 2022), *A. armata* (Roque et al., 2019), and *A. taxiformis* (Stefenoni et al., 2021), suggesting that the contribution of macronutrients (e.g., protein, fiber) from algal feeds to meet amino acids and energy requirements of dairy cows is small. On the other hand, up to 6% (diet DM basis) of *C. crispus* was fed to dairy cows (Brito's Lab unpublished), and with similar or greater inclusion rate, diets need to be formulated considering the seaweed contribution to cows' nutritional requirements. Seaweeds bioaccumulate minerals as shown by their high concentration of ash (Table 2), which may require careful ration formulation to not

overfeed certain minerals (e.g., iodine) while avoiding interactions between minerals and other dietary compounds in the gastrointestinal tract, which can ultimately impair mineral absorption (Goff, 2018).

Table 2. Nutrient composition [% of dry matter (DM), unless otherwise noted] of *Ascophyllum nodosum*, *Asparagopsis* species, and *Chondrus crispus* used in studies conducted with lactating dairy cows.

	Seaweeds			
Nutrient	*A. nodosum*[1]	*A. armata*[2]	*A. taxiformis*[3]	*C. crispus*[4]
Crude protein (CP)	10.2, 10.3, 7.65	18.3	14.6	12.8
Soluble CP, % of CP	57.0, 54.0, 39.5	-	-	22.7
Neutral detergent fiber	53.9, 39.2, 46.8	27.2	18.5	40.5
Acid detergent fiber	39.9, 20.8, 31.9	10.9	11.3	7.73
Lignin	20.0, 12.2, 16.3	2.83	-	2.60
Neutral detergent insoluble CP	5.50, 5.60, 5.40	-	-	-
Acid detergent insoluble CP	5.31, 5.10, 4.75	-	-	-
Ether extract	2.30, 2.40, 3.40	0.32	0.89	2.53
Starch	0.70, 0.40, 0.70	-	0.80	-
Ethanol soluble carbohydrates	3.30, 3.90, 0.95	-	-	-
Ash	25.9, 26.1, 22.9	50.4	55.5	36.9
Calcium	1.31, 1.28, 1.12	4.47	3.31	3.74
Phosphorus	0.25, 0,21, 0.16	0.27	0.22	0.23
Magnesium	0.69, 0.80, 0.89	1.38	1.56	0.91
Potassium	3.53, 2.57, 2.51	-	2.48	2.47
Sodium	3.90, 3.59, 3.42	9.36	10.2	4.40
Sulfur	2.84, 2.71, 3.37	-	-	5.40
Chloride	4.70, 4.73, 3.16	-	-	-
Iron, mg/kg of DM	287, 403, 234	1,188	4,964	1,570
Zinc, mg/kg of DM	9.00, 11.0, 33.5	66.3	21.0	53.7
Copper, mg/kg of DM	3.00, 4.00, 2.50	13.3	7.00	2.00
Manganese, mg/kg of DM	20.0, 24.0, 24.5	62.3	92.0	106
Arsenic, mg/kg of DM	28.3, -, 14.9	-	-	-
Iodine, mg/kg of DM	820, 727, 415	-	-	394
Bromoform, mg/g of DM	-,-,-	1.32	10[5]	-

[1]Data are mean values reported by Antaya et al. (2015), Antaya et al. (2019), and Silva et al. (2022), respectively.
[2]Data are mean values reported by Roque et al. (2019).
[3]Data are mean values reported by Stefenoni et al. (2021).
[4]Data are unpublished results from Brito's Lab.
[5]Value based on Figure 3 (experiment 4) reported by Stefenoni et al. (2021).

Effect of Selected Seaweeds on Production Performance in Lactating Dairy Cows

Ascophyllum nodosum

There are few controlled studies in which DMI was measured individually in lactating dairy cows supplemented with ASCO meal. Antaya et al. (2015) reported that DMI tended to increase quadratically (17.5, 18.1, 18.1, and 17.6 kg/d) in dairy cows fed

incremental amounts (0, 57, 113, and 170 g/d) of ASCO meal. In 2 follow-up studies done at the University of New Hampshire, DMI (mean = 17.1 kg/d) did not change in dairy cows supplemented (113 g/d) or not with ASCO meal (Antaya et al., 2019) during the grazing season or in dairy cows (mean = 21.1 kg/d) receiving increasing levels (0, 57, 113, and 170 g/d) of ASCO meal or 300 mg/d of monensin (Silva et al., 2022). Similarly, Pompeu et al. (2011) feeding 56 and 132 g/d of ASCO meal and Cvetkovic et al. (2014) feeding 57 g/d of ASCO meal found no difference in DMI of dairy cows. Collectively, these results indicate that ASCO meal supplemented up to 170 g/d had no negative impact on DMI in Jersey (Antaya et al., 2015, 2019; Silva et al., 2022) or Holstein (Pompeu et al., 2011; Cvetkovic et al., 2014) cows.

Yields of milk yield, 4% fat-corrected milk (FCM), and energy-corrected milk (ECM) were not affected in dairy cows fed varying amounts of ASCO meal (Antaya et al., 2015, 2019; Silva et al., 2022), thus in agreement with Pompeu et al. (2011) and Karatzia et al. (2012). Whereas yields of 4%FCM and ECM did not change with feeding ASCO meal versus the control diet in the study of Cvetkovic et al. (2004), milk yield increased by 1.7 kg/d. Kellogg et al. (2006) reported a significant interaction between ASCO meal supplementation and breed for milk yield, with large-frame cows (mostly Holsteins) producing more milk (+2.3 kg/d) when offered ASCO meal (mean = 104 g/d) than those in the control diet, but no difference was observed for small-frame cows (mostly Jerseys, Milking Shorthorns, and Holstein × Jersey crosses). Positive milk yield responses in the experiments of Cvetkovic et al. (2004) and Kellogg et al. (2006) may be associated with beneficial effects of ASCO meal on alleviating heat stress in ruminants as this seaweed seems to regulate body temperature despite the mechanism not being fully elucidated (Allen et al., 2001). In fact, Pompeu et al. (2011) demonstrated that ASCO meal supplementation reduced body temperature to increasing ambient temperature in dairy cows during the hot summer months. Furthermore, Kellogg et al. (2006) showed decreased respiration rate in ASCO meal-fed cows over the summer. However, body temperature and respiration rate were not impacted with ASCO meal supplementation to heat-stressed (Cvetkovic et al., 2004) or grazing (Antaya et al., 2019) dairy cows, with both studies conducted in the summer. In all these experiments (i.e., Cvetkovic et al., 2004; Kellogg et al., 2006; Pompeu et al., 2011; Antaya et al., 2019), cows were not submitted to controlled heat stress conditions, thus data should be interpreted cautiously.

Concentrations and yields of milk fat and protein were not changed in cows supplemented with varying levels of ASCO meal (Antaya et al., 2015, 2019; Pompeu et al., 2011; Karatizia et al., 2012; Chaves Lopez et al., 2016; Silva et al., 2022). Cvetkovic et al. (2004) reported that milk fat concentration tended to decrease in cows offered ASCO meal possibly in response to a dilution effect caused by increased milk volume (+1.7 kg/d). In fact, milk fat yield did not change between treatments indicating no effect of diets on milk fat synthesis in mammary tissues (Cvetkovic et al., 2004). Whereas milk protein concentration was not affected by diets in the study of Cvetkovic et al. (2004), milk protein yield followed milk production and increased with ASCO meal supplementation. *Ascophyllum nodosum* is rich in phlorotannins, which are polyphenolic compounds known to make complexes with proteins and carbohydrates (Ragan and Glombitza, 1986). It is conceivable that ASCO-phlorotannins may have reduced protein degradation in the

rumen, with escaped amino acids used for milk protein synthesis in the mammary gland. Kellogg et al. (2006) observed inconsistent treatment effect on milk fat concentration as it was lower for cows fed control versus ASCO meal in July but tended to increase with feeding ASCO during August. They attributed this discrepancy to temporal changes in milk fat concentration not related to dietary treatments. In contrast, milk protein concentration was not affected by ASCO meal supplementation in the experiment of Kellogg et al. (2006). Note that Kellogg et al. (2006) did not report production of milk components in their study.

Asparagopsis armata

This author is aware of only 1 published study (i.e., Roque et al., 2019) in which lactating dairy cows were fed *A. armata*. Roque et al. (2019) fed incremental amounts (0, 0.5, and 1%; diet OM basis) of *A. armata* to dairy cows in a 3 × 3 Latin square design (21-d periods) and reported a decrease in DMI of 3.0 and 10.6 kg/d comparing the control diets with 0.5 or 1% of *A. armata* supplementation, respectively. Authors hypothesized that decreased DMI may have been associated with the high concentration of minerals supplied by *A. armata* resulting in poor palatability. They also observed that while milk yield was 4.2 kg/d lower in cows receiving 1% *A. armata* than in those assigned to the control diet, no difference was detected between 0 and 0.5% *A. armata*. Reduced DMI (-3.0 kg/d) accompanied by similar milk yield with feeding control versus 0.5% *A. armata* implies mobilization of body reserves to keep up with milk synthesis. However, body weight (BW) change did not differ between these 2 diets, which may have been caused by limitations of short-term, changeover experimental designs to discriminate treatment differences for variables that represent altered nutrient partitioning such as BW change and retained N (Zanton, 2019).

Milk fat concentration averaged 3.84% and was not affected by diets with varying levels of *A. armata* (Roque et al., 2019). Contrarily, milk protein concentration was greatest, intermediate, and lowest in cows fed 0, 0.5, and 1% *A. armata*, respectively. Although milk protein yield was not reported by Roque et al. (2019), calculated milk protein yield decreased by 17% with feeding 1 versus 0% *A. armata*. As discussed above, DMI decreased by 38% in cows supplemented with 1% *A. armata* possibly leading to a reduced supply of rumen-degradable protein, which can ultimately impair microbial protein synthesis and availability of essential amino acids for milk protein synthesis. Overall, feeding *A. armata*, particularly at the greatest level of supplementation (i.e., 1% of the diet OM) negatively affected DMI, milk yield, and milk protein concentration. Therefore, further research is needed to better understand the use of *A. armata* for high-producing dairy cows.

Asparagopsis taxiformis

Research in which lactating dairy cows were fed diets containing *A. taxiformis* is scarce. Stefenoni et al. (2021) supplemented dairy cows with incremental amounts (0, 0.25, and 0.5% of the diet DM) of *A. taxiformis* and oregano leaves and observed that feeding 0.5% *A. Armata* led to the lowest DMI (-1.8 kg/d compared with the control diet).

Both yields of milk and ECM followed DMI and decreased by 2.6 and 2.4 kg/d, respectively, in cows fed 0.5% *A. taxiformis* relative to control. According to Stefenoni et al. (2021), decreased palatability with feeding 0.5% *A. taxiformis* was likely involved with the observed depression of DMI in their experiment. They also stated that the likelihood of sorting was negligible considering that *A. taxiformis* was finely ground and mixed into the total-mixed ration, further reinforcing a potential taste avoidance response. In fact, Muizelaar et al. (2021) reported that cows either frequently refused or selected against a concentrate mix containing *A. taxiformis*, dextrose, wheat, dehydrated beet pulp, and water. Whereas the concentrations of milk fat and milk protein were not affected with feeding *A. taxiformis*, their yields decreased by 6.2 and 6.3%, respectively, comparing 0.5% *A. taxiformis* versus control (Stefenoni et al., 2021). As discussed above for *A. armata*, further research is needed to better understand the processes underpinning the negative impact of *A. taxiformis* on production performance of high-producing dairy cows.

Chondrus crispus

While both *Asparagopsis* species discussed herein are not native of the US coast, the red seaweed *C. crispus* grows in the intertidal zone of the North Atlantic including the Gulf of Maine where it is wild harvested. In fact, a 3 × 3 Latin square design study was conducted at the University of New Hampshire (Brito's Lab unpublished) to evaluate the effect of incremental amounts of *C. crispus* (0, 3, and 6% of the diet DM) on DMI, milk production, and milk composition using 18 Jersey cows fed total-mixed rations with a 65:35 forage:concentrate ratio. Feeding *C. crispus* decreased DMI linearly (20.7, 19.3, and 18.9 kg/d for 0, 3, and 6% *C. crispus*, respectively). This reduction in DMI could be associated with palatability issues or sorting. *Chondrus crispus* used in the study was dried and milled into small flakes (mean particle size = 3.2 mm) and cows may have had the opportunity to sort. In fact, it was frequently observed small, harder pieces of *C. crispus* in the orts which appear to be the seaweed stipe (i.e., stem-like structure), indicating that cows selectively refused parts of the alga material. Despite the linear reduction in DMI, milk yield did not change and averaged 18.4 kg/d across treatments. Likewise, concentration (mean = 5.51%) and yield (mean = 1.01 kg/d) of milk fat, and concentration (mean = 3.64%) and yield (mean = 0.67 kg/d) of milk true protein were similar among diets. Overall, inclusion of up to 6% *C. crispus* in the diet DM negatively affected DMI but milk yield and composition remained unchanged.

Effect of Selected Seaweeds on Enteric Methane Emissions in Dairy Cows

Ascophyllum nodosum

Previous *in vitro* research revealed that phlorotannins extracted from ASCO dosed at 500 µg/L reduced CH_4 production in batch culture with forage (barley silage plus alfalfa hay) or ground barley as substrates (Wang et al., 2008). Similarly, Belanche et al. (2016) observed a quadratic decrease in CH_4 production during an *in vitro* batch culture study with vials dosed with incremental levels (up to 2 g/L) of ASCO meal. Therefore, ASCO meal has potential to suppress enteric CH_4 production *in vivo*. Table 3 shows the effect of ASCO meal, *Asparagopsis* species, and *C. crispus* on enteric CH_4 production, as well

as mean percentage change when comparing the control diets versus those with the greatest inclusion of seaweeds.

To the best of this author knowledge, only 1 study (i.e., Antaya et al., 2019) was published to date evaluating the effect of ASCO meal on enteric CH_4 emissions in lactating dairy cows. Antaya et al. (2019) reported a diet × period interaction for enteric CH_4 production, which decreased by 10.4% in grazing dairy cows supplemented with 113 g/d of ASCO meal during the first period of the study (June) but not thereafter (July-September). This suggests a transient effect of ASCO meal on suppressing methanogenesis or an adaptation of the archaeal community to ASCO meal supply over time. Moreover, CH_4 yield and CH_4 intensity did not change and averaged 20 g/kg of DMI and 23.4 g/kg of ECM, respectively, between treatments (Antaya et al., 2019). Zhou et al. (2018) reported a linear decrease in the ruminal concentration (copies/g of DM) of archaea when rams received increasing dietary levels of ASCO (up to 5% of the diet DM), suggesting that ASCO meal should be supplemented at a greater amount than that fed by Antaya et al. (2019) to consistently inhibit ruminal methanogenesis. Nevertheless, unpublished results from Brito's Lab revealed no change in enteric CH_4 production (mean = 389 g/d), CH_4 yield (mean = 18.7 g/kg of DMI), and CH_4 intensity (mean = 13.8 g/kg of ECM) in dairy cows supplemented with 400 g/d of ASCO (~2% of diet DM) for 3 weeks. It is important to note that high dietary inclusion (>1% of the diet DM) of ASCO meal may not be feasible in commercial settings due to the risk of iodine toxicity and impairment of the thyroid function. Overall, based on limited *in vivo* research done with lactating dairy cows, it appears that ASCO meal has low enteric CH_4 mitigation potential.

Asparagopsis armata

Roque et al. (2019) reported that compared with the control diet (0% *A. amarta*), enteric CH_4 production decreased by 26.4 and 67.2% in dairy cows supplemented (diet OM basis) with 0.5 and 1% *A. armata*, respectively (Table 3). Similarly, CH_4 yield decreased by 20.3 and 42.7%, and CH_4 intensity by 18.2 and 60.1% with feeding 0.5 and 1% *A. armata*, respectively, relative to the control diet. *Asparagopsis armata* is known to bioaccumulate bromoform (Paul et al., 2006) that, in turn, has been shown to inhibit methanogenesis possibly in synergy with other halogenated or brominated compounds (Machado et al., 2018). In briefly, *A. armata* effectively suppressed CH_4 emissions, particularly at the greatest supplementation level. However, DMI, milk yield, and concentrations of milk fat and protein also decreased, which may limit the large-scale use of *A. armata* in dairy diets.

Asparagopsis taxiformis

Enteric CH_4 production (Table 3), CH_4 yield, and CH_4 intensity decreased by 34.4, 29.4, and 26.2%, respectively, in dairy cows fed (diet DM basis) 0.5% *A. taxiformis* versus the control diet (0% *A. taxiformis*) in experiment 3 of Stefenoni et al. (2021). However, no differences in enteric CH_4 production, CH_4 yield, and CH_4 intensity were observed between the control diet and 0.25% *A. taxiformis* (Stefenoni et al., 2021). According to Stefenoni et al. (2021), decreased CH_4 emissions in response to *A. taxiformis* supplementation was associated with the presence of

bromoform and possibly other halogenated and brominated metabolites that accumulate in the tissues of this red seaweed. They also observed a decrease in the molar proportion of ruminal acetate and an increase in that of ruminal propionate with feeding 0.5% *A. taxiformis* versus control, thus indicating a shift in fermentation toward propionate, which is a hydrogen sink. Authors further reported that *A. taxiformis* (0.5% of the diet DM) was highly effective to suppress CH_4 yield in periods 1 and 2 of the study (mean = -55% reduction), but no treatment difference was seen on periods 3 and 4, possibly because of bromoform losses during storage over time. In contrast, Roque et al. (2021) demonstrated consisted suppression of CH_4 production and CH_4 yield in beef steers fed 0.5% *A. taxiformis* throughout the 21-week study, thus suggesting differences in the preservation of bromoform between batches of *A. taxiformis*. Overall, *A. taxiformis* has high potential as a dietary strategy to mitigate enteric CH_4 emissions in ruminants, but reduced production performance reported by Stefenoni et al. (2021) may limit producers' adoption.

Table 3. Enteric methane (CH_4) production and mean percentage change in lactating dairy cows fed *Ascophyllum nodosum* (ASCO) meal, *Asparagopsis* species, or *Chondrus crispus*.

Breed	Basal diet[1]	Treatments	CH_4, g/d	Reference
Jersey	Pasture + pTMR	0 g/d ASCO meal	371	Antaya et al. (2019)[2]
		113 g/d ASCO meal	363	
		Mean % change[3]	-2.16	
Holstein	TMR	0% *A. armata*	396[4]	Roque et al. (2019)
		0.5% *A. armata*	291[4]	
		1% *A. armata*	130[4]	
		Mean % change[3]	-67.2	
Holstein	TMR	0% *A. taxiformis*	349	Stefenoni et al. (2021)[5]
		0.5% *A. taxiformis*	350	
		1% *A. taxiformis*	229	
		1.77% oregano leaves	374	
		Mean % change[3]	-34.4	
Jersey	TMR	0% *C. crispus*	383	Brito's unpublished
		3% *C. crispus*	352	
		6% *C. crispus*	351	
		Mean % change[3]	-8.4	

[1]pTMR = partial total-mixed ration; TMR = total-mixed ration.
[2]A diet by period interaction was observed for enteric CH_4 production, which decreased by 10.3% with feeding 113 g/d of ASCO meal on period 1 but no change between diets thereafter.
[3]Mean % change comparing control versus diets with the greatest inclusion of seaweeds.
[4]Aproximate values based on Figure 1A and percentage reduction reported in the text.
[5]Data from experiment 3.

Chondrus crispus

As for both *Asparagopsis* species discussed above, there is limited *in vivo* research in which the enteric CH_4 mitigation potential of the red seaweed *C. crispus* was evaluated. It should be noted that the amounts of *C. crispus* fed *in vivo* during a study

conducted at the University of New Hampshire (Brito's Lab unpublished) were based on results from preliminary *in vitro* research that showed moderate to high CH_4 mitigation potential in response to *C. crispus*. Cows fed incremental amounts (% of the diet DM) of *C. crispus* had a linear decrease in enteric CH_4 production (from 383 to 351 g/d; Table 3), thus in line with the linear reduction seen for DMI discussed previously. Compared with the control diet, enteric CH_4 production dropped by 8.4% with feeding 6% *C. crispus* (Table 3). However, CH_4 production was similar between 3% (352 g/d) and 6% (351 g/d) *C. crispus*, suggesting that it should not be fed in levels > 3% of the diet DM. Both CH_4 yield (mean = 18.4 g/kg of DMI) and CH_4 intensity (mean = 15.3 g/kg of ECM) did not differ across diets. Bromoform was not measured in *C. crispus* but based on a much less effective response on suppressing enteric CH_4 production compared with *A. armata* (Roque et al., 2019) or *A. taxiformis* (Stefenoni et al., 2021), it is conceivable that bromoform accumulation by *C. crispus* is minimal despite it having the enzymatic systems to synthesize this brominated metabolite (Thapa et al., 2020).

Effect of Selected Seaweeds on Milk Concentrations of Iodine and Brominated Metabolites and Human Health Implications

Milk iodine concentration

Iodine is a structural component of the thyroid hormones triiodothyronine (T3) and thyroxine (T4), and iodine deficiency is a public health concern that been linked to goiter and poor brain development as reviewed by Fuge and Johnson (2015). On the other hand, excess iodine intake can lead to thyroiditis, hyperthyroidism, hypothyroidism, and goiter in individuals with underlining thyroid issues or in vulnerable groups such as seniors, fetuses, and neonates (Pennington, 1990; Katagiri et al., 2017). Seaweeds (brown > red > green) bioaccumulate iodine through the uptake of iodide leached into the seawater based on the review of Fuge and Johnson (2015). Therefore, milk iodine concentration generally increases in response to seaweed supplementation to dairy cows. Table 4 shows the milk iodine concentration and mean percentage change comparing the control diets with those with the greatest dietary inclusion of seaweeds.

Milk iodine concentration increased linearly from 178 to 1,370 µg/L (Antaya et al., 2015) and from 383 to 1,228 µg/L (Silva et al., 2022) in dairy cows fed incremental amounts (0, 57, 113, and 170 g/d) of the brown seaweed ASCO (Table 4). Milk iodine concentration was, on average, 318% greater in grazing dairy cows supplemented with 113 g/d ASCO meal (mean = 481 µg/L) than in the diet without seaweed supplementation (mean = 118 µg/L; Antaya et al., 2019; Table 4). Similarly, feeding the red seaweeds *A. taxiformis* (Stefenoni et al., 2021) or *C. crispus* (Brito's Lab unpublished) increased the concentration of iodine in cow's milk. Specifically, Stefenoni et al. (2021) reported a 416% increase in milk iodine concentration comparing 0.5% *A. taxiformis* (mean = 2,966 µg/L) versus control (mean = 575 µg/L) as shown in Table 4. A linear increase in milk iodine concentration was observed in dairy cows fed increasing dietary levels of *C. crispus* (from 204 to 1,796 µg/L; Table 4). Furthermore, mean milk iodine concentration (1,021 µg/L) from cows fed 113 g/d of ASCO meal (~0.58% of the diet DM; Antaya et al., 2015; Silva et al., 2022) was 66% lower than that from cows fed the greatest amount (0.5% of the

diet DM; ~118 g/d) of *A. taxiformis* (Stefenoni et al. (2021). This discrepancy in milk iodine concentration between ASCO meal and *A. taxiformis* despite similar amounts fed suggest differences in seaweed iodine concentration or iodine bioavailability. Note that Stefenoni et al. (2021) did not report the iodine concentration of the *A. taxiformis* used in their study. However, Roque et al. (2021) reported a mean iodine concentration of 2,270 mg/kg for *A. taxiformis* in their study, with this value being 177 and 447% greater than that obtained by Antaya et al. (2015) and Silva et al. (2022), respectively.

Table 4. Milk iodine concentration and mean percentage change in lactating dairy cows fed *Ascophyllum nodosum* (ASCO) meal, *Asparagopsis taxiformis*, or *Chondrus crispus*.

Breed	Basal diet[1]	Treatments	Milk iodine, µ/L	Reference
Jersey	TMR	0 g/d ASCO meal	178	Antaya et al. (2015)
		57 g/d ASCO meal	602	
		113 g/d ASCO meal	1,015	
		170 g/d ASCO meal	1,370	
		Mean % change[2]	+670	
Jersey	Pasture + pTMR	0 g/d ASCO meal	118	Antaya et al. (2019)[3]
		113 g/d ASCO meal	481	
		Mean % change[2]	+308	
Holstein	TMR	0% *A. taxiformis*	575	Stefenoni et al. (2021)[4]
		0.5% *A. taxiformis*	2,966	
		Mean % change[2]	+416	
Jersey	TMR	0 g/d ASCO meal	383	Silva et al. (2022)
		57 g/d ASCO meal	729	
		113 g/d ASCO meal	1,027	
		170 g/d ASCO meal	1,228	
		300 mg/d monensin	339	
		Mean % change[2]	221	
Jersey	TMR	0% *C. crispus*	204	Brito's unpublished
		3% *C. crispus*	848	
		6% *C. crispus*	1,796	
		Mean % change[2]	+780	

[1]pTMR = partial total-mixed ration; TMR = total-mixed ration.
[2]Mean % change comparing control versus diets with the greatest inclusion of seaweeds.
[3]A diet by period interaction was observed for milk iodine concentration, with the greatest difference in milk iodine between diets (+416%) being detected in period 2.
[4]Data from experiment 3.

According to the European Food Safety Authority (EFSA, 2013), milk iodine concentration should not exceed 500 µg/L to minimize risks of iodine toxicity in humans. Figure 1 shows the distribution of milk iodine concentration (n = 128 individual observations) from cows fed ASCO meal (Antaya et al., 2015, 2019; Silva et al., 2022) or *C. crispus* (Brito's Lab unpublished). Based on Figure 1, 80% (102 out of 128) of the individual observations was above the 500-µg/L threshold considered safe for humans'

health (EFSA, 2013). Likewise, milk iodine concentration from cows fed *A. taxiformis* was 5.9-fold greater than 500 µg/L (Stefenoni et al., 2021). The 2020–2025 Dietary Guidelines for Americans recommends the consumption of 3 cups-equivalent (1 cup = 236.7 mL) of fat-free or reduced-fat milk daily for children and adolescents ages ≥ 9-18 and adults as part of a healthy diet (USHHS USDA, 2020). For examples, boys (age 9-13 years old) consuming 3 cups-equivalent of milk from cows fed 0.5% *A. taxiformis* would exceed their iodine recommended dietary allowance (i.e., 120 µg/d; US Institute of Medicine, 2001) by 17.6-fold and the iodine tolerable upper intake (i.e., 600 µg/d; US Institute of Medicine, 2001) by 3.5-fold assuming milk as the sole iodine source in their diet. Therefore, a hypothetical large-scale adoption of *A. taxiformis* by dairy producers across the US to mitigate enteric CH_4 emissions would require approaches to reduce iodine concentration of *A. taxiformis* such as washing procedures or using feeds containing goitrogenic compounds like canola meal or legumes (e.g., white clover).

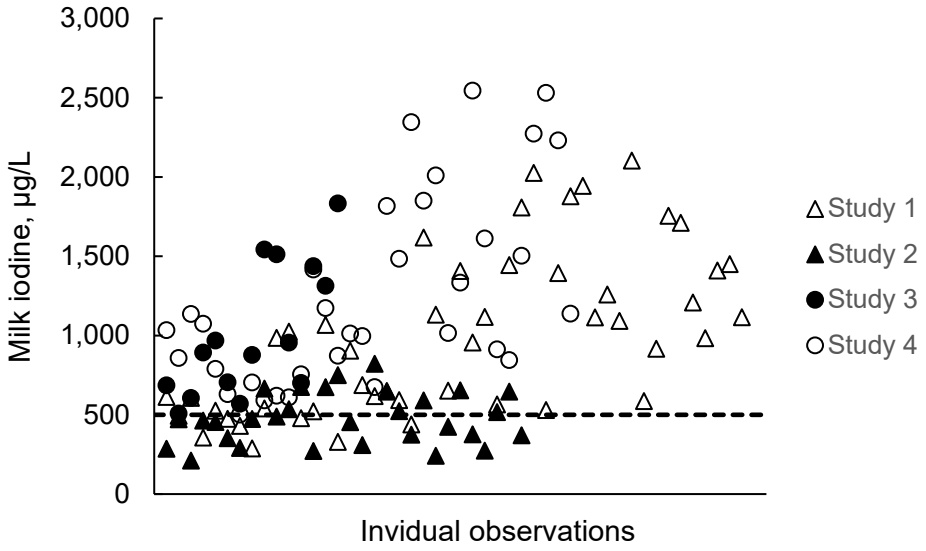

Figure 1. Individual milk iodine concentration observations (n = 128) from Jersey cows fed varying amounts of *Ascophyllum nodosum* meal (Study 1 = Antaya et al., 2015; Study 2 = Antaya et al., 2019; Study 3 = Silva et al., 2022) or *Chondrus crispus* (Study 4 = Brito's Lab unpublished). The dashed line represents the 500-µg/L threshold considered safe for human health according to the European Food Safety Authority (EFSA, 2013).

Weiss et al (2015) reported linear reductions in milk iodine concentration in dairy cows fed increasing amounts (from 0 to 13.9% of the diet DM) of canola meal and 2 levels (0.5 and 2 mg/kg) of supplemental iodine as ethylenediamine dihydroiodideiodine. On average, milk iodine concentration dropped by 43% (from 725 to 413 µg/L) and was below 500 µg/L in cows fed the greatest amounts of canola meal and supplemental iodine (Weiss et al., 2015). Antaya et al. (2019) observed that the milk iodine concentration of grazing dairy cows supplemented with 113 g/d of ASCO meal was below 500 µg/L (mean = 432 µg/L) in 2 out of 3 periods (diet × period interaction) likely associated with increased intake of goitrogenic compounds from grazed herbage. For comparison, milk iodine

concentration from dairy cows in confinement supplemented with 113 g/d of ASCO meal averaged 1,015 µg/L (Antaya et al., 2015) and 1,027 µg/L (Silva et al., 2022). In addition to seaweed washing procedures and feeds with goitrogenic compounds, it may be necessary to eliminate the use of iodine-based solutions for milking hygiene procedures and feed iodine-free mineral/vitamin premixes if cows are to be supplemented with seaweeds with high CH_4 mitigation such as *A. armata* or *A. taxiformis*.

Milk concentration of brominated metabolites

As discussed earlier, red seaweeds accumulate several brominated metabolites, particularly bromoform (Paul et al., 2006). Therefore, bromoform along with bromide can be transferred to milk, thus raising human-health concerns (Roque et al., 2019). In fact, the US Environmental Protection Agency (US EPA, 2008) and the World Health Organization (WHO, 2017) have both set maximum standard concentrations for bromoform in drinking water at 80 and 100 µg/L, respectively. Regarding bromide, it has been suggested an acceptable daily intake of 0.4 mg/kg of BW, which would result in an acceptable total daily intake of 24 mg of this metabolite for an individual weighing 60 kg (EMEA, 1997).

Roque et al. (2019) reported no treatment differences in the milk concentration of bromoform, which averaged 0.11, 0.15, and 0.15 µg/L in dairy cows fed (diet OM basis) 0, 0.5, and 1% *A. armata*, respectively, in a 3 × 3 Latin square design with 21-d periods. Stefenoni et al. (2021) observed that the milk concentration of bromoform was not affected with feeding (diet DM basis) of 0.5% *A. taxiformis* to dairy cows despite a numerical increase of 75.2% when comparing the control diet (16.5 µg/L) with that containing seaweed (28.9 µg/L) in a 4 × 4 Latin square design with 28-d periods (experiment 3; bromoform measurements done on control and 0.5% seaweed diets only). The lack of treatment effect on milk bromoform concentration in the study of Stefenoni et al. (2021) may be associated with a high variation in animal response or in the analytical procedure or both considering that the standard error of the mean was 10.6 µg/L. Interestingly, milk bromoform concentration was 196-fold greater in cows fed *A. taxiformis* than in those receiving *A. armata*, but the reason for this discrepancy is not obvious based on data reported by Roque et al. (2019) and Stefenoni et al. (2021). Muizelaar et al. (2021) reported that on d 1 of their study bromoform was detected in the milk of most cows fed (diet DM basis) 67 and 133 g/d of *A. taxiformis* (mean = 9.1 and 11 µg/L, respectively), and it was again detected in the milk of only 1 cow supplemented with 333 g/d of *A. taxiformis* on d 9 (mean = 35 µg/L). No bromoform was found above the detection limit level (i.e., 5 µ/L) in the milk of all cows on d 10 and 17, which may be partially explained by animals inconsistently consuming the seaweed treatments and, at times, completely avoiding *A. taxiformis*.

The mean milk bromoform concentration (0.15 µg/L) reported by Roque et al. (2019) in cows fed both *A. armata* diets (0.5 and 1%) was, on average, 533- and 667-fold lower than the threshold levels considered acceptable for drinking water according to the US Environmental Protection Agency (80 µg/L; US EPA, 2008) and the World Health Organization (100 µg/L; WHO, 2017), respectively. As for *A. taxiformis*, using the milk

bromoform concentration of 28.9 µg/L from cows fed the diet with 0.5% seaweed and the recommended 3 cups-equivalent of milk/d for children (USHHS USDA, 2020), it would result in a daily bromoform consumption of 20.5 µg, thus 3.9-. and 4.9-fold lower than the maximum bromoform concentrations set for drinking water by the US Environmental Protection Agency (US EPA, 2008) and the World Health Organization (WHO, 2017), respectively. Therefore, based on limited and variable data (Roque et al., 2019; Muizelaar et al., 2021; Stefenoni et al., 2021), milk bromoform concentration in dairy cows' milk appear to be safe for human consumption, but further research is needed to better understand the reasons behind discrepant milk bromoform results reported in the literature. Specifically, research is needed to improve knowledge on metabolism and pharmacokinetics of bromoform in dairy cows, while standardizing sample processing and analytical methods used to quantify bromoform across different biological matrices (e.g., milk, urine, blood, feces, tissues).

Stefenoni et al. (2021) measured bromide in milk and reported an average concentration of 40.4 mg/kg in cows fed 0.5% *A. taxiformis*, which was 692% greater than that found in milk from cows in the control diet (5.1 mg/kg). Following the recommended intake of 3 cups-equivalent of milk/d for children and adolescents ages ≥ 9-18 and adults based on the 2020–2025 Dietary Guidelines for Americans (USHHS USDA, 2020), it would result in a consumption of 28.7 mg/d of bromide if drinking milk from cows fed 0.5% *A. taxiformis*, thus slightly above the acceptable daily intake of 24 mg of bromide for an individual weighing 60 kg (EMEA, 2007). However, studies done with healthy volunteers dosed with up to 9 mg of bromide/kg of BW showed no detrimental effects on human health apart from incidental nausea episodes (Sangster et al., 1982, 1983). Further research is needed to better understand the variation in milk bromide concentration in response to varying dietary levels of *A. taxiformis*.

Effect of Selected Seaweeds on Iodine and Bromoform Metabolism in Dairy Cows

Iodine

There is scarce information on the effect of seaweeds on iodine metabolism in lactating dairy cows. Specifically, this author is aware of only 2 studies that investigated the effects of incremental amounts of seaweeds on iodine intake and output on milk, urine, and feces as shown in Table 5. On average, 140 and 61% of the iodine consumed was excreted via feces in cows supplemented with ASCO meal (Silva et al., 2022) or *C. crispus* (Brito's Lab unpublished), respectively, indicating that iodine was extensively recycled via the gastrointestinal tract as documented in earlier research (e.g., Miller et al., 1975). Interestingly, when cows were fed ASCO meal, the amount of iodine secreted on milk (mean = 19.5 mg/d) and excreted in urine (mean = 19 mg/d) was very similar. However, when iodine intake increased by an average of 7.1-fold comparing ASCO-fed with *C. crispus*-fed cows, urine became the dominant route for iodine excretion after feces (Table 5). These results suggest that the sodium-iodide symporter present in the lactating mammary gland (Cavalieri, 1997) likely saturated due to excess iodine supply shifting the output of iodine from milk to urine. It was also observed a quadratic increase in the urinary excretion of iodine in cows fed *C. crispus*, with the difference in the amount excreted

being greater between 0 and 3% *C. crispus* (+45.3 mg/d) than between 3 and 6% (+13 mg/d). On the other hand, fecal excretion of iodine increased linearly following *C. crispus* supplementation (+128 and +96 mg/d comparing 0 vs. 3% and 3 vs. 6%, respectively) implying that as iodine intake largely surpassed requirement (from 3 to 6% *C. crispus*), iodine consumed was diverted from the urinary to the gastrointestinal tract. Potential environmental implications of excreted iodine need to be further investigated, particularly in a scenario of large adoption of seaweeds to mitigate enteric CH_4 emissions in both dairy and beef industries.

Table 5. Intake, milk secretion, and fecal and urinary excretion of iodine (mg/d) in dairy cows fed incremental amounts of *Ascophyllum nodosum* (ASCO) meal or *Chondrus crispus*.

	ASCO meal (g/d)[1]					*P*-value	
Item	0	57	113	170	SEM	Linear	Quadratic
Intake	8.60	28.7	48.6	68.9	2.34	<0.001	0.96
Milk	7.30	14.4	19.5	24.6	1.78	<0.001	0.44
Urine	5.10	14.1	18.0	24.8	1.98	<0.001	0.59
Feces	20.5	48.1	60.6	86.6	7.76	<0.001	0.92

	***C. crispus* (% of the diet DM)**					*P*-value	
Item	0	3	6	-	SEM	Linear	Quadratic
Intake	28.4	233	462	-	8.73	<0.001	0.20
Milk	4.48	15.0	31.4	-	2.12	<0.001	0.25
Urine	13.1	58.4	71.4	-	4.58	<0.001	<0.001
Feces	27.3	155	251	-	15.1	<0.001	0.21

[1]Adapted from Silva et al. (2022).
[2]Brito's Lab unpublished results.

Bromoform

It appears that the study conducted by Muizelaar et al. (2021) is the only one to this author knowledge that has investigated the effect of *A. taxiformis* on bromoform metabolism. In brief, bromoform was detect in urine of cows supplemented with *A. taxiformis* on d 1 and 10 of the experiment, but not on d 17 as it was below the limit detection level (<2 µg/L). Likewise, fecal bromoform concentration was not found in fecal samples because it was below the 20-µg/kg limit detection level. Overall, data from Muizelaar et al. (2021) should be interpreted cautiously due to cows either refusing or inconsistently consuming *A. taxiformis*, indicating that further research is needed to better understand bromoform metabolism in long-term experiments.

Effect of Selected Seaweeds on Iodine Intake and Iodine Toxicity Concerns in Dairy Cows

Excessive iodine intake can lead to toxicity in ruminants and associated symptoms such as excessive nasal and ocular discharge, hyperthermia, salivation, decreased milk

production, coughing, and dry scaly coats according to the review of Paulíková et al. (2002). Iodine intake averaged 68.9 and 462 mg/d, respectively, in dairy cows fed 170 g/d of ASCO meal (Silva et al., 2022) or 6% of the diet DM as *C. crispus* (Table 5). Adequate iodine intake was calculated as 8.16 and 7.93 mg/d, respectively, for cows receiving 170 g/d of ASCO meal (mean = 450 kg of BW and 27.2 kg/d of milk) or 6% of *C. crispus* (mean = 489 kg of BW and 22.5 kg/d of milk) based on the Equation 7-34 [Dietary iodine = 0.216 × BW (kg)$^{0.528}$ + 0.1 × milk yield (kg/d] reported in the NASEM (2021). However, actual iodine intake was 744 and 5,726% greater than estimated adequate iodine intake (NASEM, 2021) when feeding 176 g/d of ASCO meal or 6% *C. crispus*, respectively. Signs of iodine toxicity have been documented in dairy cows with estimated iodine intake ranging from 250 to 785 mg/d in diets containing ethylenediamine dihydroiodideiodine and time of supplementation varying from 1 month to 7 years (Olson et al., 1984). Ong et al. (2014) reported low-grade pyrexia, nasal discharge, respiratory distress, watery stools, and enlargement of the thyroid gland in 2 adult Holstein cows with estimated iodine intake averaging 10 mg/100 kg of BW. Despite excessive iodine intake in cows fed ASCO meal (Silva et al., 2022) or *C. crispus* (Brito's Lab unpublished), cows did not show signs of iodine toxicity. In addition, serum (Silva et al., 2022) and plasma (Brito's Lab unpublished) concentrations of T3 and T4 were not affected by diets even though cows were not exposed to long-term excess iodine intake as the experimental periods last 28 d (ASCO meal study) and 24 d (*C. crispus* study).

Neither Roque et al. (2019) nor Stefenoni et al. (2021) reported the concentrations of iodine for *A. armata* and *A. taxiformis*, respectively. Assuming an iodine concentration of 2,270 mg/kg for *A. taxiformis* (Roque et al., 2021), estimated iodine intake for dairy cows receiving 0.5% *A. taxiformis* (Stefenoni et al., 2021) would be 268 mg/d. Estimated adequate iodine intake using the NASEM (2021) Equation 7-34 (see above) averaged 10.7 mg/d for cows consuming 0.5% *A. taxiformis* weighing 635 kg and producing 42.2 kg/d of milk. Therefore, this estimated iodine intake was 2,405% greater than the estimated adequate iodine from NASEM (2021). Note that Stefenoni et al. (2021) did not report any iodine toxicity symptoms in their 4 × 4 Latin square design study with 28-d experimental periods.

Effect of Selected Seaweeds on Dairy Cow Health

Studies evaluating the impact of feeding the red seaweeds *A. armata*, *A. taxiformis*, and *C. crispus* on markers of dairy cow health are limited or not available. In contrast, the brown seaweed ASCO is likely the most studied algal feed as related to animal health based on reports in the literature [see review papers from Allen et al. (2001), Evans and Critchley (2014), and Makkar et al. (2016)]. However, most published research that have documented health benefits in response to ASCO meal supplementation such as modulation of body temperature, improved immune system, and decreased shedding of *E. coli* was done with beef, sheep, and pigs (Allen et al., 2001; Evans and Critchley, 2014; Makkar et al., 2016). Data on the effect of ASCO meal on mitigating heat stress are scarce and studies were not conducted under controlled conditions (e.g., Pompeu et al., 2011).

The algal feed ASCO meal is popular very among organic dairy producers in the US (Hardie et al., 2014; Antaya et al., 2015; Sorge et al., 2016; Snider et al., 2021), with up to 72.5% of organic grassfed dairies that participated in a national survey indicating the use of ASCO (Snider et al., 2021). According to a survey reported in Antaya et al. (2015), organic dairy producers feed ASCO meal for the following reasons: (1) it improves body condition and overall animal appearance, (2) it decreases somatic cell count, reproductive problems, and incidence of "pinkeye" (i.e., infectious bovine keratoconjunctivitis), and (3) it reduces incidence of nuisance flies. However, controlled studies are needed to corroborate these anecdotal claims.

Antaya et al. (2015) reported a linear decrease in the plasma concentration of non-esterified fatty acids in early- to mid-lactation dairy cows fed incremental amounts (0, 57, 113, and 170 g/d) of ASCO meal. They also observed a tendency for a linear decrease in the serum concentration of cortisol in response to ASCO meal supplementation. Similarly, Silva et al. (2022) saw a linear decrease in the serum concentration of cortisol with feeding varying amounts (0, 57, 113, and 170 g/d) of ASCO meal. However, the mechanisms behind these changes in blood non-esterified fatty acids and cortisol are not well understood and require further research. Cows in the study of Antaya et al. (2015) and Silva et al. (2022) were exposed to winter and summer conditions, respectively, which may have led to cold and heat stress that were alleviated by ASCO meal supplementation ultimately decreasing cortisol levels. In fact, ASCO meal and ASCO extracts have been associated with body's thermoregulatory control in beef cattle and sheep with concomitant reduction in circulating cortisol (Allen et al., 2001; Archer et al., 2007). Contrarily, serum cortisol concentration did not change in grazing dairy cows receiving 113 g/d of ASCO meal despite the study being conducted during the summer months when cows are more susceptible to heat stress (Antaya et al., 2019). Furthermore, plasma activities of the antioxidant enzymes superoxide dismutase (mean = 0.40 U/mL), glutathione peroxidase (mean = 50.3 nmol/min per mL), and catalase (mean = 8.03 nmol/min per mL) were not changed in cows supplemented with up to 170 g/d of ASCO meal in an experiment done from June to November (Silva et al., 2022). Chaves Lopez et al. (2016) observed a 44.5% reduction in milk somatic cells count (from 490,000 to 272,000) with feeding 100 g/d of ASCO meal compared with the control diet, thus suggesting improvement in milk quality and mammary gland health. Note that Chaves Lopez et al. (2016) used only 22 cows (n = 11/treatment) and their results should be interpreted cautiously. In general, ASCO meal appears to have some positive health benefits, but additional studies under strict conditions (e.g., controlled humidity and ambient temperature, immune system challenge, etc.) are needed to fully address the role of ASCO meal on improving dairy cattle heath.

