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New Dietary Guidelines for Americans and Impact on Dairy

J.M. Aldrich
CSA Animal Nutrition

Introduction

Since the first edition was published in 1980, the Dietary Guidelines for Americans (DGA) have provided science-based advice on what to eat and drink to promote health, reduce the risk of chronic disease, and meet nutrient needs. In 1990, the National Nutrition and Monitoring Related Research Act required that at least every 5 years the US Department of Agriculture (USDA) and Health and Human Services (HHS) would publish a report containing nutritional and dietary information and recommendations for the general public. The DGA is used to inform several government funded programs such as the National School Lunch Program (NSLP), Supplemental Nutrition Assistance Program (SNAP) and Women Infant Children (WIC). Meal standards for these programs align with the recommendations in the guidelines and provide an important avenue for dairy product consumption. In 2019, nearly 11 billion pounds of fluid milk, 683 million pounds of cheese, and 662 million pounds of yogurt and other dairy foods moved through these federal food assistance programs (Brown, 2021), representing almost 10% of the U.S. milk production.

The Good News for Dairy In the DGA

- Dairy is included as one of the major food groups that also included vegetables, fruits, grains, protein foods, and oils.
- The guidelines emphasize that American diets should be based on the consumption of nutrient dense foods. Dairy is considered a nutrient dense food that includes milk, yogurt, cheese, low-lactose, and lactose-free dairy products.
- For most life stages 3 servings of milk daily are recommended (Infants <6 months are recommended to be fed exclusively on human milk, toddlers 12 to 23 months; ~ 2 servings, and children 2 to 8 years old; 2 to 2 ½ servings).
- Sugar sweetened beverages and beverages based on nuts or oats (e.g., almond, rice, and coconut “milks”), which often compete with milk, are not recommended because they are not nutrient dense, and their nutrient profile does not fully replicate that of dairy milk. The DGA makes it very clear that these beverages are not adequate substitutes for milk.
- The amount of fruit juice, which also competes with milk, is limited from 4 to 10 ounces daily depending on the energy (kcal) needs of an individual.
- 93 percent of Americans do not consume the recommended servings of dairy products (Fig. 1). Therefore, if more people would adhere to these guidelines, dairy consumption in the United States would increase.

- Besides protein, the guidelines point out that dairy is an excellent source of three other nutrients of public health concern, namely calcium, potassium, and vitamin D.
- Although there was no movement for full-fat dairy products, the DGA states that people should choose low-fat and fat-free dairy “most often”. This new language provides flexibility for people to consume some full-fat dairy without exceeding recommended intakes of saturated fat.

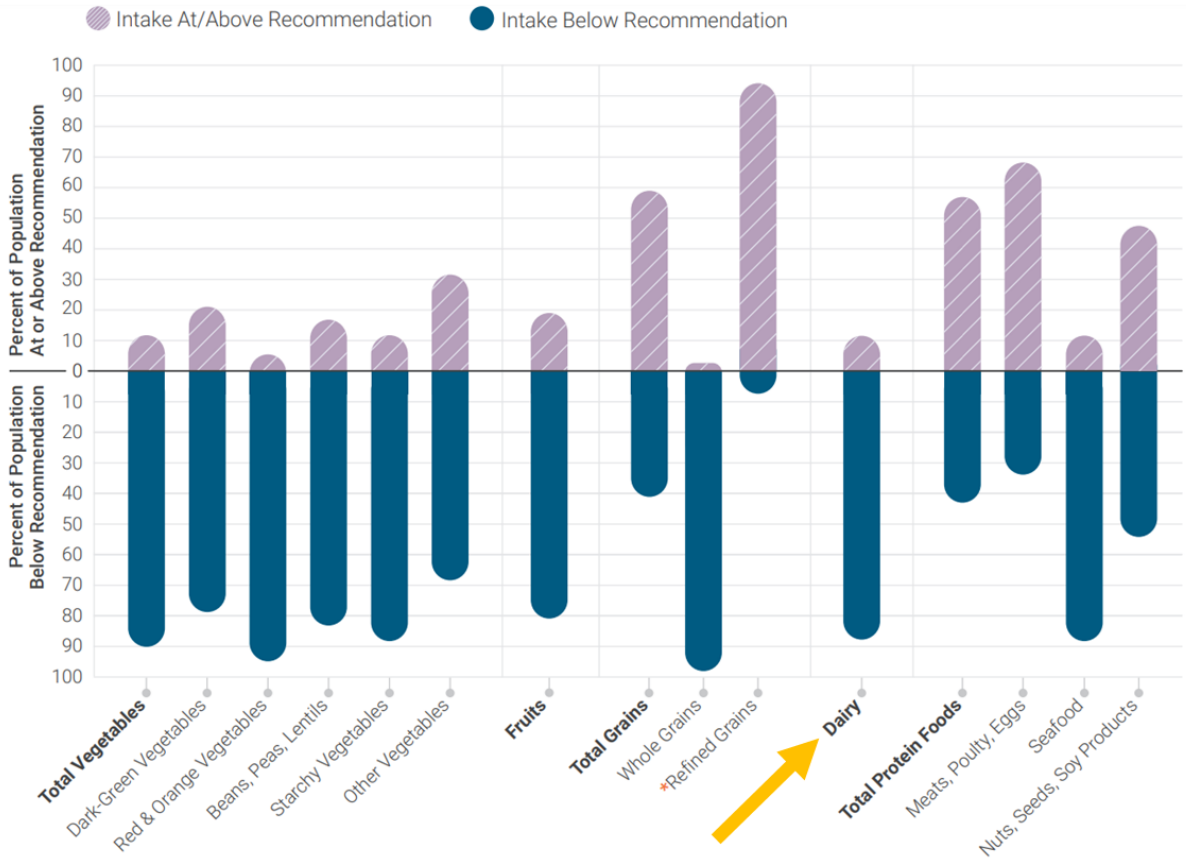

Figure 1. Dietary intakes compared to recommended intakes (USDA/HHS, 2020)

Concerns about the DGA Relative to Dairy

The DGA continues the long-held recommendation to limit saturated fat intake to less than 10% of total energy (kcal) intake. The reason for this limit was born of what became known as the diet/heart hypothesis of coronary heart disease (CHD) developed in the 1950’s and 1960’s with Ancel Keys (Keys 1953; Keys et al., 1966) playing the leading role. Others have written in great detail about the flawed underpinning science, early on (Yerushalmy and Hilleboe, 1957) and more recently (Lock and Bauman, 2011; Elliot, 2014; Teicholz, 2014; Rico and Rico, 2018). Briefly, the theory is based on the belief that dietary saturated fat increases the level of cholesterol in blood, which increases cholesterol deposition in arteries and leads to CHD. While high blood cholesterol is a well-

established risk factor for heart disease, this hypothesis concerning dietary saturated fat to increase blood cholesterol has never been agreed upon by scientists and researchers but continues to be presented as fact (Elliot, 2014). In a review of recent meta-analyses of randomized trials and observational studies (Astrup, et al., 2020), it was reported that there is no clear beneficial effect to reduce saturated fat intake to lower the risk of cardiovascular disease (CVD) and total mortality, whereas a protective effect against stroke was apparent.

The health effects of food in general and dairy in particular cannot be predicted by the content of any single nutrient group. Whole fat dairy and other foods that contain saturated fat in a complex matrix are not associated with increased risk of CVD (Astrup, et al., 2020). In fact, as reviewed by Rico and Rico (2018), recent studies suggest that dairy in general, including full-fat dairy may protect from obesity and associated chronic diseases. Conjugated linoleic acid from milk fat has been shown to have anti-carcinogenic, anti-atherogenic, anti-diabetic and other beneficial health effects in animal models (Bauman, et al., 2001; Ip, et al., 1999). Evidence in humans also support that full-fat dairy promotes satiety to reduce total daily energy consumption and helps to displace other foodstuffs with poor nutritional value (e.g., sugar sweetened beverages) that would otherwise contribute to excess energy consumption.

Nearly 1 in 3 North American children are now overweight or obese and childhood obesity has increased in the last 40 years while consumption of whole milk has been halved (Vanderhout, et al., 2020). A recent analysis suggests that higher cow-milk intake is associated with lower childhood obesity (Vanderhout, et al., 2020).

Many prefer the taste of whole fat over low fat milk. Dietary preferences throughout life are affected by what one eats in childhood (USDA/HHS, 2020). Children and adolescents who are only exposed to low fat dairy, may not continue to drink milk later in life.

When limiting calories from fat, including saturated fat, those calories are likely mostly replaced with carbohydrates. Today, there is strong evidence and growing consensus that over-consumption carbohydrates especially sugar and refined carbohydrates is the dietary factor largely responsible for obesity and risk for chronic diseases (Taubes, 2007; Taubes, 2011; DiNicolantonio et al.,2016).

Finally, due to the DGA recommendation to limit intake of saturated fat, only fat-free or low-fat dairy products are recommended. This means butter, higher fat cheese, heavy cream and other higher fat dairy products are not recommended.

Soy Beverage versus Milk

Even though most plant-based beverages are not recommended substitutes for dairy the DGA states: “…for individuals who choose dairy alternatives, fortified soy beverages (commonly known as “soy milk”) and soy yogurt – which are fortified with

calcium, vitamin A and vitamin D – are included as part of the dairy group because they are similar to milk and yogurt based on nutrient composition and use in meals."

Does the nutritional equivalency implied hold up to a more detailed evaluation? Both contain similar calories, the total fat content is similar, but the fatty acid profile differs between beverages, and total protein is also similar. Calcium is higher in soy beverage, but it is in the form of calcium carbonate which has lower bioavailability than the calcium in milk. Both are fortified with vitamins A and D. Soy beverage is fortified with a few B vitamins riboflavin (vitamin B2) and vitamin B12, whereas milk provides these as part of its native nutrient matrix. Sugar is lower in soy beverage, but the source is added cane sugar, whereas milk sugar is from lactose.

Although the total protein content between dairy milk and soy beverage is the same, the biological quality of the protein is quite different. Protein quality can be defined by the essential amino acid (EAA) composition (relative to the human requirement pattern) and the intestinal digestibility of the protein and amino acids (FAO, 2013; CVB 2016). The comparison of milk and soy protein in terms of amino acid digestibility in the small intestine (relative to the human requirement pattern) is shown in Figure 2.

Compared to requirements, note that soy meets the EAA requirement pattern, except that it is very limited in methionine (Met) in fact, it covers only 50% of the requirement. Milk, on the other hand meets or exceeds the requirement pattern for all EAA. Compared to soy, the EAA of milk proteins range from equal (tryptophan and phenylalanine) to 1.5-times higher in lysine and 2-times higher in Met. The lower level of Met in soy beverage can be compensated by consumption of other foods that have higher levels of Met, such as grains (e.g., corn or rice) but then care must be taken not to exceed total energy intake.

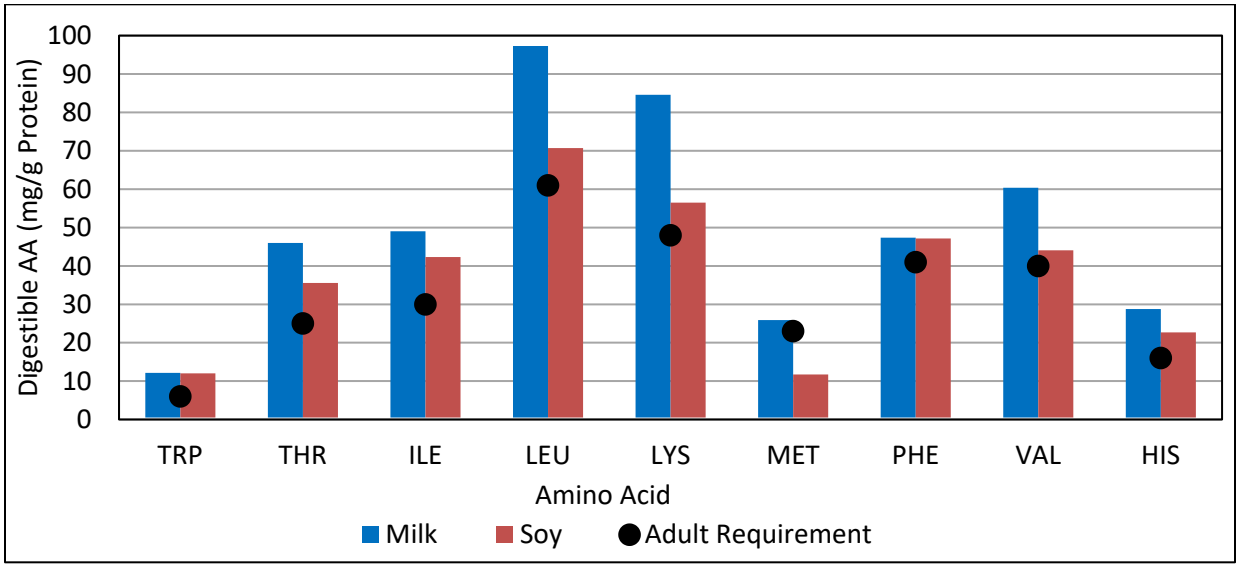

Figure 2. Comparison of milk and soy protein in terms of digestible amino acids (AA) in the small intestine relative to the adult requirement

Another item that needs to be factored into this assessment is affordability. The DGA acknowledges that a healthy dietary pattern needs to fit within budgetary constraints. Comparing costs of milk versus soy beverage using recent prices from a major Midwest supermarket showed that milk is $2.99/gallon (128 oz) whereas soy beverage costs $2.99 for 64 oz (the largest package size available). Importantly, this is only a simple comparison by volume and does not account for the greater costs that would be incurred if one aimed to match the additional EAA that would be needed from soy beverage or from the purchase of foods to achieve similar intakes of EAA. The bottom line is that soy beverage fails the test as a nutritional alternative to milk and is twice the cost of milk per serving and even more costly if one aims to match daily nutritional intakes.

Finally, even though dairy is recognized as its own food group, it is not referenced as a source of protein in the protein category, even though it is a significant contributor to protein and amino acid requirements. The recommended 3 servings of dairy/day will provide: 8 g/serving x 3 = 24 g of protein per day. Using a conservative recommended intake of 0.8 g protein per kilogram body mass, an average 70 kg adult (154 pounds) would achieve approximately 40% of their daily protein needs.

Summary

There is positive news for dairy in the most recent DGA. Dairy products are recognized as an important component of a healthy eating pattern. About 90% of the U.S. population does not meet recommended dairy consumption. Alternative plant-based and sugary soft drinks that compete with dairy are strongly discouraged by the DGA. Unfortunately, the DGA continues to limit saturated fats so only low-fat or fat-free dairy products are advised. Soy-based products are suggested as milk alternatives despite their nutritional inferiority and higher costs.

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Enteric Methane Mitigation

E. Kebreab and J. Fouts
Department of Animal Science
University of California, Davis

Introduction

The world is experiencing unprecedented extreme weather events due to climate change, caused by accumulation of greenhouse gases (GHG). Methane is a GHG with global warming potential of 28 times that of carbon dioxide over a 100-year period and more effective at trapping heat during the time it is in the atmosphere. The largest source of methane is from agriculture and waste and particularly ruminants such as cattle (NASEM, 2018). Globally, livestock are responsible for about 14.5% of GHG emissions ranging from 4% in the US to 43% in New Zealand (Gerber et al., 2013). According to the Food and Agriculture Organization of the United Nations (FAO), demand for animal products is increasing, especially in countries with growing populations and income. Due to increases in ruminant population to satisfy demand, methane emissions from livestock rose more than 50% in the last 60 years and are expected to continue rising (FAO, 2017). In the US, agricultural emissions in general are about 10% of total GHG emissions with animal agriculture contributing to about 4% of total direct GHG emissions (EPA, 2021).

Enteric methane represents about 2 billion tons of CO_2 equivalent per year or more than 4% of annual global GHG emissions. Enteric methane production contributes to 70% of GHG emissions from livestock in the US (EPA, 2021); therefore, it is key to mitigating such emissions. About a quarter of livestock related methane emissions from enteric fermentation is from dairy cattle. The majority (about 72%) comes from beef cattle. However, the majority of manure related methane is from dairy cattle followed by swine. About a third of nitrous oxide emissions is from dairy cattle while beef contribute close to half (EPA, 2021).

A number of strategies have been developed to reduce enteric methane emissions. The mitigation strategies can be classified into (1) feed manipulation, (2) rumen modifiers, and (3) increasing animal production through genetics and management (Knapp et al., 2014). The objective is to review the status of mitigation options that have high potential to reduce enteric methane emissions from livestock. The review is not intended to be exhaustive but rather highlighting potential mitigation options that can be deployed within the next 5 to 10 years.

Feed Manipulation

There are several ways to manipulate feed and diet to reduce enteric methane emissions. In this section we cover feed manipulation through addition of lipids, nitrate and improvement of forage quality.

Lipids

Added dietary lipids could decrease methanogenesis in several ways including (1) lowering the quantity of organic matter fermented in the rumen; (2) hindering the activity of rumen methanogens; and (3) through biohydrogenation of lipids rich in unsaturated fatty acids. Supplementation of dairy cow diets with lipids has been one of the most extensively experimented enteric methane mitigation strategies. A systematic review by Eugène et al. (2008) concluded that lipid supplemented diets containing, on average, 6.4% ether extract (EE) reduced methane production in lactating dairy cows by 9% (~30 g/cow per day) compared with diets containing 2.5% EE. Furthermore, they observed that this reduction was mainly a consequence of decreased DMI, although milk production and milk composition were not affected. A meta-analysis by Grainger and Beauchemin (2011) showed a persistent reduction in enteric methane per unit of DMI for dietary lipid supplementations. In another meta-analysis, Patra (2014) examined the impact of the composition of added lipids on enteric methane production and reported that fats with high concentrations of C12:0, C18:3, and polyunsaturated fatty acids had marked inhibitory effect on methane production independent of DMI in cattle. Odongo et al. (2007) fed Canadian dairy cows with myristic acid (C14:0) at 5% of dietary DM and observed methane intensity reduction of about 29% without altering DMI, milk yield, or milk fat percentage. Jayasundara et al. (2016) calculated that a one unit increase in EE from 3.0% of DM was associated with a 12.5 g/cow per day reduction in methane production, implying that total methane reduction associated with increased dietary EE from 3.0% to 6.0% of DM would be, on average, 37.5 g per cow per day.

Nitrate

Nitrate ($NO3^{-}$) is an inorganic anion and acts as an alternative hydrogen sink in the rumen. Supplementing a diet with nitrate is regarded as an effective and promising methane mitigation strategy by competing with methanogens for available hydrogen through its reduction of ammonia in the rumen. Studies have shown major reductions in methane emissions with nitrate supplementation, but with large variation in response. In a meta-analysis, Lee and Beauchemin (2014) demonstrated that nitrate is a viable candidate for feed additive that could be used to mitigate enteric methane emissions in ruminants. Similarly, van Gastelen et al. (2019) demonstrated that methane production was indeed consistently decreased when feeding nitrate to different types of ruminants. Feng et al. (2020) quantified the amount of methane emissions expected to be reduced by nitrate supplementation through a meta-analysis. The authors reported that nitrate supplementation reduced methane emissions (production in g/d) by 20.4% in dairy and 10.1% in beef cattle on average. Similarly methane yield (in g/kg of DMI) was reduced by 15.5% in dairy and 8.95% in beef cattle in a dose-dependent manner. The mitigating effect of nitrate on methane production and yield was greater in dairy than in beef cattle. However, effect of type of cattle appears to be related to slow-release nitrate use in beef cattle. A greater nitrate dose enhanced the nitrate mitigating effect on methane production and yield, whereas an increased DMI reduces the mitigating effect of nitrate on methane production.

Forage quality

Forages constitute the major proportion of dairy cow diets; however, few studies have investigated the effect of forage type on enteric methane emissions. Corn silage usually contains greater amounts of starch (e.g., 30% DM; Maizex, 2015) than silages from other forages (e.g., 9.4% DM in barley silage; Oba and Swift, 2013). Feeding more starch without compromising rumen health (i.e., acidosis) and/or production (e.g., milk fat depression) has been shown to be associated with less methane losses (Mills et al., 2003) and improved milk yields (Khorasani et al., 1994). Therefore, increasing the proportion of corn silage at the expense of cereal or legume silage is considered a promising enteric methane mitigation strategy, provided that the desired maturity stage of corn corresponding to high starch contents is achieved. It may be possible to decrease enteric methane yield by up to 15% with forage quality improvement; however, possible trade-offs from increased methane emissions from manure and implications related to increased production of whole corn silage in place of other silage crops need to be evaluated at the whole farm scale (Jayasundara et al., 2016).

Feed Additives

Recent advances in understanding of the rumen and methanogenesis has led to development of feed additives that have the potential to reduce enteric methane emissions substantially. Due to continued interest in this area, research is expected to accelerate in developing feed additives that can provide options in mitigating enteric methane emissions. In this section we will discuss mitigation options that directly affect methanogenesis in the reticulo-rumen. These include inhibitors that target methanogens or other microbes associated with methane emissions and vaccines. Innovations that have the potential to reduce enteric emissions including 3-nitrooxypropanol (3NOP) macroalgae and plant secondary compounds are discussed below.

3-Nitrooxypropanol (3NOP)

The compound, 3NOP, is a highly specific inhibitor of methanogenesis in the reticulo-rumen. Several studies using 3NOP as an additive have reported reduction in methane emissions from beef and dairy cattle up to 60%. Dijkstra et al. (2018) conducted a meta-analysis of the effectiveness of 3NOP and reported an average of 32.5% reduction; however, there was differences in type of animals. In dairy cattle, at an average 3NOP supplementation of 81 mg/kg DM, there was a 39% reduction in enteric methane while in beef cattle the emission was reduced by 22.2% with average supplementation of 144 mg 3NOP/kg DM. The authors attributed the greater efficacy of 3NOP in decreasing methane emissions in dairy cattle compared with beef cattle to the higher feed intake level in dairy cattle. Higher feed intake levels increase rumen concentrations of fermentation products, including VFA and hydrogen. Larger feed intake levels in dairy cattle than in beef cattle may be associated with relatively (i.e., per unit of feed fermented) greater alternative hydrogen sinks for rumen methanogenesis, resulting in relatively lesser concentrations of enzyme catalyzing methane formation and elevated inhibitory potential of 3NOP. 3NOP is a compound consisting of a molecule of propylene glycol and nitrate

and resembles a key molecule in methane formation - methyl-coenzyme M reductase (MCR). 3NOP specifically targets MCR, which is a nickel enzyme and only active when its Ni ion is in the +1 oxidation state (Duin et al., 2016). MCR catalyzes the methane-forming step in the rumen fermentation. 3NOP preferably binds into the active site of MCR and effectively inactivates it. 3NOP is demonstrated to inhibit growth of methanogenic archaea at concentrations that do not affect the growth of nonmethanogenic bacteria in the rumen (Duin et al., 2016). According to Hristov et al. (2015), supplementation of 3NOP did not significantly affect feed intake or milk production.

Macroalgae

Some seaweed species, particularly *Asparagopsis*, contain bromoform and bromochlormethane as active ingredients that has been shown to be effective in vitro (Machado et al., 2016). Bromochloromethane in its pure form cannot be used as it is a banned substance under the Montreal Protocol. The first in vivo trial using *Asparagopsis armata* in cattle (Roque et al., 2019a) reported up to 67% reduction in methane production in dairy cattle with inclusion of 1% of organic matter (OM). The authors reported a decline in feed intake, particularly at the high level of inclusion, which might have compromised milk production. Kinley et al. (2020) reported that methane emissions in Brangus cattle declined 98% with inclusion of only 0.02% (OM basis) of *Asparagopsis taxiformis*. Additionally, they reported no reduction in feed intake or loss of productivity. Roque et al. (2021) conducted a longer-term study to investigate the potential for adaptation by microbes and interaction with feed quality. The authors reported that there was no evidence of microbial adaptation when steers were fed for up to 5 months. The efficacy was dependent on fiber concentration and ranged from about 50% in high NDF diets to 80% under feedlot conditions. Analysis of meat quality in supplemented groups showed that there was no interactive effect between treatments and time on the shelf life of steaks (Bolkenov et al., 2021). The efficacy of methane reduction appears to correlate with the concentration of bromoform compounds, which appear to be the main active ingredients although other yet to be identified substances may contribute to methane reduction as well (Vijin et al., 2020). Analysis of the meat from seaweed supplemented animals did not show any bromoform residue. The main barriers for adoption of macroalgae as a mitigation option are 1. Regulatory approval and 2. Scaling up production enough to feed cattle around the world. Further research and removal of barriers is required before widespread adoption.

Plant Secondary Compounds

Tannins

Tannins are soluble, phenolic compounds that accumulate within plant tissues likely due to ongoing metabolic processes and contribute to the plant defense system (Swanson, 2003). The methane mitigation mechanisms of tannins are not well understood but may be due to a combination of various factors. Several mechanisms have been proposed for the anti-methanogenic activity of tannins including direct inhibition of methanogens and the protozoa population associated with methanogens; decreasing

hydrogen production through inhibition of fibrolytic bacteria and fiber digestibility, and acting as an alternative hydrogen sink to methanogenesis (Aboagye and Beauchemin, 2019). Jayanegara et al. (2012) conducted a meta-analysis describing the relationship between rumen methane formation and the level of dietary tannin (hydrolysed or condensed) inclusion between in vivo and in vitro models. These authors reported that low levels of inclusions of tannins in animal experiments often yielded inconsistent results on methane production, but that variability seemed to diminish at higher doses, leading to setting the threshold for detecting treatment differences in animals to be >20 g/kg DM of tanniferous inhibitors. Furthermore, reduction in methane production was often followed by a suppression in OM and fiber digestibility. Care should be taken when supplementing tannins as several studies have shown that tannins bind and interact with dietary proteins in the gastrointestinal tract, which reduces nitrogen availability to the animal (Waghorn, 2008).

Essential oils and blends

Essential oils are naturally occurring chemical ompounds extracted from plants and used in fragrances and cosmetics and, to a lesser extent, pharmaceutical products for humans and animals (Honan et al., 2021). Volatile in nature, they contribute to the phenotypic expression of the plant including color and scent (Benchaar et al., 2008). Consumption of essential oils affects rumen microbial communities and fermentation patterns in a varying manner, depending on the source (Benchaar and Greathead, 2011). Many essential oils hold a high affinity for lipid and bacterial membranes, leading to disruption, but the broad antimicrobial effect is likely to be due to a combination of mechanisms (Helander et al., 1998). Numerous plants such as cinnamon, lemongrass, ginger, garlic, juniper berries, eucalyptus, thyme, citrus, oregano, mint, rosemary and coriander have been screened in vitro (Becnhaar et al., 2008; Nanon et al., 2015). However, only few have been studied in vivo.

Some studies have used an essential oil ‘blend’ or ‘complex’ containing extracts from multiple plants. For example, Mootral is synthesized from natural products including garlic- and flavonoid-containing citrus extract and has demonstrated anti-methanogenic properties (Eger et al. 2018; Vrancken et al., 2019). The garlic component in Mootral targets methanogenic archaea populations and protozoal communities in the rumen and has led to nearly complete inhibition of methane production in vitro at a dosage of 2 g experimental mixture/day, without compromising bacterial population (Eger et al., 2018). A 23.2% decrease in methane yield (26.8% expressed in methane production) was observed in Angus x Hereford crosses after 12 weeks of treatment by supplementing Mootral at 1.58 g/kg DM (Roque et al., 2019b). Adverse effects on DMI, ADG and feed efficiency were not detected over the 12-week trial. Lactating cattle offered Mootral incorporated in pellets at a rate of 0.64 g/kg DM for Holstein-Friesian and 1.21 g/kg DM for Jersey herd experienced suppression of methane of 20.7% and 38.3%, respectively (Vrancken et al., 2019). Additionally, 3–5% increase in milk yield across breeds was observed with increased feed efficiency in the Jersey cattle.

