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Biomarkers of Residual Feed Intake in Holstein Dairy Cows

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Biomarkers of Residual Feed Intake in Holstein Dairy Cows

By

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THESIS

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List of Abbreviations

RFI – Residual feed intake

LFE - Least feed-efficient

MFE – Most feed-efficient

DIM – Days in milk

PUFA – Polyunsaturated fatty acids

SFA – Saturated fatty acids

DGLA - Dihomo-γ-linolenic acid

IOFC – Income over feed cost

RDP – Rumen degradable protein

DMI – Dry matter intake

BW – Body weight

BCS – Body condition score

GHG – Greenhouse gas

NH_3 - Ammonia

MPS – Microbial protein synthesis

MUN – Milk urea nitrogen

NPN – Non-protein nitrogen

RUP – Rumen undegradable protein

CP – Crude protein

CHO – Carbohydrate

NSC – Non-structural carbohydrate

N – Nitrogen

GDH – Glutamate dehydrogenase

FA – Fatty acids

TMR – Total mixed ration

NRC – Nutrient requirements of dairy cattle

FAME – Fatty acid methyl esther

SPE – solid phase extraction

NE – Net energy

ΔBodyE – Body energy changes

ΔBW – Body weight changes

$BW^{0.75}$ – Metabolic body weight

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ABSTRACT

The present thesis consists of two chapters. The first chapter focuses on reviewing previously conducted research pertaining to feed efficiency, nitrogen metabolism, and fatty acids utilization in dairy cows to underscore the key points investigated in the research conducted during my master's. The second chapter describes a study aimed to identify biomarkers associated with residual feed intake (RFI) and improve predictive models for feed efficiency. The study used a subset of 24 lactating Holstein cows representing extremes of least feed-efficient (LFE, n = 12, RFI = 2.44) and most feed-efficient (MFE, n = 12, RFI = -2.69) that had no difference in 3 energy sinks (body weight change, metabolic body weight, and energy secreted in milk) from a population of 454 genotyped cows with full phenotype for feed efficiency. Rumen fluid and serum samples were collected between 60 and 90 DIM. Rumen fluid samples were collected using an oroesophageal tubing procedure. Serum samples were used to measure fatty acids using a two-step assay. The fatty acid methyl ester was performed using solid phase extraction and quantified using chromatographic peak area and internal standard-based calculations. Ruminal ammonia nitrogen was performed using a phenol-hypochlorite assay, while serum urea was measured using a commercial ELISA test validate for the bovine species. Cows in the MFE group had higher ruminal ammonia nitrogen concentrations than cows in the LFE group. There were no differences in serum urea concentration between MFE and LFE cows. Serum fatty acids concentration differed between groups, with myristic acid, palmitic acid, cis-heptadecenoic acid, stearic acid, and total saturated fatty acids in greater concentrations in the MFE group than in the LFE group. Total polyunsaturated fatty acids concentration was lower in the MFE group than in the LFE group. Myristic acid, palmitic acid, total polyunsaturated fatty acids (PUFA), and saturated fatty acids (SFA) were correlated with RFI. A model incorporating myristic acid, palmitic acid, palmitoleic acid, ante iso heptadecanoic acid + palmitoleic acid, oleic acid, cis-vaccenic acid, petroselinic acid,

stearic acid, linoleic acid, dihomo-γ-linolenic acid (DGLA), cis-monounsaturated fatty acids, omega 6 PUFA, total PUFA, total SFA, other and unknown fatty acids resulted in a $R^2 = 0.74$. When rumen ammonia nitrogen was added to the previous model, an improvement was observed (Full $R^2 = 0.84$). The findings of the current study provide evidence that ruminal ammonia and fatty acids in serum are correlated with RFI, and the integration of these metabolites may improve the prediction of RFI.

Keywords: feed efficiency, residual feed intake, rumen nitrogen metabolism, polyunsaturated fatty acids, dairy cows

Chapter 1: Literature Review

INTRODUCTION

Feed efficiency in dairy cows is described as the amount of product produced (e.g., milk) per unit of feed intake, measuring the effectiveness of converting nutrients into requirements for a dairy cow maintenance, and the synthesis of milk and its components. Feed efficiency includes metrics such as mass, energy, protein, or economic value(VandeHaar et al., 2016).

Feed efficiency in dairy cows reflects the effectiveness of converting feed into milk production. Various measurement methods, including gross feed efficiency and income over feed cost (IOFC), have been utilized. However, residual feed intake (RFI) is currently considered the gold standard (VandeHaar et al., 2016), and it was included as a trait for selection by the Dairy Cattle Breeding Council. RFI calculates the disparity between actual and predicted feed intake, providing a standardized assessment (Koch et al., 1963; VandeHaar et al., 2016).

Factors affecting feed efficiency encompass a wide range, from rumen microbial populations to external conditions like inflammation and environmental factors. Improving feed efficiency is crucial for economic viability in dairy farming, given the significant portion of total expenses attributed to feed costs (*USDA ERS - Milk Cost of Production Estimates*, 2021).

Genetic selection based on RFI presents challenges due to the difficulty of measuring this trait, as it requires individual intake recording of the cows. Additionally, the limited population with phenotype data for RFI has limited RFI reliability. Therefore, it is necessary to develop new approaches to improve RFI prediction. Nevertheless, enhancing feed efficiency is a strategy that might also help mitigate greenhouse gas emissions in dairy farming, as more feed-efficient cattle tend to produce less manure and methane (Bell et al., 2012; Dechow et al., 2004; Korver, 1988).

Understanding nitrogen metabolism in ruminants is essential for economic profitability and environmental sustainability. Dietary protein quality significantly impacts nutritional value,

with various factors influencing its digestibility. The utilization of rumen-degradable dietary protein (RDP) affects microbial protein synthesis and ammonia production, emphasizing the importance of balanced dietary formulations (Chen et al., 1987; Hristov et al., 2004).

Another crucial point is the breakdown of proteins into peptides and amino acids in the rumen is crucial for microbial protein synthesis. Ensuring optimal energy availability and balanced breakdown rates is essential for efficient nitrogen metabolism and microbial protein synthesis (Wallace et al., 1997). Linked with protein, urea and ammonia are also pivotal for ruminant nitrogen metabolism. The breakdown of urea in the rumen is facilitated by urease-producing bacteria. Ammonia, which is the primary nitrogen source for protein synthesis, is produced through different enzymatic mechanisms. These processes influence nitrogen utilization and, consequently, affect nitrogen utilization efficiency (Gibbons & McKarthy, 1957; Hespell, 1984; John et al., 1974; Pilgrim et al., 1970).

Microbes may also play an important role in nitrogen metabolism, and lipids and fatty acids supplementation have been shown to influence microbial activities and fermentation (Jenkins, 1993), impacting nitrogen metabolism. Understanding these interactions is important for optimizing ruminant nutrition.

Ammonia and circulating fatty acids are suggested to be important in differentiating most feed-efficient (MFE) cows from least feed-efficient (LFE) cows and may have the potential to improve models for predicting RFI in the future.

FEED EFFICIENCY

Definition and methods of measurement

Feed efficiency in dairy cows is described as the amount of product produced (e.g., milk) per unit of feed intake, measuring the effectiveness of converting nutrients into requirements for a dairy cow maintenance, and the synthesis of milk and its components. Feed efficiency can include metrics such as mass, energy, protein, or economic value (VandeHaar et al., 2016).

Different methods of measuring feed efficiency in dairy cows have been investigated throughout the years, including gross feed efficiency, income over feed cost **(IOFC)**, residual solids production, and RFI, which is currently the usual method to measure feed efficiency in dairy cows (Martin et al., 2021). Gross feed efficiency is expressed by the ratio of milk output to feed input, which can be normalized to energy-corrected milk and dry matter or energy intake, respectively. The heritability of gross feed efficiency traits is relatively moderate (0.14 to 0.37) and depends on the lactation stage when it is measured (Spurlock et al., 2012; Vallimont et al., 2011; Van Arendonk et al., 1991). The IOFC, which consists of the difference between sales milk income and feed costs, is easy to calculate but limited due to milk price fluctuations; therefore, it is not widely used for calculating feed efficiency. Assessing the feed efficiency of dairy cows with residual solids production comprises calculating the difference between the actual versus predicted production of milk solids, considering a given DMI, body size, and body condition. This method involves regressing milk solids yield against cow DMI, metabolic body weight ($BW^{0.75}$), change in BW, and body condition score (Coleman et al., 2010). Lastly, RFI, even though it has its limitations (Old et al., 2024), the method most used for feed efficiency calculations, is calculated statistically by the difference between the actual intake and the predicted feed intake based on models developed for growing beef cattle (Koch et al., 1963).

Factors Impacting Feed Efficiency

Factors influencing feed efficiency are diverse and encompass aspects ranging from rumen microbial populations (Monteiro et al., 2022) to feeding behavior (Richardson & Herd, 2004). Differences in rumen microbial populations have been studied in relation to feed efficiency (Elolimy et al., 2022; Monteiro et al., 2022; Rius et al., 2012; Zhang et al., 2022) , along with feeding behavior (Connor et al., 2013; Green et al., 2013; Williams et al., 2011), physical activity (Connor et al., 2013), and physiological differences (Xi et al., 2016).

Maintenance requirement is an important aspect to be considered regarding feed efficiency, since the energy for work functions, cell component synthesis and membrane transport are considerable (Baldwin et al., 1985) and body weight is positively correlated with maintenance costs, with smaller cows having less maintenance costs (VandeHaar et al., 2016). Cows with similar levels of milk production could have around 20% maintenance net energy (McNamara, 2015) and by increasing feed intake and milk production, a dilution of maintenance occurs, leading to increased feed efficiency (Bauman et al., 1985).

Studies have shown that feeding behavior plays a significant role in feed efficiency. For instance, a study suggested that feeding patterns contribute to approximately 2% of the variation in RFI (Richardson & Herd, 2004). Additionally, research on Holstein–Friesian heifers revealed that more efficient heifers exhibited slower eating rates and fewer meals compared to less efficient cows (Green et al., 2013; Williams et al., 2011).

Furthermore, the rate of feed consumption impacts feed digestibility, with increased passage rates associated with decreased digestibility (Connor, 2015). More feeding time in dairy cows can represent less time resting and ruminating, negatively impacting the production (Grant & Albright, 1995). Additionally, Holstein cows in a low RFI group were linked to reduced feeding time and slower feeding rates (Connor et al., 2013).

External factors, such as inflammation and environmental conditions, also impact feed efficiency. Inflammation status can divert nutrients away from production (Bertoni et al., 2008; Loor et al., 2005), while exposure to extreme temperatures can further depress feed efficiency in dairy cattle (Rhoads et al., 2009; Wheelock et al., 2010).

Advances in genetics, nutrition, management, and health have contributed to an overall improvement in dairy operations, leading to increased milk production. However, improvements in feed efficiency by increasing milk production may become less feasible as marginal gains diminish over time (VandeHaar et al., 2016). This highlights the importance of finding new approaches to enhance feed efficiency, which is essential for understanding the nutrient utilization of dairy cattle, which reflects the proportion of nutrients consumed that contribute to milk production (Bach et al., 2020).

Feed efficiency and profitability

Enhancing economic efficiency is a primary objective for most dairy farms. With feed expenses accounting for up to 54% of total costs in dairy operations (USDA-ERS, 2021), improving feed efficiency is essential for enhancing overall economic performance. Increasing production output without proportionally increasing feed input can help mitigate fixed costs. However, optimizing economic efficiency must be assessed at the farm level, considering constraints such as barn capacity, land availability, manure management, and labor resources (Mosheim & Lovell, 2009).

Managing costs associated with non-lactating heifers and dry cows, which can constitute up to 25% of the dairy herd, is crucial as they do not directly contribute to income, impacting overall farm profitability. Research has shown significant differences in feed intake among heifers based on residual feed intake (RFI), a measure of feed efficiency. Heifers in the lowest 10% for

RFI (more feed-efficient) consumed 15% to 20% less feed compared to those in the top 10% for RFI (less feed-efficient), translating to substantial cost savings for the farm (Waghorn et al., 2012; Williams et al., 2011).

Moreover, subsequent studies have indicated that selecting only more feed-efficient heifers does not compromise lactation performance. For instance, during days 75 to 195 of their first lactation, no differences were observed in feed intake, milk yield, milk components, body weight change, or body condition score among heifers categorized by RFI (Waghorn et al., 2012; Williams et al., 2011). This suggests that targeting feed-efficient genetics can lead to reduced feed costs without detrimental effects on milk production or cow health, thus bolstering the economic efficiency of dairy farming operations.

Genetic selection through RFI and limitations

To enhance feed efficiency through genetic programs targeting RFI, it is pivotal to consider the heritability of this trait, which has been reported at 0.22 to 0.38 in growing heifers (Korver, 1988; Pryce et al., 2012) and around 0.32 in lactating dairy cows (Veerkamp et al., 1995). These heritability levels are notably lower compared to those of milk production traits in Holsteins and Jerseys (0.78 and 0.61, respectively) from Australia (Erbe et al., 2012). The challenge in obtaining genotyped individuals and phenotypic data for RFI traits underscores the difficulty in achieving high genomic selection accuracy (Daetwyler et al., 2008).

When selecting animals for improved feed efficiency, attention must be paid to potential impacts on fertility, as body condition score (BCS) tends to negatively correlate with fertility. This correlation can be attributed to the mobilization of body fat to meet energy demands for milk production (Bastin et al., 2010; Dechow et al., 2004). In summary, advancing the reliability of RFI for genetic selection faces several limitations, including challenges in collecting accurate

phenotypic data, the extended periods required to calculate RFI, and the need for large populations to ensure robust estimates (Connor, 2015). Overcoming these hurdles is crucial for optimizing genetic selection strategies aimed at improving feed efficiency and overall dairy farm profitability.

Feed efficiency and sustainability

In 2021, agriculture contributed approximately 10.6% of the total greenhouse gas (GHG) emissions in the United States, with 88% of these emissions attributed to methane and nitrous oxide (USDA, 2021). The sources of these emissions are primarily linked to enteric fermentation and manure management from ruminant livestock (US EPA, 2015). A study found that feed production, enteric methane, and manure as the primary contributors to GHG emissions within agriculture (Thoma et al., 2013).

Studies by Grainger et al. (2007) and de Haas et al. (2011) have demonstrated that methane emissions are influenced by feed intake and residual feed intake (RFI) in cattle under different feeding regimes (de Haas et al., 2011; Grainger et al., 2007). Consequently, enhancing feed efficiency has been proposed as a strategy to mitigate GHG emissions in dairy farming (Bell et al., 2012). Moreover, more feed-efficient cattle tend to produce less manure compared to less feed-efficient counterparts due to reduced dry matter intake (DMI). Thus, improving feed efficiency represents a promising approach to reducing GHG emissions in agriculture.

NITROGEN METABOLISM IN RUMINANTS

Understanding nitrogen metabolism in ruminants is extremely important to meet ruminant requirements and avoid nitrogen losses that could impact dairy farmers' profitability since protein is the most expensive nutrient in a diet, and environmental concerns with air and water pollution caused by excess nitrogen excreted in feces and urine (Hristov & Jouany, 2005).

