ABSTRACT

FARRICKER, MIRANDA, JANELLE. The Role of Fiber and Malic Acid in Ruminant Function and Microbial Fermentation. (Under the direction of Dr. Vivek Fellner).

Maintaining a healthy rumen environment is essential to provide a constant pattern of nutrient supply to dairy cows and optimize efficiency of nutrient use and animal performance. Lactating cow diets may include different grains as a source of energy. The ratio of concentrates and fermentable fiber can influence production response and gut health. Greater amounts of physically effective neutral detergent fiber (peNDF) seen in higher forage diets improved chewing behavior, rumination, and NDF turnover. In ruminant species, chewing contributes to fiber degradation that allows for nutrient and energy extraction and maintains the saliva that buffers rumen pH with each food bolus that returns to the rumen during rumination, making it a vital contributor to gut health and animal performance. Chapter 1 aims to merge the physical and chemical components of dietary fiber into one measurement termed physically effective undegradable NDF at 240 hours of *in vitro* fermentation (peuNDF240) which is calculated by multiplying the physical effectiveness factor (pef; % particles ≥1.18-mm sieve) and undegradable NDF (uNDF) following 240 h of *in vitro* digestion. Dry matter intake (DMI) and energy corrected milk (ECM) have a negative relationship with peuNDF240, meaning that as it increases, there is a decrease in kg/d of dietary intake as well as in the animal's milk yield. The impact of gut health on animal performance is further explored with a focus on sub-acute ruminal acidosis (SARA) in Chapter 2. Although a drop in rumen pH is an inevitable event during fermentation, a drop between 5.2 and 5.5 or prolonged periods under 5.8 can cause distress to the animal that eventually leads to decreases in performance factors that impact profitability. In this study, we varied the forage-to-concentrate ratio of three different corn silage

and ground corn diets to induce a drop in pH to simulate acidotic behaviors. As the quantity of ground corn concentrate increases, the amount of peNDF in the diet decreases so there is less fiber content to stimulate buffering. Previous studies have shown that ruminal pH increased with dietary malate when animals were fed a corn-based diet. Four levels of malic acid were added to each diet to determine if it may mitigate production and health deficits such as SARA, and if so, the optimal level of inclusion. Due to the primary relation of SARA to the depression in pH, the role of malate is to effectively alleviate acid accumulation and pH decline. Increased ground corn concentrate in the diet induced a drop in pH while increasing inclusion levels for malic acid alleviated those symptoms and raised the culture pH within the two lower forage diets. Forage quality, quantity, and particle size all contribute to a stable and productive rumen microbial environment that improves animal production and profitability.

The Role of Fiber and Malic Acid in Ruminant Function and Microbial Fermentation

by
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DEDICATION

This thesis is dedicated to my grandpa, Raymond Medley, Jr. I will always remember standing in the laboratory with you talking about chemistry and seeing your eyes light up over our shared passion for science and discovery. Thank you for indulging yourself in my research interests and being one of my greatest supporters through so many endeavors. You have set an exceptionally high standard for kindness and professionalism in my life and will always be my role model.

BIOGRAPHY

Miranda Janelle Farricker began her undergraduate study in biochemistry at Barrett, The Honors College at Arizona State University in August 2016. She transferred to North Carolina State University in August 2018 to pursue a degree in animal science. While at both universities and throughout graduate school, she was a scholarship student-athlete in Women's Softball, involved in community outreach and youth mentorship, worked as an undergraduate and graduate teaching assistant, and was inducted into the Alpha Epsilon Delta, National Society of Collegiate Scholars, and Chi Alpha Sigma honor societies. She also served as a team representative for the NC State Student-Athlete Advisory Committee and was recognized as an Academic All-American three times by the National Fastpitch Coaches Association. She graduated *Cum Laude* with a Bachelor of Science in Animal Science and a concentration in Veterinary Bioscience in May of 2020. In August 2020, Miranda began her Master of Science in Animal Science and Nutrition under the guidance of Dr. Vivek Fellner at NC State and received a research grant from the North Carolina Dairy Producers Association. She completed an additional graduate research project under the guidance of Dr. Rick Grant at the William H. Miner Agricultural Research Institute in Chazy, NY during the summer of 2021. In August 2022, she began a PhD in Animal Science at Cornell University under the guidance of Dr. Joseph McFadden.