Improved Efficiency

Animal efficiency has been a goal for decades for both beef and dairy cattle to produce more with less input. Increasing animal production efficiency is accompanied by decreases in methane emission intensity (methane emitted per unit of milk produced, often measured as CH_4 g/kg of milk) (Hristov et al., 2013). Factors affecting methane intensity include forage-to-concentrate ratio, forage quality, forage type, grazing management, and breeding strategies.

Forage-To-Concentrate Ratio

Decreasing the forage-to-concentrate ratio has been shown to reduce methane emissions and intensity (Tyrell and Moe, 1972). Aguerre et al. (2011) conducted an *in vivo* study to investigate the effects of different forage-to-concentrate ratios (47:53, 54:46, 61:39, 68:32) on emissions and production responses in dairy cattle. The lowest forage-to-concentrate ratio resulted in a 17% decrease in methane emissions (g/d) compared to the highest ratio. The authors also found that methane emissions per kilogram DMI, milk, and 3.5% ECM had a positive, linear relationship with forage level in the diet. Starch fermentation shifts VFA production away from butyrate and acetate and towards propionate (Ungerfeld, 2020). Butyrate and acetate are associated with hydrogen production, leading to more hydrogen available for methanogenesis. Conversely, propionate uses hydrogen and is a competitive sink against methanogenesis. (Benchaar et al., 2001; Ungerfeld, 2020). In addition, starch decreases the pH of the rumen environment, inhibiting methanogens and decreasing hydrogen availability, thus decreasing the digestibility of fiber in the diet and the production of methane (Van Kessel and Russell, 1996). Although concentrates reduce methanogenesis, excessive starch in the diet leads to subacute ruminal acidosis, laminitis, and decreased milk fat, compromising animal health and productivity.

Forage Quality

Improving forage quality is a potential strategy in reducing methane emissions and intensity (Hristov et al., 2013). When DMI increases with highly digestible feeds, methane produced per unit of feed consumed decreases (Hristov et al., 2013). Forage quality is determined by maturity, climate, and plant species (Eugène et al., 2021). As a plant matures, its fiber and lignin content increase, resulting in lower digestibility and higher enteric methane when fed to cattle (Jung and Allen, 1995). Lower fiber content, and thus higher digestible feeds, leads to faster fermentation and increased propionate production (van Gastelen et al., 2019; Ungerfeld, 2020). The impact of forage quality on methane emission and intensity varies between studies and animals (Hristov et al., 2013). van Gastelen et al. (2019) used 19 studies to assess the effects of increased digestibility (expressed as OM digestibility) of grass silage on dairy and beef cattle. In dairy cattle, the authors found that an average of 25% OM digestibility improvement resulted in an average decrease of 10% for methane yield (g/kg DMI) and 19% for methane intensity (g/kg milk). However, beef cattle saw no effects of improved digestibility on yield or intensity. The authors attribute this difference in animals to differences in feed intake

relative to body weight, as well as the composition of the diets. Most of the dairy studies incorporated concentrates in the diet, which contributed to the reduction in methane emissions.

Forage Type

Methane emissions also have the potential to be reduced when corn silage replaces grass silage (Hristov et al., 2013). van Gastelen et al. (2019) compared the effects of using corn silage in the place of grass silage in beef or dairy cattle across 23 studies, finding an average of 8% decrease in methane intensity from replacing grass silage with corn silage. The authors found a smaller effect of this dietary strategy in beef cattle compared to dairy, attributing this difference to the different responses in DMI and apparent total-tract digestibility. Dairy cattle fed corn silage increased their DMI, leading to increased fermentation and more propionate production (van Gastelen et al., 2019). Although replacing grass silage with corn silage in the diet shows promise in reducing enteric emissions, manure methane could increase from corn silage, and carbon dioxide emissions from the soil are greater for corn compared to grass silage (Eugène et al., 2021). Therefore, more research is needed on the whole-system effects of corn silage on the environment.

Legume forages have also been shown to reduce methane emissions (Hristov et al., 2013). When considering dietary energy, the replacement of timothy hay with alfalfa showed a 21% reduction in methane emissions within a modelling approach (Benchaar et al., 2001). The lower fiber content of legumes and faster passage rate allows for decreased methane production. Some legumes also contain tannins (van Gastelen et al., 2019; Eugène et al., 2021), which were discussed previously as a plant secondary compound capable of reducing enteric methane emissions. Legume silages also have the added benefit of lower nitrogen input from fertilizer and more nitrogen provided to the animal, increasing animal productivity while decreasing ammonia emissions (Eugène et al., 2021). However, excessive intake of legumes with high concentrations of protein (such as red or white clover) increases the risk of bloat.

Grazing Management

Grazing contributes to 45 and 57% of total beef and milk production throughout the world (Zubieta et al., 2021), so its management is an important consideration in methane mitigation. Rotational grazing allows for more efficient use of forage, therefore, it is a proposed strategy for reducing methane intensity in dairy and beef cattle (Hristov et al., 2013). DeRamus et al. (2003) found that annual methane emissions under rotational grazing decrease by 22% compared to continuous grazing. However, Zubieta et al. (2021) emphasized that the impact of rotational or continuous grazing on methane intensity is based on stocking rate, herbage allowance, and herbage mass. Although limited studies assess the impact of rotational grazing on methane intensity, its additional environmental benefits include a preservation of biodiversity and reduction of soil erosion (Thomspon and Rowntree, 2020). Other grazing strategies include concepts discussed previously: graze at ideal maturity/quality of forages, include leguminous forages (Archimede et al.,

2011), supplement with concentrates (Zubieta et al., 2021), and incorporate plant secondary compounds (Thompson and Rowntree, 2020).

Breeding Strategies

In addition to management and nutritional approaches, breeding for more efficient and low methane emitting animals has the potential to reduce methane emissions and intensity. Genetic traits that contribute to more efficient dairy cattle, and thus lower methane emission intensity, include milk protein, milk fat, survival, and calving interval. In addition, methane production is considered a heritable trait, with a heritability ranging from 0.21 to 0.35 (De Haas et al., 2011; Lassen and Løvendahl, 2016). By actively selecting for lower methane emitting cows, methane production and intensity can be reduced. De Haas et al. (2021) quantified the impact of adding methane emissions in the Dutch breeding strategy, finding that selective breeding has the potential to reduce methane intensity between 13 and 25% by 2050. Limitations to breeding for low methane emitters include the lack of knowledge on the full biological implications and impractical methane measurement techniques on the animal level, hindering direct selection and collection of data (De Haas et al., 2011). Once these limitations are overcome, breeding for low emitting animals could provide cost-effective, cumulative, and permanent effects on methane reduction (De Haas et al., 2011; 2021).

Summary

Enteric methane production contributes to most of the GHG emissions from livestock; therefore, it is key to mitigating such emissions. A number of strategies have been developed to reduce enteric methane emissions. These vary from those that directly target methanogenesis to indirectly reducing emissions by improving feed efficiency. Recent advances in understanding of the rumen and methanogenesis has led to development of feed additives that have the potential to reduce enteric methane emissions substantially. Overall, more research is needed on feed additives to understand methane reduction, rumen adaptation, and cattle health implications in the long-term. Due to continued interest in this area, research is expected to accelerate in developing feed additives that can provide options in mitigating enteric methane emissions. Increased animal production efficiency or improved reproduction would also indirectly reduce methane emissions as it reduces methane intensity (methane produced per unit of product). Improved efficiency could be achieved through better forages (such as high sugar/starch or low fiber) or better management particularly in grazing systems. Breeding for low methane emissions has also shown a promise in selecting breeds for reduced enteric methane emissions.

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Ruminant Farm Systems Model: Development Progress and Applications

K. F. Reed
Department of Animal Science
Cornell University

Model Background

Simulation models are a tool that can guide policy, support farm decisions, and evaluate novel technologies. Models can estimate multiple outcomes that result from of management changes or adoption of new technologies and provide a more robust, systematic evaluation than isolated research experiments. Examples of whole-farm models for dairy production include the Integrated Farm Systems Model (Rotz et al., 2013), DairyMod (Johnson et al., 2008), DyNoFlo (Cabrera et al., 2006), and SIMS(DAIRY) (Del Prado et al., 2011). However, wide-scale industry adoption of these models has not occurred due to limitations in model applications for current and future scenario analysis. Existing model structure and code bases prevent model adaptation or development of features like data integration and novel management scenarios that would encourage widespread. Thus, we are developing a new farm simulation model that can adapt to changing technologies and support sustainable dairy production (Kebreab et al., 2019).

The Ruminant Farm Systems Model (RuFaS, Figure 1) applies modern computer coding practices centered around clarity and adaptability to respond to evolving technologies in the dairy industry. RuFaS embraces the key characteristics for next-generation agricultural systems models described by Jones et al. (2017) so that it can be adaptable as new technology arises, be interoperable with other software and models, and meets user needs by continuous interaction with stakeholders during the development process.

Our development team includes scientists from 5 universities and several USDA-ARS stations who represent a range of disciplines. Rather than relying on research scientists to fill the role of translating their model equations and algorithms into computer code, we work closely with computer scientists to develop the modular codebase. We emphasize thorough documentation at all steps of model development. The scientists develop detailed pseudocode that provide heuristic descriptions of the model processes, literature references, and the mathematical equations. Similarly, the computer scientists provide in-code comments that describe the flow of information and references to equation numbers from the pseudocode to link the computer code directly to the scientific documentation.

The Ruminant Farm System (RuFaS) model is based on a foundation of four biophysical modules (animal, manure handling, crop + soil, and feed storage) that represent the main components of a dairy farm as shown in Figure 1. The simulation inputs include the desired length of the simulation, herd characteristics, manure management strategy, crop characteristics, and other elements of farm management. We

use a tiered file structure for inputs that separates inputs that designate whole farm and simulation structure from inputs specific to each of the modules with increasing level of detail associated with inputs at lower tiers. Model outputs are exported to CSV files, graphic images, and an SQL database. The model uses a daily time-step and is programmed in Python, an adaptable and easy to read computer programming language.

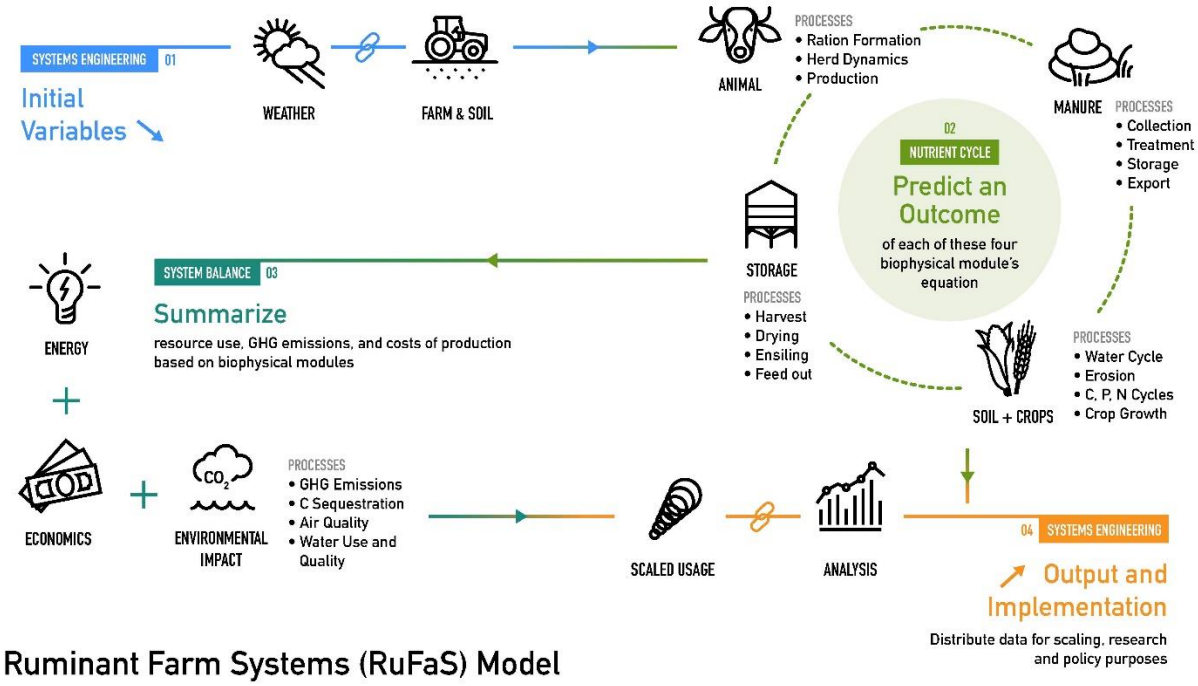

Figure 1. Conceptual diagram of the Ruminant Farm Systems model

Progress Updates

Model Inputs and Management Options

Through the model inputs, the user defines the farm management and environment for each simulation scenario. At the farm level, the user specifies the target lactating cow number, the replacement rate and growing herd size, the housing type and size, purchased and growing feeds, field number and size, and provides the weather (temperature, solar radiation, precipitation) during the simulation period. At the herd level, the user can specify the breed and reproduction protocols. The model primarily uses the Wood's lactation curve to estimate the baseline milk production for each cow on each day of lactation and this baseline production can be adjusted to fit desired farm or total lactation production by modifying the lactation curve parameters. Other animal characteristics that can be modified by the user include parameters that define the bodyweight distribution, reproductive efficiency, and probability of disease.

The model user defines a manure management strategy for each animal pen to provide the flexibility to represent different manure compositions and handling methods based on the animal group. The method and frequency of manure collection, treatment

and processing methods, and storage length and type are all set by the user for each pen or group of pens.

The Crop and Soil Module has similar flexibility to represent a range of crop production practices. The user can specify any number of fields, each with its own size, soil properties, crop rotation, and tillage, fertilization, planting, and harvest practices. Crop growth in RuFaS is based on the methods used in the Soil and Water Assessment Tool (Neitsch et al., 2011) and currently has the ability to simulate corn, alfalfa, soybeans, rye, winter wheat, meadow fescue, and beets.

The feed storage module is much simpler than the rest of the model in its current state and provides only empirical estimates of forage composition change, emissions, and leachate for silage and hay storage. This module estimates changes in forage composition during storage once per season which is the only part of the model that does not function on a daily timestep.

Nutrient Cycling and Outputs

RuFaS simulates transformation, export, and loss of biomass, agriculturally significant elements (N, P, and K) and H_2O as they cycle through the 4 modules that represent a dairy farm. The Feed Storage module tracks the composition and inventory of farm grown feeds. This information is passed to the Animal Module and used, in combination with purchased feeds, by the automated, least-cost diet formulation algorithms to simulate feed delivery each day. The diet formulation algorithms are currently based on the NRC (2001) Nutrient Requirements for Dairy Cattle though we hope to update them soon. The Animal Module uses a Monte Carlo stochastic framework to simulate each individual animal as they move through their lifecycle on the farm which is represented by 5 distinct animal classes. Detailed descriptions of the ration formulation methods and the life events simulated by the Animal Module are provided in our recent publication (Hansen et al., 2021). The simulated intake, diet composition and characteristics of each individual animal drive the estimates for partitioning of the diet N, P, and K into milk, body mass, and manure to maintain a mass balance of these important elements at the animal level. Manure organic matter and enteric methane emissions are also estimated. Daily production of manure from each animal is summed per pen and the total manure mass and composition (DM content, volatile solids, degradable volatile solids, N, K, P, soluble P, and ammonia concentration) are passed to the Manure Module.

The Manure Module first simulates ammonia emissions from the barn floor after excretion and before cleaning and then combines the bedding, flush water, and parlor cleaning water into a simulated reception pit. Currently the model can represent both flushing and scraping cleaning systems from tie stall and free stall pens. Compost bedded-pack barns and dry-lot housing systems are still in development. After the reception pit, the model simulates movement of the combined manure and wastewater to either long term storage or for processing. Current options for manure processing include mechanical solid-liquid separation and anaerobic digestion. Manure emissions and composition change are estimated at each daily step during manure handling, processing, and storage.

On the days when the Crop and Soil Module simulates manure application to fields, the Manure Module passes information about the amount and composition of the manure in storage and subtracts the mass of the simulated manure application from the stored quantities. The Crop and Soil Module then simulates daily biogeochemical nutrient and water transformation, crop uptake, and loss from the soil profile based on a combination of the SWAT (Neitsch et al., 2011), SurPhos (Vadas, 2009) and DayCent (Del Grosso et al., 2011) models. Crop growth rate and composition is based on solar radiation, temperature, and water and N availability. At harvest, above ground crop biomass is partitioned into crop residue that remains on the field and that which is transferred to the Feed Storage Module to inventory management, completing the dairy farm nutrient cycle.

Model Applications

One of the features that sets RuFaS apart from other farm simulation models is the objective for the model to be used for both research and as a decision support tool for the dairy industry. The detailed documentation and use of Python language will facilitate research use by empowering future scientists to understand, modify, and update the model as part of their research program. For industry applications, the flexibility built into the model structure and multiple options for each management decision will support the industry need to estimate current environmental footprints and to inform sustainable decision making.

For example, one type of management decision that RuFaS could support is determination of the reproduction protocols. A recent case study compared two different synchronization protocols (5dCoSynch and OvSynch56) under two different voluntary waiting periods. By including these options in a farm system model, RuFaS is able to provide estimates of the impact of these decisions on the expected feed consumption, enteric methane production, and manure production. For example, in a preliminary comparison, we found that a shorter voluntary waiting period reduced the enteric methane intensity of milk production but that the improved conception rate of the OvSynch56 protocol, did not appear to reduce the methane intensity in comparison to the 5dCoSynch protocol.

Farm system impacts of diet changes or improvements in feed efficiency are another example of an application of RuFaS to inform management decisions. In the case study we presented in Hansen et al. (2021) we demonstrate that RuFaS is able to compare changes in feed efficiency by assigning a stochastic residual feed intake (RFI, kg/d) value to individual animals. As expected, improved efficiency and reduced RFI decreased enteric methane and manure production. However, the percent decrease in both enteric methane and manure emissions were not equivalent to the percent increase in feed efficiency due to non-linearities in the system. Thus, RuFaS can provide estimates of expected environmental benefits from nutrition and breeding programs to improve feed efficiency that account for interactions between the diet, animals, herd dynamics, and downstream farm management choices.

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Should We Consider Lysophosphatidylcholines as a Potential Immunotherapy in Dairy Calves?

B. N. Tate, M. M. Deys, and J. W. McFadden
Department of Animal Science
Cornell University

Introduction

The neonatal bovine immune system is characterized as immunonaive at birth. The calf is highly dependent on maternal immunoglobulins, cytokines, and immune cells consumed in colostrum for immune protection (i.e., passive immunity). Unfortunately, poor quality, inadequate absorption, or feeding of insufficient amounts of colostrum may lead to failure of passive transfer of immunity from the dam to the calf, which causes the calf to become susceptible to early-life diseases. As a result, there is a window in time pre-weaning when the calf is highly susceptible to disease, termed the “gap in immunity” or “window of susceptibility”. During this time, the calf’s innate and adaptive immune systems (i.e., active immunity) are unable to provide sufficient immune protection in instances of pathogenic challenge (Chase et al., 2008). The development of a calfhood illness can negatively impact growth performance and potentially milk production later in life (Urie et al., 2018; Abuelo et al., 2021). Antibiotic therapy is one common approach to manage calfhood morbidity and prevent mortality; however, antibiotic use is often mismanaged and potentially contributes to the development of bacterial resistance (Langford et al., 2003; Walker et al., 2012). Consequently, there is a need for the development of safe, efficacious interventions that bolster immune function and thus protect against early-life disease in neonatal dairy calves. Non-antibiotic immunomodulators, that either enhance or suppress immune cell function, are a promising alternative to prevent or treat disease in young calves.

The delivery of the lysophospholipid lysophosphatidylcholine (LPC) is a promising potential strategy to bolster immunity and reduce antibiotic usage in young dairy cattle. In non-ruminants, bioactive LPC have been shown to modulate key bactericidal mechanisms in immune cells, such as neutrophils, and protect against morbidity and mortality caused by a systemic infection (Yan et al., 2004; Hong et al., 2010; Smani et al., 2015). While the mechanisms by which LPC causes these effects have received some attention in rodents and humans (Liu et al., 2020), our understanding of whether and how LPC influences immune function remained unexplored in dairy cattle. We aim to review immunity of the neonatal calf, LPC metabolism, and the modes of action by which LPC may modulate immunity in non-ruminants and ruminants. We also discuss a recent study at Cornell University that investigated the effects of LPC administration on bactericidal mechanisms in neutrophils isolated from neonatal Holstein heifer calves.

Bovine Neonatal Immunity and Disease

In mammals, the innate immune system is the body's first line of defense against disease (Turvey et al., 2010). It is a fast-acting and semi-specific form of immunity that is broadly distributed. Cellular components of the innate immune system include neutrophils, macrophages, dendritic cells, and natural killer cells. Neutrophils are an abundant and motile phagocyte that are “first responders” at the site of pathogen invasion. The ability of neutrophils to destroy microbes involves increases in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, hydrogen peroxide (H_2O_2) production (i.e., oxidative burst), and discharge of cytosolic granules containing proteins with bactericidal and permeabilizing properties. Macrophages encounter, identify, and engulf invading pathogens. At the surface of macrophages and dendritic cells, bacterial lipopolysaccharide (LPS) and viral double-stranded ribonucleic acid trigger ligation of toll-like receptors to stimulate type I interferon (e.g., IFNα and IFNβ) production. In turn, type I IFNs activate natural killer cells, which survey their environment with activating and inhibitory receptors, cytokine and chemokine receptors, and adhesion molecules. Natural killer cells also prevent infection by secreting pore-forming perforin and cytotoxic granzymes to lyse infected cells rapidly without antigen specificity.

Adaptive immunity is a much slower acting and longer lasting type of immunity (Turvey et al., 2010). The lymphocytes of the adaptive immune system include B cells and 3 major types of T cells including helper T cells, effector T cells, and suppressor T cells. B cells are stimulated by helper T cells to produce antibodies that will recognize pathogens and notify the phagocytes to destroy. Helper T cells stimulate cytotoxic T cells to develop, which kill the infected cells. Suppressor T cells deactivate both B and T cells. Memory B and T cells recall the antibody response to fight infection with reoccurrence.

The maternal womb is an environment that protects calves from pathogen exposure (Chase et al., 2008). Because the placenta of cows does not allow transmission of immunoglobulins from dam to fetus, the newborn calf relies on antibodies (e.g., IgG, IgA, and IgM), cytokines (e.g., interleukin-6 [IL6]), and leukocytes (i.e., T and B cells) provided in colostrum to enhance their immunologic protection. This passive immunity is important because the neonate experiences decreases in complement activity, neutrophil and macrophage activity, interferon production, natural killer cell functionality, and dendritic cell generation (Chase et al., 2008). The neonate is also born with no memory T or B cells, decreased lymphocyte responsiveness, and low antibody production (Chase et al., 2008). Neonates are dependent on the innate immune system to prime adaptive immunity. Because of finite passive immunity and slow development of active immunity, pre-weaned calves experience a high-risk “gap in immunity” or “window of susceptibility” spanning one to five weeks of age.

Calves are highly susceptible to infection because innate and adaptive immunity are underdeveloped. The consequence is diarrhea, septicemia, or bovine respiratory disease. Calf diarrhea (i.e., scouring) is a common early-life disease and cause of

mortality and economic loss for producers. Diarrhea is attributed to enteric pathogens including bacteria. Enterotoxigenic *Escherichia coli (E. coli)* is a common cause of neonatal diarrhea in farm animals (Dubreuil et al., 2016). Septicemia is a systemic infection in which bacteria and LPS enter the bloodstream. Umbilical cord infection is often the cause. Most septicemia cases occur in calves with *E. coli* infection and diarrhea, and calves with septicemia are prone to developing meningitis (inflammation of the meninges). Bovine respiratory disease is another condition caused by pathogens including viruses and bacteria (e.g., bovine respiratory syncytial virus). This disease causes pneumonia and fever. Dairy calf pneumonia most often afflicts calves from 2 to 6 months of age with peak incidence occurring at ~5 weeks of age (Ames, 1997). Previous studies estimate calfhood morbidities such as these at ~35% with incidence of diarrhea and bovine respiratory disease ranging from 10 to 35% (Waltner-Toews et al., 1986; Wells et al., 1997; Donovan et al., 1998; Hill et al., 2009). Unfortunately, morbidity increases mortality, reduces growth, and increases age and difficulty at first calving (Sivula et al., 1996; Rossini, 2004; Stanton et al., 2012). The risk for dairy calf mortality during the first year of life may range between 2 and 12% (mean of 5 to 11% based on cow parity) depending on the year, twins, region, age of calves, and management (Waltner-Toews et al., 1986; Del Río et al., 2007; Walker et al., 2012).

The industry standard to enhance calf immunity and prevent disease is to feed colostrum immediately after birth. However, failure of passive transfer of immunity occurs when calves absorb an inadequate amount of immunoglobulin caused by delayed feeding or when calves are fed low quality colostrum. The prevalence of failure of passive transfer in US dairy heifer calves is estimated at ~19% (Beam et al., 2009). Although calves with inadequate passive transfer are at heightened risk for infection, all calves are immunosuppressed and at risk for disease. The common approach to decrease calf morbidity and mortality is antibiotic administration; however, the extensive and potentially mismanaged use of antibiotics and the development of antibiotic resistant bacteria are major industry and societal concerns. Studies demonstrate that calves harbor highly resistant *E. coli* and prior systemic antibiotic therapy are associated with the fecal recovery of more resistant *E. coli* (Khachatryan et al., 2004; Berge et al., 2005. Consumers are concerned because pathogenic-resistant organisms propagated in livestock may enter the food supply (Landers et al., 2012). The development of novel non-antibiotic therapeutic tools that bolster immunity in livestock including dairy calves deserve consideration.

Lysophosphatidylcholine Metabolism and Immunomodulation

Lysophosphatidylcholines are bioactive lysophospholipids composed of a glycerol backbone, a single fatty acyl chain that varies in carbon length and saturation, and phosphocholine. Although the intestinal absorption of LPC is possible, secretory phospholipases A2 and lecithin:cholesterol acyltransferase control the cleavage of phosphatidylcholine to facilitate the endogenous production of LPC. Lysophosphatidylcholine can be converted to phosphatidylcholine via the actions of LPC acyltransferase and the availability of acyl-conenzyme A. Alternatively, LPC may

be degraded by lysophospholipases or autotaxin. In non-ruminants, the concentration and type of LPC is highly dependent upon the tissue and disease status (Liu et al., 2020). In mammals including humans and cows, the most abundant LPC are typically palmitoyl-LPC (LPC-16:0), stearoyl-LPC (LPC-18:0), oleoyl-LPC (LPC-18:1), and linoleoyl-LPC (LPC-18:2).

Lysophosphatidylcholines play many roles in regulating cellular function and disease development (see review by Liu et al., 2020). For instance, it is generally discussed that LPC are key components of bile that assist with the emulsification of neutral lipids in the intestine. LPC are also key components of cellular membranes and lipoproteins including a main constituent of oxidatively damaged low-density lipoproteins. More recently, bioactive properties of LPC have received attention. For example, LPC have been shown to modulate insulin-stimulated glucose disposal, endothelial calcium ion mobilization, cellular proliferation and apoptosis, and immune cell functionality including chemotaxis, phagocytosis, migration, and inflammation (Liu et al., 2020). In dairy cattle, our understanding of LPC is rudimentary. Our lab has discovered that circulating LPC status is lowest at parturition in dairy cattle transitioning from gestation to lactation (Rico et al., 2021), that endotoxin administration decreases circulating LPC concentrations in lactation cows (e.g., LPC-16:0, -18:0, and -18:1; McFadden et al., 2019), and extreme heat increases total and individual LPC in post-weaned Holstein calves experiencing heat stress (e.g., LPC-16:0, -18:0, and 18:2; unpublished); however, the importance of these findings remains uncertain. Because the transition cow experiences inflammation (Bradford et al., 2015), endotoxemia triggers immune activation, and heat stress is characterized by impairment of cellular immune response (Bagath et al., 2019), we aim to consider the role of LPC within bovine immunity.