Data on the effect of the red seaweeds *A. armata* and *A. taxiformis* on health of lactating dairy cows are limited. Roque et al. (2019) did not evaluate markers of animal health and did not report any adverse effect of *A. armata* on the health of 12 lactating dairy cows used in their experiment. Muizelaar et al. (2021) euthanized 2 lactating dairy cows that consistently consumed 67 g/d of *A. taxiformis* and observed loss or absence of papillae on parts of the ruminal wall in both cows. They also saw signs of inflammation in the ruminal wall of the 2 euthanized cows after histological examination of the ruminal papillae (Muizelaar et al., 2021). These histopathological changes in the ruminal wall and

papillae of 2 cows were comparable to those found on 5 out of 10 sheep supplemented with increasing levels (0, 0.5, 1, 2, and 3%; diet OM basis) of *A. taxiformis* (Li et al., 2016). In contrast, no histopathological abnormalities and signs of inflammation were seen in 2 out of 2 euthanized sheep that had no access to *A. taxiformis* (i.e., control diet; Li et al., 2016). However, these histopathological changes detected in the ruminal wall and papillae of sheep and dairy cows could not be conclusively associated with *A. taxiformis* supplementation according to Li et al. (2016) and Muizelaar et al. (2021). Although Stefenoni et al. (2021) did not report any detrimental health effect in response to various levels of *A. taxiformis* supplementation to dairy cows, blood activity of the enzyme alanine aminotransferase decreased by 22.8% (from 57.5 to 44.4 U/L) with feeding 0.5% *A. taxiformis* versus control. This enzyme has been used as a marker of liver health and increased activity of alanine aminotransferase may be associated with liver damage and metabolic or infectious diseases as discussed by Stefenoni et al. (2021). Therefore, *A. taxiformis* may have some hepatoprotective effect, but Stefenoni et al. (2021) stated that they were not able to offer a reasonable explanation for the marked reduction seen for alanine aminotransferase activity in cows fed 0.5% *A. taxiformis*. It is clear based on these few reports that long-term studies are needed to properly assess the effect of *A. taxiformis* on health of high-producing dairy cows.

Summary and Implications

Feeding the brown seaweed ASCO meal had no negative effect on DMI. Milk yield response to ASCO meal supplementation varied, with some studies showing no effect on milk yield, whereas others resulting in improved milk production. In contrast, feeding the red seaweeds *A. armata*, *A. taxiformis*, and *C. crispus* decreased DMI, particularly when cows were fed the greatest amount of each seaweed. Milk yield followed DMI and decreased with feeding both *Asparagopsis* species, but not when cows were fed *C. crispus*. *Ascophyllum nodosum* meal did not consistently reduce or did not reduce enteric CH_4 production *in vivo*, but further research may be needed to fully address its effect on ruminal methanogenesis. On the other hand, enteric CH_4 production decreased in dairy cows receiving *A. armata*, *A. taxiformis*, and *C. crispus* even though the magnitude of CH_4 suppression varied with *A. armarta* ranking first (- 67.2%), and *A. taxiformis* (-34.4%) and *C. crispus* (-8.4%) second and third, respectively. Milk iodine concentration generally increased above the 500-µg/L threshold considered safe for human consumption when cows received seaweeds (i.e., ASCO meal, *A. taxiformis*, *C. crispus*) in their diets. Therefore, technologies to reduce iodine in seaweeds, especially in those with high CH_4 mitigation potential such as *A. taxiformis* and *A. armata* would be needed to reduce the risk of excess iodine intake in humans assuming large adoption of algal-based feeds by dairy producers. There are scarce data on the impact of ASCO, *Asparagopsis* species, and *C. crispus* on dairy cow health and results (either positive or negative) are not conclusive. Data obtained from cows fed the selected seaweeds reviewed in this paper came from short-term, changeover design studies, thus indicating the need for long-term, continuous design experiments to better understand the impact of these algal sources on production performance, enteric CH_4 production, and cow health. Costs and availability of seaweeds, producer adoption, environmental impact (e.g., urinary and fecal excretion of iodine and bromoform), governmental policies and subsidies, and consumers'

willingness to pay premiums for dairy products with reduced carbon footprint will all interact to shape the success (or failure) of algal-based feed for high-producing dairy cows in the US and overseas.

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Epidemiology of Bovine Colostrum Production in New York Holstein Herds: Cow, Management, Nutritional, and Environmental Factors

T. A. Westhoff[1], T. R. Overton[2], S. Mann[1]

[1]Department of Population Medicine and Diagnostic Sciences, and
[2]Department of Animal Science,
Cornell University, Ithaca, NY

Introduction

Ingestion of an adequate volume of high-quality colostrum is essential for the growth and health of newborn calves (Godden et al., 2019). Colostrum quality and yield have been reported to vary by cow, month of calving, and season (Conneely et al., 2013, Gavin et al., 2018, Borchardt et al., 2022); however, the mechanisms regulating colostrum production in dairy cattle remain poorly understood. Cows entering parity ≥ 3 are reported to have higher immunoglobulin G (IgG) concentration compared to cows in parity 1 or 2 (Bartier et al., 2015). Shortening the dry period does not appear to affect colostrum quality but reduction in colostrum yield has been reported (Mansfeld et al., 2012, Mayasari et al., 2015, O'Hara et al., 2019). Maximum temperature humidity index (THI) and photoperiod (Gavin et al., 2018) as well as season (Conneely et al., 2013, Borchardt et al., 2022) have been associated with changes in colostrum production and quality. Other studies, however, have shown colostrum quality not to be associated with season or month of calving (Pritchett et al., 1991, Bartier et al., 2015, Dunn et al., 2017). In addition, experimental manipulation of the photoperiod during the dry period did not affect colostrum yield or IgG concentration (Morin et al., 2010).

Prepartum nutrition and management affect postpartum health and production (Van Saun and Sniffen, 2014, Cardoso et al., 2020), yet we lack knowledge of the influence of these nutritional and management strategies on colostrum yield and Brix %. Feeding a controlled energy or low starch prepartum diet has been reported to increase colostrum IgG concentration and numerically decrease colostrum yield (Mann et al., 2016, Fischer-Tlustos et al., 2021). Altering prepartum protein supply does not appear to affect colostrum yield or IgG concentration in beef and dairy cattle (Farahani et al., 2017, Farahani et al., 2019, Hare et al., 2019). Previous authors reported DCAD does not influence IgG concentration; however, results on colostrum yield are mixed (Weich et al., 2013, Martinez et al., 2018, Graef et al., 2021). Additionally, colostrum yield has been weakly positively correlated with postpartum BHB at 1 and 7 DIM ($r = 0.19$ and 0.16), respectively (Sawall and Litherland, 2013). There is a lack of data available to understand variables that are associated with colostrum production in herds with management systems typical to commercial dairies in the United States.

We hypothesized that colostrum yield and Brix % are associated with cow, farm management, nutritional, and environmental factors. Our objectives of this work performed on NY Holstein dairy farms were to 1) describe colostrum production and 2)

identify individual cow, herd management, nutritional, and environmental factors associated with colostrum production.

Materials and Methods

Farm selection, enrollment, and data collection

A list of farm contacts was compiled by the investigators based on previous NY statewide research projects, as well as by contacting NY veterinarians and nutritionists. Inclusion criteria included: 1) ability to collect and record individual colostrum weight or volume and a composite sample Brix % reading, 2) minimum herd size of 500 lactating Holstein cows, 3) use of dairy management software DairyComp 305 (DC305, Valley Ag Software), and 4) heifer calves housed on site for at least the first week of life. During an enrollment period, owners or herd managers were asked, by phone or email, if the farm met the inclusion criteria and if they were interested in participating in the study. At the end of the enrollment period, a convenience sample of 19 New York Holstein dairy farms were included in this observational study between October 2019 and February 2021.

Farm personnel harvested individual cow colostrum according to existing farm protocols. Colostrum yield was either collected as a volume or as a weight. Weight was measured on a digital scale and volume was determined using volume markers on commercial bottles or milking buckets. For farms choosing to collect colostrum yield in weight (n = 15), colostrum collection buckets were labeled, and empty weights were recorded for each bucket. Farm personnel were then instructed to record the bucket ID and the total weight of the colostrum bucket such that the colostrum yield could be calculated. A digital Brix refractometer was used for composite sample Brix % reading. Colostrum record binders were provided to each farm to record cow ID, date and time of colostrum harvest, bucket ID, colostrum yield, Brix %, notes, and the initials of the individual responsible for colostrum collection. Final colostrum yield was calculated by subtracting the recorded weight of the bucket from the total weight. For farms collecting colostrum yield as volume (n = 4), farm personnel recorded volume in pints or liters. Colostrum volume was converted to liters then to weight using the equations $L = P\ x\ 2.1134$ and $kg = L\ x\ 1.0524$, where L = colostrum volume in liters, P = colostrum volume in pints, kg = colostrum weight in kilograms, and 1.0524 as the density of Holstein colostrum (Morin et al., 2001).

Two environmental data loggers measuring light intensity (Lum/ft^2) and ambient temperature/relative humidity (HOBO Models MX2202/MX2301A, respectively, Onset Computer Corp.) were mounted facing the length of the barn, approximately 3 m above ground, directly above the resting area in the close-up dry cow pen at each farm. Light intensity and temperature/relative humidity were recorded in 15- and 30-min intervals during the entire study period, respectively. Temperature-humidity index (THI) and Lux were calculated for heat and humidity exposure and light intensity, respectively.

Farms were visited 4 times, approximately 3 months apart, during the data collection period. At each visit, colostrum records and a DC305 backup was collected. Diets fed to animals from -60 to 0 d relative to parturition were evaluated for particle size using a Penn State Particle Separator (PSPS) and submitted to a commercial laboratory (Dairy One Cooperative Inc.) for analysis of chemical composition by near-infrared reflectance spectroscopy and wet chemistry analysis of minerals. Physically effective NDF (peNDF) was calculated by multiplying diet aNDF (% of DM) by the proportion of the diet ≥ 4 mm. Stocking density was recorded for the far-off and close-up pens as the number of cows in the pen divided by the number of useable stalls or by 9.3 m^2 of lying space in a bedded pack (Nordlund, 2009). Blood samples were collected from a convenience sample of 8 primiparous and 16 multiparous postpartum cows (3-14 DIM) to determine BHB concentrations on a handheld meter (Nova Biomedical).

Analytical Approach

For primiparous cows, animal-level variables considered for associations with colostrum yield and Brix % included sex of the calf, age at first calving, whether the calf was a stillbirth (defined as DC305 code “dead on arrival” (DOA)), colostrum yield and Brix %, gestation length, heat and humidity exposure, and light intensity. For multiparous cows, animal-level variables included in univariable screening for associations with colostrum yield and Brix % included sex of the calf, whether the calf was a stillbirth, parity, colostrum yield and Brix %, gestation length, days dry, heat and humidity exposure, light intensity, and previous lactation length and 305ME. Close-up pen-level variables evaluated for associations with colostrum yield and Brix % included pen stocking density, proportion of fresh cows with BHB ≥ 1.2 mmol/L, if the pen housed primiparous and multiparous cows, parity (1 vs ≥ 2), and diet starch, aNDF, crude protein, DCAD, peNDF, and proportion of the diet in ≥ 19-mm sieve of the PSPS.

Continuous variables were first assessed for a linear relationship with colostrum yield and Brix %, respectively. If the assumption of a linear relationship was not fulfilled (defined as an absolute correlation coefficient ≥ 0.20), variables were categorized for subsequent analysis. Individual cow records with a recorded gestation length greater or less than 15 d of the mean were removed to limit inclusion of animals with incorrect records of breeding dates or abortions (Norman et al., 2009). Gestation length was categorized for both primiparous (PP) and multiparous (MP) into 3 categories: short (PP=261-271, MP=263-273 d), normal (PP=272-280, MP=274-282 d), or long (PP=281-291, MP=283-293 d). Brix % was grouped into 4 categories: ≤ 22.0, 22.1-24.4, 24.5-27.0, and > 27.0%. Colostrum yield was dichotomized at < 6 and ≥ 6 kg as the amount of colostrum needed for two colostrum feedings (3.78 and 1.89 L at first and second feeding). Age at first calving and dry period length were grouped into 3 categories: (≤ 20, 21-24, > 24) m and (< 47, 47-67, > 67) d, respectively. Quartiles 1 and 3 were used as cut points for 3 categories of previous lactation length (< 297, 297-344, > 344) d and previous lactation 305ME (≤ 13,090, 13091-15,862, > 15,862) kg. Due to fewer animals entering parities 6 to 10 they were grouped together resulting in parity categories 1, 2, 3, 4, or ≥ 5 (5+). Given the low number of twin calvings, twins were not further categorized by sex, resulting in the three calf categories singleton female, singleton

male, or twins. Pen stocking density, proportion of fresh cows with BHB ≥ 1.2 mmol/L, and proportion of the diet in ≥ 19-mm sieve in the PSPS were grouped into 4 categories: (≤ 80, 81-100, 101-120, > 120) %, (≤ 5.0, 5.1-10.0, 10.1-15.0, > 15.0) %, and (≤ 11.2, 11.3-15.2, 15.3-19.1, > 19.1) %, respectively. Diet composition was categorized for starch (≤ 18.5, 18.6-22.5, > 22.5) % of DM, aNDF (≤ 39.0, 39.1-43.5, > 43.5) % of DM, crude protein (≤ 13.5, 13.6-15.5, > 15.5) % of DM, DCAD (≤ -16.0, -15.9 to -8.0, > -8.0) mEq/100g, and peNDF (≤ 27.0, 27.1-32.0, > 32.0).

To account for the total exposure to light intensity (lux), as well as heat and humidity exposure (THI) during the close-up period, total area under the curve (AUC) was calculated separately for 5 different periods in the last 3 wk of the prepartum period: -21 to -1, -14 to -1, -21 to -15, -14 to -8, and -7 to -1 d relative to calving. Light intensity AUC was categorized into 3 groups using quartiles 1 and 3 as cut points for each prepartum period. Heat and humidity exposure (THI) AUC was categorized for each prepartum period for an average THI per 30-m interval of ≤ 40.2, 40.3-50.1, 50.2-60.0, 60.1-69.2, and > 69.2. To prevent multicollinearity, each prepartum period was screened in univariable models with dependent variables colostrum yield and Brix %. The prepartum period with the lowest Akaike information criterion (AIC) was then selected for further analysis.

Following univariable screening, animal-level mixed effects multivariable models were generated in PROC MIXED (SAS 9.4, SAS Institute Inc.), for the following four outcomes of interest: colostrum yield from primiparous cows, Brix % from primiparous cows, colostrum yield from multiparous cows, and Brix % from multiparous cows. All mixed models included the random effects of herd and month of calving. All variables with $P \leq 0.10$ in univariable screening were included in the initial multivariable model. Stepwise manual backwards elimination was used until a final model was defined as all remaining variables having $P < 0.05$. Biologically plausible 2-way interactions were then tested and retained in the model if $P < 0.05$. Tukey's post hoc test was used to adjust pairwise comparisons for the number of multiple comparisons. Data reported as least squares means (LSM) and 95 % confidence interval where different superscripts differ ($P < 0.05$; Tukey's test).

For pen-level analysis, mixed effects multivariable models with repeated measures were generated using PROC MIXED (SAS v. 9.4) for the outcome variables colostrum yield and Brix %. Explanatory variables were applied to all animals calving ± 14 d of the farm visit. Models included the repeated effect of visit, subject of farm, and the random effects of month of calving and month of visit. Far-off pen stocking density, if the pen housed primiparous and multiparous cows, and far-off diet starch, crude protein, aNDF, DCAD, peNDF, and proportion of the diet in the ≥ 19-mm sieve of the PSPS were included as covariates. Variables with $P \leq 0.20$ in univariable screening entered the initial multivariable model. Stepwise manual backwards elimination was used until all remaining variables had $P \leq 0.05$. Biologically plausible 2-way interactions were then tested and retained in the model if $P < 0.05$.

Results

Monthly colostrum yield from 5,790 primiparous and 12,553 multiparous cows is illustrated in Figure 1. Median (range) colostrum yield was 4.1 (0.1-38.6) kg for primiparous and 5.0 (0.1-43.8) kg for multiparous cows. Average (± SD) colostrum Brix % was 24.6 ± 3.9 and 25.7 ± 4.4 for primiparous and multiparous cows, respectively.

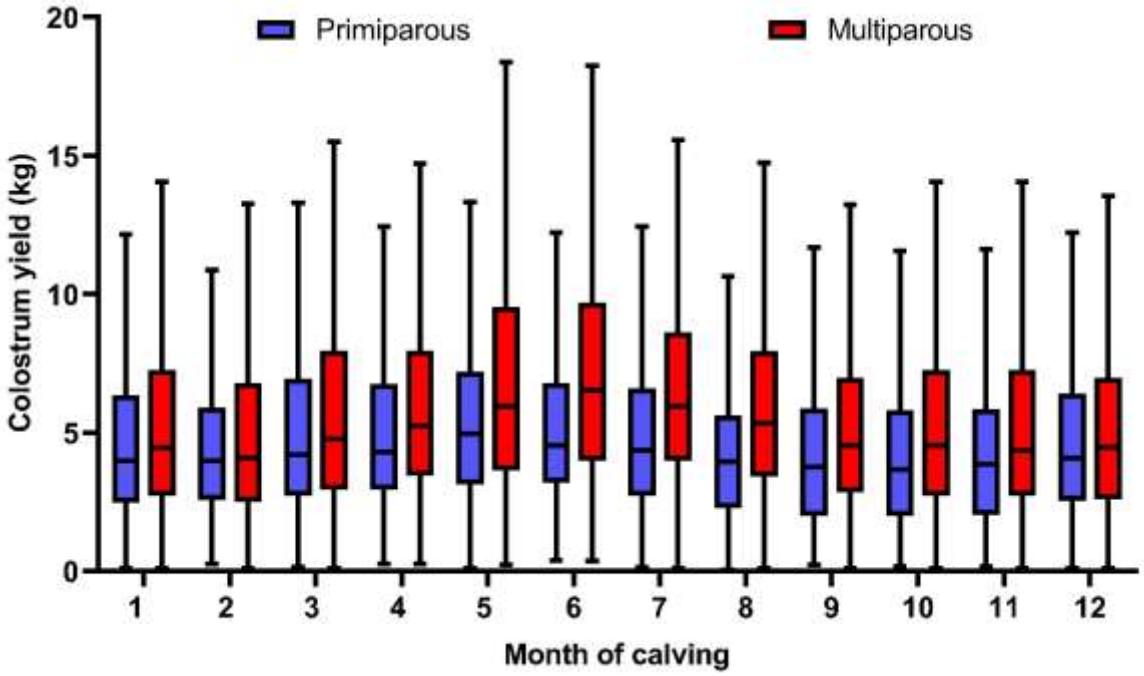

Figure 1. Box and whisker plot of monthly colostrum yield (kg) from 5,790 primiparous and 12,553 multiparous Holstein cows from 18 NY farms.

Animal-level analysis

A total of 5,790 primiparous cows from 18 NY farms were included in the final analysis. Colostrum yield from primiparous cows was associated with sex of the calf ($P < 0.01$) and categorized colostrum Brix % ($P < 0.01$). Colostrum yield, LSM (95 % CI), for twin, bull, and heifer calves were 4.9 (3.7-6.4)^{xy}, 4.1 (3.5-4.8)^{x}, and 3.9 (3.4-4.5)^{y} kg, respectively. Colostrum yield for categorized colostrum Brix % ≤ 22.0, 22.1-24.4, 24.5-27.0, and > 27.0 % were 3.9 (3.3-4.6)^{z}, 4.5 (3.8-5.3)^{xy}, 4.5 (3.8-5.3)^{x}, and 4.2 (3.6-5.0)^{y} kg, respectively. Colostrum Brix % from primiparous cows was associated with sex of the calf ($P < 0.01$), whether the calf was a stillbirth ($P = 0.01$), and categorized light intensity AUC 14 d before calving ($P = 0.01$). Colostrum Brix % for twin, bull and heifer calves were 25.2 (23.7-26.7)^{xy}, 24.6 (23.8-25.5)^{x}, and 24.2 (23.4-25.1)^{y} %, respectively. A dead calf was associated with a lower Brix % compared to an alive calf [24.4 (23.4-25.4) vs. 25.0 (24.1-25.9) %; $P = 0.01$], respectively. Brix % for an average light intensity per 15-min interval 14-d before calving of ≤ 64.0, 64.1-154.2, and >154.2 lux were 25.0 (24.0-25.9)^{x}, 24.5 (23.6-25.5)^{y}, and 24.6 (23.6-25.5)^{xy} %, respectively.

Final mixed effects multivariable models for variables associated with colostrum yield and Brix % from 12,553 multiparous cows are reported in Table 1. Colostrum yield from multiparous cows was associated with sex of the calf ($P < 0.01$), whether the calf was a stillbirth ($P < 0.01$), parity ($P < 0.01$), categorized colostrum Brix % ($P < 0.01$), dry period length ($P < 0.01$), previous lactation 305ME ($P < 0.01$), gestation length ($P < 0.01$), previous lactation length ($P < 0.01$), heat and humidity exposure AUC 7 d before calving ($P < 0.01$), and light intensity AUC 14 d before calving ($P = 0.01$). Greater colostrum yields were associated with 2nd parity, twins, alive calves, and increasing dry period and gestation length categories. Colostrum Brix % from multiparous cows was associated with whether the calf was a stillbirth ($P < 0.01$), parity ($P < 0.01$), heat and humidity exposure AUC 7 d before calving ($P < 0.01$), dry period length ($P < 0.01$), previous lactation 305ME ($P < 0.01$), gestation length ($P = 0.01$), and colostrum yield ($P < 0.01$). Brix % was lowest in 2nd parity, with colostrum yield ≥ 6 kg, and with a dry period ≤ 67 d.

Table 1. Mixed effects multivariable models for variables associated with colostrum yield (kg) and Brix % in Holstein multiparous (n = 12,553) cows from 18 NY farms.

	Colostrum yield (kg)[1]		Colostrum Brix %	
Variable	LSM (95% CI)[2]	*P*	LSM (95% CI)[2]	*P*
Colostrum yield (kg)				<0.01
<6			26.7 (25.9-27.5)[a]	
≥6			25.1 (24.3-25.9)[b]	
Brix %		<0.01		
≤22	6.0 (5.3-6.8)[a]			
22.1-24.4	5.7 (5.0-6.5)[b]			
24.5-27	5.0 (4.4-5.7)[c]			
≥27	4.1 (3.6-4.6)[d]			
Calf Sex		<0.01		
Female	4.6 (4.0-5.2)[c]			
Male	5.0 (4.4-5.7)[b]			
Twin	6.0 (5.2-7.8)[a]			
Dry period length (d)		<0.01		<0.01
<47	4.2 (3.7-4.7)[c]		25.6 (24.7-26.4)[b]	
47-67	5.1 (4.5-5.7)[b]		25.7 (24.9-26.5)[b]	
>67	6.5 (5.7-7.4)[a]		26.4 (25.6-27.3)[a]	
Prev. lactation 305ME (kg)		<0.01		<0.01
≤13,090	4.9 (4.4-5.6)[b]		26.0 (25.2-26.9)[a]	
13,091-15,862	5.2 (4.6-5.9)[a]		25.9 (25.1-26.8)[a]	
>15,862	5.3 (4.7-6.0)[a]		25.7 (24.9-26.5)[b]	
Gestation length (d)		<0.01		0.01
263-273	4.8 (4.2-5.4)[c]		25.8 (25.0-26.7)[ab]	
274-282	5.2 (4.6-5.8)[b]		26.0 (25.2-26.9)[a]	
283-293	5.5 (4.9-6.3)[a]		25.8 (25.0-26.6)[b]	
Stillbirth		<0.01		<0.01
Alive	5.5 (4.9-6.1)[a]		26.2 (25.4-27.0)[a]	
Dead	4.9 (4.2-5.6)[b]		25.6 (24.6-26.5)[b]	

Prev. lactation length (d)		<0.01		
<297	4.9 (4.3-5.5)[b]			
297-344	5.0 (4.4-5.7)[b]			
>344	5.6 (4.9-6.3)[a]			
Parity		<0.01		<0.01
2	5.4 (4.8-6.2)[a]		24.4 (23.6-25.2)[d]	
3	5.2 (4.6-5.9)[b]		25.6 (24.7-26.4)[c]	
4	4.9 (4.3-5.5)[c]		26.3 (25.5-27.2)[b]	
5+	5.0 (4.4-5.7)[bc]		27.3 (26.4-28.1)[a]	
Heat and humidity exposure AUC 7 d before calving (Average THI per 30 min interval)[3]		<0.01		<0.01
≤40.2	4.7 (4.1-5.4)[c]		26.3 (25.5-27.2)[a]	
40.3-50.1	4.9 (4.3-5.5)[c]		26.2 (25.4-27.0)[a]	
50.2-60.0	5.1 (4.5-5.8)[bc]		25.7 (24.9-26.6)[b]	
60.1-69.2	5.4 (4.7-6.1)[ab]		25.7 (24.9-26.6)[ab]	
>69.2	5.7 (5.0-6.5)[a]		25.5 (24.6-26.3)[b]	
Light intensity AUC 14 d before calving (Average Lux per 15 min interval)[4]		0.01		
≤64.0	5.0 (4.4-5.7)[ab]			
64.1-154.2	5.0 (4.5-5.7)[b]			
>154.2	5.4 (4.7-6.1)[a]			

[1]Data natural logarithm transformed before analysis. Reported as back-transformed LSM (95% CI).

[2]LSM (95% CI) with different superscripts differ (P < 0.05; Tukey's test). Model included random effects of herd and month of calving.

[3]Area under the curve (AUC) was calculated for temperature-humidity index (THI) 7 d before calving in 30-min intervals. THI was collected from the close-up dry cow pen at each farm.

[4]Area under the curve (AUC) was calculated for light intensity (Lux) 14 d before calving in 15-min intervals. Lux was collected from the close-up dry cow pen at each farm.

Pen-level analysis

Cows (n=4,396) from 17 farms were included in the pen-level analysis. Variables associated with colostrum yield and Brix % are reported in Table 2. Colostrum yield was associated with diet starch (P = 0.01), peNDF (P = 0.04), proportion of the diet in ≥ 19-mm sieve of the PSPS (P < 0.01), crude protein (P < 0.01), DCAD (P = 0.03), proportion of fresh cows with BHB ≥ 1.2 mmol/L (P < 0.01), and parity (P < 0.01). Colostrum yield was numerically lowest in 1st parity, and with diet starch ≤ 18.5 % of DM, and peNDF ≤ 27.0. Colostrum Brix % was associated with diet starch (P < 0.01), peNDF (P < 0.01), DCAD (P < 0.01), stocking density (P < 0.01), and parity (P < 0.01). Colostrum Brix % was numerically greatest with diet starch ≤ 18.5 % of DM, stocking density ≤ 80.0 %, and in 2nd or greater parity.

Table 2. Mixed effects multivariable models for close-up diet and pen level variables associated with colostrum yield and Brix % in Holstein cows (n=4,396) from 17 NY farms.

	Colostrum yield (kg)[1]		Colostrum Brix %[2]	
Variable	LSM (95% CI)[3]	*P*	LSM (95% CI)[3]	*P*
Starch (% of DM)		0.01		< 0.01
≤ 18.5	4.0 (3.3-4.8)[b]		26.5 (25.5-27.6)[a]	
18.6-22.5	4.7 (4.0-5.6)[a]		25.2 (24.2-26.1)[b]	
> 22.5	4.3 (3.5-5.2)[ab]		24.9 (23.8-25.9)[b]	
peNDF[4]		0.04		< 0.01
≤ 27.0	3.9 (3.3-4.7)[b]		25.4 (24.4-26.5)[ab]	
27.1-32.0	4.5 (3.8-5.3)[a]		26.0 (25.0-26.9)[a]	
> 32.0	4.6 (3.8-5.5)[ab]		25.2 (24.1-26.2)[b]	
19 mm PSPS (% AF)[5]		< 0.01		
≤ 11.2	4.4 (3.6-5.4)[ab]			
11.3-15.2	4.8 (4.0-5.7)[a]			
15.3-19.1	3.9 (3.3-4.7)[b]			
> 19.1	4.2 (3.5-5.0)[ab]			
Crude protein (% of DM)		< 0.01		
≤ 13.5	4.3 (3.6-5.2)[b]			
13.6-15.5	5.0 (4.2-5.9)[a]			
> 15.5	3.7 (3.1-4.5)[b]			
DCAD (mEq/100g)		0.03		< 0.01
≤ -16.0	4.1 (3.4-5.0)[ab]		25.0 (24.0-26.1)[b]	
-15.9 to -8.0	4.1 (3.4-4.8)[b]		26.0 (25.1-27.0)[a]	
> -8.0	4.8 (4.0-5.8)[a]		25.5 (24.5-26.5)[ab]	
BHB ≥ 1.2 mmol/L (%)[6]		< 0.01		
≤ 5.0	4.0 (3.3-4.7)[b]			
5.1-10.0	4.0 (3.3-4.8)[b]			
10.1-15.0	5.0 (4.1-6.0)[a]			
> 15.0	4.4 (3.6-5.4)[ab]			
Stocking density (%)[7]				< 0.01
≤ 80			26.1 (25.1-27.2)[a]	
81-100			25.2 (24.3-26.2)[bc]	
101-120			24.8 (23.7-25.8)[b]	
> 120			25.9 (24.8-27.1)[ac]	
Parity		< 0.01		< 0.01
1	4.1 (3.4-4.8)[b]		24.8 (23.9-25.8)[b]	
2+	4.6 (3.9-5.4)[a]		26.2 (25.2-27.1)[a]	

[1]Data natural logarithm transformed before analysis. Reported as back-transformed LSM (95% CI).
[2]Colostrum yield was included as a covariate.
[3]LSM (95% CI) with different superscripts differ (P < 0.05; Tukey's test). Model included random effects of month of calving and month of farm visit with a repeated effect of farm visit with subject of farm. Far-off pen level variables stocking density, percent of diet in the 19 mm sieve of the Penn State Particle Separator, if the pen housed primiparous and multiparous cows, and far-off diet starch, aNDF, crude protein, DCAD, and peNDF were included as covariates.
[4]Physically effective NDF calculated by multiplying diet aNDF by percent of diet ≥ 4 mm.
[5]Percent of diet in the 19 mm sieve of the Penn State Particle Separator.

[6]Percent of fresh cows (3-14 DIM) with BHB ≥ 1.2 mmol/L.
[7]Stocking density calculated by dividing number of cows by number of usable stalls or 9.3 m^2 of lying space in the pen during the farm visit.

Conclusions and Implications

Colostrum yield and Brix % were associated with cow, farm management, nutritional, and prepartum environmental factors. Although future studies are needed to determine the cause-effect relationship between the observed variables that were associated with changes in colostrum yield and Brix %, recognizing these factors associated with colostrum production remains a strategic opportunity to review current nutritional and management practices on farms struggling with period of low colostrum supply. In agreement with other authors (Mansfeld et al., 2012, Mayasari et al., 2015, O'Hara et al., 2019), these data suggest colostrum yield is reduced when shortening the dry period. Our results support a numerical decrease in colostrum yield when feeding low starch prepartum diets reported previously (Mann et al., 2016). Additionally, other nutritional and management variables associated with colostrum production should be considered.

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Varying Proportions of Alfalfa and Corn Silage for Lactating Dairy Cows

R. J. Grant[1], S. Y. Morrison[1], and L. E. Chase[2]
[1]William H. Miner Agricultural Research Institute, Chazy, NY
[2]Department of Animal Science, Cornell University

Introduction

Alfalfa and corn silage are the predominant forages in the US, but their acreage is moving in opposite directions. Between 1982 and 2012, corn silage production increased by 33% while alfalfa hay production declined 75% (Martin et al., 2017). Decisions about raising alfalfa and corn silage revolve around the relative difficulty of growing alfalfa and its lower yield potential balanced against the benefits of legumes for soil health, nitrogen fixation, and the long-term sustainability of dairy-forage systems. Intensification of the US dairy industry has driven greater reliance on corn silage as the primary forage rather than perennials, but greater dairy-forage system productivity has come at the expense of soil carbon (Gamble et al., 2021). The bottom line is that the percentage of alfalfa in dairy cow rations has dwindled over recent decades. For example, since 1999 in California, alfalfa inclusion in high-producing dairy cow rations slipped approximately 50%, from 28 to 14% of the dietary dry matter (DM; Robinson, 2014).

Alfalfa and Corn Complementarity

Corn silage and alfalfa are nutritionally complementary forages in many ways. Their respective content and rumen degradability of fiber, protein, and starch can be leveraged in ration formulation to enhance organic matter fermented and microbial protein synthesis. Alfalfa has lower neutral detergent fiber (NDF) content, more undegraded NDF at 240 hours of in vitro fermentation (uNDF240), but a faster rate of rumen NDF digestion than corn silage (Raffrenato et al., 2019). Associated with differences in anatomical structure, alfalfa tends to break into cuboidal fragments when chewed. In contrast, grasses break into longer pieces more easily entangled in the rumen digesta mat. Overall, alfalfa has a more rapid rumen turnover rate, is less filling, and consequently promotes greater dry matter intake (DMI) than grasses. However, the measured intake response relative to corn silage has been variable (see later discussion).

In addition to fundamental differences between alfalfa and corn silage in fiber characteristics, alfalfa also has a much higher cation exchange capacity than corn silage, reflective primarily of greater pectin and secondarily of lignin. Alfalfa hay has 80% greater cation exchange capacity than corn silage, contributing to higher rumen pH conditions (McBurney et al., 1981; Robinson, 2014). Alfalfa also contains more sodium and potassium than corn silage and therefore has greater dietary cation-anion difference. Together with the physical effectiveness of the NDF in alfalfa stems, alfalfa may help to stabilize rumen pH and boost milk fat percentage when cows are fed higher corn silage rations (Mertens, 1997; Robinson, 2014).

Immature alfalfa contains a greater content of crude protein (CP; 20 to 22% of DM) than corn silage (6 to 8% of DM) and many other common forages. Depending on prevailing feed prices, it can be an economical forage source of CP compared with purchased feed ingredients in corn silage-based rations. The high rumen degradability of CP (RDP) in alfalfa complements the high starch content of corn silage. This higher RDP is a double-edged sword, however, and may limit the inclusion of alfalfa in dairy rations and elevate the risk of high milk urea nitrogen (MUN) values and excessive urinary nitrogen (N) excretion. Alfalfa also contains more lysine (4.4% of metabolizable protein, MP) than corn silage (2.5% of MP; Zang, 2021).

There is considerable potential to optimize the nutritional interactions between alfalfa and corn silage, particularly between RDP and rumen fermentable starch to enhance microbial protein production. Ration formulation strategy and complementarity with other dietary ingredients will affect any synergy between alfalfa and corn silage. Nonetheless, economic, environmental, and social considerations encourage the use of higher fiber, higher forage diets in the dairy industry (Martin et al., 2017). With the prevalence of corn silage-based rations fed to dairy cattle and the downward trend in alfalfa use, we need to reconsider alfalfa in ration formulation and nutrient management programs. Two nutritional questions loom large for dairy nutritionists: 1) can we successfully feed more alfalfa in dairy rations than is commonly done, and 2) is there a nutritional benefit of feeding more alfalfa?

Miner Institute Study: Optimizing Alfalfa and Corn Silage Ratios

We recently conducted a study aimed at identifying potential associative effects between alfalfa and corn silage on milk component output (mainly milk fat and protein) and their efficiency of production in high-producing Holstein cows. Our study was unique given the wide range of dietary alfalfa-to-corn silage ratios fed (90:10 to 10:90, DM basis) and the high level of milk production, which makes the results directly applicable to progressively managed dairy herds.

Over two enrollments, we fed 105 cows (45 primiparous, 60 multiparous) in a randomized complete block design with a 1-wk covariate (50:50 alfalfa to corn silage, DM basis) followed by a 4-wk feeding period. Following the covariate period, cows were blocked and assigned to 1 of 5 diets: 90:10, 70:30, 50:50, 30:70, and 10:90 alfalfa hay-to-corn silage (DM basis; Table 1 provides the simplified ingredient composition of the five experimental diets). All diets contained 62% forage on a DM basis, and water was added to the 50:50, 70:30, and 90:10 diets to increase moisture content. All rations fell between approximately 45 and 60% DM.

We used alfalfa hay rather than silage in this study because there was no available source of alfalfa silage that met our specifications for NDF (i.e., approximately 35% of DM). Consequently, we sourced sufficient alfalfa hay from one location in Ohio for the entire study that then had to be chopped prior to feeding. As an experimental model hay was judged to be our best option because it ensured more uniformity and consistency

during the study than silage would have. Even though many dairy farmers feed silage rather than hay, the dietary model that we used should be largely applicable to silage systems in terms of the cow's lactation responses. Previous research comparing alfalfa hay and silage found they were often similar in the DMI and fat-corrected milk (FCM) responses elicited (Broderick, 1985; Broderick, 1995). In general, practical on-farm considerations for feeding hay versus silage include potential leaf losses when baling and processing, the challenge of chopping and feeding dry alfalfa hay versus similar quality silage, and whether to add water to the ration (as we did in our study).

Dietary CP content was allowed to vary with the goal of having a similar MP supply among all five diets, as predicted by the Cornell Net Carbohydrate Protein System model (CNCPS v. 6.55). However, when we used cow data and feed analyses measured during the study (i.e., wk 4 for cow responses and wk 3 and 4 for feed analyses), we found that MP supply increased from 107 to 112 g/kg of DMI as the ratio of alfalfa hay to corn silage increased. As the proportion of alfalfa hay in the diet increased, the supply of lysine also increased by about 5.7% for the highest alfalfa diets. This change reflected the relative concentration of lysine in corn versus alfalfa protein (Park et al., 2020).

Table 1. Ingredient and dietary composition (% of ration DM) of diets with varying proportions of alfalfa hay and corn silage.

Ingredient and dietary composition	Alfalfa-to-corn silage ratio (DM basis)				
	10:90	30:70	50:50	70:30	90:10
Corn silage	56.4	43.5	31.0	18.6	5.7
Alfalfa hay	5.7	18.6	31.0	43.5	56.4
Concentrate	37.9	37.9	38.0	37.9	37.9
DM, %	45.0	50.0	52.5	59.4	60.4
CP	15.7	15.6	16.4	17.1	17.6
aNDFom[a]	30.6	29.3	28.3	26.7	25.5
Starch	26.5	27.9	26.3	26.2	26.0
Sugar (ESC)[a]	5.6	5.3	5.6	5.6	5.6
Ether extract	5.1	4.6	4.6	4.9	4.6
MP supply, g/kg DMI[a]	107	107	110	111	112
Lysine, g/d	194	198	198	207	205
Methionine, g/d	71	73	73	77	76
ECM/ME intake, kg/Mcal[a]	0.70	0.70	0.70	0.68	0.71

[a]aNDFom = amylase-modified neutral detergent fiber on organic matter basis; ESC = ethanol soluble carbohydrates; MP = metabolizable protein; ECM = energy-corrected milk; ME = metabolizable energy.

Overall, these five total mixed rations (TMR) were much smaller in particle size than silage-based diets typically fed to lactating cattle in the US (Table 2). But they were similar in particle distribution to diets commonly fed in the Parma region of Italy where dry forage diets predominate in the production of Parmigiano Reggiano cheese (Heinrichs et al., 2021). As alfalfa proportion increased relative to corn silage, the dietary physical effectiveness factor (pef) decreased while the uNDF240 content increased, reflecting the

physical and chemical characteristics of the alfalfa hay. Combining both measures into physically effective uNDF240 (peuNDF240), the range among the five diets shrank to about one percentage unit. Still, the highest alfalfa diet contained less peuNDF240 than the highest corn silage diet.

Table 2. Particle size measured using Penn State Particle Separator and undegraded fiber characteristics of diets varying in proportion of alfalfa hay and corn silage.

Measure	Alfalfa-to-corn silage ratio (DM basis)				
	10:90	30:70	50:50	70:30	90:10
Particle size distribution, % as fed					
>19 mm	4.4	5.8	5.9	7.1	8.9
8-19 mm	45.8	37.6	31.7	23.4	15.6
4-8 mm	11.6	11.4	11.5	11.5	11.6
Pan	38.1	45.2	50.9	58.0	64.0
pef[a]	0.62	0.55	0.49	0.42	0.36
peNDF (pef x aNDFom)	18.9	16.0	13.9	11.2	9.2
uNDF240, % of DM[a]	9.5	10.2	10.1	12.1	12.5
peuNDF240, % of DM	5.7	5.6	4.9	5.1	4.7

[a]pef = physical effectiveness factor measured as % of as-fed particles retained on ≥4.0-mm sieve of Penn State Particle Separator; peNDF = physically effective neutral detergent fiber; uNDF240 = undegraded neutral detergent fiber at 240 h of in vitro fermentation; peuNDF240 = physically effective uNDF240.

Lactational Performance Responses

Dry matter intake was not affected by diet (Table 3). In fact, as the ratio of alfalfa to corn silage ranged between 10:90 and 90:10 (DM basis) DMI only varied by 0.5 kg/d and averaged about 3.90% of BW. Previous studies have reported variable responses in DMI as ratio of alfalfa to corn silage varied, with many finding no effect on DMI (Dhiman and Satter, 1997; Wattiaux and Karg, 2004: Erdman et al., 2011; Arndt et al., 2015); some showing increased DMI as alfalfa increased (Brito and Broderick, 2006; Mullins et al., 2009; Weiss et al., 2009); and one finding a positive effect of corn silage on DMI (Uddin et al., 2020). When evaluating these previous studies, ration formulation strategy clearly played an important role in determining the relative intake and milk yield response of cows to varying proportions of alfalfa and corn silage (e.g., forage percentage in the ration, carbohydrate content, and use of forage or non-forage sources of fiber). But with our formulation approach and using alfalfa hay, DMI was unaffected across a wide range of alfalfa hay to corn silage ratios.

Table 3. Lactation and rumination responses to diets varying in proportion of alfalfa hay and corn silage.

Measure	Alfalfa-to-corn silage ratio (DM basis)				
	10:90	30:70	50:50	70:30	90:10
Dry matter intake, kg/d	26.3	26.6	26.7	26.8	26.4
Dry matter intake, % of BW	3.82	3.85	3.86	3.91	3.91
ECM yield, kg/d[a]	47.9	48.7	48.2	47.0	48.3
ECM/DMI, kg/kg[a]	1.82	1.83	1.81	1.76	1.83
Milk fat, %	4.08	4.06	4.02	4.01	4.22
Milk fat, kg/d	1.80	1.82	1.79	1.75	1.83
Milk true protein, %	3.01	3.07	3.01	3.02	3.05
Milk true protein, kg/d[b]	1.33	1.37	1.35	1.31	1.33
Milk urea nitrogen, mg/dl[c]	9.8	8.5	10.4	11.0	12.0
De novo milk fatty acids, g/100 g FA[a,d]	24.76	25.86	25.82	25.22	25.58
Rumination time, min/d[e]	499	477	462	449	396

[a]ECM = energy-corrected milk; DMI = dry matter intake; FA = fatty acid.
[b]Significant cubic ($P = 0.04$) effect.
[c]Significant linear ($P < 0.001$) and quadratic ($P = 0.002$) effect.
[d]Significant quadratic ($P = 0.03$) effect.
[e]Significant linear ($P < 0.001$) effect.

Yield of ECM was unaffected by the ratio of alfalfa and corn silage (Table 3). Efficiency of ECM production was also unaffected by the ratio of the forages. As with DMI, previous reports on the effect of alfalfa-to-corn silage ratio on milk yield and its efficiency of production have been variable. Many studies have observed no effect of the ratio on ECM or FCM yield (Kleinschmit et al., 2007; Mullins et al., 2009; Erdman et al., 2011) and a few have shown a positive response of greater corn silage (Groff and Wu, 2005; Uddin et al., 2020). A reasonable conclusion would be that a blend of alfalfa and corn silage that avoids the extremes seems to be desirable to maximize ECM yield. As examples, Arndt et al. (2015) found a quadratic effect of the ratio of alfalfa silage to corn silage between 20:80 and 80:20 (DM basis) on fat- and protein-corrected milk yield, with the predicted maximum being at 50:50 alfalfa to corn silage. Weiss et al. (2009) observed that ECM yield was maximized for diets containing 75:25 alfalfa silage to corn silage (DM basis). Dhiman and Satter (1997) concluded that corn silage and alfalfa silage in a ratio between 1/3 to 2/3 corn silage was optimal for milk yield and most efficient use of dietary N.