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CHAPTER 1: Comparison of Two Methods for Calculating Physically Effective Undegradable Neutral Detergent Fiber over 240 hours (peuNDF240)

Abstract

Undegradable neutral detergent fiber following 240 h of *in vitro* fermentation (uNDF240) can be multiplied by the fraction of feed particles ≥ 1.18 mm with dry sieving (physical effectiveness factor; pef) to calculate physically effective uNDF240 (peuNDF240). Previous research indicates that peuNDF240 better predicts dry matter intake (DMI) and energy-corrected milk (ECM) than uNDF240. Our first objective was to expand existing data on the relationships between uNDF240 and calculated peuNDF240 with DMI and ECM. Twenty-two treatment means from 7 experiments conducted at the William H. Miner Agricultural Research Institute in Chazy, NY were analyzed using linear regression in R version 3.6.3. Dietary forages included corn silage, hay crop silage, timothy hay, and wheat straw. Cows in the studies averaged (mean ± SD) 100.27 ± 14.54 d in milk, 27.98 ± 1.19 kg/d DMI, and 49.56 ± 2.49 kg/d ECM yield. Our second objective was to measure the difference between calculated peuNDF240 and the directly measured uNDF240 content of the pef fraction. Three studies from the database with existing diet samples (10 treatments) were analyzed for *in vitro* uNDF240 content of the pef fraction to determine a directly measured peuNDF240. The dietary uNDF240 had a moderately negative (P<0.001) relationship with DMI and ECM (r = -0.69 and -0.73 respectively). Linear analysis was used to predict DMI and ECM, of which uNDF240 accounted for 47 and 53% of the variance, respectively. Calculated peuNDF240 had a stronger relationship with DMI (y = -1.044x+33.3; r = -0.85, P<0.001) but slightly weakened the relationship with ECM (y = -1.697x+58.2; r = -0.66, P<0.001). The peuNDF240 accounted for 71 and 43% of the variance in DMI and ECM, respectively. For the second objective, there was a strong linear relationship (y = 1.79; r = 0.87, P=0.001) suggesting that measured peuNDF240 may be reliably predicted from the calculated value. Theoretically, the measurement should be equal to calculated peuNDF240;

however, we observed a slope of ~1.8. More data with a wider range of dietary NDF sources such as alternative grasses or legume-based diets is required to confirm this relationship. However, the use of peuNDF240 to predict DMI and ECM, whether calculated or directly measured, shows promise as a means to combine the effects of dietary particle size and degradability.

Literature Review

Characterizing the Rumen

There is an enduring need within the dairy industry to determine an improved method of optimizing forages to stimulate chewing, thereby promoting nutrient access and providing energy to lactating cows. The dairy cow has a unique four-compartment stomach consisting of the rumen, reticulum, omasum, and abomasum, which together facilitate anaerobic microbial fermentation at a temperature of approximately 39°C and a pH of 6 (Kraff, 2014).The rumen is an essential component of the digestive process for ruminant animals within a four-compartment stomach that serves as the primary site for microbial fermentation of feedstuffs (Kumar et al., 2014; Henderson et al., 2015). This fermentation is a critical step in the conversion of feed into milk (Nocek, 1997; Firkens et al., 2001). Occupying the majority of the left abdominal cavity, the rumen is the largest compartment in lactating dairy cattle, with a volumetric capacity of up to 80 liters in adult cattle (McDonald et al., 2002). The rumen is a complex microbial ecosystem consisting of billions of microbes, including bacteria, protozoa, and fungi, that work together to break down complex carbohydrates and other nutrients into simpler compounds that can be absorbed by the cow (Oba and Allen, 2003). The microbial population of the rumen is highly diverse and can adapt to changes in diet composition, feed intake, and other environmental factors (Jami and Rashi, 2019). The fermentation of feed in the rumen produces volatile fatty acids (VFAs), with the most important VFAs being acetate, propionate, and butyrate, which serve as the primary energy source for the ruminant animal (Van Soest, 1994; McDonald et al., 2011). The efficiency of rumen fermentation is influenced by a variety of factors, including the physical and chemical properties of the feed, the type and density of the microbial population, and the overall health and function of the rumen (Krehbiel et al., 2003). The dietary requirements of lactating dairy cattle are largely dictated by the needs of the rumen microbes, and providing a diet that can support their growth and fermentation can help to maximize milk production and support the overall health and well-being of the animals (Reynolds et al., 2003; Bauman, 2011; Tirado-Gallegos et al., 2016).