Dietary effects

Dietary protein is the main nitrogen source in many diets and significantly impacts dietary nutritional value. Digestibility of protein is correlated with solubility (Chen et al., 1987); however, it is also influenced by multiple factors such as molecule structure (Wallace & Kopecny, 1983), artificial cross-links inhibiting hydrolysis (Friedman & Broderick, 1977), heat and chemical treatments changing the solubility and degradation properties (Kaufmann & Lüpping, 1982), changes in pH and fermentation (Calsamiglia et al., 2008). Treatments like heating and formaldehyde application, altering solubility and cross-linking, can protect proteins from rumen degradation, providing bypass protein to the lower digestive tract (Kaufmann & Lüpping, 1982). The efficiency of dietary nitrogen utilization is defined by the milk protein yield divided by the nitrogen intake and can be higher or lower depending on the feeding strategy and it varies in ruminants with an average nitrogen efficiency around 25% (Huhtanen & Hristov, 2009; Kohn et al., 2005). Ammonia is a major nitrogen source for ruminal bacteria, particularly cellulolytic ones (Russell et al., 1992). Rumen bacteria derive a significant portion of their nitrogen from NH_3-N, ranging from 38% to 70–80% (Hristov & Broderick, 1996; Koenig et al., 2000; Leng & Nolan, 1984; Mathison & Milligan, 1971; Oldham et al., 1980). Adequate NH_3 levels are crucial for optimizing microbial protein synthesis **(MPS)** in the rumen. However, the efficiency of NH_3 utilization in the rumen significantly impacts ruminant production's economic cost and environmental footprint.

Ruminal NH_3 levels positively correlate with milk urea nitrogen **(MUN)** concentration in dairy cows, and increased NH_3 levels can lead to elevated non-protein nitrogen **(NPN)** content in milk (Broderick & Clayton, 1997; Moorby & Theobald, 1999). Improving NH_3-N utilization in the rumen can reduce MUN content and enhance milk processing quality (Martin et al., 1997). Ruminal NH_3 concentration depends on the rate of ruminal degradability and the concentration of

rumen-degradable dietary protein **(RDP)** relative to microbial needs and available dietary energy (Hristov & Jouany, 2005).

Various factors, including bioactive compounds and fatty acids, can influence specific groups of rumen microorganisms and alter RDP/NH_3 utilization in the rumen(Armentano et al., 1993; Santos et al., 1998). Dietary crude protein (CP), excess RDP, and ruminally undegradable protein **(RUP)** supplementation can affect ruminal fermentation and production, particularly in dairy cows. Increasing dietary CP concentration may lead to greater milk production, but it also increases ruminal NH_3 and urinary nitrogen losses. High-yielding dairy cows may not benefit from increased CP concentration in the diet (Bach et al., 2000).

The utilization of RDP for MPS is energy-dependent, and if not utilized, RDP is likely to be degraded to NH_3 and detoxified in the liver (Lobley et al., 1995). Increasing RDP concentration in the diet may increase ruminal NH_3 concentration and decrease the efficiency of utilization of dietary nitrogen for milk protein synthesis (Christensen et al., 1993). Excess dietary RDP above requirements do not increase MPS or its efficiency in the rumen but reduce the efficiency of utilizing ruminal NH_3-N for milk protein synthesis in dairy cows (Bach et al., 2005; Hristov et al., 2004).

The concentration of ammonia in the rumen can vary significantly depending on factors such as diet, feeding time and frequency, animal factors, and likely other variables to be discovered. This variation can lead to decreased efficiency in microbial ammonia capture and ultimately result in nitrogen wastage (Hoover & Stokes, 1991). The extent to which ammonia is utilized in the rumen primarily depends on the rate of release and the balance of carbohydrate (CHO) and nitrogen availability (Hristov & Jouany, 2005).

Carbohydrate availability dictates the rumen's microbial growth rate and the ruminal ammonia utilization efficiency (Heldt et al., 1999; Hristov et al., 1997; Newbold & Rust, 1992;

Schwab et al., 2005). If energy is limited, ruminal microorganisms degrade feed proteins to ammonia, suppressing ammonia uptake (Hristov et al., 1997; Nocek & Russell, 1988). Therefore, carbohydrate supplementation, as well as the source and degradability of starch and the synchronization of ruminal energy and nitrogen release, are crucial factors in improving the efficiency of ruminal ammonia and overall dietary nitrogen utilization in ruminants.

The use of non-protein nitrogen (NPN) sources, like urea, as substitutes for feed protein in cattle diets. Molasses and starch enhanced dietary NPN utilization, with starch showing slightly greater utilization of urea compared to cellulose, xylan, or pectin (Belasco, 1956; Mills et al., 1944). Carbohydrate (CHO) supplementation consistently reduced ruminal NH_3 concentration, while microbial protein synthesis (MPS) in the rumen was often enhanced (Chamberlain et al., 1985; Rooke et al., 1987).

Huhtanen (1987) observed a linear increase in MPS with increasing levels of intra-ruminal CHO infusion in cattle, with sucrose having a greater effect than xylose. Similarly, Khalili & Huhtanen (1991) reported reduced NH_3 concentration and increased MPS with sucrose supplementation in dairy cows. However, Feng et al. (1993) found conflicting results, with increased NH_3 levels and decreased MPS in dairy cows fed high nonstructural CHO (NSC) diets.

The CHO source also influenced outcomes. Heldt et al. (1999) observed decreased ruminal NH_3 concentration with pure starch compared to glucose or fiber in beef steers. Maltodextrin and sucrose supplementation, for instance, reduced and have no effect to ruminal NH_3 concentrations in dairy cows, respectively (Kim, 1999; Kim et al., 1999).

Overall, CHO supplementation, particularly with starch or sugars, could reduce ruminal NH_3 concentration and improve N usage. More studies are needed to understand the mechanisms behind N utilization and develop feeding strategies that consider multiple factors for optimal N efficiency in livestock.

Peptide and amino acid digestion

The breakdown of proteins in the rumen by microbial enzymes starts with the release of oligopeptides, which are further broken down into smaller peptides and eventually into individual amino acids. This process is essential for incorporating amino acids into microbial protein synthesis. When sufficient energy is available, amino acids are efficiently utilized for microbial protein production, and the breakdown of peptides is not considered a significant inefficiency. However, if there is a lack of energy or the rate of peptide breakdown exceeds the rate of assimilation, peptide catabolism can lead to excessive ammonia production and poor nitrogen retention. This imbalance can result in elevated ruminal ammonia concentrations, negatively affecting nitrogen utilization efficiency in ruminants. Therefore, ensuring optimal energy availability and maintaining a balanced peptide breakdown and assimilation rate is crucial for efficient nitrogen metabolism and microbial protein synthesis in the rumen (Wallace et al., 1997). Amino acids derived from protein breakdown undergo further breakdown processes. Initially, oligopeptides are hydrolyzed into smaller peptides by microbial enzymes. These peptides are then enzymatically degraded into individual amino acids (Hristov et al., 2004). This breakdown of peptides into amino acids is a vital step in the process of microbial protein synthesis, as amino acids serve as the building blocks for microbial protein production.

When sufficient energy is available, rumen microbes efficiently utilize amino acids for protein synthesis. However, if energy availability is limited or if the rate of amino acid breakdown exceeds the rate at which they can be assimilated, it can lead to excessive ammonia production (Wallace et al., 1997). This surplus ammonia can have detrimental effects on nitrogen retention and overall nitrogen utilization efficiency in ruminants.

Therefore, maintaining an adequate energy supply and ensuring a balanced rate of amino

acid breakdown and assimilation are crucial for efficient nitrogen metabolism and microbial protein synthesis in the rumen (Wallace et al., 1997). By optimizing these processes, nutrient utilization, and overall productivity in ruminants can be enhanced.

Urea

Urea breakdown in the rumen is a rapid process facilitated by urease, which hydrolyzes urea into ammonia (Roffler & Satter, 1975; Virtanen, 1966). The breakdown of proteins in the rumen by microbial enzymes starts with the release of oligopeptides, which are further broken down into smaller peptides and eventually into individual amino acids. This process is essential for incorporating amino acids into microbial protein synthesis. When sufficient energy is available, amino acids are efficiently utilized for microbial protein production, and the breakdown of peptides isn't considered a significant inefficiency.

This enzyme, inhibited by acetohydroxamic acid, is primarily associated with bacterial populations in the rumen (Gibbons & McKarthy, 1957; Jones et al., 1964). While specific microbes responsible for urea hydrolysis are not conclusively identified, anaerobic bacteria like Lactobacillus, Peptostreptococcus, and Ruminococcus have been isolated (Gibbons & Doetsch, 1959; Slyter et al., 1968). Facultatively anaerobic bacteria such as Streptococcus and Staphylococcus are also implicated (Cheng & Costerton, 1980). It has been suggested that urease-producing bacteria reside on the rumen wall rather than in the rumen fluid (Hobson & Stewart, 1988). The urease enzyme, partially purified, exhibits variable activity influenced by factors like dietary nickel and ammonia levels (Cook, 1976; John et al., 1974). Regulation of urease activity remains poorly understood, impacting the efficient utilization of urea as a nitrogen source in ruminant diets (Czerkawski & Breckenridge, 1982). Despite its rapid breakdown, urea remains a valuable nitrogen source for ruminants, though its efficient utilization requires a better

understanding of urease regulation (John et al., 1974; Smith & Bryant, 1979). Additionally, urea recycling plays a significant role in nitrogen economy, especially in low-protein diets. This process allows ammonia absorbed through the rumen wall to be converted back to urea in the liver and recycled to the rumen via saliva or direct transfer, enhancing nitrogen utilization efficiency (Helmer & Bartley, 1971).

Ammonia absorption

Ammonia serves as the primary nitrogen source for protein synthesis in the rumen, with between 42% and 100% of microbial nitrogen derived from it (Al-Rabbat et al., 1971b, 1971a; Mathison & Milligan, 1971; Nolan et al., 1976; Pilgrim et al., 1970). Enzymatic mechanisms for ammonia uptake into amino acids vary, with different affinities for their substrates, likely changing as ruminal ammonia concentrations fluctuate (Hespell, 1984). Glutamine synthetase-glutamate synthase is the highest-affinity system for ammonia assimilation, primarily active at low ammonia concentrations (Brown et al., 1974). Other systems with lower affinity include NADP-glutamate dehydrogenase (NADP-GDH), NAD-glutamate dehydrogenase (NAD-GDH), and alanine dehydrogenase (Wallace, 1979). NAD-GDH is the most active ammonia-assimilating enzyme in rumen contents, mucosa, and bacteria attached to the rumen wall (Hoshino et al., 1966; Lenártová et al., 1985). Various aminotransferase activities disperse bound ammonia throughout the amino acid pool (Bhatia et al., 1979; Chalupa et al., 1976). Although it is abundant in the amino acid pool, alanine may not necessarily be the primary product of ammonia assimilation and its role remains unclear (Blake et al., 1983; Shimbayashi et al., 1975). Enzymatic mechanisms for ammonia uptake likely reflect the varied niches occupied by rumen microorganisms, with the glutamate synthase system preserved for conditions of ammonia limitation (Erfle et al., 1977; Hespell, 1984). The microenvironment within the rumen likely varies, affecting the efficiency of

ammonia assimilation and explaining the differential effects of ammonia concentration on the fermentation rates of different feeds (Níkolić & Filipović, 1981; Odle & Schaefer, 1987).

Fatty acids

The effect of dietary lipids and individual fatty acids (FA) on nitrogen metabolism in the rumen is multifaceted and essential for optimizing ruminant nutrition, particularly in dairy cows. Jenkins (1993) notes that supplementing ruminant diets with free or protected long-chain fatty acids aims to increase energy density while minimally disrupting ruminal fermentation and digestion, as well as manipulating milk and meat FA composition. However, dietary lipids undergo significant transformations in the rumen, profoundly affecting ruminal protozoa, microbial activities, fermentation, digestion, and intake (Doreau & Ferlay, 1994; Faverdin, 1999; Jenkins, 1993).

Studies have shown that certain fatty acids, particularly medium-chain saturated FA, inhibit ruminal protozoa, thereby decreasing ruminal ammonia concentration and altering fermentation patterns (Ha et al., 2001; Matsumoto et al., 1991). Long-chain unsaturated fatty acids can impact MPS efficiency and reduce methane production in the rumen (Giger-Reverdin et al., 2003; Hristov et al., 2004).

In dairy cow diets, various fatty acid sources have been studied. In steers, tallow (C18:1, C16:0 and C18:0) and soy oil (C18:2 and C18:1) did not affect microbial protein synthesis (MPS) but increased its efficiency compared to Ca-soap treatments (Jenkins & Palmquist, 1984). Lecithin (C18:2, C16:0, and C18:1) and maize oil (C18:2, C18:1, and C16:0) reduced ruminal ammonia concentration and had no effect on MPS but increased its efficiency (Jenkins & Fotouhi, 1990). Rapeseed oil (C18:1, C18:2 and C16:0) decreased ruminal NH_3 and butyrate concentrations and increased MPS efficiency (Tesfa, 1993). However, the effects of rapeseed oil supplementation on

ruminal NH_3 concentration vary among studies (Doreau et al., 1991; Doreau et al., 1993). Piantoni et al. (2013) found that supplementation of palmitic acid (C18:0) can increase milk production and milk fat yield. Results agree that other fatty acids supplemented to dairy cows, such as C16:0 also have the potential to increase milk yield and milk components (Piantoni et al., 2015).

Studies have also investigated the effects of saturated and unsaturated fatty acids on ruminal protozoa and fermentation. Medium-chain saturated fatty acids, such as capric and lauric acids, strongly affect ruminal fermentation and can eradicate protozoa (Hristov et al., 2004). Long-chain unsaturated fatty acids, like linolenic and linoleic acids, reduce protozoal numbers and NH_3 concentration, though their effects on fermentation are less pronounced compared to medium-chain saturated fatty acids (Hristov et al., 2004)

Chapter 2: Characterization of biomarkers of feed efficiency in dairy cows

INTRODUCTION

Feed costs have been identified as one of the most important expenses in a dairy farm, and it is estimated to be up to 54% of the total costs in a dairy operation (VandeHaar & St-Pierre, 2006). In the last 20 years, research efforts have been intensified towards the development and advancement of strategies to optimize the conversion of feed nutrients into milk and its components, underscoring that optimizing feed efficiency is paramount to promoting the sustainability of the dairy sector (Bach et al., 2020; Connor et al., 2013). Moreover, the anticipated benefit of better nutrient utilization is recognized as a major path to help reduce carbon footprint and nitrogen waste, two of the major pillars to support the improvement of dairy environmental stewardship (Kebreab et al., 2001; Tamminga, 1992)

Residual feed intake (RFI) became the gold standard measurement of feed efficiency in dairy cows, and recently, it was incorporated as a genomic trait in the U.S. National Dairy C*attle* evaluation (Hardie et al., 2017; Li et al., 2020; VandeHaar et al., 2016). The RFI is calculated by the difference between observed dry matter intake (DMI) and predicted DMI adjusted for the energy sinks as NE_L secreted in milk, metabolic BW, body energy changes, and parity. Although RFI is one of the main measurements for feed efficiency, there is a limitation due to the necessity of measuring the energy sinks, such as individual DMI, body weight, body condition score, and net energy secreted on milk for an extended period to collect enough data to calculate RFI accurately, which is challenging and expensive, mostly restricted to studies conducted in research farms equipped with individual intake capabilities. Furthermore, RFI exhibits a moderate to low heritability (Li et al., 2020), highlighting the necessity to investigate novel approaches that can help improve predictive models for feed efficiency.