When we focus specifically on milk composition, diet did have an effect. Content and production of milk fat was high and unaffected by diet, averaging about 4.0% and 1.8 kg/d. But there was a significant cubic ($P = 0.04$) effect of diet on milk true protein output. The high milk fat content in all diets indicates healthy rumen conditions as rumen pH and milk fat have been reported to be positively related (Allen, 1997). Mirroring the change in true protein output there was a significant linear ($P = 0.001$), quadratic ($P = 0.002$), and cubic ($P = 0.002$) effect of diet on MUN. Milk urea nitrogen was reduced between the 10:90 and 30:70 alfalfa-to corn silage diets, and then it increased incrementally for the 50, 70, and 90 alfalfa diets. Although the difference in MUN among the five TMR was

relatively small, it may be that the greater soluble protein of alfalfa hay complemented the rumen fermentable starch provided by the corn silage, resulting in a stimulation of microbial protein production in the rumen. This would make sense given that milk true protein was greatest for the 30:70 alfalfa-to-corn silage diet and MUN was the lowest. For the higher alfalfa diets (50, 70, and 90 alfalfa), MUN increased likely reflecting an oversupply of RDP, although milk protein output generally remained similar to the 10:90 alfalfa-to-corn silage diet. A small but significant quadratic ($P = 0.03$) effect of diet on de novo fatty acids as alfalfa proportion increased suggests an optimal ratio of alfalfa hay and corn silage between 30:70 and 50:50. Greater proportion of de novo fatty acids in milk fat and lower unsaturation index (data not shown) both indicate better conditions for rumen fiber fermentation and synthesis of milk fat (Woolpert et al., 2016).

There was a significant linear ($P < 0.001$) effect on rumination (Table 3). The amount of time that cows spent rumination per day decreased from 499 to 396 min/d from the 10:90 to the 90:10 alfalfa-to-corn silage diet. Overall, these rumination times are greater than previously reported for finely chopped alfalfa hay diets (443 min/d; Cavallini et al., 2018) except for the 90:10 alfalfa-to-corn silage diet. For lactating dairy cows fed a wide range of diets the average range of rumination has been reported as being between 420 to 520 min/d (Haan, 2020). Overall, even though the peNDF content of these diets was less than ordinarily fed in the US, rumination activity (except for the 90:10 alfalfa diet) and milk fat content fell within desirable ranges.

Perspectives on Feeding Alfalfa

Factors in addition to the cow response to diet will ultimately determine optimal amounts of corn silage and alfalfa that will be grown or purchased and fed on any given dairy farm. These factors include relative cost of production for alfalfa versus corn silage; agronomic differences between the forages; acreage required for N in manure; differences in water use; variability in nutrient composition across cuttings for alfalfa versus one harvest for corn; and relative costs of protein sources and other feed ingredients. The best answer will require a whole-farm modeling approach that integrates rations with factors such as manure management and crop rotations. Such models are under development but unavailable today. On-going work with whole-farm models will allow us to optimize forages from a nutritional, agronomic, and economic perspective.

For now, though, the five diets were evaluated using the CNCPS model (AMTS Cattle Pro 4.16.9.1) with these inputs:

- Nutrient composition of feeds was from samples collected and analyzed during the study.
- Federal Order 1 milk component prices from May 2021 (when study was conducted) were used to calculate milk price for each diet. No adjustments for producer price differential or somatic cell premiums were made.
- Alfalfa hay and corn silage price was set using the May 2021 Penn State feed price listing.
- Other feed prices were based on the Penn State listing and a feed price list from a commercial feed company.

- Farm-produced feeds were defined as only corn silage (PF1) or both corn silage and alfalfa hay (PF2).
- Urinary urea-nitrogen and ammonia emissions were calculated using equations from Burgos et al. (2007; 2010).
- Corn silage yields and N fertilization rates were from the Miner Institute farm records. Yields of 18 tons/acre for corn silage and 4.5 tons/acre for hay on an as-fed basis were used.

Total feed cost increased with higher levels of alfalfa hay in the diet (50, 70, and 90 alfalfa; Table 4). Purchased feed cost when alfalfa was purchased off farm (PF1) increased as less corn silage was fed. However, purchased feed cost decreased with higher levels of home-grown alfalfa hay in the diet (PF2). Similarly, income over purchased feed cost was generally higher with a greater proportion of alfalfa when both forages were home grown.

Total pounds of manure produced and fecal N excretion were similar for all diets. Urinary N excretion and ammonia emissions increased in the diets with the two highest alfalfa hay amounts but were least for the 30:70 alfalfa-to-corn silage TMR. Methane emissions were slightly elevated with the higher levels of alfalfa hay in the diet.

The nutrient management aspect of crop acres and manure N application rates needs to be an integral component of evaluating the results of this project. It is beyond the scope of this paper to conduct a complete analysis due to variability of the key factors required. These include soil type, soil fertility, number of years that corn has been planted on the field, manure storage type, manure application and incorporation process, and target N application rates. The total as-is pounds of manure from one cow are about 155 lb/d. However, literature data indicate that there are highly variable N losses between the cow and uptake by the plant. Given these variables, it is difficult to calculate the crop acres needed per cow on a N basis for corn silage. It was assumed that manure is not applied to alfalfa fields. The potential imbalance of manure N available and crop acres required will be greater as the amount of corn silage in the diet decreases. This is an area that needs more in-depth analysis. Overall, about 1 acre/cow for corn silage and 0.6 acres/cow for alfalfa hay would be required to feed the 30:70 alfalfa-to-corn silage TMR for a year. This calculation assumed a loss of 20% for corn silage from harvest to feed-out and a 10% loss for alfalfa hay.

Table 4. Diet evaluation using Cornell Net Carbohydrate Protein System model (AMTS Cattle Pro 4.16.9.1).

	Alfalfa-to-corn silage ratio (DM basis)				
	10:90	30:70	50:50	70:30	90:10
Costs and returns					
Milk, $/cow/d	19.71	20.11	19.84	19.27	19.90
Total feed cost, $/d/cow	9.85	9.74	9.99	10.30	10.15
PFC1, $/cow/d[a]	6.91	7.45	8.45	9.31	9.85
PFC2, $/cow/d[a]	6.49	6.06	6.01	6.03	5.65
IOTFC, $/cow/d[a]	9.86	10.37	9.85	8.97	9.75
IOPFC1, $cow/d[a]	12.80	12.66	11.39	9.96	10.05
IOPFC2, $/cow/d[a]	13.22	14.05	13.83	13.24	14.25
Manure production, N and P excretion, CH_4 emissions					
Manure, lb/cow/d	152	151	155	160	157
Fecal N, g/cow/d	251	252	255	263	258
Urine N, g/cow/d	208	193	214	253	238
Urinary urea-N, g/cow/d	164	146	173	182	196
Total manure N, g/cow/d	459	445	469	516	496
NH_3 emission, g/cow/d	84	78	87	90	95
P excretion, g/cow/d	47.7	44.0	43.6	45.3	40.8
CH_4, L/cow/d	694.5	683.7	709.9	716.5	714.4
Forage needs					
Corn silage, tons/cow/yr	18.9	14.8	10.5	6.3	1.9
Corn silage, acres/cow	1.26	0.99	0.70	0.42	0.13
Alfalfa hay, tons/cow/yr	0.7	2.2	3.7	5.2	6.7
Alfalfa hay, acres/cow	0.19	0.61	1.02	1.43	1.85

[a]PFC1 = Purchased feed cost with only corn silage grown on farm; PFC2 = purchased feed cost with both corn silage and alfalfa hay grown on farm; IOTFC = income over total feed cost; IOPFC1 = income over purchased feed cost with only corn silage grown on farm; IOPFC2 = IOPFC with both forages grown on farm.

Conclusions

Overall, our results suggest that cows will perform well on diets containing as much as 90% of the forage as alfalfa with minimal corn silage compared with high corn silage rations. An optimal ratio of the two forages where milk true protein is maximized, MUN is minimized, and milk fatty acid metrics are optimized is about 30:70 to perhaps 50:50 alfalfa hay and corn silage. Based on our study and previously published research, this translates into diets containing between 20 to 25% alfalfa and up to 35% alfalfa in the ration dry matter. Factors in addition to cow response to the diet will factor into forage decisions on farm. Nonetheless, based on these dairy performance results and our knowledge of the agronomic advantages of alfalfa, sustainable dairy-forage programs can utilize higher alfalfa-to-corn silage ratios than is commonly practiced today within the dairy industry.

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Modelling of Greenhouse Gas Emissions, Nitrogen Losses and Economic Performance Under Differing Farm Systems, Diets and Land Use Scenarios for a New Zealand Dairy Farm

H. Tacoma, B. Rys, S. Davison, and W. Morritt.
Headlands Consultancy, New Zealand

Introduction

Over the last two decades there has been a large amount of research into strategies to reduce greenhouse gas (GHG) emissions, both domestically and internationally. Current advice to the dairy industry is indicating that the mechanism for reducing GHG emissions should be to reduce reliance on concentrate feeds/byproducts and for the industry to move back to a lower stocked, pasture /forage-only system.

The New Zealand dairy industry has historically been a predominantly pasture-based system. However, over the past two decades, NZ farmers have introduced concentrate feeds into their farming systems to optimise the productivity of their cows and land. Nevertheless, the New Zealand dairy industry is still largely pasture based, with approximately 85% of feed grown on farm, and 15% of feed imported from outside the farm (Ledgard et al., 2020).

Given the large (approx. 37%) contribution of enteric methane production from ruminants to New Zealand's national GHG emissions, there is particular focus on reducing enteric methane production. Methane is naturally produced during fermentation in the rumen as it is an end product in the fermentation of carbohydrate feed sources (Beauchemin et al., 2008; O'Neill et al., 2011). Nitrogen (N) losses are also an important environmental factor requiring optimisation. N losses from NZ dairy systems can be very high due to the high quantities of soluble and degradable protein in high quality pasture (Higgs et al., 2013). The challenge before New Zealand dairy farmers is to reduce their environmental footprint whilst maintaining or increasing productivity and profitability.

The primary objective of this investigation was to use modelling software (Udder, Red Sky, and Overseer) to analyse productivity and profitability, as well as GHG emissions and N outputs through modelling a series of multi-factor alterations to the average farm in the Waikato region as defined by the 2018 /2019 DairyNZ economic survey (DairyNZ, 2019). The secondary objective of this investigation was to use CNCPS software to verify the accuracy of the trends in GHG emissions obtained from modelling through Overseer. Given the large contribution of enteric methane production to total GHG emissions in NZ dairy systems, using a separate, internationally respected model is important to corroborate the results from the Overseer model.

Materials and Methods

Udder Models

A whole-farm model was developed in the farm modelling software, Udder to represent an 'average farm' in the Waikato, based on information from the 2018-19 DairyNZ economic survey (DairyNZ, 2019). This farm model ("control") consisted of a 117 ha, Spring calving dairy farm with a start of calving date of 15th July, 344 milking cows at peak, a calving period of approximately 11 weeks, an annual heifer replacement rate of 25% of peak cow numbers and a feeding system consisting of mainly ryegrass /white clover pasture plus smaller amounts of imported silage (pasture silage was chosen for this exercise) and palm kernel expeller (PKE).

Pasture grazing decision rules are discussed in detail by Macdonald et al. (2010). The decision rules used in the current modelling was in accordance with these rules, with the aim of optimising quality and quantity of pasture production. Rotation lengths were set in accordance with a template designed to reach the end of the first grazing round by approximately September 25. The rotation length from there on was primarily designed with the intention to graze plants at the 2.5 – 3 leaf stage for the majority of the season, maximizing pasture harvested without impacting pasture quality as set out by Fulkerson and Donaghy (Fulkerson and Donaghy, 2001). The exception to this rule was during the period of seedhead accumulation (Oct to Jan), in which a 2-2.5 leaf stage was targeted.

Financial Analysis

The economic performance of the control farm was extrapolated from Red Sky Farm Performance Financial Analysis software (version 5.04.02); this program provides a platform for analysing the financial performance of a farm and the opportunity to benchmark different farms or farm systems against one another. The financial analysis was performed in a spreadsheet, extrapolating the expenses from the control scenario and allocating costs on a per-cow or per-hectare basis in accordance with the method used by Macdonald et al. (2011) (Table 1).

Table 1: Various costs used within the models

Milk Price	$6.50 /kg MS	(plus variations)
Concentrate price	$500 /t DM	(plus variations)
Imported forage	$350 /t DM	
Home-made forage	$120 /t DM	
Nitrogen	$1,850 /t N	

Analysis of Environmental Parameters

GHG emissions and N losses were calculated using Overseer farm modelling software (version 6.4.0). Overseer calculates total farm GHG emissions by estimating methane, nitrous oxide and carbon dioxide (CO_2) emissions, presented as CO_2 equivalents. Global warming potential (GWP) on a 100-year basis and standard Intergovernmental Panel for Climate Change (IPCC) 2007 factors of 25 and 298 kg

CO_2 equivalent /kg respectively were used for methane and nitrous oxide. GHG emissions from young stock (YS) and concentrate production were included in the analysis.

N losses calculated in Overseer take into account the N losses from leaching, volatilisation, and denitrification on the milking platform. N losses which occurred during the rearing of the replacement heifers off the dairy platform were calculated separately and reported for and added to each scenario. More detailed descriptions of Overseer and the GHG section of the Overseer model are given by Wheeler et al. (2006) and Wheeler et al. (2008). N losses from land outside the farm growing the supplementary feed (forage or concentrate) were not accounted for in the current modelling. Under New Zealand rules, and in contrast to its GHG emissions, N losses from the production of concentrate feeds are allocated to the nutrient- and environmental budgets of the farms where they are physically occurring (the accompanying GHG losses are duplicated to the dairy farm, apparently double-counting these). N losses associated with the consumption of the concentrate feeds are accounted for in the current modelling. There was no feed-pad or barn on the farm as modelled. Therefore, the majority of the dung and urine was deposited directly onto pasture. Liquid effluent collected from the yard is collected in a pond, stirred, and spread regularly throughout the year.

Scenarios 1-4

Using the same software programs and methodology used for the control farm, four alternative scenarios were modelled. In these scenarios, the physical farm parameters were kept the same as the control farm and milk production was controlled, by way of milker numbers and concentrate inputs, to remain as close as possible to the control farm model. Variations in cow size, genetic merit, stocking rate and the level of concentrate feeding were incorporated into the systems (Table 2).

Table 2: Metrics of the control farm and the five scenarios

	Control	**Sc. 1**	**Sc. 2**	**Sc. 3**	**Sc. 4**	**Sc. 5**
# of peak cows	344	306	258	247	220	293
Farm Area (ha)	117	117	117	114	107	117
Cow Live Weight (kg)	450	475	500	500	550	450
kg Live Weight / ha	1,323	1,242	1,103	1,083	1,131	1,127
Relative cow genetic merit	100%	101%	104%	105%	107%	100%
Total feed consumed(t DM) *	1,943	1,881	1,762	1,738	1,688	1,686
Feed consumed vs. control		-3.2%	-9.3%	-10.6%	-13.1%	-13.2%
Stocking Rate (cows/ha)	2.94	2.62	2.21	2.17	2.06	2.5
Farm production (kg MS**)	124,890	124,839	124,819	124,941	124,954	111,308

* Including young stock

** kg MS = kg's milk fat + kg's milk protein. 1 kg MS equals approx. 15.7 litres US milk.

Initial and final average body condition score (BCS) of the herd was the same in each scenario to ensure annual milk production wasn't at the cost of body fat reserves. Final average pasture cover (APC) was equal to the initial APC. Both conditions were put in place to ensure that the model is feasible and has long term sustainability.

After modelling in Udder, these alternative farm system scenarios were then modelled through the Overseer program and the financial spreadsheet, using the same methodology and decision rules as in the control scenario. In scenario 1, the concentrate included in the ration was 100% maize grain. In scenarios 2-4, the concentrate was a blend of soybean hull (42%), maize grain (42%), and dried distillers grain (16%).

Scenario 5

Scenario five was created to represent current industry advice for reducing GHG emissions through reduced stocking rate (15% reduction) and reduced imported feed input, using pasture and forage-based supplements only (Climate Change Commission, 2021) The physical farm parameters were the same as those in control and scenarios 1-4. As a result of a 15% reduction in stocking rate and the use of forage-supplements only, the production level of this scenario was 11% lower than that of the other scenarios.

All Farm Model Scenarios

In each of the five alternative scenarios modelled in Udder, the same base pasture growth rates, per-ha pasture production, pasture quality parameters and per-hectare level of N- and other fertiliser was applied as in the control farm. Important differentiations between the scenarios, apart from cow numbers and cow size- and quality were: the amount of concentrates fed, the timing and area of silage harvesting, and the N applications (Figure 1). Where dairy platform land area was reduced, total N- and other fertiliser use was reduced proportionally to maintain the same per-hectare application rate; similarly, the area of each soil type modelled in the control Overseer model was adjusted proportionally to ensure the percentage of each soil type on the farm was maintained in each of the Overseer models.

In all scenarios (including control), the rising 1-year-old calves grazed off-farm from 1st December, returned as rising 2-year-old heifers on June 1 the following season. This is considered standard industry practice in the Waikato region. Cow size and genetic merit were important variable factors (Table 2). For each scenario, BCS at the end of the season was very similar to the beginning of the season. This was approximately BCS of 5 for scenarios 2, 3 & 4, and BCS 4.5 for scenarios 1 & 5, and control.

The diet on the control farm consisted of pasture, conserved forage, and PKE; approximately 80% standing pasture, 12% imported pasture silage on dry matter (DM) basis annually, and 8% imported PKE, the exact amounts being fed varying through the year. This diet was designed to be representative of an average Waikato farm using DairyNZ survey data (DairyNZ, 2019). In scenario 4**,** the annual diet consisted of approx. 72 % standing pasture, 10% silage (home-grown pasture silage only) and 18.5% imported concentrates. Scenario 5 consisted of approx. 84% standing pasture, 11% imported- and 5% home-grown silage.

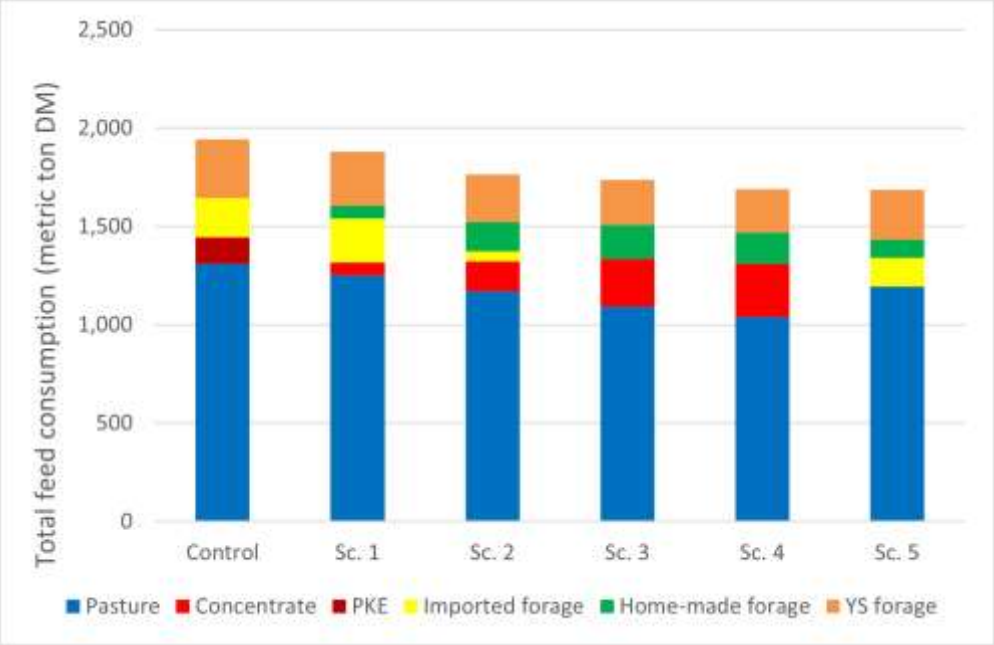

Figure 1: Total feed consumption per scenario (including young stock

Methane Emissions and CNCPS

The Cornell Net Carbohydrate and Protein System (CNCPS) was used to model methane emissions in three of the model scenarios (control, scenario 4 and scenario 5).

Results and Discussion

Whole Farm Systems Analysis

Each of the four alternative scenarios (1-4) progressively increased the per-cow production, the milk production as a percentage of liveweight, the feed conversion efficiency (FCE) and the percentage of feed partitioned to milk production (Table 3).

Table 3. Production responses from changing system parameters

	Control	Sc. 1	Sc. 2	Sc. 3	Sc. 4	Sc. 5
Peak Cow Numbers	344	306	258	247	220	293
Production/cow (kg MS)	363	408	484	506	568	380
Production/cow as % liveweight	81%	86%	97%	101%	103%	84%
FCE (kg DM feed per kg MS) *	15.6	15.1	14.1	13.9	13.5	15.1
FCE % improvement over control		3.2%	9.6%	10.9%	13.5%	3.2%
% Feed energy partitioned to MS*	44.6%	46.7%	50.1%	51.2%	52.6%	45.7%

* Including young stock

Simultaneously, the GHG emissions and N losses in scenarios 1-5 both decreased, and profitability increased in comparison with the control farm (Tables 4-6).

Table 4: Imported feeds and operating profit (OP)

	Control	**Sc. 1**	**Sc. 2**	**Sc. 3**	**Sc. 4**	**Sc. 5**
Concentrate imported (t)	0	66	150	239	272	0
Concentrate as % of diet	0%	4.0%	9.9%	15.8%	18.5%	0%
PKE imported (t)	133	0	0	0	0	0
PKE as % of diet	8.1%	0%	0%	0%	0%	0%
Forage imported (t DM)	202	224	51	0	0	148
Home grown silage (t DM)	0	66	150	175	159	91
Farm area retired	0%	0%	0%	2.6%	8.5%	0%
Operating Profit	$270,777	$291,263	$331,657	$315,970	$330,970	$250,753
Change OP vs. control*		7.6%	22.5%	16.7%	22.2%	-7.4%

*Milk price of $ 6.50/kg MS, concentrate cost of $ 500/t.

Table 5: Overseer GHG emissions* and N losses, excluding young stock.

	Control	**Sc. 1**	**Sc. 2**	**Sc. 3**	**Sc. 4**	**Sc. 5**
Methane (t eCO2 /yr)	925	888	841	814	803	814
N2O (t eCO2 /yr)	304	288	272	258	251	279
CO2 (t CO2 /yr)	222.5	179.9	180.6	198.6	204.7	150.5
Total GHG (t eCO2 /yr)	1,452	1,356	1,293	1,270	1,258	1,243
Total GHG emissions vs. control		-6.6%	-10.9%	-12.5%	-13.3%	-14.4%
Total GHG (kg eCO_2) per kg MS	11.6	10.9	10.4	10.2	10.1	11.2
N loss (kg N /yr)	5,199	5,225	4,853	4,842	4,670	4,754
N loss vs. control		+0.5%	-6.7%	-6.9%	-10.2%	-8.6%

*Overseer includes embodied emissions of imported supplements in its GHG calculations.

Table 6: Overseer GHG emissions* and N losses, including young stock.

	Control	**Sc. 1**	**Sc. 2**	**Sc. 3**	**Sc. 4**	**Sc. 5**
Methane (t eCO_2/yr)	1095	1049	977	951	929	959
N_2O (t eCO_2/yr)	378	357	331	317	303	342
CO_2 (t CO_2 /yr)	245	202	199	217	218	171
Total GHG (t eCO_2/yr)	1718	1609	1507	1485	1450	1,472
Total GHG emissions vs. control		-6.4%	-12.3%	-13.6%	-15.6	-14.4%
Total GHG (kg eCO_2) per kg MS	13.8	12.9	12.1	11.9	11.6	13.2
Total kg GHG/kg MS vs. control		-6.3%	-12.2%	-13.6%	-15.7%	-3.9%
N loss (kg N/yr)	6,829	6,661	6,114	6,067	5,769	6,165
N loss vs. control		-2.5%	-10.5%	-11.2%	-15.5%	-9.7%

*Overseer includes embodied emissions of imported supplements in its GHG calculations.

By progressively decreasing the stocking rate but increasing cow size and genetic potential in each of the scenarios, the total DM consumed reduced progressively through scenario 1 to 4 (Table 2 and Figure 1). As stocking rate decreased and cow size and genetic merit increased, the total system energy inflow and the percentage of energy ingested required for cow maintenance decreased. Additionally, he percentage of feed energy partitioned towards milk production increased, resulting in increased feed conversion efficiency (FCE; Table 3).

There were inverse relationships between milk production per cow as % LW and methane production, and between concentrate fed (% of diet) and methane production (Table 3, Table 4 & Table 5). Inverse relationships were also observed between concentrate imported (t) and total GHG (t eCO2 /yr), and between concentrate imported (t) and total kg GHG (eCO2) /kg MS until methane efficiency plateaued for scenarios 3 & 4 (Table 4, Table 5 & Table 6).

Scenario 4 showed the largest reduction in total farm GHG emissions and N losses compared with the control farm; 15.7% and 15.5% respectively (including young stock, Table 5). Scenario 4 utilised a diet with the highest concentrate inclusion (18.5%) and the largest, most genetically capable cows of the scenarios examined, with a lower stocking rate (SR) than any of the other scenarios (Table 2). This resulted in a decrease in total farm feed requirements (incl. YS) of 13.2% compared to the control farm. Due to the lower SR, the concentrate feed inputs and the higher genetic capacity, the cows in scenario 4 had the highest milk solids (MS) production per cow, and the lowest methane production per kg MS produced (Table 3 & Table 5).

The main mechanism involved in reducing GHG emissions and N losses in the modelled scenarios is the reduction of total DM consumed on each farm (Table 2). Reducing the total quantity of feed consumption is a commonly accepted method for reducing enteric methane production (O'Neill et al., 2011). As a lower percentage of the feed energy was partitioned to maintenance and more towards milk production over the scenario sequence, the increased production per cow resulted in almost identical total farm milk production, achieved with fewer cows, and less total feed. As total feed consumption progressively reduced, GHG production and N losses also decreased. This result is in accordance with the concept described by Hristov et al. (2013) who reported that on a per cow basis, whilst methane emissions increase as feed intake increases, the efficiency of methane emissions per kg of DMI also increases with increasing feed intake above maintenance level. Therefore, a lower stocking rate combined with higher production per cow, as is the case with scenario's 2, 3 and 4, the maintenance energy requirements are diluted by higher DMI per cow and both methane production per kg MS and total farm methane production decrease. This concept is also supported by Knapp et al. (2014) and Boadi et al. (2004) who reported that lower methane production in scenarios where milk production remains constant with reducing cow numbers should be expected.

For scenarios 2 to 4, N losses were inversely correlated with concentrate feed % in the diet and the stocking rate (Table 4, 5 & 6). The concentrate feed was formulated to have lower average crude protein (CP) content than the pasture (Overseer default of 3.7% N for pasture). As concentrate proportion of the diet increased, the overall CP content of the diet decreased, which reduced the N losses. Lower stocking rates result in fewer urine spots, thus decreasing the potential for N

losses from urine. Higgs et al. (2013) reported that a primary method of reducing N losses is through reducing N content in feed. With increasing levels of concentrate in scenarios 2 to 4, N losses progressively reduced from a 10.5% reduction from control for scenario 2 to a 15.5% reduction for scenario 4 (including YS). Scenario 1 had high N losses due to the high reliance on pasture silage as a supplementary feed.

Scenario 4 showed the second largest improvement in operating profit (OP) (22.2%) compared with the control farm (Table 4), notwithstanding the fact that 8.5% of the productive farmland was able to be retired from dairy production in this scenario.

Scenario 5 was designed to reflect the implications of current NZ recommendations for reducing GHG emissions. A 15% reduction in SR from control was implemented and no concentrate feed was imported; cow size and genetic quality were left unchanged. Whilst scenario 5 did reduce total farm GHG emissions by 14.4%, farm milk production was reduced by 11% (Table 2 & Table 6). This resulted in methane efficiency similar to the control; 13.8 vs. 13.2 kg GHG (e CO2) /kg MS for control and scenario 5 respectively, whilst scenarios 2, 3 and 4 reduced total GHG emissions to 12.9 –11.6 kg GHG (eCO2) /kg MS. Furthermore, profitability of Scenario 5 was approximately 7.4% lower than the control farm and 20-24% lower than that of Scenarios 2-4.

Scenarios 2, 3 & 4 had a similar or stronger reduction in N losses compared with scenario 5, whilst achieving 20-24% higher OP. The total land area for the milking platform was able to be reduced, maintaining productivity in scenarios 3 & 4, whereas the full land allocation was required to produce the results of scenario 5 (Table 2). These results are summarised in Figure 2:

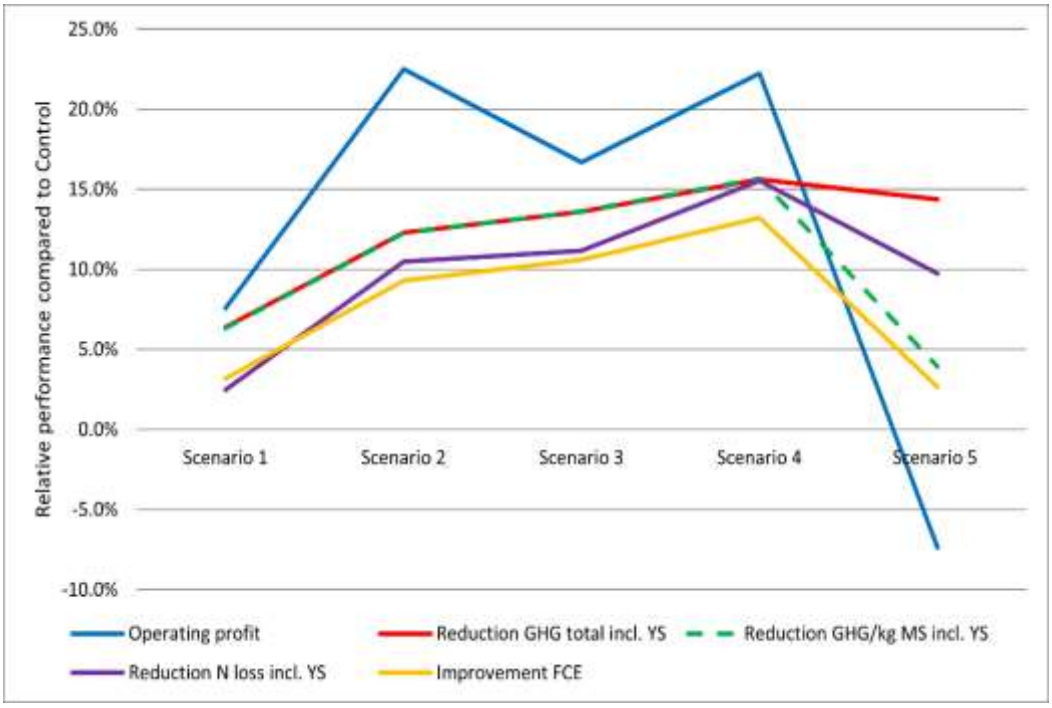

Figure 2: Relative performances of systems 1 through to 5 compared to control.

The intensity of NZ dairy emissions have decreased over the past three decades due to increased milk production from a reduced number of cows (Clark & Journeaux, 2021). Scenario's 1-4 progressively reduce the intensity of GHG emissions per unit of milk production through increased FCE and optimising milk production on the land area and feed available, as well as reducing the total farm GHG emissions. Scenario 5 only marginally reduces the intensity of GHG emissions (kg e CO_2) per unit of milk produced compared to control, and still has a higher intensity than scenario 1, the least optimal of the 4 alternative scenarios in terms of GHG (kg e CO_2) /kg MS.

An important distinction to make is that in our research, concentrate feeds are not being used to increase the stocking rate. Instead, concentrates are being utilised to optimise the per-cow production, allowing a lower SR but maintaining farm milk production. This optimisation of per-cow production is where scenarios 3 and 4 have major advantages over scenario 5. Whilst scenario 5 does achieve environmental benefits, it is at the expense of milk production and profit. In terms of the global food supply, New Zealand milk has a low carbon footprint compared to internationally produced milk (Knapp et al., 2014; Ledgard et al., 2020). Therefore, it is preferable to maximise efficiency of NZ milk production as in scenarios 3 and 4 rather than achieve similar environmental goals by sacrificing milk production as in scenario 5, effectively shifting milk production elsewhere globally.

It is pertinent to note that many feeds utilized in the stock feed industry are by-products from the manufacturing or processing. Alternative disposal of by-products potentially results in negative environmental implications (Russomano et al., 2012); hence it should be considered that utilizing these products as stockfeed increases the efficiency of the global food system.

Financial Analysis

The OP for scenarios 1-4 showed robust profit margins at variable concentrate prices and milk pay outs, when compared with scenario 5. Scenario 1 was more profitable than scenario 5 at all calculated concentrate prices for a milk pay-out of $5.00 and higher, and at a milk pay-out of $4.50 until the concentrate price reached $650 /t. Scenario 2 was more profitable than scenario 5 at all calculated milk pay-outs and concentrate prices. Scenarios 3 & 4 were more profitable than scenario 5 at most concentrate and milk prices. It was only when there was a combination of the milk price being very low and the concentrate price being very high that system 5 would exceed the profitability of scenarios 3 & 4. An example is shown below:

OP Sc 4, pay-out vs conc. price	$ 350	$ 400	$ 450	$ 500	$ 550	$ 600	$ 650	$ 700	$ 750
$4.00	$ 59,385	$ 45,785	$ 32,185	$ 18,585	$ 4,985	-$ 8,615	-$ 22,215	-$ 35,815	-$ 49,415
$4.50	$ 121,862	$ 108,262	$ 94,662	$ 81,062	$ 67,462	$ 53,862	$ 40,262	$ 26,662	$ 13,062
$5.00	$ 184,339	$ 170,739	$ 157,139	$ 143,539	$ 129,939	$ 116,339	$ 102,739	$ 89,139	$ 75,539
$5.50	$ 246,816	$ 233,216	$ 219,616	$ 206,016	$ 192,416	$ 178,816	$ 165,216	$ 151,616	$ 138,016
$6.00	$ 309,293	$ 295,693	$ 282,093	$ 268,493	$ 254,893	$ 241,293	$ 227,693	$ 214,093	$ 200,493
$6.50	$ 371,770	$ 358,170	$ 344,570	$ 330,970	$ 317,370	$ 303,770	$ 290,170	$ 276,570	$ 262,970
$7.00	$ 434,247	$ 420,647	$ 407,047	$ 393,447	$ 379,847	$ 366,247	$ 352,647	$ 339,047	$ 325,447
$7.50	$ 496,724	$ 483,124	$ 469,524	$ 455,924	$ 442,324	$ 428,724	$ 415,124	$ 401,524	$ 387,924
$8.00	$ 559,201	$ 545,601	$ 532,001	$ 518,401	$ 504,801	$ 491,201	$ 477,601	$ 464,001	$ 450,401
$8.50	$ 621,678	$ 608,078	$ 594,478	$ 580,878	$ 567,278	$ 553,678	$ 540,078	$ 526,478	$ 512,878

Methane Emissions, CNCPS

The use of a pasture-forage-concentrate system in scenario 4, with 18.5% of the diet as concentrate feed reduced the milking platform methane production by 13.9% compared with the control farm (Tables 6a and 6b, excludes young stock)). Scenario 5 resulted in a 9.6% reduction in methane production compared with the control farm (Tables 6a and 6c, excludes young stock), but there was also an 11% reduction in milk production (Table 2).

Table 6a: CNCPS-predicted monthly methane production; control.

Month	Cow numbers	Milk production (kg FCM /cow)	Methane produced (g/kg milk)	Total Methane (kg /month /herd)
July	11	17.44	24.23	51
August	168	22.50	19.76	2,315
September	307	26.10	17.75	4,267
October	344	27.09	17.57	5,076
November	344	24.08	19.46	4,836
December	334	20.68	22.04	4,719
January	329	18.11	25.36	4,685
February	329	16.45	27.78	4,210
March	329	15.25	29.37	4,568
April	268	14.97	32.92	3,963
May	56	15.12	34.19	289
Total kg CH_4				38,979
Average grams methane /L FCM				22.51

Table 6b. CNCPS-predicted monthly methane production; scenario 4.

Month	Cow numbers	Milk production (kg FCM /cow)	Methane produced (g/kg milk)	Total Methane (kg /month /herd)
July	7	23.74	21.52	39
August	104	31.16	16.74	1,682
September	197	36.76	14.57	3,165
October	220	37.63	14.48	3,716
November	220	33.97	15.67	3,513
December	218	29.59	18.12	3,623
January	217	26.21	18.95	3,341
February	217	24.21	21.31	3,134
March	217	23.75	23.69	3,785
April	217	22.68	25.92	3,827
May	197	20.72	30.45	3,729
Total kg CH_4				33,555
Average grams methane /L FCM				19.14
Total methane vs. control.				-13.9%

Table 6c. CNCPS-predicted monthly methane production; scenario 5.

Month	Cow numbers	Milk production (kg FCM /cow)	Methane produced (g/kg milk)	Total Methane (kg /month /herd)
July	9	16.88	25.78	43
August	141	21.94	20.46	1,962
September	262	26.10	18.27	3,748
October	293	27.09	17.72	4,360
November	293	24.30	19.18	4,096
December	290	20.46	20.46	3,763
January	288	17.78	25.59	4,061
February	288	16.33	28.56	3,762
March	288	15.25	30.33	4,129
April	255	15.24	30.93	3,605
May	159	14.45	36.77	1,689
Total kg CH_4				35,219
Average grams methane /L FCM				22.94
Total methane vs. control.				-9.6%

There was a 15% reduction in methane production per kg FCM for scenario 4 whilst in scenario 5, there was a 1.9% increase in methane production per kg FCM. The decrease in methane production per kg FCM in scenario 4 corresponds with increased levels of concentrate in the diet which resulted in an increase in milk production per cow and decreased energy (%) partitioned towards maintenance. The level of methane produced per kg FCM is at its lowest when cows are at peak levels of milk production, consuming a diet of pasture and concentrates without supplementary forages (in October, scenario 4).

The increase in methane production per kg FCM in scenario 5 is due to the reduction in milk production and the low feed conversion efficiency in this scenario.

The significant reductions in methane production for scenario's 4 & 5 compared with the control farm found in the CNCPS calculations align with the trends observed in the Overseer modelling. The modelling of the systems through CNCPS also confirms the feasibility of the systems which were originally modelled through Udder.

Conclusions

The modelling undertaken shows that a multi-faceted approach to tackling environmental problems on New Zealand dairy farms will yield the most beneficial outcomes, with substantial reductions in GHG emissions and N losses whilst improving profitability and land use efficiency.

Progressive improvements in environmental parameters can be achieved with the incorporation of concentrates into the farm system in conjunction with reducing stocking rate and land area employed and increasing size and genetic merit of cows to optimise intake and production on a per cow basis. This results in lower total feed consumption for similar milk production, resulting in reduced GHG emissions and N losses. Utilising concentrates in the diet enabled high DMI and high milk production

per cow, which dilutes maintenance requirements and increases the efficiency of methane production per unit of milk produced.

Carefully designed and executed pasture-forage-concentrate (PFC) systems improved economic performance over a wide range of pay-outs and concentrate prices and increased the productive efficiency of land and animals without reducing farm production. Designing these PFC systems requires a whole-system approach, analysing various levels of concentrate feed inputs, stocking rates, cow liveweight, cow genetic merit and land use to achieve the most efficient milk production whilst maintaining or improving profitability on farm.

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Effects of Ambient Heat Exposure and Dietary Organic Acids and Pure Botanicals on Gut Permeability and Milk Production

A. B. P. Fontoura and J. W. McFadden
Department of Animal Science
Cornell University

Introduction

Heat stress compromises the gastrointestinal barrier in non-ruminants. However, it is unclear whether exposure to environmental conditions that cause heat stress enhance gastrointestinal permeability in ruminants. Dietary supplementation of organic acid and pure botanicals (OA/PB) have been shown to improve animal performance by enhancing gastrointestinal health in swine and poultry species. Thus, it is reasonable to hypothesize that heat stress will progress with increased gastrointestinal permeability in lactating cows, which can reduce milk production and that OA/PB supplementation may prevent these consequences. Dietary OA/PB supplementation also represents a promising strategy to support and reduce antibiotic usage in livestock production systems. These compounds have unique antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties, which have potential to improve gastrointestinal health by controlling bacterial pathogen growth and enhancing barrier function. Organic acid and pure botanical feeding are a common practice for swine and poultry species and have been recently tested in heat-stressed weaned dairy calves; however, these additives have received minimal attention for lactating ruminants. This conference proceeding aims to review the fundamental feeding practices for heat-stressed lactating dairy cattle and concepts related to OA/PB feeding in domestic animals. The composition and properties of OA (i.e., citric and sorbic acids) and PB (i.e., thymol and vanillin) are summarized. The effects of heat stress and dietary OA/PB supplementation on gastrointestinal permeability and milk production are discussed. A recent study conducted at Cornell University that investigated the effects of heat stress conditions and dietary OA/PB supplementation in lactating cows on gastrointestinal permeability and milk production is presented in support.

Definitions and functions of organic acid and pure botanicals

Citric acid: A weak organic acid that is produced as an intermediary metabolite by mitochondria during the citric acid cycle. It is antimicrobial in nature. The proposed mechanism of action involves a lower pH within the bacterial cell, causing damage to enzymatic activity, protein, DNA, and extracellular membranes (Mani-López et al., 2012).

Sorbic acid: A short-chain unsaturated fatty acid that inhibits microbial enzymes and decouples nutrient transport in the cell, making it an antimicrobial and antifungal agent (Roth and Kirchgessner, 1998).

Thymol: A natural monoterpenoid phenol that has antioxidant properties and promotes bactericidal activity and membrane permeabilizing actions towards pathogens such as *Salmonella enterica* (Trevisi et al., 2007).

Vanillin: A phenolic aldehyde that increases the palatability of feeds. This compound has antimicrobial, anti-inflammatory, and antioxidant properties (Fitzgerald et al., 2004).

Exposure to hot ambient temperature and its physiological consequences

Heat stress is a limiting factor to efficient animal production, and it negatively impacts health and development during all lifecycle stages. Heat stress is deleterious to dairy cattle health, well-being, growth, fertility, milk production, and is of major concern for dairy systems. Decreased feed intake, increased sweating, respiration rate, and body temperature are well known physiological responses to exposure of ambient heat (Collier et al., 1982; Baumgard and Rhoads, 2013). In addition, it has been proposed that heat-stressed mammals shunt blood flow from the visceral organs towards the body periphery (Hall et al., 1999), which consequently, causes ATP depletion, acidosis, altered ion pump activity, and oxidative stress in the intestinal epithelium (Hall et al., 1999, 2001). This insult provokes paracellular permeability and tight junction opening (Lambert, 2009). Increased intestinal permeability results in the leakage of microbial-derived metabolites (i.e., lipopolysaccharide [LPS]) into the circulation, and stimulates local and systemic immune response (Ghosh et al., 2020).

Studies in humans and non-ruminant models have observed that intestinal permeability induced by diet and heat stress evolves with microbial dysbiosis (Brown et al., 2012; He et al., 2019). Importantly, these changes disrupt host immunity processes and contribute to pathogenesis of several metabolic diseases (Kinross et al., 2011; Rooks and Garrett, 2016). In dairy cattle, metabolic diseases throughout the life cycle are a substantial challenge faced by high-producing cows (Sordillo, 2016) and heat stress may exacerbate these conditions (Koch et al., 2019). For years researchers in the field of ruminant physiology have been postulating that heat stress may provoke systemic inflammation (Baumgard and Rhoads, 2013; Dahl et al., 2020; Most and Yates, 2021; Ríus et al., 2022). Recently, the inflammation caused by immune activation was highlighted as an important player in the pathophysiology and progression from heat stress to lethal heat stress (Burhans et al., 2022). Because the immune system relies on glucose availability for rapid cell proliferation and action (Palsson-McDermott and O'Neill, 2013; Abdel-Haleem et al., 2017) and considering the evidence that an activated immune system prioritizes nutrient utilization at the expense of processes like lactation and fetal development (Johnson, 1997), milk production is jeopardized during heat stress. In addition, even though recent studies demonstrated that LPS administration has the capability to halt milk production through immune activation (Kvidera et al., 2017), we still need to validate heat stress can induce similar responses in dairy cattle.