Lactating Dairy Cattle Dietary Requirements

An evergreen point of investigation in the literature is which and what percentage of ingredients should constitute the ideal dairy cow feed. Previous studies (e.g., Mertens, 1997; NRC, 2001) recommend dietary NDF content around 25-30% to promote optimal rumen function and overall cow health in this diet. Grant (2021) defines fiber as the slowly degraded or indigestible feed components that occupy space in the digestive tract. However, the presence of fiber alone does not suffice for optimal feed because digestibility is dependent on the source and quality of the forage fiber (Bell et al., 2001). To facilitate digestion of fiber, feed must be masticated at intake to begin breaking down the fibrous cell wall composed of pectin-bound cellulose, hemicellulose, and lignin (Van Soest, 1994). Despite being time-intensive for cows to pulverize, cows must still consume a sufficient quantity of fiber in their diet to promote overall gut microbiome stability (Grant, 2021). As a natural byproduct of routine microbial fermentation in the rumen, acid builds up regardless of the nutritional profile of a given dietary ration. Chewing behavior triggers the production of saliva that is then swallowed with each returned feed bolus to the rumen, which helps to maintain the proper pH and microbial population in the rumen (Beauchemin, 1991). Dairy cows are recommended to spend an average of around 8-10 hours per day ruminating (Grant, 2021), which is composed of smaller 30-60 second bouts in which the cow regurgitates and re-masticates many small feed boluses (Beauchemin, 1991). The bicarbonate in saliva then acts as a natural pH buffer, neutralizing the stomach. Rumination is thus essential to stabilizing the microbial population and gut health, contributing to overall cow well-being.

To support high levels of milk production, lactating dairy cattle require a balanced diet that is high in energy and protein, with a balance of carbohydrates and other nutrients that can support the needs of the rumen microbes (Van Soest, 1994). The balance of these nutrients can be influenced by factors such as the forage to concentrate ratio in the diet, the particle size and degradability of the feedstuffs, and the presence of additives such as buffers or direct-fed microbials (Hristov et al., 2017). In addition to the rumen, the digestive tract of lactating dairy cattle includes the reticulum, omasum, and abomasum, which work together to further break down feedstuffs and absorb nutrients (NRC, 2001). The abomasum serves as the true stomach where digestion of proteins and other nutrients occurs. To ensure optimal milk production, animal health, and reproduction, lactating dairy cattle have specific dietary requirements for energy, protein, fiber, minerals, and vitamins. The National Research Council (NRC) provides guidelines for these dietary requirements based on the cow's body weight and milk production level i.e. an early lactation dairy cow would require approximately 16.5-17.5% crude protein, 30-35% neutral detergent fiber, and approximately 1.6 Mcal of net energy for lactation (NE_L) per kilogram of dry matter (NRC, 2001). Meeting these dietary requirements is essential for optimal milk production, animal health, and reproduction.

Metabolic versus Physical Control of Feed Intake

The rumen of dairy cattle plays a crucial role in the initial digestion of food and serves as a reservoir for fermentative microorganisms. Two methods can be used to fill the rumen: chemical fill and physical fill. Metabolic, or chemostatic fill, of the rumen is a feeding strategy that involves adding easily digestible carbohydrates, such as grains or other concentrates, to the diet of dairy cattle to achieve higher milk production (Grant and Albright, 1995; Conrad, 1964). **Figure 1.1** (Conrad, 1964) illustrates the turning point where intake potential is regulated by distension (physical fill) versus chemostatic fill. These feedstuffs are rapidly fermented by the microorganisms in the rumen, leading to a rapid decrease in motility and pH (Beauchemin et al., 2009). While this can result in a temporary increase in milk production, it may also lead to drop in rumen pH that can cause a range of health problems further explored in Chapter 2 (Plaizier et al., 2008). On the other hand, the physical fill of the rumen is achieved by consuming fibrous feedstuffs, such as hay or pasture. These feedstuffs take longer to digest and provide a slower release of energy, leading to a more stable rumen pH and increased rumen motility (Beauchemin et al., 2009). Studies have shown that the physical fill of the rumen can help prevent acidosis and promote better animal health and milk production (Hristov et al., 2004; Zebeli et al., 2012). For example, dairy cows fed a high-fiber diet had higher milk production and improved milk fat and protein content compared to those fed a low-fiber diet (Yan et al., 2020). Similarly, cows fed a diet high in corn silage and low in concentrates had improved feed efficiency and milk production compared to cows fed a high-concentrate diet (Cerutti et al., 2021). Therefore, it is important for dairy farmers to carefully balance their animal's diets to ensure optimal rumen health and milk production.