Our research group has been working on understanding the interplay of the rumen microbiome and feed efficiency. In a recent study, our findings suggested that more feed-efficient cows have a greater concentration of protozoa than least-efficient cows (Monteiro et al., 2023). An increased presence of protozoa has been linked to a more consistent and stable microbial ecosystem in the rumen

of sheep (Koenig et al., 2000) and cows (Firkins et al., 2007), promoting a better synergism with live bacteria to improve starch degradation (Fondevila & Dehority, 2001) and increase urea cycle and nitrogen recycling in dairy cows (Recktenwald & Van Amburgh, 2009). Moreover, a recent study identified a greater concentration of serum fatty acids in cows in the lowest quartile for RFI (Top 25% feed-efficient cows) when compared to the highest quartile for RFI (Bottom 25% - feed-efficient cows) in dairy cows (Nehme Marinho & Santos, 2022), where the RFI value were already corrected for body energy changes and had no correlation with parity, milk production, milk components and body weight, which suggest that more feed efficient cows may have more energy available that can be used to enhance milk production. It is important to emphasize that this energy would not come from fat tissue mobilization to support lactation since the body energy changes were accounted in the model.

These findings led us to design a study to test the hypothesis that biomarkers from ruminal fermentation, such as ruminal ammonia nitrogen, and serum metabolites, such as serum urea and serum fatty acids, are associated with RFI and can improve the reliability of RFI predictive models. Our objective was to assess the association of serum fatty acids, serum urea, and ruminal ammonia with RFI and integrate these biomarkers to improve predictive models for RFI.

MATERIALS AND METHODS

All animal care and experimental procedures for this study were approved by the Institute of Animal Care and Use Committee from the University of California, Davis (protocol #21864), the University of Florida (protocol #201910673), and the University of Guelph Animal Care Committee (protocol #4064).

Animals, Housing, Diets, and Experimental Design

The study was performed concurrently with other 6 experiments that totaled 19 treatments (which were accounted in our statistical models) at the University of Florida Dairy Unit (Alachua, FL, United States; *n*=238) and the Ontario Dairy Research Centre (ODRC; Elora, ON, Canada; *n*=216) between March 2019 and May 2021. For the study, we selected 24 cows (14 multiparous and 10 primiparous) from the 454 genotyped primiparous and multiparous Holstein cows, varying only in RFI, DMI and net energy of lactation (NEL) intake (Table 1). The subset group (*n*=24) did not differ in body weight (BW), metabolic BW ($BW^{0.75}$), body weight changes (BWC), body condition score (BCS), and body energy changes (ΔBodyE). Cows were milked twice a day and were housed in free stall barns in both facilities from the U.S. and Canada. A total mixed ration (TMR) was fed twice daily, and the animals had free access to feed and water. The TMR was formulated to meet or exceed nutrient recommendations for lactating dairy cows based on the NRC, 2001 (Nutrient Requirements of Dairy Cattle, 2001) and the chemical composition of the diet was described by Monteiro et al. (2024) (Table 2). All cows had their DMI, body weight, and production data recorded between 56 (SD ± 15) and 105 (SD ± 12) days in milk. Individual feeding gates (Calan Broadbent Feeding System, American Calan Inc., Northwood, NH) were used to measure the DMI in U.S. cows, and Canada facilities used automated feed bins (Insentec B.V., Hokofarm Group, Emmeloord, AX, Netherlands). The DMI was calculated through the difference between offered TMR and refusals multiplied by the dry matter content of the diet. During milking, the milk yield was recorded daily using electronic milk flow meters in the United States herd (AfiFlo, S.A.E. Afikim, Israel) and Canada herd (DeLaval, Tumba, Sweeden). Samples were collected once a week in Canada and twice a week in the U.S. from both milking times for milk fat, true protein, and lactose analyses. Milk composition was analyzed at the Southeast Milk Inc. laboratory (Belleview, FL) for the U.S. cows and at the Lactanet Guelph Analysis Center

laboratory (Guelph, ON) for the Canada cows. The milk components for each cow were calculated using the yields of milk fat, true protein, and lactose from each milking. After milking, the animals had their BW recorded with a walk-through scale in the U.S. (AfiWeigh, SAE Afikim, Israel) and in Canada (DeLaval, Tumba, Sweden). The BCS was measured weekly by trained personnel on a 1-5 scale with intervals of 0.25 units, following the method of Elanco BCS chart (Elanco Animal Health, 2009). Body energy changes calculation was accessed through the NRC equation: BEC = [2.88 + (1.036 x BCS week)] x BW change (kg/d).

Ruminal fluid collection

Ruminal fluid samples were collected from 454 cows at 62 (SD ± 3) d postpartum using the oro-esophageal tubing procedure, as described (da Cunha et al., 2023). After the morning diet delivery, the ruminal content was collected between 2 and 6 hours later. The oro-esophageal sampling device was connected to a vacuum pump using a tube approximately 200 cm long and 2.5 cm in diameter. Vacuum pressure was built in the tube, and then the first two samples were discarded, avoiding contamination of rumen contents by saliva and mucus. After being collected, the rumen content was immediately placed in sterile conical tubes and snap-frozen in liquid nitrogen.

Blood sampling

Blood samples were collected at 60 ± 3 d postpartum by venipuncture of the coccygeal blood vessels into evacuated blood collection tubes. After collection, samples were placed on ice until arrival to the laboratory and then centrifuged at 2000 × for 15 minutes at 4°C, and two aliquots of 2 mL of serum were frozen at -20°C until analysis.

Ruminal ammonia nitrogen analyses

The rumen fluid samples were ship from University of Guelph and University of Florida conserved in dry ice to University of California (Davis, CA, United States), where the analyses were conducted. Ruminal ammonia nitrogen concentrations were assessed using a phenol-hypochlorite assay

(Broderick & Kang, 1980) with an adapted protocol to plate readers as described previously (Monteiro et al., 2021). The principle of the assay consisted in the reaction of the ammonia (NH_3) with alkaline hypochlorite and phenol (C_6H_6O) in the presence of sodium nitroprusside – $C_5H_4N_6Na_2O_3$ – to form indophenol, with blue color, in a Berthelot reaction. The concentration of ammonia is directly proportional to the absorbance of indophenol, which is measured using a mass spectrophotometer with a wavelength of 620 nm.

Serum urea and serum fatty acids analyses

The blood samples were ship from University of Guelph and University of Florida conserved in dry ice to University of California (Davis, CA, United States), where the analyses were conducted. The urea concentration in the serum was measured using ELISA (QuantiChrom Urea Assay Kit). Fatty acids content was analyzed in a two-step assay. First, the method of fatty acid methyl ester (FAME) purification (Christie, 1998) was done using solid phase extraction (SPE). The FAME (1mg) extracts were previously eluted with 95:5 hexane/ethyl ether and loaded into silica gel SPE columns for purification using a positive pressure manifold processor (PPMP; Agilent technologies). The eluate was dried under nitrogen and dissolved in hexane for gas chromatography analysis. Then, the FAME was analyzed using the 175 °C plateau temperature program described previously (Kramer et al., 2008) using a CP-Sil88 column (100 m, 0.25 mm ID, 0.2 μm film thickness, Agilent Technologies, Santa Clara, CA, USA). To identify FAME for serum, standard No. 603 from Nu-Check Prep Inc. was used. The FAME was quantified using chromatographic peak area and internal standard-based calculations.

Calculations

Metabolic body weight was calculated as $BW^{0.75}$, changes in $BW^{0.75}$ (ΔBW) as the weekly changes across approximately 49 experimental days, body energy changes as $\Delta BodyE = [(2.88 + 1.036 \times BCS) \times \Delta BW]$, and net energy (NE) secreted in milk as NE secreted in milk = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)].

Residuals of feed intake (RFI; kg/d) were assessed for all 454 lactating dairy cows enrolled in the study by fitting the following model from the NRC 2001 (Nehme Marinho & Santos, 2022; NRC, 2001; VandeHaar et al., 2016) predict DMI:

$$DMI\ (kg/d) = \mu + Parity + BW^{0.75} + \Delta BodyE + NE\ secreted\ in\ milk + Treatment + \varepsilon$$

Parity (primiparous vs. multiparous) and the main energy sinks of lactating dairy cows ($BW^{0.75}$, ΔBodyE, NE secreted in milk) were fixed effects, and the previous treatment to which cows were exposed during experiments, and the residual error were considered as random effects. The predicted DMI was subtracted from the observed DMI to assess residuals, which were named RFI (kg/d). Cows that were predicted to have a higher DMI than the observed values, thus, with a negative RFI, were deemed efficient. Cows with the inverse, which had a lower predicted DMI than observed, were deemed not efficient. All 454 cows were ranked by their RFI, and the top 10% (LFE, n = 45) and bottom 10% (MFE cows, n =45) were used for later analyses. After selecting the top and bottom 10% cows for RFI, 12 cows from the least efficient group (LFE) and 12 from the most efficient group (MFE). The location of cows according to feed efficiency groups was similar from cows the University of Guelph (LFE, n = 7; MFE, n = 7) and University of Florida (LFE, n=5; MFE, n=5). Likewise, the parity according to feed efficiency was similar in primiparous (LFE, n = 5; MFE, n = 5) and multiparous (LFE, n = 7; MFE, n = 7) cows. As part of experiment design, cows were paired to not differ in any of the energy sinks (net energy of lactation, BCS, BW, metabolic BW, BW changes, body energy changes), but only DMI and, consequently, RFI (Table 1). Lastly, location and season were also accounted in the model.

Statistical analyses

Statistical analyses were performed in the MIXED procedure of SAS (SAS/STAT version 9.4; SAS Institute Inc., Cary, NC).

An analysis of variance was performed in the MIXED procedure of SAS to test the effects of DMI, Parity, $BW^{0.75}$, ΔBodyE, NE secreted in milk, milk production, and its components, and others on

the final Least (n = 12) and Most (n =12) efficient cows. The model only contained the fixed effect of the RFI group and the random effect of residual error. Thus, these cows only differed on feed intake, and all other factors that could impact RFI were not different (Table 1).

All serum urea and serum fatty acids concentrations evaluated in the study and their association with RFI were tested in ANOVA using the PROC MIXED procedure of SAS. Also, biomarkers that differed or tended to differ between LFE and MFE cows were fitted in a linear regression to investigate a possible correlation between RFI and serum fatty acids. All statistically significant biomarkers found to differ between LFE and MFE cows were individually fitted in a simple linear regression analysis with RFI to assess their coefficient of determination (R^2). Then, a full model was fitted by including all significant variables (based on ANOVA) together and using backward elimination to determine how much variation these variables would explain when used together based on the lowest AICC and adjusted R^2. Throughout all statistical analysis, significance was declared when $P \leq 0.05$ and tendency was declared when $0.05 < P \leq 0.10$.

RESULTS

Intake measurements, lactation, and animal performance

As per the study's design, cows enrolled in the MFE group had a lower RFI, DMI and NEL intake compared to LFE cows (Table 1). Also, as established by the experimental design, the MFE and LFE groups had no differences in dietary NE_L, lactation performance measures (milk, fat, protein and lactose yield, milk fat, protein, and lactose percentage, energy corrected to milk and NE secreted in milk) and animal performance (BW, metabolic BW, BW changes, BCS, and body energy changes). (Table 1).

Ruminal ammonia-N and Serum Urea-N Concentration

Cows in the MFE group had a higher concentration of ruminal ammonia-N (P = 0.05) than cows in the LFE group (Figure 1A). However, the serum urea-N concentration did not differ (P = 0.31) between the LFE and MFE groups (Figure 1B).

Fatty acids profile

Differences were found between the MFE and LFE cows in six different groups of fatty acids analyzed. There were greater concentrations of the following fatty acid groups in MFE than in LFE cows: myristic acid (P = 0.02), palmitic acid (P = 0.02), cis-heptadecenoic acid (P = 0.04), stearic acid (P = 0.05), and total saturated fatty acids (SFA) (P = 0.01) (Figure 2). Conversely, the concentration of total polyunsaturated fatty acids (PUFA) was lower (P = 0.02) in MFE than in the LFE cows (Figure 2) (Table 3).

A tendency to be different was found in six different fatty acids, with the concentration of palmitoleic acid (P =0.08), oleic acid (P =0.06), and cis-monounsaturated fatty acids (P = 0.09) tending to be greater in the MFE than LFE cows. The concentration of linoleic acid (*P* = 0.06), γ-linolenic acid (*P* = 0.08), and omega 6 PUFA (P = 0.06) tended to be lower for MFE than for LFE cows (Table 3).

Associations between RFI and Fatty Acids

Myristic acid (*P* = 0.04), palmitic acid (*P* < 0.01), total PUFA (*P* = 0.04), and total SFA (*P* = 0.01) were correlated with RFI, while cis-heptadecenoic acid (*P* = 0.07) and stearic acid (*P* = 0.08) tended to be correlated with RFI (Figure 3).

Models for the Prediction of RFI

A model using the myristic acid, palmitic acid, palmitoleic acid, ante iso heptadecanoic acid + palmitoleic acid, oleic acid, cis-vaccenic acid, petroselinic acid, stearic acid, linoleic acid, dihomo-γ-linolenic acid (DGLA), other and unknown fatty acids, cis-monounsaturated fatty acids, omega 6 PUFA,

total PUFA and total SFA were used to predict the RFI leading to tendency ($P = 0.08$) and an adjusted R^2 of 0.74. A second model including ruminal ammonia nitrogen the previously listed fatty acids fatty acids (myristic acid, palmitic acid, palmitoleic acid, ante iso heptadecanoic acid plus palmitoleic acid, oleic acid, cis-vaccenic acid, petroselinic acid, stearic acid, linoleic acid, dihomo-γ-linolenic acid (DGLA), other and unknown fatty acids, cis-monounsaturated fatty acids, omega 6 PUFA, total PUFA and total SFA were used to predict the RFI leading to tendency ($P = 0.07$) and an adjusted R^2 of 0.84. (Figure 4).