Dietary management of the heat-stressed dairy cow: Lessons learned from the past and a look into the future

The feeding management of heat-stressed dairy cows is not a new concern for the dairy industry and has been a topic of discussion for many years (West, 1999). However, with global warming, climate change, and our advancements in ruminant nutrition, the dietary management of the heat-stressed cow is a timely discussion, and it is important for overall profitability of dairy farms. The reduction in dry matter intake (DMI) caused by heat stress is a challenge to nutritionists. Meeting the nutrient requirements for both thermoregulation and milk synthesis during periods of heat stress is difficult to achieve, but the following strategies and/or combination of dietary changes discussed below are aimed at providing support to the heat-stressed dairy cow.

One of the feeding recommendations for heat-stressed dairy cattle is the reduction of fiber content of the diet (West et al., 1999). This is because there is greater heat production associated with acetate metabolism relative to propionate (Reynolds et al., 1991). West et al. (1999) observed a higher milk volume and fat-corrected milk yield from cows fed 30% neutral detergent fiber (NDF) compared to 42% when exposed to a temperature humidity index (THI) range from 72.1 to 83.7. The authors attributed this to the greater heat increment of production from dietary fiber associated with acetate formation in the rumen as opposed to propionate formation from concentrate diets. Thus, it became a common management strategy to reduce fiber inclusion in the diet during the summer months (West, 1999). However, it is important to note that this approach may be taken with caution, considering that an increased inclusion of concentrate feeds with lower fiber content in the diet may lead to ruminal acidosis (Neubauer et al., 2020). In addition, the suggested reduction in fiber content is more pertinent to diets with a higher forage inclusion, as West et al. (1999) also noted that inclusion of fiber from up to 35% did not markedly reduce dry matter intake when cows were exposed to high THI. Therefore, for forage-based diets, monitoring the chemical composition and digestibility of forages may be a better strategy for the heat-stressed dairy cow. Feeding a higher quality (i.e., lower $uNDF_{240}$) and highly digestible forage is preferred because it may lower the heat increment and increase the energy value for the heat-stressed cow.

In relation to protein feeding during exposure to heat stress, as reviewed by Conte et al. (2018), it is important to increase the level of protein in the diet in attempt to counterbalance the reduced DMI experienced by heat-stressed animals. However, this must be done with caution, as the higher inclusion of nitrogen (N) in the diet might lead to an excess N intake. This is undesirable from many perspectives, but especially in heat stress situations, because of the high energy cost (i.e., 7.2 kcal/g of N) associated for metabolizing excess N (Tyrrell et al., 1979). In addition, the high energy cost to transform N into urea may, in turn, further suppress milk synthesis (Huber et al., 1994). Thus, it has been suggested that milk yield is adversely affected by excessive intake of rumen degradable protein and that energy expenditure for urea synthesis might be partially responsible for the depressed milk yield of cows fed with surplus of crude protein (CP; Higginbotham et al., 1989). It is possible that the increase in protein content of the diet must come from rumen undegradable proteins (Conte et al., 2018). This suggestion

stems from the fact that diets richer in rumen undegradable protein fed during periods of hot weather allowed for increased milk yield and lower plasma urea concentrations. Interestingly, Arieli et al. (2004) observed that low protein diets (e.g., 15.3 and 15.1% CP) presented improved milk protein efficiency ratio and overall CP efficiency, and reduced milk urea-N when compared to high protein diets (e.g., 17.3 and 16.7% CP). In addition, balancing for amino acids might help circumvent the issue of excess nitrogen in diets fed to heat-stressed dairy cattle. This work has been initiated in lactating cattle (Higgs and Amburgh, 2016; LaPierre et al., 2019), but concepts have yet to be tested in the context of heat stress.

Increasing the energy density of the diet is also important because of the reducing effects heat stress exerts on feed intake. The inclusion of whole oilseeds (e.g., soybean and cottonseed), animal-sourced fats (e.g., fish meal, lard, and tallow) and commercially available supplemental fats (e.g., C16:0, C18:0 fatty acids [FA]) are examples of high-energy sources with an increased efficiency of utilization and low heat increment associated with digestion and assimilation (Baldwin et al., 1980). However, balancing the amount of fat feeding with the fiber content of the diet and ensuring that supplemental fats such as the mixes from unsaturated FA are protected against ruminal degradation (e.g., calcium salts or mixed prills) and therefore biohydrogenation is warranted (Jenkins and Harvatine, 2014). This is important to maximize feed intake, FA digestion, and limit adverse effects on ruminal health and milk fat synthesis. Importantly, more research is needed to better understand the effects of dietary FA in heat-stressed cattle, on milk yield, milk fatty acid composition, and health as this area remains relatively unexplored (Bionaz et al., 2020).

Heat stress exposure markedly perturbs electrolyte and blood acid-base chemistry balance and this imbalance comes as a consequence of the increases in sweating and respiration rates experienced by heat-stressed cows (Burhans et al., 2022). During heat stress exposure, sodium and potassium are lost via sweat, as well as impacted by decreases in circulating aldosterone levels (El-Nouty et al., 1980). Through increased panting, carbon dioxide is lost via pulmonary ventilation, causing blood pH imbalance to trigger respiratory alkalosis (Sanchez et al., 1994). To overcompensate, bicarbonate excretion via the kidneys is heightened, leading to a decline in blood bicarbonate concentrations. Schneider et al. (1988) demonstrated that heat-stressed cows have a diurnal pattern of alkalosis that shifts into compensated acidosis during the evening when temperatures are cooler, as the animal tries to adjust for the alkalotic condition caused by hyperventilation, which may inadvertently cause metabolic acidosis due to excessive urinary bicarbonate excretion. The reduced concentrations of blood bicarbonate may also compromise the buffering capability associated with the bicarbonate system, which may be critical for periods where animals are exposed to bouts of heat, as it may predispose them to experience sub-acute ruminal acidosis (Abdela, 2016). Diets formulated with increased levels of sodium and potassium have resulted in greater DMI and milk yields (West, 1999). The supplementation of bicarbonate can improve ruminal pH and aid in fiber digestion.

Water is an essential component in the diet of all mammals, and it is also especially important for heat-stressed cattle because it is required for thermoregulation. Water can be supplied in three main forms: as a processes from metabolism (i.e., water absorbed from the products of digestion in the omasum), ingested through feed consumed (i.e., water content in the diet), and freely consumed drinking water (Beede and Collier, 1986). Importantly, a common response after exposure to heat stress is to increase water consumption in the form of drinking water (West, 2003). This is also important because, during lactation, water intake is extremely important for milk synthesis (Murphy et al., 1983). Although water intake is an important component to the heat stress response and is vital for milk synthesis, it is common for this measurement to be neglected in research trials. While the general consensus is that heat-stressed cows increase water consumption (West, 2003), recently, Collier et al. (2019) proposed that water consumption diverges based on production level, with higher producing cows (i.e., > 30 kg/d of milk) tending to reduce their water intake, and lower producing cows (i.e., < 25 kg/d) drinking more water. Regardless of the reasonings behind this divergence, it is important to supply clean and cool water during periods of exposure to heat stress conditions, as increased water intake has the capacity to improve weight gain (Ittner et al., 1951) and milk yield (Milam et al., 1986).

Feed additives used to improve heat stress resilience in lactating dairy cattle

There are a plethora of nutritional strategies using feed additives available and in a broader sense, these supplements are aimed at improving inflammation, oxidative stress, and intestinal health. For example, supplementation of zinc (Zn) is reported to improve intestinal morphology (i.e., jejunum villi height) and ameliorate inflammation (i.e., decreased circulating concentrations of serum amyloid A) of heat-stressed steers (Opgenorth et al., 2021). And although the mode of action for this improvement is unclear, Caco-2 cell models suggest that this could be due to upregulation of tight junction proteins (Wang et al., 2013), but also might be due to a redistribution of tight junction proteins as suggested by wild-type human colonic T84 intestinal epithelial cell models (i.e., redistribution of claudin-4, Sarkar et al., 2019).

Supplementation of rumen-protected methionine (Met) also represents a potential approach to reduce inflammation and oxidative stress in dairy cows, because Met can be metabolized to *S*-adenosyl methionine, an important methyl donor that is also a main constituent of the very low-density lipoproteins (i.e., phosphatidylcholine). Methionine metabolism can generate intracellular antioxidants such as glutathione and taurine (Brosnan and Brosnan, 2006). In addition, because transcription and translation of RNA are inhibited during heat exposure and thus there is a reduction of milk protein synthesis (Sonna et al., 2002). The supplementation of Met may be beneficial as Met is one of the major limiting amino acids for dairy cows (Schwab et al., 1992). This is important to consider because inflammation and oxidative stress are thought to occur when animals are exposed to heat stress environment. Pate et al. (2020), while evaluating Met supplementation (i.e., 1.05 g/kg of DMI) in heat-stressed cows during mid-lactation, observed a tendency for increased milk components (i.e., fat and protein), although no changes were observed in DMI, milk and component yields.

Betaine is a methyl donor with multiple functions that may decrease the effects exerted by heat stress exposure. In growing pigs, it was shown that betaine can act as an osmolyte and can decrease the basal heat production and maintenance requirements (Schrama et al., 2003), which could be due in part to a decrease in the need for cellular ion pumping (Dunshea et al., 2019). This may translate into improved rumen fermentation activity and gains in nutrient absorption (Mahmood et al., 2020). Importantly, Dunshea et al. (2019) while evaluating betaine supplementation in dairy cows during the summer months, when temperatures are increasingly higher, have reported improved milk volume (i.e., 6% increase) and milk fat and protein yields (i.e., +48 g/d and +42 g/d, respectively).

Supplementation of lipoic acid has also been highlighted as a potential nutritional supplementation during heat stress (Rhoads et al., 2013). This is because lipoic acid can serve as a cofactor of mitochondrial enzymes that perform oxidative decarboxylation and can also scavenge reactive oxygen and nitrogen species (Ambrosi et al., 2018) and is capable of regeneration of both enzymatic (e.g., glutathione peroxidase, and Coenzyme Q10) and non-enzymatic antioxidants (e.g., vitamins E and C; Abadi et al., 2013). Furthermore, it was suggested that alpha lipoic acid supplementation improved antioxidant status in peripheral blood mononuclear cells of water buffaloes from the Murrah breed after exposure to 40 °C (Samad et al., 2019).

The supplementation of vitamins (e.g., A, B, C, and E) is also considered as a viable strategy for heat-stressed cattle due to their anti-oxidative capacities (Castillo et al., 2013), which can strengthen immunity and health of heat-stressed cattle. For example, dietary rumen-protected niacin (i.e., vitamin B3) supplementation is a promising approach to enhance thermotolerance in cows. Niacin is known to induce skin vasodilatation and increase peripheral heat loss (Di Costanzo et al., 1997). The vasodilation effect is because niacin induces the secretion of prostaglandin D2 (Morrow et al., 1989), which in turn, improves heat loss. Indeed, Zimbelman et al. (2010), while supplementing niacin (i.e., 12g/d) to mildly heat-stressed dairy cows (i.e., THI > 72 for 12 h/d, during 7 d), observed a reduction in rectal and vaginal temperatures. Although niacin seems to be effective to improve heat dissipation and reduce rectal temperature in lactating dairy cows, the effects on milk production remained relatively constant compared to unsupplemented animals (Di Costanzo et al., 1997).

Dietary supplementation of OA (e.g., citric and sorbic acids) and PB (e.g., thymol and vanillin) represents a promising strategy to support and reduce antibiotic usage in livestock production systems (Rossi et al., 2020). These natural compounds have unique antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties, which when combined, have potential to improve gastrointestinal health by controlling bacterial pathogen growth and enhancing barrier function (Tugnoli et al., 2020). Organic acids are characterized as weak and short-chain acids that are widely distributed in nature. Although there is a plethora of OA and PB blends that can be fed to cattle, swine, and poultry (Hassan et al., 2010; Ma et al., 2021), the mode of action of OA is centered on the acidification of the gastrointestinal tract. The OA undissociated form can penetrate bacterial cells, which possess a neutral pH, and dissociate causing a reduction in

intracellular pH, while inhibiting enzymatic reactions and nutrient transport (Mani-López et al., 2012). By restricting the growth of pH-sensitive and pathogenic bacteria, supplementation of OA blends has been shown to improve weight gain and feed conversion ratio in broiler chicks (Hassan et al., 2010). Pure botanicals are single components of plant essential oils and oleoresins. These compounds are also reported to possess pH-reducing properties against bacteria, and provide anti-inflammatory, antioxidant and immunomodulatory properties (Rossi et al., 2020). In vitro, thymol, a direct extract from thyme, has been shown to reduce the growth and expression of virulence genes in *Escherichia coli* K88 (Bonetti et al., 2020). The extract from vanilla beans vanillin showed bacteriostatic action when tested against specific bacteria (e.g., *E. coli*, *Lactobacillus plantarum*, and *Listeria innocua*; Fitzgerald et al., 2004). Dietary OA/PB supplementation promoted greater average daily gain and body weights in pigs. The study also involved the collection of ileal and jejunal tissue samples post-weaning for Ussing chamber analysis of transepithelial electrical resistance, intermittent short-circuit current, and dextran flux. Results indicated that pigs fed OA/PB at 5 g/kg of body weight tended to have reduced intermittent short-circuit current in the ileum, which suggests improved intestinal barrier. These findings were supported by increased trans-epithelial resistance in Caco-2 cells grown in the presence of OA/PB (0.2 or 1 g/L; Grilli et al., 2015). The authors were also able to demonstrate that feeding OA/PB downregulated the ileal gene expression of inflammatory cytokines including interleukin-12 and transforming growth factor-β) in pigs. This may mean that dietary OA/PB may ensure the integrity of the intestinal barrier by minimize inflammation.

Evaluating the effects of ambient heat exposure and dietary OA/PB supplementation on gut permeability and milk production in dairy cattle

In ruminants, it is unclear whether exposure to environmental conditions that cause heat stress enhances gastrointestinal permeability. In addition, it has been shown that animal performance has been improved by feeding OA/PB to non-ruminants to improve gastrointestinal health. To test the hypothesis that 1) heat stress will progress with increased gastrointestinal permeability and decreased milk production in lactating cows, and 2) that dietary OA/PB supplementation will prevent these outcomes, our lab recently completed a study (Fontoura et al., 2022).

Forty-six Holstein cows (208 ± 4.65 d in milk [mean ± SD], 3.0 ± 0.42 lactations, 122 ± 4.92 d pregnant) were enrolled in a study with a completely randomized design. Following a 7 d acclimation in thermoneutrality (temperature-humidity index [THI] 68), cows were assigned to 1 of 4 groups: thermoneutral conditions (TN-Con, n = 12), HS conditions (HS-Con, n = 12; diurnal THI 74 to 82), TN conditions pair-fed to match HS-Con (TN-PF, n = 12), or HS fed OA/PB (HS-OAPB, n = 10; 75 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride) for 14 d. Cows were milked twice daily and fed a corn-silage based total mixed ration top-dressed without (triglyceride only) or with OA/PB. Acute and chronic changes in gastrointestinal permeability were evaluated in vivo using the paracellular permeability marker Chromium (Cr)-EDTA in a 180 mM solution on d 3 and 13, respectively. Blood samples for Cr analysis were collected at 0, 1, 2, 4, 8, 12, 18, and 24 h relative to Cr-

EDTA administration by coccygeal venipuncture into an evacuated tube containing dipotassium EDTA for total Cr determination using ^{53}Cr isotope analysis. Blood for plasma and serum separation was collected on d -1, 1, 2, 3, 7, and 14 and analyzed for metabolic and health marker concentrations. Data were analyzed using a mixed model including fixed effects of treatment, time, their interaction, and the random effect of cow. Planned contrasts included HS-Con vs. TN-Con, HS-Con vs. TN-PF, and HS-Con vs. HS-OAPB. Main effects were declared significant at $P \leq 0.05$ and trending towards significance at $0.05 < P \leq 0.15$. Interactions were declared significant at $P \leq 0.15$.

Exposure to heat stress conditions for 14 d markedly increased rectal temperatures (40.7 and 40.7 vs. 38.6 and 38.1 °C, $P < 0.01$) and respiration rates (95 and 94 vs. 59 and 50 resp/min, $P < 0.01$) of cows housed in heat stress conditions (HS-Con and HS-OAPB, respectively) compared to cows housed in thermoneutrality (TN-Con and TN-PF, respectively). We observed that HS-Con cows had greater plasma Cr area-under-the-curve (AUC; $P = 0.05$) and tendency for greater Cr AUC ($P = 0.12$) on d 3, relative to TN-Con and TN-PF cows, respectively. HS-Con cows also had greater plasma Cr concentrations from h 4 to 24 on d 3, relative to TN-Con cows (Treatment × Time, $P < 0.11$; Figure 1A). HS-Con cows had similar plasma Cr AUC on d 13, relative to TN-PF and TN-Con; however, TN-PF cows tended to have greater plasma Cr concentrations from h 12 to 24 post bolus, relative to TN-Con (Treatment × Time, $P = 0.13$; Figure 1B). Importantly, HS-Con cows had increased circulating levels of lipopolysaccharide-binding protein relative to all other treatments ($P < 0.05$). HS-Con had greater water intake, and lower yields of milk and milk lactose and protein, relative to TN-PF ($P < 0.01$). Plasma total fatty acid concentrations were reduced while insulin concentrations were increased in HS-Con, relative to TN-PF ($P < 0.05$). HS-OAPB cows had greater water intakes ($P = 0.05$) and tendency for increased dry matter intake, relative to HS-Con ($P = 0.14$). In addition, HS-OAPB also had greater energy-corrected milk yields relative to HS-Con cows ($P = 0.05$). This increase could be explained by the greater milk protein yield ($P = 0.05$) and tendency for greater milk yield ($P = 0.12$) of HS-OAPB cows. Milk urea N and plasma urea N concentration were lower in HS-OAPB cows, relative to HS-Con ($P < 0.01$), which suggests improved N efficiency. Overall, the present results highlight important mechanisms that might account for milk production losses and health impairments caused by heat stress independent of reductions in feed intake. In addition, dietary OA/PB supplementation represents a means to partially restore milk production and improve N efficiency in dairy cattle experiencing heat stress, and thus can be incorporated into already existing feeding strategies to optimize production of heat-stressed cattle.

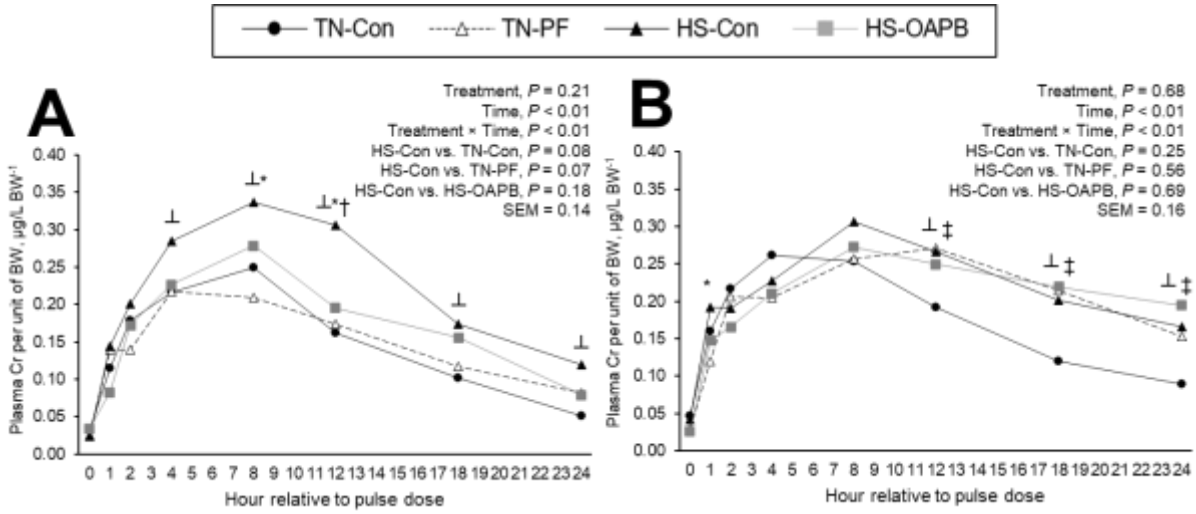

Figure 1. Effects of heat stress and dietary organic acid and pure botanical supplementation on gastrointestinal permeability measured by Cr concentrations in plasma after a pulse dose of Cr-EDTA in pregnant multiparous lactating Holstein cows. Figure A and B are relative to Cr-EDTA challenge on day 3 and 13 of heat stress conditioning, respectively. $^{\perp}$HS-Con vs. TN-Con, Treatment × Time, $P < 0.15$. *HS-Con vs. TN-PF, Treatment × Time, $P < 0.15$. ‡TN-PF vs. TN-Con, Treatment × Time, $P < 0.15$. †HS-Con vs. HS-OAPB, Treatment × Time, $P < 0.15$.

Summary

Heat exposure compromises the gastrointestinal barrier and leads to inflammation in non-ruminants. Our results indicate that heat stress increases gut permeability and inflammation markers rapidly and independently of dietary intake. However, our findings suggest that the quickly developed increase in gastrointestinal permeability reduce with time. Dietary organic acid and pure botanical supplementation is common practice in swine and poultry production, and science now suggests that we consider the practice in lactating dairy cows. The justification is the ability of OA/PB feeding to enhance feed intake, intestinal functionality, and reduce gastrointestinal bacterial pathogens. This said, microencapsulation of OA/PB to avoid rumen degradation of these compounds is likely needed in dairy cattle to elicit benefits in the lower gut. Our findings in heat-stressed lactating Holstein cows are early evidence that dietary microencapsulated OA/PB feeding is a means to partially restore feed intake, milk production and N incorporation in milk. On-going investigations are examining gastrointestinal bacterial profiles in relation to differing environments and whether dietary OA/PB influences the gastrointestinal bacteria profile in relation to exposure to heat stress environment.

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Lethal Heat Stress in Dairy Cattle: Unrecognized, Misdiagnosed, Needs Research

W. S. Burhans, C. A. Rossiter-Burhans and L. H. Baumgard
Dairy-Tech Group, S. Albany, VT

Introduction

Heat Stress (**HS**) affecting dairy cows is widely recognized in the dairy industry in terms of production and management, but not in terms of mortality or diagnosis of potential lethality. Cattle deaths from HS can be extensive. A high mortality rate occurred in heat events in the US this summer where 2 to 10 thousand cattle died in Kansas feedlots (Chappell, 2022, Guilfoil, 2022, Laborie, 2022). Extreme cattle mortality has occurred in previous years as well, in California (2017) 5-10K, Iowa (1995) 3.75K, and Nebraska (1999) 5K (Osgood, 2017, Lees et al., 2019). Mortality due to HS is also reported internationally (Morignat et al., 2014, Vitali et al., 2015). Clearly HS can kill cows in large numbers, which tends to be recognized only because of the enormous unexpected excess mortality associated with extreme heat events, as opposed to diagnosis of risk or cause of death by veterinarians.

While pathological outcomes of HS can include death (lethality), the pathobiology of lethal heat stress **(LHS)** in bovines is not described in prominent current veterinary textbooks nor research literature. Better awareness and understanding of LHS is needed. Perhaps the major reason literature describing differential diagnostics in bovines does not include LHS is that unlike humans or dogs, which are frequently hospitalized for heat stroke, cattle are not hospitalized during heat events, so there is a deficit of observed pathology indicative of LHS. Differential diagnosis of severe HS is also not considered because it typically presents like a nutritional problem, and veterinarians, producers and nutritionists are generally unaware of the disorder, or its presentation or pathology. Consequently, alternate differential diagnoses are pursued, typically nutritional, such as acidosis, mycotoxicosis, or fatty liver. In less severe heat events HS commonly presents with diarrhea, suggesting a gastrointestinal tract (**GIT**) disorder. Severe HS also can present as “downer cow syndrome” which has multiple possible and differential causes (Grünberg, 2022). These include signs that can be present in LHS, including severe hypokalemia, hypophosphatemia, and systemic illnesses or infections such as toxic mastitis or metritis, and hepatic lipidosis or liver failure. Other signature HS related clinical signs are discussed later. However, its initial presentation often suggests that the underlying problem is nutritional.

Additional reasons contribute to lack of awareness of LHS. It is not a recognized pathology in cattle, whereas heat stroke is widely recognized in dogs and humans. There are no pathognomonic signs (i.e., no definitive indicators or diagnostics exclusive to LHS). Unlike LHS occurring with major heat events, it can occur in a low number of cases in a herd with less severe heat / humidity exposure. Important risk factors for LHS, including the intensity and duration of heat and humidity, and whether the exposure is abrupt or cattle are acclimatized, are often not considered.

Ultimately, failure to recognize LHS occurs because it is a complex disorder that is the cumulative outcome of numerous physiological processes and pathogenic pathways that are incompletely understood in human and veterinary medicine. Recognition occurs when a heat event is overwhelmingly obvious, such as the Kansas feedlot deaths, June 2022 (Chappell, 2022, Guilfoil, 2022, Janousek, 2022). However, the veterinarians attending those feedlots described a diagnosis based on the atypical abrupt heat and humidity conditions immediately prior to those deaths, and not so much on understanding specific pathologies of death by heat. However, HS severe enough to be lethal also occurs in dairy cows, but is essentially unrecognized.

The authors have been involved in a number of cases of LHS which were puzzling and misdiagnosed by the veterinarians and nutritionists involved. Four of the cases, described below, provide insight into the pathology and diagnostic challenge of LHS on dairy farms. Each demonstrated different factors that contribute to LHS and illustrate the lack of awareness of LHS by dairymen, veterinarians, and nutritionists.

Case 1:

A dairy milking over 1,000 cows in Idaho had multiple cases of diarrhea, down cows in established lactation, and cow deaths. The dry lot dairy had eleven corrals but only one corral had a shade. The herd veterinarian and the nutritionist made presumptive diagnoses of acidosis. Diets were revised even though the source of “acidosis” was not clear; rations had adequate fiber and were not excessively fermentable. Diagnostics included necropsies by two veterinarians and multiple clinical chemistries. Observations of hyperemia, petechia, ecchymosis, and fibrin at necropsy revealed severe systemic inflammation and coagulopathy. During one of the necropsies the dairyman asked the veterinarian to examine the family dog, which had just collapsed on the lawn. The veterinarian made a presumptive diagnosis of heat stroke, and advised taking the dog immediately to a clinic for cooling, fluids, and electrolyte support. The dog died at the clinic; cause diagnosed as heat stroke. At the time, the veterinarians, nutritionist, and dairyman made no association of the dog with cow deaths, which had numbered approximately 20 in the previous several weeks. No diagnosis for the cow deaths was determined.

Case 2:

A dairy near case 1 had cows with similar presentations, including oddly colored diarrhea, recumbent cows in established lactation, and cow deaths. Also a dry lot dairy, all of the pens had shades. Although this dairy had nearly three thousand cows, it had far fewer cow deaths. Again, necropsies were done but the health problems and cow deaths remained undiagnosed.

On both Idaho dairies the deaths stopped after a relatively short period of time. In spite of the efforts of four experienced veterinarians no definitive diagnosis was made at either dairy. The difference in death rate likely reflects the difference in solar radiation

exposure (shades). The authors later attended a conference where Dr. Lance Baumgard described “Leaky Gut,” a condition initiated during severe heat exposure which causes the gastrointestinal tract (GIT) epithelium to become permeable and “leak” it’s content, including endotoxin. The condition Dr. Baumgard described as heat stress seemed to fit as a reasonable explanation of the HS events at the Idaho dairies.

Case 3:

Several years later (2018) an excellent nutritionist in the Northeast had a herd (~350 cows) that experienced down cows in established lactation accompanied by deaths from mid-June to early July. For each of 4 weeks the author was consulted by the nutritionist about interpretation and plausibility of diagnostics and differentials being proposed. These included clinical chemistries (results were inconclusive), rumenocentesis to assess acidosis risk, liver biopsies and mycotoxin analyses. In the fifth week the nutritionist commented that “The cows might just be too darn hot!”. “Aha!” declared the consultant. The dairy’s freestall barn had a high ceiling, was located on a breezy knoll and had good natural ventilation. The dairy had installed an automated milking system the previous year. The change included construction of “robot rooms” which completely closed in the sidewalls of all four quadrants of the barn, which also had insufficient fans and no sprinklers. The conclusion was that LHS was responsible for unexplained cow deaths based on two contributing risk factors. 1. The closed sidewalls and paucity of fans left the barn poorly ventilated with little heat abatement. 2. The weather had been very cool in May and early June, but in mid-June it suddenly turned very hot, with temperatures in the high nineties (^{o}F) and high humidity. The cows were not acclimatized and adapted to the heat.

Case 4

The fourth case occurred midsummer in Northern New England, again on a dairy that experienced relatively cool conditions in early summer followed by sudden high temperatures for several days. Several cows died; initially necropsies and clinical chemistries did not suggest a diagnosis. However, this dairy had a serious water problem, in that congested water system pipes did not always provide adequate water for the cows. Also, access to waterers was poor. In this case the histories, clinical chemistry, and necropsies early on were helpful in suggesting LHS as the putative cause of the deaths, and possibly limited the number of deaths. In both of these Northeast dairies the lack of acclimatization was a contributing factor, and was exacerbated by ventilation and heat abatement on one dairy, and inadequate water access and dehydration on the other. Learning the history and context was critical in arriving at a diagnosis at both dairies; diagnostics alone were insufficient.

These four cases are important because they reflect that LHS and its pathology are not recognized within the dairy industry. Necropsies, clinical chemistries and other diagnostics were performed in these cases. Knowledge was lacking about how the results, coupled with the environmental conditions, suggested LHS was occurring. These cow death events and the lack of awareness that LHS could be a differential diagnosis

stimulated the initiative to describe the putative pathology and pathogenesis of LHS. The dairies chronicled here were well managed, which suggests that many dairies experience LHS deaths and do not recognize it. Dairy advisors and producers are not familiar with the environmental risk factors, presentation and pathogenesis of LHS, or its potential lethality. Popular press and scientific literature address HS consequences related to productivity and reproduction and management actions like heat abatement, nutritional strategies or additives. There is information about why HS events may be a reason cows die in a herd.

The primary objective of this paper is to increase awareness of LHS in cattle for veterinarians and nutritionists (dairy and beef). The second objective is to contribute to basic understanding of its presentation, recognition, and pathogenesis. There are no detailed published works about LHS or its diagnosis in cattle and more research is needed.

Pathogenesis of LHS

The pathogenesis of LHS is more likely to occur under specific conditions, including high ambient temperatures concurrent with high humidity, poorly ventilated micro environments, exposure to direct solar radiation, and sustained periods without nighttime cooling. The onset of severe HS is also a function of acclimatization status; cows in warmer environments that are gradually exposed to a heat load become acclimatized physiologically whereas cows not gradually exposed do not become heat adapted. This is why in Northern temperate regions in the US, cows, like humans, can be unexpectedly affected by LHS (cows) or heat stroke (people, dogs) (Kadzere et al., 2002, Nienaber and Hahn, 2007, Leon and Kenefick, 2011). Cows that are not acclimatized are at higher risk of LHS pathologies when suddenly exposed to a high heat and humidity. This is especially the case in extreme heat midsummer when the spring and early summer have been cool. Other factors can increase risk of LHS in cattle, such as fatness, high production and dry matter intake, breed, genetics, coat color, overcrowded facilities, preexisting health conditions, extent and efficacy of heat abatement, and hydration status (Brown-Brandl et al., 2006, Sullivan and Mader, 2018). These factors are well known, with published research describing their effects so they are not addressed in this paper. The origins of HS tissue and organ damage that become lethal involve multiple complex systems, including cardiac, vascular, immune, hemostatic, metabolic, redox, renal, respiratory, hepatic and other systems. The complex pathology of severe HS in bovines has not been described, but appears to be similar to and substantially conserved across other mammalian species. Here LHS is described based on research on heat stroke in other mammalian species. The label LHS is used instead of heat stroke because heat stroke includes neurological pathology, which has not been reported in cattle.

The basic pathogenesis of LHS can be described as a four stage progression:

- **Stage 1.** Heat exposure above thermoneutral and the corresponding behavioral and acclimatization responses.
- **Stage 2.** Prolonged severe heat exposure resulting in physiological dysfunction.

- **Stage 3.** Physiological dysregulation and systemic degeneration including concurrent counteracting pro- and anti- inflammatory, oxidative, hemostatic system responses.
- **Stage 4. Lethality** arising from organ dysfunction, respiratory dysfunction, and septic shock.

LHS Pathogenesis Stage 1: Behavioral and Evaporative Cooling Responses

Stage 1 of HS pathogenesis has two elements: Adaptive behavior and evaporative cooling. The stage 1 elements are primarily HS responses, not pathologies, although as heat load severity increases, they contribute to pathologies of stage 2.

Adaptive behavior:

Adaptive changes include reduced dry matter intake (reduces metabolic heat production), changes in meal patterns (eating less during hotter daytime hours and more during cooler nighttime hours), increased time standing, increased bunching, and increased water intake (Burhans et al., 2022).

Evaporative Cooling:

Evaporative cooling exposes body water containing body heat energy (sweat or exhaled air) to cooler drier air where it diffuses into the air and disperses heat energy as it changes from liquid to gas phase (water vapor). Bovine evaporative cooling has three mechanisms: 1) sweating, 2) increased respiration rate (**RR**), and 3) peripheral cooling associated with blood flow redistribution. Sweating is stimulated by elevated skin temperature and provides evaporative cooling (Gebremedhin et al., 2010); sweating also causes loss of potassium in sweat (Kadzere et al., 2002). To a lesser extent, sweating increases sodium loss by renal excretion due to reduced aldosterone level, which is a mechanism to conserve potassium from renal excretion. Evaporative cooling also occurs from increased RR (Robertshaw, 2006, White, 2006). As the heat load intensifies, RR progresses to panting and hypersalivation, which result in salivary loss by drooling, exacerbating loss of sodium and potassium. Panting and hyperventilation cause respiratory alkalosis as increased CO_2 is expired and blood CO_2 levels decrease; respiratory alkalosis stimulates renal excretion of bicarbonate (**HCO_3^-**) resulting in compensatory metabolic acidosis. Overall, the elevated RR causes an alternating acid/base disturbance with alkalosis in the hotter daytime hours and metabolic acidosis in the cooler nighttime. Increased core body and skin temperature stimulate redistribution of blood flow from the core to the periphery to achieve peripheral cooling (Lambert et al., 2002, Cronje, 2005, Wang et al., 2011, Baumgard and Rhoads, 2013). This redistribution involves vasodilation of peripheral vasculature, accompanied by vasoconstriction of the gastrointestinal tract (**GIT**) to maintain systemic blood pressure. These stage 1 mechanisms have been extensively researched and are relatively well recognized and understood in cattle.

LHS Pathogenesis Stage 2: Prolonged Severe Heat, Physiological Dysfunction

When heat load is severe and prolonged, cows become hyperthermic which progresses to physiological dysfunctions, sequelae to stage 1 adaptive heat responses (blood flow redistribution, acid/base disturbances, and electrolyte derangement).

GIT Permeability:

The most consequential dysfunction sequela of stage 1 HS is the development of GIT hyperpermeability, colloquially termed “leaky gut” (Wang et al., 2011, Baumgard and Rhoads, 2013, Koch et al., 2019). While the occurrence of GIT hyperpermeability has been clearly demonstrated in many mammalian species, the primary causal mechanism and specific location of GIT “leakiness” in ruminants is not known definitively (Burhans et al., 2022). Multiple causal mechanisms for GIT hyperpermeability have been suggested, including heat exposure alone (Dokladny et al., 2006), thermal damage to tissues, hypoxia (inadequate blood oxygen) due to hypoperfusion (Salzman et al., 1994), oxidative and nitrosative stress, epithelial damage due to hyper-osmolality and cell swelling, ruminal histamine, splanchnic mast cell activation, GIT endotoxins, and mast cell secretions such as proteases and histamine, increased cortisol, and tissue acidosis (Burhans et al., 2022). Rumen pH decrease might also contribute; pH appears to vary from normal during HS, although the variability, diurnal pattern, range, or duration of rumen pH changes during HS has not been well investigated nor definitively profiled (Burhans et al., 2022). Reduced rumen motility (Attebery and Johnson, 1969) might contribute to pH decrease during HS.

Endotoxin Translocation:

Hyperpermeability of the GIT during HS facilitates translocation of endotoxins out of the GIT into systemic circulation. Endotoxins (lipopolysaccharide, i.e., **LPS**) are the “structural parts of gram negative bacteria cell wall, are potent immune stimulating antigens, and are comprised of three major regions: a side chain, core polysaccharides, and lipid A” (Andersen, 2003). The side chain of repeating units of oligosaccharides differs between Gram-negative bacterial strains and is unique to specific strains. The location of LPS efflux from the GIT in ruminants is not definitively known (Gao et al., 2022). An experiment in goats utilizing a 24 hour exposure to HS (35^{o}C/95^{o}F) concluded that net efflux of LPS occurs from both the intestines and the rumen (Wang et al., 2011), consistent with earlier in vitro work in bovine epithelia (Emmanuel et al., 2007). A small amount of endotoxin escapes the GIT normally; the net amount that appeared in the portal vein during HS increased 228%. The net LPS absorption measured by Wang in the mesenteric vein was only 20% of the net portal vein flux. Thus it appears that most of the efflux is primarily from the rumen, consistent with speculation based on earlier research (Cronje, 2005). The efflux proportions from these locations were the same during thermoneutral conditions, suggesting that location of LPS efflux from the GIT may not change or differ during HS.

Rumen pH:

Panting, open mouth panting, drooling and saliva loss increase as heat intensity and duration of exposure increase. Saliva loss results in Na^+ and bicarbonate (**HCO_3^-**) loss, concurrent renal excretion of both Na^+ and HCO_3^- increases these losses, presumably decreasing supply (the authors are unaware of studies which have quantified this). Both Na^+ and HCO_3^- are needed for VFA absorption from the rumen lumen; compromised supply would presumably reduce VFA absorption out of the rumen and reduce ruminal buffering (Aschenbach et al., 2011, Burhans et al., 2022). Saliva loss from drooling and renal HCO_3^- excretion during respiratory alkalosis could potentially result in reduced rumen pH. As noted above, this remains plausible but has not been investigated.

Permeability & Rumen pH:

Two things occur in the rumen when rumen pH is reduced during acidosis. First, there is an increase in ruminal endotoxin content due to high starch fermentability or low effective fiber, (Gozho et al., 2005, Li et al., 2012). An increase in colonic endotoxin occurs when hind gut acidosis results from high colonic starch loads. Second, it is known that there is an increase in ruminal histamine production with greater ruminal fermentability (Sanford, 1963, Garner et al., 2002); higher fermentability decreases rumen pH. Increasing histamine flux from the rumen occurs at lower pH (Plaizier et al., 2008), likely by stimulating epithelial inflammation (Sun et al., 2017) or facilitated by low rumen pH (Aschenbach and Gäbel, 2000). If and how these consequences of increased RR, panting and drooling contribute to HS effects is not definitely known, but potentially they could contribute to the development of hyperpermeability of the GIT epithelium; research is needed.

Hepatic Overload:

Endotoxin and intact bacteria translocated from the GIT are conveyed by the portal vein to the liver where they are degraded by Kupfer cells. Kupfer cells are hepatic macrophages. Activated by endotoxin and bacteria they release cytokines (immune system proteins providing immune regulation and communication), prostanoides (inflammation mediators), nitric oxide and reactive oxygen species (pro-oxidants) that degrade bacteria and detoxify LPS (Bilzer et al., 2006, Dixon et al., 2013). Normally the liver is presented with only a small amount of “leaked” LPS and detoxifies it. But as the GIT epithelium becomes increasingly permeable the efflux delivered to the liver increases and can overwhelm the capacity of the Kupfer cells, and then the flux of endotoxin becomes systemic (Wang et al., 2013).

Renal Dysfunction & Tubular Necrosis:

Kidney damage is common during heat stroke and LHS. Causes include core body hypoperfusion due to blood flow redistribution, although given the retroperitoneal locations of the kidneys direct thermal damage might also be a factor. Damage can also be caused by myoglobin exposure resulting from myofibrilar (muscle) protein degradation

(Bruchim et al., 2006, Leon and Kenefick, 2011, Gordon, 2017, Iba et al., 2022). Myofibrilar degradation can occur during LHS due to tissue catabolism stimulated by the need for glucogenic substrate, and as a result of hyper-inflammation as discussed in stage 3 below. There are no published case reports of LHS or associated observations of renal deterioration in mature cattle (Sullivan and Mader, 2018). Experimental induction of LHS in yearling Holstein steers did show degeneration of renal tubules, glomeruli mesangial cells, and urinary bladder and adrenal parenchyma congestion (Terui et al., 1980). Renal tubule degeneration has been reported in cases of LHS in other ruminants (young sheep) (Sula et al., 2012, Sprake et al., 2013). Renal dysfunction is commonly associated with heat stroke in dogs (Bruchim et al., 2017a) and in humans (Leon and Helwig, 2010).

LHS Pathogenesis Stage 3: Systemic Dysregulation & Degeneration:

Hyper-inflammation:

In addition to pro- and anti-inflammatory cytokines, pro-oxidants are generated due to hypoxia in ischemic tissues, thermal damage to tissues, and endotoxins. Systemic inflammatory response syndrome **(SIRS)** is a condition of systemic hyper-inflammation that occurs in LHS. It is characterized as a "cytokine storm", where the pro- and anti-inflammatory immune responses compete and create an imbalanced state that is often termed "out of control". In LHS, hyper-inflammation is caused in part by high systemic levels of endotoxin. Toll like receptors **(TLR)** are immune system proteins that recognize and bind pathogens. Endotoxin binds to toll like receptor 4 (**TLR4**) which stimulates many different cytokines, including tumor necrosis factor-α (**TNF**-α), interleukins (**IL**) IL-1 and IL-6 (Seeley et al., 2012). Many, many immune system modulators are involved in HS and endotoxin responses; both TLR4 and TNF-α exemplify this type of concurrent counteracting, causing as well as inhibiting immune responses. For instance, TNF-α, strongly pro-inflammatory, also induces anti-inflammatory activity such as IL-6 which can effect both pro-inflammatory and anti-inflammatory responses.

Oxidative Stress:

TNF-α primes phagocytes, including Kupfer cells to produce pro-oxidants, both reactive oxygen species (**ROS**) and reactive nitrogen species, (**RNS**). At low amounts these pro-oxidants have an important role in cellular signal transduction and redox regulation. In modest amounts these pro-oxidants are protective against cell damage by pathogens, but at prolonged elevated levels such as in endotoxemia they can trigger cell death (Halliwell and Gutteridge, 2015). In a short term HS study (24 hours), ongoing HS resulted in an increased systemic load of LPS (Wang et al., 2011) and net decrease (consumption) of antioxidants available in splanchnic tissues. Wang et al. cite similar HS associated reduction of anti-oxidant activity in other studies in goats, pigs, broilers, and dairy cows. However, Wang et al. note that some previous studies incorrectly measured increases in oxidative indicators, and therefore incorrectly concluded that oxidative stress increased during HS. Wang et al. attribute those errors (conclusions of increased oxidative stress) to measuring concentrations in blood, without considering simultaneous

decreases of blood flow, which could mean net increases may not have occurred in those studies. Wang et al. also conclude that in the trial they report net oxidative stress decreased when both concentration and flux are considered. Clearly, further assessment is needed of the effects of duration and severity of HS on oxidative stress, and of the measurement approaches used in Wang and other studies. Importantly, examining the appropriate level of dietary antioxidants supplied during severe HS in cattle should be a high priority.

Dysregulated Inflammation (SIRS):

As severe HS continues, increasing systemic endotoxin levels, ongoing hypoxia, and thermal tissue damage all facilitate progression of immune and inflammatory responses to pathological dysfunction levels that increase the risk of death. Systemic Inflammatory Response Syndrome (SIRS) is a hyper-inflammatory immune response that develops in cattle exposed to prolonged and severe hyperthermia. Similar to sepsis, SIRS differs in that it is defined with an expanded set of causes (Jaffer et al., 2010, Berg and Gerlach, 2018). Sepsis is the response to systemic infection caused by biological pathogens like microbes or viruses, whereas SIRS causes include the infectious causes of sepsis, but also non-biological causes of tissue damage such as trauma, burns, thermal damage, major surgery, or HS. The “out of control” (Jaffer et al., 2010) hyper-inflammatory response is a hallmark of SIRS. The severity and impact of dysregulation of the immune response is proportional to the extent to which pro- and anti- inflammatory cytokine counter-activation is systemic, as opposed to localized only to areas of cellular damage (Seeley et al., 2012). Dysregulation extent also depends on the magnitude of the cytokine responses because there can be thermal and hypoxic tissue damage generating immune responses in many tissues simultaneously.