In dairy cow nutrition, the composition of feed and forage is crucial in determining the rate and extent of ruminal fermentation and digestion. Neutral detergent fiber (NDF) and solubles (NDS) are two analytical measures commonly used to determine the composition of feed and forage (Zebeli and Ametaj, 2009; **Figure 1.2**). NDF indicates fiber that may be fermentable in the rumen and, when chemically assayed, fractionates into cellulose, hemicellulose, and lignin (Van Soest, 1994). NDF represents the total plant cell wall content, including hemicellulose, cellulose, and lignin, that forms structural rigidity termed "bulkiness" of a diet (Van Soest, 1994). NDF is commonly used to estimate feed digestibility and physical fill, as its components employ the chewing behavior necessary to fully break down the forage and increase the surface area over which microbial attachments can occur. NDF also influences the digestibility of a feedstuff; for instance, a feed with 20% NDF would be more digestible than a feed with 30% NDF. Researchers now have the laboratory capacity to fractionate NDF and quantify its structural components. Percentages of NDF in a lactating dairy cow diet tend to fall between 30 and 80% NDF (Buxton and Redfearn, 1997), the wide range encompassing variable forage quality and animal requirements.

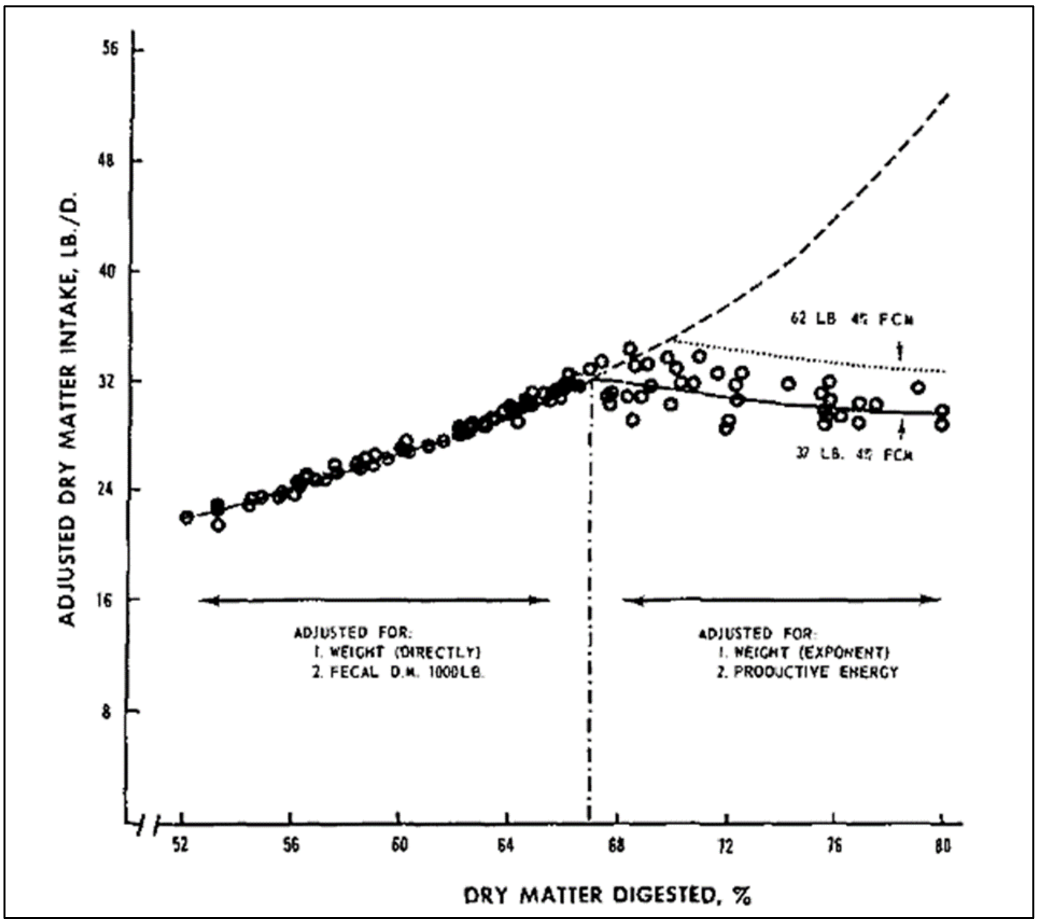


Figure 1.1. Intake as a Function of Digestibility, as Limited by Chemostatic Mechanisms and Distension of Rumen (via Conrad, 1964).

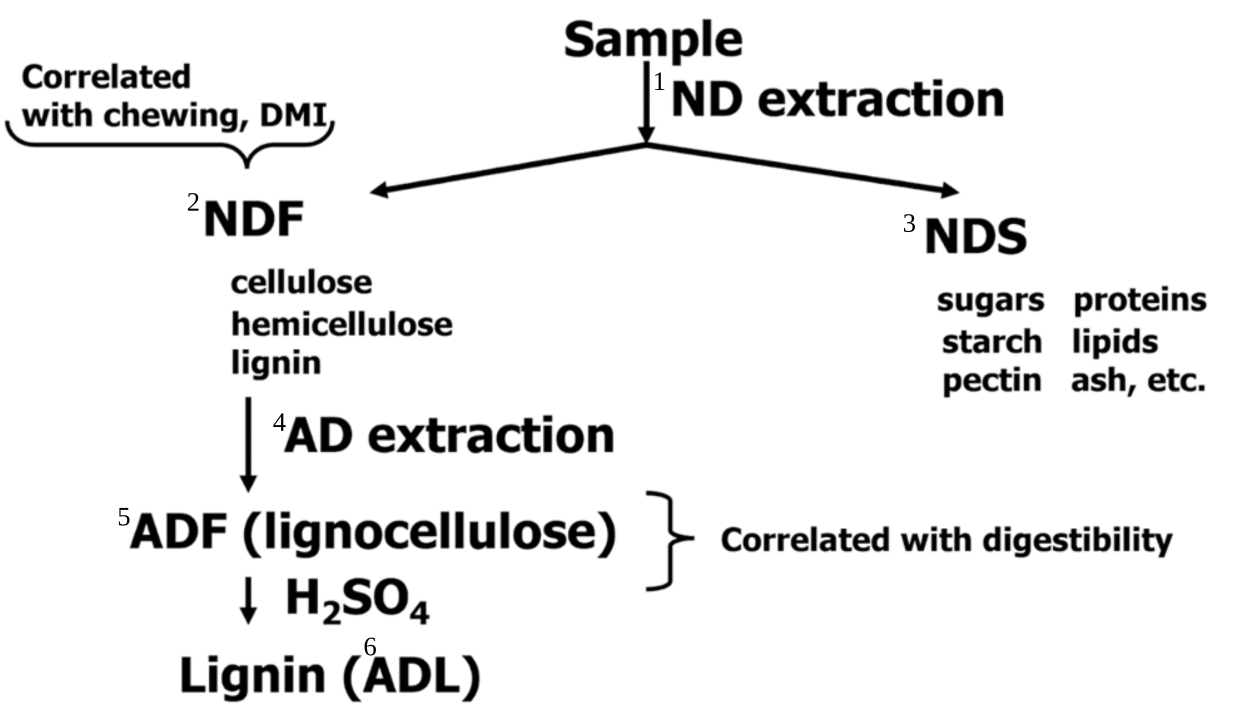
Analytical fractions and chemical constituents of dairy cow feed and forage composition

Alternatively, NDS represents the soluble, readily fermentable carbohydrate fraction of the plant material, including simple sugars, starch, and some pectin (Mertens, 2002). The chemical constituents of dairy cow feed and forage composition, including carbohydrates, proteins, lipids, minerals, and vitamins, can vary widely depending on various factors (Hristov et al., 2013). Carbohydrates, particularly starch and sugars, are the most important component of dairy cow feed, providing energy for maintenance, growth, and milk production (Jenkins et al., 2008). Proteins are essential for milk production and tissue growth, while lipids are another important energy source, especially in early lactation (Eastridge, 2006). Minerals and vitamins are also vital for the cow's health and milk production (Shaver, 2018).