Lysophosphatidylcholine and the development of sepsis in non-ruminants

In non-ruminants, a role for LPC has been considered during the development of sepsis. Septic patients have lower total plasma concentrations of LPC than healthy patients (Drobnik et al., 2003) and one corollary study suggests that circulating LPC are predictive of 28-day mortality in patients with severe sepsis (Park et al., 2014). Low circulating LPC concentrations may be due to downregulated secretory phospholipase A2 and lecithin-cholesterol acyltransferase activity (Ahn et al., 2017). These findings suggest that low LPC status enhances an individual's risk to succumbing to severe infection and increasing LPC status could be protective. This is supported by Yan and coworkers (2004). Specifically, in mice that undergo cecal ligation puncture (CLP) to induce experimental sepsis, mortality is nearly certain within ~10 d of the procedure; however, subcutaneous long-chain and saturated LPC-16:0 or LPC-18:0 effectively protect against sepsis-induced mortality caused by CLP or intraperitoneal *E. coli* administration. This response is less evident or non-existent with unsaturated or short-chain LPC (e.g., LPC-18:1 or LPC-6:0, respectively).

The ability of LPC to protect against sepsis-induced mortality appears to involve a direct role of LPC to modulate immune function. The action of LPC targets both the innate and adaptive immune systems. First, LPC triggers mechanisms that enhance phagocytic activity of neutrophils. Treatment of neutrophils with LPC-18:0 increases cytotoxic H_2O_2 production, increases phagocytic clearance of *E. coli*, and blocks neutrophil deactivation, negating the oxidative burst dysfunction often caused by experimentally-induced sepsis (Yan et al., 2004; Smani et al., 2015). Lysophosphatidylcholine therapy also inhibits the ability of LPS to induce tumor necrosis factor-α (TNFα; a key mediator of septic shock) release from neutrophils and promote mortality in mice (Yan et al., 2004). Lysophosphatidylcholine (i.e., LPC-16:0) treatment has also been shown to increase IFN-γ secretion from natural killer cells or T cells (Huang et al., 1999). Interferon-γ serves in part to activate macrophages (Ma et al., 2003). Lysophosphatidylcholines may also help promote B-cell antibody production. For example, treating human peripheral blood mononuclear cell cultures with LPC-18:0 increased immunoglobulin production (i.e., IgM, IgA, and IgG; Huang et al., 1999).

It remains unclear how LPC elicit their effects on neutrophil functionality. Lysophosphatidylcholines are hypothesized to bind to a G protein-coupled receptor found on immune cells called G2A to induce immune cell activation (Kabarowski, 2009); albeit, the anti-septic action of LPC may require G2A cooperativity with adenosine receptor type 2b (Li et al., 2019). One study found that LPC-18:0 increased bactericidal activity of neutrophils (i.e., increased cytotoxic H_2O_2 production), enhanced *E. coli* killing and blocked deactivation of neutrophils within a model of experimentally induced sepsis but were attenuated by blocking the G2A receptor with an anti-G2A antibody (Chen et al., 2005; Hong et al., 2010). Alternatively, toll-like receptors may mediate LPC action (Liu et al., 2020).

Lysophosphatidylcholine therapy also appears to attenuate the effects of infection in part by bactericidal-independent mechanisms. For example, LPC may suppress the activation and release of inflammatory elements such as high-mobility group box-1 (HMGB1) and caspase-11 (Chen et al., 2005; Li et al., 2018). High-mobility group box-1 is a ubiquitous nuclear protein secreted by monocytes and macrophages, and it is considered a stimulator of proinflammatory cytokine release from immune cells and late mediator of sepsis (Stevens et al., 2017). Injection of anti-HMGB1 antibodies, or treatment with agents that inhibit its release, such as LPC-18:0, have been found to protect mice against sepsis (Yang et al., 2004; Chen et al., 2005).

Lysophosphatidylcholine enhances bactericidal mechanisms in neutrophils isolated from pre-weaned Holstein heifer calves

There is a need to develop non-antibiotic interventions that prevent and address the development of early-life illnesses in neonatal calves in order to reduce industry antibiotic use and improve animal health. Therefore, our lab performed a study to investigate the effects of LPC on bactericidal mechanisms in neutrophils isolated from dairy calves. Polymorphonuclear leukocytes were isolated from Holstein heifer calves (2

to 5 wk of age) via Ficoll gradient double-density centrifugation and re-suspended in Roswell Park Memorial Institute (RPMI)-1640 media. The resulting cell suspension was composed of ~95% neutrophils. These neutrophils were treated in the absence or presence of 50 μM LPC-16:0, -18:0, or -18:1 in a 1:2 molar ratio with bovine serum albumin for varying lengths of time at 37°C and 5% CO_2. We performed tests to assess neutrophil functionality including H_2O_2 production to evaluate the oxidative burst, TNFα and IL6 secretion in the absence or presence of LPS (i.e., *E. coli* O55:B5), and *E. coli* killing capacity (i.e., *E. coli* cell suspensions followed by Luria broth agar plating to count colony-forming units). Statistical analyses were carried out using the mixed model procedure of SAS (v9.4, SAS Institute Inc., Cary, NC) with the model including the fixed effect of treatment and the random effect of calf and replicate within treatment. For each experiment, 3 calves were used with biological and technical replicates performed in duplicate.

We first determined that LPC did not overtly modify neutrophil viability. We then confirmed that phorbol myristate acetate (PMA), an agonist of NADPH oxidase, stimulated H_2O_2 production as quantified by a luminol chemiluminescence assay, relative to an unsupplemented control ($P < 0.01$). We then discovered that LPC-16:0 and -18:0 robustly increased H_2O_2 production, relative to unsupplemented controls ($P < 0.001$). The effect was less robust for LPC-18:1 but still significant ($P < 0.001$). We then compared the effects of LPC-18:0 versus LPC-18:1 on neutrophil TNFα and IL6 secretion in the absence of presence of LPS using ELISA. No change in cytokine secretion was observed in response to LPC in the absence of LPS; however, LPC-18:0 (but not LPC-18:1) potentiated the ability of LPS to stimulate TNFα and IL6 secretion ($P < 0.05$). Indeed, neutrophils in the absence of LPC were able to kill *E. coli* when co-cultured with live *E. coli* in a ratio of 1:10, respectively, relative to cultures with just *E. coli* ($P < 0.01$). The presence of LPC-18:0 was able to enhance the ability of neutrophils to kill *E. coli*, relative to neutrophils and *E. coli* co-cultured in the absence of LPC ($P < 0.001$). This effect was not observed for LPC-18:1. The ability of LPC-18:0 to directly kill *E. coli* in the absence of neutrophils was also investigated but proved insignificant. Collectively, our findings indicate that saturated LPC (i.e., LPC-18:0) induce neutrophil activation. It appears possible that LPC-18:0 helps neutrophils kill *E. coli* in part by inducing the oxidative burst. The ability of LPC-18:0 to induce pro-inflammatory cytokine secretion in the presence of LPS is also intriguing; however, we were unable to assess what effect this may have on immunity using this in vitro approach. It is likely that this response would elicit effects on other host immune cells. Whether the totality of responses that we observed are due to the ability of LPC-18:0 to act via the G2A receptor should be considered. Future studies that study the effects of LPC in pre-weaned Holstein dairy calves also has scientific merit. New approaches to enhance immune function in dairy cattle could be revealed.

Summary

Lysophosphatidlycholines are effective immunomodulators in non-ruminants. Science suggests that LPC acts upon both the innate and adaptive arms of the host

immune system, upregulating mechanisms involved in pathogen clearance and immune protection. In dairy cattle experiencing endotoxemia or parturition, we have revealed that circulating concentrations of LPC are low. Moreover, our in vitro data indicates that LPC do activate neutrophils isolated from pre-weaned Holstein heifer calves. We can only hypothesize that the observed increase in the oxidative burst, cytokine secretion, and *E. coli* killing have the potential to translate into heightened immune response in young calves. However, this will require careful consideration of mode of delivery, LPC type, dose, and duration. The identification of novel immunotherapies that could replace antibiotics and prevent disease deserves our attention considering the calf's susceptibility to infection and disease. The effects of LPC in older animals including periparturient and heat-stressed dairy cattle that may also experience bouts of endotoxemia also requires consideration.

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Relationships Between Transition Cow Nutrition and Management Strategies and Outcomes in Large Dairy Herds in the Northeastern US

A. L. Kerwin[1], W. S. Burhans[2], D. V. Nydam[3], and T. R. Overton[1]

[1]Department of Animal Science, Cornell University
[2]Dairy-Tech Group, South Albany, VT
[3]Department of Population Medicine and Diagnostic Sciences, Cornell University

Introduction

Many management factors contribute to cow success during the transition period including minimizing management related stressors, ration formulation and feeding strategies, monitoring and treatment of health disorders, and facilitating cow comfort (Nydam et al., 2017). Nutritional strategy recommendations during the transition cow period are often driven by results from controlled research trials or anecdotal observations. Although research exists evaluating transition cow nutritional strategies, large-scale data availability is limited, particularly for the periparturient and fresh cow periods. In addition, controlled research trials are often completed in tiestall barns, removing many influences of environment and management, potentially resulting in varying outcomes in freestall herds.

The adoption of a controlled energy diet throughout the dry period has increased amongst the dairy industry and has been supported by controlled research trials for improving postpartum health (Janovick et al., 2011; Mann et al., 2015; Richards et al., 2020); however, some studies have demonstrated decreased milk production in animals fed a controlled energy prepartum diet (Vickers et al., 2013). It has been proposed that feeding a lower starch diet during the fresh period might result in cows having improved milk production compared to cows fed higher levels of fermentable starch during the fresh period (Allen et al., 2009); however, data are lacking or have not fully supported this theory (McCarthy et al., 2015; Rockwell and Allen, 2016).

Limited field data exist that describe different management strategies that contribute to cow success in commercial farm settings. Therefore, recommendations are often driven by field experience from concepts established through controlled research with comparatively small numbers of cows. Bach et al. (2008) reported a 13.2 kg/d range in the mean milk production across 47 herds that were fed the exact same TMR and determined that 50% of the observed variation was attributed towards non-nutritional management factors. Limited research and field observations have demonstrated that non-nutritional management factors, such as stocking density, pen moves, and commingling of primiparous and multiparous cows, can impact health and milk production (Cook and Nordlund, 2004; von Keyserlingk et al., 2008; Huzzey et al., 2012); however, besides heat abatement, little research has evaluated the impact of management factors on reproductive performance. Controlled research trials typically attempt to evaluate the change in one management factor while minimizing changes with additional management

factors; therefore, the magnitude of the impact of management factors has not been fully elucidated. In addition, most studies evaluating management factors have not been conducted during the transition period. Evaluating management and nutritional factors with outcomes on commercial farms, such as health, blood biomarkers, milk production, and reproductive performance may provide an understanding of how these factors contribute to transition cow success across a range of farm practices.

Our first objective was to identify relationships between dry period and periparturient period nutritional strategies as characterized by ration contents of starch, forage NDF, or both, and biomarkers of energy metabolism [nonesterified fatty acids (**NEFA**) and β-hydroxybutyrate (**BHB**)] and inflammation [haptoglobin (**Hp**)], disease, milk production, and reproductive performance. Our second objective was to evaluate relationships between putative periparturient management factors at the pen- and herd-level with blood biomarkers, disease, milk production and reproductive performance.

Experimental Design

A prospective cohort study was conducted from a convenience sample of 72 farms located in New York and Vermont between November 2012 and August 2015. Inclusion criteria for herds were 1) Holstein herds, 2) ≥ 400 milking cows, 3) free-stall housing, 4) TMR-fed herds, and 5) enrolled in monthly DHI testing or have on-farm milk recording with record management by Dairy Comp 305 (DairyComp 305, Valley Ag Software, Tulare, CA) or PCDART (PCDART, Dairy Records Management System, Raleigh, NC). Farms that met these inclusion criteria were enrolled based upon their willingness to participate. Farms were visited 3 times for data collection focused on the same cohort of animals during the far-off dry (28 to 49 d prior to expected parturition), close-up dry (0 to 21 d prior to expected parturition; 4 wk after the first visit), and fresh (0 to 21 DIM; 16 to 21 d after the second visit) periods. Cows were observed for health disorders of interest in the first 30 DIM by farm personnel and case definitions for the health disorders were provided to the farm for recording and consistency purposes.

Blood samples were collected from a convenience sample of 11 to 24 cows within each herd at the close-up visit and from the same cohort of cows at the fresh period visit. To reflect herd demographics, approximately one-third of the cows sampled were primiparous cows. Postpartum whole blood was measured for BHB. Prepartum and postpartum plasma was analyzed for NEFA and postpartum plasma was analyzed for Hp on cows 0 to 12 DIM.

The formulated diets fed to the cows observed at the time of the visit were collected from the nutritionist or herd manager. The forages fed to the observed group of cows were sampled at each visit and analyzed by near-infrared spectroscopy at a commercial laboratory (Green Mountain Feed Testing Laboratory, Newport, VT) and evaluated for particle size with the 3-sieve Penn State Particle Separator (**PSPS**; Cumberland Valley Analytical Services, Maugansville, MD). Physically effective NDF (**peNDF**) was calculated by multiplying the proportion of TMR above the 4-mm sieve by the average analyzed ration NDF on a DM basis. Physically effective undigested NDF after 240 h of in vitro

fermentation (**peuNDF240**) was calculated by multiplying the proportion of TMR above the 4-mm sieve by the average analyzed ration undigested NDF after 240 h of in vitro fermentation on a DM basis (**uNDF240**; Miller et al., 2020). The formulated diets with analyzed forage samples were inputted into the Cornell Net Carbohydrate and Protein System (CNCPS v. 6.1, Cornell University, Ithaca, NY). The ration CNCPS files were imported into the Nutritional Dynamic System Professional (NDS Professional version 3.8.10.06, RUM&N Sas, Reggio Emilia, Italy) for nutrient extraction.

For each visit, the farms were retrospectively dichotomized within parity group into different nutritional strategies as determined by starch, forage NDF, or both, based on the CNCPS-formulated diet. For the far-off period, farms were characterized as feeding a controlled energy diet (**CE**; <16.5% starch and ≥40% forage NDF) or not CE (**NCE**; ≥16.5% starch or <40% forage NDF or both). For the close-up period, farms were characterized as feeding a higher forage NDF (**HF**; ≥40% forage NDF) or lower forage NDF diet (**LF**; <40% forage NDF) and for the fresh period, farms were characterized as feeding a lower starch (**LS**; <25.5% starch) or higher starch diet (**HS**; ≥25.5% starch).

For the management pen-level analysis, we assessed management explanatory variables during the far-off, close-up, and fresh period visits. Stall stocking density was calculated as the number of cows in the pen at the time of the visit divided by the number of usable stalls in the pen. If the pen was a bedded pack, a stall was considered 11 m^2 of pack space (Nordlund, 2009). Bunk stocking density was calculated as the number of cows in the pen at the time of the visit divided by the number of headlocks. If a pen did not have headlocks, a headlock was considered 61 cm of rail space (NFACC, 2009). The feed pushup frequency within each day for a pen was dichotomized as < 5×/d or ≥ 5×/d (Miller-Cushon and DeVries, 2017). The feeding frequency within each day was not evaluated during the prepartum period due to few observations in which the pen was fed more than once per day. Commingling of primiparous and multiparous cows was also assessed at the pen-level.

For the management herd-level analysis, explanatory variables assessed included whether cows were routinely vaccinated in the calving pen and fresh pen, whether the herd utilized a maternity pen or a calving pen, the number of pen moves during the prepartum and postpartum period, time spent in the calving pen and fresh pen, and time spent locked up in the fresh pen. A maternity pen was classified as a pen cows moved into at least 0 to 3 d prior to expected calving while a calving pen was classified as a pen cows move into when exhibiting signs of labor. The prepartum pen moves were the number of pen moves from dry off or 60 d prior to expected calving to parturition for primiparous and multiparous cows, respectively (> 2 vs. ≤ 2). The move from the lactating pen to the far-off dry cow pen was included in this measure for multiparous cows. The postpartum pen moves were the number of pen moves from parturition to 90 DIM (> 2 vs. ≤ 2). The time spent in the calving pen before moving to the fresh pen after parturition was dichotomized as ≤ 8 h or > 8 h. The time spent in the first pen moved into after parturition was dichotomized as ≤ 10 d or > 10 d. Time spent locked up in fresh pen for health checks was categorized as: 1) locked up < 1×/d for < 1 h, 2) locked up daily for < 1 h, or 3) locked up daily for ≥ 1 h.

The outcomes of interest were: 1) prevalence of elevated prepartum circulating NEFA concentration in multiparous cows (≥0.17 mmol/L), 2) prevalence of elevated postpartum circulating NEFA (≥0.59 mmol/L), 3) prevalence of postpartum circulating BHB (≥1.2 mmol/L), 4) prevalence of elevated postpartum circulating Hp (≥0.45 g/L), 5) disorder incidence of one or more of displaced abomasum, clinical ketosis, or metritis within 30 DIM (**DI**), 6) herd average milk production at 4 wk of lactation (**WK4MP**), 7) herd average 305-d mature equivalent milk yield at approximately 120 DIM (**ME305**), 8) 21-d herd pregnancy rate (**PR**), 9) herd risk of conceiving as identified by pregnancy (**CR**), and 10) the pregnancy risk to first service (**PRFS**). Biomarker thresholds were chosen as they were the herd alarm levels associated with an increase in disorder incidence for primiparous and multiparous cows. The prevalence of elevated prepartum NEFA concentrations were only evaluated for multiparous cows since a herd-alarm level was not identified for primiparous cows. The 21-d PR was determined by averaging the two-21 d periods after the herd VWP for the group of cows that calved within the same time frame as the cows sampled. The CR was determined by averaging the conception risk, as identified by pregnancy, for the first 2 estrus cycles after the VWP for the group of cows that calved within the same time frame as the cows sampled. Cows that were never bred were removed from the PRFS analysis (n = 155). All outcomes were calculated by parity within a farm due to some farms feeding different diets to the multiparous and primiparous cows and multiparous and primiparous cows being housed separately.

All statistical analyses were calculated using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). For objective 1, mixed effects linear models were generated using PROC MIXED for all outcomes by parity group at the herd-level. Nutritional strategies were assessed during the dry period and the periparturient period using two models for each outcome: A) the main effects of the nutritional strategy during the far-off and close-up dry periods, and B) the main effects of the nutritional strategy during the close-up dry and fresh periods. Calving season [cool (October through April) vs. warm (May through September)] and the interaction between the nutritional strategy main effects were included in the full models. Multiparous and primiparous cows were initially analyzed separately. If the association between the nutritional strategies and outcome of interest were similar between parity groups, then multiparous and primiparous cows were combined and parity group was included as a covariate and herd was included as a random effect. A manual backwards stepwise elimination was used to remove the interaction term if $P \geq 0.15$ and season and parity group if $P \geq 0.10$. Comparisons to farms that fed NCE during the far-off period and HF in the close-up period were not assessed due to a limited number of observations, as this is not a common nutritional strategy amongst farms in the Northeastern United States.

For objective 2, a simple linear regression (PROC REG or PROC GLM) was conducted on all possible continuous explanatory variables and categorical explanatory variables that occurred before the outcome of interest to determine the univariable association for the pen level-management analysis. Explanatory variables with a $P < 0.2$ were offered to a multivariable general linear model (PROC GLM) for each outcome. Calving season was included as a covariate if $P < 0.10$ in a univariate analysis and a manual backwards stepwise elimination process ensued until all variables had a $P < 0.1$.

The far-off period was not assessed for primiparous cows due to too many missing observations. For the herd-level management analysis, a simple linear regression analysis (PROC GLM) was conducted on all possible explanatory variables and included parity, the interaction with parity, and the random effect of farm. Calving season was included in the simple linear regression analysis as a covariate if season was associated with the outcome. Explanatory variables with a $P < 0.2$ were offered to a multivariable general linear model (PROC MIXED) for each outcome with herd as a random effect and a manual backwards stepwise elimination process ensued until all variables had a $P < 0.1$.

Nutritional Strategy Results

Prevalence of Elevated Biomarkers

We found no evidence that there was a difference in the prevalence of elevated prepartum NEFA concentration between the far-off ($P = 0.97$) or close-up ($P = 0.25$) nutritional strategies for multiparous cows.

We found no evidence that there was a difference in the prevalence of elevated postpartum NEFA concentration for the dry period nutritional strategies for multiparous and primiparous cows nor for the periparturient period nutritional strategies for multiparous cows. We observed an interaction between the close-up and fresh period nutritional strategies for primiparous cows ($P = 0.05$) such that herds that were fed HF × HS had a higher prevalence of elevated NEFA (28.7 ± 6.5%) than herds that were fed LF × HS (11.7 ± 4.3%; $P = 0.14$), but not different than herds fed HF × LS (16.1 ± 6.7%; $P = 0.54$) or LF × LS (21.9 ± 5.1%; $P = 0.84$).

For the prevalence of elevated BHB concentration analysis, multiparous and primiparous cows were separated for the dry period nutritional strategy analysis due to dissimilar results. We observed an interaction between the far-off and close-up nutritional strategy for primiparous cows ($P = 0.10$); however, we found no evidence that there was a difference in the prevalence of elevated postpartum BHB concentration for the typical nutritional strategies observed in the Northeastern US based on the Bonferonni test. For the dry period model for multiparous cows, we observed a lower prevalence of elevated BHB concentration for HF fed herds during the close-up period than LF fed herds (13.0 ± 3.6 vs. 21.1 ± 2.6%; $P = 0.07$) and there was no evidence for a difference in the prevalence of elevated BHB concentration for the far-off nutritional strategies ($P = 0.59$). Primiparous and multiparous cows were combined for the periparturient model due to similar results. We observed a lower prevalence of elevated BHB concentration on HF fed herds versus LF fed herds (11.1 ± 2.8 vs. 16.6 ± 2.0%; $P = 0.11$) during the far-off period and HS fed herds versus LS fed herds (10.0 ± 2.3 vs. 17.8 ± 2.5%; $P = 0.02$) during the close-up period.

For the prevalence of elevated Hp concentration analysis, we found no evidence that there was a difference in the prevalence of elevated postpartum Hp concentration for the periparturient period nutritional strategies for multiparous cows. For the dry period nutritional strategy, we found no evidence that there was a difference in the prevalence

of elevated Hp concentration for the far-off nutritional strategy (P = 0.77); however, we observed a difference in the prevalence of elevated Hp concentration for the close-up nutritional strategy for primiparous and multiparous cows such that HF fed herds had a higher prevalence of elevated Hp concentration that LF fed herds (P = 0.14). We observed a difference in the prevalence of elevated Hp concentration for the fresh nutritional strategy for primiparous cows such that LS fed herds had a lower prevalence of elevated Hp concentration that HS fed herds (P = 0.06).

Postpartum Health, Milk Yield, and Reproductive Performance Outcomes

We found no evidence that there was a difference in DI for the dry period nutritional strategies for multiparous and primiparous cows. We observed an interaction between the close-up and fresh nutritional strategies for multiparous and primiparous cows (P = 0.009) such that cows fed HF close-up followed by a LS fresh diet or LF close-up followed by a HS fresh diet had the highest DI (18.9 ± 4.0%)I; however, we found no evidence that the DI differed between any of the nutritional strategies based on the Tukey honest significance difference test *(*P > 0.19).

We found no evidence that there was an association between different nutritional strategies and either WK4MP or ME305.

For the 21-d PR analysis, multiparous and primiparous cows were separated for the dry period nutritional strategy analysis due to dissimilar results. For multiparous cows, there was no evidence that the 21-d PR differed between far-off nutritional strategies (P = 0.69); however, the 21-d PR was slightly higher in LF fed herds during the close-up period compared to HF fed herds (24.7 ± 1.0 vs. 22.2 ± 1.4%; P = 0.14). We observed an interaction between the far-off and close-up period nutritional strategies for primiparous cows (P = 0.07); however, we found no evidence that there was a difference in the 21-d PR for the typical nutritional strategies observed in the Northeastern United States, based on the Bonferroni test (P > 0.26). Multiparous and primiparous cows were also separated for the periparturient period nutritional strategy analysis due to dissimilar results. Similar to the dry period nutritional strategy model for multiparous cows, the 21-d PR was slightly higher in LF fed herds during the close-up period compared to HF fed herds (24.7 ± 1.0 vs. 22.1 ± 1.3%; P = 0.14); however, there was no evidence that the 21-d PR differed between the fresh period nutritional strategies. We found no evidence that the 21-d PR differed between the close-up (P = 0.49) or fresh period (P = 0.22) nutritional strategies for primiparous cows.

For the CR analysis, multiparous and primiparous cows were separated for the dry period nutritional strategy analysis because the results were dissimilar; however, we found no evidence that there was an association between different dry period nutritional strategies and CR for multiparous and primiparous cows. For multiparous cows, there was no evidence that the CR differed between the periparturient nutritional strategies. For primiparous cows, we observed an interaction (P = 0.14) between the close-up and fresh period nutritional strategies such that HF × HS fed herds (50.1 ± 2.7%) had a higher CR

than HF × LS fed herds (40.6 ± 2.8%; *P* = 0.08), LF × LS fed herds (40.2 ± 2.3%; *P* = 0.03), and LF × HS fed herds (42.5 ± 1.9%; *P* = 0.11).

For the PRFS analysis, multiparous and primiparous cows were combined in the dry period and periparturient period models due to similar results. We found no evidence that there was an association between different nutritional strategies and PRFS.

Management Strategy Results

Prevalence of Elevated Biomarkers

Only multiparous cows were evaluated for the prevalence of elevated prepartum NEFA concentrations since we only identified a herd-alarm level for multiparous cows. For the herd-level analysis, we found no evidence that prepartum pen moves was associated with the prevalence of elevated prepartum NEFA concentrations. For the pen-level analysis, no explanatory variables remained in the far-off model. For the close-up period, a 1-percentage unit increase in the proportion of particles on the 4-mm sieve of the PSPS for the close-up period pens resulted in a 1.2-percentage unit increase in the proportion of multiparous cows with elevated prepartum NEFA concentration (R^2 = 0.06; *P* = 0.03).

For the prevalence of elevated postpartum NEFA concentrations, no explanatory variables remained in the models for primiparous cows nor in the far-off period model for multiparous cows. For the pen-level analysis, a 1-percentage unit increase in the proportion of particles in the PSPS pan during the close-up and fresh periods resulted in a 1.0-percentage unit decrease (*P* = 0.04) and 0.7-percentage unit increase (*P* = 0.09) in the proportion of multiparous cows with elevated postpartum NEFA concentration, respectively. A 1-percentage unit increase in bunk stocking density for multiparous cows during the fresh period resulted in a 0.15-percentage unit increase in the proportion of multiparous cows with elevated postpartum NEFA concentration (*P* = 0.06). For the herd-level analysis, herds that kept cows in the calving pen for > 8 h after parturition had a greater proportion of cows with elevated postpartum NEFA concentration compared to herds that kept cows in the calving pen for ≤ 8 h (43.6 ± 6.0 vs. 21.0 ± 2.3%; *P* < 0.001).

For the prevalence of elevated postpartum BHB concentrations, no explanatory variables remained in the BHB models for the far-off period for primiparous cows or the close-up period for primiparous and multiparous cows. For the pen-level analysis, a 1-percentage unit increase in the proportion of particles on the 8-mm PSPS sieve during the fresh period resulted in a 1.2-percentage unit decrease in the proportion of primiparous cows with elevated postpartum BHB concentrations (*P* < 0.001). Commingled fresh period pens had a greater proportion of primiparous cows with elevated BHB concentrations compared to non-commingled fresh period pens (16.2 ± 2.6 vs. 6.2 ± 3.6%; *P* = 0.03). Fresh period pens that were fed >1×/d had a lower proportion of primiparous cows (7.1 ± 3.7 vs. 15.3 ± 2.5%; *P* = 0.08) and multiparous cows (21.7 ± 4.9 vs. 40.1 ± 3.2%; *P* = 0.08) with elevated BHB concentrations compared to pens that were fed ≤1×/d. A 1-cm per cow increase in water space during the far-off period resulted

in a 3.7-percentage unit decrease in the proportion of multiparous cows with elevated postpartum BHB concentration (P = 0.04). For the herd-level analysis, we observed an interaction between parity group and the time spent in the calving pen after parturition such that herds that kept multiparous cows in the calving pen for more than 8 h had a lower proportion of multiparous cows with elevated postpartum BHB concentration (4.7 ± 7.0 vs. 17.7 ± 2.0%; P = 0.08) and herds that kept primiparous cows in the calving pen for more than 8 h had a higher proportion of primiparous cows with elevated postpartum BHB concentration (26.6 ± 5.4 vs. 7.2 ± 2.1%; P = 0.001) compared to herds that kept primiparous or multiparous cows in the calving pen for ≤ 8 h after parturition.