DISCUSSION

This study was carried out to assess the potential of biomarkers to predict RFI, and the findings corroborate that rumen nitrogen metabolism markers and serum fatty acids differ between the least and most efficient dairy cows and can be used to improve RFI predictive models. The results of ruminal ammonia nitrogen revealed a greater concentration for most feed-efficient cows, leading us to analyze the urea nitrogen concentration in serum due to the necessity of the animal to detoxify ammonia by converting it into urea (Tan & Murphy, 2004). However, there was no difference in serum urea nitrogen. The reason why the most feed-efficient cows do not also have a greater concentration of serum urea nitrogen is unclear, but one possible explanation is that MFE cows may have a higher urease activity and, consequently, more urea, coming from either feed or recycled from blood, being hydrolyzed to ammonia (Pearson & Smith, 1943; Rekib & Sadhu, 1968). Therefore, resulting in equal concentrations for most and least feed-efficient groups, which combined with the greater ruminal nitrogen concentration may suggest that most feed-efficient cows recycle nitrogen more efficiently. Another point to consider is that the cows were sampled after the morning milking and fed only after the collections, experiencing an interval of around 1-2h without feed, affecting concentrations of urea that can be rapidly converted to ammonia within 30 to 2 hours (Rekib & Sadhu, 1968).

The serum fatty acids were also an alternative to assess RFI biomarkers, and our findings indicated that linoleic acid, γ-linoleic acid, and total omega-6 polyunsaturated fatty acids tended to be greater in the least feed-efficient cows compared to the most feed-efficient ones. In general, MFE cows had greater concentrations of saturated and monounsaturated coupled with lower concentrations of polyunsaturated, which suggests a positive relationship between feed efficiency and biohydrogenation. As well characterized in cattle, the biohydrogenation process leads to the hydrolyzation of dietary lipids, non-esterified FA released in the rumen, for instance, conversion of linoleic acid, γ-linoleic acid, and cis-vaccenic acid into saturated fatty acids such as stearic acid (Polan et al., 1964) which also were present in greater concentration in most feed-efficient cows when compared to the least efficient cows.

Myristic acid, found in higher serum concentrations for the most feed-efficient cows, has been shown to increase apparent diet digestibility and body nitrogen retention when supplemented to dairy cows (Dohme et al., 2004), which may influence the mechanism of energy and protein usage in cows more feed-efficient. Also, myristic acid was tested for the reduction of methane emissions in the past in dairy cows and sheep, and it was effective in inhibiting the activity of methanogens in ruminants (Machmüller et al., 2003; Odongo et al., 2007). Besids being a pollutant, methane can represent a significant energy loss, from 2-12% of gross energy intake (Johnson & Johnson, 1995; Moe & Tyrrell, 1979). Even though in the current study, the methane emissions were not measured, these potential functions of reducing methane production could be associated with improved usage of dietary nutrients by reducing the losses of energy for methane production; for this reason, the cows with a greater concentration of myristic acid could potentially be more feed efficient.

A study found that gamma linolenic acid was in lower concentrations for most feed-efficient cows, while oleic acid was greater for most feed-efficient cows (Martin et al., 2021). Likewise, our findings indicate that the most feed-efficient cows tended to have a greater concentration of oleic acid. In the previous study, researchers found that the bile acids concentration was greater in most feed-

efficient cows compared to the least efficient, and suggested that it might be due to improved lipid digestion (Martin et al., 2021). The concentration of oleic acid-acylcarnitine was also greater in most feed-efficient cows compared to least feed-efficient cows, which could be explained by alterations in rumen biohydrogenation, complete oxidation, or lipid digestion and absorption (Martin et al., 2021).

Supplementing a specific fatty acid in the diet can increase the fatty acid concentration in the plasma of ruminants (Khalilvandi-Behroozyar et al., 2023; Zachut et al., 2010). Palmitic acid (C16:0) is the most common saturated fatty acid found both in plants and animals, having palm, kernel, coconut oil, and milk fat as its main sources (Loften et al., 2014). Supplementation of C16:0 in the diet of dairy cows can increase milk fat percentage (Steele & Moore, 1968) and enhance fiber digestibility (Sears et al., 2024). The most feed-efficient group had a greater concentration of C16:0 compared to the least feed-efficient group, which suggests that most feed-efficient cows may have an improved mechanism in using palmitic acid.

The negative effects of supplementing fat sources such as C16:0 on DMI are not consistent, and studies suggest that when fatty acid inclusion is considered carefully, diets supplemented with fatty acids have no effect on DMI (Mathews et al., 2016; Piantoni et al., 2013). Indeed, studies found that fatty acids supplementation could increase DMI as a result of increased milk yield of cows supplemented with fatty acids (de Souza & Lock, 2018; Piantoni et al., 2015). The increase in DMI by the effect of C16:0 was suggested to be linked to fibrolytic bacteria performance since palmitic acid is a component of fibrolytic bacteria cell membranes (de Souza et al., 2018; Mackie et al., 1991). Fibrolytic bacteria such as *Butyriovibrio fibrisolvens* can lead to better NDF digestibility (Gobius et al., 2002), but further research is needed to elucidate how the C16:0 concentrations found in the rumen correlate to serum C16:0 and its impact on the overall feed efficiency of dairy cows.

The most feed-efficient cows also had a higher concentration of stearic acid (C18:0) when compared to the least feed-efficient group. Stearic acid has been shown to have a greater amount flowing

into the duodenum than the amount supplied by the diet (Loor et al., 2004; Wu et al., 1991), which can be another sign that most feed-efficient cows may have an improved mechanism for biohydrogenation compared to least feed-efficient cows. Another scenario that could explain this difference in efficiency between the two groups is a greater capacity of most feed-efficient cows in the absorption of C18:0 due to a tolerance in the saturation of intestinal sites of C18:0 absorption (Kucuk et al., 2004). Stearic acid also undergoes unsaturation to C18:1, which tended to be greater in most feed-efficient cows than in least feed-efficient cows in our results.

Additionally, the isomer cis9-C17:1, which is the predominant isomer in milk and intramuscular fat of ruminants (Alves et al., 2006), was found to be higher in MFE cows than in LFE cows in our study. It is important to highlight that data from gas chromatography analysis that reports finding C17:1 should be reported as the isomeric composition cis9-C17:1 (Alves et al., 2006). Moreover, C17:1, together with C17:0, was found to be a metabolite with the potential to improve predictions of microbial protein flow to the duodenum, which was related with milk secretion of C17:1 and C17:0 (Vlaeminck et al., 2005). Since microbial protein is a large contributor to the amino acids in the small intestine (Clark et al., 1992), cows with greater concentration of cis9-C17:1 may have a different flow of microbial protein in the duodenum. Thus, further research on cis9-C17:1 and its impact on dairy cows' feed efficiency is needed.

Another study indicated that a greater concentration of circulating fatty acids may increase the expression of the enzyme pyruvate carboxylase mRNA. This enzyme maintains oxaloacetate for the tricarboxylic acid cycle and gluconeogenesis, contributing to energy partitioning during the transition period (White et al., 2011). The data from the previous study suggested that MFE cows may have an improved mechanism of energy partitioning. One of the most promising findings of the current study aligns with this conceptual idea, revealing that incorporating fatty acids and ruminal ammonia can potentially help to predict RFI and shed light on a path to finding biomarkers or improving genomic

selection for feed efficiency. Future studies need to investigate further nitrogen and fatty acid metabolites association when exploring in a larger population can improve the prediction of RFI and lead to the identification of biomarkers and pathways to improve selection for feed efficiency.

CONCLUSION

In conclusion, our findings emphasize the importance of rumen nitrogen metabolism markers such as ruminal ammonia, and serum fatty acids in distinguishing between the MFE and LFE dairy cows. Ruminal ammonia nitrogen and serum fatty acids revealed distinct profiles between the MFE and LFE cows and may be potential biomarkers for RFI in dairy cows that can be incorporated into predictive models to enhance our ability to identify and select more feed-efficient animals, thereby promoting sustainable and environmentally responsible dairy farming practices. The role of serum urea still needs further research to elucidate mechanisms of urea recycling in MFE cows, which would provide a better understanding of how nitrogen metabolism and, specifically, urea recycling impact feed efficiency. Future research with ruminal and circulating fatty acid measurements may provide new steps for enhancing feed efficiency in dairy herds.

Tables and Figures

Table 1. Description of intake measures, and lactation and animal performance characteristics

Items	RFI Group		SEM	*P*-value
	Least	Most		
Intake measures				
RFI[1]	2.44	-2.69	0.20	< 0.001
DMI[2]	26.1	20.7	0.98	< 0.001
NE_L[3] intake	44.3	35.1	1.59	< 0.001
Diet NE_L	1.7	1.7	0.009	0.99
Lactation performance				
Milk Yield	39.6	40.1	2.13	0.85
Milk Fat, %	3.81	3.62	0.16	0.44
Milk Protein, %	3.06	2.93	0.071	0.22
Milk Lactose, %	4.84	4.83	0.039	0.85
Fat Yield	1.50	1.44	0.09	0.67
Protein Yield	1.20	1.17	0.06	0.76
Lactose Yield	1.92	1.94	0.10	0.88
Energy corrected milk	40.5	39.8	2.10	0.81
NE secreted in milk	28	27.4	1.44	0.78
Animal performance				
BW[4]	668	677	25.8	0.81
MBW[5]	131	132	3.81	0.82
BWC[6]	0.388	0.256	0.13	0.50
BCS[7]	3.27	3.19	0.09	0.58
BEC[8]	2.56	1.76	0.87	0.53

[1]RFI = Residual feed intake
[2]DMI = Dry matter intake
[3]NEL = Net energy of lactation
[4]BW = Body weight
[5]MBW = Metabolic body weight
[6]BWC = Body weight changes
[7]BCS = Body condition score
[8]BEC = Body energy changes

Table 2. Experimental summary and chemical composition of the diets used in the study

			United States				
Item	Mean ± SD	UOG[1]	UF[1]	UF[2]	UF[3]	UF[4]	UF[5]
n	454 (total)	216	35	40	51	12	100
Rumen sampling, *DIM*	62 ± 3	64	60	60	60	66	60
First day, *DIM*	56 ± 15	42	50	50	50	85	61
Last day, *DIM*	105 ± 12	91	100	105	100	125	110
Total collection days	50 ± 3	49	51	56	51	40	49
Chemical composition							
OM	92.7 ± 0.61	93.4	92.1	92.5	92.4	93.7	92.8
CP	17.0 ± 0.88	15.8	16.8	17.7	18.4	16.3	16.9
RDP[1]	10.9 ± 0.47	10.7	11.9	10.1	11.0	11.4	11.4
RUP[1]	5.22 ± 0.13	5.10	5.40	4.90	5.20	5.00	5.20
NDF	28.6 ± 4.07	29.3	29.8	34.2	23.5	26.8	25.3
Forage NDF	23.0 ± 2.63	25.6	18.4	19.3	21.6	18.2	22.5
Starch	30.5 ± 1.61	27.1	31.1	27.9	31.7	31.8	31.5
ADF	16.0 ± 3.02	19.4	16.6	18.3	15.7	10.7	15.1
NFC	43.1 ± 5.37	45.1	41.4	33.4	45.7	48.6	46.1
Ether extract	4.64 ± 1.59	3.63	4.10	7.27	5.44	4.26	4.26
NEL, *Mcal/kg of DM*	1.69 ± 0.03	1.68	1.67	1.74	1.70	1.73	1.65

Adapted from Monteiro et al. (2024).

OM = organic matter, CP = crude protein, RDP = rumen degraded protein, RUP = rumen undegraded protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, NFC = non-fibrous carbohydrates, NEL = net energy required of lactation, and Mcal = megacalories, SD = standard deviation, UOG = University of Guelph, UF = University of Florida.

[1]Mion et al. (2023), DOI: https://doi.org/10.1093/jas/skad041

[2]Zimpel et al. (2021), DOI: https://doi.org/10.3168/jds.2021-20486

[3]Unpublished.

[4]Oyebade et al. (2023), DOI: https://doi.org/10.3168/jds.2022-22898

[5]Lobo et al. (2023), DOI: https://doi.org/10.3168/jds.2022-22583

Table 3. Analysis of variance on serum fatty acids from lactating cows varying in residual feed intake (RFI).

Fatty Acids	RFI group		SEM	*P*-value
	Least	Most		
Saturated Fatty Acids				
Lauric Acid (C12:0)	0.284	0.355	0.058	0.40
Myristic Acid (C14:0)	0.466	0.551	0.023	0.02
Pentadecanoic Acid (C15:0)	0.536	0.568	0.044	0.61
Palmitic Acid (C16:0)	11.6	13.1	0.417	0.02
Isovaleric Acid (C17:0 iso)	0.127	0.157	0.027	0.43
Stearic Acid (C18:0)	12.4	13.9	0.49	0.05
Anteiso Heptadecanoic Acid (C17:0 ante iso) + Palmitoleic Acid (t13-16:1)	0.342	0.257	0.039	0.14
Heptadecanoic Acid (C17:0) + Cis-13-Hexadecanoic Acid (C13-16:1)	0.564	0.561	0.050	0.97
Total Saturated Fatty Acids	25.3	28.4	0.783	0.01
Monounsaturated Fatty Acids				
Palmitoleic Acid (trans9-16:1)	0.124	0.186	0.023	0.08
Sapienic Acid (cis7-16:1)	0.563	0.582	0.036	0.72
Palmitoleic Acid (cis9-16:1)	2.21	2.83	0.381	0.26
Cis-Heptadecenoic Acid (cis9-17:1)	0.253	0.389	0.043	0.04
Vaccenic Acid (trans11-18:1)	0.346	0.430	0.043	0.18
Oleic Acid (cis9-18:1)	7.21	8.18	0.354	0.06
Cis-Vaccenic Acid (cis11-18:1)	0.761	0.939	0.087	0.16
Petroselinic Acid (cis12-18:1)	0.588	0.517	0.045	0.27
Cis-Monounsaturated Fatty Acids	11.6	13.4	0.733	0.09
Polyunsaturated Fatty Acids				
Linoleic Acid (C18:2n-6)	46.8	41.5	1.88	0.06
γ-Linolenic Acid (C18:3 n-6)	1.11	0.901	0.08	0.08
α-Linolenic Acid (C18:3 n-3)	4.31	4.10	0.593	0.81
Rumenic Acid (cis9,trans11-CLA[1])	0.355	0.363	0.063	0.93
Dihomo-γ-Linolenic Acid (DGLA[2]) (C20:3n-6)	2.34	1.99	0.156	0.12
Arachidonic Acid (C20:4n-6)	1.97	1.89	0.128	0.66
Eicosapentanoic Acid (C20:5n-3)	0.554	0.638	0.098	0.55
Docosapentaenoic Acid (DPA[3]) (C22:5n-3)	0.432	0.439	0.064	0.94
Omega 6 Polyunsaturated Fatty Acids	52.2	46.3	2.11	0.06

Omega 3 Polyunsaturated Fatty Acids		5.29	5.18	0.722	0.91
Total Polyunsaturated Fatty Acids		57.5	51.5	1.72	0.02
Others/unknowns	3.74	4.72	0.472	0.15	
Trans-Fatty Acids	0.825	0.979	0.103	0.30	

[1]CLA = Conjugated Linolenic Acid
[2]DGLA = Dihomo Gamma Linolenic Acid
[3]DPA = Docosapentaenoic Acid

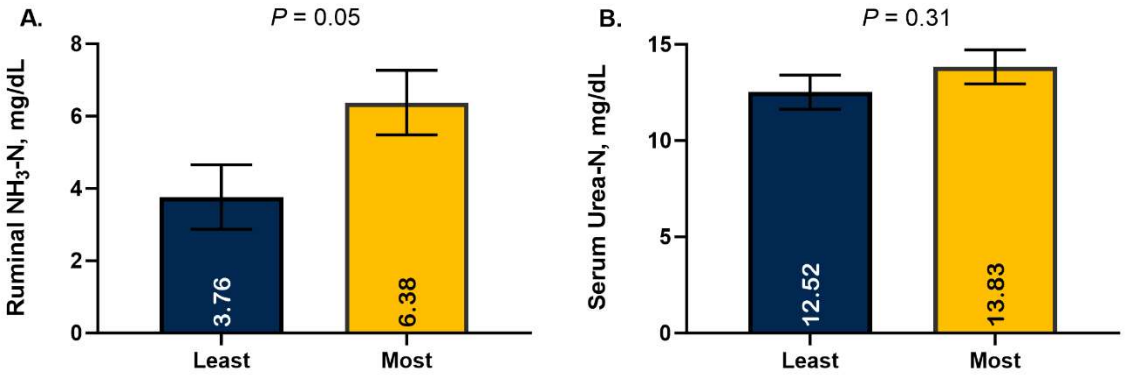

Figure 1. (A) Ruminal ammonia nitrogen concentration and (B) serum urea nitrogen concentration for least (n=12) and most (n=12) feed efficient dairy cows based on their residual feed intake.