Understanding ideal feed and forage composition is necessary for optimizing cow health and milk production. Former literature demonstrates that feed digestibility is strongly related to the composition of feed and forage, particularly the cell wall fractions (Bargo et al., 2002). For instance, cellulose, the most indigestible fraction of the cell wall, is highly resistant to microbial degradation due to its crystalline structure (Jung and Allen, 1995). Hemicellulose, on the other hand, is more easily degraded than cellulose due to its less organized structure (Van Soest, 1994). Lignin, which is highly resistant to microbial degradation, is often considered the most limiting factor to the digestibility of cell wall material (Jung et al., 2018) and is fractionated as acid detergent lignin (ADL). On the other hand, excessive amounts of NDS can lead to subacute ruminal acidosis (SARA), as the rapid fermentation leads to the production of excessive amounts of lactic acid and a drop in rumen pH (Oba and Allen, 2003). Therefore, understanding NDF and NDS composition and their impact on ruminal fermentation and digestibility is critical in designing optimal dairy cow diets.

Fractionation of NDF: Plant Cell Wall Components of Feed and Forages

The digestibility and passage rates of feed in the rumen of lactating dairy cows are affected by the fractionation of plant cell wall components (Huhtanen et al., 2015). The three primary components of the plant cell wall components include cellulose, hemicellulose, and lignin (Jung and Allen, 1995). Hemicellulose is more easily degraded due to its less-organized structure (Van Soest et al., 1991). It forms the amorphous matrix of the cell wall and includes xylose, arabinose, and other sugars (Jung et al., 2018). Lignin, a non-carbohydrate polymer, acts as a glue and is highly resistant to microbial degradation, increasing the structural rigidity of the cell wall (Latham, 1999; Kumar, 2018).



[1]ND = neutral detergent *[3]NDS = ND soluble* *[5]ADF = AD fiber*
[2]NDF = ND fiber *[4]AD = acid detergent* *[6]ADL = AD lignin*

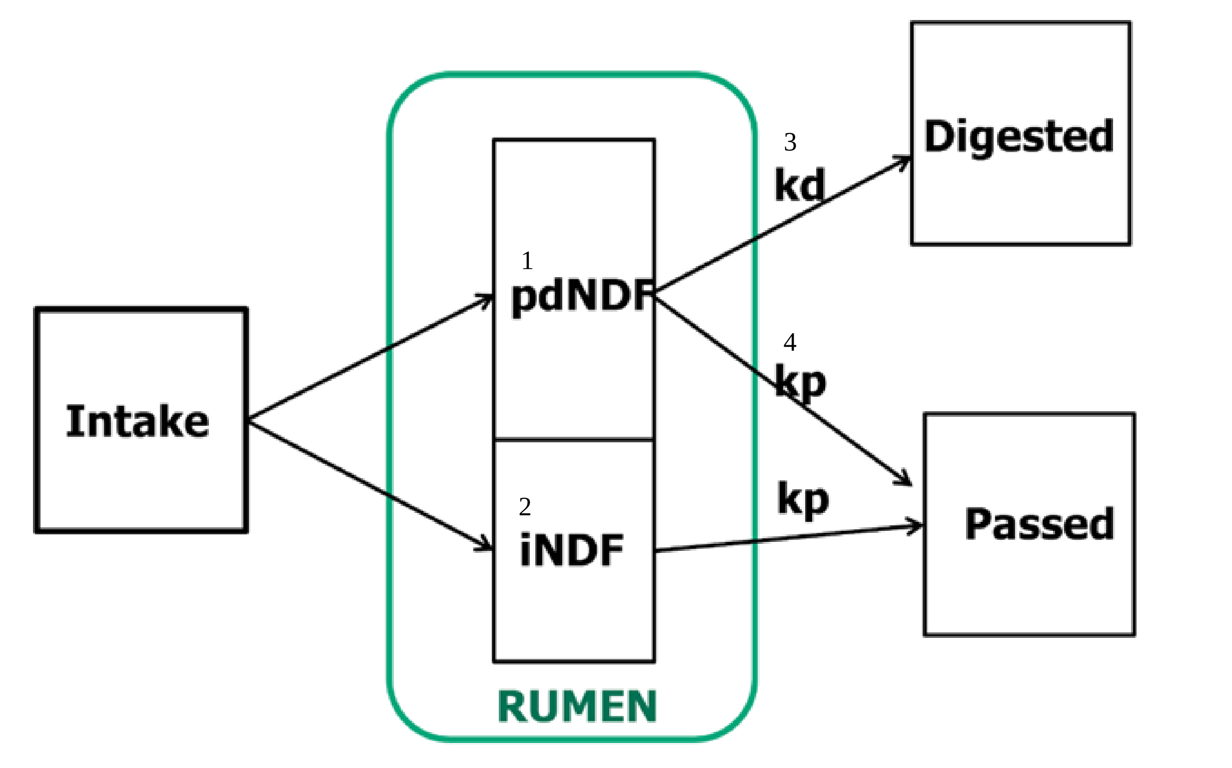
Figure 1.2. Simplified Depiction of Fiber Fractionation based on Degradability (Mertens and Ely, 1979; Mertens, 2011 via Grant, 2021).