For the prevalence of elevated postpartum Hp concentrations, no explanatory variables remained in the model for the close-up period for multiparous cows. For the pen-level analysis, pushing up feed ≥ 5×/d during the close-up and fresh periods resulted in a 22.9% (R^2 = 0.08; P = 0.07) and 22.7% (R^2 = 0.08; P = 0.06) increase in the proportion of primiparous cows with elevated Hp concentration, respectively. A 1-percentage unit increase in the proportion of particles on the 19-mm PSPS sieve during the far-off period resulted in a 0.4-percentage unit decrease in the proportion of multiparous cows with elevated Hp concentration (R^2 = 0.04; P = 0.10). Commingled fresh period pens had a lower proportion of multiparous cows with elevated Hp concentration compared to non-commingled fresh period pens (36.0 ± 2.5 vs. 48.8 ± 4.3%; P = 0.01). For the herd-level analysis, we observed an interaction between the number of prepartum pen moves and parity (P = 0.07). Herds that moved primiparous cows ≤ 2× from 60 d prior to expected calving to parturition had a lower proportion of primiparous cows with elevated Hp concentrations compared to herds that moved primiparous cows > 2× (57.1 ± 5.2 vs. 69.0 ± 5.6%; P = 0.04). There was no evidence that the proportion of multiparous cows with elevated Hp concentration in herds that moved cows ≤2× from dry-off to parturition versus herds that moved multiparous cows > 2 times differed (P = 0.50). We also observed an interaction between parity group and the time in the calving pen after parturition (P = 0.002). Herds that kept primiparous cows in the calving pen for >8 h had a greater proportion of primiparous cows with elevated Hp concentrations compared to herds that kept primiparous cows in the calving pen for ≤ 8 h (79.4 ± 8.3 vs. 46.7 ± 3.5; P < 0.001). There was no evidence that the proportion of multiparous cows with elevated Hp concentrations differed between herds that kept cows in the calving pen for >8 h versus ≤ 8 h (P = 0.57). We also observed an interaction between parity group and the time locked in the fresh pen (P = 0.09). There was no evidence that the proportion of multiparous cows with elevated Hp concentrations differed between herds that had multiparous cows locked up for different periods of time (P = 0.56). Herds that had primiparous cows locked up daily for ≥ 1 h had the lowest proportion of primiparous cows with elevated Hp concentration (53.3 ± 7.9%) though there was no evidence that it differed from herds that had primiparous cows locked up daily for < 1 h (72.3 ± 5.7%; P = 0.11) or herds that had primiparous cows locked up for < 1×/d for < 1 h (63.6 ± 5.6%; P = 0.72).

Postpartum Health, Milk Yield, and Reproductive Performance Outcomes

For the DI pen-level analysis, a 1-percentage unit increase in bunk stocking density during the close-up period resulted in a 0.13-percentage unit increase in DI for primiparous cows (R^2 = 0.09; *P* = 0.03). Fresh period pens that had feed pushed-up ≥ 5×/d had a higher DI for primiparous cows than pens that had feed pushed-up < 5×/d (14.0 ± 2.5 vs. 0.0 ± 7.5%; *P* = 0.08). Caution should be used when interpreting the fresh period model for primiparous cows as only 6 observations remained in the <5×/d feed pushup frequency category. A 1-percentage unit increase in the proportion of particles on the PSPS 19-mm sieve during the far-off period and particles on the 8-mm sieve during the close-up period resulted in a 0.3-percentage unit decrease (*P* = 0.08) and 0.5-percentage unit increase (*P* = 0.02) in DI for multiparous cows, respectively. Fresh period pens that were fed >1×/d had a lower DI for multiparous cows than pens that were fed ≤1×/d (7.5 ± 2.9 vs. 14.8 ± 1.8%; *P* = 0.04). For the herd-level model, herds that had cows vaccinated in the calving pen had a higher DI than herds that did not (26.1 ± 5.0 vs. 13.5 ± 2.0%; *P* = 0.02). Herds that did not lock up cows daily had a lower DI (14.4 ± 3.2%) compared to herds that had cows locked up daily for < 1 h (22.7 ± 3.5%; *P* = 0.06); however, there was no evidence that herds that had cows locked up < 1×/d for < 1 h differed from herds that had cows locked up daily for ≥ 1 h (22.2 ± 4.5%; *P* = 0.25).

For the WK4MP analysis, no explanatory variables remained in the far-off model for multiparous cows. A 1-percentage unit increase in the proportion of particles on the PSPS 19-mm sieve for the close-up pen rations resulted in a 0.1-kg/d increase in WK4MP for primiparous cows (R^2 = 0.06; *P* = 0.07). A 1-percentage unit increase in the fresh pen stall stocking density resulted in a 0.03-kg/d increase in WK4MP for primiparous cows (*P* = 0.07). Primiparous and multiparous cows in fresh period pens and multiparous cows in close-up pens that had feed pushed up ≥ 5×/d produced less WK4MP than pens that had feed pushed up < 5×/d (fresh pen for primiparous: 33.4 ± 0.4 vs. 35.2 ± 1.0 kg/d; *P* = 0.09; close-up pen for multiparous: 46.5 ± 0.5 vs. 48.6 ± 1.1 kg/d; *P* = 0.08; fresh pen for multiparous: 46.6 ± 0.4 vs. 49.1 ± 1.0 kg/d; *P* = 0.03). Primiparous cows commingled with multiparous cows in the fresh period pens produced less WK4MP than primiparous cows in non-commingled pens (33.4 ± 0.4 vs. 35.2 ± 1.0 kg/d; *P* = 0.03). A 1-percentage unit increase in uNDF240 (%DM) in the fresh period pen TMR resulted in a 0.9-kg/d decrease in WK4MP for multiparous cows (P = 0.01). For the herd-level analysis, we observed a calving pen vaccination by parity group interaction (P = 0.05). Herds that had multiparous cows vaccinated in the calving pen produced less WK4MP than herds that did not have multiparous cows vaccinated in the calving pen (42.7 ± 1.8 vs. 46.8 ± 0.4 kg/d; *P* = 0.04); however, we found no evidence that there was a difference in WK4MP for primiparous cows that were vaccinated in the calving pen versus not vaccinated (*P* = 0.80).

For the ME305 analysis, no explanatory variables remained in the close-up period model for primiparous cows nor for the far-off period model for multiparous cows. For the pen-level analysis, a 1-percentage unit increase in peuNDF240 during the fresh period resulted in a 468-kg (R^2 = 0.15; *P* = 0.002) and 278-kg (R^2 = 0.07; *P* = 0.02) decrease in ME305 for primiparous and multiparous cows, respectively. A 1-percentage unit increase in stall stocking density during the close-up period resulted in an 8-kg increase in ME305

for multiparous cows (P = 0.06). A 1-percentage unit increase in the proportion of particles on the PSPS 4-mm sieve for the close-up period TMR resulted in an 86-kg increase in ME305 for multiparous cows (P = 0.01). Multiparous cows that were commingled with primiparous cows in the close-up period pens produced less ME305 than multiparous cows that were not commingled (12,414 ± 166 vs. 13,129 ± 253 kg; P = 0.02). For the herd-level analysis, there was an interaction between postpartum pen moves and parity (P = 0.06) such that herds that had primiparous cows moved ≤ 2× within the first 90 DIM produced more ME305 milk than herds that moved primiparous cows > 2× (12,950 ± 199 vs. 12,231 ± 214 kg; P = 0.01). We found no evidence for a difference in ME305 for herds that moved multiparous cows ≤ 2× versus > 2× within the first 90 DIM (P = 0.32).

For the 21-d PR herd-level analysis, we observed an interaction between parity and the use of a maternity pen, such that herds that moved primiparous cows into a maternity pen where they were expected to calve in the next 0 to 3 d had a lower 21-d PR compared to herds that move primiparous cows into a calving pen when the primiparous cow was showing signs of labor (26.4 ± 1.8 vs. 29.7 ± 1.3%; P = 0.10). We did not observe a difference between multiparous cows (P = 0.32).

For the herd-level results for the CR analysis, herds that had cows stay in the calving pen for > 8 h after parturition had a lower CR than herds that had cows in the calving pen for ≤ 8 h (35.8 ± 2.4 vs. 40.3 ± 0.9; P = 0.09).

For the PRFS pen-level analysis, a 1-percentage unit increase in stall stocking density during the close-up period for primiparous cows and far-off period for multiparous cows resulted in a 0.15- (P = 0.06) and 0.19-percentage unit (P = 0.03) increase in PRFS, respectively. A 1-percentage unit increase in the proportion of particles on the PSPS 8-mm sieve during the close-up period for primiparous cows and close-up period for multiparous cows resulted in a 0.9- (P = 0.002) and 0.5-percentage unit (P = 0.007) increase in PRFS, respectively. Multiparous cows in the far-off and close-up period pens that had feed pushed up ≥ 5×/d had a higher PRFS than for pens that had feed pushed up < 5×/d (far-off: 35.2 ± 1.9 vs. 27.9 ± 3.1%; P = 0.05; close-up: 34.5 ± 2.0 vs. 23.3 ± 4.4%; P = 0.02). For the herd-level analysis, we observed an interaction between the amount of time locked in the fresh pen for fresh cow health checks and parity group (P = 0.08); however, we found no evidence that PRFS differed within each parity group (multiparous: P = 0.25; primiparous: P = 0.16). Herds that kept cows in the calving pen for > 8 h after parturition had a lower PRFS than herds that kept cows in the calving pen for ≤ 8 h (24.5 ± 4.2 vs. 38.2 ± 1.8%; P = 0.003).

Conclusions and Implications

This study provides further epidemiological evidence that nutritional and management factors both influence transition cow outcomes. In general, the nutritional strategy results of our study support feeding cows a high forage NDF close-up and high starch fresh diet to minimize excessive prevalence of elevated BHB concentration and reduce disease incidence in the early postpartum period. Similarly to the multiparous cows, the results of our study support feeding primiparous cows a controlled energy far-

off, high forage NDF close-up, and high starch fresh diet to maximize reproductive performance, minimize excessive prevalence of elevated BHB, and to reduce disease incidence in the early postpartum period. From a management perspective, our results support maximizing bunk space and adequate water space per cow, avoiding commingling and increasing the feeding frequency during the fresh period, increasing the proportion of particles on the PSPS 19-mm sieve for the prepartum rations, not increasing the peuNDF240 of the fresh ration too much, avoiding vaccination in the calving pen, move cows to the calving pen when showing signs of labor versus 0 to 3 days prior to expected calving, reducing the number of prepartum and postpartum pen moves, and reducing the amount of time spent in the calving pen after parturition. Due to limited data and contradicting results, further research should evaluate short- and long-term effects of the amount of time locked up in the fresh pen, peuNDF240 during the transition period, particle size, feed pushup frequency, the use of a calving pen versus a maternity pen, and time spent in the calving pen.

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Memoriam for Peter J. Van Soest (1929 – 2021)
His Revolutionary Impact on the Science and Education of Fiber Nutrition

D. R. Mertens
Mertens Innovation & Research LLC

Introduction

Few, if any, animal scientists have the legacy of Peter J. Van Soest. He permanently changed the chemical and in vitro analysis of feeds and the understanding of herbivore nutrition throughout the world. His ideas were well-developed, comprehensive, and often, transformational. His novel and revolutionary methods of analysis provide the foundation for current methods of feed evaluation that are used worldwide.

I could write a book about my experiences with this brilliant man who became a foster father to me in many ways. I was lucky to spend many hours and days in the lab with him learning by observing, listening, and asking questions, and, more importantly, with him discussing his thoughts and asking me questions – and he usually didn't provide the answers. My job was to figure things out myself. I will tell a few stories (to the best of my recollection) to describe the man behind the accomplishments. He was personally as interesting and influential as he was professionally. It took me a while to understand his philosophy of science, but I think we were kindred spirits.

I first talked to Peter Van Soest during a telephone call on a Friday afternoon. I had visited five universities to find a compatible advisor and program. Peter was away from Cornell when I visited, and the previous day, I had decided to work with Dale Bauman at Illinois even though I did not like the course requirements there. Peter began the telephone conversation saying that I had made a good impression at Cornell and that he had an assistantship to offer after I completed my Purina Fellowship. Although I had taken the inorganic, organic and biochemistry series, I was hesitant because I had heard about how brilliant he was, and was concerned that I wouldn't meet his expectations. Dr. Van Soest said he would like to ask two questions. I thought "Oh no, here is the test!" But his first question was "Did I like course work?" I replied that I would be happy if I never sat in another lecture. He said "Fine" and promised that he would make sure I could take the minimum courses necessary. Then he asked, "Do you like to work in the lab?" I said that I loved lab work and it was one of the reasons I continued my education, so that I could understand the why and how of what I was measuring. He said "Great!" and that he expected me to be in the lab with him. I accepted his offer without hesitation. I would learn later that his philosophy was that I had to think on my own, do my own work, and learn at the hand of the master.

My goal was to obtain my Ph.D. before I turned 26, which meant I had three years to finish my degree. So, the first week I was at Cornell, I asked Dr. Van Soest what topic I should work on? He looked at me directly and said very bluntly that a Ph.D. was independent work and I was to pick the topic. I asked how my research would be supported and he said, “It is my job to get the funds, but it is your job to pick a suitable project.” I thought, “How great is this, I get to pick something that I am interested in and Dr. Van Soest is going to pay for it.” So, I went to the library to search the literature and picked a topic. At the end of the week, I presented it to Dr. Van Soest. He rubbed his beard thoughtfully and picked a green USDA lab notebook from his bookshelf, turned to the right page and said, “I looked at that in 1963 and it is not a fruitful avenue of work.” For the next 10 weeks, the same result occurred, Dr. Van Soest had either already studied my idea or knew someone who had. Sometimes he gave me a paper or list of references to read and I came to the conclusion it was not the right topic. I was getting desperate. But I realized that I was learning more and more about intake, NDF and digestion kinetics. So, on a Friday before Thanksgiving, I told Dr. Van Soest that I thought there should be mathematical relationships between intake, digestibility, and NDF digestion kinetics and I proposed to develop a model to do this. Peter stared into space in deep thought and said, “Now that would be interesting.” I ran out of his office before he could change his mind!

Not until later did I fully understand that he taught me several valuable philosophical lessons. Important science was not about single experiments, but about large bold ideas that required thought and experimentation. He also taught me that asking the correct question was the key to success. In addition, I learned that you search the literature before you do your dissertation work, or any research, and that you save a lot of time and effort, and home in on the best question, by learning what other people have done before you. These valuable experiences taught me a lot about Peter Van Soest, and now I will discuss his talents and accomplishments. At his CNC honorary symposium, I presented a thorough review of the detergent system of fiber analysis (Mertens, 2003) and I will only give a broad overview of his research in this paper.

Biographical Sketch

Dr. Peter J. Van Soest, one of the most influential animal scientists and professors of the last century, died on March 21, 2021. Dr. Van Soest was born June 30, 1929 and grew up on a dairy farm in Snohomish, Washington. He graduated from Washington State University with a B.S. in Dairy Husbandry in 1951, and a M.S. in 1952. He obtained his Ph.D. from the University of Wisconsin in 1955 and was drafted into the Army where he served as a biochemist at Walter Reed Institute of Research. In 1957, he was hired by Dr. Lane Moore to join the Dairy Cattle Research Branch of the Animal Husbandry Research Division at the USDA-Agriculture Research Service in Beltsville, MD, and was given the mission to develop nutritionally relevant fiber analyses that would replace crude fiber (CF). In 1968, he joined the Animal Science Department at Cornell University, where he spent the remainder of his distinguished career as a scientist, teacher and cherished personality.

Van Soest – The Scientist and Creative Thinker

Although its deficiencies were well known, CF had been used since the 1860s, and replacing it was no simple challenge. Dr. Van Soest initially focused on the measurement of lignin, the indigestible component of fiber. Nitrogen and hemicellulose contamination of lignin was a serious problem, and he used acid and detergents to remove them. This led to the acid detergent fiber (ADF) method, which was a preparatory step for the measurement of acid detergent lignin. His ADF method became the replacement for CF very quickly. His paper (Van Soest, 1963b) became a Citation Classic in April 1979 with 345 citations (it has many more now). In the Citation, Peter indicated that Dr. Lane Moore was certain that fiber was a crucial component in feeds, and that, "Without Lane Moore's faith and support of my work, the story of fiber may well have turned out differently." When explaining this work he stated "In developing my work on fiber it appeared that the central problem was the convenient separation of plant protein from lignin, both of which are alkali soluble. To solve this problem, I explored the ability of various kinds of detergents to remove protein . . . ." In another article, he remembered his work in the Army using chelating dyes to detect traces of minerals. He thought that perhaps the binding properties of detergents would change the way constituents could be dissolved from forages. This resulted in a revolutionary change in fiber analysis that did not evolve from anything previously done. A great mind at work!

Dr. Van Soest knew that ADF was not a measure of total fiber and created the neutral detergent fiber (NDF) method as an estimate of plant cell walls and a measure of total insoluble fiber in feeds and foods. His next Citation Classic (June 1992) was for the original NDF method (Van Soest and Wine 1967), his development of a comprehensive system of feed evaluation (Van Soest, 1967), and the first edition of his book (Van Soest 1982), Science Citation Index indicated that his book had 730 citations at the time, and the two papers had 915,320 citations – an incredible number. I wonder if the latter number was Peter's total lifetime citations in 1992 because the Citation stated "These publications represent the developments of a lifetime. Originating with improved methods for the analysis of dietary fibers, the methods have been widely applied in agronomy, ruminant, nonruminant and human nutrition, and the forage ecology of wild herbivores." To date, Van Soest et al. (1991) has nearly 25,000 citations, and Goering and Van Soest (1970) has over 14,000 citations (M. B. Hall, pers. comm.). I am sure the number of citations for the 1970 handbook is greatly undercounted because it is cited in so many different ways. Few researchers have a publication with 1,000 citations, yet these publications are only a part of the impact of Peter Van Soest.

Not content with these breakthrough analyses of fiber, Dr. Van Soest focused his efforts on the variable digestibility of fiber and total dry matter in feeds. He was the first to propose that dry matter digestibility was a function of the digestible NDF and digestible neutral detergent soluble matter (Van Soest and Moore, 1965), and that true digestibility could be measured by neutral detergent extraction of in vitro residues (Van Soest, Wine, and Moore, 1966). This in vitro method, developed with input from microbiologist Marvin Bryant at Beltsville, was included in the USDA handbook of analyses (Goering and Van Soest, 1970). He pioneered the concept of true digestibility, ideal nutritive entities, and a

summative equation that are the basis for our current evaluation of available energy in feeds (Van Soest, 1967). He postulated that NDF was the feed component that limited overall digestibility of feeds because it was the component with the greatest variability in true digestibility. These classic papers summarize his thoughts on those important concepts. His most important papers are listed in Appendix 1 (I have added a few to Peter's list). I believe that the papers in bold font should not just be cited, but read by everyone involved in animal nutrition.

Dr. Van Soest started his career trying to accurately isolate lignin, which was thought to define indigestible fiber at the time. He completed the circle of this seminal contribution at the end of his career by demonstrating that lignin was the major fiber component defining indigestible NDF and that lignin prevented other cell wall constituents from being fermented in the rumen. Indigestible NDF, or undigested NDF measured after long fermentation times in vitro, is one of the most important feed components currently used for feed evaluation. His last publication (Van Soest, Robertson, Hall, and Barry; 2020) focused on the unsuitability of Klason lignin for nutritional use.

For his efforts, he received numerous awards, including: American Feed Manufacturers Nutrition Award from American Dairy Science Association (1967); Hoblitzelle National Award in Agriculture (1968); Merit Award of the American Grassland Council (1969); Fellow of the America Institute of Chemists (1970); American Society of Animal Science Award in Nutrition Research (1983); Honorary Research Fellow, Institute of Grassland and Animal Production, UK (1985-92); Farma Foods International Fibre Prize (1991); International Dairy Production Award from American Dairy Science Association (1992); Pioneer Hibred Forage Award from American Dairy Science Association (1993); Washington State University Distinguished Graduate Award (1995); Fellow of the American Society of Nutritional Sciences (1995); and Morrison Award American Society of Animal Science (2001). In 1992, he received an honorary Doctor of Science in Animal Production from the University of Milan.

What set Peter Van Soest apart was that he was a thinker and a creator of new ideas. I have met several people who thought they were geniuses because they knew things, but they had little to no understanding or wisdom. Peter was a true genius who not only knew, but also understood, and he understood so well that he could explain the most complicated concepts to the rest of us. Dr. Van Soest had a seemingly insatiable curiosity and the passion to learn, in great detail, about that which interested him – ranging from music, to languages, to architecture, to the influence of wild flowers in Sicily imparting flavors to cheeses (B. Mahanna, pers. com.). But he always wanted more than to know; he wanted to understand the how and the why. In my opinion this is what distinguished him from most other scientists. He was a deep and determined thinker about what he observed and learned. He also wanted to understand the history of a subject and would typically trace an idea to its origin, sometimes in the native language of the authors!

In the wee hours of the morning, we graduate students would often discuss what made Peter so different. His brilliance was easily recognized by anyone who spent 30 minutes with him. But how did he know and remember so much information, how could

he explain things so clearly, and how could he easily jump from one thought to the next so effortlessly and describe connections that we never expected? It was my opinion that everything he knew and understood was interconnected in a mental model that started with a big overall picture and progressed to the smallest detail. He also connected aspects of one subject to another (translational thinking). But there were no random (unexplained) or extraneous bits of information in his mind. He never got lost in details. He formed his own opinion about everything he read and added it to his mental model if it aided his understanding. If after careful review, the information made no sense he discarded it, typically after finding the "fatal flaw" in the paper. He constantly tested the limitations of his understanding.

Dr. Van Soest's philosophy of science can be stated in his own words.

- "The danger facing the progress of nutritional research is the advancement of ***inadequate theories*** and methods by persons who are too anxious to produce a practical test without fully ***examining the limitations of their point of view***" (Van Soest, 1964).
- ". . . . a ***comprehensive theory*** regarding the availability of the dry matter of forages. . . .The ***principle*** upon which the ***new system*** is founded is that the dry matter of forages may be divided into a readily available soluble fraction and a fibrous fraction of partial availability" (Van Soest and Moore, 1965).
- "Progress in forage research, as in any science, is dependent on ***basic knowledge which leads to understanding true relationships***" (Van Soest, 1967).

The italics are mine, but you can see that Peter thought in terms of comprehensive theories developed from basic knowledge that led to understanding of true relationships, and in critical examination of his and other's points of view. Peter practiced mental meta-analysis! He had little respect for those who only wanted to know, got stuck on small details, and did not expend the effort to comprehend and understand.

He also gave little credence in using statistics to tell you what the data meant. You had better have your understanding of relationships and hypothesis in place before you did statistical analysis. "One must conclude that the size of correlation is an inefficient tool for discerning basic relationships . . . ." (Van Soest, 1967). Although I cannot find the direct quotes, there are other comments Peter made in which he indicated that the sign and magnitude of regression coefficients must make sense (fit a mental model) for a statistical relationship to be an acceptable reflection of reality. He also was very suspicious of multiple regression for interpreting data because he felt that interactions often made the results uninterpretable. Thinking first, statistics later!

Van Soest – The Teacher

As great as he was as a scientist, Peter Van Soest was also a consummate teacher and mentor – he loved to teach at every opportunity. Innumerable students benefited from his knowledge and ability to explain complicated concepts in ways that made them easy to understand. Thanks to Mike Van Amburgh and others, he was still informally teaching

graduate students at Cornell in his 90th year! His textbook, “Nutritional Ecology of the Ruminant,” is and will remain the definitive work on the concepts of digestion and metabolism, physiological relationships, feed characteristics, and feed evaluation principles that are crucial to our current understanding of ruminant and herbivore nutrition. His broad knowledge and deep understanding about the chemistry of feeds and principles of animal nutrition were inspiring. At times, his knowledge of chemistry could be daunting – he not only understood the basis for the periodic table, he could explain it to you! He was dedicated to helping his students and colleagues understand concepts and their applications to nutrition.

He taught by example, by experience, by questioning, by informal discussions (often after hours) and by lectures. I think he derived great satisfaction in sharing his knowledge and thoughts, and in seeing the light come on in the minds of those around him. I also believe that he enjoyed, if not needed, company to stimulate his thinking. There were so many carefully intertwined thoughts in his mind, that you never quite knew where the discussion or lecture was going to go. That was the fascination and excitement, you just knew that a conversation with Peter was going to be informative and would broaden your horizons.

This leads to two stories about the “teaching” relationship between my future wife, Carolyn, and Peter. I took Carolyn to a party at Peter’s home and noticed that they had a discussion. On the way back to her apartment, Carolyn said to please not leave her alone with Peter in the future because his intelligence was intimidating, and she didn’t want to say or ask something stupid. She had mentioned eating oatmeal and got a lecture on fiber! I told her that Peter never felt a question was stupid as long as you were interested and wanted to understand.

At the next party, she reminded me to stay close by. I did my best, but I briefly left the room and when I returned there was no Carolyn or Peter in sight. I finally found them touring Peter’s back yard. They seemed to be getting along so I decided that discretion was the better part of valor. Later, I asked Carolyn how it went, and she said, “I now understand why you admire and respect him so. I made a comment about a large stone in a flowerbed and Peter told me that it was millions, maybe billions, of years old and came from the bed rock of the earth’s crust up in Canada. He then described how it was shaped and delivered by glaciers. Next, he told me what elements and chemicals it was made of and how it was formed. I also made a comment about a flower and Peter then described the pigments, and their synthesis and purpose in the plant. It was all interesting and fascinating.” Peter had another convert, and they became good friends!

Another memorable event with Peter happened in Sicily. Peter and I were among the speakers and, on this occasion, Carolyn came to the conference with me. I asked her if she would like to attend Peter’s presentation and she said yes, but wondered if she would understand it. As I recall, it was an interesting talk about fiber and how it was digested by ruminants and other herbivores. She enjoyed it immensely and told me that she was happy that she understood most of it. After the talk, Peter asked us to take a walk around Ragusa. I was reticent, but Peter insisted. What an afternoon it turned out to

be, we got a personal tour by an astounding guide who described the geology of the city, its history and architecture, and the artwork in the many churches and chapels. Peter's knowledge was amazing and he wanted to teach us what he knew. It is a memory of him that we will both cherish.

As a teacher he was engaging, motivating, inspiring, passionate, and incredibly thought provoking. He wanted you to know and understand what he was presenting, and he had a knack for making the complex seem simple and attainable. But occasionally, he would go off-script. I took his graduate course the second time he taught it. He had developed a set of notes and I soon discovered that we had talked about everything in the course during our discussions in the lab and often much more. Occasionally, Peter would start thinking during a lecture and end up several concepts away from where he had planned. The students would typically stop by my office to have me explain what happened during the lecture. This taught me two things: you never know a subject until you try to explain it to someone and there is great personal satisfaction in teaching.