Coagulopathy:

During severe HS progression to LHS involves development of dysregulated coagulopathy (clotting disorders)., Endotoxin stimulates endothelial (blood vessel wall) injury which activates hyper pro-coagulation elements causing thrombi that in turn activate anti-coagulation processes that inhibit thrombosis (clot) formation, causing bleeding. The effect is initially a compensated coagulation (coagulants offset the coagulation) followed by decompensated and unregulated hemostasis as anticoagulants are consumed. Then excessive coagulation occurs resulting in systemic disseminated intravascular coagulation **(DIC)** (Bruchim et al., 2017b). DIC is characterized by both systemic vascular thrombosis, and as clotting factors are consumed, vascular hemorrhage also. Very high heat alone (43^{o}C / 109^{o}F) activates some coagulation (Gader et al., 1990, Mohanty et al., 1997, Bruchim et al., 2017a). Both pro- and anti- coagulant factors, along with an important role of platelets (thrombocytes), contribute to vascular hyper-permeability. Endotoxins bind to a receptor complex,TLR4 and MD-2, (Ohto et al., 2012) which activates platelets and promote adhesion of platelets and neutrophils to endothelial cells (cells lining the walls of the blood vessels). Endotoxin also stimulates TLR in endothelial cells, enhancing coagulation, increasing platelet accretion, and resulting in the formation of microthrombi (small aggregates of platelets, fibrin, and red

blood cells, i.e., tiny clots). Microthrombi impair perfusion through small vasculature, including arterioles, capillaries, and venules. As the continuously increasing endotoxin load becomes overwhelming, endothelial cell activation causes endothelial cell death, which results in vascular permeability, which further intensifies both inflammation and coagulation. This ongoing and conflicted vascular response to endotoxemia contributes to intravascular coagulation (DIC) systemic organ dysfunction, and ultimately death.

Disseminated Intravascular Coagulation (DIC):

Opposing processes of pro- and anti- coagulation generate DIC, known historically as “consumptive coagulation”. Systemic inflammatory responses stimulate cytokine production (Bruchim et al., 2008), especially the immune modulators interleukins (IL-1) and (IL-6) and tumor necrosis factor (TNF). Systemic DIC occurs predominantly in the microvasculature, and can result in organ tissue ischemia and organ dysfunction (Boral et al., 2017). Tissue factor (thromboplastin, an enzyme that converts prothrombin to thrombin) initiates DIC by causing excessive thrombin production (Stokol, 2012, Boral et al., 2017). Thrombin is a protease enzyme that converts soluble fibrinogen to fibrin, facilitating blood clot formation; an excess of thrombin results in microvascular occlusion in arterioles and capillaries. Acute DIC consumes platelets, resulting in a low platelet count (thrombocytopenia) and potentially increasing prothrombin time (**PT**) and activated thromboplastin (**aPTT**) times (longer PT and aPTT indicate extensive clotting). As the extent of excessive intravascular coagulation increases, activity of a main anti-coagulant factor, antithrombin (**AT**), is inhibited. However, as more fibrin clots are created, fibrinolysis is stimulated by the enzyme plasmin and begins to break down fibrin clots (Bruchim et al., 2008, Chapin and Hajjar, 2015). The net effect of platelet consumption and fibrinolysis is an increase in extravascular bleeding. Vascular permeability also may result in an efflux of fibrin into the extravascular space; this efflux may also be promoted by histamine (Burhans et al., 2022). Concurrent dysregulation of coagulation and fibrinolytic systems, i.e., hyper-coagulation (clotting) and hyper-fibrinolysis (bleeding), is a hallmark sign of DIC coagulopathy (Iba and Levy, 2020). Common clinical indicators of DIC are petechia and purpura (tiny and small hemorrhages) visible in mucous membranes and organs post mortem. Like processes during SIRS, the body’s conflicting regulatory processes are competing, in this case pro- and anti- coagulation.

LHS Pathogenesis Stage 4: Lethality: Multiple Organ Dysfunction Syndrome, Acute Respiratory Distress Syndrome, Septic Shock

Multiple Organ Dysfunction Syndrome (MODS):

Complex, MODS is not uniformly or precisely defined in human medicine (Burhans et al., 2022), even less so in veterinary species (Osterbur et al., 2014), especially large animal production medicine. Precipitated by HS, consequences including thermal trauma, SIRS, DIC, and sepsis, result in MODS, as reported in many species, including humans, dogs, and laboratory animals. As LHS progresses through SIRS and DIC, multiple body systems become dysregulated and dysfunctional to the extent they are unable to maintain homeostasis without intervention (Nyström, 1998, Osterbur et al., 2014). Largely a

sequela to vascular endothelium activated to be pro-inflammatory, the primary cause of MODS is DIC hyper-coagulation in the microvasculature. However, organ tissue ischemia and thermal damage also contribute to MODS pathogenesis (Iba and Levy, 2020). Elevated levels of both pro- and anti-inflammatory cytokines are correlated with organ failure and death in animal and human heat stroke. Occurring in multiple organs, MODS especially affects the lungs, kidney, heart, liver, adrenals, and GIT (Bouchama et al., 2005, Osterbur et al., 2014, Boral et al., 2017).

Acute Respiratory Distress Syndrome (ARDS):

Acute Respiratory Distress Syndrome (ARDS) has been linked to heat stroke in humans (el-Kassimi et al., 1986, Bouchama et al., 1996, Tulapurkar et al., 2012) and in dogs (Bruchim et al., 2009). Like SIRS, DIC, and MODS, ARDS pathophysiology involves pro- and anti-inflammatory immune responses to inflammation which become unbalanced responses. Triggering events for ARDS can be SIRS or sepsis, presumably originating from the endotoxemia in LHS. Tissue damage from other causes as described above likely contribute. Similar to DIC coagulopathy, ARDS is precipitated by vascular injury (Matthay and Zemans, 2011) and activation of the pulmonary vascular endothelium resulting in derangement of coagulation. This generates hyper-coagulation in pulmonary and alveoli microvasculature (Dunkel, 2015). Activated platelets, neutrophils, and macrophages leak out from the microvasculature into extravascular tissue, and release pro-oxidants, proteases, and cytokines which intensify ongoing inflammation. This causes degeneration of both the alveolar endothelial and epithelial barriers, which induces pulmonary edema. Damage to the pulmonary interstitium and alveolar walls further impairs pulmonary function, causing hypoxia and hypercapnia (abnormally high blood CO_2). Severe damage to the alveolar epithelium is associated with respiratory failure and high mortality. Mortality risk from ARDS increases with the extent of MODS and other extant comorbidities (Matthay et al., 2012). Like SIRS or DIC, there is a paucity of research on ARDS pathogenesis in ruminant LHS. Neither pulmonary epithelial injury nor alveolar edema occurred in sheep dosed with endotoxin (Wiener-Kronish et al., 1991, Matthay and Zemans, 2011). However, Wiener-Kronish et al. used a 4 and a 24 hour exposure to endotoxin, but suggested that a more prolonged exposure (as during LHS) could possibly result in alveolar barrier function injury and edema. Whether the alveolar epithelium of bovines is resistant to degradation when exposed to LPS remains a research need.

Septic Shock (SS):

Septic shock (SS) is a subcategory of sepsis and SIRS distinguished by extreme circulatory, cellular, and metabolic abnormalities which intensify mortality risk (Singer et al., 2016); SS can be a terminal consequence of LHS. Septic shock is one of several types of cardiovascular shock, which has several different causes (Mosier, 2022). The form of SS in LHS is maldistributive shock (Constable et al., 2017b) associated with initial blood redistribution to the periphery stimulated by the need for cooling described earlier. Septic shock induced by uncontrolled endotoxemia generally has two phases, an initial hyper-dynamic phase of increased cardiac output, and a later hypo-dynamic phase of

reduced cardiac output (Constable et al., 2017a). Early on, cardiac output is increased by increased heart rate with a stroke volume similar to that prior to heat exposure, and by increased cardiac contractility (greater fraction of cardiac volume is ejected). The later deteriorating phase is characterized by systemic DIC, MODS, ARDS, reduced venous return volume, decreased cardiac contractility, decreased cardiac output, increased systemic arterial hypoxemia, and decreased arterial pressure. Loss of cardiac function leads to a moribund state. Septic shock in LHS is an outcome of systemic hyper-inflammation induced by endotoxin- stimulated TNF-α, IL-1 and other cytokines (Mosier, 2022) as described above in stage 3. These dynamic disorders in LHS do not occur in every case, nor in a consistent order. End stage SS can occur as a consequence of SIRS and ARDS, or of prolonged direct cardiac damage, as seen in human cases (Zahger et al., 1989, Marchand and Gin, 2022). These systemic disorders (i.e. SIRS, ARDS, and SS) are similar manifestations of a common underlying syndrome of diffuse, nonlocalized multi-organ dysfunction or failure (Armstrong et al., 2018) that ultimately is caused by the systemic and dysregulated hyper-immune response. In the end, the tissue hypoxia, “cytokine “storms”, and overwhelmingly dysregulated responses induce organ dysfunction and ensuing cellular and tissue injury in essentially all organ systems, then death results.

Suggested Epidemiologic, Clinical, and Diagnostic Information For Assessing LHS Probability In Cattle

LHS is a complex disorder in cattle veterinary medicine that currently has no working definition. Recognition that heat causes cattle deaths is only made when large numbers of cattle deaths are associated with a simultaneous severe heat event, as in the thousands of such deaths referenced here in the introduction. Death attributed to heat stress in cattle is made by association, without examining “How does heat cause cattle to die?” There are no published reports or studies that have investigated the specific signs, pathogenesis, clinical pathology, or post-mortem findings in mature cattle exposed to HS conditions that die. There are no cattle specific information on which to base valid, practical differential diagnostic approaches to LHS. Nonetheless, until research data is available, there is a need to review and suggest contexts, epidemiologic and observable signs, and diagnostics that can be useful. Such information will improve recognition of LHS as a potential differential diagnosis, and help assess the probability that LHS may or may not be occurring in individual animals, thus in a herd. No criteria or tests are definitive or pathognomonic (unique or decisive for a specific disorder) for diagnosis of LHS in cattle. Assessing probability of LHS requires compiling three types of information: 1) thorough history and context information, 2) clinical assessment and extent of signs in both affected and non-affected animals, and 3) triaging select situation-specific diagnostic tests that can be the most useful. Diagnostics and evidence of DIC coagulopathy can be useful when LHS is suspected. However, test panel results are not specific (Bruchim et al., 2008, Stokol, 2012), and vary with extent and progression of LHS at the time samples are obtained for testing.

Based on diagnosis of heat stroke in dogs and humans potential diagnostic options for LHS in a herd are listed below. Heat related injury (HRI) and DIC in animal species

other than bovine are well-studied disorders in veterinary medicine (Stokol, 2012, Bruchim et al., 2017a, Bruchim et al., 2017b, Hall et al., 2022). The published studies are useful in suggesting diagnostic information for HRI and predicting mortality in those species (Hall, E.J 2021). Although some diagnostic tests need to be validated and have reference intervals established for cattle; this could be accomplished in the near future if prioritized. Meanwhile, diagnostic decisions of LHS in cattle should be based on veterinary consultation, with a bovine veterinarian, a veterinary diagnostic laboratory, veterinary support specialists, and veterinary pathologists. Suggestions below are targeted at situation, context, and herd level assessment, which are essential to establish a probability that LHS is present. Suggestions listed for individual animal information are useful to support herd level information.

Suggested LHS Related Information: Epidemiologic, Clinical, and Diagnostic

History, Context, and Environment

1. History and context are essential and should include a detailed description of pertinent details and include a timeline highlighting the beginning and progression of the heat environment and cattle signs and behaviors: daily temperatures, humidity, THI (Zimbelman et al., 2009), night temperature (hot or cool?) wind speed if available. Do for A) 4 to 6 weeks prior to the heat event, and B) same as above for the 'runup' period (days marking the heat start) and for the duration of the heat event.
2. Micro environment of cattle location: Same data as above, actual or estimated
3. Micro environment of the cattle housing: daily-evening temperatures, ventilation, water access, cattle space/stocking density, quantify & rate these (Excellent, Adequate, Inadequate, Unsatisfactory).
4. Outside environment use: surface? duration? solar exposure? shade availability? water?
5. Animals bunching?

Affected Animals: Characteristics & Clinical Signs

1. Affected animals: Age, breed, DIM, haircoat color, location/pen, approximate # total affected in the group/pen, group/pen size.
2. Respiration Rate, panting score.
3. Physical examination, including demeanor, body temp, auscultation & heart rate
4. Recumbent / downer animals in established lactation?
5. Timeline of signs in animals: start, # affected over days/time.

Non-affected Animals: Characteristics & clinical signs?

1. Same as above on a set of clinically *non-affected animals* in same facility, but that may be representative of either less exposed or less affected baseline values.

Pen Observations: (10+ apparently affected cattle and suspect herdmates):
1. Prevalence of Respiration rate (_# elevated> 100? >120? >150?)?

2. Prevalence of _# open mouth panting? _# tongue extended? _# neck extended?
3. Percentage of fecal drops that are diarrhea (< 1 inch high, no defined margin).
4. Abnormal fecal characteristics: unusual color; blood present?: frank (red-hematochezia) or occult (black-melena).

Ante-mortem Diagnostic Options: Samples from clinical and clinical suspects:

1. Clinical chemistry (usually a standard bovine chemistry panel): liver & muscle enzymes, (gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), sorbitol dehydrogenases (SDH), aspartate aminotransferase (AST), creatine kinase (CK)), albumin, blood urea nitrogen (BUN), creatinine, bilirubin. Alkaline phosphatase (ALP) (request if not on the standard large animal panel.)
2. Optional- separate cardiac troponin (cTnI).
3. CBC (Complete Blood Count) both whole blood in anticoagulant and dried blood smear slides for platelet count.
4. Coagulation Panel (interpretation requires the 1st 3 be run together): Fibrinogen, PT (prothrombin time), aPTT (activated partial thromboplastin time), D-dimer or FDP (fibrin degradation products), ATA (Antithrombin activity). Note: a single set of coagulation panels is likely inadequate; individual animals being monitored should be tested at initial veterinary evaluation and again 12 and 24 hours later. Results vary with extent, stage, and degree of damage/progression. Results are poor sensitivity early in heat stress injury and have good sensitivity as pathology progresses to severe HS. (Bruchim et al., 2017b).

Necropsy Gross Pathology Observations:

1. Key organs to assess: Rumen, GIT intestines and lumen contents, heart, lungs, kidney, liver, all mucous membranes, organ external and internal surfaces and tissue.
2. Organ circulatory pathology present?: vascular hyperemia; tissue congestion, swelling edema; hemorrhage, petechial, ecchymosis of organ external / internal surfaces; extravascular tissue edema; abdominal/thoracic/extravascular fluid or fibrin.
3. GIT epithelial and mucosal intraluminal lesions, i.e., ulcers, necrosis, mucosal hemorrhage, bloody fluid contents.

Necropsy Histopathology Tissue Submissions:

1. Key organs to assess: Heart, lungs, kidney, liver, intestine, muscle.
2. Organ and circulatory histopathology present?: micro-thromboses, fibrin, inflammatory cellular infiltration, necrosis, vascular dilation/engorgement, tissue congestion, tissue edema, muscle fiber degeneration, microscopic petechia, ecchymosis.

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A New Method for Monitoring Forage Variability Improves Feed Efficiency and Profitability

J. A. Barrientos-Blanco and K. Reed
Department of Animal Science
Cornell University

Introduction

Forage composition variability increases the risk of underfeeding and overfeeding lactating cows by contributing to uncertainty and inconsistency of the delivered dietary nutrients at the feed-bunk. Inconsistency in the diet can lead to drops in milk production, decreases in feed efficiency and income over feed cost (IOFC), and impacts on cow welfare. However, adjusting diets according to changes in forage components can decrease the uncertainty and improve the consistency of the dietary nutrients delivered to cows. Forage variability can be monitored using industrial process control-charts to identify changes in forage components that require diet balance adjustments. We developed a control-chart application for monitoring forage variability and an algorithm for optimizing sampling practices. We estimated the optimal sampling practices (optimum number of samples, sampling frequency, limits of variation) using the renewal reward model as the objective function for the genetic algorithm optimizer suggested by (St-Pierre and Cobanov, 2007) and used them as inputs for the control-chart application. We hypothesize that adjusting diets according to changes in forage components signaled by the control-chart application increases diet consistency and accuracy and will result in increased feed efficiency and IOFC. The objective of our study is to measure the impact of implementing a diet reformulation protocol using our control chart application on diet accuracy, feed efficiency, and IOFC of NY dairy farms.

To achieve our objective, we implemented the optimal sampling and diet formulation protocol (treatment protocol) and comparing responses with the current diet formulation practices (control protocol). For the treatment protocol, we optimized the sampling practices for 3 enrolled farms (avg. herd size 750, 1,000, and 2,000) and monitored changes in starch and NDF of corn silage and CP and NDF of alfalfa-grass haylage using our control-chart application. Corn silage, alfalfa-grass haylage, and TMR were sampled 3x a week for 16 weeks between May 9th and August 26th, 2022. When the application signaled a change in the composition of corn silage or haylage, we requested a diet adjustment from the nutritionist using the average composition of the forages within the new variation period. The study is a crossover structure design with two periods of 8-weeks and two treatments. The observational units are pens with 1, 1, and 5 pens enrolled in the study from the 750, 1,000, and 2,000 high-cows farms, respectively. Practical constraints prevent balancing the control and treatment protocols between periods because 1) only the 2,000-cow dairy has the ability to implement both protocols in the same 8-week period, and 2) we were unable to initiate a treatment protocol on either the 750, or 1,000 cow dairies during the first period.

Main Findings

On the 2,000-cow dairy, the diet of the treatment protocol was adjusted 13x and the control protocol diet was adjusted 5x during the 16-weeks of the study. Preliminary results from the analysis of the diet composition, feed intake, and milk production data from the 2,000-cow farm suggest an increase in IOFC of $0.12 per head per day but no difference in feed efficiency (Table 1). Further, diet cost was $0.12 per cow per day higher for the treatment protocol. Therefore, the resulted difference in IOFC was related to 2.72 lbs/head higher ECM for the treatment protocol. There was a numerical but not a significant difference in DMI between protocols, so the increase of ECM is likely related to accurate formulated diet for the treatment protocol due to higher reformulation frequency. This result is in alignment with the theoretical outcomes reported in a simulation study by White and Capper (2014) who reported an increase in milk yield but not effect on DMI with more frequent reformulation of diets.

Table 1. Average and standard deviation of DMI, ECM, FE, diet cost, and IOFC of high-production cows tested with treatment and control protocols

Parameter	Treatment protocol	Control protocol	P-value
DMI (lbs/hd)	56.41 ± 3.70	55.68 ± 2.76	0.290
ECM (lbs/hd)	104.39 ± 7.47	101.67 ± 5.50	0.053
FE (lbs ECM / lbs DMI)	1.88 ± 0.17	1.87 ± 0.27	0.948
Diet cost ($/hd)	7.70 ± 0.67	7.58 ± 0.59	
IOFC ($/hd/day)	4.30 ± 1.34	4.18 ± 1.23	

We quantified accuracy of a delivered diet as the deviation of delivered nutrients from the formulated diet nutrients. Our analysis of diet accuracy reported in Table 2 showed that delivered diet for the pens managed with the treatment protocol was more accurate than the delivered diet for the control protocol. Values of Table 2 were calculated subtracting the DM, NDF, CP, and Starch content of the formulated diet from the delivered diet at the feed bunk. Consequently, values closer to zero means more accuracy of the delivered diet. In our analysis, DM and CP content of the formulated diets for the treatment protocol were 1.09% and 0.10% which were significantly closer to the delivered diet than those of the control protocol (Table 2). However, the NDF content of the formulated diet for the treatment protocol was not significantly closer to the delivered diet. The starch content of the formulated diet for the treatment protocol was numerically less accurate but not significantly different than the formulated diet for the control protocol. Higher accuracy of DM and CP of the delivered diet for the treatment protocol can be explained by more frequent adjustments signaled by the control chart application and further analysis is needed to understand why a similar improvement in accuracy was not achieved for NDF and starch.

Table 2. Difference between the DM, NDF, CP, and Starch content of formulated diet and the diet delivered at the feed bunk.

Formulated diet - delivered diet	Treatment protocol	Control protocol	P-value
DM %	0.83 ± 1.03	1.92 ± 0.87	< 0.001
CP %	0.11 ± 0.42	-0.21 ± 0.34	< 0.001
NDF %	-4.72 ± 0.98	-4.81 ± 1.02	0.646
Starch %	0.40 ± 0.96	0.17 ± 0.91	0.249

Take Home Message

More accurate diet decreases the risk of uncertainty and improves the consistency of the delivered nutrients at the feed bunk. Adjusting the diet formula when control chart application signals change in haylage and corn silage components may improve the accuracy of the delivered diet to dairy cows. However, further study to understand the impact of this protocol on multiple nutrient outcomes and practical considerations for implementation on farm needs to be done.

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Mitigation of Enteric Methane Emissions: A Down-to-Earth Perspective

J. W. McFadden[1] and A. N. Davis[2]
[1]Department of Animal Science, Cornell University, Ithaca, NY
[2]State University of New York at Cortland, Cortland, NY

Introduction

Methane is an anthropogenic greenhouse gas (GHG) and potent climate pollutant that has contributed ~0.5°C to observed global warming from the years 1850-1900 to 2010-2019 (IPCC, 2021). Although cattle are part of a natural biogenic carbon cycle involving carbon recycling between animals and plants, livestock are estimated to contribute ~30% of global anthropogenic methane emissions (Tian et al., 2016; Jackson et al., 2020; Saunois et al., 2020). The additional public concern is that the global livestock population has tripled over the past century; which is expected to expand in the future (Godfray et al., 2018; Henchion et al., 2021). Global animal protein supply is anticipated to increase 17% from the year 2017 to 2050 under a "business as usual" scenario (Henchion et al., 2021). Therefore, methane emissions from livestock are expected to increase. Attention has centered on the development and application of dietary feed additives that act as rumen environment modifiers (e.g., essential oils) or direct inhibitors (e.g., 3-nitroxypropanol [3-NOP] or halogen-containing seaweed) of enteric methane production. Dietary approaches to inhibit ruminal methanogenesis have strong scientific merit to reduce methane emissions from cattle and slow the progression of climate change in the short-term; however, limited evidence supports the ability of any single approach to enhance the energetic efficiency of milk production in a consistent manner with a clear mode of action.

Although feed additives to reduce enteric methane production in ruminants are exciting to consider, we cannot ignore the reality that a large disparity exists between developed and developing countries with regard to methane emissions and production efficiency. Zhang and coworkers (2022) provided an analysis of a 130-year global inventory of methane emissions from livestock. In the 1890s, developed and developing countries accounted for 44% and 56% of total methane emissions (Tg CH_4 yr^{-1}), respectively. In the 2010s, 18% and 82% of global methane emissions were derived from developed and developing regions, respectively. The greatest increases in methane emissions between the 2010s and 1890s were in South Asia (29% of total), Brazil (12%), Northern Africa (12%), and China (11%). This is problematic when we consider that South Asia is expected to experience a 64% increase in energy-corrected milk production by 2030, relative to levels observed in 2017 (Henchion et al., 2021). At the country level, India was the top emitter in the 2010s at 24.0 Tg CH_4 yr^{-1}, as compared to the United States of America at 8.3 Tg CH_4 yr^{-1}. From a dairy perspective, milk production efficiency in cattle (or buffaloes) in the developing world pales in comparison to developed nations. This proceeding will examine the energetics and efficiency of milk production in cattle including a focus on feed ingredients that can substantially inhibit enteric methane production. A case study of India will explore the

challenges and potential solutions that require immediate consideration to enhance milk production efficiency and reduce the intensity of methane emissions.

Energetics of methane and milk production

The 8th Revised Edition of the Nutrient Requirements of Dairy Cattle summarizes the classical energy flow system (NASEM, 2021). Gross energy (GE), or the amount of energy in the feed, minus fecal energy is digestible energy (DE). Digestible energy is divided into six fractions: digested neutral detergent fiber [NDF], digested starch, digested fatty acid, digested residual organic matter (i.e., sugars, pectins, gums, glycerol of triacylglycerol, and fermentation acids), supplemental nonprotein nitrogen on a crude-protein equivalent basis, and digested crude protein. Energy lost in urine and via methane is subtracted from DE to obtain ME. Urinary energy can be estimated from urinary N excretion. Gaseous energy is a function of dry matter intake (DMI) and the content of fatty acids and digested NDF in the diet. Net energy is ME minus heat production from digestive and metabolic processes (i.e., heat increment). The conversion of ME to net energy of lactation is 0.66.

The use of indirect calorimetry has demonstrated that methane losses vary from 2 to nearly 12% of GE intake (Johnson et al., 1993). The average loss being ~5 to 6%. Johnson and Johnson (1995) stated that as diet digestibility increases, variability in methane loss also increases. The authors describe two primary modes of action that cause variability in methane production by cattle. First, the amount and type of dietary carbohydrate fermented in the reticulorumen, which involves a balance between rate of carbohydrate fermentation and passage of feed. Second, the regulation of propionate production (a hydrogen sink), as opposed to acetate, regulates hydrogen supply and methane production. The authors further postulated that if the acetate to propionate ratio was 0.5, the loss of substrate energy as methane would be 0%; however, Wolin and Miller (1998) suggested that if all carbohydrates were fermented to acetate, and none to propionate, then methane energy loss would be 33%. This is significant when we consider that the acetate to propionate ratio in rumen fluid can range from less than 1:1 to greater than 4:1 in low and high forage diets, respectively (Russell, 1998).

In a retrospective analysis of data derived from 20 energy metabolism studies involving 579 lactating dairy cows, Yan and coworkers (2010) evaluated methane energy output in relationship to factors that define energetic efficiency. Methane energy per unit of GE intake and methane energy per unit of milk energy output (i.e., emissions intensity) were lowered with increasing feeding level (ME intake/ME requirement for maintenance), milk energy per unit of metabolic body weight ($kg^{0.75}$), as well as intakes of GE, DE, or ME per unit of metabolic body weight. The authors estimated that methane energy is 8.5% of GE intake at maintenance feeding level but can decrease by half if feeding level is high. Moreover, a reduction of energy expenditure on maintenance as a proportion of ME intake from 100 to 40% could decrease methane energy from 7.6 to 3.6%, respectively. Indeed, high-yielding cows produce less methane per unit of milk energy because of “dilution of maintenance”. Gains of 100 kg of milk per lactation are predicted to result in a 7.3% decrease in methane per unit of energy-

corrected milk at a production level of 7,000 kg; which is in contrast to 3.1% for cows producing 13,000 kg without changes in rumen fermentation or nutrient digestibility (Kebreab et al., 2008; Knapp et al., 2014). Feeding high-quality (i.e., more energy-dense and digestible) to cattle that have a higher genetic merit for milk production, and better management, have the confirmed potential to reduce methane energy output as a proportion of GE intake and dilute the cost of maintenance.

Approaches to reduce conversion of gross energy to methane energy

It is often argued that the inhibition of methanogenesis in the ruminant has the potential to enhance milk production. Reducing the conversion of GE to gaseous energy could enhance the conversion of GE to DE and ME. As described by Beauchemin and colleagues (2020), cows fed diets with 70% digestible energy, a moderate decrease (e.g., 25%) may only increase ME by 0.75% to 4.25%. Because the efficiency of converting ME to net energy for lactation is 0.66, it may be difficult to observe improvements in milk production (especially in small population sizes often used for scientific research). The authors state that more severe inhibition of methane production beyond 50% without compromised DMI or digestibility may be required to observe substantial increases in milk production. Two dietary feed ingredients that have potential to inhibit methanogenesis 30% or more include 3-NOP or halogen-containing seaweed.

3-nitrooxypropanol (3-NOP)

The competitive inhibitor 3-NOP reduces enteric methane production by 20 to 80% in beef cattle, dairy cattle, and sheep (average of 30%; Martínez-Fernández et al., 2014; Hristov et al., 2015; Lopes et al., 2016; Vyas et al., 2016). As a structural analog of methyl coenzyme M, 3-NOP blocks the active site of methyl-coenzyme M reductase, which inhibits the last step of methanogenesis (Duin et al., 2016). A plethora of studies have assessed the impacts of 3-NOP on methane and hydrogen production, milk production and composition, DMI, feed efficiency, methanogen growth, digestibility, and energetics (Jayanegara et al., 2018; Almeida et al., 2021).

The effects of 3-NOP on methane and hydrogen emissions are consistent. In a 12-week study of lactating dairy cows, Hristov and colleagues (2015) reported a 30% reduction in methane production (assessed using GreenFeed system [C-Lock Inc., Rapid City, SD]) when 3-NOP was included in the ration at 40 to 80 mg/kg of dry matter [DM]. Melgar and colleagues (2021) also used a GreenFeed system to measure enteric methane production in dairy cows fed 3-NOP (60 mg/kg of DM) for 15 weeks. They observed that 3-NOP reduced emission yield and intensity by 27% and 29%, respectively. van Gastelen and coworkers (2020) used respiration chambers to measure enteric methane production in early lactation dairy cows fed 3-NOP (51 mg/ kg of DM) for 16 weeks. In this study, 3-NOP reduced methane emission at 55 and 111 days in milk by an average of 18.5%. The observed decrease in methane production develops with a consistent increase in H_2 emissions. Normal production of methane by methanogenic archaea involves reduction of CO_2 to CH_4 with H_2. Increases in ruminal H_2 concentrations has potential to lead to the down-regulation of H_2-generating

pathways and up-regulation of H_2-consuming pathways. An increase in H_2 emissions with 3-NOP treatment has been observed by Melgar et al. (2021) and van Gastelen et al. (2022). These collective changes on CH_4 and H_2 emissions by 3-NOP have been confirmed by recent meta-analyses (Jayanegara et al., 2018; Almeida et al., 2021).

There have been a number of recent studies examining the interactions of 3-NOP feeding with other dietary ingredients to identify strategies to maximize methane inhibition. van Gastelen and coworkers (2022) concluded that 3-NOP inhibited methanogenesis (and increased H_2 emissions) more when lactating cows were supplemented with corn silage as compared with cows fed grass silage. Schilde and coworkers (2021) confirmed that feeding high concentrates in combination with 3-NOP synergistically lowered methane yield, more than low concentrate diets containing 3-NOP. Feeding beef cattle a high-forage diet (90% barley silage) containing 3-NOP (200 mg/kg of DM) and canola oil (50 g/kg of DM) suppressed ruminal methanogenesis more than when either were offered alone (Gruninger et al., 2022). The authors concluded that 3-NOP inhibited the hydrogenotrophic methanogenesis pathway, whereas oil caused changes in the rumen microbial community to alter rumen fermentation. The addition of monensin to beef cattle rations supplemented with 3-NOP was unable to lower methane yield more than 3-NOP alone (Vyas et al., 2018). It is conceivable that co-supplementation strategies involving 3-NOP and alternative inhibitors of methanogenesis (with different modes of action) will be required to inhibit methane production more than 50% and thus favorably impact milk production in dairy cattle.

The effects of methane inhibition by 3-NOP on milk production and composition, DMI, feed efficiency, and bodyweight have received attention. van Gastelen and colleagues (2020) found no effect on any of these variables in early lactation dairy cows fed 3-NOP (51 mg/kg of DM) for 16 weeks. These findings are consistent with Reynolds and colleagues (2014), who found no change in DMI, yields of milk or fat-corrected milk, or milk energy in lactating dairy cows administered 3-NOP (500 or 2,500 mg/d delivered into the rumen via fistula; 2x daily before feeding) for 5 weeks. Similarly, Melgar and coworkers (2021), as well as Lopes and colleagues (2016), observed no changes in DMI or milk yield in lactating dairy cows, except for an increase in milk fat concentration with 3-NOP feeding. These findings are consistent with the results from Hristov and colleagues (2015), who found that neither DMI or milk production were affected by 3-NOP supplementation (40 to 80 mg/kg feed DM) in lactating cows supplemented for 12 weeks; however, milk protein and lactose yields, as well as bodyweights were increased by 3-NOP treatment. They also observed that methane emission per unit of DMI or per unit of energy-corrected milk were about 30% less for the cows treated with 3-NOP, relative to unsupplemented cows. The increase in bodyweight was also observed in mid-lactation dairy cows administered 2,500 mg of 3-NOP per day for 28 days with a 38% forage diet, which occurred without changes in DMI or milk production (Haisan et al., 2014 and 2017). The ability of 3-NOP to reduce energy lost as methane appears to spare energy to support milk component synthesis or body tissue accretion; albeit, the impact of 3-NOP on nutrient utilization and partitioning is likely influenced by stage of lactation and the homeorhetic mechanisms of the cow.

An investigation by van Gastelen and colleagues (2020) observed that 3-NOP promoted positive effects on total-tract digestibility of nutrients, including a greater ME intake to GE intake ratio. An improved total tract digestibility with 3-NOP feeding has been observed in other studies (Hristov et al., 2015; Haisan et al., 2017; Melgar et al., 2020). van Gastelen and colleagues (2020) hypothesized that improved digestibility may result in more efficient rumen fermentation due to greater availability of propionate, relative to acetate. A decrease in the ratio of acetate to propionate in response to 3-NOP consistently develops with 3-NOP treatment (Haisan et al., 2014; Martínez-Fernández et al., 2014; Romero-Perez et al., 2014; Romero-Perez et al., 2015; Lopes et al., 2016; Haisan et al., 2017; Martínez-Fernández et al., 2018; van Gastelen et al., 2020). Greater propionate availability in response to 3-NOP may also explain frequently observed increases in bodyweight. Propionate is the main glucogenic precursor in ruminants, and promotes the release of insulin, which promotes fat storage and reduces mobilization of body reserves (van Knegsel et al., 2007). The observed increase in H_2 emissions with 3-NOP treatment does not appear to negatively impact rumen function. This could be a potential concern since an increase in H_2 partial pressure in the rumen is known to cause negative feedback on rumen fermentation, feed intake or digestibility (Leng, 2014). This said, 3-NOP treatment has been shown to increase fecal nitrogen excretion and decrease nitrogen digestibility to decrease body nitrogen balance (Reynolds et al., 2014), which should be examined further considering nitrous oxide emissions from manure is another concern for climate change.

Seaweed

In coastal regions, seaweeds have been a part of livestock diets since initial agricultural practices began (Heuzé et al., 2017). Members of the red micro algae genus *Asparagopsis*, particularly *A. taxiformis* and *A. aramata*, have gained considerable attention because of their ability to inhibit enteric methanogenesis (Machado et al., 2014). These seaweeds contain a high abundance of bioactive compounds, called halogenated methane analogues (HMAs), that inhibit the activity of methanogens. Examples of these HMAs include bromochloromethane (Machado et al., 2014; Heuzé et al., 2017; Stefenoni et al., 2021), bromoform (Brooke et al., 2020), chloroform (Abbott et al., 2020), and dichloromethane (de al Moneda et al., 2019). Their specific mode of action is to bind with reduced vitamin B_{12}, blocking the cobamide-dependent methyltransferase reaction required for formation of methyl-coenzyme M (Wood et al., 1968). In *Asparagopsis*, the most abundant bioactive constituent and most important contributor to its antimethanogenic activity is bromoform, followed closely by dibromochloromethane (Paul et al., 2006).

Dietary supplementation with *A. taxiformis, A. aramata,* or isolated HMAs (i.e., bromochloromethane or chloroform) consistently reduces methane emissions in sheep (Li et al., 2016), goats (Mitsumori et al., 2012), and cattle (Johnson et al., 1972; Roque et al., 2019; Kinley et al., 2020; Roque et al., 2021; Stefenoni et al., 2021). Johnson and colleagues (1972) observed a complete inhibition of methane production (100%) in steers administered 5.5 g of bromochloromethane per d for 28 days. Kinley and colleagues (2020) observed a 40% and 98% reduction in enteric methane production

(assessed by respiration chambers) in steers supplemented with *A. taxiformis* at 0.10% and 0.20% of organic matter, respectively, for 90 d. In lactating dairy cows administered *A. aramata* at 0.5% or 1% of organic matter for 21 d, methane production (assessed using the GreenFeed system) was reduced by 26% and 67%, respectively (Roque et al., 2019). Similarly, Stefenoni and coworkers (2021) observed a 34% reduction in methane emissions (assessed using the GreenFeed system) in lactating dairy cows supplemented with *A. taxiformis* at 0.5% of DM for 28 d. Supplementing lactating goats with 0.66 mg/kg BW bromochloromethane per d for 70 d resulted in a 32% reduction in methane emissions, which was assessed using respiration chambers (Abecia et al., 2012). Across studies, the reduction in methane emissions by red microalgae or its bioactive compounds consistently increases H_2 production (Kinley et al., 2020; Roque et al., 2019 and 2021; Stefenoni et al., 2021).

The effect of reduced methane emissions by seaweed on productivity (i.e., bodyweight gain or milk production) varies greatly. The reduction in methane emission observed by Kinley and colleagues (2020) in steers was accompanied by weight gain increases of 53% and 42% for the 0.10% and 0.20% of organic matter inclusion of *A. taxiformis,* respectively, with no changes in DMI or feed conversion efficiency. Although Johnson and colleagues (1972) observed a complete inhibition of methane production by bromochloromethane, only a numerical increase in average daily gain was observed (i.e., not statistically significant). Roque and coworkers (2019) observed an 12% reduction in milk yield, and lower DMI, in lactating cows administered *A. aramata* at 1% of organic matter for 21 d. Similarly, Stefanoni and colleagues (2021) observed a 6.5% reduction in DMI, milk yield, and energy-corrected milk yield in lactating dairy cows supplemented with *A. taxiformis* at 0.5% of DM for 28 d. In contrast, Abecia and colleagues (2012) observed a 36% increase in milk yield in lactating goats.

While digestibility and energetics data are limited in studies of seaweed feeding to cows, Johnson and coworkers (1972) found no effect of bromochloromethane on the digestibility of DM, energy, or acid detergent fiber in steers. Reducing methane emissions by supplementing *A. taxiformis, A. aramata,* or isolated HMAs consistently results in a decrease in the proportion of acetate to propionate in the rumen (Abecia et al., 2012; Roque et al., 2019; Kinley et al., 2020; Roque et al., 2021; Stefenoni et al., 2021). Safety concerns do exist for seaweed feeding. Milk iodine concentrations are elevated in cows fed a seaweed mix of *Ascophyllum nodosum* and *Laminaria digitate* (Newton et al., 2021). Although bromoform does not appear to accumulate in tissue, the compound does appear to be excreted in urine and milk (Muizelaar et al., 2021). Signs of inflammation, hemorrhages and ulcers have also been document following the histological examination of the rumen wall and papillae of cows fed *Asparagopsis taxiformis* (Muizelaar et al., 2021). These findings are concerning and further testing should be required before seaweed feeding is adopted as farm practice. Such an effort will help be beneficial to ensure consumer acceptance of the technology if proven safe.

Why we must prioritize enhancing feed efficiency in developing nations: A case study of India

Developing nations such as India are major contributors to global anthropogenic emissions of GHG. In recent years, the share of Indian agriculture was 7% of global emissions (CO_2 equivalent [CO_2e]) from agriculture (Pathak, 2015). By 2050, the number of milk-consuming households in India is projected to increase from 185 million to 349 million (Gupta and Dasgupta, 2020). It is logical that India is the world's largest producer of milk, producing 195 million metric tons in 2020 or ~22% of global production (FAO, 2020). The milk produced in India contributes to a major portion of the gross income of rural households and most of the livestock sector gross domestic product. In stark contrast to North America and Europe, low-producing buffaloes (*Bubalus bubalis*) yield more than half of the milk in India, followed by indigenous cows (*Bos indicus*) and indigenous cows crossbred with exotics (e.g., Holstein, Jersey, etc…). It is estimated that India currently has over 50 million dairy cows or ~18% of the world's total population (Steensland, 2019). Moreover, countries of the Indian subcontinent manage 37% of the world's dairy goats and produce 41% of the world's goat milk (Pulina et al., 2018). The vast population of ruminants in India is a concern because enteric production of methane by ruminants is the largest anthropogenic source in agriculture; therefore, in India, as the demand for and production of milk rises, we can expect an increase in GHG emissions from ruminants.

The milk revolution, often referred to as the White Revolution, was key to increase milk production by 400% from 1968-1969 to 2003-2004 (Deka et al., 2015). Operation Flood was a government-sponsored program that promoted crossbreeding, improved access to feed and veterinary services, and enhanced markets, and milk processing and preservation infrastructure to avoid chronic milk shortage in India. The program augmented rural incomes and provided milk and dairy products at fair prices for the consumer. Today, Indian farming primarily consists of traditional smallholder production systems managing 1 to 5 animals that produce approximately 5 liters of milk per day; albeit, the number of larger commercial systems is gradually increasing. In an evaluation of cattle production in Eastern India (Gupta et al., 2014), the majority of farmers were unable to spare land for fodder production, followed their own feeding practices, were unsatisfied with milk production levels, required training in diet formulation, and desired a transition from natural breeding to artificial insemination to increase the population of crossbred animals with higher milk production and heat tolerance.

Although the White Revolution was a success for Indian agriculture, the country is faced with an agrifood challenge in an era of climate change. Milk and dairy products are a major source of affordable and nutritious food for millions of Indians (Ohlan, 2012). For Indians that consume animal-sourced foods, milk provides the highest proportion of total caloric intake. This is especially important for growing infants and lactating mothers. Following UN Population Division forecasts, the human population in India is expected to increase 194 million between 2015 and 2030 (Liu et al., 2018). This is the highest rate of growth in Silk Road Economic Belt and Maritime Silk Road

countries, and it is anticipated that India will surpass China as the most populous in the world by 2030 (Liu et al., 2018). In parallel, the FAO estimates per capita consumption of meat and milk in India will increase 94% between 2006 and 2050 (highest increase in the world; Searchinger et al., 2018). Indeed, an increase in dairy imports has potential to offset demand for domestic production. Total food imports including dairy are expected to increase 8.5 to 18.3% by 2050 (Hamshere et al., 2014); but, these projections will be influenced by government policies toward animal agriculture. Domestic milk production will need to increase to meet projected demand. Despite producing the most milk of any country in the world, milk productivity in India remains one of the lowest (Bardhan and Sharma, 2013). Such poor efficiency is unlikely to meet the future demand for milk. This is especially concerning when we consider that India ranks 97 out of 118 on the Global Hunger Index and 39% of children under five in India are defined as 'stunted' due to malnutrition (of below average height; von Grebmer et al., 2016; Ritchie et al., 2018).

In India, it is estimated that 90% of total methane emissions from enteric fermentation are contributed by buffalo and cattle and the remainder from small ruminants (e.g., goats) and other domestic animals (Swamy and Bhattacharya, 2006). In 2006, approximately 48% and 35% of enteric and manure methane emissions were derived from indigenous cattle and buffaloes, respectively. Enteric and manure methane density (Gg/sq. km/y) is highest in Northern states such as Bihar, Uttar Pradesh, Rajasthan, and Punjab. As compared to dairy production in North America or Europe, methane yields per kg of protein produced by dairy ruminants are greater in South Asia countries including India (Chang et al., 2021). Crossbreeding taurine and indicine cattle has potential to increase milk production efficiency. Across all Indian states and territories, mean GHG intensity per unit of milk for crossbred cows is 1.21 kg of CO_2e kg^{-1} milk versus 2.96 kg of CO_2e kg^{-1} milk for indigenous cows (Patra, 2017). For perspective, the average enteric methane emissions intensity in the United States is ~0.25 kg of CO_2e kg^{-1} milk (Tricarico et al., 2020). A similar situation is observed when evaluating emissions on the basis of milk energy. For example, mean intensity for crossbred cows is 0.41 kg of CO_2e MJ^{-1} milk energy versus 1.00 kg of CO_2e MJ^{-1} milk energy for indigenous cows. Crossbred cows have lower GHG intensity per unit of milk when compared to buffaloes (1.21 versus 1.85 kg of CO_2e kg^{-1} milk); albeit, intensity of GHG emissions is comparable on the basis of milk energy. Enhanced regional utilization of agro-industrial byproducts, feeding nutrient-balanced diets, and accelerated adoption of artificial insemination and crossbreeding efforts are promising approaches to enhance efficiency and reduce methane emissions from cattle; however, such strategies are needed within the framework of existing religious and socio-economic challenges with government support.