Lignin is often considered the most limiting factor to the digestibility of cell wall material because of its hydrophobicity and chemically cross-linked matrix structure, which makes it resistant to microbial fermentation (Huhtanen et al., 2015, Raffrenato et al., 2010) and is considered the most indigestible fraction of the cell wall (Mertens, 1997). The ruminal fermentation and digestion of plant cell wall components in dairy cows can be improved by manipulating their physical and chemical properties, such as by altering the particle size and composition of the feedstuffs, or by using chemical or biological agents that can modify the cell wall components to improve their digestibility (Beauchemin et al., 1994; Jung et al., 2018). This is important for optimizing the nutritional value of dairy cow feed and for maximizing milk production.

Indigestible NDF (iNDF) Characterized by Digestibility and Passage Rates in the Rumen

There are both physical and chemical evaluations of NDF that account for particle size and digestibility of fiber. If a high total of NDF were fed to an animal but had a slow rate of digestion (k_d), the animal would consume a lower amount of fiber as opposed to a fast-digesting forage that readily supplies nutrients (Ball et al., 2001; **Figure 1.2**). The National Research Council (NRC, 2001) recommends that dairy cows be fed between 28-32% NDF, but never below 25%.

Neutral Detergent Fiber (NDF) is a commonly used term in animal nutrition, and it can be divided into two fractions - indigestible NDF (iNDF) and potentially digestible NDF (pdNDF). While cellulose, hemicellulose, and lignin are all considered to be NDF, lignification of forages inhibits the facility of microbial attachment and the cell wall carbohydrates that are most extensively crosslinked with lignin are thus categorized as indigestible (iNDF). As the concentration of lignin increases over the span of a fiber source's life, the woodiness of the fiber source rises as well (**Figure 1.2, Figure 1.3**). High prevalence of lignin, present in legumes and mature forages, consequently limits microbial degradation of cellulose and hemicellulose fiber fractions (Ball et al., 2001; Goeser, 2020). Thus, the iNDF fraction is the portion of NDF that is indigestible in the rumen and will pass out of the animal without being utilized for nutrient absorption.



[1]*pdNDF = potentially digestible neutral detergent fiber NDF)*
[2]*iNDF = indigestible NDF*
[3]*kd = rate of digestion*
[4]*kp = rate of passage*

Figure 1.3. Simplest Model of Digestion and Passage in the Rumen (Allen and Mertens, 1988 via Grant, 2021).