Van Soest – The Unique Person and Character

Dr. Van Soest was a one-of-a-kind scientist and professor, but he was also a unique individual with a myriad of interests – a modern renaissance man. His curiosity knew few bounds and he had the passion and intellect to pursue whatever interested him. He could not only discuss the characteristics of the rare earth elements and the modeling of carbohydrate digestion and passage, but he could also describe the chemistry of plant pigments, the ecological interaction of herbivores with plants, the nutrition of zoo animals, the role of fiber in human diets, the heat damaged proteins in breakfast cereals, the nutrition of donkeys and elephants, and the evolutionary development of digestion in dinosaurs. In addition, Peter loved classical music (see his daughter, Anne's, comments in Appendix 2), geology, art, and history. What a treat for a dairy farm boy from Missouri, whose music background involved pickup trucks, honky tonks, and broken hearts, to listen to classical music from an incredible stereo system and have his major professor describe who wrote the piece, who their patron was, why the piece was composed the way it was, and the history of the era in which it was written. Wow! He was also a connoisseur of wild mushrooms and single malt whiskey, and loved to cook ethnic foods and curries. It was always informative and entertaining to have a conversation with Peter, and they invariably would involve a "teachable" moment.

Some years ago, I bought an expensive bottle of scotch for Peter. Naturally, I did not buy his favorite style. So, he got several partially empty bottles from his cabinet and arranged them in order. We sipped each one while he explained their character, the differences in the distilling processes, and location of origin for each. After the lesson, he put the bottles away and we spent the evening and night discussing his latest thoughts. I stayed overnight and was given three papers to read so that we could discuss them in the morning. Ever the teacher, ever the graduate student!

Peter was certainly a memorable character. He was at times socially awkward or unaware, but he was always approachable, even engaging. He could talk to anyone from a farmer to a renowned colleague and communicate in a language that each could understand. His style of dress was unusual, if not eclectic, and I never knew if this was a conscious decision, a tweaking of social conventions, or if the denim jacket was just comfortable! Peter was somewhat unassuming and never pretentious or presumptuous. For most of my life, I called him Dr. Van Soest. I was taught to respect your elders and give honor to those who deserved it. I never felt his equal and respected the work he did to become an esteemed scientist, scholar, and professor. Eventually, he became aggravated with me and demanded that I called him Peter. Even though he was a dear friend to many of us, at times, it still feels awkward for me to call him Peter or Pete.

Peter detested mindless bureaucrats and administrators, and meaningless rules and paperwork. If you couldn't defend your policies and ideas, he had little respect for your position and loved to thwart silly rules. Lane Moore at Beltsville "protected" his scientists, especially Peter because he was so different. When Peter was engrossed in an idea, he might spend 16 hours working and then rest on a cot in his office before getting back to work. After several days of intense work, Peter would go home and rest for a few days. But federal scientists still had to fill out a timesheet indicating that they worked 8 hours each day (brain ON at 8am and brain OFF at 4:30pm)! A secretary began tracking Peter to document where he was. Peter learned that she was afraid of rodents, so he promptly got a pet white rat, named Fritz, that he kept in his lab coat pocket so she wouldn't bother him in the lab. He would release Fritz into his office when he was away to keep her out of it as well. When he moved to Cornell, they found some "do not staple, bend or mutilate" punch-card paychecks in his office that had been chewed by Fritz and had to be replaced.

At times, Peter had little patience with colleagues that just didn't get it. You had better not be superficial or absorbed in your own ideas without inspecting their limitations. He could get very animated and was willing to debate anyone at any time. While he may have conceded some small points, he never lost an argument on the bigger picture. I would never bet against him in a scientific argument! He often put more thought into a competitor's idea than the originator. He was that thorough in his thinking.

But Peter was perhaps most known for his enormous powers of concentration. Because of this he was the epitome of the absent-minded professor. We graduate students joked that, when Peter was thinking about something, Morrison Hall could explode and he wouldn't notice unless someone told him! His ability to block everything out and focus all of his mind on one thing until he figured it out was phenomenal. We were all envious and wished we had a fraction of his ability. But his concentration and focus did lead to some interesting situations. One day, Peter stopped by my office grumbling that he had to walk home in the rain because he forgot his umbrella. Fifteen minutes later, Mrs. Van Soest was looking for him and I told her that he had left to walk home in the rain. She said, "But he just called 20 minutes ago and asked me to pick him up!"

The last story involves a professor of mine from Missouri (Dr. Fred Martz) who came for a sabbatical with Peter. We shared an office and I told him about the many rare reprints, copies and papers I had organized for Peter when his office got too cluttered. So, Dr. Martz asked if he could review and copy some of the materials. He collected quite a stack of material on his desk, and asked Peter to show him where the copy machine was. I had alerted Dr. Martz that Peter's secretary had strict bounds on what she would do (copy) for anyone other than "her" professors. One day Dr. Martz asked Peter again to show him where the copier was. Dr. Martz picked up a portion of the papers and he followed Peter to the third-floor elevator. They went down to the basement where the copy machine was, and walked past it, to the door at the end of the hallway. Then they went outside, up the street and turned down Tower Road. At this point Dr. Martz said, "No wonder your secretary doesn't like to copy things, this is quite a hike." To which Peter replied, "Oh, I forgot where we were going." Dr. Martz wondered where they would have ended up, if he hadn't said something. I remarked, "I have no idea, but it would have been an interesting journey!" How could you not love and enjoy a person like that?

Conclusions

We will mourn the loss of a great scientist, teacher, mentor and friend, but our sadness is diminished by our appreciation of a life well-lived. It is impossible to contemplate what the current state of herbivore nutrition, feed evaluation, forage improvement and the ecology of plant-animal interactions would be without the efforts of Peter J. Van Soest. For those who knew him, he will be remembered for his awesome intellect, the intensity of his curiosity, the power of his concentration, his passion for scientific understanding, and his ability to share his knowledge and understanding with others.

On behalf of all of us, I want to thank his family for the sacrifices they must have made to allow Peter to become the great scientist that he was, and for sharing his time with us. It can be said that the value of a person is in the problems they solved, the friends they advised, and the family they left behind. Without a doubt, Peter was priceless!

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Appendix 1

(My comments in italics)

Interesting early papers by P. J. Van Soest

Van Soest, P. J. and T. H. Blosser. 1954. A detailed study of levels of certain blood constituents in normally calving dairy cows and in dairy cows with parturient paresis. J. of Dairy Sci 37:185-194. *Peter's MS research*

Van Soest, P.J. 1955. Interrelationships between composition and physical condition of feeds, rumen fermentation products, blood constituents and milk fat of ruminants. Ph.D. dissertation, Univ. of Wisconsin, Madison.

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Van Soest, P. J. 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency. A review. J. of Dairy Sci. 46: 204-216. *Peter's first review paper – a classic paper demonstrating his approach to scientific understanding*

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Appendix 2

Comments from Peter's daughter, Anne Van Soest, to clarify his forays into music

"Peter was an accomplished musicologist and music historian. He taught himself the recorder and he collected and played all instruments in the recorder family (bass, tenor, alto, soprano, sopranino and even the Garklen-Flotlein). It was impossible for him to play the latter as it was only 6 inches long and his fingers were too big. His favorites were the alto and bass. There are some lovely photos of him playing the bass in the lab as a young man. He was particularly fond of the recorder concertos by George Frederick Handel which he practiced at the family home.

In terms of ensembles, Peter organized a group at his church in Washington and again at the episcopal church at Cornell. He wrote, arranged and conducted music for whatever musicians were available. The period of music was baroque and renaissance. Peter was adept at arranging parts for the available instruments as well as the player's ability. He was keenly aware of fingerings and/or string crossings so as to avoid awkward or unplayable passages. These were not just transcriptions but artful arrangements. At

the height of the group's heyday in the early 70's the group played various Terpsichore dances by Michael Praetorius.

He had a lifelong love of music and art, which he instilled in his children. The family can recall many listening sessions on his audiophile quality hi-fi system. His favorite composer was Franz Joseph (aka Papa) Hayden, who is often referred to as the father of the symphony and the string quartet. I think Peter saw Hayden as a kindred spirit because of his groundbreaking innovations in the world of classical music.

Just moments before he left us for the last time just after 8:00am on Sunday, I played for him Hayden's string quartets known as "the sun" opus 20 no. 4-6. It cannot be overlooked that Peter died on Johan Sebastian Bach's 336th birthday. Bach was another favorite of Peter's due to Bach's mathematical and artful genius in counterpoint, cannon and fugue."

Appendix 3
Memoriam by Italian Colleagues

Peter J. Van Soest, one of the greatest scientists ever in animal nutrition, left us. He changed the way of looking at feeds, fiber and its analysis, ruminants and their nutrition and ecology and who determined a turning point in the study of ruminants, bringing it to the level of great science compared to a previous prevailing empiricism. However, our intent is not to summarize his career, his studies, his discoveries; they are too many and would distract us from the man and scientist that he was.

Capable of furious battles with some colleagues whose ideas, but even more the scientific spirit, he did not share, he was always available to motivate young people, whether they were his students or not, and found ways to make them feel important and stimulate their scientific curiosity. He transmitted in a simple way, that sometimes could appear naive, his immense intelligence and passion for knowledge, his availability and human generosity and his will to study and explore science always in a free and critical way.

He was an eclectic and highly cultured man, a master of animal science, ancient music, art, ethnic cuisine and much more. It was hard not to love him, as you can only love a dear relative, for those who had the opportunity to know him well and be close to him. His numerous students scattered all over the world and all those who had the chance to meet him, even occasionally, by listening to a lecture at a conference or a chat with breeders (whom he loved and by whom he was loved), have a very dear memory of him, clearly visible from the moved and not formal participation that his death has aroused all over the world.

As Italian scientific community we are particularly grateful to him. Many researchers, professionals and breeders had the good fortune to know him personally during his many visits to Italy, where he was at home, albeit his second home, and where, perhaps for this reason, he left a very strong scientific imprint. Italy has recognized his

greatness conferring him an Honorary Degree (Laurea Honoris Causa) of the University of Milan and the honorary citizenship of the city of Ragusa, in Sicily. Many Italians have also had the opportunity to study with him at Cornell University. Even a few months in his laboratories, following his courses and participating in his jovial after hours, were enough to understand, amazed, his greatness and be marked indelibly.

In reality, we are all a little bit his students. Even those who did not know him personally, have certainly breathed deeply his thought and research in their university studies at all levels, in their research or technical activities.

We therefore say goodbye to him, as Italian scientific community of animal science, with great emotion, comforted by the thought that his human and scientific legacy will keep his memory vivid forever.

Antonello Cannas
Stefania Carpino
Giuseppe Licitra

Meadow Fescue Grass Varieties for Optimal Forage Quality in Dairy Production Systems

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

Introduction

Achieving optimal forage quality on dairy farms is key towards supporting high milk production. In New York State, over 85% of alfalfa sown is done in combination with a perennial grass, a unique practice in dairy production systems. Inter-seeding a grass species into the alfalfa (Medicago sativa L.) stand can increase the neutral detergent fiber digestibility (NDFD), an important forage quality to support high milk production yields. Meadow fescue (Festuca pratensis) (MF), a grass species recently been brought to the attention of forage extension specialists in the MidWest at the U.S. Dairy Forage Research Center, Dr. Michael Casler and at Cornell University, Dr. Jerry Cherney in the School of Integrated Plant Sciences, and Dr. Debbie Cherney in the Department of Animal Science. Originating from northern Europe, and well established as a high-quality forage for lactating dairy cows, show potential as a high-yielding, winter-hardy, high quality grass to be adopted into the dairy forage systems. The objective of this study was to achieve the highest possible quality at spring harvest for the grass at the optimum harvest date for alfalfa, and to compare varieties over the two spring growing seasons of 2020 and 2021. Pure stands of nineteen meadow fescue varieties were evaluated for forage quality and the rate of change in quality during spring growth.

2020 and 2021 Growing Seasons and Take-Home Message

Grass quality with respect to the optimal alfalfa harvest date for both seasons is best quantified by evaluating the NDFD value and assessing the rate of NDF decline before heading of each grass variety. Optimal alfalfa harvest date for alfalfa-grass mixtures is earlier than for pure alfalfa stands and is when the alfalfa crop is about 32-35% NDF. A 10-day difference in the optimal alfalfa harvest date was observed between the two seasons, June 2nd in 2020 and May 24th in 2021. The delayed grass growth in 2020 was due to drought conditions early in the season that slowed plant development. Average nutritive value of MF varieties changed linearly and in a similar fashion in both years where NDFD significantly decreased from 90% to 74% (R^2 = 0.99) in 2020 and from 87% to 75% (R^2 = 0.99) in 2021 over the 8-day period prior to the typical harvest date. Rates of NDFD decline were 1.4% units/day and 1.2% units/day in 2020 and 2021, respectively. Neutral detergent fiber (NDF) also increased over both seasons, with NDF levels increasing from 43% to 52% (R^2 = 0.84) in 2020 and from 43% to 55% (R^2 = 0.99) in 2021. Consistent over both spring harvest seasons, the SW Revansch variety ranked with the highest NDFD, whereas the Liherold variety had the lowest NDFD values. Research consistently highlights the potential economic advantage of improved grass to alfalfa forage quality where a 1% unit increase in NDFD translates to a 0.5 to 1 lb milk/cow/day increase in milk production. With significant differences in NDFD among MF varieties, current results emphasize the importance of selecting high quality grass varieties and optimizing time of harvest to achieve optimal forage quality and yield.

Optimizing Sampling Practices at NYS Dairy Farms

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

Introduction

Current standard forage sampling practices may not yield samples that accurately represent the quality of the delivered forages in dairy diets. Sampling frequency, number of samples, and allowed deviation in the change of forage quality (Control limits) can be optimized according to the farm characteristics to improve interpretation of forage samples and diet accuracy. For a period of 16 weeks in the winter of 2020 and spring of 2021, we collected corn silage and haylage samples in duplicate, 3 days per week before diet mixing from 8 NYS dairy farms with 3 silage storage methods (bunker, bag, and drive over pile). Lactating herd size was recorded to be used as an input in the optimization analysis. We also recorded the change of the volume of each silo, the sampling date, and identified spatially explicit methods to define the field(s) of origin of the silage within the bags (1 dimensional) and bunker silages (2 dimensional) to estimate feeding rate (ft/d and ft^3/d) and feeding time for each field of origin (days per field of origin). The herd size records and the field-of-origin feeding time (average feeding d/field) were used as inputs in the renewal reward model (RRM) described by St-Pierre and Cobanov (2007) to define the farm size and in-control time parameters for each farm, respectively. Then, we applied a genetic algorithm optimization method using the RRM as objective function to estimate the optimum sampling frequency, sample number, and control limits of each farm. In our study context, the in-control time parameter refers to the length of time haylage and corn silage quality stays stable without a major change. The objective of this study is to develop a method to increase sampling efficiency and diet accuracy by optimizing sampling practices at eight different NYS case study dairy farms.

Main Findings

Feedout data records reported in table 1 show a small-scale farm A (±118 cows) and large-scale farm B (±1,229 cows) using bags and medium-scale farm C (±517 cows) and D (±656 cows) using bunkers for haylage and corn silage. The feeding rate (ft^3/d) of farm A and C was lower than B and D for haylage and corn silage. The feeding rate (ft^3/d) of all farms was related to the farm size, ensiling method, bag and bunker dimensions, and forage type. Field-of-origin feeding time (d/field) not only depends on feeding rate (ft/d) and (ft^3/d), and silage face area (ft^2/d), but also depends on the field area and number of fields harvested per farm.

St-Pierre and Cobanov (2007) identify in-control time of the RRM as one of relevant parameters for optimizing sampling practices. They proposed 30 days as the in-control time parameter for the RRM based on expert opinion. However, in a previous study, our mixed-model analysis of the haylage and corn silage at harvest and feedout

identified field-of-origin as a primary contributor to variation in forage composition. Thus, haylage and corn silage composition is expected to change with the field of origin. For this reason, we propose the average field-of-origin feeding time (d/field) as in-control time (d) parameter of the RRM.

Table 1. Farm herd size, ensiling method, feeding rates, and silage area of sampled haylage and corn silage.

Ingredient	Farm	Number of cows	Ensiling type	Feeding rate (ft/d)	Silage face area (ft^2)	Feeding rate (ft^3/d)	Field-of-origin feeding time (d/field)
Haylage	A	118	Bag	1.7	64	109	7 (1 - 16)
	B	1229	Bag	3.7	154	554	4 (1 - 13)
	C	517	Bunker	1.0	271	301	13 (5 - 16)
	D	656	Bunker	1.7	518	601	6 (1 - 2)
Corn Silage	A	118	Bag	3.4	64	215	18 (5 - 33)
	B	1229	Bag	11.4	154	1762	6 (1 - 13)
	C	517	Bunker	1.3	438	632	7 (1 - 24)
	D	656	Bunker	0.8	1251	1,131	6 (1 - 13)

Optimum sampling practices estimated by optimizing the RRM were different for each farm when we set the in-control time equal to the average field-of-origin feeding time (d/field) and farm herd size equal to the average lactating cow number (Table 2). Consistent with the number of samples proposed by St-Pierre and Cobanov (2007), our results suggest the optimum number of samples is 2 samples per sampling time for all farms, ensiling methods, and forage ingredients. A larger number of cows and shorter in-control time increased the optimum sampling frequency. The optimum sampling frequencies using the field-of-origin feeding time to parameterize the RRM were consistent with sampling frequencies suggested by St-Pierre and Cobanov (2007). However, the optimum control limits were lower than the proposed by St-Pierre and Cobanov (2007). Lower control limits increase the number of samples out of the stable composition range and but could yield more accurate and consistent diets. Total quality cost ($/d) in table 2 refers to the sum of the costs required to collect samples, sample analyze sample composition, and adjust diets, as well as expected changes in milk production. Consistent with St-Pierre and Cobanov (2007), the total quality cost ($/d) increased with the increase of herd size. However, the total quality cost estimated using the field-of-origin feeding time were higher than the total quality cost estimated by St-Pierre and Cobanov (2007). This result is due to the shorter in-control time inputs that we estimated based on the expected frequency of changing the field-of-origin.

Take Home Message

The optimal number of samples for each farm, regardless to the herd size, field feeding time, ensiling method, and type of forage is 2 samples per sampling time. Optimal sampling frequency increases with the increase in the herd size. Shorter values for the in-control parameter decreases the allowed deviation in the change of forage quality. Our estimates of the field-of-origin feeding time suggest that these values vary between

farms and support farm specific estimates for the in-control time parameter needed by the RRM model.

Table 2. Optimum estimates and range of in-control time, number of samples, control limits, and total quality cost calculated with three different optimization methods in farms with different lactating herd sizes and two ensiling methods.

Herd Size and in-control time data source	Ingredient	Farm	Number of cows	Ensiling method	In-control time (d)	Number of samples	Sampling frequency (d)	Control limits (SD)	Total quality cost ($/d)
NYS Farms	Haylage	A	118	Bag	7 (1 - 16)	2 (1 - 2)	14 (12 - 19)	0.56 (0.00 – 1.11)	$67 ($59 - $76)
		B	1229	Bag	4 (1 - 13)	2 (1 - 2)	3 (2 - 3)	0.80 (0.00 - 1.04)	$574 ($451 - $777)
		C	517	Bunker	13 (5 - 16)	2 (2 - 2)	4 (4 - 5)	0.99 (1.00 - 1.20)	$229 ($196 - $275)
		D	656	Bunker	6 (1 - 2)	2 (1 - 2)	4 (3 - 4)	0.84 (0.38 - 1.07)	$312 ($265 - $377)
	Corn Silage	A	118	Bag	18 (5 - 33)	2 (1 - 2)	13 (12 - 19)	0.86 (0.00 - 1.16)	$58 ($47 - $76)
		B	1229	Bag	6 (1 - 13)	2 (1 - 2)	3 (2 - 3)	0.80 (0.00 – 1.04)	$574 ($451 - $777)
		C	517	Bunker	7 (1 - 24)	2 (2 - 2)	5 (4 - 5)	0.95 (0.00 – 1.21)	$227 ($171 - $340)
		D	656	Bunker	6 (1 - 13)	2 (1 - 2)	4 (3 - 7)	0.75 (0.00 - 1.10)	$324 ($258 - $427)
St-Pierre and Cobanov (2007)	All forage ingredients	A	118	Bag	30	2 (-)	13 (-)	1.14 (-)	$48 (-)
		B	1229	Bag	30	2 (-)	3 (-)	1.32 (-)	$338 (-)
		C	517	Bunker	30	2 (-)	5 (-)	1.26 (-)	$158 (-)
		D	656	Bunker	30	2 (-)	5 (-)	1.11 (-)	$194 (-)

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Effect of Prepartum DCAD Strategy and Level of Dietary Calcium on Postpartum Calcium Status and Performance of Multiparous Holstein Cows

G.M. Graef[1], A.L. Kerwin[1], L.N. Ferro[1], S. Ordaz-Puga[1], C.M. Ryan[1], T.A. Westhoff[2], D.M. Barbano[3], K.M. Glosson[4], K.P. Zanzalari[4], J.D. Chapman[4], T.R. Overton[1]

[1]Department of Animal Science, Cornell University, Ithaca, NY
[2]Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY
[3]Department of Food Science, Cornell University, Ithaca, NY
[4]Phibro Animal Health Corporation, Teaneck, NJ

Introduction

The transition period, three weeks prior to and post calving, provides opportunity for disruptions of homeostasis. Commonly, the rapidly increased demand for calcium (**Ca**) for parturition, colostrum production, and milk production may leave the cow at risk for hypocalcemia. Low blood calcium, or hypocalcemia, is recognized as a notable detriment to health and productivity of dairy cows (Curtis et al., 1983). Subclinical hypocalcemia (**SCH**) can predispose cows to an increased risk of other health disorders, decreased milk production, and decreased reproductive performance (Kimura et al., 2006; Reinhardt et al., 2011; Caixeta et al., 2017; McArt and Neves, 2020). Circulating concentrations of Ca in cattle are generally well maintained by a complex homeostatic mechanism involving parathyroid hormone (**PTH**) and 1,25-dihydroxyvitamin D_3 to manage functions of Ca ruminal and intestinal absorption, renal excretion and retention, and Ca resorption of the bone (Goff, 2008). When Ca demand increases abruptly around parturition, the homeorhetic systems' ability to meet this demand, via these mechanisms coupled with Ca intake, will determine the presence and degree of hypocalcemia in the cow.

It is understood that there are dietary interventions to support the cow's homeostatic mechanisms and either prevent or reduce the severity of Ca imbalances at the time of calving. In the United States, a common method for managing hypocalcemia is by manipulating the dietary cation anion difference (**DCAD**) in the close-up period prior to calving (Ender et al., 1971; Goff et al.,1995). Feeding a negative DCAD diet creates a state of compensated metabolic acidosis within the cow, resulting in decreased urine pH and increased urinary Ca excretion, thereby improving tissue sensitivity to homeostatic signaling (Goff, 2008; Leno et al., 2017). While feeding a negative DCAD diet is a generally accepted method of reducing risk of SCH and milk fever, the level of anionic supplementation remains a point of debate (Santos et al., 2019). Recent meta-analyses observed that lower levels of DCAD reduced the risk of milk fever and overall disease while improving performance of parous cows (Lean et al., 2019; Santos et al., 2019).

Dietary Ca manipulation in conjunction with some level of DCAD can be used to improve plasma Ca status and cow health; however, the improvement in milk production has been debated. Ryan et al. (2020) and Glosson et al. (2020) reported that negative DCAD (prepartum urine pH 5.5-6.0) with supplemental dietary Ca had positive effects on

cow health, production, and reproduction parameters. Goff and Koszewski (2018) recommend restricted or low dietary Ca with a negative DCAD (prepartum urine pH 6.5-7.0) to mitigate hypocalcemia and improve overall production. A meta analysis by Santos et al. (2019) included experiments with varying Ca supplementation, found that positive and negative DCAD programs targeting different urine pH levels with increased dietary Ca was associated with an increase in risk of milk fever. More work needs to be conducted to elucidate the level of DCAD and dietary Ca supplementation to optimize dairy cow health and performance.

The objective of this study was to compare the effects of two levels of prepartum DCAD, two levels of dietary Ca, and their interactions on the parameters of Ca metabolism, health, and milk performance of transition dairy cows. We hypothesized that cows fed a lower level of DCAD (evaluated by urine pH) and higher dietary level of Ca would have improved Ca status and greater overall performance than alternative experimental diets.

Materials and Methods

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee prior to the beginning of the experiment. Multiparous cows (n = 98) were enrolled between October 2019 and July 2020 in a completely randomized design, restricted to balance for parity (entering 2nd lactation vs. 3rd and greater), body condition score (**BCS**), and previous 305-d mature equivalent milk production. Cows diagnosed with twins or entering their first parity were excluded from this study. Cows were housed in sawdust bedded, individual tie-stalls and fed with individual feed bins at the Cornell University Ruminant Center (Harford, NY). All cows were moved in weekly approximately 35 d prior to expected date of parturition and fed a standard far-off or control diet for a 7d covariate period. At 26 d prior to expected parturition, cows were assigned to one of four dietary treatments until parturition. All diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS, v 6.55, Cornell University, Ithaca, NY). Diets were identical except for the main effects of DCAD level (PART: -2.6 mEq/100 g DM or FULL: -10.3 mEq/100 g DM) and dietary Ca concentration (1.50% or 0.70% DM). After calving, all cows were fed the same fresh cow diet. Ingredients and analyzed composition of the diets are presented in Table 1 and 2.

Cows were fed daily, feed offered and refused amounts were recorded. Prepartum cows were fed between 0900 and 1100 h and postpartum cows were fed between 0700 and 0900 h. Weekly TMR samples and feed ingredients were collected to evaluate DM and calculate daily DMI. Forages and TMR samples were dried, ground, and composited at 4-week intervals over the course of the study. Composites were submitted to a commercial laboratory for wet chemistry analysis (Cumberland Valley Analytical Services, Waynesboro, PA). Body weights and BCS (Edmonson et al., 1989) were measured weekly from enrollment until 63 DIM.

Prepartum urine pH (**UpH**) was collected and recorded 3x/week using a portable glass electrode pH meter to monitor compensated metabolic acidosis through urine pH (PART: 6.5-7.0 pH or FULL: 5.5-6.0 pH). Free-catch, midstream urine samples were collected at -25, -14, -7, +1, 2, 3, 4, 5, 7, 14 d relative to parturition and stored at -20ºC prior to analysis of ammonium the Cornell University Animal Health and Diagnostic Center (Ithaca, NY). Urine sample collection occurred between 1800 and 1900 h.

Table 1. Ingredient composition of the prepartum diets and the common postpartum diet.

	Prepartum				
Ingredient, % of DM	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**Postpartum**
Corn silage	43.33	43.33	43.33	43.33	44.96
Hay crop silage	—	—	—	—	16.89
Wheat straw	30.00	30.00	30.00	30.00	—
Corn grain, finely ground	2.00	2.00	2.00	2.00	16.20
Soybean meal	5.00	5.00	5.00	5.00	4.66
Amino Plus	8.00	8.00	6.97	6.97	3.70
Soybean Hulls, ground	0.67	0.67	4.67	4.67	3.64
Animate[1]	4.33	3.33	4.33	3.33	—
Ca DiCal	0.80	0.80	0.80	0.80	—
Blood meal	1.67	1.67	1.67	1.67	2.24
Dextrose	—	—	—	—	1.61
Calcium carbonate	3.33	3.33	0.43	0.43	1.53
Palmit 80[2]	—	—	—	—	0.98
Sodium sesquicarbonate	—	—	—	—	0.78
Salt	0.37	0.37	0.33	0.33	0.47
Selenium	0.03	0.03	0.03	0.03	—
Molasses	—	—	—	—	0.32
Bypass fat	—	—	—	—	0.32
Magnesium oxide	0.33	0.33	0.33	0.33	0.23
Calcium sulfate	—	—	—	—	0.22
Mono-Dicalcium phosphate	—	—	—	—	0.11
Smartamine[3]	—	—	—	—	0.04
Dairy ADE[4]	0.03	0.03	0.03	0.03	0.02
Vitamin E[5]	0.03	0.03	0.03	0.03	0.00
Rumensin[6]	0.01	0.01	0.01	0.01	0.01
Corn distillers, ethanol	—	—	—	—	0.98
Trace mineral premix	0.04	0.04	0.04	0.04	0.10
Mineral oil	—	—	—	—	0.02
Filler[7]	—	1.00	—	1.00	—

[1]Commercial dietary anion supplement, (Phibro Animal Health Corp., Quincy, IL).
[2]Commercial high palmitic acid fat; Global Agri-trade Corporation (Rancho Dominguez, CA).
[3]Met, physically protected with pH-sensitive coating; Adisseo (Antony, France).
[4]Vitamin mix; Cargill Animal Nutrition (Minneapolis, MN). Contains 30,073 kIU/kg vitamin A, 5,783 kIU/kg vitamin D, and 92,534 IU/kg vitamin E.
[5]Contains 510,750 IU/kg vitamin E.
[6]Premix contained 26,400 g/t of monensin; Elanco Animal Health (Greenfield, IN)
[7]Filler contained ground rice hulls (47.4%), corn distillers ethanol (35.5%), urea (7.1%), Mg oxide (7.0%), Ca carbonate (2.6%), and Na bicarbonate (0.4%).