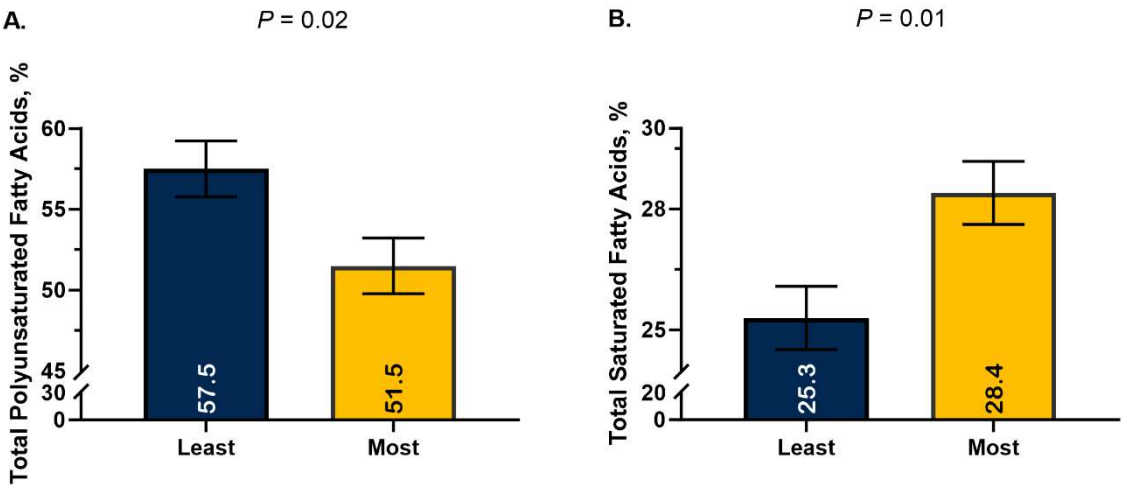

Figure 2. Concentration of total polyunsaturated fatty acids (A) and concentration of total saturated fatty acids (B) from serum for least (n=12) and most (n=12) feed efficient dairy cows.

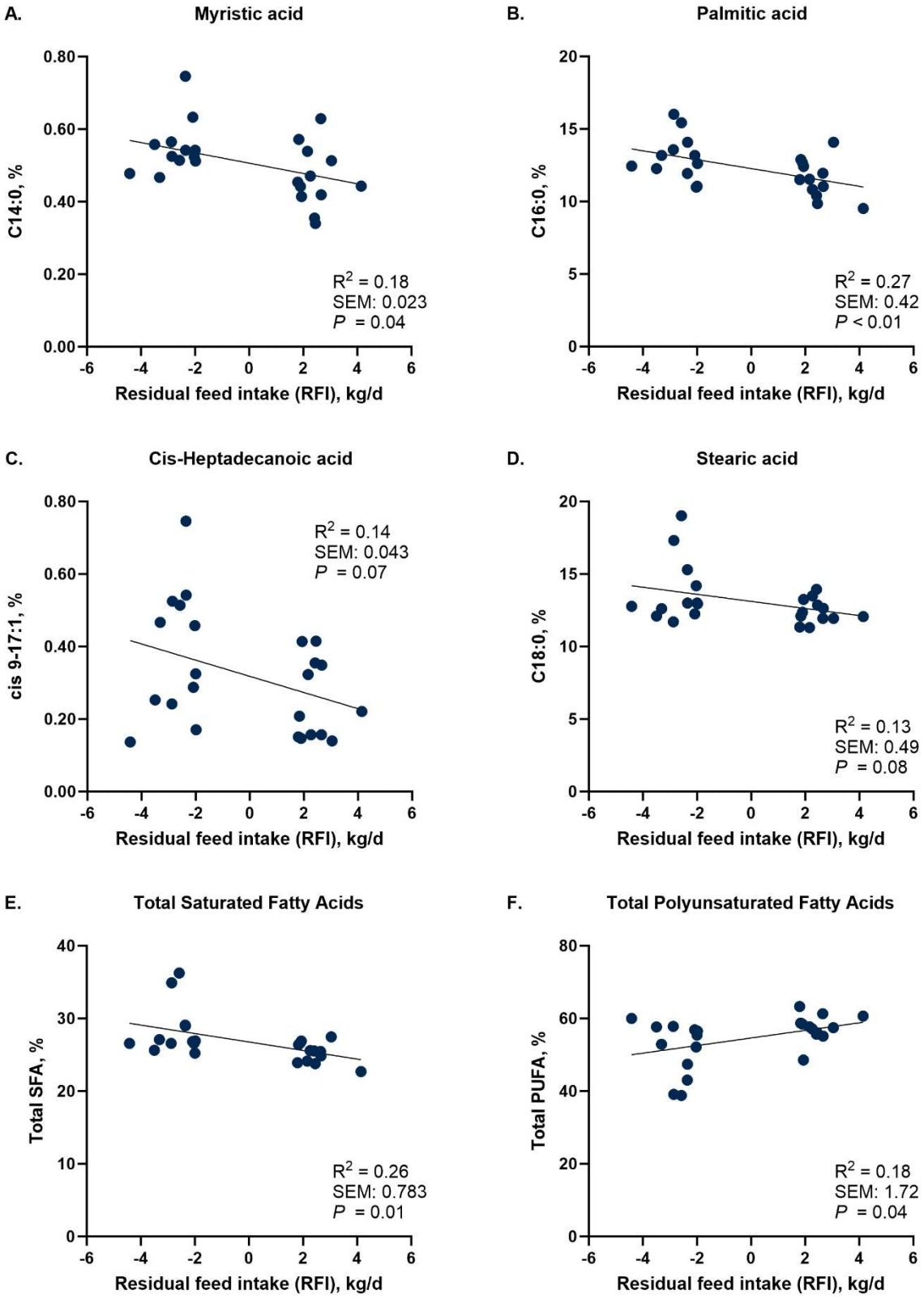
A.
Myristic acid
C14:0, %
0.80
0.60
0.40
0.20
0.00
R2 = 0.18
SEM: 0.023
P = 0.04
-6 -4 -2 0 2 4 6
Residual feed intake (RFI), kg/d
B.
Palmitic acid
C16:0, %
20
15
10
5
0
R2 = 0.27
SEM: 0.42
P < 0.01
-6 -4 -2 0 2 4 6
Residual feed intake (RFI), kg/d
C.
Cis-Heptadecanoic acid
cis 9-17:1, %
0.80
0.60
0.40
0.20
0.00
R2 = 0.14
SEM: 0.043
P = 0.07
-6 -4 -2 0 2 4 6
Residual feed intake (RFI), kg/d
D.
Stearic acid
C18:0, %
20
15
10
5
0
R2 = 0.13
SEM: 0.49
P = 0.08
-6 -4 -2 0 2 4 6
Residual feed intake (RFI), kg/d
E.
Total Saturated Fatty Acids
Total SFA, %
40
30
20
10
0
R2 = 0.26
SEM: 0.783
P = 0.01
-6 -4 -2 0 2 4 6
Residual feed intake (RFI), kg/d
F.
Total Polyunsaturated Fatty Acids
Total PUFA, %
80
60
40
20
0
R2 = 0.18
SEM: 1.72
P = 0.04
-6 -4 -2 0 2 4 6
Residual feed intake (RFI), kg/d

Figure 3. Correlation between the percentage of myristic acid (A), palmitic acid (B), cis-heptadecanoic acid (C), stearic acid (D), total saturated fatty acids (E) and total polyunsaturated fatty acids (F) in the serum with residual feed intake (kg/d).

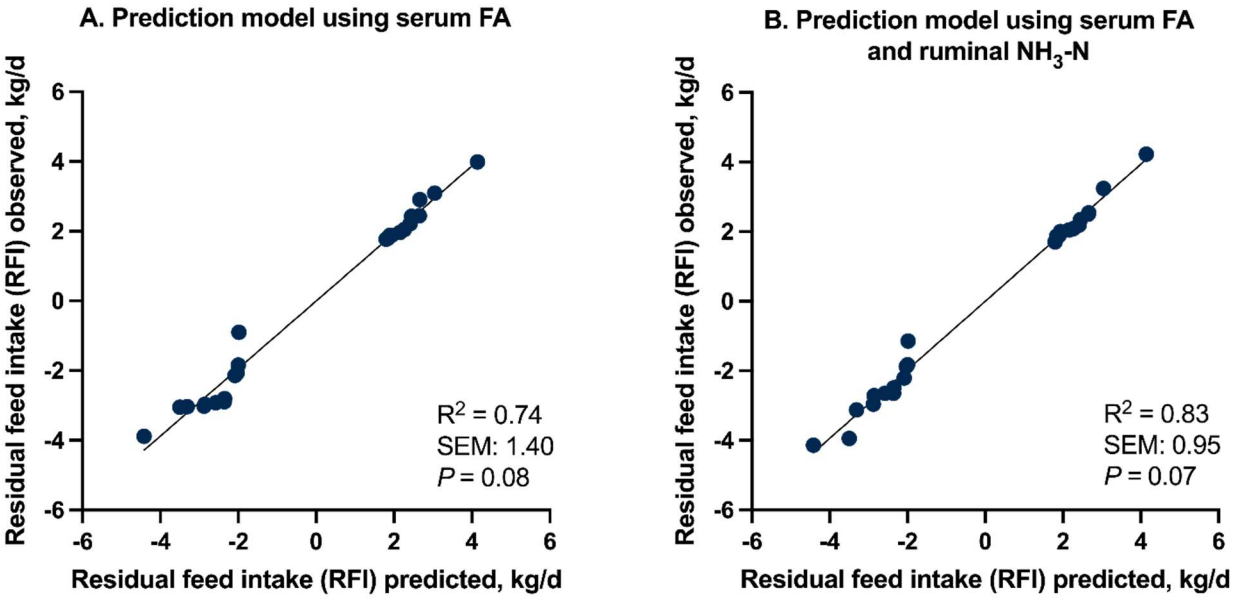

Figure 4. Model using ruminal ammonia nitrogen, and serum fatty acids such as myristic acid, palmitic acid, palmitoleic acid, ante iso heptadecanoic acid + palmitoleic acid, oleic acid, cis-vaccenic acid, petroselinic acid, stearic acid, linoleic acid, dihomo-γ-linolenic acid (DGLA), other and unknown fatty acids, cis-monounsaturated fatty acids, omega 6 polyunsaturated fatty acids, total polyunsaturated fatty acids, and total saturated fatty acids to predict the RFI**.**

REFERENCES

Al-Rabbat, M. F., Baldwin, R. L., & Weir, W. C. (1971a). In Vitro 15Nitrogen-tracer Technique for Some Kinetic Measures of Ruminal Ammonia1. *Journal of Dairy Science*, *54*(8), 1150–1161. https://doi.org/10.3168/jds.S0022-0302(71)85993-3

Al-Rabbat, M. F., Baldwin, R. L., & Weir, W. C. (1971b). Microbial Growth Dependence on Ammonia Nitrogen in the Bovine Rumen: A Quantitative Study1. *Journal of Dairy Science*, *54*(8), 1162–1172. https://doi.org/10.3168/jds.S0022-0302(71)85994-5

Alves, S. P., Marcelino, C., Portugal, P. V., & Bessa, R. J. B. (2006). Short communication: The nature of heptadecenoic acid in ruminant fats. *Journal of Dairy Science*, *89*(1), 170–173. https://doi.org/10.3168/jds.S0022-0302(06)72081-1

Armentano, L. E., Bertics, S. J., & Riesterer, J. (1993). Lack of Response to Addition of Degradable Protein to a Low Protein Diet Fed to Midlactation Dairy Cows1. *Journal of Dairy Science*, *76*(12), 3755–3762. https://doi.org/10.3168/jds.S0022-0302(93)77718-8

Bach, A., Calsamiglia, S., & Stern, M. D. (2005). Nitrogen Metabolism in the Rumen*. *Journal of Dairy Science*, *88*, E9–E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Bach, A., Huntington, G. B., Calsamiglia, S., & Stern, M. D. (2000). Nitrogen Metabolism of Early Lactation Cows Fed Diets with Two Different Levels of Protein and Different Amino Acid Profiles1. *Journal of Dairy Science*, *83*(11), 2585–2595. https://doi.org/10.3168/jds.S0022-0302(00)75152-6

Bach, A., Terré, M., & Vidal, M. (2020). Symposium review: Decomposing efficiency of milk production and maximizing profit. *Journal of Dairy Science*, *103*(6), 5709–5725. https://doi.org/10.3168/jds.2019-17304

Baldwin, B. R., Forsberg, N. E., & Hu, C. Y. (1985). Potential for Altering Energy Partition in the Lactating Cow. *Journal of Dairy Science*, *68*(12), 3394–3402. https://doi.org/10.3168/jds.S0022-

0302(85)81252-2

Bastin, C., Loker, S., Gengler, N., Sewalem, A., & Miglior, F. (2010). Genetic relationships between body condition score and reproduction traits in Canadian Holstein and Ayrshire first-parity cows. *Journal of Dairy Science*, *93*(5), 2215–2228. https://doi.org/10.3168/jds.2009-2720

Bauman, D. E., McCutcheon, S. N., Steinhour, W. D., Eppard, P. J., & Sechen, S. J. (1985). Sources of Variation and Prospects for Improvement of Productive Efficiency in the Dairy Cow: A Review. *Journal of Animal Science*, *60*(2), 583–592. https://doi.org/10.2527/jas1985.602583x

Belasco, I. J. (1956). The Role of Carbohydrates in Urea Utilization, Cellulose Digestion and Fatty Acid Formation. *Journal of Animal Science*, *15*(2), 496–508. https://doi.org/10.2527/jas1956.152496x