Balanced ration formulation is a means to increase milk production efficiency in India. The FAO (2012) suggests that balanced ration formulation can increase daily income from rearing livestock by ~10% in India. Blümmel and coworkers (2009) estimated that milk yield per animal in India can increase from 3.6 to 9 L/d by feeding cows nutrient-balanced diets. Moreover, increasing milk yield per animal from 3.6 to 12 L/d would reduce the number of livestock by 70%, feed required by 48%, and methane

production by 46%; albeit, this is dependent upon increasing the energy density of diets in a country with limited availability of concentrates and poor quality fodder. Goswami and coworkers (2013) demonstrated the feasibility of reducing feed cost by 19% compared to a routine feeding plan when formulating diets for crossbred dairy cows yielding 5 to 10 kg of milk/d in central India. Therefore, advanced ruminant nutrition will enhance the efficiency of nutrient use for milk production in India, which will decrease nutrient requirements for maintenance, GHG emissions, and ruminant animals required per unit of milk or milk energy.

Summary and Future Directions

The use of feed additives such as 3-NOP or seaweed to inhibit ruminal methanogenesis has merit; however, we must temper enthusiasm, and be rational and transparent. For 3-NOP, we require studies that investigate the use of 3-NOP over full lactations, assess varies modes of delivery to ensure that cattle in non-confinement management scenarios (e.g., grazing systems) also benefit, and assess its impact on nutrient flow to the duodenum. It is also apparent that the magnitude of efficacy for 3-NOP is highly influenced by the diet. We must continue to examine the interaction of 3-NOP with dietary ingredients that influence rumen fermentation and methanogen activity. For seaweed, we must be confident that this approach won't increase the presence of iodine, bromine, arsenic, and other halogenated compounds in meat or milk to limits of human safety concern. We need to carefully consider how long-term feeding of seaweed impacts the health and productive lifespan of the animal, examine stability, bioavailability, and safety of seaweed compounds, and require complete life cycle assessments that consider the production, processing, transport, and use of the product to ensure that the net impact of the technology on our environment is positive and economically competitive. We must also consider how methane inhibitors influence the emissions of other GHG from the rumen or manure including nitrous oxide, better understand how rumen ecology adapts (or doesn't), examine compound replacement or additivity on methane emissions, consider early-life methane-inhibitor interventions that have long-term benefit, and better define energetic conversion of GE to milk energy. We must also aggressively challenge and acknowledge the limitations of the scientific methods that we utilize to define efficacy of methanogenesis inhibitors.

Feed additives that inhibit methanogenesis may not be cost-effective or practical in developing nations with unique production systems. It is highly likely that technologies that inhibit ruminal methanogenesis, if approved for use, are more likely to be adopted in developed regions of the world. Therefore, the use of dietary approaches to lower enteric methane production will need to be high in order to enhance the conversion of gross energy to milk (and not methane) but also compensate for cattle that don't receive such interventions on a global scale. This is why we must continue to enhance the productive efficiency of cattle (and buffaloes) in regions of the world that are far-behind North America and Europe.

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Inflammation During the Transition Period

S. Rodriguez-Jimenez, M. A. Abeyta, B. M. Goetz, J. Opgenorth, A. D. Freestone, G. J. Combs, T. A. Flemming and L. H. Baumgard
Iowa State University, Ames, IA; baumgard@iastate.edu

Introduction

Optimizing cow health and productivity during the transition period represents a significant hurdle to the dairy industry. During early lactation inadequate nutrient consumption is coupled with increasing milk energy output; a scenario that creates a negative energy balance (NEB; Drackley, 1999). Therefore, milk yield during NEB is prioritized by alterations in carbohydrate, lipid, protein, and mineral metabolism. Traditionally, excessive adipose tissue mobilization, the ensuing hyperketonemia and the magnitude of hypocalcemia were thought to be the pathological foundation of transition cow problems and immunosuppression. However, high producing healthy cows may also present high NEFA, hyperketonemia and transient subclinical hypocalcemia. These are key homeorhetic adjustments that cows employ to prioritize milk synthesis at the expense of tissue accretion. Further immune activation also markedly influences metabolism and mineral trafficking, and these adjustments are utilized to prioritize an activated immune system. Thus, an inflamed cow also has a very similar bioenergetic and mineral metabolism footprints as a high producing healthy cow. We believe that altered NEFA, ketones, and calcium are due to one of two reasons: 1) high producing healthy cows are naturally adjusting metabolism during NEB to emphasize milk synthesis, or 2) unhealthy cows in which metabolic alterations reflect immune activation and subsequent hypophagia. The difference in these two models is more than an academic debate, since this nuance has large economic implications for the producer.

Correlation is Unequal to Causation

Dairy cow lactation maladaptation has extensively been researched for more than five decades and this is primarily because the incidence of health problems is highest in the first two months of lactation. The periparturient period certainly has more dynamic variations in bioenergetics (NEFA, glucose, ketones, insulin, glucagon, BUN, etc.) and minerals (Ca and P) than during established lactation. Importantly, these temporal patterns are often occurring while negative health events are detected. Correlation and causality are sometimes incorrectly assumed to be equal in regard to the events that occur during the transition period and are claimed to be inevitable rather than coincidental. Most of the assumptions have been largely based on associations and not cause-and-effect relationships garnered from controlled and intervening experimentation. Even from a relationship perspective, assessing the strength or robustness of the associations is difficult due to variability in analysis and statistical methods. In particular, different metabolite thresholds are biasedly set for different outcomes and time points among observational studies. Additionally, inconsistent association metrics (e.g., odds ratio, relative risk, hazard ratio) are used to assess these relationships. The inconsistency

and inaccuracy of using correlation to interpret causation creates suspect on-farm decision-making and unnecessary farm expenses. More detailed description of this area is covered in our recent review (Horst et al., 2021).

Traditional Dogmas:

Long-standing tenets describe a causal role of hypocalcemia, increased NEFA, and hyperketonemia in the incidence of transition diseases and disorders (Figure 1). Hypocalcemia has traditionally been considered a gateway disorder leading to ketosis, mastitis, metritis, displaced abomasum, impaired reproduction, and decreased milk yield (Curtis et al., 1983; Goff, 2008; Martinez et al., 2012; Chapinal et al., 2012; Riberio et al., 2013; Neves et al., 2018a,b). The proposed mechanisms by which hypocalcemia leads to these ailments include impaired skeletal muscle strength and gastrointestinal motility (Goff, 2008; Oetzel, 2013; Miltenburg et al., 2016; Goff, 2020), decreased insulin secretion (Martinez et al., 2012, 2014), and the development of immunosuppression (Kimura et al., 2006). Like hypocalcemia, increased NEFA and hyperketonemia are presumed causative to illnesses such as DA, retained placenta, metritis, reduced lactation performance, poor reproduction, and an overall increased culling risk (Cameron et al., 1998; LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010; Chapinal et al., 2011; Huzzey et al., 2011). Excessive NEFA mobilization and the affiliated increase in hepatic lipid uptake, triglyceride (TG) storage, and ketone body production has been traditionally believed to be the driving factor leading to ketosis and fatty liver (Grummer, 1993; Drackley, 1999). Additionally, elevated NEFA and ketones are thought to compromise immune function (Lacetera et al., 2004; Hammon et al., 2006; Scalia et al., 2006; Ster et al., 2012) and suppress feed intake (Allen et al., 2009). Thus, the magnitude of changes in NEFA, BHB and Ca have traditionally been purported as predictors of future performance.

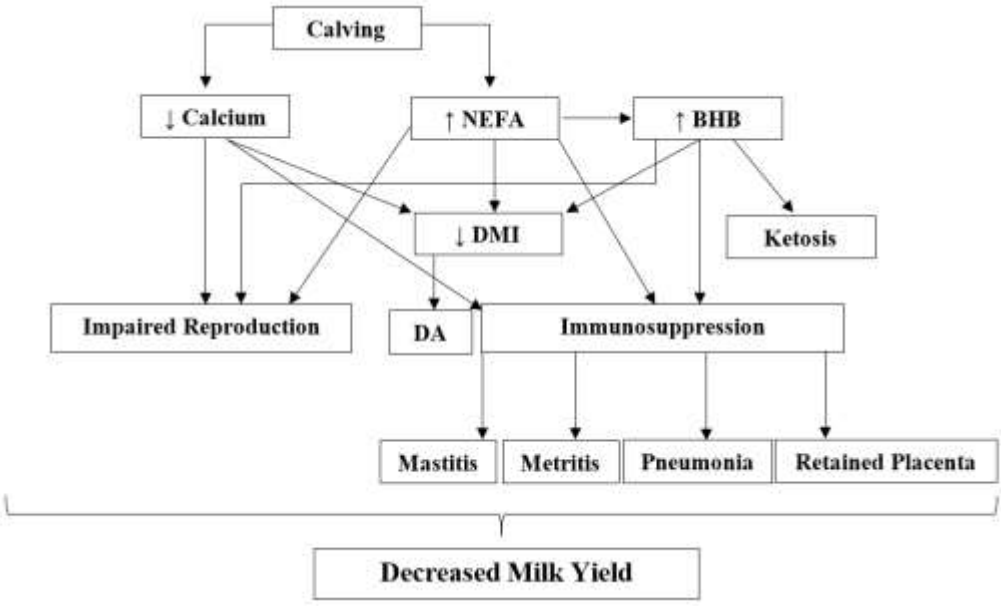

Figure 1. Traditional mechanisms by which hypocalcemia and increased NEFA and ketones are thought to cause poor transition cow health and performance

Culling Trends

A cow's entire lactation and the opportunity to have an additional lactation are heavily dependent on how successfully she adapts throughout the transition period. There is a disproportionate amount of health care and culling that occurs within 60 days after parturition. Minimizing large increases in NEFA and hyperketonemia and preventing subclinical hypocalcemia have been a key strategy in an attempt to improve overall herd health (because the dogma is that they are causal to disease). However, despite our industry's veterinary and scientific endeavors, herd health has arguably not improved with time (Table 1). The question then begs asking: "are we medicating the wrong problems"?

Table 1. National Animal Health Monitoring Systems

Culling Reason	NAHMS (1996)	NAHMS (2002)	NAHMS (2014)
Voluntary Reasons	21.3	19.3	21.1
Reproduction	25.3	26.5	24.2
Mastitis	25.1	25.9	24.4
Injury	4.1	6.0	5.2
Death	3.8	4.8	4.2
Disposition	0.9	0.9	-
Lameness	14.2	16.3	16.8
Other	3.9	4.1	-

Inflammation in the Transition Period

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

Although an overt inflammatory response is present around calving, numerous reports have described a reduction in immune competence during this time (Kehrli et al., 1989; Goff and Horst, 1997; Lacetera et al., 2005). Traditionally, hypocalcemia and hyperketonemia have been primary factors considered responsible for periparturient immunosuppression (Goff and Horst, 1997; Kimura et al., 2006; LeBlanc, 2020), however,

recent evidence suggests this is more complex than originally understood and that the systemic inflammatory milieu may be mediating the immune system to become “altered” and not necessarily “suppressed” around calving (Trevisi and Minuti, 2018; LeBlanc, 2020). Whether or not the “immune incompetence” frequently reported post-calving is causative to future illnesses or is a consequence of prior immune stimulation needs further attention.

The Importance of Glucose

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

Ketogenesis occurs when glucose is in short supply. This can come from a combination of factors including lack of substrate (i.e., reduced feed intake and ruminal fermentation) or high glucose utilization by other tissues (i.e., the immune system or mammary gland). When glucose demand is high, the TCA cycle intermediate oxaloacetate leaves the cycle to supply carbon for gluconeogenesis (Krebs, 1966). Oxaloacetate is also the molecule that combines with acetyl CoA (the end-product of adipose-derived NEFA) to allow the TCA cycle to continue progressing. If the TCA cycle is limited in its progression due to lack of oxaloacetate, acetyl CoA enters into ketogenesis. The link between onset of lactation, immune system activation, and lack of glucose leading to ketogenesis may help explain the metabolic footprint of a poorly transitioning dairy cow.

Metabolism of Inflammation

Inflammation has an energetic cost which redirects nutrients away from anabolic processes (see review by Johnson, 2012) and thus compromises productivity. Upon activation, most immune cells become obligate glucose utilizers via a metabolic shift from oxidative phosphorylation to aerobic glycolysis (not anaerobic glycolysis typically learned about in biochemistry classes), a process known as the Warburg effect. This metabolic shift allows for rapid ATP production and synthesis of important intermediates which support proliferation and production of reactive oxygen species (Calder et al., 2007; Palsson-McDermott and O’Neill, 2013). In an effort to facilitate glucose uptake, immune cells become more insulin sensitive and increase expression of GLUT3 and GLUT4

transporters (Maratou et al., 2007; O'Boyle et al., 2012), whereas peripheral tissues become insulin resistant (Poggi et al., 2007; Liang et al., 2013). Furthermore, metabolic adjustments including hyperglycemia or hypoglycemia (depending upon the stage and severity of infection), increased circulating insulin and glucagon, skeletal muscle catabolism and subsequent nitrogen loss, and hypertriglyceridemia occur (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005). Interestingly, despite hypertriglyceridemia, circulating BHB often decreases following LPS administration (Waldron et al., 2003a,b; Graugnard et al., 2013; Kvidera et al., 2017a). The mechanism of LPS-induced decreases in [BHB] has not been fully elucidated but may be explained by increased ketone oxidation by peripheral tissues (Zarrin et al., 2014). Collectively, these metabolic alterations are presumably employed to ensure adequate glucose delivery to activated leukocytes.

Energetic Cost of Immune Activation

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

Inflammation and Metabolic Disorders

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptions to support glucose sparing for milk production. Early lactation dairy cows enter a normal physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and typically enter negative energy balance (NEB; Drackley, 1999; Baumgard et al., 2017). During NEB, cows mobilize NEFA in order to partition glucose for milk production in a

homeorhetic strategy known as the “glucose sparing.” However, increasing evidence suggests that chronic inflammation may be an additional energy drain that initiates the sequence of these disorders (Bertoni et al., 2008; Eckel and Ametaj, 2016) and this is supported by human, rodent, and ruminant literature which demonstrate effects of lipopolysaccharide (LPS) and inflammatory mediators on metabolism and hepatic lipid accumulation (Li et al., 2003; Bradford et al., 2009; Ilan et al., 2012; Ceccarelli et al., 2015). We and others have demonstrated that cows which develop ketosis and fatty liver postpartum have a unique inflammatory footprint both pre- and post-partum (Ohtsuka et al., 2001; Ametaj et al., 2005; Abuajamieh et al., 2016; Mezzetti et al., 2019; Figure 3). Because the activated immune system has an enormous appetite for glucose, it can exacerbate a glucose shortage by both increasing leukocyte glucose utilization and reducing exogenous gluconeogenic substrates by inhibiting appetite. Reduced DMI is a highly conserved response to immune activation across species (Brown and Bradford, 2021) which can further increase NEFA mobilization and hepatic ketogenesis (Figure 3).

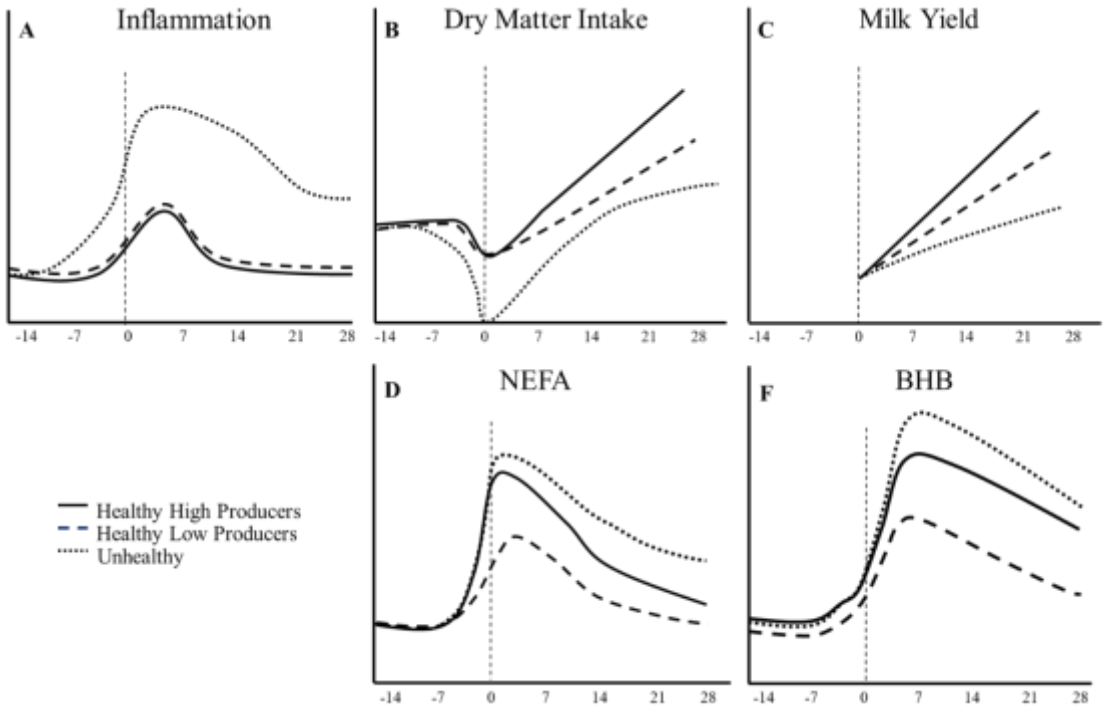

Figure 2. Transition period patterns inflammation (A), dry matter intake (B), milk yield (C), NEFA (D) and BHB (F) in healthy high producers (solid line), healthy low producers (dashed line) and unhealthy (dotted line).

Inflammation and Subclinical Hypocalcemia

Subclinical hypocalcemia (SCH) remains a prevalent metabolic disorder afflicting ~25% of primiparous and ~50% of multiparous cows in the United States (Reinhardt et al., 2011). Although no overt symptoms accompany SCH, it has been loosely associated with poor gut motility, increased risk of DA, reduced production performance (i.e., milk yield and feed intake), increased susceptibility to infectious disease, impaired

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

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

In addition to SCH, there are on-farm milk-fever situations that are biologically difficult to explain. For example, even while strictly adhering to a pre-calving calcium strategy, there remains a small percentage (~<1%) of cows that develop clinical hypocalcemia. Additionally, reasons for why a mid-lactation cow develops milk-fever are

not obvious. Further, there appears to be an undecipherable seasonality component to clinical hypocalcemia in the southwest and western USA that coincides with the rainy season. Inarguably, there remain some aspects of Ca homeostasis that continue to evade discovery.

Conclusion

New evidence and thinking around inflammation are challenging the traditional dogmas surrounding hypocalcemia, elevated NEFA, and hyperketonemia as the causative factors in transition cow disease. We suggest, based upon the literature and on our supporting evidence, that activation of the immune system may be the causative role in transition cow failure (rather than the metabolites themselves) as inflammation markedly alters nutrient partitioning and these metabolites as a means of supporting the immune response (Figure 3). More research is still needed to understand the causes, mechanisms, and consequences of immune activation and how to prevent immune activation or support its efficacy to provide foundational information for developing strategies aimed at maintaining productivity.

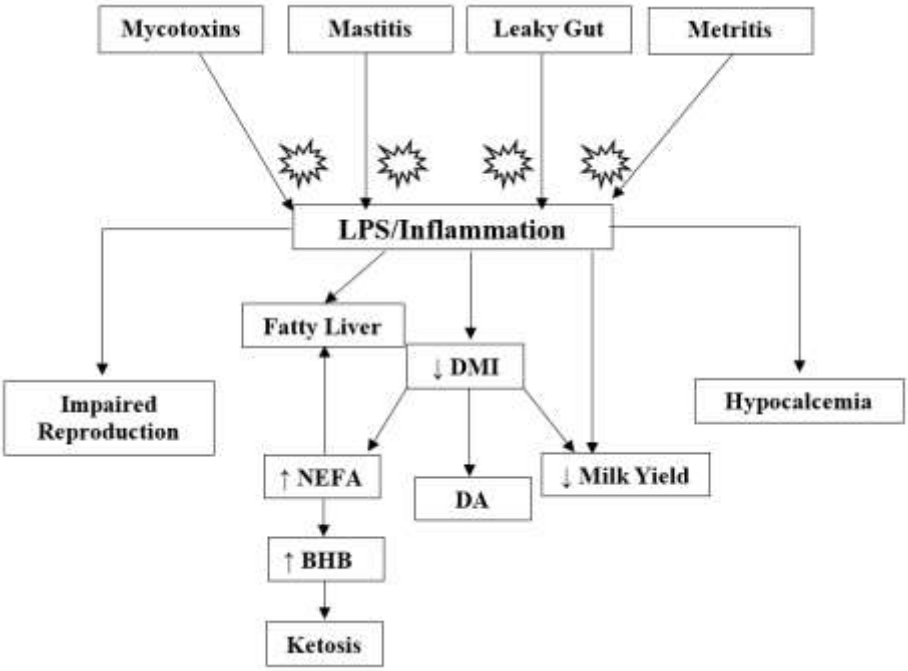

Figure 3. Potential downstream consequences of immune activation. In this model, decreased feed intake, hypocalcemia, excessive NEFA, hyperketonemia and hepatic lipidosis are not causative to poor transition cow performance and health, but rather a reflection of prior immune stimulation.

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Effects of Dietary Methionine and Calcium-Salts Enriched in Omega-3 Fatty Acids in Periparturient Dairy Cows: A Trial Update

T. L. France[1], A. Javaid[1], K. S. Juarez-Leon[1], and J.W. McFadden[1]
[1]Department of Animal Science, Cornell University, Ithaca, NY

Introduction

The transition period (3 weeks before through 3 weeks postpartum), is a critical life event for the dairy cow. Around calving, cows involuntarily reduce feed intake, and nutrient demands increase to support fetal growth and milk synthesis. A systemic inflammatory response occurs at parturition, which can develop with fatty liver disease and ketosis. Nutritional strategies that improve health and milk production for the transition dairy cow are of interest. Two nutrients that deserve attention include methionine (Met) and omega-3 fatty acids (n3FA) because of their role in hepatic transmethylation (McFadden et al., 2020). Specifically, Met is utilized by Met adenosyltransferase to generate *S*-adenosylmethionine, which donates methyl groups to phosphatidylethanolamine to form phosphatidylcholine (PC) by the actions of phosphatidylethanolamine-*N*-methyltransferase (PEMT). In non-ruminants, evidence suggests that PEMT prefers phosphatidylethanolamine enriched in very long chain fatty acids (FA) such as docosahexaenoic acid (C22:6; DHA; DeLong et al., 1999). PC synthesis is a critical component of very-low-density lipoproteins, which aid in reducing fatty liver while partitioning lipids to the mammary gland (Watkins et al., 2003). We suspect that this pathway is downregulated during the transition period due to insufficient dietary supply of Met and n3FA. The objective of this study was to investigate the effects of dietary Met and calcium-salts (CS) of FA enriched without or with eicosapentaenoic acid (C20:5; EPA) and DHA on milk production, and hepatic methyl donor metabolism and function in periparturient cows.

Materials and Methods

In a randomized complete block study design, 79 multiparous Holstein cows were balanced by parity and previous 305-day ME and assigned to 1 of 4 dietary treatments (n = 19/treatment): 1) Met unsupplemented (-Met) with CS of palm oil not enriched in n3FA (-n3FA; 0% EPA and DHA; EnerGII; Virtus Nutrition,Corcoran, CA), 2) Met supplemented (+Met; Smartamine M; Adisseo Inc., Antony, France) with -n3FA, 3) -Met with CS enriched in n3FA (+n3FA; 3.2% of EPA and DHA; EnerG-3; Virtus Nutrition), or 4) +Met with +n3FA from wk -3 prior to expected calving through wk 4 of lactation. Cows were fed corn silage-based total mixed rations, pre- and postpartum, which were formulated to provide Met at ≤ 0.96 or ≥ 1.13 g Met/Mcal metabolizable energy for -Met and +Met, respectively. CS were fed at 1.5% FA (% ration dry matter) for all treatments pre and postpartum. Liver biopsies were performed at -1, +1, and +3 wk, relative to expected or actual parturition. Blood was collected weekly. Cows were milked thrice daily and milk samples were collected twice a week. Pre- and postpartum data were analyzed using PROC MIXED of SAS v9.4. Pre-planned contrasts included:

1) effect of Met (-Met vs. +Met), 2) effect of n3FA (-n3FA vs. +n3FA), and 3) effect of co-supplementation (+Met/+n3FA vs. +Met/-n3FA and -Met/+n3FA).

Results

Although prepartum dry matter intake (DMI) was not modified by diet, +Met and +n3FA cows had greater postpartum DMI, relative to cows unsupplemented with Met or n3FA, (P = 0.01 and 0.03, respectively). Met intakes were greater in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA prepartum (P = 0.01; 25.6 vs. 22.7 g/d, respectively) and postpartum (P < 0.01; 62.4 vs. 48.7 g/d, respectively). Cows fed +n3FA consumed more EPA ad DHA pre- and postpartum as compared to -n3FA (P < 0.01). Yields of energy-corrected milk (ECM) were greater in +Met and +n3FA compared to -Met and -n3FA (P = 0.01 and 0.05, respectively). Energy-corrected milk yield had an overall increase of 5.53 kg/d in +Met/+n3FA compared to -Met/-n3FA. Similar results were observed for yields of fat-corrected milk, milk fat, and milk protein. Milk protein % were greater (P = 0.03) in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA (3.15, 3.11 and 2.95%, respectively). Milk fat % tended to be greater (P = 0.10) in +Met/+n3FA, relative to +Met/-n3FA and -Met/-n3FA (5.41, 5.25, and 5.06%, respectively).

Postpartum body weight change (wk 1 to 4) was lower for +Met supplemented cows, relative to -Met cows (P = 0.01). Circulating creatinine, globulin, total FA, aspartate transaminase, serum amyloid A, oxidized and reduced glutathione, and total cholesterol concentrations were modified by time ($P \leq 0.01$) but not treatment. At calving, -Met/-n3FA had greater plasma triglyceride concentrations, relative to +Met/-n3FA and -Met/+n3FA ($P \leq 0.01$), whereas +Met/+n3FA tended to have greater plasma glucose concentrations, relative to rest (P = 0.09). Serum total protein and albumin concentrations were greater in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA (P = 0.03 and 0.06, respectively). Liver *S*-adenosylhomocysteine concentrations tended to be greater in +Met/-n3FA and -Met/+n3FA diets at +3 wk postpartum, relative to -Met/-n3FA (P = 0.05 and 0.09, respectively). These data suggest enhanced activation of PEMT. Postpartum liver functionality index (Bertoni and Trevisi, 2013) values tended to be greater for +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA (P = 0.08). These findings suggest enhanced liver function in cows supplemented with Met and n3FA, relative to cows supplemented with Met or n3FA alone.

Conclusion

In conclusion, transition cows supplemented with RP-Met or CS enriched in EPA and DHA experienced increased milk yield, ECM, and FCM as well as fat and protein yields with RP-Met or EPA/DHA supplementation, increased milk protein content with RP-Met, enhanced body weight (EPA/DHA) or reduced body weight loss (RP-Met), enhanced liver *S*-adenosylhomocysteine concentrations with RP-Met or EPA/DHA, and enhanced liver Met concentrations with RP-Met feeding. Co-supplementation of RP-Met and EPA/DHA increased milk fat and protein content, plasma glucose concentrations at calving, and liver function (LFI), relative to cows fed RP-Met or EPA/DHA alone. Future

research should consider how changes in the FA feeding level and composition of close-up diets, notably EPA and DHA content, influences postpartum health outcomes and milk production in dairy cattle.

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Effects of Heat Stress and Dietary Organic Acids and Botanicals on Hepatic One-carbon Metabolism

V. Sáinz de la Maza-Escolà[1,2] and J. W. McFadden[1]
[1]Department of Animal Science, Cornell University, Ithaca, NY.
[2]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy.

Introduction

Reductions in milk protein content and yield motivated to investigate the impact of heat exposure on protein metabolism in dairy cows (Gao et al., 2017). McGuire et al., (1989) confirmed that heat stress (HS) reduces the intestine absorptive capacity of amino acids (AA), and this is probably explained by the loss in intestinal integrity (Koch et al., 2019). Very recently, our group has demonstrated that heat-stressed lactating dairy cows develop with an increased total-tract gut permeability (Fontoura et al., 2022). This condition leads to leakage of bacteria and their endotoxin (e.g., lipopolysaccharide [LPS]) into the bloodstream, which in turn triggers an immune response. This is associated with hepatic removal and utilization of AA to produce acute phase and heat-shock proteins (Rius et al., 2019). In addition, the activation of the immune system increases glucose consumption (Kvidera et al., 2017). It is well known that heat-stressed dairy cows have reduced feed intake, which partially explains the lowered production responses (Baumgard and Rhoads, 2013). Despite this hypophagia, increasing levels of circulating insulin concentrations are common in heat-stressed cows (Wheelock et al., 2010; Fontoura et al., 2022). Insulin is a hormone that inhibits lipolysis and might induce muscle protein breakdown to support gluconeogenesis. The end product of AA catabolism is urea. Robust increases in plasma levels of urea-nitrogen are a repeatedly observed response in heat-stressed dairy cows (Wheelock et al 2010; Gao et al., 2017; Fontoura et al., 2022).

Excessive circulating urea can cause toxicity, even in ruminants (Whitehair, 1989). Urea can damage cells by disrupting the osmotic balance and as a consequence require osmoprotective responses to counteract it. Research from human and rodent species tells us that under hyperosmotic conditions, liver and kidney cells accumulate methylamine osmolytes such as betaine or glycerophosphocholine (GPC; Okazaki et al., 2018). The abundance of betaine transporters increases under osmotic stress (Kempson et al., 2014). In response to changing levels of NaCl and urea, Burg and Gallazzini (2009) identified a reduction in the activity of glycerophosphocholine phosphodiesterase (GPC-PDE), the enzyme that degrades GPC to choline, and as a result they observed an intracellular accumulation of GPC. The literature reports higher accumulations of GPC rather than betaine, and presumably it is due to a lower metabolic cost. The inhibition of an enzyme doesn't require extra energy whereas betaine transporters are against gradient concentration (Burg and Peters, 1998).

GPC is synthesized from the degradation of phosphatidylcholine (PC) and broken down into choline and α-glycerophosphate. The inhibition of GPC-PDE can

reduce choline recovery and negatively affect the CDP pathway to support PC synthesis. Choline also has a one-carbon unit that is called methyl group, which can be used in the one carbon metabolism. Choline can enter the methionine cycle through the oxidation into betaine. The methionine cycle is coupled to the folate cycle to drive the synthesis of *S*-adenosyl methionine (SAM; the Universal Methyl Donor). SAM can then provide methyl groups to be used for DNA synthesis, PC synthesis via the PEMT pathway or to maintain the redox status through the transsulfuration pathway (McFadden et al., 2020).

We need to develop nutritional strategies to mitigate heat stress effects and gut-liver axis consequences. Dietary supplementation of organic acid and pure botanicals (OA/PB) has been shown to improve animal performance by enhancing gastrointestinal health in swine and poultry species (Hassan et al., 2020, Grilli et al., 2015b). Dietary OA/PB supplementation was also investigated in dairy calves experiencing moderate heat stress (Fontoura, 2022b), and it was observed that dietary OA/PB supplementation partly restored dry matter intake (DMI).

A recent study conducted at Cornell University investigated the effects of heat stress conditions and dietary OA/PB supplementation in lactating Holstein dairy cows (Fontoura et al., 2022). In this study, OA/PB supplementation tended to elevate DMI and restore milk yield and energy-corrected milk. OA/PB was able to have a higher protein yield and lower milk and plasma urea, showing that it was able to improve N incorporation in the milk. OA/PB also showed a modest but real improvement in total-tract gut permeability and an improved intestinal health supported by a reduced concentration of plasma LPS-binding protein, compared to their HS control counterparts. We hypothesized that HS will develop with accumulation of glycerophosphocholine (GPC) in the liver and that dietary OA/PB will prevent it. Our objective was to evaluate the effects of HS and dietary OA/PB supplementation on liver one-carbon and phospholipid metabolism.

Materials and Methods

Liver samples from the trial Fontoura et al., (2022) were used for these analyses. Briefly, forty-six Holstein cows (208 ± 4.65 d in milk [mean ± SD], 3.0 ± 0.42 lactations, 122 ± 4.92 d pregnant) were enrolled in a study with a completely randomized design. Following a 7 d acclimation in thermoneutrality (temperature-humidity index [THI] 68), cows were assigned to 1 of 4 groups: thermoneutral conditions (TN-Con, n = 12), HS conditions (HS-Con, n = 12; diurnal THI 74 to 82), TN conditions pair-fed to match HS-Con (TN-PF, n = 12), or HS fed OA/PB (HS-OAPB, n = 10; 75 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; Aviplus® R; Vetagro S.p.A) for 14 d. Cows were milked twice daily and fed a corn-silage based total mixed ration top-dressed without (triglyceride only) or with OA/PB. Liver biopsies were sampled at d 6 of acclimation (baseline) and d 13 of environmental conditioning and analyzed by liquid-chromatography mass-spectrometry (LC-MS; Division of Nutritional Sciences; Cornell University). Data were analyzed using a general linear mixed model including fixed effects of treatment and block, the random effect of

cow, and lactation, days in milk and baseline values included as covariates. Planned contrasts included HS-Con vs. TN-Con, HS-Con vs. TN-PF, and HS-Con vs. HS-OAPB. Main effects were declared significant at $P \leq 0.05$ and trending towards significance at $0.05 < P \leq 0.15$.

Results

Hepatic choline concentrations were reduced in HS-Con compared to TN-Con ($P = 0.02$) and TN-PF ($P = 0.05$). No changes were observed in hepatic phosphocholine or lysophosphatydilcholine concentrations, but HS-Con increased PC compared to TN-PF ($P < 0.01$). In agreement with our hypothesis, HS-Con accumulated greater amounts of GPC compared to thermoneutrality ($P < 0.01$) and OAPB feeding was able to significantly prevent this accumulation ($P = 0.02$). Similar results were obtained for the GPC:choline ratio (negatively correlated to the activity of the GPC-PDE), where HS-Con had greater values compared to thermoneutrality ($P < 0.01$) and HS-OAPB tended to lower the ratio ($P < 0.14$). We did not see changes in methionine or dimethylglycine but instead, betaine was increased in TN-PF group compared to HS-Con ($P < 0.01$). SAM tended to decrease in HS-Con compared to TN-Con ($P < 0.10$), which could be a consequence of the lower choline concentration. Although no differences were detected in *S*-adenosyl homocysteine (SAH), HS-Con had a lower ratio SAM:SAH compared to TN-Con ($P = 0.05$) and HS-OAPB was able to restore it ($P = 0.06$). This ratio is the marker that indicates the remethylation capacity of the liver.

Conclusion

We conclude that heat stress develops with methyl donor deficiency in parallel with an impaired N metabolism and that supplementation of OA/PB improves the remethylation capacity in the liver. On-going transcriptomic analyses will provide a better understanding of the hepatic metabolism of dairy cows exposed to heat stress.

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Hand-held NIR Devices Used to Predict Grass Percentage in Alfalfa-grass Mixtures

Tacoma-Fogal, R., M. Boggess, J.H., Cherney, M. Digman, and D.J.R. Cherney.
Department of Animal Science
Cornell University

Introduction

Recent advancements in the field of infrared reflectance spectroscopy (NIRS) have generated interest in the agricultural industry regarding the development of handheld spectrometers to estimate forage nutritive value (Borba et al., 2021; Giussani et al., 2022; Rego et al., 2020). Though NIRS has been used to evaluate forage nutrients since the 1980's, advances in spectrometers that minimize background noise and enhance stability of measurements have allowed the possibility of NIR spectroscopy use at the farm level (Digman et al., 2022; Gorla et al., 2022). Over half a dozen handheld NIR scanners have become more readily available over the past decade and this number is expected to increase significantly as the technology develops, providing a rapid, accurate, cost-effective method for the average consumer to conduct their field analyses (Beć et al., 2021). However, a major drawback associated with NIRS involves the need for calibration development, a procedure that requires robust assessment to capture the wide variation of forage quality that exists (Evangelista et al., 2021; Gorla et al., 2022). Continued evaluation of spectrometers and calibration equations is therefore needed to validate the performance of handheld NIR instruments in the agriculture sector (Beć et al., 2020; Catunda et al., 2022; Rukundo et al., 2020).

The Neo Spectra Scanner is one of the few hand-held NIR units that allows users to access the spectra and develop calibrations. This device accurately predicts forage nutritional value in dried, ground alfalfa and grass samples (Digman et al., 2022; Gorla et al., 2022). Other handheld NIR devices have been used to analyze different nutritive parameters of fresh forages (Carreira et al., 2021; Thomson et al., 2022), but calibrations estimating grass percentage in grass:aflafa mixes have not appeared in the literature. Additionally, various scanning techniques have been utilized, but no study has made specific comparisons of methodology.

On-farm NIR analysis will be particularly important for alfalfa-grass producers, to improve field management and optimize nutrient management by reducing variability in dairy rations, thus creating opportunities for more sustainable dairy farm production systems (Cherney et al., 2020). With over 84% of alfalfa sown in New York State grown in combination with a perennial grass, providing farmers with the tools to estimate grass content of mixtures using a hand-held unit will be a cost-effective solution to the problem of evaluating and managing variability in alfalfa-grass composition (Karayilanli et al., 2016). The Neo Spectra Scanner has great potential with a wide NIR spectral range of 1,350 to 2,500 nm, given all other hand-held instruments have a narrower NIR scanning range (Beć et al., 2021; Giussani et al., 2022). The objective of this study is to determine

a scanning technique between two alternatives, and to develop a calibration equation for the Neo Spectra Scanner for estimating grass percentage in alfalfa-grass fresh mixtures.

Materials and Methods

Sample Collection and Preparation

Samples of either grass and alfalfa were collected during the growing seasons of 2021 and 2022 between May and October. Forage samples were collected from six privately owned, commercially operated dairy farms located within a 40-mile radius around Ithaca, NY. Samples consisted of a wide variety of forage maturities in an attempt to cover the range of grass:alfalfa stands that can exist on a dairy farm in the NE. Several different alfalfa cultivars were included, along with grass cultivars from seven grass species: tall fescue (*Lolium arundinaceum*), meadow fescue (*Schedonorus pratensis*), orchardgrass (*Dactylis glomerata L*.), reed canarygrass (*Phalaris arundinacea L.*), Brome grass (*Bromus inermis*), Quackgrass (Elymus repens), and timothy (*Phleum pratense L.).* Approximately three kilograms of each pure forage stand was hand-harvested at a 10cm stubble height with a battery-powered clipper, from an area roughly 2 m^2 that varied depending on the growth stage. Samples were coarsely chopped as soon as possible using a HEGE 44 Laboratory chopper (Wintersteiger, Salt Lake City, UT).

Mixed samples were made up of pure chopped grass and pure alfalfa with an increasing proportional mix of fresh grass to alfalfa. Mixed samples that ranged from approximately 20% to 80% grass were made and combined subsamples were mixed well before being scanned.

Instrument and Scanning Procedure

A NeoSpectra-Scanner (Si-Ware-Systems, Cairo, Egypt) was used to analyze the fresh chopped forage samples. Spectra collected were in the range of 1350-2550 nm (257 wavelengths) using the proprietary application provided on an Android tablet. Each sample was distributed uniformly in a 410 x 40 x 13 cm deep rectangular container, maintaining a thickness of at least 5 cm (Figure 1). The stationary scanning procedure involved placing the scanner firmly on the forage sample for four seconds per scan and taking four scans at different locations in the container. The sliding scan involved direct contact of the scanner with the forage material for four seconds as the device was dragged over the forage sample. Between each sliding scan, approximately 2cm was removed from the top layer to ensure scanning of a different part of the sample. As with the stationary scanning technique, four sliding scans were taken for each sample.

Figure 1: The Neo Spectra instrument and sample in preparation for scanning.

Determination of Grass Percentage

Grass percentage of a mixed sample was estimated by a multi-step procedure. After each forage collection, one pure sample of grass and one of alfalfa, each weighing approximately 250g, were oven dried for 48 hours. The proportion of dry matter of each pure fresh forage type was determined. These proportions were utilized to estimate the dry matter weight of both the grass and the alfalfa part of a mixed sample. To estimate the grass percentage of a mixed sample the dry matter weight of the grass was divided by the sum of the dry matter weights for grass and alfalfa, and then multiplying by 100.

Model Development

All data manipulation and plotting were performed in MATLAB version 9.12 R2022a (MathWorks, Inc., Natick, MA, USA). Spectra included two datasets, stationary and sliding. The four spectra from repeated scans were averaged to obtain one spectra per sample. For each of these two datasets, a randomly selected portion (25%) was held out for external validation.

Calibration models were built in PLS Toolbox version R9.1 (2022 Eigenvector Research, Inc., Manson, WA, USA) using partial least squares (PLS) regression. The dependent variable was the percentage of grass, and the independent variables were the averaged spectra. Preprocessing methods applied were mean-centering (MC), mean-centering and Savitzky-Golay smoothing (SG), both of which have been previously applied in NIR forage research (Berzaghi et al., 2021; Digman et al., 2022; Gorla et al., 2022; Rego et al., 2020). Five-fold cross-validation was used to determine the optimal number of latent variables for the PLS analysis. The performance of each calibration model was evaluated by external validation. The model obtained using the calibration

data was fit to the held-out data and the root mean squared error on the held-out data was determined, which is known as the root mean square error of prediction (RMSEP). For each model, R-squared was also reported and used as a model performance measure.

Results

Spectral Data

In total, 534 samples were scanned resulting in 4272 scans. Variability in spectral data of stationary scans was greater than that of sliding scans (Fig. 2).

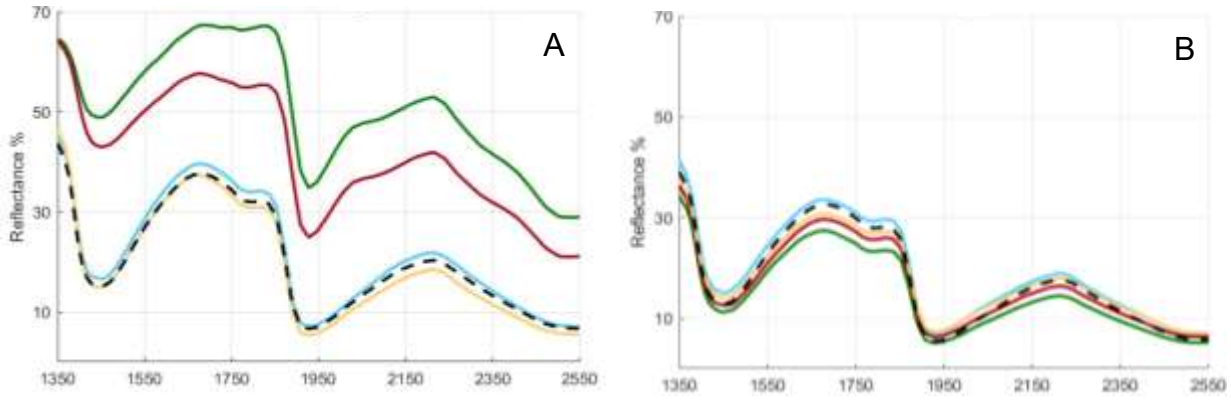

Figure 2: Reflectance spectra for the four stationary (A) and four sliding (B) scans for sample #414.

Of the 534 averaged spectra, 133 were held out for external validation; the same sample numbers were held out for both stationary and sliding scanning techniques. Average reflectance of each sample mean-centered (Fig. 3), for both scanning techniques reveal that there is considerably more variability in spectra across the 100% grass samples (red) than with 100% alfalfa samples (blue). The pure alfalfa spectra are typically below zero in these figures, while the 100% grass spectra are largely above zero, illustrating the capacity of the NIR spectrometer to predict grass % in a grass:alfalfa fresh mix.

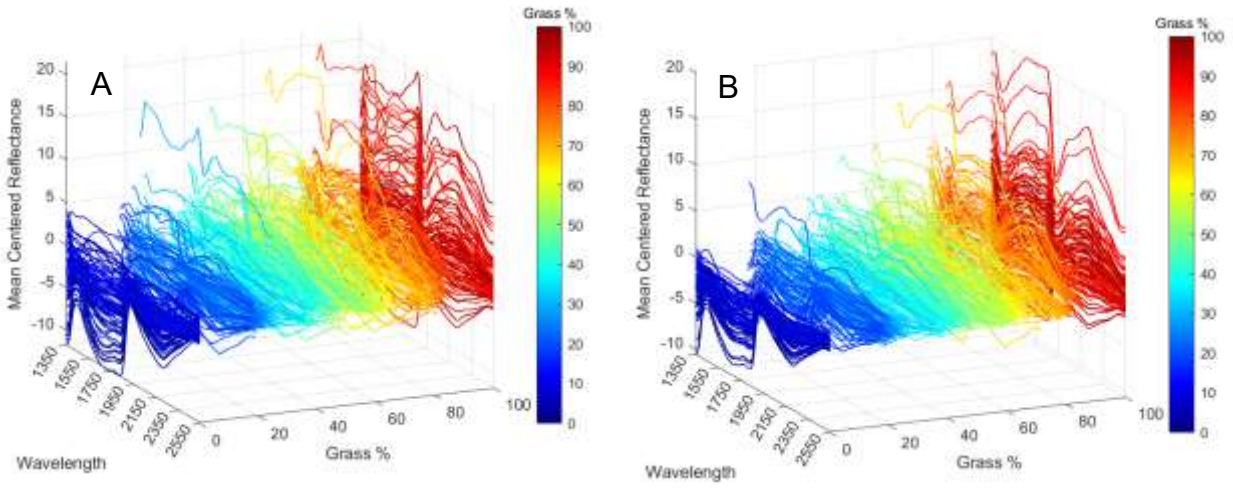

Figure 3: Mean centered average reflectance by wavelength and grass percentage using the stationary technique (A) and the sliding technique (B) (n=401).