Blood samples were collected via coccygeal venipuncture between 0600 and 0730 h once a week prior to treatment assignment, twice weekly until 1 week prior to expected parturition, and then daily until parturition. After calving, 4 samples were collected every

12 h and categorized as 0.5, 1, 1.5, and 2 d postpartum samples. Samples were also collected at 3, 5, 7, 14, 21, and 28 d postpartum. One aliquot was stored at -20ºC to be submitted for analysis of total Ca (**tCa**), P, and Mg. Ionized calcium (**iCa**) was measured in whole blood collected in lithium heparin vacutainers on d -25 (covariate), -7, -3, -1, +0.5, 1, 1.5, 2, 3, and 5, relative to parturition using iSTAT CG8+ cartridges (Abbott Laboratories, Lake Bluff, IL).

Within approximately 2 h of parturition, the first colostrum was weighed, evaluated for BRIX, and a sample was subsequently collected and frozen for immunoglobulin G (**IgG**) analysis (Cornell University Animal Health Diagnostic Center, Ithaca, NY). All cows were milked 3x/d until 60 DIM and daily milk weights were recorded. Weekly milk samples were taken at 3 consecutive milkings for the duration of the study and analyzed for milk composition in the Barbano lab at Cornell University using Fourier transform mid-infrared techniques (Barbano et al., 2014).

Table 2. Analyzed nutrient composition (mean ± SD, % of DM unless otherwise noted) for the prepartum diet and the common postpartum diet.

	Prepartum				
Nutrient	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**Postpartum**
CP	12.9 ± 0.2	12.6 ± 0.2	12.6 ± 0.2	12.8 ± 0.2	14.5 ± 0.2
NDF	44.1 ± 0.6	44.0 ± 0.6	45.0 ± 0.6	45.9 ± 0.6	30.9 ± 0.4
Starch	18.1 ± 0.5	18.0 ± 0.5	17.7 ± 0.6	17.7 ± 0.5	29.2 ± 0.4
Sugar	4.5 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	4.21 ± 0.3	3.9 ± 0.2
Ash	9.08 ± 0.29	9.58 ± 0.27	7.86 ± 0.29	7.69 ± 0.29	7.83 ± 0.20
Ca	1.44 ± 0.04	1.53 ± 0.04	0.74 ± 0.04	0.67 ± 0.04	1.03 ± 0.03
P	0.34 ± 0.01	0.35 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.31 ± 0.01
Mg	0.50 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.47 ± 0.02	0.31 ± 0.01
K	1.06 ± 0.03	1.05 ± 0.03	1.06 ± 0.03	1.07 ± 0.03	0.29 ± 0.02
Cl	0.90 ± 0.03	0.71 ± 0.03	0.88 ± 0.03	0.70 ± 0.03	0.45 ± 0.02
DCAD, (mEq/100g DM)	-8.47 ± 1.80	-2.01 ± 1.68	-11.86 ± 1.79	-2.86 ± 1.79	29.04 ± 1.27

Statistical Analysis

Prepartum and postpartum samples were analyzed separately. Statistical analyses were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC). Continuous measures that were not repeated over time underwent ANOVA using PROC MIXED with fixed effects of Ca level, DCAD level, parity, and all possible interactions. Data evaluated over time underwent repeated measures ANOVA using PROC MIXED and the repeated measures statement for time. Fixed effects included in the model were Ca level, DCAD level, time, parity, and interactions with the random effect of cow nested within Ca and DCAD level. Covariate measures were included in the model when pretreatments were available.

Results

A total of 98 cows were included in final analysis. Urine pH and ammonium results are reported in Table 3. Urine ammonium pre- and postpartum is illustrated in Figure 1.

Cows fed FULL had lower UpH and significantly higher rate of ammonium concentration excreted than cows fed PART (5.64 vs. 6.71 ± 0.10; *P* < 0.001 and 0.65 vs. 0.34 ± 0.09 mg/L; *P* < 0.001, respectively). Dietary Ca did not affect UpH ((*P* = 0.27); however, cows fed higher Ca tended (*P* = 0.10) to have lower concentrations of ammonium in urine. Pre- and postpartum DMI are reported in Table 4 and Figure 2. Cows fed FULL had lower prepartum DMI than cows fed PART (13.1 vs. 14.1 ± 0.3 kg/d; *P* = 0.04). Dietary Ca did not affect prepartum DMI (*P* = 0.21). Analysis of postpartum DMI from wk 1 to 9 showed that DMI tended to be increased for those cows fed ~1.5% Ca (21.8 vs. 20.9 ± 0.5 kg/d; *P* = 0.07); prepartum DCAD did not affect postpartum DMI (*P* = 0.70).

Table 3. Least squares means and SEM of prepartum urine pH and ammonium (mg/L) concentration.

	Treatment					*P*-value	
Variable	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**SEM**	**Ca**	**DCAD**
Prepartum UpH	5.68	6.81	5.59	6.62	0.13	0.27	<0.001
Prepartum NH4+	0.61	0.30	0.69	0.39	0.13	0.10	<0.001

Pre- and postpartum serum mineral concentrations are reported in Table 5 and iCa is presented in Figure 3. Postpartum circulating iCa and P from d 0 to 3 tended to be increased for cows fed FULL compared to PART (iCa: 0.98 vs. 0.94 ± 0.02 mM; *P* = 0.07 and P: 1.51 vs.1.42 mM ± 0.04; *P* = 0.09). Cows fed ~1.5% Ca had lower postpartum circulating iCa and tCa from d 0 to 3 (iCa: 0.97 vs. 1.01 ± 0.02 mM; *P* = 0.02, tCa: 2.09 vs. 2.17 ± 0.04 mM; *P* = 0.04). There was no effect of the interaction between Ca and DCAD on cow mineral status pre- or postpartum.

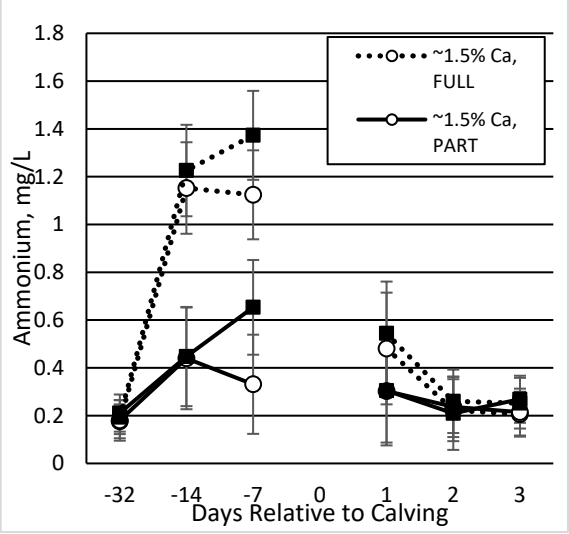

Figure 1. Urine ammonium excretion pre- and postpartum by treatment.

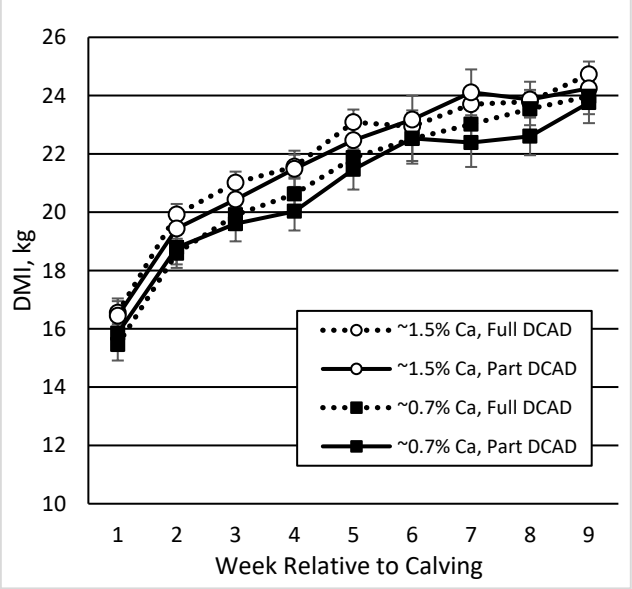

Figure 2. Weekly average postpartum DMI reported by treatment.

Colostrum measurements, milk yield, and milk composition are presented in Table 6 and Figure 4. We observed an effect of DCAD level on colostrum production (kg) such that cows fed FULL produced more colostrum than cows fed PART (7.70 kg vs. 5.43 kg ± 0.11; P = 0.02). There was also a trend for an interaction favoring ~1.5% Ca, FULL. There was no effect of Ca or DCAD on IgG concentrations. When evaluating milk yields for wk 1 to 9, a trend (P = 0.10) for an interaction of DCAD, dietary Ca, and week existed such that cows fed the PART and ~0.7% Ca had the lowest milk yield and cows fed the FULL and ~1.5% Ca had the highest milk yield. Cows fed ~1.5% Ca generally had higher milk yields in wk 1 to 3 postpartum (40.2 vs. 38.7 ± 0.9 kg/d; P = 0.02). There was no evidence that there was a difference in components between Ca, DCAD, or the interaction between Ca and DCAD for wk 1 to 9 or wk 1 to 3. There was a slight increase in lactose percentage for cows fed ~1.5% Ca prepartum (4.66% vs. 4.60% ± 0.03; P = 0.05).

Table 4. Least squares means and SEM for pre- and postpartum DMI, BW, and BCS.

	Prepartum					*P*-value		
Variable	~1.5% Ca, FULL	~1.5% Ca, PART	~0.7% Ca, FULL	~0.7% Ca, PART	SEM	Ca	DCAD	Ca*DCAD
Prepartum								
DMI (kg/d)	13.5	14.2	12.6	14.0	0.50	0.21	0.04	0.47
BW (kg)	759	782	765	764	6.91	0.37	0.10	0.07
BW Change (kg)	17.43	22.57	9.97	6.35	0.48	0.03	0.81	0.38
BCS	3.58	3.61	3.58	3.58	0.03	0.49	0.65	0.67
BCS Change	0.20	0.19	0.24	0.17	0.10	0.85	0.39	0.48
Postpartum								
DMI (kg/d)	21.92	21.74	21.06	20.78	0.50	0.07	0.60	0.92
BW (kg)	685	678	685	680	12	0.93	0.59	0.92
BW Change (kg)	-31.90	-65.74	-24.92	-58.19	12.07	0.61	0.02	0.98
BCS	3.10	3.13	3.15	3.12	0.05	0.69	0.88	0.49
BCS Change	-0.59	-0.59	-0.31	-0.60	0.10	0.13	0.10	0.11

Table 5. Least squares means and SEM for iCa, tCa, P, and Mg pre- and postpartum (d 0 to 3).

	Treatment					*P*-value			
Variable	~1.5% Ca, FULL	~1.5% Ca, PART	~0.7% Ca, FULL	~0.7% Ca, PART	SEM	Ca	DCAD	Ca* DCAD	Ca* DCAD* Parity
Prepartum									
iCa (mmol/L)	1.14	1.11	1.16	1.15	0.03	0.32	0.49	0.15	0.47
tCa (mmol/L)	2.43	2.45	2.42	2.48	0.02	0.62	0.05	0.49	0.13
P (mmol/L)	6.21	6.18	6.19	6.08	0.09	0.44	0.39	0.65	0.89
Mg (mmol/L)	2.27	2.31	2.32	2.33	0.04	0.36	0.52	0.49	0.25
Postpartum									
iCa (mmol/L)	0.91	0.98	0.96	1.01	0.02	0.02	0.07	0.44	0.30
tCa (mmol/L)	2.11	2.07	2.18	2.15	0.04	0.04	0.40	0.88	0.05
P (mmol/L)	1.48	1.42	1.54	1.42	0.06	0.56	0.09	0.58	0.89
Mg (mmol/L)	0.97	1.01	0.98	0.97	0.02	0.55	0.42	0.28	0.11

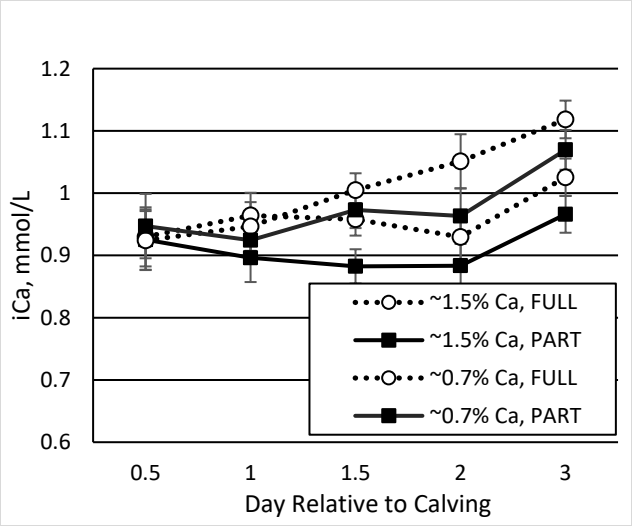

Figure 3. Average iCa concentration by treatment for d 0.5, 1,1.5, 2, and 3.0.

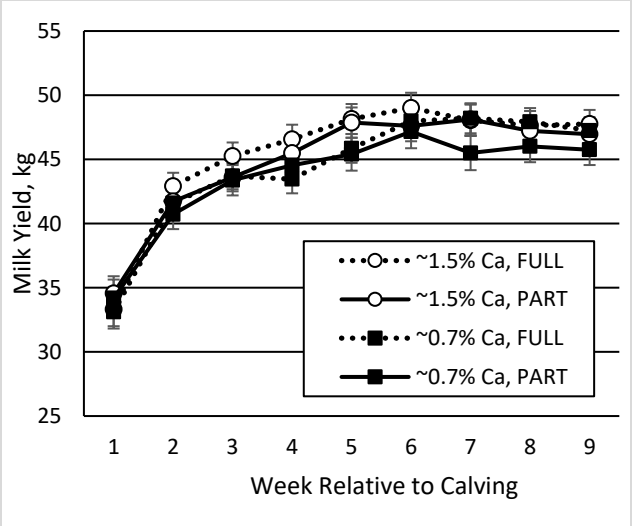

Figure 4. Average weekly milk yields and SE for wk 1 to 9 postpartum.

Table 6. Least squares means and SEM for colostrum measurements, milk yield, and milk composition from wk 1 to 9 of lactation.

	Treatment					*P*-value			
Variable	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**SEM**	**Ca**	**DCAD**	**Ca* DCAD**	**Ca* DCAD *Wk**
Colostrum (kg)	9.62	5.29	6.17	5.56	1.16	0.19	0.02	0.10	-
Colostrum IgG (mmol/L)	375	393	357	378	1.08	0.56	0.48	0.94	-
Milk Yield (kg/d)	45.4	44.8	44.3	43.6	1.02	0.24	0.48	0.96	0.10
Fat (%)	4.41	4.38	4.47	4.59	0.14	0.25	0.73	0.52	0.75
Fat (kg/d)	0.70	0.66	0.66	0.71	0.04	0.90	0.91	0.18	0.53
3.5% FCM (kg/d)	53.8	51.6	51.2	53.7	1.7	0.82	0.92	0.10	0.72
Protein (%)	2.75	2.73	2.76	2.74	0.04	0.66	0.62	0.98	0.82
Protein (kg/d)	1.29	1.36	1.22	1.28	0.10	0.43	0.47	0.93	0.93
Lactose (%)	4.66	4.66	4.64	4.57	0.03	0.05	0.15	0.14	0.65
Lactose (kg/d)	2.19	2.11	2.06	2.09	0.06	0.13	0.55	0.26	0.68
TS (%)	12.94	12.86	12.98	13.01	0.16	0.47	0.88	0.66	0.82
TS (kg/d)	6.08	5.82	5.74	5.95	0.17	0.47	0.84	0.10	0.71
ECM (kg/d)	51.9	49.8	49.5	51.6	1.6	0.82	0.99	0.10	0.61
MUN (mg/dL)	6.14	6.92	6.98	7.36	0.50	0.15	0.20	0.95	0.16
SCS	0.99	0.84	1.08	1.32	0.38	0.49	0.92	0.63	0.42

Conclusions and Implications

Cows fed the lower Ca diet did recover their normal iCa levels more quickly but didn't meet the production levels of their ~1.5% Ca, FULL counterparts. Cows fed ~0.7% Ca, PART were slower to return to normal iCa concentration post calving without marked increases in milk yields over time. This may warrant more investigation. Overall, feeding a higher Ca diet in conjunction with a more negative DCAD (5.5 ≤ UpH ≤ 6.0) reduced the blood calcium level of cows postpartum but numerically improved production of cows over the first 3 wk of lactation.

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Brief Introduction to the NASEM (formerly known as NRC) 8th Revised Edition of the Nutrient Requirements of Dairy Cattle

W. P. Weiss
Department of Animal Sciences
Ohio Agricultural Research and Development Center
The Ohio State University, Wooster

Introduction

After 20 years, a new "Dairy NRC" is about to be released albeit with new name. The 8th revised edition of the Nutrient Requirements of Dairy Cattle will now be designated as a product of the National Academies of Science, Engineering, and Medicine (NASEM) rather than the National Research Council (NRC). The Academies have always been the governing unit of the NRC. Although the name has changed, the procedures related to development of the revised edition remained the same. A committee of experts are chosen by the Academy that represents a broad range of expertise and geography, and the committee is vetted for potential conflicts of interest. The final committee was comprised of Rich Erdman (co-chair), Bill Weiss (co-chair), Mike Allen, Lou Armentano, Jim Drackley, Jeff Firkins, Mary Beth Hall, Ermias Kebreab, Paul Kononoff, Helene Lapierre, and Mike Vandehaar.

The main charge of the committee was "to (conduct) a comprehensive analysis of recent research on the feeding and nutrition of dairy cattle, including research on the amounts of amino acids (AA), lipids, fiber, carbohydrates, minerals, vitamins, and water needed by preweanling, growing, reproducing, and lactating dairy cattle. . . and to … evaluate new information to improve the accuracy of predicting animal performance from nutrient input and of predicting nutrient input when animal performance is known." The committee was also charged with developing a computer model that reflected the discussion and equations in the text.

It is far beyond the scope of this paper to discuss everything that has been revised (the final book will likely exceed 500 pages). Rather this brief review will discuss some major revisions from NRC (2001) and their implications and will be limited to lactating cows even though the chapters on transition cows, calves and heifers have been modified extensively. The amount of text dedicated to different sections does not reflect the importance or magnitude of the changes made, but rather reflects this author's areas of expertise. Details on equations and software will be available when the revision is published in December 2021.

Estimating Dry matter Intake

The dry matter intake (DMI) equation in NRC (2001) used only animal factors (milk production, body weight, and days in milk). Because milk yield is strongly related to DMI, the equation was fairly accurate on estimating DMI when production measures were

known. The equation did not work as well when a diet was formulated without knowing actual production. The new NRC includes an improved animal factor only equation (based on more data and data from higher producing cows) and an animal and diet factor equation. Primary dietary factors that influence DMI are forage NDF (negatively related to DMI), in vitro NDF digestibility (positively related to DMI) and the primary source of fiber in the diet estimated using the ADF/NDF ratio (high ratio indicates a legume-based diet and a lower ratio indicates a grass-based diet). The new equations will be more accurate with today's higher producing cows and reflect the impact of diet on DMI. Users are cautioned that when using the diet factor equation, entered milk yields must be reasonable because milk yield is still the major driver of DMI. Equations to estimate DMI for dry and prefresh cows, calves and heifers were also updated and include dietary NDF (except for the calf equations).

Energy

The NRC (2001) was the first revision of the Dairy Requirements series that calculated energy values (i.e., net energy for lactation, NEL) from the nutrient composition of the feeds. Prior to that revision, NEL values of feeds were fixed. In the 2001 system, digestible energy (DE) was calculated for feeds by estimating the energy provided by digestible portions of NDF, CP, fatty acids (FA), and nonfiber carbohydrate (100 – NDF – CP – FA – ash). The DE of the diet was calculated as a weighted mean from feed values, and the diet DE was then discounted based on DM intake (DMI) and TDN concentration of the diet. TDN concentration was essentially a proxy for diet starch concentration. One issue that was identified regarding NRC (2001) was that energy balance (NEL supply minus NEL requirements for maintenance, milk, growth, and reproduction) was underestimated for high producing cows. Because it was a problem with high producing, high DMI cows, the source of the error was assumed to be an overestimation of lactation NEL requirements and/or an underestimation of NEL concentration of the diet likely caused by the discount factor.

Research published after NRC (2001) indicated that the greatest source of error was indeed the discount factor. Dry matter digestibility did not decrease as much with increasing DMI and diet TDN as the NRC 2001 equation calculated. One meta-analyses (de Souza et al., 2018) found the NRC (2001) discount was about 3 times greater per unit of DMI as a percentage of body weight) than suggested by current data (Figure 1). One reason for the error is that NRC (2001) used a cow fed at maintenance (approximately 7 kg of DM) as the base and discounted from there. Usually, the base was from nonlactating cows fed at restricted intakes. This resulted in substantial extrapolation and assumed linearity starting at a very low and restricted DMI. DeSouza et al. (2018) developed discount equation from digestibility data collected from lactating cows with DMI ranging from about 1.7 to 4.6% of BW. The average DMI of the dataset was about 3.5% of BW and that was set as the base in the new NRC; therefore, extrapolation is much less than with the old equation. Because increased dietary starch can depress NDF digestibility, its effect was also included (the base was set at 26% starch which was approximately the mean concentration in the dataset used. This approach is much more theoretically accurate than using TDN as done previously.

The improved discount equation should correct most of the underestimation of NEL balance in high intake cows by NRC (2001). However the NEL required for lactation also was likely overestimated slightly (Moraes et al., 2015) which contributed to the problem. This issue was addressed by increasing the metabolizable energy (ME) to NEL efficiency from 0.64 used in NRC (2001) to 0.66 as determined by Moraes et al (2018).

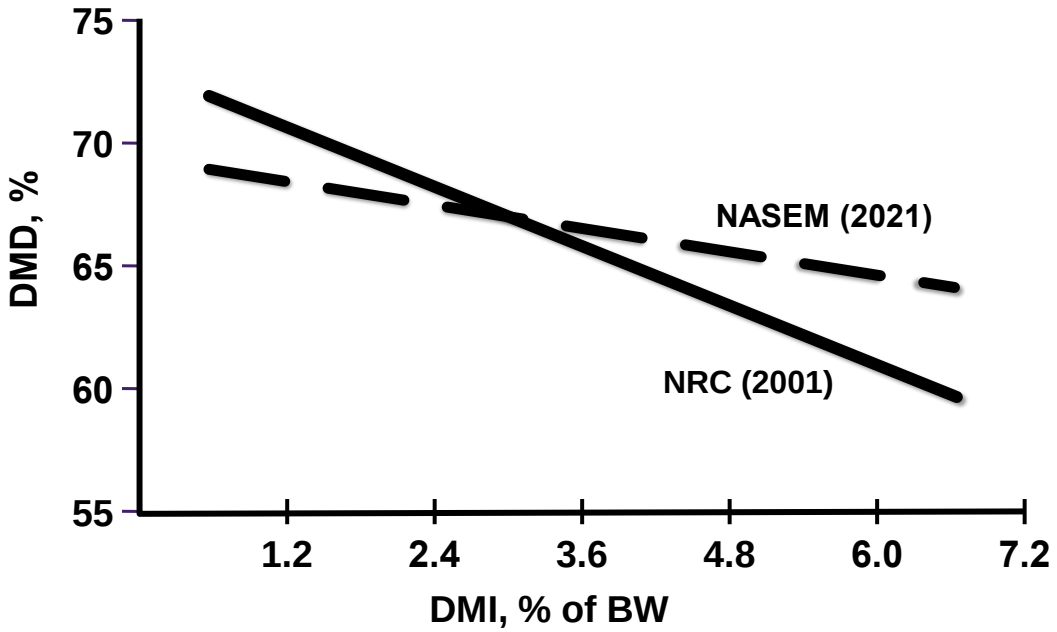

Figure 1. The effect of increasing dry matter intake (DMI) expressed as % of body weight (BW) on dry matter digestibility (DMD) using the NRC (2001) discount equation and the discount equation in the new NASEM (2021) model. For NRC (2001) diet TDN was set at 72% and for the NASEM line, dietary starch was set at 26%. Overall, the effect of DMI on digestibility (i.e., digestible energy) is about 3 times greater using NRC (2001) than in the updated NASEM book.

Other changes made to the energy prediction equation would be considered fine-tuning. The NFC fraction was replaced with starch and residual organic matter (ROM; i.e., NFC – starch) as outlined by Weiss and Tebbe (2018) and Tebbe et al. (2017). This allows better estimation of the energy provided by a variety of starch sources (e.g., different grind sizes of corn grain, high moisture vs dry corn, different maturities of corn silage). The true digestibility of ROM was set at 96% (Tebbe et al., 2018) and starch digestibility values are constants based on the feed (Table 1). Users can choose to use a lignin-based equation as in NRC (2001) or 48 h in vitro NDF digestibility. An equation is used to convert in vitro digestibility into estimated in vivo digestibility.

Table 1. Starch digestibility coefficients used in the new NRC for selected feeds (not all feeds are shown).

Feed	Starch digestibility
Default	0.91
Corn grain, dry, fine grind (<1250 μm)[2]	0.92
Corn grain, dry, medium grind (1500 um to 3250 μm)	0.89
Corn grain, dry, coarse grind (>3500 μm)	0.77
Corn grain, high-moisture, fine grind (<2000 μm)	0.96
Corn grain, high-moisture, coarse grind (>2500 μm)	0.90
Corn grain, steam flaked	0.94
Sorghum grain, dry, ground	0.83
Sorghum grain, steam flaked	0.94
Corn silage <30% DM	0.91
Corn silage 32 – 37% DM	0.89
Corn silage >40% DM	0.85
Barley, ground	0.91
Wheat	0.93

Another change was to the true digestibility coefficient used for FA. In NRC (2001) the true digestibility of FA was assumed to be 100% at maintenance DMI (92% for a typical lactating cow). This was based on very limited data because at that time, FA was not commonly measured. Over the past 2 decades a substantial database of FA digestibility was developed and allowed better estimation of the true digestibility of FA. Two meta-analyses have been conducted (Weiss and Tebbe, 2018, Daley et al., 2020) and both derived essentially the same true digestibility value (73%) with no metabolic fecal FA (i.e., intercept was not different from 0). In the new NRC, digestible FA are calculated as 0.73* FA (% of DM). This is substantially lower than the 0.92*FA (% of DM) used in NRC (2001) but the difference is not as great as it appears because in NRC (2001), FA contributed to metabolic fecal energy but not in NRC (2021). However, the DE concentration of feeds with appreciable concentrations of FA will be lower in the new NRC than in NRC (2001).