Bell, M. J., Jones, E., Smith, J., Smith, P., Yeluripati, J., Augustin, J., Juszczak, R., Olejnik, J., & Sommer, M. (2012). Simulation of soil nitrogen, nitrous oxide emissions and mitigation scenarios at 3 European cropland sites using the ECOSSE model. *Nutrient Cycling in Agroecosystems*, *92*(2), 161–181. https://doi.org/10.1007/s10705-011-9479-4

Bertoni, G., Trevisi, E., Han, X., & Bionaz, M. (2008). Effects of Inflammatory Conditions on Liver Activity in Puerperium Period and Consequences for Performance in Dairy Cows. *Journal of Dairy Science*, *91*(9), 3300–3310. https://doi.org/10.3168/jds.2008-0995

Bhatia, S. K., Pradhan, K., & Singh, R. (1979). Microbial Transaminase Activities and Their Relationship with Bovine Rumen Metabolites. *Journal of Dairy Science*, *62*(3), 441–446. https://doi.org/10.3168/jds.S0022-0302(79)83264-6

Blake, J. S., Salter, D. N., & Smith, R. H. (1983). Incorporation of nitrogen into rumen bacterial fractions of steers given protein- and urea-containing diets. Ammonia assimilation into intracellular bacterial amino acids. *British Journal of Nutrition*, *50*(3), 769–782. https://doi.org/10.1079/BJN19830148

Broderick, G. A., & Clayton, M. K. (1997). A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *Journal of Dairy Science*, *80*(11), 2964–2971. https://doi.org/10.3168/jds.S0022-0302(97)76262-3

Broderick, G. A., & Kang, J. H. (1980). Automated Simultaneous Determination of Ammonia and Total Amino Acids in Ruminal Fluid and In Vitro Media1. *Journal of Dairy Science*, *63*(1), 64–75. https://doi.org/10.3168/jds.S0022-0302(80)82888-8

Brown, C. M., Macdonald-Brown, D. S., & Meers, J. L. (1974). Physiological Aspects of Microbial Inorganic Nitrogen Metabolism. In A. H. Rose & D. W. Tempest (Eds.), *Advances in Microbial Physiology* (Vol. 11, pp. 1–52). Academic Press. https://doi.org/10.1016/S0065-2911(08)60268-4

Calsamiglia, S., Cardozo, P. W., Ferret, A., & Bach, A. (2008). Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH1. *Journal of Animal Science*, *86*(3), 702–711. https://doi.org/10.2527/jas.2007-0146

Chalupa, W., Patterson, J., Chow, A., & Parish, R. (1976). *Deaminase inhibitor effects on N-utilization*. *43*(1), 316–317.

Chamberlain, D. G., Thomas, P. C., Wilson, W., Newbold, C. J., & Macdonald, J. C. (1985). The effects of carbohydrate supplements on ruminal concentrations of ammonia in animals given diets of grass silage. *The Journal of Agricultural Science*, *104*(2), 331–340. https://doi.org/10.1017/S0021859600044002

Chen, G., Sniffen, C. J., & Russell, J. B. (1987). Concentration and Estimated Flow of Peptides from the Rumen of Dairy Cattle: Effects of Protein Quantity, Protein Solubility, and Feeding Frequency1. *Journal of Dairy Science*, *70*(5), 983–992. https://doi.org/10.3168/jds.S0022-0302(87)80103-0

Cheng, K.-J., & Costerton, J. W. (1980). Adherent rumen bacteria—Their role in the digestion of plant material, urea and epithelial cells. In Y. Ruckebusch & P. Thivend (Eds.), *Digestive Physiology and Metabolism in Ruminants: Proceedings of the 5th International Symposium on Ruminant Physiology, held at Clermont—Ferrand, on 3rd–7th September, 1979* (pp. 227–250). Springer Netherlands. https://doi.org/10.1007/978-94-011-8067-2_11

Christensen, R. A., Lynch, G. L., Clark, J. H., & Yu, Y. (1993). Influence of Amount and Degradability of Protein on Production of Milk and Milk Components by Lactating Holstein Cows1. *Journal of Dairy Science*, *76*(11), 3490–3496. https://doi.org/10.3168/jds.S0022-0302(93)77688-2

Christie, W. W. (1998). Gas chromatography-mass spectrometry methods for structural analysis of fatty acids. *Lipids*, *33*(4), 343–353. https://doi.org/10.1007/s11745-998-0214-x

Clark, J. H., Klusmeyer, T. H., & Cameron, M. R. (1992). Microbial Protein Synthesis and Flows of Nitrogen Fractions to the Duodenum of Dairy Cows1. *Journal of Dairy Science*, *75*(8), 2304–2323. https://doi.org/10.3168/jds.S0022-0302(92)77992-2

Coleman, J., Berry, D. P., Pierce, K. M., Brennan, A., & Horan, B. (2010). Dry matter intake and feed efficiency profiles of 3 genotypes of Holstein-Friesian within pasture-based systems of milk production. *Journal of Dairy Science*, *93*(9), 4318–4331. https://doi.org/10.3168/jds.2009-2686

Connor, E. E. (2015). Invited review: Improving feed efficiency in dairy production: challenges and possibilities. *Animal*, *9*(3), 395–408. https://doi.org/10.1017/S1751731114002997

Connor, E. E., Hutchison, J. L., Norman, H. D., Olson, K. M., Van Tassell, C. P., Leith, J. M., & Baldwin, R. L., VI. (2013). Use of residual feed intake in Holsteins during early lactation shows potential to improve feed efficiency through genetic selection1. *Journal of Animal Science*, *91*(8), 3978–3988. https://doi.org/10.2527/jas.2012-5977

Cook, A. R. (1976). Urease Activity in the Rumen of Sheep and the Isolation of Ureolytic Bacteria. *Microbiology*, *92*(1), 32–48. https://doi.org/10.1099/00221287-92-1-32

Czerkawski, J. W., & Breckenridge, G. (1982). Distribution and changes in urease (EC 3.5.1.5) activity in Rumen Simulation Technique (Rusitec). *British Journal of Nutrition*, *47*(2), 331–348. https://doi.org/10.1079/BJN19820042

da Cunha, L. L., Monteiro, H. F., Figueiredo, C. C., Canisso, I. F., Bicalho, R. C., Cardoso, F. C., Weimer, B. C., & Lima, F. S. (2023). Characterization of rumen microbiome and metabolome from oro-esophageal tubing and rumen cannula in Holstein dairy cows. *Scientific Reports*, *13*(1), 5854. https://doi.org/10.1038/s41598-023-33067-5

Daetwyler, H. D., Villanueva, B., & Woolliams, J. A. (2008). Accuracy of Predicting the Genetic Risk of Disease Using a Genome-Wide Approach. *PLOS ONE*, *3*(10), e3395. https://doi.org/10.1371/journal.pone.0003395

de Haas, Y., Windig, J. J., Calus, M. P. L., Dijkstra, J., de Haan, M., Bannink, A., & Veerkamp, R. F. (2011). Genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. *Journal of Dairy Science*, *94*(12), 6122–6134. https://doi.org/10.3168/jds.2011-4439

de Souza, J., Preseault, C. L., & Lock, A. L. (2018). Altering the ratio of dietary palmitic, stearic, and oleic acids in diets with or without whole cottonseed affects nutrient digestibility, energy partitioning, and production responses of dairy cows. *Journal of Dairy Science*, *101*(1), 172–185. https://doi.org/10.3168/jds.2017-13460

Dechow, C. D., Rogers, G. W., Sander-Nielsen, U., Klei, L., Lawlor, T. J., Clay, J. S., Freeman, A. E., Abdel-Azim, G., Kuck, A., & Schnell, S. (2004). Correlations Among Body Condition Scores from Various Sources, Dairy Form, and Cow Health from the United States and Denmark. *Journal of Dairy Science*, *87*(10), 3526–3533. https://doi.org/10.3168/jds.S0022-0302(04)73489-X

Dohme †, F., Machmüller, A., Sutter ‡, F., & Kreuzer, M. (2004). Digestive and metabolic utilization of lauric, myristic and stearic acid in cows, and associated effects on milk fat quality. *Archives of Animal Nutrition*, *58*(2), 99–116. https://doi.org/10.1080/00039420410001667485

Doreau, M., & Ferlay, A. (1994). Digestion and utilisation of fatty acids by ruminants. *Animal Feed Science and Technology*, *45*(3), 379–396. https://doi.org/10.1016/0377-8401(94)90039-6

Doreau, M., Ferlay, A., & Elmeddah, Y. (1993). Organic matter and nitrogen digestion by dairy cows fed calcium salts of rapeseed oil fatty acids or rapeseed oil1. *Journal of Animal Science*, *71*(2), 499–504. https://doi.org/10.2527/1993.712499x

Doreau, M., Legay, F., & Bauchart, D. (1991). Effect of Source and Level of Supplemental Fat on Total and Ruminal Organic Matter and Nitrogen Digestion in Dairy Cows. *Journal of Dairy Science*, *74*(7), 2233–2242. https://doi.org/10.3168/jds.S0022-0302(91)78396-3

Elolimy, A. A., Liang, Y., Wilachai, K., Alharthi, A. S., Paengkoum, P., Trevisi, E., & Loor, J. J. (2022). Residual feed intake in peripartal dairy cows is associated with differences in milk fat yield,

ruminal bacteria, biopolymer hydrolyzing enzymes, and circulating biomarkers of immunometabolism. *Journal of Dairy Science*, *105*(8), 6654–6669. https://doi.org/10.3168/jds.2021-21274

Erbe, M., Hayes, B. J., Matukumalli, L. K., Goswami, S., Bowman, P. J., Reich, C. M., Mason, B. A., & Goddard, M. E. (2012). Improving accuracy of genomic predictions within and between dairy cattle breeds with imputed high-density single nucleotide polymorphism panels. *Journal of Dairy Science*, *95*(7), 4114–4129. https://doi.org/10.3168/jds.2011-5019

Erfle, J. D., Sauer, F. D., & Mahadevan, S. (1977). Effect of Ammonia Concentration on Activity of Enzymes of Ammonia Assimilation and on Synthesis of Amino Acids by Mixed Rumen Bacteria in Continuous Culture1. *Journal of Dairy Science*, *60*(7), 1064–1072. https://doi.org/10.3168/jds.S0022-0302(77)83989-1

Faverdin, P. (1999). The effect of nutrients on feed intake in ruminants. *Proceedings of the Nutrition Society*, *58*(3), 523–531. https://doi.org/10.1017/S0029665199000695

Feng, P., Hoover, W. H., Miller, T. K., & Blauwiekel, R. (1993). Interactions of Fiber and Nonstructural Carbohydrates on Lactation and Ruminal Function1. *Journal of Dairy Science*, *76*(5), 1324–1333. https://doi.org/10.3168/jds.S0022-0302(93)77463-9

Firkins, J. L., Yu, Z., & Morrison, M. (2007). Ruminal nitrogen metabolism: Perspectives for integration of microbiology and nutrition for dairy. *Journal of Dairy Science*, *90 Suppl 1*, E1-16. https://doi.org/10.3168/jds.2006-518

Friedman, M., & Broderick, G. A. (1977). Protected Proteins in Ruminant Nutrition. In Vitro Evaluation of Casein Derivatives. In M. Friedman (Ed.), *Protein Crosslinking: Nutritional and Medical Consequences* (pp. 545–558). Springer US. https://doi.org/10.1007/978-1-4757-9113-6_31

Gibbons, R. J., & Doetsch, R. N. (1959). PHYSIOLOGICAL STUDY OF AN OBLIGATELY ANAEROBIC UREOLYTIC BACTERIUM. *Journal of Bacteriology*, *77*(4), 417–428. https://doi.org/10.1128/jb.77.4.417-428.1959

Gibbons, R., & McKarthy, R. (1957). Obligatory anaerobic urea–hydrolyzing bacteria in the bovine

rumen. *Maryland Agric. Exp. Sta. Misc. Publ*, *291*, 12–16.

Giger-Reverdin, S., Morand-Fehr, P., & Tran, G. (2003). Literature survey of the influence of dietary fat composition on methane production in dairy cattle. *Livestock Production Science*, *82*(1), 73–79. https://doi.org/10.1016/S0301-6226(03)00002-2

Gobius, K. S., Xue, G. -P., Aylward, J. H., Dalrymple, B. P., Swadling, Y. J., McSweeney, C. S., & Krause, D. O. (2002). Transformation and expression of an anaerobic fungal xylanase in several strains of the rumen bacterium Butyrivibrio fibrisolvens. *Journal of Applied Microbiology*, *93*(1), 122–133. https://doi.org/10.1046/j.1365-2672.2002.01662.x

Grainger, C., Clarke, T., McGinn, S. M., Auldist, M. J., Beauchemin, K. A., Hannah, M. C., Waghorn, G. C., Clark, H., & Eckard, R. J. (2007). Methane Emissions from Dairy Cows Measured Using the Sulfur Hexafluoride (SF6) Tracer and Chamber Techniques. *Journal of Dairy Science*, *90*(6), 2755–2766. https://doi.org/10.3168/jds.2006-697

Grant, R. J., & Albright, J. L. (1995). Feeding behavior and management factors during the transition period in dairy cattle. *Journal of Animal Science*, *73*(9), 2791–2803. https://doi.org/10.2527/1995.7392791x

Green, T. C., Jago, J. G., Macdonald, K. A., & Waghorn, G. C. (2013). Relationships between residual feed intake, average daily gain, and feeding behavior in growing dairy heifers. *Journal of Dairy Science*, *96*(5), 3098–3107. https://doi.org/10.3168/jds.2012-6087

Ha, J. K., Lee, S. S., Gao, Z., Kim, C.-H., Kim, S. W., Ko, J. Y., & Cheng, K.-J. (2001). The Effect of Saturated Fatty Acids on Cellulose Digestion by the Rumen Anaerobic Fungus, Neocallimatix frontalis C5-1. *Asian-Australasian Journal of Animal Sciences*, *14*(7), 941–946. https://doi.org/10.5713/ajas.2001.941

Hardie, L. C., VandeHaar, M. J., Tempelman, R. J., Weigel, K. A., Armentano, L. E., Wiggans, G. R., Veerkamp, R. F., de Haas, Y., Coffey, M. P., Connor, E. E., Hanigan, M. D., Staples, C., Wang, Z., Dekkers, J. C. M., & Spurlock, D. M. (2017). The genetic and biological basis of feed efficiency in mid-lactation Holstein dairy cows. *Journal of Dairy Science*, *100*(11), 9061–9075.

https://doi.org/10.3168/jds.2017-12604

Heldt, J. S., Cochran, R. C., Mathis, C. P., Woods, B. C., Olson, K. C., Titgemeyer, E. C., Nagaraja, T. G., Vanzant, E. S., & Johnson, D. E. (1999). Effects of level and source of carbohydrate and level of degradable intake protein on intake and digestion of low-quality tallgrass-prairie hay by beef steers. *Journal of Animal Science*, *77*(10), 2846–2854. https://doi.org/10.2527/1999.77102846x

Hespell, R. (1984). Influence of ammonia assimilation pathways and survival strategy on ruminal microbial growth. *Herbivore Nutrition in the Subtropics and Tropics/Edited by FMC Gilchrist and RI Mackie*.