Calibration and Validation Performance

Comparison of RMSE calibration to RMSE cross validation indicated that using 15 latent variables did not result in over fitting and therefore 15 latent variables were chosen for the analysis (Table 1). Preprocessing methods performed on both calibration and prediction datasets that resulted in the highest R-squared and lowest RMSE were MC. Calibration model performance was good with an R-squared of 85% for sliding and 72% for stationary. Reduction in R-squared from calibration to prediction was 7% for both stationary and sliding illustrating that the calibration model adequately estimates grass percentage in a grass:alfalfa mix sample.

Table 1:Calibration, cross-validation and prediction R-squared and RMSE for sliding and stationary techniques for reflectance spectra.

	Calibration		**Cross Validation**		**Prediction**	
	R-squared	**RMSE**	**R- squared**	**RMSE**	**R-squared**	**RMSE**
Reflectance						
Stationary						
MC	71.8%	18.308	63.3%	20.927	65.1%	19.796
SG	71.8%	18.331	63.6%	20.928	65.1%	19.806
Sliding						
MC	85.0%	13.373	80.6%	15.259	78.0%	15.613
SG	85.0%	13.392	80.6%	15.262	77.9%	15.619

Notes: RMSE=root mean square error; MC=mean-centered; SG= mean-centering and Savitzky-Golay smoothing.

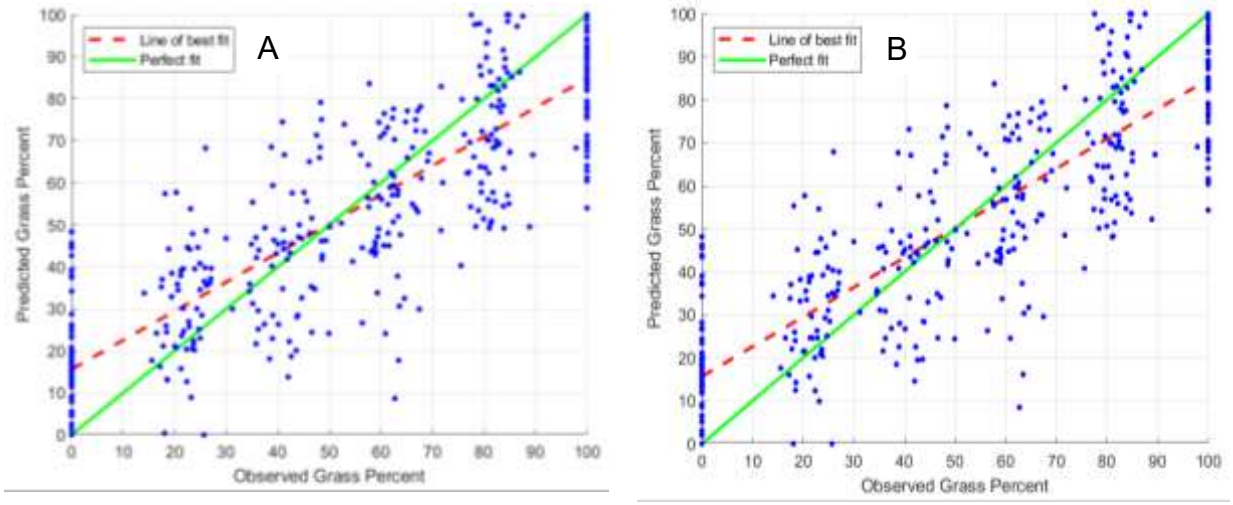

Figure 4: Scatter plot of observed and predicted grass percentage for stationary (A) and sliding (B) scanning technique.

A scatterplot of the actual grass percentage and the grass percentage predicted by the calibration equation indicates that the correlation between the observed and the predicted is 72% for stationary and 85% for sliding (Fig. 4). The red dashed line of best fit is below the green perfect fit line for the higher percentages of grass suggest that the calibration equation is underestimating the grass percentages in mixtures that contain proportionally more grass compared to alfalfa. Similarly, at the low end when there is a low percentage of grass in the forage mix, the model overestimates the amount of grass with about a 10% error.

Discussion

The sliding technique provided a less variable spectra and performed better than the stationary technique in modelling. A calibration equation was developed that results in a correlation in excess of 90% on the calibration data and an R-squared close to 80% on data that was not used in the development of the equation (Table 1). No previous research has been published on the estimation on the percentage of grass within a fresh mixed grass:alfalfa sample using handheld NIRS technology. This technology has however, been applied to predicting the nutritive value of fresh forage, specifically neutral detergent fiber (NDF) and crude protein (CP). Carreira et al. (2021) developed a calibration equation using a handheld NIR device on 85 pasture samples. They achieved moderate predictive precision to estimate the NDF and CP of the grass (R^2 = 0.69 and 0.84, respectively) on the calibration data. Murphy et al. (2022) evaluated fresh forages in Ireland where they developed NIRS calibrations of the dry matter (DM) and CP content of fresh perennial ryegrass in which they achieved R-squared of 86% and 84%, respectively, on held out data. However, the fresh grass samples are all from a single variety grown on a research facility and a benchtop spectrometer was used. It has been previously reported that handheld NIR instruments are subject to several sources of variability that are not associated with benchtop spectrometers (Gorla et al., 2022). More recently, Thompson et al. (2022) sampling ryegrass on a commercially run dairy farm

evaluated the efficacy of four different handheld NIR devices in comparison with a benchtop spectrometer, the industry gold standard to estimate forage nutritive value (Thomson et al., 2022). Given that our results had a predictive precision over 75% when using the sliding scanning technique, these previous studies indicate that our calibration equation compares favorably when estimating grass percentage in grass:alfalfa mixtures.

To date, comparative analysis of scanning techniques have not appeared in the literature and results from this study provide the first insight into this type of assessment. Numerous studies over the last decade have been published investigating the use of handheld NIR spectrometers in the agricultural industry and methods have included either stationary or sliding scanning techniques of forage or vegetable material (Berzaghi et al., 2021; Borba et al., 2021; Cherney et al., 2021; Digman et al., 2021; Digman et al., 2022; Digman & Runge, 2022; Rukundo et al., 2020), but none have directly compared the two methods discussed here. One comparative study of scanning concentrated only on the amount of time spent scanning and concluded that five seconds was adequate (Gorla et al., 2022).

Practical implications from this research include providing producers and nutritionists with tools to accurately record forage stand composition. This serves to improve the ability for alfalfa-grass producers to optimize field management and reduce variability in dairy rations, resulting in more environmentally and economically sustainable farming systems. Previously producers have had to rely on visual inspection to estimate forage composition and this research demonstrates the functionality and feasibility of on-farm hand-held NIRS devices offers the opportunity for real-time evaluation of forage composition that are rapid and cost-effective.

Our study is the first to estimate the percentage of grass in a fresh grass:alfalfa mixture using hand-held NIR devices. Compared to similar studies also using handheld NIR devices to analyze forages, our sample size was large (n=534) and may have contributed to the relatively high correlation between the observed and predicted grass percentage using the sliding scanning technique. However, these data also demonstrate that technique is rugged over a wide range of samples. Questions remain about whether transforming to absorbance may lead to a better calibration equation. In the literature, various statistical methods identifying outliers have been employed, and this is worth further investigation.

This research has demonstrated that hand-held NIR technology can assist producers to estimate grass percentage in grass:alfalfa forage mixtures.

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Abomasal Infusions of Essential and Non-Essential Amino Acids to Evaluate Productive Efficiencies, and Energy and Amino Acid Utilization in Lactating Dairy Cattle

A. F. Ortega[1], A. Zanotti[1], R. Coombe[1], N. Fox[1], P. A. LaPierre[1], J. Marumo[1], C. Coon[2], D. M. Barbano[2], M. E. Van Amburgh[1]
[1]Department of Animal Science
[2]Department of Food Science
Cornell University

Introduction

Over the past decades, dairy cattle have become more efficient at producing nutrient-rich foods for human consumption for multiple reasons. Genetic selection over the last 100 years has had a profound effect on the productivity of cattle to the point that selection for just production has been modified to include other traits related to productivity (Miglior et al., 2017). Nutritionally, the advancement of diet formulation models, like the NASEM (2021) and Cornell Net Carbohydrate and Protein System (CNCPS), has allowed nutritionists and producers to offer a diet balanced to meet the animal's nutrient requirements, as well as the requirements of the ruminal microbiome. One of those requirements is nitrogen (N) which can be obtained through crude protein, or more specifically through its building blocks- amino acids (AA). In ruminants, N feeding is complicated as the rumen requires ammonia from the diet, urea recycling and AA, especially branch chain AA (BCAA) for fiber digestion (Bryant, 1973). Whereas the cow requires AA that are supplied from dietary, microbial, and endogenous sources.

Lately, there has been increasing pressure to improve cattle productive efficiency to meet global environmental goals and increase income over feed cost (IOFC). The updated version of the CNCPS (v.7) (Higgs, 2014, Dineen, 2020, LaPierre, 2021), has been modified to account for protein transactions on a N basis, allowing the model to better predict an essential AA (EAA) supply and it does this by determining the optimum amount of EAA on a gram basis per megacalorie of metabolizable energy (ME; g EAA/ Mcal ME). This approach has been evaluated in recent experiments, and when EAA supplies were formulated to meet the expected ME allowable requirement, energy-corrected milk (ECM) significantly increased (LaPierre et al., 2019, LaPierre et al., 2020, Benoit et al., 2021). The model's mechanistic rumen sub-model estimates microbial growth and supply, which can often be more than 50% of the metabolizable AA supply. This has allowed for less N to be fed to high producing lactating cows, as well as decreasing the amount of N being excreted in the manure. Predicted metabolizable protein (MP) supply is the summation of EAA and non-essential AA (NEAA), with a breakout of EAA into nine individual AA supplies and one conditionally essentially AA (Arg) supply but no such breakdown of NEAA supply. Most studies evaluating AA supply in cattle diets have focused solely on EAA formulation independent of the overall metabolizable protein (MP) supply, with little consideration for the supply of individual NEAA, which are not entirely dispensable AA. Besides being the building blocks of protein, NEAA play an extensive metabolic role throughout the body. Further, there are a

lack of data to determine the dietary requirements for nutritionally relevant NEAA (Wu et al., 2013, Wu et al., 2014), although the critical functions of NEAA have been reiterated for metabolism, lactation, growth, reproduction, and health (Wu, 2009, 2010, 2022) mainly in monogastric animals with limited data on ruminants.

The concept of NEAA requirements could seem counterintuitive given that they have traditionally been thought to not be necessary for diet formulation due to the animals' capability to synthesize NEAA in appropriate levels de novo (Wu et al., 2013). However, synthesis of de novo NEAA occurs from the C and N of several substrates, including the EAA, which are taken up in excess by the mammary gland (MG; Doepel and Lapierre, 2010), and in other tissues for other purposes (Wu, 2014). To maintain whole body homeostasis, all AA are required to interact with each other and other metabolites, across all tissues to regulate metabolic pathways, gene expression, immunity, oxidative defense, secretagogues, protein turnover, and cell signaling and physiology (Wu, 2009). Therefore, even though the body can produce NEAA from other substrates, they are metabolically required, and their synthesis can be energetically unfavorable. If other substrate resources are limiting, the need to synthesize NEAA might be a limiting step.

Nearly all AA are considered glucogenic, except for Lys and Leu, because they are involved in the production of glucose through gluconeogenesis, but some are used more extensively than others (Brosnan, 2003, Wu, 2022). Wolff and Bergman (1972) infused five radiolabeled individual NEAA to investigate their metabolic fate. It is important to note that this technique tends to underestimate the contribution of AA to glucose because they don't consider isotope dilution (Lobley, 1992). Of the five, Ala and Glu were demonstrated to contribute the most C for glucose synthesis, with about 20.8% and 17.6% of their turnover being used for this purpose, respectively. Although the conversion of Asp to glucose wasn't very high, it accounted for a quarter of its turnover. It is not a surprise Ala is used in high amounts for glucose synthesis, due to its involvement in the glucose-Ala cycle, which transports pyruvate from skeletal muscle to the liver for gluconeogenesis (Felig et al., 1970). Also, Glu, with its related AA (Gln, Asp, Asn), is more complex to explain given their many roles in the body. These AA are essential carriers of C- and N-skeletons throughout the body for DNA synthesis, TCA cycle intermediates, ammonia detoxification, and antioxidants, such as glutathione formed with Glu, Gly and Cys (Wu, 2022). And Glx (Glu + Gln) are an important source of energy for immune cells and intestinal cells, as shown by the high disappearance of these AA in the GIT of ruminants and non-ruminants (Berthiaume et al., 2001, Burrin and Stoll, 2009, Rhoads and Wu, 2009). And glucose, besides being used for lactose synthesis, is catabolized to acetyl-CoA, which is oxidized through the TCA cycle. The TCA cycle is important for production of NAD(P)H and $FADH_2$, which are used in the electron transport chain for ATP production. In addition, NADPH is required to synthesize fatty acids (FA) de novo (DNFA) and their elongation. In ruminants, optimal rumen function is needed for production of acetate, which, along with glucose, provide the C (acetyl-CoA) for de novo synthesis of FA to occur and their elongation (Palmquist, 2006). This synthesis is highly dependent on the enzymatic action of acetyl-CoA carboxylase, which uses a biotin carrier protein covalently bound to a Lys side chain (Cronan, 2001, Palmquist, 2006, Barbano et al., 2014). Moreover, Ser and Gly are also involved in milk fat metabolism and related to

complex lipid synthesis of phospholipids and sphingolipids (Palmquist, 2006, McFadden et al., 2020). These two AA, with Cys and Met, are needed for one-carbon metabolism to provide methyl donors and antioxidants (McFadden et al., 2020). In summary, all 20 AA, their products and their interactions vary across different organs and physiological states and should be considered when deriving AA requirements for diet formulation.

There have been numerous infusion studies of EAA, either of all EAA, mixtures of EAA, and the individual EAA, particularly of the most limiting EAA in lactating ruminant diets Met, Lys, and His (Schwab et al., 1976, Appuhamy et al., 2011, Zanton et al., 2014). Moreover, since casein makes up most of the protein in milk protein, it has been used in infusion studies on lactating dairy cows and transition cows, with modest milk protein or milk yield response (Schwab et al., 1976, Choung and Chamberlain, 1993, Larsen et al., 2014). Earlier studies in ruminants had some focus on a few NEAA (Mepham and Linzell, 1966, Schwab et al., 1976) but mainly regarding glucose catabolism and metabolic fate of AA (Black et al., 1955, Black et al., 1968, Wolff and Bergman, 1972). And most of the data on NEAA metabolism have been generated in studies with monogastric animals (Wu, 2009, Wu et al., 2013, Wu et al., 2014). Most recently the AA profile of casein, or milk protein, were used to estimate the requirement for the EAA and NEAA for evaluation, also seeing minimal response (Metcalf et al., 1996, Doepel and Lapierre, 2010). In the current work, we have the capacity to formulate the diets with much more precision and accuracy than has been done previously (Higgs, 2014, LaPierre, 2021). The application of the CNCPS v7 allows dietary formulations to target a more precise EAA MP supply, which by calculating their difference should provide a better estimate of the NEAA supply and requirements on a macro scale. Our objectives for this study were to investigate if and how productive efficiency of a lactating cow can be changed when both EAA and NEAA requirements are met, and if an excess of NEAA supply would result in an increase or decrease in the efficiency of both energy and AA. The approach using ME as the basis of estimating AA requirements will provide an updated procedure to quantitatively determine whether NEAA are critical AA requirements of the lactating dairy cow. Also, this will allow for the evaluation of whether a quantitative NEAA supply is necessary to report in diet formulation for AA balancing. Our hypothesis is that varying NEAA supply could alter the efficiency of use of EAA and ME by decreasing the need to be synthesized endogenously from other substrates and could increase energetic and productive efficiency in the lactating dairy cow.

Methods and Materials

Experimental Design and Treatments

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee. Cows were surgically implanted with rumen cannulas at the beginning of their dry period from October – December 2021. The experiment was conducted at the Cornell University Ruminant Center (Harford, NY) from January – August 2022. Starting at an average of 49 DIM, twelve ruminally cannulated multiparous Holstein cows (n=12) in their second or third lactation (2^{nd} lactation n=10; 3^{rd} lactation n=2), were assigned into one of two blocks based on DIM (Block 1 = 75 DIM ±

27, Block 2 = 66 DIM ± 13), and each cow was randomly given a unique sequence of treatments in a replicated 6 x 5 balanced incomplete block design with 18-d periods. Cows were housed in individual metabolism stalls, fed TMR once daily at 0630 h targeted for 5% refusals. All cattle were fed the same diet for at least 18 d to serve as the covariate period of the study, where EAA, MP and ME supply are 100% of assumed requirement, targeting 48 kg of daily milk production (Cov diet). Samples were taken on d 16-18 of the covariate period. Immediately following the covariate period, all cows were fed a diet formulated at 100% ME requirements (Targeting 48 kg/d milk yield) and meeting 90% of EAA and NEAA requirements via MP balance (Exp Diet; CNCPS v.7) for 18 d. The assumption is that NEAA supply would be 90% of perceived requirements if both MP and EAA supply were formulated for 90% of animal requirements. In a previous study (LaPierre et al., 2019)**,** a 10% difference in EAA and MP supply was shown to significantly affect ECM and this served as a template for this study. Due to a forage change after Period 1 of the experiment, two Exp Diets are described throughout this paper.

Simultaneous to being offered the experimental diet, cattle received one of five abomasal infusion treatments: 1) Water to meet 90% requirement of EAA and NEAA (**90AA**), 2) EAA mix at 184.8 g/d to meet 100% requirement of EAA and 90% requirement of NEAA (**100EAA**), 3) NEAA mix at 158 g/d to meet 90% requirement of EAA and 100% requirement of NEAA (**100NEAA**), 4) EAA and NEAA mix at 344.1 g/d to meet 100% requirement of EAA and NEAA (**100AA**), 5) EAA and double NEAA mix at 501.9 g/d to meet 100% requirement of EAA and 110% requirement of NEAA (**110NEAA**). To calculate specific AA amounts for each treatment, several steps were taken. First, using CNCPS (v.6.55 and v.7), the Cov Diet was formulated to meet optimal g EAA/Mcal of ME, according to Higgs and Van Amburgh (2016), and 100% ME requirements, and the Exp Diet was formulated to meet 90% of the MP supply of the Cov Diet while maintaining 100% of ME requirements. Both the current commercially available (v.6.55) and the updated (v.7) versions of the CNCPS were used for formulation, allowing for a diet which met the AA requirements for cattle under the recommendations by v.7 to be related back to an commercially applicable version of the model. Secondly, the grams of MP supply from the Exp Diet were subtracted from the grams of MP supply of the Cov Diet, to calculate total AA (TAA) that needed to be infused. Since the model provides specific EAA amounts, the same subtraction was done to calculate EAA amounts to be infused. For NEAA amounts, we used data from our laboratory using multiple time hydrolysis (Van Amburgh et al., 2017, Ortega et al., unpublished) to generate an AA profile for microbial and dietary protein. Due to analytical procedures, Glu and Gln are analyzed as one (Glx), as well as Asp and Asn (Asx). The portion of MP belonging to NEAA was calculated by subtracting the EAA amounts from the TAA, and subsequently multiplied by the percentage of each NEAA from the AA profile. And because Tyr was very insoluble, 75% of Tyr was replaced with Phe, and for similar reasons, Glx was partitioned as 33% Gln and 67% Glu, and Asx was 25% Asn and 75% Asp. A detailed supply of AA amounts from diet MP and abomasal infusions are presented in Table 1.

AA Mixture Preparation and Abomasal Infusion Setup

All AA mixtures were prepared in Nalgene bottles using hot water at least 2 d before delivery to the farm. The 100EAA and 100NEAA mixtures were prepared every 4 d in 21.6 L/cow (infused at 258 mL/h), the 100AA mixture was prepared every 3 d in 32.1 L/cow (infused at 510 mL/h), and the 110NEAA was prepared every 2 d in 21.4 L/cow (infused at 510 mL/h). The treatments were infused continuously, except when cows went to the parlor, using Masterflex LS Standard Digital Drive Pumps installed with L/S Easy-Load 3 peristaltic pump heads. The tubing was then attached to a modified version of a stainless-steel infusion device described in Westreicher-Kristen and Susenbeth (2017).

Data Collection

Dry matter intake (DMI) and refusals for each cow were measured daily throughout the experiment. Samples of TMR were obtained 3 times per week in each period and composited. During d 15 - 18 of each period, TMR, refusals and forages were collected daily and composited. Grain mix ingredients were collected when a new batch of grain mix was delivered to the farm. All samples were sent Cumberland Valley Analytical Services (CVAS; Waynesboro, PA) using near-infrared reflectance spectroscopy (TMR, refusals, forages) or by wet chemistry (mix ingredient) for chemical analysis of nutrient composition. Cows were milked three times per day (0600, 1400, and 2200 h) and milk weights were recorded at each milking (Del Pro Farm Manager; De Laval). On d 15 - 18 of each period, milk samples were collected at every milking and analyzed for milk components, using Fourier transformed infrared (FTIR) milk analysis, including DNFA, preformed FA (PFFA), mixed FA (MFA), FA chain length and double bonds and FA profile, and milk urea nitrogen (MUN; Barbano et al., 2014). Milk component yield was calculated as the sum-product of daily milk yields at each milking throughout a given day and the analyzed component values of the same day. The ECM was calculated according to Tyrrell and Reid (1965). Eight fecal samples were collected each period on d 15 - 17 (d 15: 1100 h, 1700 h, 2300 h, d 16: 0500 h, 1400 h, 2000 h, d 17: 0200 h, 0800 h), composited by cow and period, dried in an air-forced oven and ground to 1 mm, and used to estimate total tract NDF digestibility using uNDFom240 as an internal marker (Huhtanen et al., 1994, Raffrenato et al., 2018). Body weights (BW) and body condition scores (BCS; 1-5 scale) were measured and recorded five times during each period on d 1, 5, 10, 14, and 18. Three blood samples were drawn from each cow twice per day from d 15 - 18 of each period. Cows were bled at 0500 h before feeding and first milking session, and 8 h after feeding from coccygeal vein into sodium and lithium heparinized Vacutainers, and Vacutainer serum tubes (Becton Dickinson, Rutherfurd, NJ). Blood analyses have not been performed at this time, but we will perform analysis for plasma urea N (PUN), non-esterified FA (NEFA), glucose, insulin, and AA.

Diet Composition, ME, MP, and MP-EAA Analysis

Assessment of diet composition, MP, ME, and MP-EAA values presented in this paper was predicted and estimated by the CNCPS v.6.5.5, AMTS.Cattle.Professional (AMTS, LLC; v. 4.16.6) using the following method: Assessment was performed using

observed model parameters to show data that reflected precisely what the animals were presented with, for the model to be better predict animal performance. First, A ‘cattle group’ was created for each period and the observed animal inputs were recorded. Additionally, all CVAS analyses were imported directly into the model. A ‘recipe’ was created for each period using the specific period’s chemical analyses of the forages and six individual grain mix ingredients. From the farm’s feed intake program, one® by Milc group (https://onemilc.com/), the inclusions of the two forages and the grain mix were extracted and inputted into the recipe. Finally, inclusion of the three ingredients were adjusted using the average observed DMI for each cow in all periods.

Statistical Analysis

The statistical analysis was performed using R (version 4.2.0; R Core Team, 2022). The production data were analyzed with a mixed model using the function “lmer” and “anova” of the “lmerTest” package (Kuznetsova et al., 2017) with fixed effects of covariate measurements, period, treatment, and the interaction of period and treatment, as well as the random of effect of block and cow nested within block. Post-hoc pairwise comparison testing was conducted with the packages “emmeans” (Lenth, 2022). Least-square treatment means (LSmeans) from this package are presented in this paper. Values generated from chemical analyses, diet composition, and CNCPS outputs are raw means. Statistical significance was reported as $P \leq 0.05$ and tendencies as $0.05 < P \leq 0.10$. For the preparation of this paper, two cows were dropped from the statistical analysis. One cow was milk fat depressed and the other was considered an exceptional outlier, producing on average 60.14 kg of milk and 3.95 kg of components which, by standard deviation, more than 2x greater than the other cattle.

Table 1. Daily amounts of MP AA in the diet and daily amounts of AA abomasally infused into the cows for each treatment.

	Diet MP Supply[1]			Infusion Supply[3]				
AA, g/d	Cov Diet[2]	Exp Diet 1[2]	Exp Diet 2[2]	90AA	100EAA	100NEAA	100AA	110NEAA
Arg	187.7	168.0	173.1	-	18.4	-	18.3	18.3
His	85.8	74.6	76.7	-	16.0	-	15.9	15.9
Ile	144.1	131.8	135.9	-	9.34	-	9.29	9.31
Leu	241.7	213.5	220.2	-	36.7	-	36.5	36.5
Lys	221.8	196.0	201.3	-	26.8	-	26.7	26.7
Met	83.4	71.7	74.8	-	15.7	-	14.5	12.9
Phe	153.7	136.0	140.1	-	21.5	9.04	30.7	40.0
Thr	144.8	130.7	134.7	-	15.0	-	14.9	14.9
Trp	43.5	38.9	40.1	-	4.29	-	4.91	4.91
Val	174.8	155.6	160.2	-	21.1	-	24.0	24.0
Total EAA	1481.3	1316.8	1357.0	0.00	184.8	9.04	195.6	203.4
Ala	ND	ND	ND	-	-	20.7	20.7	41.4
Asx[4]	ND	ND	ND	-	-	33.6	33.6	67.2
Cys	ND	ND	ND	-	-	3.92	3.56	8.04
Glx[4]	ND	ND	ND	-	-	38.7	38.7	77.4
Gly	ND	ND	ND	-	-	18.0	18.0	35.9
Pro	ND	ND	ND	-	-	16.0	16.0	32.0
Ser	ND	ND	ND	-	-	15.2	15.2	30.4
Tyr[4]	ND	ND	ND	-	-	3.04	2.76	6.23
Total NEAA	1550.3[5]	1386.9[5]	1430.0[5]	0.00	0.00	149.2	148.6	298.6
Total AA	3031.6	2703.7	2787.1	0.00	184.8	158.2	344.1	501.9
			Total AA Exp Diet 1	2703.7	2888.5	2861.9	3047.8	3205.6
			Total AA Exp Diet 2	2787.1	2971.9	2945.3	3131.2	3289.0

[1] Predicted using AMTS.Cattle.Professional (AMTS, LLC; v. 4.16.6)

[2] Same grain mix was used for both experimental diets, forages in Exp Diet 1 were corn silage and haylage bunk 6, and forages in Exp Diet 2 were BMR and haylage bunk 8

[3] EAA calculated using CNCPS, and NEAA by microbial and dietary AA profile determined from multiple time hydrolysis (see text)

[4] Asx = 25% Asn + 75% Asp; Glx = 33% Gln + 67% Glu; Tyr = 75% Phe

[5] NEAA calculated by the difference between Total AA and Total EAA

ND = Not

Results and Discussion

Dietary and Chemical Composition

Ingredient composition of the Cov Diet and the two Exp Diets are in Table 2. Due to a lower inventory of corn silage than anticipated, a forage switch had to be done starting in Period 2. Given the different chemical composition, especially switching from conventional corn silage to BMR, minor adjustments had to be done to the inclusion rate of all diet ingredients. To achieve the 10% drop in MP supply in the Exp Diet, we included lower amounts of the ingredients with higher rumen undegradable protein (RUP): AminoPlus, bloodmeal, Smartamine M, Smartamine ML, and haylage. To offset the decrease in these ingredients, ingredients such as urea, wheat middlings, soybean meal, corn meal, and citrus pulp were increased.

The chemical analysis and model predictions, presented in Table 3, were consistent with the expected results comparing the Cov Diet to both Exp Diets. The Cov Diet had higher CP, RUP, which is related to higher predicted MP supply by the model. The ME was formulated to be similar in all diets and this was reinforced by predictions with the observed model inputs, although predictions of Exp Diet 2 showed a higher ME due to the change in corn silage. Moreover, in the Cov Diet, as formulated, MP and ME were close to each other in terms of % required (94.9 and 92.6, respectively) and allowable milk (41.8 and 40.5 kg, respectively) but ME was lower than originally formulated. ADICP, in the Cov Diet, was lower than original formulation and this is attributed to a low ADICP in one of the ingredients of the grain mix. Since ADICP is the protein associated to the acid detergent portion of the diet, the diet had less protein available to the animal. This caused the cows to have a lower milk protein % than originally expected for this period. The change in forages after the first period is noticeable from the chemical analyses. Consistent with the difference between a corn silage and a BMR there was higher observed aNDFom and lower uNDFom240, lignin, and starch. This allows for better rumen function, since there is more fiber available to the microbes. The starch content of the diet was lower than it is desired but there were no signs that the cows had any acidosis. CP was lower for the Exp Diet 2, RUP was similar in Exp Diet 1 and NDICP was higher in Exp Diet 2, and the model predicted higher MP in the Exp Diet 2. The forage changes also showed higher overall FA, ether extract, and the model estimated higher ME. These predictions are consistent with the higher milk composition observed during the periods being fed Exp Diet 2.

Table 2. Ingredients inclusion in the covariate and experimental diets used to evaluate model predictions of MP, ME, NEAA requirements, and efficiency of use of EAA relative to ME.

Ingredient, %DM	Cov Diet[1]	Exp Diet 1[2]	Exp Diet 2[2]
Period	Covariate	1	2 to 5
Corn Silage / BMR	40.2	40.1	40.6
Haylage Bunk 6 / Bunk 8	19.2	17.6	17.5
Corn meal	13.1	14.6	14.4
AminoPlus[3]	6.10	2.54	2.51
Citrus Pulp	4.18	4.89	4.84
Wheat middlings	4.18	5.34	5.28
Dextrose	4.07	4.61	4.57
Soybean meal	2.03	3.49	3.45
Blood meal	1.83	1.27	1.26
Energy Booster 100[4]	1.01	1.07	1.06
Palmit 80[5]	1.01	1.07	1.06
Urea 281	0.10	0.46	0.46
Smartamine M[6], g/d	27.0	17.0	17.0
Smartamine ML[6], g/d	29.0	22.0	22.0
Levucell SC[7], g/d	8.90	9.50	9.55
Rumensin[8], g/d	2.00	2.10	2.13
Vitamin and Minerals	2.64	2.81	2.78

[1] All cows were fed the same diet during the corresponding period regardless of treatment
[2] Same grain mix was used for both experimental diets, forages in Exp Diet 1 were corn silage and haylage bunk 6, and forages in Exp Diet 2 were BMR and haylage bunk 8
[3] Ag Processing Inc, Omaha, NE
[4] Milk specialties, Eden Prairie, MN.
[5] Global Agri Trade Corporation, Rancho Dominguez, CA
[6] Adisseo USA Inc, Alpharetta, GA
[7] Lallemand Inc, Milwaukee, WI.
[8] Elanco Animal Health, Greenfield, IN

Table 3. Observed nutrient composition of the Cov and Exp diets used to evaluate model predictions of MP, ME, NEAA requirements, and efficiency of use of EAA relative to ME.[1]

Observed chemical composition	Cov Diet	Exp Diet 1	Exp Diet 2
Period	Covariate	1	2 to 5
DM, %As-Fed	49.9 ± 1.4	50.0 ± 1.2	45.8 ± 2.0
CP, %DM	16.4 ± 1.5	15.8 ± 0.6	14.9 ± 0.2
NDICP, %CP	18.0 ± 2.0	17.2 ± 1.7	18.8 ± 0.5
ADICP, %CP	6.68 ± 0.3	7.22 ± 0.4	7.07 ± 0.1
Soluble protein, %CP	43.5 ± 3.9	47.8 ± 3.4	47.8 ± 1.5
RUP, %CP	28.2 ± 2.0	26.1 ± 1.7	26.1 ± 0.8
Sugar, %DM	7.00 ± 1.8	7.04 ± 1.7	8.34 ± 0.2
Starch, %DM	26.7 ± 0.8	25.8 ± 2.0	22.2 ± 1.2
NFC, %DM	45.3 ± 1.5	44.9 ± 1.1	43.1 ± 0.5
NSC, %DM	33.7 ± 1.9	32.8 ± 2.0	30.5 ± 1.1
ADF, % DM	18.1 ± 1.4	18.9 ± 1.5	20.3 ± 0.8
Lignin, % DM	2.84 ± 0.3	2.86 ± 0.3	2.45 ± 0.1
Ether Extract, %DM	4.58 ± 0.4	4.56 ± 0.2	4.60 ± 0.1
C18:0, Total FA	7.23 ± 0.4	7.36 ± 0.6	7.80 ± 0.1
C18:1, Total FA	13.8 ± 1.7	16.3 ± 1.7	17.0 ± 0.6
C18:2, Total FA	17.3 ± 5.2	18.0 ± 2.8	18.8 ± 1.4
C18:3, Total FA	13.4 ± 2.7	14.4 ± 2.5	17.8 ± 1.6
Nel, Mcal/lb	0.77 ± 0.01	0.77 ± 0.01	0.77 ± 0.01
Ca, %DM	0.77 ± 0.05	0.69 ± 0.1	0.62 ± 0.03
Mg, %DM	0.30 ± 0.04	0.27 ± 0.01	0.27 ± 0.01
P, %DM	0.37 ± 0.03	0.37 ± 0.02	0.36 ± 0.01
K, %DM	1.70 ± 0.2	1.80 ± 0.1	1.78 ± 0.07
Ash, % DM	7.83 ± 0.4	7.34 ± 0.3	6.98 ± 0.2
aNDFom, % DM[3]	28.8 ± 1.3	32.6 ± 1.3	33.6 ± 1.4
uNDF240om, % aNDFom[3]	31.8 ± 2.0	29.2 ± 1.1	22.1 ± 1.3
MP, g/d[4]	3031.6	2703.7	2787.3 ± 21.8
MP, % Required[4]	94.9	83.9	86.4 ± 1.1
MP Allowable Milk, kg[4]	41.8	34.6	36.2 ± 0.7
ME, Mcal/kg[4]	2.68	2.69	2.75 ± 0.01
ME, % Required[4]	92.6	93.5	95.5 ± 1.8
ME Allowable Milk, kg[4]	40.5	41.0	42.2 ± 1.1
Productive N: Urinary N[4]	1.38	1.67	1.70 ± 0.04

[1] Performed by NIR at Cumberland Valley Analytical Services, unless otherwise stated
[2] Same grain mix was used for both experimental diets, forages in Exp Diet 1 were corn silage and haylage bunk 6, and forages in Exp Diet 2 were BMR and haylage bunk 8
[2] All cows were fed the same diet during the corresponding period regardless of treatment
[3] Analysis performed in-house at Cornell University
[4] Predicted using AMTS.Cattle.Professional (AMTS, LLC; v. 4.16.6)

Lactation Performance

Although, there were no significant treatment (Trt; *P*-value < 0.05) responses in the lactation performance parameters, except for MUN (Table 4), there were numerical differences among treatments. The MUN showed significant differences among the treatments, increasing when higher amounts of AA were infused. For milk yield, the 100AA Trt, in which all AA were infused at what was calculated to be the optimal amounts, had the greatest yield among the response variables. For ECM (Trt *P*-value = 0.27), the 100AA treatment produced 1.5 kg/d of ECM more than the 90AA treatment, where no AA were infused. A milk yield response was also reported in other studies where all AA were infused in the abomasum (Metcalf et al., 1996, Doepel and Lapierre, 2010). The ECM and milk yield response was significant in early lactation cows infused with casein and AA (Larsen et al., 2014, Bahloul et al., 2021). Postpartum cows have a much higher demand for energy, which could be provided by the AA being infused.

In addition, Metcalf et al. (1996) showed the highest milk protein percentage for the treatment that only had EAA infused, while Doepel and Lapierre (2010) reported the highest value for the treatment where all AA were infused. In our study, milk protein percent (Trt *P*-value = 0.53) was numerically higher for the treatments infusing all AA, 100AA and 110NEAA (3.21% and 3.23%, respectively) compared to the other three treatments, 90 AA, 100EAA, and 100NEAA (3.18%, 3.16%, and 3.18%, respectively). In the current study, since the requirements for NEAA were supposedly met, there should be a higher availability of these NEAA as well as EAA for protein synthesis. One example is that of Pro which is produced from Arg in the MG (Trottier et al., 1997, Lapierre et al., 2012, Wu, 2022). Previous infusion studies (Metcalf et al., 1996, Doepel and Lapierre, 2010) showed the highest milk fat yield for the treatment that only infused EAA, while in the present study the same treatment, 100EAA, had the second highest milk fat yield but almost identical to the 100AA (1.94 kg/d vs 1.95 kg/d, respectively). Milk fat percent was consistently high in the current study whereas in previous infusion studies (Metcalf et al., 1996, Doepel and Lapierre, 2010) this was observed only when infusing EAA (100EAA = 4.87%). The lowest milk fat percent in the past infusion studies (Metcalf et al., 1996, Doepel and Lapierre, 2010) was observed in the infusions of all AA, while in the present study this was observed where the theorical requirements of NEAA were met at 110% (110NEAA = 4.63%). This decreased milk fat percent translated into reduced fat and solids yield (Trt *P*-value = 0.21) in the 110NEAA treatment. Among treatments, BW (Trt *P*-value = 0.73) and DMI (Trt *P*-value = 0.84) were similar. The highest ECM feed efficiency was observed in with the 100AA treatment, due to having the lowest DMI (27.21 kg/d) and the highest ECM (48.75 kg/d).

Milk fat responses were further explored by analyzing various FA production metrics using FTIR (Barbano et al., 2014; Table 5). To our knowledge, this is the first study to report these responses in relation to abomasal infusions of all 20 EAA and NEAA. Consistent with the milk fat percentage and yield responses, total FA (TFA) % (Trt *P*-value = 0.40) were highest for the 100EAA treatment and lowest for the 110NEAA (4.52% vs 4.32%, respectively), while TFA yield (Trt *P*-value = 0.43) was highest for the 100AA (1.82 kg/d) and lowest for the 110NEAA and 100NEAA (1.77 kg/d and 1.76 kg/d,

respectively). Barbano et al. (2014) reported higher de novo FA (DNFA), mixed FA (MFA), and preformed FA (PFFA), in g/100 g milk (%), with higher bulk tank milk fat percent, but these relationships do not seem to hold true in the present study where cows are fed an isocaloric diet and varying amounts of different AA mixes. The most significant differences in overall milk FA production were observed between the 100EAA and the 110NEAA treatment. While there were no statistical differences among treatments in DNFA % (Trt *P*-value = 0.91) and yield (Trt *P*-value = 0.51), the 110NEAA treatment had a trend for higher relative production of DNFA (Trt *P*-value = 0.12) compared to the 100EAA treatment (27.88 vs 27.15 g/100 g FA, respectively). In the present study, this outcome could be related to higher AA availability. Since all EAA and theoretical NEAA requirements were met, the excess of NEAA could have been catabolized into glucose and TCA cycle intermediates to produce more reducing equivalents (NAD(P)H, $FADH_2$) and acetyl-CoA for production of DNFA, by mechanisms explained in the introduction. This FA response can be further seen with the relative production of MFA (Trt *P*-value = 0.09), where the 110NEAA had a trend for higher production compared to the 100EAA treatment (42.47 vs 41.40 g/100 g FA, respectively). The MFA can come from either dietary fat or de novo synthesis, with the relative contributions of each depending on the energy status of the cow, and since the cows in this study are in positive energy balance, there is a higher contribution from de novo synthesis (Palmquist, 2006, Barbano et al., 2014). In the results for all PFFA: g/100 g milk (Trt *P*-value = 0.02), g/d (Trt *P*-value = 0.06), and g/100 g FA (Trt *P*-value = 0.03), were all significantly lower for the 110NEAA (1.29 g/100 g milk, 525.15 g/d, and 29.59 g/100 FA) compared to other treatment, especially 100EAA where all metrics were highest (1.43 g/100 g milk, 576.60 g/d, and 31.43 g/100 FA). This response is also clear with the individual PFFA % and yield of C18:0 (Trt *P*-value = <0.01 and Trt *P*-value = 0.02, respectively) and C18:1 *cis-9* (Trt *P*-value = 0.01 and Trt *P*-value = 0.04, respectively). PFFA are derived from dietary sources and endogenous FA catabolized from adipose tissue (Palmquist, 2006, Barbano et al., 2014). In this study the lower PFFA values could possibly be explained by the increased glucogenic AA which lead to higher amounts of glucose and, subsequently, more insulin that is associated with lower lipolysis.

Summary

Abomasal infusions of an AA profile consisting of both EAA and NEAA, formulated with the CNCPS v.6.5.5 and v.7, and the most current and accurate AA chemical composition of dietary ingredients, resulted in the cows being more energetically efficient as shown by producing the highest ECM, especially when compared to a treatment with no AA infusions. The provision of more NEAA with the optimal AA profile, caused cows to produce lower milk fat, driven by a significant drop in PFFA. Further work needs to be performed on the blood analyses in these cattle to get a complete picture of the metabolic status of the cows to see if the theories postulated for the responses observed are correct. Overall, the model predictions of the requirements of the animals appear to have been met given the consistent responses predicted and observed. Incorporating NEAA in the model could allow for better production predictions. The negative responses in milk FA due to additional NEAA infusions, shows the necessity of building a FA sub model and developing metabolic interactions between FA and AA.

Table 4. Effect of abomasal infusion AA treatment on lactation performance, body weight, body condition score, and dry matter intake (LS-means).

	Treatment[1]						*P*-value		
	90AA	100EAA	100NEAA	100AA	110NEAA	SEM	Trt	Period	Trt*Period
Milk Production, kg/d									
ECM[2]	47.3	47.8	47.5	48.8	48.1	1.45	0.27	0.03	0.04
Milk Yield	40.1	40.0	40.1	41.0	41.0	1.37	0.18	<0.01	0.31
True Protein Yield	1.27	1.26	1.26	1.31	1.32	0.08	0.15	0.89	0.49
Fat Yield	1.89	1.94	1.89	1.95	1.90	0.08	0.46	0.13	0.09
Lactose Yield	1.85	1.85	1.84	1.90	1.90	0.07	0.27	<0.01	0.65
Solids Yield	5.47	5.49	5.44	5.62	5.58	0.19	0.21	0.04	0.15
Solids-not-Fat Yield	3.57	3.55	3.54	3.66	3.68	0.17	0.17	0.04	0.55
Milk Composition, %									
True Protein	3.18	3.16	3.18	3.21	3.23	0.05	0.53	<0.01	0.79
Fat	4.73	4.87	4.83	4.79	4.63	0.17	0.32	0.01	0.32
Lactose	4.61	4.60	4.61	4.63	4.63	0.03	0.61	0.16	0.44
Solids	13.6	13.7	13.7	13.8	13.6	0.13	0.69	<0.01	0.45
Solids-not-Fat	8.89	8.64	8.88	8.94	8.96	0.07	0.32	<0.01	0.83
MUN, mg/dL	8.27[a]	9.24[ab]	9.00[ab]	10.32[bc]	10.99[c]	1.96	<0.01	<0.01	0.88
Body measurements, Intake, and Efficiency, kg/d									
Body Weight	710.7	708.8	712.0	712.7	712.6	4.25	0.73	<0.01	0.20
BCS, 1-5 Scale	2.84	2.87	2.84	2.83	2.82	0.03	0.49	0.43	0.78
DMI	27.3	27.1	27.5	27.2	28.0	0.93	0.84	0.63	0.85
ECM Feed Efficiency	1.75	1.77	1.75	1.82	1.73	0.06	0.46	0.15	0.89

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

[1] 90AA = Water, no AA infused; 100EAA = EAA infusion at 184.83 g/d; 100NEAA = NEAA infusion at 158.22 g/d; 100AA = EAA and NEAA infusion at 344.14 g/d; 110NEAA = EAA and 2x NEAA infusion at 501.91 g/d.