In NRC (2001), metabolizable energy (ME) was calculated directly from DE using an equation that was developed several decades ago. That equation did not correctly account for the effect of protein or fat on ME. The new NRC will estimate methane using a published equation based on DMI and dietary concentrations of FA (negative effect on methane) and digestible NDF (positive effect on methane). Urinary energy is estimated by estimating urinary N excretion (g/d) and multiplying that value by 0.0143 Mcal/g (Morris et al., 2021). Both methane and urinary energy are calculated for a diet, not a feed. Therefore, feeds will not have ME or NEL values. The change in the method to calculate ME will result in higher ME values for diets with high FA concentrations and lower ME values for higher fiber diets and diets with excess CP. In the previous NRC, NEL was approximately .64*ME. Based on a re-analysis of Beltsville calorimetry data, Moraes et al. (2018) determined that 0.66 was more accurate and that value is used to convert diet ME into NEL concentrations of diets.

Energy requirements were also evaluated and modified as necessary. The greatest change was in the maintenance requirement. Several papers published over the past 15 years determined that the standard equation for maintenance (which has been used for more than 30 years) underestimated the maintenance requirement of modern dairy cows. Using an average from several newer studies, the maintenance requirement was increased from 0.08*MBW to 0.10*MBW (where MBW is metabolic body weight in kilograms). This change is a 25% increase in maintenance or about 2.5 Mcal of NEL/day for a 650 kg cow). The equation to calculate gestation energy requirements changed to better model fetal growth but the change did not appreciably alter gestation NEL requirements. Lactation energy requirements changed slightly because the efficiency coefficient (0.66) changed from 0.64. Equations to estimate NEL requirements for grazing cows were updated based on newer data and generally activity requirements will be less when calculated using the new NRC than when using NRC (2001).

Protein and Amino Acids

This section underwent the greatest change as compared to NRC (2001) and the complexity of the model precludes a detailed discussion in this paper. Microbial protein is estimated based on estimated rumen digested starch and fiber (these are estimated based on diet composition, not digestion rates). Rumen undegradable protein is based on the A, B, C fraction scheme described in NRC (2001); however rather than estimating rate of passage based mostly on intake as done in NRC (2001), constant rates of passage are used (one for concentrates and one for forages). Significant improvements were made in the estimates for the digestibility of the rumen undegraded protein because the data base was much larger allowing greater screening for spurious values. Supplies of metabolizable protein (MP) and metabolizable AA are the sum of digestible microbial AA or true protein and digestible rumen undegraded AA or true protein. In NRC (2001) endogenous protein was included in MP supply; however, this was an error because endogenous protein does not cause a net increase in MP supply. Therefore, endogenous protein is considered a requirement rather than a supply function in the new NRC.

For lactating cows, maintenance requirements are mostly based on both net protein and amino acids. The requirement for metabolic fecal protein was changed markedly and is now a function of dietary fiber. The calculation for endogenous urinary CP was also changed. In addition, rather than using a classic requirement model for milk protein (e.g., to produce 1200 g of milk protein you need X grams of MP or specific AA) a response model is used (based on AA and energy supplied by the diet, the cow should be able to produce X grams of milk protein). The response function for milk protein yield is based on DE supply (the DE is from components other than CP) and supply of lysine, methionine, leucine, isoleucine, histidine, and total essential AA. The equation to estimate milk protein yield illustrates that an almost infinite array of AA profiles can result in similar milk protein yields. Efficiency of converting metabolizable AA to milk protein is not fixed as it was for MP in NRC (2001). The function includes a quadratic term for total essential AA which means efficiency decreases as supply of essential AA increases.

Minerals

The same basic approach to establish mineral requirements used in NRC (2001) was used in the new NRC. However, a term used to describe human nutrient requirements was introduced to reflect the uncertainty associated with requirement calculations for many minerals. If the committee deemed that the data was not adequate to establish a requirement for an essential mineral, the term ‘adequate intake’ or AI was used. Basically, when that term is used, it means the committee thinks that if most cows eat this amount of mineral she will function normally. Requirements were calculated for all macrominerals and for copper and zinc. Adequate intake was used for cobalt, manganese, iron, iodine, and selenium.

Overall changes in dietary requirements or AI were small for most minerals although equations may have changed appreciably. For example, the maintenance requirement for absorbed Ca increased substantially; however, this was countered by a substantial increase in the absorption coefficients (AC) for Ca. For some electrolytes, endogenous fecal excretion increased while endogenous urinary excretion decreased (or vice versa) resulting in little overall change in requirements. These changes may not alter diet formulation, but they better reflect routes of excretion and more accurately reflect absorption. Phosphorus requirement (both absorbed or dietary) did not change greatly but the new NRC calculates the AC for P from the chemical form (inorganic or organic) of P within the feedstuff. This should improve overall accuracy. Magnesium requirements increased slightly but AC were changed substantially. The AC for Mg supplements were reduced by more than 50% while the AC for basal feeds increased about 50%. These changes were based on a large database that was not available in 2001. In addition, the new NRC includes an equation to adjust the AC based on dietary potassium.

Most trace mineral requirements or AI did not change greatly or at all and only Cu and Mn will be discussed. The requirement and AC for Cu underwent rigorous evaluation because of increased concerns about high liver Cu and Cu toxicity in dairy cows. For the average lactating cow, the dietary Cu requirement in NRC (2001) appears to be correct; however, partitioning of requirements between maintenance and lactation were incorrect.

Based on new data, the maintenance requirement for Cu is about twice as high as in NRC (2001) which means dietary requirements for dry cows and low producing cows will increase. However, the lactation requirement (per kilogram of milk) was more than 3 times too high. Therefore, Cu requirements for higher producing cows will be slightly less than in NRC (2001). The AI for Mn was also evaluated rigorously because an experiment with pregnant beef heifers fed diets that met NRC (2001) requirements resulted in calves born expressing clinical Mn deficiency (Hansen et al., 2006). Based on very limited data (Weiss and Socha, 2005), the maintenance AI for Mn was increased about 30% and the AC was reduced by about 40%. The net result was dietary AI for Mn about doubled for dry and lactating cows.

Vitamins

The new NRC established AI for vitamins A, D and E and in most situations, values were the same as in NRC (2001). The new NRC maintained the base requirement for vitamin A of 110 IU/kg of BW but included an additional requirement of 1000 IU/kg of milk greater than 35 kg/d (based on the retinol concentration in milk). Therefore, for a 650 kg cow producing 35 kg of milk, the vitamin A AI is 71,500 IU/d but for the same cow producing 40 kg of milk/d, the AI is 76,500. The vitamin D AI for lactating cows was increased from 30 IU/kg to 40 IU/kg of body weight. For other animals the 30 IU/kg body weight AI was maintained. The change for lactating cows was based on maintaining blood plasma concentrations of 25-OH vitamin D at 30 ng/ml. The AI for vitamin E was not changed for dry and lactating cows (1.6 and 0.8 IU/kg body weight). An increased AI was set for late gestation cows (i.e., prefresh) at 3 IU/kg of body weight or about 2000 IU/d.

Conclusions

The 8th revised edition of the NASEM (formerly NRC) Nutrient Requirements of Dairy Cattle reflects the current state of knowledge for applied dairy nutrition. All facets of nutrition for calves, heifers, dry cows, and lactating cows were reviewed and changes in requirements were made when appropriate. The book also contains up to dates reviews on numerous topics relevant to feeding dairy cattle. This article is only a brief introduction to the changes made since NRC (2001) but attempted to highlight important (but definitely not all) changes made. People desiring more details will need to purchase the book (I do not receive any royalties from book sales).

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Effect of Increasing Monensin Concentration on the Performance of Lactating Dairy Cows Fed Contemporary Diets

A. C. Benoit[1], P. A. LaPierre[1], G. D. Mechor[2], D. M. Barbano[3], and M. E. Van Amburgh[1]
[1]Department of Animal Science, Cornell University, Ithaca, NY 14853
[2]Elanco Animal Health, Greenfield, IN, 46140
[3]Department of Food Science, Cornell University, Ithaca, NY 14853

Introduction

Monensin is a carboxylic polyether ionophore produced naturally by *Streptomyces cinnamonensis* and fed to dairy cattle to alter rumen microbial population and fermentation by reducing gram-positive bacteria and enhancing gram-negative metabolism (McGuffey et al., 2001; Vasquez et al., 2021). This shift in microbial population increases propionate production in coordination with the disposal of H_2 due to reduced methane production (Russell and Strobel, 1989; Fellner et al., 1997). Through these changes, feed efficiency improves because of the increased availability of propionate for glucose production in the liver that can be used by the mammary gland to increase milk production (Ipharraguerre and Clark, 2003; Duffield et al, 2008b). Although the mode of action of monensin is well understood, treatment effects reported in previous studies have been inconsistent (Phipps et al., 2000; Dubuc et al., 2009; McCarthy et al., 2015). A meta-analysis by Duffield et al. (2008b) reported a 0.7 kg/d increase in milk production and a 0.3 kg/d reduction in dry matter intake (DMI) across monensin studies, but treatment effects were influenced by stage of lactation, diet type, and dose level.

Although monensin is associated with improved feed efficiency, negative effects on milk fat production and synthesis have been previously reported. Monensin altered the content of saturated and unsaturated fatty acids (FA) in ruminal fermenters through inhibition of biohydrogenation (Fellner et al., 1997), thus it is hypothesized that the mode by which monensin decreases milk fat is through an accumulation of conjugated FA in the rumen that inhibit milk fat synthesis (Alzahal et al., 2008; Baumgard et al., 2000). More recently, the effect of monensin on milk fat production was greatest in studies that fed diets high in unsaturated FA (Alzahal et al., 2008; He et al., 2012), and a reduction in milk fat synthesis was predicted to be caused by an accumulation of long chain FA in the rumen that inhibit de novo FA synthesis (Dubuc et al., 2009). Further, monensin in high starch diets has been associated with a decrease in milk fat production due to a reduction in biohydrogenation caused by monensin and high levels of rumen fermentable starch that decrease ruminal pH (Bradford and Allen, 2004; Van Amburgh et al., 2008). And more recently, Akins et al. (2014) reported a numerical decrease in milk fat content with monensin feeding in average starch (27%) diets, but not in reduced starch (21%) diets.

Using diet formulation systems such as Cornell Net Carbohydrate Protein System (CNCPS), nutritionists can monitor rumen unsaturated FA load (RUFAL), dietary fat, starch, and NDF content to help minimize diet induced milk fat depression, and therefore understand how to optimize the use of monensin in lactating dairy cows. Previous studies

that reported a decrease in milk fat production with monensin feeding were performed decades ago when dietary nutrients in dairy diets were not as well understood as they are today, and more recent monensin studies have reported no effect on milk fat production (Akins et al., 2014; Hagen et al., 2015; Vasquez et al., 2021).

The FDA has approved the use of monensin in lactating dairy cattle diets at levels of 11 g/ton to 22 g/ton (DM basis), but recently, few studies have been conducted evaluating lactation performance at various monensin concentrations using more contemporary diets formulated with refined nutrient requirements and supplies. Therefore, the amount of monensin in the diet needed to effect milk production and composition, intake, and shifts in milk FA profile is of interest. The objective of this study was to evaluate increasing dietary monensin (Rumensin, Elanco Animal Health, Greenfield, IN) concentration on milk performance, milk FA profile, and production efficiencies (component-corrected milk/ DMI) in lactating dairy cows fed contemporary diets. We hypothesized milk performance and feed efficiency would improve with increasing levels of dietary monensin with no negative effects on milk component yield or shifts in FA profile.

Materials and Methods

Experimental Design and Treatments

The experiment was conducted from September to December 2020 at the Cornell University Ruminant Center (Harford, NY), and all procedures were approved by Cornell University Animal Care and Use Committee. One-hundred ninety-two cows (120 ± 50 DIM; mean ± standard deviation) were stratified by parity, DIM, and pre-trial milk production, and assigned to 1 of 12 pens housing 16 cows per pen (12 multiparous and 4 primiparous) in a 91-day longitudinal study with a 29 day covariate and 62 day experimental period. All cows were fed 11 g/ton (DM basis) monensin for the adaptation and covariate period. Following the covariate period, pens were randomly assigned 1 of 4 treatment diets stratified by milk performance and BW data collected in the covariate period. Cattle were housed in freestall pens with 16 headlocks and sand-bedded stalls, and had free access to feed, water, and bedding. Cows were milked three times daily at 0700h, 1500h, and 2300h in a double-16 parallel parlor. Feed was delivered once daily as a TMR at 0600h ad libitum to allow for 5% refusals.

Diets were formulated to meet or exceed nutrient demands for high producing lactating dairy cows using CNCPS (v6.55; Van Amburgh et al., 2015). Methionine and lysine were balanced using the latest information on requirements and supply as generated in the studies of LaPierre et al. (2020) where amino acid requirements are described on a gram per unit of ME basis (Higgs and Van Amburgh, 2016). For diet formulation, the methionine requirement was set at 1.19 g methionine per Mcal ME and lysine was set at 3.21 g per Mcal ME (or 2.7 times the grams methionine). All diets consisted of (DM basis) 34.9 % corn silage, 19.4 % grass haylage, 18 % corn meal, 6.8 % soybean meal, and 21 % pre-mix containing monensin (Purina Animal Nutrition, Caledonia, NY; Table 1). Treatments were 0 g/ton monensin (CON), 11 g/ton monensin

(R11), 14.5 g/ton monensin (R14.5), and 18 g/ton monensin (R18) on a DM basis, and monensin intake was formulated to be 305 mg/d, 404 mg/d, and 515 mg/d for R11, R14.5, R18, respectively.

Table 1. Ingredient composition of experimental diets

	Diet[1]			
Ingredient, % of DM	CON	R11	R14.5	R18
Corn silage	34.9	34.9	34.9	34.9
Grass haylage	19.4	19.4	19.4	19.4
Corn meal	18.0	18.0	18.0	18.0
Soybean meal	6.81	6.81	6.81	6.81
SoyPass[2]	5.83	5.83	5.83	5.83
Citrus pulp	4.49	4.49	4.49	4.49
Wheat middlings	4.49	4.49	4.49	4.49
Dextrose	1.60	1.60	1.60	1.60
Bloodmeal	1.00	1.00	1.00	1.00
Berga fat F100[3]	0.60	0.60	0.60	0.60
Energy Booster 100[4]	0.60	0.60	0.60	0.60
Ground limestone	0.54	0.54	0.54	0.54
Min AD[5]	0.45	0.45	0.45	0.45
Sodium bicarbonate	0.42	0.42	0.42	0.42
White salt	0.27	0.27	0.27	0.27
Vitamin and mineral mix[6]	0.22	0.22	0.22	0.22
Magnesium oxide	0.11	0.11	0.11	0.11
Smartamine M[7]	0.10	0.10	0.10	0.10
Smartamine ML[7]	0.10	0.10	0.10	0.10
Levucell SC[8]	0.05	0.05	0.05	0.05
Rumensin 90[9]	-	0.006	0.008	0.01

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Lignotech USA, Rothschild, WI.
[3]Berg + Schmidt America LLC, Libertyville, IL.
[4]Milk specialties, Eden Prairie, MN.
[5]Calcium (22%) and magnesium (12%) supplement (Min-AD, Winnemucca, NV).
[6]Contained (DM basis) 27.4% Ca; 223 ppm Fe; 24,997 ppm Zn; 5,765 ppm Cu; 18,473 ppm Mn; 134.5 ppm Se; 568 ppm Co; 568 ppm I; 2021 KIU/kg vitamin A; 562 KIU/kg vitamin D; 9660 IU/kg vitamin E)
[7]Adisseo Inc, Alpharetta, GA.
[8]Lallemand Inc, Milwaukee, WI.
[9]Monensin, 90.7 g/lb. (Elanco Animal Health, Greenfield, IN).

Forages and TMR were sampled twice weekly, composited, and sent to Cumberland Valley Analytical Services (Waynesboro, PA) once per week for nutrient analysis. Additionally, FA profile was determined on TMR samples. Grains were sampled once weekly, and a 4 wk composite was sent once monthly for chemical analysis. Grain mixes were sent for determination of monensin concentration upon delivery of a new batch (Eurofins Food Chemistry Testing US, Inc, Greenfield, IN). Feed DM was determined twice weekly for diet adjustment and calculation of DMI. Pen level intake was obtained daily using Feedwatch (Valley Agricultural Software, Tulare, CA), and determined using observations of feed offered and feed refused.

Milk production was recorded at every milking (Delpro, DeLaval Inc, Kanas City, MO) and milk samples were taken at 3 consecutive milk sessions once weekly during the last two weeks of the covariate period and every week of the experimental period. Samples were analyzed for fat, true protein, anhydrous lactose, and MUN using a FTIR spectrophotometer (Lactoscope model FTA, Delta Instruments, Drachten, the Netherlands) at the Department of Food Science at Cornell University (Ithaca, NY). De novo, mixed-origin, and preformed FA were analyzed by FTIR on all milk samples according to PLS prediction models described by Woolpert et al. (2016) and calibration was carried out using gas-liquid chromatography reference chemistry described by Wojciechowski and Barbano (2016). The same calibration set was used for milk components and FA analysis with concentrations ranging from 0.05 to 1.4 g/100g milk de novo FA, 0.08 to 2.2 g/100g milk mixed FA, and 0.06 to 1.9 g/100g milk preformed FA. In addition, FA chain length (mean carbon number per FA) and unsaturation (double bonds per FA) were measured as previously described by Wojciechowski and Barbano (2016). Body weight (BW) was obtained once weekly following the 1500h milk session as well as body condition score (BCS) using a 5-point scale according to Wildman et al. (1982). Blood samples were collected once weekly via the coccygeal vein into tubes containing sodium heparin. Samples were centrifuged (3,000 × *g* for 20 min at 4˚C), and plasma was harvested and frozen at -20 ˚C for urea nitrogen analysis (No. 640, Sigma-Aldrich, St. Louis, MO). Finally, rumination time (minutes per day) was obtained from cows with a pre-existing Smartbow ear tag (Zoetis, Parsippanny, NJ; CON: n = 34, R11: n = 38, R14.5: n = 42, and R18: n = 42).

Statistical Analysis

All data, excluding BCS, were analyzed through SAS version 9.4 (SAS Institute Inc., Cary, NC) using PROC MIXED and LSMEAN statements to compare treatment means. When individual cow variables with covariate structure and repeated weekly measurements (milk production, milk composition and FA profile, BW, rumination, and PUN) were analyzed, pen was the experimental unit and cow was the observational unit as previously described by Fessenden et al. (2020) and Bellow et al. (2016), and the following model was used:

$$Y_{ijklm} = \mu + T_i + W_j + TW_{ij} + P_{k:i} + B_{l:k:i} + BX_{lik} + \varepsilon_{ikklm},$$

where Y_{ijklm} = dependent variable, μ = overall mean, T_i = fixed effect of treatment i, W_j = fixed effect of week j, TW_{ij} = fixed interaction of treatment i and week j, $P_{k:l}$ = random effect of pen k within treatment i, $B_{l:k:i}$ = random effect of cow within pen k within treatment i, BX_{lik} = the covariate adjustment for each cow, and ε_{ikklm} = residual error. An auto-regressive structure [AR(1)] was used to analyze repeated measurements with cow in pen within treatment. For pen level variables (DMI and production efficiencies), a random effect of pen within treatment was used. Three cows did not complete the experiment due to health issues (1 and 2 cows from R14.5 and CON, respectively). The BW data from wk 6 to 9 of the experimental period were removed from statistical analysis due to scale malfunctions during extreme cold weather conditions, with wk 5 BW was used as final BW to determine BW change. Degrees of freedom were determined using Kenward-Roger option and least square means were adjusted by Tukey method for multiple comparison tests. Body condition score data was analyzed using a non-parametric analysis (PROC NPAR1WAY) with treatment as the classification variable. Statistical significance was reported as $P \leq 0.05$ and tendencies as $0.05 < P \leq 0.10$.

Results and Discussion

Ingredient composition and chemical analysis of the diets are in Table 1 and 2, respectively, and chemical analysis of the forages and concentrate mixes are in Table 3. The analyzed monensin concentration for all treatment pre-mixes, on a DM basis, are as follows: CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, and R18 = 19.3 g/ton monensin. The actual monensin intake was 0, 384, 465, and 589 mg/d for CON, R11, R14.5, and R18, respectively. Lactation performance results are in Table 4. We observed a numerical increase in DMI in the R18 group compared to CON, R11, and R14.5 (27.7 vs. 26.9, 26.8, and 26.7 kg/d, respectively). Monensin treatment tended to have a quadratic effect on DMI ($P = 0.10$) where R11 and R14.5 had slightly decreased DMI compared to CON, but DMI increased in the R18 group. This finding is not consistent with previous studies as increasing dietary monensin has been associated with either no change or a slight decrease in DMI (Akins et al., 2014; Hagen et al., 2015), although Recktenwald et al. (2014) reported a trend for increased DMI in cows fed monensin compared to none in diets high and low in starch and protein content. Milk yield was not affected by monensin treatment in agreement with experiments of Alzahal et al. (2008) and Hagen et al. (2015) (Table 4). The lack of an adaptation period for the CON group following the covariate diet of 11 g/ton monensin was predicted to decrease the ability to detect treatment effects because we observed a decrease in milk yield in the CON group compared to all monensin treated groups from wk 4 to 9 (data not shown) indicating cows were still adjusting to the removal of monensin in the beginning 3 wk of the experimental period. This is consistent with lactose production data as we observed a decrease in lactose yield in the CON group compared to all monensin treated groups following wk 3 of the experimental period (data not shown). In agreement, Akins et al. (2014) reported an increase in milk yield in cows fed monensin from wk 4 to 12, but not from wk 1 to 3, suggesting cows were still adapting to monensin changes in the diet.

Table 2. Analyzed nutrient composition (mean ± SD) of experimental diets

	Diet[1]			
Item	CON	R11	R14.5	R18
DM, % as-fed	43.4 ± 1.5	44.0 ± 1.2	43.5 ± 1.3	44.1 ± 1.4
CP, % of DM	15.3 ± 0.3	14.9 ± 0.6	15.0 ± 0.6	15.4 ± 0.6
ADF, % of DM	19.4 ± 1.6	20.4 ± 1.6	19.7 ± 1.0	18.8 ± 1.4
aNDF, % of DM	32.0 ± 1.4	32.8 ± 0.9	31.7 ± 1.1	31.3 ± 1.7
Sugars, % of DM	5.7 ± 0.3	5.7 ± 0.7	5.8 ± 0.2	5.9 ± 0.4
Starch, % of DM	25.6 ± 1.6	24.9 ± 1.0	25.3 ± 0.9	26.2 ± 1.2
Ether extract, % of DM	4.4 ± 0.2	4.2 ± 0.3	4.4 ± 0.2	4.2 ± 0.3
Ash, % of DM	7.2 ± 0.3	7.0 ± 0.3	7.1 ± 0.4	7.1 ± 0.3
NFC, % of DM	43.7 ± 1.2	43.7 ± 0.9	44.5 ± 1.6	44.6 ± 1.4
NSC, % of DM	31.3 ± 1.5	30.5 ± 1.1	31.1 ± 0.8	32.1 ± 1.1
ME, Mcal/kg[2]	2.7	2.7	2.7	2.7
FA, % of DM				
Total	3.56 ± 0.31	3.47 ± 0.11	3.73 ± 0.27	3.78 ± 0.28
16:0	1.12 ± 0.13	1.04 ± 0.03	1.14 ± 0.11	1.19 ± 0.10
18:0	0.33 ± 0.05	0.31 ± 0.03	0.33 ± 0.06	0.35 ± 0.05
18:1 *cis*-9	0.50 ± 0.07	0.49 ± 0.02	0.53 ± 0.05	0.54 ± 0.06
18:2 *cis*-9, *cis*-12	1.13 ± 0.08	1.11 ± 0.05	1.20 ± 0.07	1.20 ± 0.07
18:3 *cis*-9, *cis*-12, *cis*-15	0.31 ± 0.04	0.34 ± 0.02	0.33 ± 0.04	0.32 ± 0.03
RUFAL[3]	1.94	1.94	2.06	2.06

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Predicted using the Cornell Net Carbohydrate and Protein System v6.5 (Van Amburgh et al., 2015).
[3]Rumen unsaturated fatty acid load = 18:1 + 18:2 + 18:3 from the chromatographic analysis of the diets.

Table 3. Nutrient analysis (mean ± SD) of diet ingredients

Item	Corn Silage	Grass Haylage	CON Mix	R11 Mix	R14.5 Mix	R18 Mix
DM, % as-fed	29.3 ± 0.7	39.5 ± 4.0	90.5 ± 0.3	90.7 ± 0.9	90.5 ± 0.4	90.4 ± 0.3
CP, % of DM	7.5 ± 0.4	15.7 ± 0.7	21.9 ± 0.5	23.9 ± 1.9	21.2 ± 1.3	22.4 ± 1.5
ADF, % of DM	24.1 ± 1.1	34.4 ± 1.4	14.7 ± 2.0	14.0 ± 2.6	14.8 ± 2.9	14.3 ± 2.4
aNDF, % of DM	39.7 ± 1.7	52.0 ± 1.9	22.7 ± 3.3	22.1 ± 3.6	23.4 ± 3.5	22.5 ± 2.2
Sugars, % of DM	0.4 ± 0.2	3.4 ± 0.6	17.5 ± 0.9	16.2 ± 2.0	18.3 ± 0.9	18.4 ± 1.0
Starch, % of DM	34.5 ± 1.6	1.4 ± 0.3	5.0 ± 0.7	5.3 ± 3.9	5.4 ± 1.7	5.8 ± 3.3
Ether extract, % of DM	3.2 ± 0.1	3.7 ± 0.3	6.7 ± 0.9	5.6 ± 1.3	6.8 ± 1.7	6.9 ± 2.2
Ash, % of DM	3.4 ± 0.3	8.5 ± 0.5	13.0 ± 1.9	12.1 ± 2.5	12.8 ± 0.3	13.2 ± 1.2
NFC, % of DM	46.8 ± 1.4	23.1 ± 1.4	38.8 ± 2.0	35.1 ± 2.5	39.6 ± 3.1	40.4 ± 1.5
NSC, % of DM	34.9 ± 1.6	4.8 ± 0.6	22.5 ± 0.7	21.5 ± 3.6	23.7 ± 1.4	24.2 ± 2.5

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.

Additionally, the experimental period for Akins et al. (2014) was 3 wk longer than the current study, allowing for greater detection of monensin effects on milk yield over time.

No significant treatment effects were observed for milk fat concentration or yield; however, milk fat percentage increased numerically with increasing monensin concentration (4.60, 4.67, 4.71, and 4.66 for CON, R11, R14.5, and R18 respectively; Table 4). The numerical increase in milk fat was most likely an effect of monensin on de novo FA synthesis as there was a linear increase ($P < 0.05$; Table 5) in de novo and mixed fat content with increasing levels of monensin. Previous research has shown monensin decreases milk fat concentration with increasing monensin levels (Dubuc et al., 2009; Duffield et al., 2008b), while others (Martinez et al., 2009; McCarthy et al., 2018) have reported no effect on milk fat. More recently, monensin has been shown to interact with other dietary factors such as starch content and unsaturated oils to reduce milk fat, rather than causing milk fat depression independently (McCarthy et al., 2018). Van Amburgh et al. (2008) also reported monensin diets high in starch content and unsaturated oil might have a stepwise negative effect on milk fat production, whereas rumen unsaturated FA increase, the risk of milk fat depression increases with monensin. In the current study, monensin concentration had no negative effect on milk fat production, rather milk fat content increased with monensin treatment due to the change in de novo and preformed fat synthesis. This finding is consistent with the expected increase in propionate production which would provide more energy for productive functions in the gland (Prange et al., 1978; Van Maanen, et al., 1978).