Hobson, P. N., & Stewart, C. S. (1988). *Rumen microbial ecosystem*. Springer Science & Business Media.

Hoover, W. H., & Stokes, S. R. (1991). Balancing Carbohydrates and Proteins for Optimum Rumen Microbial Yield1. *Journal of Dairy Science*, *74*(10), 3630–3644. https://doi.org/10.3168/jds.S0022-0302(91)78553-6

Hoshino, S., Sarumaru, K., & Morimoto, K. (1966). Ammonia Anabolism in Ruminants. *Journal of Dairy Science*, *49*(12), 1523–1528. https://doi.org/10.3168/jds.S0022-0302(66)88130-4

Hristov, A., McAllister, T., & Cheng, K.-J. (1997). *Effect of carbohydrate level and ammonia availability on utilization of proportional to-amino nitrogen by mixed ruminal microorganisms in vitro*. *48*, 186–189.

Hristov, A. N., & Broderick, G. A. (1996). Synthesis of Microbial Protein in Ruminally Cannulated Cows Fed Alfalfa Silage, Alfalfa Hay, or Corn Silage1. *Journal of Dairy Science*, *79*(9), 1627–1637. https://doi.org/10.3168/jds.S0022-0302(96)76526-8

Hristov, A. N., Etter, R. P., Ropp, J. K., & Grandeen, K. L. (2004). Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. *Journal of Animal Science*, *82*(11), 3219–3229. https://doi.org/10.2527/2004.82113219x

Hristov, A. N., & Jouany, J. P. (2005). Factors affecting the efficiency of nitrogen utilization in the rumen. In E. Pfeffer & A. N. Hristov (Eds.), *Nitrogen and phosphorus nutrition of cattle: Reducing the environmental impact of cattle operations* (1st ed., pp. 117–166). CABI Publishing.

https://doi.org/10.1079/9780851990132.0117

Hristov, A. N., & Jouany, J.-P. (n.d.). *Factors affecting the efficiency of nitrogen utilization in the rumen.* Retrieved November 10, 2023, from https://www.cabidigitallibrary.org/doi/epdf/10.1079/9780851990132.0117

Huhtanen, P. (1987). The effects of intraruminal infusions of sucrose and xylose on nitrogen and fibre digestion in the rumen and intestines of cattle receiving diets of grass silage and barley. *Agricultural and Food Science*, *59*(5), Article 5. https://doi.org/10.23986/afsci.72274

Huhtanen, P., & Hristov, A. N. (2009). A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *Journal of Dairy Science*, *92*(7), 3222–3232. https://doi.org/10.3168/jds.2008-1352

Jenkins, T. C. (1993). Lipid Metabolism in the Rumen. *Journal of Dairy Science*, *76*(12), 3851–3863. https://doi.org/10.3168/jds.S0022-0302(93)77727-9

Jenkins, T. C., & Fotouhi, N. (1990). Effects of lecithin and corn oil on site of digestion, ruminal fermentation and microbial protein synthesis in sheep. *Journal of Animal Science*, *68*(2), 460–466. https://doi.org/10.2527/1990.682460x

Jenkins, T. C., & Palmquist, D. L. (1984). Effect of Fatty Acids or Calcium Soaps on Rumen and Total Nutrient Digestibility of Dairy Rations1. *Journal of Dairy Science*, *67*(5), 978–986. https://doi.org/10.3168/jds.S0022-0302(84)81396-X

John, A., Isaacson, H. R., & Bryant, M. P. (1974). Isolation and Characteristics of a Ureolytic Strain of Selenomonas ruminantium. *Journal of Dairy Science*, *57*(9), 1003–1014. https://doi.org/10.3168/jds.S0022-0302(74)85001-0

Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science*, *73*(8), 2483–2492. https://doi.org/10.2527/1995.7382483x

Jones, G. A., MacLeod, R. A., & Blackwood, A. C. (1964). Ureolytic rumen bacteria: I. characteristics of the microflora from a urea-fed sheep. *Canadian Journal of Microbiology*, *10*(3), 371–378. https://doi.org/10.1139/m64-050

Kaufmann, W., & Lüpping, W. (1982). 4—PROTECTED PROTEINS AND PROTECTED AMINO ACIDS FOR RUMINANTS. In E. L. Miller & I. H. Pike (Eds.), *Protein Contribution of Feedstuffs for Ruminants* (pp. 36–75). Butterworth-Heinemann. https://doi.org/10.1016/B978-0-408-11151-5.50008-6

Kebreab, E., France, J., Beever, D. E., & Castillo, A. R. (2001). Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems*, *60*(1), 275–285. https://doi.org/10.1023/A:1012668109662

Kh, K. (1999). Effects of varying degree of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in cattle consuming grass silage. *Journal of the Science of Food and Agriculture*, *79*, 833–838.

Khalili, H., & Huhtanen, P. (1991). Sucrose supplements in cattle given grass silage-based diet. 1. Digestion of organic matter and nitrogen. *Animal Feed Science and Technology*, *33*(3), 247–261. https://doi.org/10.1016/0377-8401(91)90064-Y

Khalilvandi-Behroozyar, H., Mohtashami, B., Dehghan-Banadaky, M., Kazemi-Bonchenari, M., & Ghaffari, M. H. (2023). Effects of fat source in calf starter on growth performance, blood fatty acid profiles, and inflammatory markers during cold season. *Scientific Reports*, *13*(1), 18627. https://doi.org/10.1038/s41598-023-45956-w

Kim, K. H., Choung, J.-J., & Chamberlain, D. G. (1999). Effects of varying the degree of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in lactating dairy cows consuming a diet of grass silage and a cereal-based concentrate. *Journal of the Science of Food and Agriculture*, *79*(11), 1441–1447. https://doi.org/10.1002/(SICI)1097-0010(199908)79:11<1441::AID-JSFA385>3.0.CO;2-Z

Koch, R. M., Swiger, L. A., Chambers, D., & Gregory, K. E. (1963). Efficiency of Feed Use in Beef Cattle. *Journal of Animal Science*, *22*(2), 486–494. https://doi.org/10.2527/jas1963.222486x

Koenig, K. M., Newbold, C. J., McIntosh, F. M., & Rode, L. M. (2000). Effects of protozoa on bacterial nitrogen recycling in the rumen. *Journal of Animal Science*, *78*(9), 2431–2445.

https://doi.org/10.2527/2000.7892431x

Kohn, R. A., Dinneen, M. M., & Russek-Cohen, E. (2005). Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats1. *Journal of Animal Science*, *83*(4), 879–889. https://doi.org/10.2527/2005.834879x

Korver, S. (1988). Genetic aspects of feed intake and feed efficiency in dairy cattle: A review. *Livestock Production Science*, *20*(1), 1–13. https://doi.org/10.1016/0301-6226(88)90049-8

Kramer, J. K. G., Hernandez, M., Cruz-Hernandez, C., Kraft, J., & Dugan, M. E. R. (2008). Combining Results of Two GC Separations Partly Achieves Determination of All *cis* and *trans* 16:1, 18:1, 18:2 and 18:3 Except CLA Isomers of Milk Fat as Demonstrated Using Ag-Ion SPE Fractionation. *Lipids*, *43*(3), 259–273. https://doi.org/10.1007/s11745-007-3143-4

Kucuk, O., Hess, B. W., & Rule, D. C. (2004). Soybean oil supplementation of a high-concentrate diet does not affect site and extent of organic matter, starch, neutral detergent fiber, or nitrogen digestion, but influences both ruminal metabolism and intestinal flow of fatty acids in limit-fed lambs. *Journal of Animal Science*, *82*(10), 2985–2994. https://doi.org/10.2527/2004.82102985x

Lenártová, V., Holovská, K., Havassy, I., Javorský, P., & Rybosová, E. (1985). Ammonia-utilizing enzymes of adherent bacteria in the sheep's rumen. *Physiologia Bohemoslovaca*, *34*(6), 512–517.

Leng, R. A., & Nolan, J. V. (1984). Nitrogen Metabolism in the Rumen. *Journal of Dairy Science*, *67*(5), 1072–1089. https://doi.org/10.3168/jds.S0022-0302(84)81409-5

Li, B., VanRaden, P. M., Guduk, E., O'Connell, J. R., Null, D. J., Connor, E. E., VandeHaar, M. J., Tempelman, R. J., Weigel, K. A., & Cole, J. B. (2020). Genomic prediction of residual feed intake in US Holstein dairy cattle. *Journal of Dairy Science*, *103*(3), 2477–2486. https://doi.org/10.3168/jds.2019-17332

Lobley, G. E., Connell, A., Lomax, M. A., Brown, D. S., Milne, E., Calder, A. G., & Farningham, D. a. H. (1995). Hepatic detoxification of ammonia in the ovine liver: Possible consequences for amino acid catabolism. *British Journal of Nutrition*, *73*(5), 667–685. https://doi.org/10.1079/BJN19950072

Loften, J. R., Linn, J. G., Drackley, J. K., Jenkins, T. C., Soderholm, C. G., & Kertz, A. F. (2014). Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. *Journal of Dairy Science*, *97*(8), 4661–4674. https://doi.org/10.3168/jds.2014-7919

Loor, J. J., Dann, H. M., Everts, R. E., Oliveira, R., Green, C. A., Guretzky, N. A. J., Rodriguez-Zas, S. L., Lewin, H. A., & Drackley, J. K. (2005). Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. *Physiological Genomics*, *23*(2), 217–226. https://doi.org/10.1152/physiolgenomics.00132.2005

Loor, J. J., Ueda, K., Ferlay, A., Chilliard, Y., & Doreau, M. (2004). Biohydrogenation, Duodenal Flow, and Intestinal Digestibility of *Trans* Fatty Acids and Conjugated Linoleic Acids in Response to Dietary Forage:Concentrate Ratio and Linseed Oil in Dairy Cows. *Journal of Dairy Science*, *87*(8), 2472–2485. https://doi.org/10.3168/jds.S0022-0302(04)73372-X

Machmüller, A., Machmüller, A., Soliva, C. R., & Kreuzer, M. (2003). Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. *The British Journal of Nutrition*, *90*(3), 529–540. https://doi.org/10.1079/bjn2003932

Mackie, R. I., White, B. A., & Bryant, M. P. (1991). Lipid Metabolism in Anaerobic Ecosystems. *Critical Reviews in Microbiology*, *17*(6), 449–479. https://doi.org/10.3109/10408419109115208

Martin, B., Coulon, J. B., Chamba, J. F., & Bugaud, C. (1997). Effect of milk urea content on characteristics of matured Reblochon cheeses. *Le Lait*, *77*(4), 505–514. https://doi.org/10.1051/lait:1997436

Martin, M. J., Pralle, R. S., Bernstein, I. R., VandeHaar, M. J., Weigel, K. A., Zhou, Z., & White, H. M. (2021). Circulating Metabolites Indicate Differences in High and Low Residual Feed Intake Holstein Dairy Cows. *Metabolites*, *11*(12), Article 12. https://doi.org/10.3390/metabo11120868

Martin, P., Ducrocq, V., Gordo, D. G. M., & Friggens, N. C. (2021). A new method to estimate residual feed intake in dairy cattle using time series data. *Animal*, *15*(2), 100101. https://doi.org/10.1016/j.animal.2020.100101

Mathews, A. T., Rico, J. E., Sprenkle, N. T., Lock, A. L., & McFadden, J. W. (2016). Increasing palmitic

acid intake enhances milk production and prevents glucose-stimulated fatty acid disappearance without modifying systemic glucose tolerance in mid-lactation dairy cows. *Journal of Dairy Science*, *99*(11), 8802–8816. https://doi.org/10.3168/jds.2016-11295

Mathison, G. W., & Milligan, L. P. (1971). Nitrogen metabolism in sheep. *British Journal of Nutrition*, *25*(3), 351–366. https://doi.org/10.1079/BJN19710100

Matsumoto, M., Kobayashi, T., Takenaka, A., & Itabashi, H. (1991). Defaunation Effects of Medium-Chain Fatty Acids and Their Derivatives on Goat Rumen Protozoa. *The Journal of General and Applied Microbiology*, *37*(5), 439–445. https://doi.org/10.2323/jgam.37.439

McNamara, J. P. (2015). TRIENNIAL LACTATION SYMPOSIUM: Systems biology of regulatory mechanisms of nutrient metabolism in lactation1,2. *Journal of Animal Science*, *93*(12), 5575–5585. https://doi.org/10.2527/jas.2015-9010

Mills, R. C., Lardinois, C. C., Rupel, I. W., & Hart, E. B. (1944). Utilization of Urea and Growth of Heifer Calves with Corn Molasses or Cane Molasses as the Only Readily Available Carbohydrate in the Ration. *Journal of Dairy Science*, *27*(7), 571–578. https://doi.org/10.3168/jds.S0022-0302(44)92634-2

Moe, P. W., & Tyrrell, H. F. (1979). Methane Production in Dairy Cows. *Journal of Dairy Science*, *62*(10), 1583–1586. https://doi.org/10.3168/jds.S0022-0302(79)83465-7

Monteiro, H. F., Figueiredo, C. C., Mion, B., Coelho Jr, W. M., Bisinotto, R. S., Marinho, M. N., Santos, J. E. P., Peñagaricano, F., Ribeiro, E. S., Schenkel, F. S., Weimer, B. C., Guan, L., Neves, A., Brown, C. T., & Lima, F. S. (2023). A multi-omics approach to characterize the role of the rumen microbiome on feed efficiency in dairy cows. *Abstracts of the 2023 American Dairy Science Association® Annual Meeting*, *106*, 142.