[2] Calculated according to Tyrrell and Reid (1965)

Table 5. Effect of abomasal infusion AA treatment on de novo, mixed, and preformed fatty acid production (LS-means).

	Treatment[1]						*P*-value		
	90AA	100EAA	100NEAA	100AA	110NEAA	SEM	Trt	Period	Trt*Period
Total Fatty Acids									
g/100 g milk	4.40	4.52	4.50	4.49	4.32	0.15	0.40	0.01	0.27
g/d	1766.0	1812.7	1763.5	1822.3	1765.8	76.1	0.43	0.19	0.11
De Novo Fatty Acids[2]									
g/100 g milk	1.22	1.25	1.24	1.24	1.22	0.02	0.91	<0.01	0.47
g/d	488.8	496.5	488.6	506.6	501.4	20.2	0.51	0.02	0.08
g/100 g FA	27.4	27.2^{x}	27.5	27.7	27.9^{y}	0.49	0.12	0.00	0.47
Mixed Fatty Acids[2]									
g/100 g milk	1.85	1.90	1.90	1.89	1.86	0.04	0.77	<0.01	0.33
g/d	740.9	755.2	744.7	769.3	760.7	29.7	0.56	0.01	0.06
g/100 g FA	41.5	41.4^{a}	41.9	41.9	42.5^{b}	0.71	0.09	0.00	0.29
Preformed Fatty Acids[2]									
g/100 g milk	1.38	1.43^{a}	1.40^{x}	1.38	1.28^{by}	0.10	0.02	0.70	0.09
g/d	556.4	576.6^{a}	547.3	563.1	525.2^{b}	51.8	0.06	0.15	0.21
g/100 g FA	31.2^{x}	31.4^{a}	30.7	30.5	29.6^{by}	1.21	0.03	<0.01	0.23
Cis and Trans Fatty Acid									
Cis, g/100 g milk	1.03	1.06^{a}	1.04	1.04	0.97^{b}	0.07	0.03	0.65	0.07
Cis, g/d	411.3	427.0^{x}	407.1	422.4	396.3^{y}	33.0	0.07	0.05	0.22
Trans, g/100 g milk	0.048	0.053	0.052	0.050	0.050	0.003	0.34	0.02	0.70
Trans, g/d	20.8	21.2	20.4	20.9	20.6	0.77	0.99	0.04	0.22
Fatty Acids									
C16:0, g/100 g milk	1.74	1.79	1.79	1.78	1.75	0.04	0.79	<0.01	0.32
C16:0, g/d	698.3	711.9	701.0	724.4	715.5	27.4	0.62	0.02	0.07
C18:0, g/100 g milk	0.42^{a}	0.43^{a}	0.43^{a}	0.41^{x}	0.38^{by}	0.04	<0.01	0.02	0.02
C18:0, g/d	169.7^{x}	174.2^{a}	167.6	169.5^{x}	156.2^{by}	18.0	0.02	0.15	0.08
C18:1 *cis-9*, g/100 g milk	0.73	0.77^{a}	0.75^{x}	0.75^{x}	0.69^{by}	0.06	0.01	0.46	0.02
C18:1 *cis-9*, g/d	295.0	309.0^{a}	292.2	304.2	282.2^{b}	28.	0.04	0.01	0.19
Average fatty acid chain length	14.2	14.2	14.2	14.2	14.2	0.03	0.44	0.21	0.75
Degree of Unsaturation	0.23	0.23	0.23	0.23	0.23	0.00	0.68	0.01	0.28

[ab] Means within a row differ with different superscripts ($P < 0.05$); [x, y] ($0.05 < P < 0.1$).

[1] 90AA = Water, no AA infused; 100EAA = EAA infusion at 184.83 g/d; 100NEAA = NEAA infusion at 158.22 g/d; 100AA = EAA and NEAA infusion at 344.14 g/d; 110NEAA = EAA and 2x NEAA infusion at 501.91 g/d.

[2] De Novo = C4:0 to C14:0; Mixed = C16:0, C16:1, C17:0; Preformed = C18:0 and longer (Barbano et al., 2014)

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Effects of Poor Maternal Nutrition on Pre- and Post-natal Growth and Metabolism

K. E. Govoni, S. A. Reed, and S. A. Zinn
Department of Animal Science
University of Connecticut

Introduction

The human population is expected to reach 9.8 billion by 2050 (Nations, 2017); therefore, it is imperative that we identify methods to improve the efficiency of food production to provide adequate, affordable, and high quality animal protein to consumers. Livestock are huge contributors to the global food supply as milk, meat, and eggs provide approximately 18% of energy and 34% of protein consumed globally (FAO, 2018). Therefore, the identification of methods to improve production efficiency is necessary to increase protein availability for human consumption. Inadequate nutrition during gestation impairs fetal growth and metabolism, which can lead to reduced productivity and quality of the product [e.g., meat, milk, fiber (Du et al., 2010a; Du et al., 2015)] in the offspring. Impaired tissue growth during prenatal development can extend into early postnatal growth and through adulthood, thereby hindering the animal's ability to develop adequate protein (i.e., muscle). Poor maternal nutrition, reduced or excess nutrition, during gestation reduces fetal growth (McMillen and Robinson, 2005; Wu et al., 2006; Reynolds et al., 2010), impairs muscle development (Du et al., 2011; Reed et al., 2014), reduces bone density (Lanham et al., 2008a; Lanham et al., 2008b), increases fat accretion (Du et al., 2010b; Du et al., 2011), alters metabolism (Wu et al., 2006; Reynolds et al., 2010), and impairs stem cell function (Oreffo et al., 2003; Pillai et al., 2016; Raja et al., 2016) in the offspring. Numerous studies in livestock, rodents, and humans have demonstrated that these negative effects can contribute to reduced efficiency of growth and altered metabolism (Du et al., 2010a; Ford and Long, 2011; Long et al., 2012; Hoffman et al., 2014; Reed et al., 2014; Hoffman et al., 2016a). Maternal nutrient restriction and over-feeding during gestation causes metabolic dysregulation and alters key metabolic pathways in offspring which are associated with reduced efficiency of growth and poor health outcomes (Wu et al., 2006; Ford et al., 2007; Ford and Long, 2011; Hoffman et al., 2016a). Current research in the field of fetal programming focuses on identifying mechanisms that contribute to these long-term, persistent negative effects of poor maternal nutrition during gestation.

Fetal Programming

Fetal programming is an important process that occurs during in utero development to ensure proper development and survival of the fetus after birth (Barker, 1995). When adverse events occur during gestation, such as reduced or excess nutrient consumption by the mother, this leads to negative programming effects on the offspring in terms of production, health, and metabolic outcomes. A classic example of the impact of restricted maternal nutrition during gestation is the Thrifty Phenotype Hypothesis proposed by Hales and Barker (Hales and Barker, 2001). Offspring born to mothers

exposed to nutrient restriction during the Dutch famine demonstrated metabolic dysregulation, increased obesity, and insulin resistance in adulthood. These outcomes are likely the result of programming during gestation to survive in an environment with limited nutritional resources. However, when the postnatal environment (adequate or excess nutrition) did not match the fetal environment, the fetal programming led to increased risk of metabolic dysregulation which ultimately reduces efficiency of growth. Specifically, maternal nutrition can negatively impact adipose, muscle, liver, pancreas, brain, and cardiovascular system, all of which can contributed to metabolic dysregulation in the fetus and postnatal offspring (Symonds et al., 2009).

Effects of Poor Maternal Nutrition on Growth and Metabolism

Models of Poor Maternal Nutrition

Poor maternal nutrition can result from excess or reduced nutrient intake including overall total energy, protein, and/or micronutrients in the diet. These are often practical problems for producers depending on their geographical location. For example, in drought conditions or during winters, forage may be reduced in quantity and/or quality. In addition, certain regions are susceptible to excess or limited micronutrients and therefore proper supplements are necessary. Variations in the quality and quantity of available feed and forage can result in periods of sub-optimal nutrition for livestock. Specifically, a lack of food and/or specific nutrients often occurs for a period of gestation, or often all of it, in many parts of the US. The timing and duration of the nutritional insult also affects the outcomes in the fetus and offspring. In our model of poor maternal nutrition in sheep, we evaluate the effects of restricted and over-feeding based on a total feed deficit or excess. Our control animals are fed a complete feed at 100% of NRC requirements. The restricted animals are fed 60% of control, based on TDN and the over-fed are provided 140% of control. This model has provided us with the advantage to compare the impact of both restricted and over-feeding in the same study.

Growth

The maternal environment can have immediate and long-lasting consequences on offspring fetal and post-natal growth. Poor maternal nutrition is known to impact fetal growth and can lead to reduced body weight at birth, but this is dependent on the timing, duration, and type of nutritional insult (Wu et al., 2006; Du et al., 2010a; Ford and Long, 2011; Reed et al., 2014; Govoni et al., 2019). As we previously summarized (Govoni et al., 2019), nutrient restriction during gestation can lead to intrauterine growth restriction and reduced birth weight; however, several studies of restricted nutrition during gestation also report no effect on offspring body weight at birth (Govoni et al., 2019). Similarly, over-feeding during gestation can lead to increased body weight at birth, but more often does not impact offspring body weight during this time (Govoni et al., 2019). Maternal diet can also impact postnatal offspring growth with compensatory gain occurring in offspring of restricted-fed ewes (Morrison et al., 2010). However, this is not desirable as it often leads to increased adipose tissue and not increased muscle mass (Hornick et al., 2000). Based on the variability in the impact of maternal diet on offspring body weight at birth and

postnatal growth, caution is needed when using birth weight as an indicator of 'healthy' offspring since these offspring can have similar birth weight, but often have differences in body composition and metabolic factors that lead to poor growth, health, and product quality as they mature.

Several proteins in the circulation and local growth factors are associated with altered growth of offspring from mothers consuming a poor diet during gestation. The growth hormone (GH)/insulin-like growth factor (IGF) axis, which is critical for fetal and postnatal development of muscle, adipose, and bone tissue, is altered. Specifically, in offspring that are born small for gestational age due to disease or limited maternal nutrient availability there is reduced circulating IGF-I and IGF binding protein (BP)-3, and increased GH and IGFBP-2 (de Zegher et al., 1997); a hormonal pattern associated with reduced growth or size in cattle (Rausch et al., 2002) and wildlife (Govoni et al., 2010). Furthermore, intrauterine administration of IGF-I in sheep increases fetal growth rate in growth-retarded fetuses (de Boo et al., 2008). Changes in these important circulating growth factors demonstrate one mechanism by which the negative effects of maternal diet alter offspring growth.

Muscle and Adipose

Muscle is the primary product in meat producing animals and adipose tissue is important in product quality. Muscle tissue is not only the end product, but also a key metabolic tissue. In addition, muscle fiber number is set at birth so insults during gestation can lead to persistent effects into adulthood resulting in decreased product quality and quantity, and metabolic dysfunction in offspring. In our sheep model of poor maternal nutrition, nutrient restriction and over-feeding lead to increased muscle fiber cross-sectional area (CSA) in offspring at birth, but at 3 months of age, smaller CSA in both treatment groups relative to control (Reed et al., 2014). These changes in muscle were associated with altered function of satellite cells (e.g., muscle progenitor cells) such that early differentiation and a reduced fusion index may account for the reduced CSA in restricted offspring at 3 months of age (Raja et al., 2016) due to precocial differentiation of myoblasts. Similarly, in cattle, muscle CSA was altered in response to early- and mid-gestation nutrient restriction (Zhu et al., 2004). Within the muscle tissue of restricted- and over-fed offspring there was increased fat accumulation demonstrating a negative impact of both maternal diets on offspring muscle growth and composition. Similarly, others report that nutrient restriction during early or late gestation results in fewer muscle fibers in lambs (Costello et al., 2008) and an increased number of glycolytic fibers (Zhu et al., 2006), which can negatively impact meat tenderness (Oury et al., 2009; Kang et al., 2011). Lambs from obese ewes have decreased abundance of the IGF-I receptor (R) coupled with decreases in Akt, mTOR, and 4EBP1 phosphorylation in the muscle, indicating suppressed signaling for protein synthesis (Yan et al., 2011). Moreover, fetal muscle (gestational day 135) in lambs from obese ewes have decreased muscle fiber diameter and increased collagen content (Huang et al., 2010; Yan et al., 2011), which persist into adulthood (Yan et al., 2011; Huang et al., 2012).

Offspring of mothers that are obese or over nourished during gestation are prone to increased fat deposition and insulin-resistance (Neri and Edlow, 2015; Pankey et al., 2017). In addition to programming the immediate offspring (F1 generation), there is mounting evidence that these effects can pass on to subsequent generations (e.g. F2, F3), even when those F1 offspring consume a normal diet. Specifically, Shasa et al. (Shasa et al., 2015) demonstrated that, despite similar birth weights, F1 and F2 offspring of F0 ewes (mothers) that were over-fed during gestation exhibited increased body fat, decreased insulin sensitivity, increased plasma cortisol, and no increase in early postnatal leptin. Similarly, F0 maternal obesity resulted in increased basal glucose and insulin concentrations in F2 females but not males, resulting in insulin resistance in the females (Pankey et al., 2017). These data demonstrate the multigenerational effects of poor maternal nutrition on offspring metabolism, insulin sensitivity, and growth.

Metabolism

It is well-established that poor maternal nutrition leads to impaired glucose sensitivity, insulin resistance, and leptin resistance in offspring, which likely contribute to increased adipose deposition and inefficient utilization of nutrients (Ford et al., 2007; Gao et al., 2014; Hoffman et al., 2016b). In support of increased adiposity with poor maternal nutrition, leptin synthesis is increased in offspring from mothers that were restricted-fed (Tzschoppe et al., 2011) or over-fed (Hoffman et al., 2014) during gestation. Maternal obesity and/or over-feeding leads to leptin resistance or increased circulating leptin later in life. This may be due to reduced peak of leptin during first week of life as demonstrated in sheep (Shasa et al., 2015). Similarly, maternal restricted feeding increased circulating leptin which was associated with increased body weight and feed intake in offspring (George et al., 2012). Leptin is important in appetite regulation and metabolic activity; therefore, alterations in circulating leptin concentrations or the body's ability to respond to leptin is one example of metabolic dysregulation that persists into adulthood and has been passed to subsequent generations (Shasa et al., 2015).

Both maternal restricted- and over-feeding impair insulin sensitivity in offspring, which can lead to decreased efficiency of growth and poor health due to increased fat accumulation (Ford et al., 2007; Hoffman et al., 2016a). Offspring from restricted- and over-fed ewes demonstrate increased insulin:glucose during an in vivo glucose tolerance test (Hoffman et al., 2016a). Maternal obesity and nutrient restriction are associated with increased type II diabetes in humans demonstrating the programming of offspring insulin production and/or response. Based on the insulin resistance in offspring, we and others have further explored the impact on pancreas development in the offspring. Restricted- and over-feeding decreased pancreatic islet number and increased islet size with reduced beta-cell proliferation (Peterson et al., 2021). Further, these changes were associated with sex-specific differentially methylated regions in response to diet. DNA methylation is a mechanism that controls gene expression. Altered methylation will impact the gene and proteins expressed and ultimately cell function, but not alter the DNA sequence. Specifically, there was a greater increase in differentially methylated regions in offspring of restricted-fed ewes and fewer in over-fed offspring. In the male offspring, there was a greater decrease in differentially methylated regions in both the restricted and over-fed

groups relative to female offspring (Peterson et al., 2021). These findings are consistent with previous sex-specific effects of poor maternal nutrition on insulin sensitivity in F2 offspring (Pankey et al., 2017). In addition, this highlights the importance of including both sexes when evaluating impact of poor maternal nutrition.

In addition to changes in circulating metabolic factors, we used metabolomic and proteomic analyses to identify metabolic changes at the tissue level in muscle and liver, two highly metabolic tissues. In a study evaluating maternal nutrient restriction effects on fetal development, we demonstrated a change in lipid abundance of cholesterol esters, ceramides, diacylglycerols, free fatty acids, sphingomyelin, and triacylglycerols in offspring blood, liver, and muscle at day 130 of gestation (Smith et al., 2022). Consistent with the changes observed in metabolite abundance, proteomics analysis demonstrated similar changes in proteins involved in glucose metabolism and glycogen synthesis in liver of fetal offspring (Smith et al., 2022). In offspring longissimus dorsi from nutrient restricted mothers, we observed changes in lipid and amino acid metabolites as early as day 90 of gestation using global metabolomics analysis. At day 135 of gestation, we see a shift such that glutamate, which was increased at day 90, is reduced at day 135, and ceramides which were decreased at day 90, are increased at day 135 (Martin et al., 2019), demonstrating a response to maternal diet that is specific to the stage of gestation. In the same analysis in offspring of over-fed ewes, we observed increases in several amino acids at day 90 of gestation, whereas at day 135 fatty acids increased; again, demonstrating stage of gestation and diet-specific responses of muscle metabolites (Martin et al., 2019). Based on changes in skeletal muscle growth, lipid composition, and metabolite abundance in response to maternal nutrient restriction and over-feeding, we further explored changes in protein abundance using proteomics. In offspring of over-fed ewes, protein synthesis was repressed at day 90 of gestation and there was a decrease in protein degradation at day 90 of gestation and at birth (Reed et al., 2022). Maternal nutrient restriction decreased protein degradation at day 90 of gestation, while increasing protein turnover (Reed et al., 2022). These findings are consistent with delayed secondary myogenesis observed in these fetuses (Gauvin et al., 2020), and our previous report that both restricted and over-feeding decrease muscle fiber growth (Reed et al., 2014); demonstrating changes in key protein pathways in fetal development.

Summary

Proper nutrition is a critical part of livestock management and production. The evidence that poor maternal nutrition (too much or too little) during gestation negatively impacts not only fetal development but can persist into adulthood and subsequent generations highlights the critical need to understand the mechanisms. Our recent metabolomics and proteomics analyses demonstrate the potential programming of key pathways involved in lipogenesis, glucose and glycogen metabolism, and protein synthesis and degradation contribute to altered tissue growth and metabolism in offspring. More importantly, these negative outcomes can occur even following a short duration of poor nutrition and with proper feeding after birth. Based on persistent effects into adulthood and subsequent generations, we and others are actively investigating the programming occurring at the tissue level (e.g., muscle, liver, pancreas, gut) to fully

understand how maternal diet programs offspring growth and metabolic dysregulation. Ideally producers would reach target feeding programs; however, this is not always realistic so there is a critical need to identify the mechanisms involved and ideal management of offspring subjected to poor maternal nutrition during gestation.

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The Cornell Net Carbohydrate and Protein System version 7: What is Taking So Long?

M. E. Van Amburgh and P. A. LaPierre
Dept. of Animal Science, Cornell University

Introduction

The development of the Cornell Net Carbohydrate and Protein System (CNCPS) has been discussed in many previous conference proceedings. The intent of this paper and presentation is to provide a description of the latest updates to the model, specifically regarding version 7, and to provide a timeline for the launch of this project into the industry. As with all things related to model development, there are many aspects which can impact the speed with which developers build, evaluate, modify, and publish the equations or processes within the system to ensure it provides appropriate and useful answers when determining what nutrient(s) are limiting productive functions in growing heifers or high producing lactating cattle. With the release of the eighth edition of the nutrient requirements of dairy cattle from the National Academies of Sciences, Engineering and Medicine (NASEM) committee (NASEM, 2021), the dichotomy between how a NASEM publication and model is developed and how the CNCPS has been approached is more apparent. Because the committee evaluates nearly all supplies of nutrients, and nutrient updates from other sources are limited, the NASEM updates provide relevant information to be included in future CNCPS updates, particularly in the areas of vitamins and minerals, fatty acids, and water intake. Nevertheless, differences in the modeling process exists and are discussed within this paper.

There are three distinct differences between the development of a new edition for the NASEM model and updates made toward the CNCPS which greatly impacts the speed with which updates are released. The first is the NASEM is comprised of a committee where each member is assigned to one or two topics to review the current literature, compile relevant data, assess, modify, and/or develop new equations while writing their assessment in book chapters. Each committee member brings expertise that is complimentary and slightly unique to ensure an effective and comprehensive assessment and update to nutrient requirements and supply. There are managers, timelines and support staff that contribute to the success of the NASEM effort. Upon the conclusion and publication of these updates, the committee provides their updated recommendations to nearly all relevant nutrients that are formulated in a dairy diet and will concomitantly describe research ‘areas of opportunity’ for the industry to focus their efforts on before the next committee is convened. The development process for the CNCPS involves efforts by graduate students, postdoctoral associates and visiting faculty, with the help of interested faculty within, and beyond, the Department of Animal Sciences. As with the NASEM process, there is a reliance on literature data, data base construction and statistical evaluation, but it is usually in the context of another project or hypothesis. These researchers typically have a specific area of nutrition that they study, whereby they evaluate current literature to understand limitations in the data, perform

experiments aimed at providing new and relevant information to lessen these limitations, and integrate these findings by assessing, modifying, and/or developing new equations within the model structure. An exception to this process in the last 12 years was the graduate program of Dr. Ryan Higgs, where his program support allowed for a focused effort on updating version 6 which, after realizing the need to redesign the calculation process of the CNCPS due to limitations in disaggregating feed and microbial nutrient supplies, provided for the translation of version 6.5 and development of it into version 7 (Higgs and Van Amburgh, 2016).

One of the shortcomings of the NASEM process is that once the publication and its associated model is released, the committee is disbanded, and updates are only made when the next committee is convened. Given the rate of newly published research in the field it can be difficult to create model which provides robust predictions with an evolving knowledgebase. As such, the frequency of which a model should be updated should reflect the needs of the industry and their understanding of nutrition. The disbandment of the committee can also be problematic from the sense that there is minimal testing and evaluation of the system in prospective animal studies. This process is left to the users, other academics, and future committees, leaving little opportunity to modify and update what the committee developed outside of literature data, compiled datasets, and statistical evaluation. Over the last few iterations of the NRC/NASEM, the published models have essentially started over with new data, a new approach and generally improved statistical approaches when analyzing current literature datasets. This leads to two distinct differences compared to the development of the CNCPS. First, the architectural and computational structure of the CNCPS model has been conserved for from the model's inception in 1990 (Fox et al., 1992, Russell et al., 1992, Sniffen et al., 1992, O'Connor et al., 1993) to 2015 (Van Amburgh et al., 2015a, Van Amburgh et al., 2015b), a 25-year period where incremental changes (Van Amburgh et al., 1998, Fox et al., 2004, Lanzas et al., 2007, Tylutki et al., 2008) were made to predictive equations of both cattle requirements and nutrient supplies through the rumen and gastro-intestinal tract, as well as refinements and additions to the model's feed library. These updates to the model have been more frequent throughout these 25 years and the methodical updating of equations based on model performance feedback and new data have allowed for the refinement of equations that have not predicted well, resulting in a more robust prediction and reconciliation of nutrient supply and requirements for cattle. This refinement in the model can be a slow, painstaking at times, process; however, it is important to note that in an integrated model, one permutation in an equation or system usually illuminates an offset in the next system or set of equations. This process of working through updated and new equations can turn into a proverbial game of "whack-a-mole", where each update leads to another unveiling of an offset which requires more work and time. This becomes more of an issue with models that exhibit greater complexity, as demonstrated in version 7, where more time is warranted, relative to v.6.5.5, to ensure that accurate predictions relative to observed data.

Given the use and distribution of the model throughout the industry, this group has felt obligated to evaluate the predictions of CNCPS v.7 in several prospective cattle studies to ensure that the predictions of requirements and supply are consistent with

observed cattle performance. These observations are in no way a means of validating the systems predictions, rather this deviation from previous versions of the model require a series of evaluations to ensure that predictions are within an acceptable range for accuracy and precision. One technique of model evaluation involves boundary testing to understand the model's limitations and if the predictions are true and consistent with higher yielding cattle than what might be found in the literature used to build the model. This boundary testing has resulted in several revisions, and subsequent delays, of version 7 due to the elucidation of biases involving rumen protozoal flows and subsequent microbial interactions. Further, the testing of boundaries for a new concept for metabolizable amino acid requirements, related to the supply of metabolizable energy, has required extensive vetting to understand how energetic efficiency impacts nitrogen and amino acid metabolism when cattle are lactating. Lastly, the procurement of time and resources to design, program, and deploy a packaged architecture for this new version that allows for a smooth integration of version 6 and version 7 systems in an industry setting has been challenging as our group looks to revamp the system of deployment and updates to users of the model.

The focus of this paper will highlight changes in supply predictions that are significantly different than v6.5.5, discuss the boundary testing which provided additional revisions to version 7, and outline the steps our group has taken to deploy this version in an appropriate timeframe. For a more mechanistic review of CNCPS v7, please refer to Higgs and Van Amburgh (2016).

Updated Nutrient Supply Predictions

Nutrient supply predictions within the updated version of CNCPS build upon ruminal and intestinal transactions that are reported in previous model versions and further describe their dynamic flow starting at the mouth, ending at the rectum, and providing pool size and flux predictions for the rumen, omasum, and small and large intestines (Table 1). This disaggregation of compartmental modeling will utilize a similar feed fractionation scheme, with a greater description of fiber carbohydrates and revisions on how intestinal digestibility of protein in feeds which contain little to no fiber are calculated. A more descriptive report becomes useful during formulation as it will allow the user to understand total tract digestibility of fiber and if feed inventory and costs allow, make modifications to enhance digestibility and energy availability. This will also provide useful information about ruminal digestibility of aNDFom as its digestion will be explicitly quantitative. The total tract digestibly estimations have been tested on four prospective studies, three of which were formulated to North American specifications and one using an Irish grazing system. On average, the resolution of predicted aNDFom total tract digestibility was within 7%, or 2.9 units, of observed total tract digestibility. This group will continue to use future studies to evaluate the accuracy of this predictions and will modify equations when biases present themselves under varying fiber feeding conditions.

Table 1. Intake, degradation, digestion and excretion by digestive compartment of carbohydrate pools from both forage and concentrate sources according to CNCPS v7 calculations.

	Digestion by compartment[1] (g/d)					
				Neutral Detergent Fiber		
	Sugar	Starch	Soluble Fiber	Fast Degrading	Slow Degrading	Undegradable
Proportion of diet, % DM	4.2	30.5	3.7	18.5	5.0	7.1
Forage ingredients, g						
Intake	181	6212	424	3481	1122	1629
Rumen degraded	105	5037	340	2954	615	0
Rumen pool[2]	15	488	35	1241	1193	3802
Rumen passage	76	1175	84	528	507	1629
Small intestine digested	76	877	0	0	0	0
Small intestine passed	0	298	84	528	507	1629
Large intestine degraded	0	207	57	226	71	0
Fecal excretion	0	91	27	302	437	1629
Apparent total tract digestion, %	100	98.5	93.7	91.3	61.1	0
Concentrate ingredients, g						
Intake	998	3078	626	1706	283	358
Rumen degraded	730	2116	445	1290	172	0
Rumen pool	50	329	62	821	216	709
Rumen passage	269	961	180	416	110	358
Small intestine digested	269	754	0	0	0	0
Small intestine passed	0	208	180	416	110	358
Large intestine degraded	0	120	107	131	22	0
Fecal excretion	0	88	73	285	88	358
Apparent total tract digestion, %	100	97.2	88.3	83.3	68.8	0

[1] Cattle consumed an average of 28.0 kg of DMI from this diet .

[2] Defines the residual quantity of each carbohydrate fraction which resides in the rumen and has not been degraded or passed.

There are two aspects to this pool size data on aNDFom which will become relevant to the user as the steady state rumen pool size of the potentially digestible aNDFom and the uNDF will be a determinant of potential dry matter intake (DMI) for the animal (Table 2). This approach is meant to complement existing equations provided within previous versions of the CNCPS, in addition to equation published in the NASEM (2021) model, providing users with an additional tool to troubleshoot and reconcile predicted and observed DMI on farm. The recommended intake and rumen fill values are based on the work conducted at Miner Institute, University of Bologna and Cornell University (Cotanch et al., 2014) using the intake metrics developed by Mertens (2010). This information was one of the outcomes of the Informal Fiber Working Group that has been meeting at the nutrition conference for over ten years.

The model will provide predictions for bacterial protein flows, as in previous versions, based on the fiber (Feed fractions CHO B3 and CHO C; FC) vs non-fiber carbohydrate (Feed fractions CHO A1, A2, A3, A4, B1, and B2; NFC) characteristics, with many of the existing metabolic coefficients, including maintenance and growth potentials, remaining intact. Ruminal protozoal relationships have been studied, quantified, and published, including the uptake of free peptides and amino acids (AA), predation and engulfment of bacteria, and lysis/excretion of nutrients back into their environment. The CNCPS v.7 can capture these relationships, where predictions for protozoal growth and flow will be quantified as a source of microbial nitrogen, carbohydrates, and fatty acids (Table 3 and Table 5). Recreation of previously fed diets and formulation of prospective studies have elucidated a supply of protozoal MP that ranges between 10 and 20% of the total metabolizable microbial supply in most Northeastern US diets. In the study by Dineen et al. (2020) cattle were fed high quality Irish pasture grass, resulting in protozoal contributions representing 23% of microbial supply. It is plausible that cattle fed these highly degradable grasses, with high sugar content, maximize microbial growth and thereby represent the upper limit of protozoal contributions between 22-25% of total microbial yield. The addition of protozoal metabolism also provides insights on the microbial yield response when varying the supply of other carbohydrate fractions to a diet, particularly regarding protozoal growth, and subsequent microbial MP supply, when sugar is increased in a diet. Previous versions of the CNCPS were not sensitive enough to capture the full microbial yield response when sugar was added, only modestly improving NFC degrading bacteria growth. Further efforts to quantify microbial metabolism in the rumen will refine the effect other carbohydrates have on the proliferation of varying microbial communities.

In this version of the model, rumen ammonia levels are estimated based on a sub-model which predicts ammonia production, subsequent hepatic urea production and full urea recycling back to the gastrointestinal tract. This updated approach has at least two benefits. First, it will provide a more stochastic approach to estimating rumen ammonia as the flux generally displays a large amplitude throughout the day but recycling of nitrogen into the rumen is generally constant (Reynolds and Kristensen, 2008). It is important to note that behavioral patterns, including meal frequency and cow time budgets, in conjunction with dietary composition, including carbohydrate digestibility and nitrogen solubility, can interact to cause large swings in rumen ammonia, which can be

problematic throughout periods of the day where its concentration could drop below 5.5 mg/dL and causing microbial growth depression. Figure 1 describes the rumen ammonia concentration for a North American based diet that is formulated for 68% forage DM which uses various concentrate feedstuffs to provide other required nutrients. Two of these ingredients, soybean meal and canola meal, are fed at varying levels to provide a different soluble and degradable protein supply in the rumen. As with previous versions of the CNCPS, version 7 can calculate an average ammonia concentration for this diet; however, a static evaluation of this concentration may not provide a meaningful explanation if microbial growth is depressed. For instance, the diet which splits 2.5 kg of DM into equal parts of soybean meal and canola meal has an average ammonia concentration of 6.5 mg/dL which can raise some concerns but does not flag microbial growth depression within the model. Conversely, if a user was to describe the feeding behavior of the target animal, in this case an 8 meal/day behavior was designated, the model would provide a more dynamic form of rumen ammonia concentration that would indicate periods throughout the day where this concentration would be fall below 6.0 md/dL and microbial growth would be marginally depressed. Users will also be provided with a summarized table (Table 4) indicating both average and range of rumen ammonia concentration and microbial growth depression. Depression of microbial growth will become more pronounced with the associated decrease in carbohydrate digestion, specifically regarding potentially digestible aNDFom (pdaNDFom) as we expect the fiber degradation to be disproportionately decreased under N limiting conditions.

Another quantitative addition to the updated version of CNCPS is the inclusion of endogenous transactions which occur ubiquitously throughout the gastro-intestinal tract (Ouellet et al., 2007, Ouellet et al., 2010). The inclusions of these flows do not add an appreciable increase in the supply of metabolizable protein, as the majority of endogenous secretions that are quantified in the model are offset by the maintenance requirement calculated for the loss of these endogenous fractions. This, however, does not mean that these fractions should be left unquantified, given that the remains of salivary proteins, ruminal secretions, and sloughed cells can all be utilized by microbial populations within the rumen to proliferate and further alter the supply of amino acids flowing out of the rumen. Contributions of endogenous proteins within the CNCPS v.7 include salivary proteins (Yisehak et al., 2012), sloughed ruminal, omasal, and abomasal cells (Larsen et al., 2000), omasal and abomasal secretions (Ørskov et al., 1986), pancreatic secretions (Hamza, 1976, Larsen et al., 2000), bile secretions (Larsen et al., 2000), and small and large intestinal sloughed cells and secretions(Larsen et al., 2000, Jansman et al., 2002).

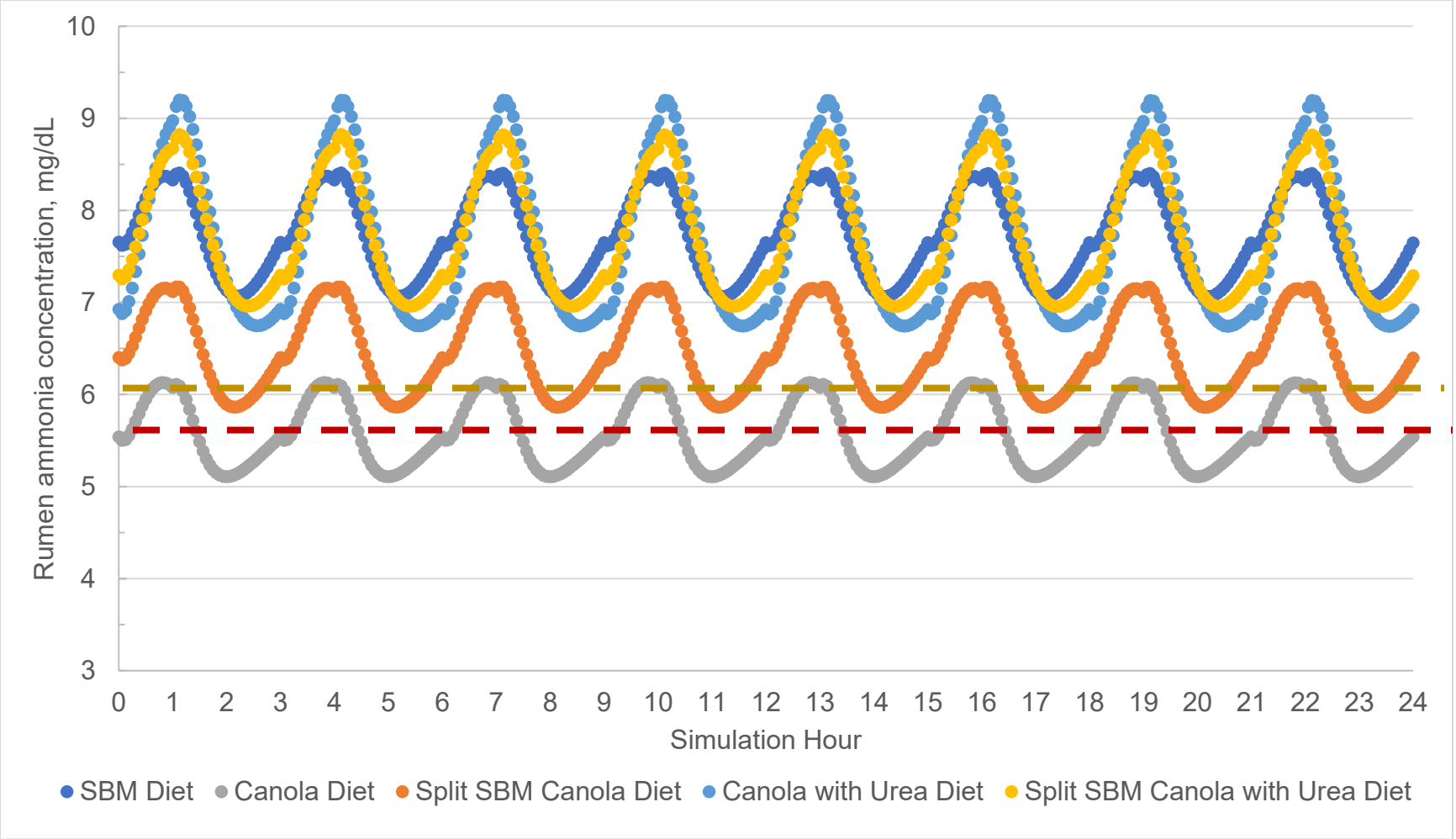

Figure 1. Rumen ammonia concentration, according to CNCPS v.7 after feeding a high forage diet (68% DM) with either A. 2.5 kg of soybean meal (SBM) included; B. 2.5 kg of canola meal included; C. 1.25 kg of SBM and 1.25 kg of canola meal included; D. 2.5 kg of canola meal with 125 grams of urea included; and E. 1.25 kg of SBM and 1.25 kg of canola meal with 125 grams of urea included. Within CNCPS v.7, microbial growth depression begins when ammonia concentration falls below 6.0 mg/dL and is significantly impactful when falling bellowing 5.5 mg/dL. Feed library values from the CNCPS were used to describe all feeds within this ration

Table 2. Output from CNCPS v.7 describing the flux and pool size of fiber fractions within the rumen. Outcomes aid in the determination of dry matter intake according to pdNDF or uNDF fill limits.

Fiber Fraction	Flux, $g \cdot d^{-1}$	Flux, kg $BW^{-1} \cdot d^{-1}$	Rumen pool size, g	Rumen pool Size, kg BW^{-1}
CHO B3; Fast	5187	0.69%	2070	0.28%
CHO B3; Slow	1405	0.19%	1421	0.19%
CHO B3; Total	6593	0.88%	3318	0.47%
NDF Recommendations[1]	-	1.27-1.47%	-	-
CHO C	1987	0.26%	4596	0.61%
uNDF Recommendations[1]	-	0.39-0.48%	-	0.48-0.62%

[1] Recommendations according to Cotanch et al. (2014)

Table 3. Metabolizable protein predictions from feed, bacteria, and protozoa under CNCPS v.7 predictions.

Metabolizable protein flows	Quantity
Feed MP, g	1349
Bacterial MP, g	1343
Protozoal MP, g	325
Feed MP, %	45.0%
Microbial MP, %	55.0%
Protozoal MP, % microbial supply	19.5%

Table 4. Rumen ammonia concentrations and associated microbial growth depression, both with provided minimum and maximums predicted over a day according to CNCPS v.7 predictions.

Rumen N concentrations	Mean	Max	Min
Rumen ammonia, mg/dL	9.3	11.1	8.1
Microbial growth depression			**% Depression**
Mean depression			0.0%
Minimum depression			0.0%
Maximum depression			0.1%

Table 5. Nitrogen supply transactions throughout the gastro-intestinal according to CNCPS v.7 predictions.

Parameter	Quantity	Parameter	Quantity
Ruminal transactions, g		**Duodenal flows, g**	
Feed		Non-ammonia nitrogen	777
Intake	664	Non-ammonia, non-microbial nitrogen	358
Degradation	359	Microbial nitrogen	506
Passage	224	**Small intestinal transactions, g**	
Free peptide and amino acids (PAA)		**Digested and absorbed**	
Degradation to ammonia	278	Feed	216
Uptake by NFC degrading bacteria	160	FC degrading bacteria	132
Uptake by protozoa	27	NFC degrading bacteria	194
Passage	38	Protozoa	79
Urea and Ammonia		Endogenous	38
Intake	81	Ammonia	29
Recycled	208	**Passage**	
Absorption	207	Feed	36
Passage	29	FC degrading bacteria	38
Uptake by FC degrading bacteria	191	NFC degrading bacteria	56
Uptake by NFC degrading bacteria	145	Protozoa	9
Excretion by protozoa	5	Endogenous	48
Microbial		Urea	96
FC degrading bacteria passage	170	**Large intestinal transactions, g**	
NFC degrading bacteria passage	250	Free PAA degraded to ammonia	13
Protozoal passage	87	Free PAA uptake by NFC degrading bacteria	13
Protozoal lysis and excretion	11	Ammonia absorption	163
Endogenous		FC degrading bacteria growth	20
Secretions	146	NFC degrading bacteria growth	27
Degradation	134	Feed excreted	36
Passage	12	Ruminal FC degrading bacteria excreted	37
		Ruminal NFC degrading bacteria excreted	56
		Ruminal protozoa excreted	9
		Endogenous excreted	33

Excretion and Productive Use

There is undoubtedly more pressure on dairy producers to evaluate and decrease nitrogen excretion, while maintaining productivity. As with the current version of the model, there will be excretion predictions for N and because of the model architecture, the user will be provided more information about the sources of N excretion and what typical values are and what can be modified (Table 5). This group aims to have users reference the breakout of nitrogen recycling along the gastro-intestinal tract, as partitioning of urea will be quantified in the rumen, small intestine, and large intestine. In doing so, users are encouraged to feed lower protein diets that will capture the native ability of a ruminant to recycle nitrogen, while minimizing excessive nitrogen loss in manure and maintain productive responses. A comprehensive outline of nitrogen excretion, including the sourcing of excreted nitrogen back to its origin, as well as quantifying metabolic urinary and urea urinary N, will provide the means to explicitly quantify and report excretion numbers for stakeholders and affiliated industries looking to inventory emissions and excretions on dairy farms. Our intent is to provide upper and lower boundaries for these excretion values and incorporate them into the current calculations based on grams of urinary urea N per unit of productivity N.

Efficiency of use has also become a means to measure productive efficiency of cattle, maximizes the productive output of cattle using more targeted nutrient supplies relative to predicted requirements. Amino acid efficiency of use, particularly describing with essential amino acids, has been made a priority within the CNCPS v.7. In addition to calculating the metabolizable gram amount of each essential amino acid, this supply is related to the metabolizable energy supply of the diet (Higgs and Van Amburgh, 2016). Efficiencies of use for each amino acid that are considered energetically optimum have been calculated and used to provide recommendations for the grams of metabolizable amino acid relative to metabolizable energy needed to achieve this efficiency. Users of the new version will be provided with these targets to formulate towards; however, the regressions used to calculate the optimum supply of amino acid relative to metabolizable energy will also provide the efficiency of use for varying supplies of amino acids which might not meet the recommended targets. This is to ensure that in the event nutritional or financial constraints or limitations in feed inventory are preventing the desired amino acid supply, the model will appropriately calculate an efficiency of use for these amino acids and allow the user with a better indication of productive expectations. Conversely, this system will produce marginal improvements in productive outputs if amino acids are supplied in excess, resulting in increased excretion of nitrogen relative to its intake.

Short- and Long-Term Goals

Not surprisingly, our contentment with this model is never satisfied and had it been, it would have reached the commercial space long before now. Currently, the CNCPS v.7 is being programed and packaged so that license holders may begin integrating this system into their existing software platforms. The development of the CNCPS, up until version 7, has existed in a spreadsheet environment that provides a ‘good-enough’ methodology for biologists to evaluate, modify, and update existing equations, while also

building new equations in parallel. Version 7 of the model was built in a more spatial environment, allowing for the construction of a system that was more comprehensive and could function dynamically as it integrated biological relationships over a simulated day and as programmed over 10 days. This environment also placates to those who are more visually adept at understanding these concepts; however, it has become computationally burdensome to host a version on this platform. As such, our short-term goal of programming version 7 into a packaged system that retains all of its functional capabilities while be computationally efficient is of utmost importance.

Beyond the computational goals of this system, we continually aim to improve the nutrient supply and predicted requirements for cattle at all stages of life. Given the rate in which fatty acids research is expanding in dairy cattle, it is apparent that the expansion of the fatty acid sub-model is warranted. The further disaggregation of feed fractions to provide better resolution of their supply, particularly regarding five and six carbon sugars, soluble fibers and proteins, and perhaps a fractionation of starch to better define its degradability. Lastly, and perhaps of greatest importance, is the quantification of behavior and its changes over time on nutrient supply. Figure 1 provides a dynamic concentration of rumen ammonia over the course of a day; however, the CNCPS v.7 predicts this concentration cycle as redundantly symmetrical, implying that cattle eat the same amount of dry matter at all meals. This obvious departure from cattle behavior is one that would provide a more robust insight into the way nutrient flows, and by extension the deficiencies of those flow relative to requirements, change throughout a day if they were corrected. Future updates of the model will look to include a behavioral sub-model which will utilize current and new animal inputs provided by the user to provide a more accurate prediction of nutrients flows and productive outputs. Overall, the intent of the updated model is to provide better information about functions that should help nutritionists improve their understanding of what might be limiting milk yield through improved mechanistic solutions.

Now, if only we could get this model out quicker…thank for your continued patience, especially you, Dr. Sniffen.

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