Milk FA profile results are in Table 5. The de novo and mixed FA concentration linearly increased in cattle fed monensin compared to CON but yields were not significantly different ($P = 0.21$) although there was a trend for a linear increase in both de novo ($P < 0.06$) and mixed FA (0.09). Both Duffield et al. (2008b) and Alzahal et al. (2008) reported a significant decrease in de novo FA concentration per total FA with monensin treatment, so the results of this experiment are not consistent with previous observations. The mixed FA yield and percent of total FA did not differ among treatment groups ($P < 0.10$), but mixed FA content linearly increased compared to CON ($P = 0.02$). The preformed FA concentration and yield were not different among treatment groups nor was preformed FA as a percentage of total FA. Alzahal et al. (2008) also found monensin treatment had no effect on preformed concentrations as a function of total FA. There was a trend for C16 concentration and yield tended to be greater ($P = 0.09$) with a significant linear effect of monensin consistent with the mixed FA results. The C18 and *cis*-9 C18:1 concentration and yield were not affected by monensin treatment. The biohydrogenation of oleic acid to stearic acid is achieved by gram-negative bacteria (Alzahal et al., 2008; Harfoot and Hazelwood, 1988) who, unlike gram-positive bacteria, are not inhibited by monensin treatment, therefore, this theory might explain the lack of treatment effects on stearic and oleic acid in the current study. The level of unsaturation of FA decreased with increasing monensin levels and was likely due to the level of de novo and mixed FA contents of the milk across treatments ($P = 0.01$; Table 5). All monensin treated groups approached a tendency for a reduction in FA chain length compared to CON ($P = 0.11$, 0.14, and 0.16 for R11, R14.5, R18, respectively) likely due to an increase in de novo synthesis in the monensin treated groups. Alzahal et al. (2008) and Fellner et al. (1997)

suggest monensin has a role in inhibiting ruminal biohydrogenation which would reduce milk fat synthesis, but in the current study, the milk fat concentration levels, de novo FA levels, and FA unsaturation suggests that monensin treatment enhanced biohydrogenation in the rumen or had some effect on FA synthesis. An alternative observation is that monensin did not impact biohydrogenation and the increased concentration of saturated FA was related to the increase in de novo and mixed FAs which would dilute out the unsaturated FA given the level of milk fat yield. We did not measure other C18:1 or C18:2 isomers that would have given more insight into the effect of monensin on biohydrogenation, although the high levels of fat production and the reduction in FA unsaturation in monensin fed cows suggest monensin did not play a role in inhibiting biohydrogenation or milk fat synthesis in the current study.

The increase in de novo and mixed FA synthesis and yield in mid- to late lactation dairy cattle was an interesting and exciting observation and one that is not well documented. The increase in de novo and mixed FA through the feeding of monensin could be due to a couple different substrate supplies. Monensin is known to increase the supply of propionate and under certain conditions, propionate can be part of an initiation sequence where synthesis of acyl chains from carbon atoms could potentially lead to incorporation into chain elongation of FA (Palmquist, 2007). In addition, with increased propionate, there will be greater glucose and capacity for reducing equivalents which means increased NADPH +H supply which would allow for an increase in the FA synthase reaction allowing for production and elongation of FA. The protein sparing effect of monensin could increase the supply of certain amino acids, including the branched chain amino acids and their conversion to branched chain volatile FA and these could serve as precursors for chain elongation for chain lengths less than 16 carbons (Massart-Leen et al., 1981; Ha and Lindsay, 1990; Liu et al., 2018). Diets were not formulated to contain high quantities of fat, thus it is possible that with lower exogenous FA, there was less competition for certain enzymes related to glycerol production and utilization, but de novo FA synthesis could be increased. Finally, it is also possible, that some of the fat content and yield was related to the supply of methionine and lysine. In the current study, the methionine and lysine were supplied at what we believe are closer to the true requirements and, with the DMI observed, the metabolizable methionine level was approximately 85 g/d and the lysine levels were approximately ≥225 g/d, levels much higher than typically fed. This data would suggest that overcoming the limitation of at least two essential amino acids (EAA) allowed for greater milk fat synthesis in these cows. There is emerging data to suggest there is a link between mTOR signaling, EAA, and the regulation of milk fat synthesis (Li et al., 2016; Nichols et al., 2020).

Table 4. Effect of increasing dietary monensin concentration on lactation performance

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x Wk
Days in milk[3]	190	168	193	184	7.2	-	-	-	-
Monensin, mg/d	0	384	465	589	-	-	-	-	-
DMI, kg/d	26.9	26.8	26.7	27.7	0.31	0.29	0.09	0.22	< 0.01
Milk, kg/d	39.3	39.9	39.7	39.6	0.34	0.48	0.38	0.69	< 0.01
Fat, %	4.60	4.67	4.71	4.66	0.04	0.16	0.40	0.38	0.16
Fat, kg/d	1.79	1.83	1.85	1.83	0.02	0.15	0.52	0.40	< 0.01
Protein, %	3.35	3.37	3.36	3.39	0.02	0.15	0.89	0.41	< 0.01
Protein, kg/d	1.30	1.33	1.33	1.33	0.01	0.13	0.46	0.41	< 0.01
Lactose, %	4.63	4.65	4.63	4.63	0.01	0.98	0.27	0.51	< 0.01
Lactose, kg/d	1.82	1.85	1.84	1.84	0.02	0.34	0.50	0.71	< 0.01
MUN, mg/dL	8.96[a]	10.24[b]	9.61[ab]	9.52[ab]	0.28	0.12	0.04	0.05	< 0.01
PUN, mg/dL	9.11	9.13	9.04	8.89	0.17	0.42	0.42	0.72	< 0.01
ECM[4], kg/d	46.0	46.9	47.1	46.8	0.50	0.17	0.47	0.46	< 0.01
3.5% FCM[5], kg/d	46.0	46.9	47.2	46.8	0.53	0.19	0.51	0.49	< 0.01
SCM[5], kg/d	42.5	43.3	43.5	43.2	0.46	0.17	0.41	0.42	< 0.01
BW, kg	692	691	694	693	2.1	0.74	0.67	0.83	0.26
BW change, kg/d	0.16	0.27	0.16	0.44	0.09	0.07	0.33	0.08	-
BCS[6]	2.93	2.93	3.04	2.93	0.40	-	-	-	< 0.01
Rumination, min/d	647	645	639	641	6.2	0.40	0.91	0.77	0.01

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

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin

[2]Week effect for all estimates ($P < 0.01$).

[3]Average of experimental period.

[4]Calculated according to Tyrell and Reid (1965).

[5]Calculated according to NRC (2001).

[6]Largest standard deviation of treatment means.

Table 5. Effect of increasing dietary monensin concentration on de novo, mixed, and preformed fatty acid production

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x Wk
Total FA, g/100 g milk	4.33	4.39	4.43	4.37	0.04	0.22	0.34	0.41	0.31
De novo[3]									
g/100 g milk	1.13	1.16	1.17	1.16	0.01	0.05	0.32	0.17	0.35
g/d	438	452	458	454	6.3	0.06	0.46	0.21	0.06
g/100 g FA	26.1	26.4	26.2	26.3	0.11	0.24	0.54	0.41	< 0.01
Mixed[4]									
g/100 g milk	1.85	1.88	1.91	1.90	0.02	0.02	0.79	0.10	0.07
g/d	720	737	753	746	11.8	0.09	0.76	0.28	< 0.01
g/100 g FA	42.8	42.9	43.0	43.1	0.18	0.25	0.66	0.64	< 0.01
Preformed[5]									
g/100 g milk	1.34	1.35	1.36	1.33	0.02	0.95	0.27	0.61	< 0.01
g/d	520	527	533	521	7.1	0.61	0.28	0.54	< 0.01
g/100 g FA	31.0	30.7	30.8	30.6	0.21	0.15	0.98	0.46	< 0.01
Chain length	14.57	14.54	14.54	14.54	0.01	0.02	0.27	0.08	< 0.01
Level of unsaturation	0.235[a]	0.231[ab]	0.227[b]	0.227[b]	0.002	<0.01	0.94	0.01	< 0.01
Fatty acids									
16:0, g/100 g milk	1.79[y]	1.81[xy]	1.85[x]	1.83[xy]	0.02	0.02	0.74	0.09	0.07
16:0, g/d	695[y]	712[xy]	728[x]	720[xy]	9.6	0.02	0.67	0.09	< 0.01
18:0, g/100 g milk	0.36	0.36	0.37	0.36	0.01	0.80	0.33	0.60	< 0.01
18:0, g/d	140	142	145	141	2.3	0.35	0.26	0.32	< 0.01
18:1 *cis*-9, g/100 g milk	0.79	0.79	0.79	0.78	0.01	0.91	0.59	0.86	< 0.01
18:1 *cis*-9, g/d	305	308	311	306	4.0	0.57	0.42	0.66	< 0.01

[a-b]Means within a row differ with different superscripts ($P < 0.05$).
[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Week effect for all estimates ($P < 0.01$).
[3]C4 to C14 (Barbano and Melilli, 2016).
[4]C16, C16:1, and C17.
[5]Greater than or equal to C18.

There is a strong correlation between true protein yield and de novo FA content of milk (Barbano et al. 2019), demonstrating an integrated outcome of metabolism and the metabolic signaling related to nutrient supply (Lobley, 2007; Rius et al., 2010). Milk protein concentration and yield were unaffected by monensin treatment (P = 0.41; Table 4), however, milk protein content and yield were both high, and paralleled the de novo and mixed FA yields again likely due to some effects of the level of EAA fed in this study. Milk protein responses to monensin treatment have been inconsistent in many studies where some have reported a decrease (Akins et al., 2014; Martinez et al., 2009), no effect (Alzahal et al., 2008; McCarthy et al., 2015), or an increase in protein content with monensin feeding (Van Amburgh et al., 2008). A meta-analysis by Duffield et al. (2008b) found monensin reduced milk protein concentration but increased milk protein yield suggesting dilution effect might be a factor as monensin increases milk production (Alzahal et al., 2008; Ipharraguerre & Clark, 2003). Given the previously described protein sparing effect of monensin on ruminal feed digestion (Poos et al., 1979; Chen and Russell, 1991; Ruiz et al, 2001), under certain conditions it is possible when feeding monensin that more feed protein can escape fermentation and flow to the small intestine, which would provide more amino acids independent of any microbial yield effects. That outcome, combined with a shift in propionate production (Prange et al., 1978; Van Maanen, et al., 1978), could possibly result in an enhancement of milk protein yield. The milk lactose concentration and yield did not differ among treatment groups (P = 0.51 and P = 0.71, respectively; Table 4). In agreement with the current study, Akins et al. (2014) and Hagen et al. (2015) found monensin had no effect on milk lactose concentration.

Although non-significant, ECM, FCM, and SCM all increased with monensin treatment compared to CON likely from the increase in milk component production in the monensin fed groups (Table 4). Previously, experiments by He et al. (2012) and Martinez et al. (2009) found monensin had no significant effect on component corrected milk yield. We observed an average 7 kg/d increase in ECM and FCM yield compared to actual milk yield across all treatment groups, and a 3.5 kg/d increase in SCM yield, again likely a result of the diet formulation of higher EAA levels, modest fat levels and strong rumen fermentation conditions. The CON group tended (P = 0.09) to have greater feed efficiency (actual milk/DMI) and R11 and R14.5 were significantly greater than R18 (P = 0.02 and P = 0.04, respectively) than R18 treatment due to the increased DMI of the cows on the R18 treatment (Table 6). However, there was a quadratic effect on ECM/DMI, FCM/DMI, and SCM/DMI by monensin treatment due to the level of DMI in the R18 treatment (Table 6). A couple of factors impacting the ability to identify differences in production efficiency are the numerical increase in DMI of the cows on the R18 treatment and the re-adjustment to the treatment diet following the covariate period as previously outlined. Although non-significant, the 0.8 kg difference in DMI of the cows on the R18 treatment obscured the typical outcome of enhanced feed efficiency at that level of monensin intake (Akins et al., 2014; Hagen et al., 2015), and likely more relevant, the re-adjustment to the CON diet from the covariate period appeared to impact treatment effects on milk yield. In the current study, monensin had no effect on estimated diet energy while Akins et al. (2014) and Hagen et al. (2015) reported an increase in estimated diet energy in cows fed 18 g/ton monensin compared to no monensin.

Milk urea nitrogen concentration was significantly greater in R11 compared to CON (P = 0.04), but not different in R14.5 or R18 (Table 4). Martinez et al. (2009) found monensin had no effect on MUN while Akins et al. (2014) reported an increase in MUN with monensin treatment. Additionally, McCarthy et al. (2015) reported significantly higher MUN values in early lactation cows who were fed diets top-dressed with monensin. Plasma urea nitrogen was unaffected by monensin treatment, although a meta-analysis (Duffield et al., 2008a) reported blood, plasma, and serum concentration increased with monensin treatment (Table 4). Recktenwald et al. (2014) suggests monensin plays a role in retaining urea N in the blood as they observed higher PUN values and larger plasma N pools with monensin treatment; however, that was not observed in the current study. The R11 and R18 treatment groups had a nonsignificant increase in BW compared to CON with R18 approaching a tendency to be greater (P = 0.11), although this observation warrants the recognition that wk 5 BW data is used to determine final BW due to an error with the scale (Table 4). In a previous study, Phipps et al. (2000) reported a significant increase in BW change with increasing levels of monensin. In the current study, BCS was not significantly different among treatment groups. This data suggests cows with few nutritional limitations will partition as much energy and nutrients towards milk production and away from BW and BCS gain even in later lactation as many of these cows were greater than 200 DIM while on treatment and not gaining appreciable amounts of weight or BCS. This observation requires further study and suggests BW accumulation in later lactation might be partially due to inadequate nutrient supply for milk and component yield, thus nutrients are retained in the tissue at a greater rate. Monensin treatment had no effect on rumination time and the values were quite high indicating good rumen health (Table 4).

Table 6. Effect of increasing dietary monensin concentration on milk production efficiency

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x wk
Milk/DMI	1.47[a]	1.48[a]	1.48[a]	1.42[b]	0.01	0.11	< 0.01	0.02	< 0.01
ECM/DMI	1.71	1.74	1.76	1.69	0.02	0.63	0.04	0.13	0.13
3.5% FCM/DMI	1.71	1.74	1.76	1.70	0.02	0.66	0.04	0.13	0.12
SCM/DMI	1.58	1.61	1.62	1.56	0.02	0.71	0.03	0.10	0.09
Estimated diet energy[3]	1.64	1.65	1.65	1.68	0.02	0.34	0.49	0.62	-

[a-b]Means within a row differ with different superscripts (P < 0.05).
[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Week effect for all estimates (P < 0.01).
[3]Estimated diet energy content = [0.08 × BW, kg$^{0.75}$ + BW change, kg/d × 5.34 + milk, kg × (0.0929 × milk fat, % + 0.0563 × milk protein, % + 0.0395 × milk lactose, %)]/DMI, kg (NRC, 2001).

Conclusion

Overall, the milk and component yield of these mid- to late lactation cattle was high and unprecedented suggesting the conditions of evaluating monensin feeding in cattle fed more contemporary diets was achieved. Increasing the supply of monensin had no significant effects on milk yield, DMI, or production efficiencies; however, some of that lack of difference is likely due to shift from a covariate period with monensin feeding to a control diet where monensin was removed and an inadequate adjustment period. We observed a positive response to monensin treatment with linear increases in de novo and mixed FA concentration which resulted in enhanced milk fat yield. This indicates monensin can be fed at higher concentrations to achieve high milk component yields in lactating cows fed contemporary diets optimized for component yield, and more research is warranted to understand the relationship between monensin and ruminal FA synthesis, especially the de novo and mixed FA.

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Dairy Industry Sustainability- Has Progress Been Made?

L.E. Chase
Department of Animal Science
Cornell University

Sustainability is a current topic of discussion, debate, controversy, and opportunity for the dairy industry. Social, environmental, and economics are the 3 pillars of sustainability (Segerkvist et al., 2020). A recent survey indicated that >50% of consumers purchasing dairy products are interested in the availability of sustainability information (Schiano e. al., 2020). Animal welfare, carbon footprint and greenhouse gas emissions were some of the attributes of sustainability listed in this report. Other reports include water and air quality as concerns. Consumer perceptions of dairy management practices also need to be recognized and considered as the industry moves ahead to address sustainability (Naspetti et al., 2021; Widmar et al., 2017). Review papers on U.S. dairy sustainability are available (Martin et al., 2017: von Keyserlingk et al., 2013)

Changes in the U.S. dairy industry between 2007 and 2017 have been quantified (Capper and Cady, 2019). Total dairy cow numbers increased by 2.1% while energy corrected milk (ECM) per cow increased by 22%. Total U.S. ECM production increased by 25%. The authors reported changes in resource use and emissions based on producing 1 million metric tons (MMT) of ECM. The number of cows needed in 2017 was 74.8% of the cows required in 2007. Resource use in 2017 compared with 2007 was 17% less feed, 21% less land and 30% less water. The dairy industry in 2017 produced 21% less manure compared with 2007. Manure nitrogen and phosphorus excretions were 17 and 14% less than the 2007 values. Total greenhouse gas emissions in 2017 were 19% less than the 2007 values. Methane emission were 19% lower than 2007. Carbon footprint decreased by 19%. These results indicate significant progress by the dairy industry in reducing environmental impact over this time when expressed on a 1 MMT basis while increasing milk production.

A second paper examined the changes in the California dairy industry between 1964 and 2014 (Naranjo et al., 2020). Daily milk production per cow increased by 129% over the 50-year period. Water and land use needed to produce 1 kg of ECM decreased by 89.9 and 89.7%. Methane emitted per kg of ECM decreased by 56%. The authors also calculated the shift in total carbon dioxide equivalent emissions per kg of ECM. The value in 2017 was 1.16 compared with 2.11 in 1964.

Progress in New York

The New York dairy and feed industry have a long history of being environmentally conscious and responsible. The emphasis on nutrient management planning and precision feeding are examples. Several New York and Northeast dairy herds have been recognized for their sustainability efforts. Table 1 lists herds recognized for outstanding dairy farm sustainability by the Innovation Center for U.S. Dairy. Noblehurst Farms were

recognized for an outstanding achievement in community partnerships award in 2016 from the Innovation Center for U.S. dairy. Table Rock Farm received the 2021 Leopold Conservation award from the New York AEM program in 2021. Lamb Farms and the Western New York Crop Management Association were recognized as a 2021 4R advocate by the Fertilizer Institute.

Table 1. Northeast Dairy Sustainability Award Recipients

Year	Farm	State
2012	Blue Spruce Farm	Vermont
2014	Sensenig Dairy	Pennsylvania
2015	Oregon Dairy	Pennsylvania
2018	E-Z Acres	New York
2018	Reinford Farms	Pennsylvania
2020	Twin Birch Dairy	New York
2021	Goodrich Farm	Vermont
2021	Red Sunset Farm	Pennsylvania

[a] Awards from the Innovation Center for U.S. Dairy

A project was done to assess changes in milk production, ration N and P levels, and nutrient excretion on New York fairy farms between 1999 and 2019 (Chase and Reed, 2021a, b). This study was done in cooperation with the Northeast Agribusiness and Feed Alliance. The 1999 diet was 50% forage with a 1:1 ratio of corn silage to alfalfa silage (DM basis). Diet NDF was 38% and was 19% starch. The 2019 diet was 60% forage with 60% of the forage as corn silage and 40% alfalfa silage. Ration NDF was 35% and starch was 26%. Milk production per cow was 40% higher in 2019 while cow numbers dropped by 10%. Total New York milk production during this period increased by 26%. Total manure nitrogen and phosphorus excretion to the environment were reduced by 8 and 20%. Ammonia potential and methane emissions were 17 and 3% lower in 2019. These results indicate that the New York dairy and feed industry have decreased the environmental impact of the dairy industry while increasing milk production.

Trials have also been conducted on commercial dairy herds to evaluate changes in diet CP on nutrient excretion and profitability. A study in western New York used 2 dairy herds over an 8-month period (Higgs et al., 2012). This trial was done in cooperation with the nutritionists working with the herd. Diets fed were evaluated and reformulated using the CNCPS model. Diet CP was reduced about 1 unit in each herd. Daily manure nitrogen output was lowered by 12 and 6%. Income over purchased feed cost increased by $1.27 per cow per day in one herd and $0.27 in the second herd.

A second trial was done over a 3-year period using 8 herds in the Upper Susquehanna watershed to evaluate the impact of implementing a precision feed management program (Van Amburgh et al., 2019). This trial was conducted in cooperation with the nutritionists working in these herds. Diets were formulated by the herd nutritionist and evaluated with the CNCPS model. Diet changes were made after discussion between the herd nutritionist and the project leader. Milk production increased by 4 pounds per cow per day. Diet CP decreased from 17.4 to 15.8%. Manure nitrogen

excretion decreased by 14%. Income over purchased feed cost increased by $137 per cow per year.

Table 2. Changes in New York – 1999 to 2019

Item	1999	2019	Change
Milk, lbs./cow/year	17,176	24,118	+40.4%
Number of dairy cows	701,000	627,000	-10,5%
Milk, lbs./cow/day	47	66	+40.4%
Total NY Milk Production, billion pounds	12	15.1	+25.8%
Ration DMI, lbs./day	38.6	48.3	+25.1%
Ration CP, % of DM	18.5	16.5	-10.8%
Ration P, % of DM	0.48	0.39	-18.8%
Ration N Intake, g/cow/day	520	578	+11.1
Milk N, g/cow/day	112	158	+41.1
Manure N, g/cow/day	408	420	+2.9%
NY Total Manure N, tons/year	114,964	105,649	-8.1%
Ration P Intake, g/day	84	85	+1.2%
Milk P, g/day	19	27	+42%
Manure P, g/day	65	58	-10.8%
NY Total Manure P, tons/year	18,331	14,640	-20.1%
Ammonia Potential Emissions, g/cow/day	145	134	-7.6%
Total NY Potential Ammonia Emissions, tons/year	40895	33803	-17.3%
Methane Emissions, g/cow/day	389	420	+8%
Methane Emissions, g/lb. of milk	8.3	6.4	-23%
Total NY Methane emissions, tons/year	109,625	105,890	-3.4%

Whole farm mass nutrient balance (WFMB) is another tool that can be used to assess the impact of dairy farms on the environment. Changes in WFMB for 91 dairy herds in the Upper Susquehanna watershed between 2004 and 2013 were reported (Cela et al., 2017). WFMB for nitrogen decreased by 50% while phosphorus was 51% lower. If a nitrogen fixation estimate was included, the nitrogen WFMB was 29% lower in 2013.

Decreases in feed nitrogen and phosphorus imports to the farm were a primary factor for the change in WFMB.

Co-Product Feeds in Dairy Rations

The use and incorporation of co-product feeds in ruminant and dairy rations has been an accepted and widely used practice for many years. The commercial feed industry is a primary user of co-product feeds from grain milling, ethanol production, beer brewing and the rendering industry. Many commercial grain mixes and protein supplements are primarily composed of co-product ingredients. There are also an increasing number of dairy producers that purchase, store, and utilize co-products in their on-farm mixed diets. The use of co-product ingredients is attractive since they are often economically priced sources of energy, protein, fiber, and fat. Co-product feeds have been used to replace both concentrates and some forages in dairy rations. A recent paper had a list of 363 unusual and byproduct feeds that could be utilized in ruminant rations (Waller, 2020). Utilizing co-product feeds in rations decreases the need to landfill or incinerate these feeds. It was estimated that 137 million tons of co-products were available in an annual basis in the U.S. (Knapp, 2015). Less carbon dioxide was released when co-products were used in diets than if they were incinerated (Van Amburgh et al., 2019). California workers reported that co-product feeds comprised 41% of total diet dry matter. Co-product feeds were 26% of total diet dry matter in 46 high producing dairy herds (Chase, 2019). A summary of 91 diets from 70 herds found that co-product feeds were 31% of the total diet (Ven Amburgh et sl., 2019). The range was 9 to 57%.

Conversion of Human Inedible Feeds

One approach that deserves more attention is the role of ruminants in converting human inedible feeds into human edible foods. A key factor in this conversion is the capability of rumen microorganisms to convert nonprotein nitrogen compounds, like urea, into protein and amino acids (Loosli et al., 1949). A second factor is the ability of rumen bacteria and fungi to produce cellulase enzymes that can break the β 1-4 glycosidic linkage of cellulose (Van Soest, 1982: Weimer, 1996). Human and mammalian digestive enzymes are not able to break this linkage. Since cellulosic carbohydrates are a large potential supply of nutrients, this provides ruminants the mechanism to convert these carbohydrates into animal products for use in human diets.

Calculating the quantity of human edible protein (HEP) produced relative to the quantity of human edible protein consumed (HEC) is one approach that can be used (Wilkinson, 2011; Ertl et al., 2015). A ratio >1 of HEP/HEC indicates that more human edible protein is produced than consumed by an animal. A paper from Sweden compared a cereal grain, soybean meal grain mix with 3 different co-product feeding strategies (Karlsson et al., 2018). The HEP/HEC ratio for the cereal-based diet was 0.73 compared with 2.56 to 2.68 for the co-product diets. Another trial replaced cereals and pulses with byproduct feeds (Ertl et al., 2015). The HEP/HEC ratio was 1.6 on the control diet snd 4.27 for the byproduct diet. When this calculation was done on an energy basis, the ratio for the control diet was 1.3 compared with 5.55 for the byproduct diet. A trial with late

lactation dairy cows was done substituting co-product feeds for corn grain snd soybean meal (Hall and Chase, 2014). The HEP/HEC ratio was 0.78 for the diet with corn and soybean meal versus 1.94 for the co-product diet.

Refinements to this approach have been proposed. One is to include digestible amino acids in calculating the efficiency of protein conversion (Ertl et al., 2016; Patel et al., 2017). This is important since the animal proteins produced are higher in biological value than most plant proteins (Oltjen and Beckett, 1996; Patel et al., 2017). One paper reported that animal proteins have a biological value 1.4 times higher than plant proteins (Pimental and Pimental, 2003).

A second refinement is to combine both the nutrient composition and the portion of HEP that the food industry demands (Tricarico, 2016). In this approach, the human edible portion of the feed is calculated as 1-NDF. This is termed the composition coefficient and assumes that the fiber fraction is not usable by humans. If a feed has an NDF>30%, then the composition coefficient is set to 0. The demand coefficient is determined by multiplying the composition coefficient by the percent of the food used for domestic use. As an example, the composition coefficient of corn grain is 0.91. Food use of corn grain was 12% of the total U.S. grain production in 2015. This result in a demand coefficient of 0.11. In an example diet, 20% of the total dry matter was human inedible. When the demand coefficient was used, this decreased to 2.2%.

. There continues to be a perception that feeds consumed by dairy cows are competing with humans for food resources. On a global basis, it was reported that 86% of the feed consumed by ruminants was not edible by humans (Mottet et al., 2017). The California group estimated that 82% of the feed consumed by dairy cows was inedible by humans (California Dairy Research Foundation, 2016). Average human inedible portion of the ration was 84% of the total dry matter intake for 46 dairy herds not feeding high moisture shelled corn (Chase, 2019). The range was 73.5 to 97.8 % of the total ration dry matter as human inedible feed. These herds averaged 59 pounds of dry matter intake and 110 pounds of milk per cow per day.

Whole Farm Considerations

The integration of animal, land resources, crops, manure management and economics is essential in evaluating and developing strategies for dairy farm sustainability. Developing forage-based diets without considering the capability of the land resources and cropping system to supply the needed forage quantities and qualities is a problem. An example of the whole farm approach is a paper simulating best management practices (BMP) on a 1,500 cow New York dairy (Veltman et al., 2018). The simulation was done using the Integrated Forage System Model (Rotz et al., 2016). A base farm was defined in terms of crop acres, housing, management, field management and diets. Several best management practices were examined to reduce the environmental impact of the farm. Application of several BMP's resulted in a projected 11% increase in milk production and a 27% increase in net return per cow. The reactive

N and total farm losses were by 41 and 46% lower. Farm carbon footprint was 41% lower with the use of the BMP's.

Summary

1. The dairy industry has made significant progress in reducing environmental impact while increasing milk production. The carbon footprint has also been reduced
2. Using co-product feeds in dairy diets decreases disposal needs and costs by incineration or in landfills.
3. Dairy cows and other ruminants can convert human inedible feeds into high quality foods (milk, meat) for human diets.
4. The dairy industry needs to be more proactive making this information available to consumers.
5. The use of whole farm integrated models is essential to continue the progress made to date. The various component of the dairy enterprise must work in unison to integrate milk production, environmental considerations, and profitability.

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