Monteiro, H. F., Figueiredo, C. C., Mion, B., Santos, J. E. P., Bisinotto, R. S., Peñagaricano, F., Ribeiro, E. S., Marinho, M. N., Zimpel, R., da Silva, A. C., Oyebade, A., Lobo, R. R., Coelho Jr, W. M., Peixoto, P. M. G., Ugarte Marin, M. B., Umaña-Sedó, S. G., Rojas, T. D. G., Elvir-Hernandez, M., Schenkel, F. S., … Lima, F. S. (2024). An artificial intelligence approach of feature

engineering and ensemble methods depicts the rumen microbiome contribution to feed efficiency in dairy cows. *Animal Microbiome*, *6*(1), 5. https://doi.org/10.1186/s42523-024-00289-5

Monteiro, H. F., Paula, E. M., Muck, R. E., Broderick, G. A., & Faciola, A. P. (2021). Effects of lactic acid bacteria in a silage inoculant on ruminal nutrient digestibility, nitrogen metabolism, and lactation performance of high-producing dairy cows. *Journal of Dairy Science*, *104*(8), 8826–8834. https://doi.org/10.3168/jds.2021-20155

Monteiro, H. F., Zhou, Z., Gomes, M. S., Peixoto, P. M. G., Bonsaglia, E. C. R., Canisso, I. F., Weimer, B. C., & Lima, F. S. (2022). Rumen and lower gut microbiomes relationship with feed efficiency and production traits throughout the lactation of Holstein dairy cows. *Scientific Reports*, *12*(1), 4904. https://doi.org/10.1038/s41598-022-08761-5

Moorby, J. M., & Theobald, V. J. (1999). Short Communication: The Effect of Duodenal Ammonia Infusions on Milk Production and Nitrogen Balance of the Dairy Cow. *Journal of Dairy Science*, *82*(11), 2440–2442. https://doi.org/10.3168/jds.S0022-0302(99)75495-0

Mosheim, R., & Lovell, C. A. K. (2009). Scale Economies and Inefficiency of U.S. Dairy Farms. *American Journal of Agricultural Economics*, *91*(3), 777–794. https://doi.org/10.1111/j.1467-8276.2009.01269.x

Nehme Marinho, M., & Santos, J. E. P. (2022). Association of Residual Feed Intake With Blood Metabolites and Reproduction in Holstein Cows. *Frontiers in Animal Science*, *3*, 847574. https://doi.org/10.3389/fanim.2022.847574

Newbold, J. R., & Rust, S. R. (1992). Effect of asynchronous nitrogen and energy supply on growth of ruminal bacteria in batch culture. *Journal of Animal Science*, *70*(2), 538–546. https://doi.org/10.2527/1992.702538x

Níkolić, J. A., & Filipović, R. (1981). Degradation of maize protein in rumen contents Influence of ammonia concentration. *British Journal of Nutrition*, *45*(1), 111–116. https://doi.org/10.1079/BJN19810083

Nocek, J. E., & Russell, J. B. (1988). Protein and Energy as an Integrated System. Relationship of

Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *Journal of Dairy Science*, *71*(8), 2070–2107. https://doi.org/10.3168/jds.S0022-0302(88)79782-9

Nolan, J. V., Norton, B. W., & Leng, R. A. (1976). Further studies of the dynamics of nitrogen metabolism in sheep. *British Journal of Nutrition*, *35*(1), 127–147. https://doi.org/10.1079/BJN19760016

Odle, J., & Schaefer, D. M. (1987). Influence of rumen ammonia concentration on the rumen degradation rates of barley and maize. *British Journal of Nutrition*, *57*(1), 127–138. https://doi.org/10.1079/BJN19870016

Odongo, N. E., Or-Rashid, M. M., Kebreab, E., France, J., & McBride, B. W. (2007). Effect of Supplementing Myristic Acid in Dairy Cow Rations on Ruminal Methanogenesis and Fatty Acid Profile in Milk. *Journal of Dairy Science*, *90*(4), 1851–1858. https://doi.org/10.3168/jds.2006-541

Old, C. A., Lean, I. J., Rossow, H. A., & Shike, D. W. (2024). Modeling differences in feed intake and efficiency: Growing and finishing beef cattle. *Applied Animal Science*, *40*(1), 40–55. https://doi.org/10.15232/aas.2023-02443

Oldham, J. D., Bruckental, I., & Nissenbaum, A. (1980). Observations onrumen ammonia metabolism in lactating dairy cows. *The Journal of Agricultural Science*, *95*(1), 235–238. https://doi.org/10.1017/S0021859600029518

Pearson, R. M., & Smith, J. A. B. (1943). The utilization of urea in the bovine rumen. 2. The conversion of urea to ammonia. *Biochemical Journal*, *37*(1), 148–153. https://doi.org/10.1042/bj0370148

Piantoni, P., Lock, A. L., & Allen, M. S. (2013). Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. *Journal of Dairy Science*, *96*(11), 7143–7154. https://doi.org/10.3168/jds.2013-6680

Piantoni, P., Lock, A. L., & Allen, M. S. (2015). Milk production responses to dietary stearic acid vary by production level in dairy cattle. *Journal of Dairy Science*, *98*(3), 1938–1949. https://doi.org/10.3168/jds.2014-8634

Pilgrim, A. F., Gray, F. V., Weller, R. A., & Belling, C. B. (1970). Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. *British Journal of Nutrition*, *24*(2), 589–598. https://doi.org/10.1079/BJN19700057

Polan, C. E., McNeill, J. J., & Tove, S. B. (1964). Biohydrogenation of unsaturated fatty acids by rumen bacteria. *Journal of Bacteriology*, *88*(4), 1056–1064. https://doi.org/10.1128/jb.88.4.1056-1064.1964

Pryce, J. E., Arias, J., Bowman, P. J., Davis, S. R., Macdonald, K. A., Waghorn, G. C., Wales, W. J., Williams, Y. J., Spelman, R. J., & Hayes, B. J. (2012). Accuracy of genomic predictions of residual feed intake and 250-day body weight in growing heifers using 625,000 single nucleotide polymorphism markers. *Journal of Dairy Science*, *95*(4), 2108–2119. https://doi.org/10.3168/jds.2011-4628

Recktenwald, E. B., & Van Amburgh, M. E. (2009). *Refining nitrogen feeding using current knowledge of recycled urea and microbial nitrogen uptake.* 66.

Rekib, A. A., & Sadhu, D. P. (1968). Effect of feeding higher doses of urea on the rumen metabolism in goat. *Indian Veterinary Journal*, *45*, 735–739.

Rhoads, M. L., Rhoads, R. P., VanBaale, M. J., Collier, R. J., Sanders, S. R., Weber, W. J., Crooker, B. A., & Baumgard, L. H. (2009). Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin1. *Journal of Dairy Science*, *92*(5), 1986–1997. https://doi.org/10.3168/jds.2008-1641

Richardson, E. C., & Herd, R. M. (2004). Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. *Australian Journal of Experimental Agriculture*, *44*(5), 431–440. https://doi.org/10.1071/ea02221

Rius, A. G., Kittelmann, S., Macdonald, K. A., Waghorn, G. C., Janssen, P. H., & Sikkema, E. (2012). Nitrogen metabolism and rumen microbial enumeration in lactating cows with divergent residual feed intake fed high-digestibility pasture. *Journal of Dairy Science*, *95*(9), 5024–5034. https://doi.org/10.3168/jds.2012-5392

Roffler, R. E., & Satter, L. D. (1975). Relationship Between Ruminal Ammonia and Nonprotein Nitrogen Utilization by Ruminants. I. Development of a Model for Predicting Nonprotein Nitrogen Utilization by Cattle1. *Journal of Dairy Science*, *58*(12), 1880–1888. https://doi.org/10.3168/jds.S0022-0302(75)84803-X

Rooke, J. A., Lee, N. H., & Armstrong, D. G. (1987). The effects of intraruminal infusions of urea, casein, glucose syrup and a mixture of casein and glucose syrup on nitrogen digestion in the rumen of cattle receiving grass-silage diets. *British Journal of Nutrition*, *57*(1), 89–98. https://doi.org/10.1079/BJN19870012

Russell, J. B., O'Connor, J. D., Fox, D. G., Van Soest, P. J., & Sniffen, C. J. (1992). A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *Journal of Animal Science*, *70*(11), 3551–3561. https://doi.org/10.2527/1992.70113551x

Santos, F. A. P., Huber, J. T., Theurer, C. B., Swingle, R. S., Simas, J. M., Chen, K. H., & Yu, P. (1998). Milk Yield and Composition of Lactating Cows Fed Steam-Flaked Sorghum and Graded Concentrations of Ruminally Degradable Protein. *Journal of Dairy Science*, *81*(1), 215–220. https://doi.org/10.3168/jds.S0022-0302(98)75568-7

Schwab, C. G., Huhtanen, P., Hunt, C. W., & Hvelplund, T. (2005). Nitrogen requirements of cattle. *Nitrogen and Phosphorus Nutrition of Cattle: Reducing the Environmental Impact of Cattle Operations*, 13–70. https://doi.org/10.1079/9780851990132.0013

Shimbayashi, K., Obara, Y., & Yonemura, T. (1975). *Pattern of free amino acids in rumen content and blood of sheep fed diet containing urea.*

Slyter, L. L., Oltjen, R. R., Kern, D. L., & Weaver, J. M. (1968). Microbial Species Including Ureolytic Bacteria from the Rumen of Cattle Fed Purified Diets. *The Journal of Nutrition*, *94*(2), 185–192. https://doi.org/10.1093/jn/94.2.185

Smith, C. J., & Bryant, M. P. (1979). *Introduction to metabolic activities of intestinal bacteria.*

Spurlock, D. M., Dekkers, J. C. M., Fernando, R., Koltes, D. A., & Wolc, A. (2012). Genetic parameters for energy balance, feed efficiency, and related traits in Holstein cattle. *Journal of Dairy Science*,

95(9), 5393–5402. https://doi.org/10.3168/jds.2012-5407

Tamminga, S. (1992). Nutrition Management of Dairy Cows as a Contribution to Pollution Control. *Journal of Dairy Science*, *75*(1), 345–357. https://doi.org/10.3168/jds.S0022-0302(92)77770-4

Tan, Z., & Murphy, M. R. (2004). Ammonia production, ammonia absorption, and urea recycling in ruminants. A review. *Journal of Animal and Feed Sciences*, *13*(3), 389–404. https://doi.org/10.22358/jafs/67425/2004

Tesfa, A. T. (1993). Effects of rape-seed oil supplementation on digestion, microbial protein synthesis and duodenal microbial amino acid composition in ruminants. *Animal Feed Science and Technology*, *41*(4), 313–328. https://doi.org/10.1016/0377-8401(93)90005-5

Thoma, G., Popp, J., Nutter, D., Shonnard, D., Ulrich, R., Matlock, M., Kim, D. S., Neiderman, Z., Kemper, N., East, C., & Adom, F. (2013). Greenhouse gas emissions from milk production and consumption in the United States: A cradle-to-grave life cycle assessment circa 2008. *International Dairy Journal*, *31*, S3–S14. https://doi.org/10.1016/j.idairyj.2012.08.013

US EPA, O. (2015, December 29). *Sources of Greenhouse Gas Emissions* [Overviews and Factsheets]. https://www.epa.gov/ghgemissions/sources-greenhouse-gas-emissions

USDA ERS - Milk Cost of Production Estimates. (n.d.). Retrieved May 14, 2024, from https://www.ers.usda.gov/data-products/milk-cost-of-production-estimates/

Vallimont, J. E., Dechow, C. D., Daubert, J. M., Dekleva, M. W., Blum, J. W., Barlieb, C. M., Liu, W., Varga, G. A., Heinrichs, A. J., & Baumrucker, C. R. (2011). *Short communication*: Heritability of gross feed efficiency and associations with yield, intake, residual intake, body weight, and body condition score in 11 commercial Pennsylvania tie stalls. *Journal of Dairy Science*, *94*(4), 2108–2113. https://doi.org/10.3168/jds.2010-3888

Van Arendonk, J. A. M., Nieuwhof, G. J., Vos, H., & Korver, S. (1991). Genetic aspects of feed intake and efficiency in lactating dairy heifers. *Livestock Production Science*, *29*(4), 263–275. https://doi.org/10.1016/0301-6226(91)90103-W

VandeHaar, M. J., Armentano, L. E., Weigel, K., Spurlock, D. M., Tempelman, R. J., & Veerkamp, R.

(2016). Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency1. *Journal of Dairy Science*, *99*(6), 4941–4954. https://doi.org/10.3168/jds.2015-10352

Veerkamp, R. F., Emmans, G. C., Cromie, A. R., & Simm, G. (1995). Variance components for residual feed intake in dairy cows. *Livestock Production Science*, *41*(2), 111–120. https://doi.org/10.1016/0301-6226(94)00056-D

Virtanen, A. I. (1966). Milk Production of Cows on Protein-Free Feed. *Science*, *153*(3744), 1603–1614. https://doi.org/10.1126/science.153.3744.1603

Vlaeminck, B., Dufour, C., van Vuuren, A. M., Cabrita, A. R. J., Dewhurst, R. J., Demeyer, D., & Fievez, V. (2005). Use of Odd and Branched-Chain Fatty Acids in Rumen Contents and Milk as a Potential Microbial Marker. *Journal of Dairy Science*, *88*(3), 1031–1042. https://doi.org/10.3168/jds.S0022-0302(05)72771-5

Waghorn, G. C., Macdonald, K. A., Williams, Y., Davis, S. R., & Spelman, R. J. (2012). Measuring residual feed intake in dairy heifers fed an alfalfa (*Medicago sativa*) cube diet. *Journal of Dairy Science*, *95*(3), 1462–1471. https://doi.org/10.3168/jds.2011-4670

Wallace, R. J. (1979). Effect of Ammonia Concentration on the Composition, Hydrolytic Activity and Nitrogen Metabolism of the Microbial Flora of the Rumen. *Journal of Applied Bacteriology*, *47*(3), 443–455. https://doi.org/10.1111/j.1365-2672.1979.tb01205.x

Wallace, R. J., & Kopecny, J. (1983). Breakdown of Diazotized Proteins and Synthetic Substrates by Rumen Bacterial Proteases. *Applied and Environmental Microbiology*, *45*(1), 212–217. https://doi.org/10.1128/aem.45.1.212-217.1983

Wallace, R., Onodera, R., & Cotta, M. (1997). Metabolism of nitrogen-containing compounds. *The Rumen Microbial Ecosystem*, 283–328.

Wheelock, J. B., Rhoads, R. P., VanBaale, M. J., Sanders, S. R., & Baumgard, L. H. (2010). Effects of heat stress on energetic metabolism in lactating Holstein cows1. *Journal of Dairy Science*, *93*(2), 644–655. https://doi.org/10.3168/jds.2009-2295

White, H. M., Koser, S. L., & Donkin, S. S. (2011). Bovine pyruvate carboxylase 5′ untranslated region

variant expression during transition to lactation and feed restriction in dairy cows1. *Journal of Animal Science*, *89*(6), 1881–1892. https://doi.org/10.2527/jas.2010-3697

Williams, Y. J., Pryce, J. E., Grainger, C., Wales, W. J., Linden, N., Porker, M., & Hayes, B. J. (2011). Variation in residual feed intake in Holstein-Friesian dairy heifers in southern Australia. *Journal of Dairy Science*, *94*(9), 4715–4725. https://doi.org/10.3168/jds.2010-4015

Wu, Z., Ohajuruka, O. A., & Palmquist, D. L. (1991). Ruminal Synthesis, Biohydrogenation, and Digestibility of Fatty Acids by Dairy Cows1. *Journal of Dairy Science*, *74*(9), 3025–3034. https://doi.org/10.3168/jds.S0022-0302(91)78488-9

Xi, Y. M., Wu, F., Zhao, D. Q., Yang, Z., Li, L., Han, Z. Y., & Wang, G. L. (2016). Biological mechanisms related to differences in residual feed intake in dairy cows. *Animal: An International Journal of Animal Bioscience*, *10*(8), 1311–1318. https://doi.org/10.1017/S1751731116000343

Zachut, M., Arieli, A., Lehrer, H., Livshitz, L., Yakoby, S., & Moallem, U. (2010). Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. *Journal of Dairy Science*, *93*(12), 5877–5889. https://doi.org/10.3168/jds.2010-3427

Zhang, H., Elolimy, A. A., Akbar, H., Thanh, L. P., Yang, Z., & Loor, J. J. (2022). Association of residual feed intake with peripartal ruminal microbiome and milk fatty acid composition during early lactation in Holstein dairy cows. *Journal of Dairy Science*, *105*(6), 4971–4986. https://doi.org/10.3168/jds.2021-21454