iNDF may play a role in rumen fill, which can promote chewing, saliva production, and rumination. Additionally, iNDF has been shown to increase as the forage matures, whereas pdNDF decreases. Several methods have been proposed to measure iNDF and pdNDF. One of the most commonly used methods for measuring iNDF is the *in situ* method, where samples of forage are placed in nylon bags and inserted into the rumen of a cow. The bags are retrieved after a set amount of time, and the residual NDF is measured. The portion of NDF that remains after the incubation period is considered iNDF. Another method used to measure iNDF is the *in vitro* gas production method. This method is based on measuring the amount of gas produced during incubation of the forage with rumen fluid. The iNDF is calculated as the residual NDF after the incubation period. The digestibility and passage rates of each fraction have significant implications for dairy cow nutrition. The passage rate of feed through the rumen is also an important consideration, as it affects the retention time of feed in the rumen and the amount of nutrients that can be absorbed. Various management strategies in dairy cow nutrition can be targeted at altering the physical and chemical properties of the feed to improve ruminal fermentation and digestion. For example, processing feed to reduce particle size can increase the surface area available for microbial fermentation and improve digestibility. Additives such as enzymes and buffers can also be included in the diet to improve rumen function and nutrient utilization.

Potentially digestible NDF (pdNDF)

The pdNDF fraction is the portion of NDF that can potentially be digested in the rumen. The pdNDF can be calculated as the difference between the total NDF and the iNDF. One of the most widely used methods to measure iNDF is the 240-hour *in vitro* NDF digestibility assay. In this method, the forage is incubated in a buffered solution with rumen fluid for 240 hours. The pdNDF is then calculated as the difference between the total NDF and the NDF that remains after the 240-hour incubation period. Studies have shown that the ratio of pdNDF to iNDF can have a significant effect on animal performance (Ferraretto and Shaver, 2018; Hoffman et al., 2003). For example, increasing the proportion of pdNDF in the diet can increase dry matter intake and milk production in dairy cows. Previous research has demonstrated that physically effective NDF (peNDF) has a positive correlation with rumen pH, whereas indigestible NDF (iNDF) has a negative correlation (Smith et al., 2010). This is important because low rumen pH is associated with a range of metabolic disorders, including subacute ruminal acidosis. Comprehension of the properties of different fractions of plant cell wall components is crucial in dairy cow nutrition, as it affects the rate and extent of ruminal fermentation, nutrient availability, and overall digestibility of feed. This knowledge can ultimately aid in the optimization of feed formulation and improve the efficiency of milk production in dairy cows. Further research in this field can also lead to the development of innovative feed additives and supplements that can enhance the utilization of plant cell wall components and improve the overall health and well-being of dairy cows. Moreover, a deeper understanding of the characteristics and functions of iNDF and pdNDF can inform the development of management strategies aimed at optimizing rumen function and digestion. With the global demand for milk and milk products on the rise, it is increasingly important to improve the efficiency and sustainability of milk production. Knowledge of plant cell wall components and their role in dairy cow nutrition can contribute to meeting these goals while promoting animal welfare and health.

Physical Analysis of Forage Fiber (peNDF)

The physical effectiveness factor (pef) is a measure of the particle size that is large enough to stimulate chewing, which includes eating and ruminating. It is defined as the particle size that is greater than or equal to 1.18 mm, as retained on a sieve, with dry, vertical shaking, according to Van Soest (1994). The Penn State Particle Separator (Lammers et al., 1996) and Ro-Tap are commonly used in practice to determine particle size distribution. Feed particles that are smaller than 1.18 mm are considered escapable (Grant, 2012) from the rumen without further mastication, and a dietary composition that is too high in volume of such particles could make an animal susceptible to fluctuations in ruminal pH, which could lead to decreased performance. Research has shown pef particles to be highly resistant to rumen passage (Mertens and Ely, 1979), which explains the required additional chewing behavior to break down the fiber components.

Fiber particle size greater than 1.18 mm is for this reason considered physically effective NDF (peNDF). The ruminal pH naturally drops during fermentation, and if the rumen becomes too acidic, then it is no longer stable for cellulolytic bacteria responsible for breaking down feed particles. Therefore, a sufficient quantity of physically effective fiber in the diet is vital because chewing behavior reduces the particle size of feed while also producing saliva that buffers the rumen pH, maintaining a stable anaerobic environment for rumen microbes that are responsible for extracting nutrients and energy from the disrupted cell walls. The time required to chew and swallow feed during eating is approximately 300 minutes per day in cows with natural feeding patterns (Grant, 2019). Resultantly, rumination is allocated an average of 510 minutes per daily time budget of a dairy cow (Grant, 2004; Miller, 2020). Physically effective NDF (peNDF) is obtained by multiplying a feed's NDF level by its physical effectiveness factor (pef * NDF = peNDF) (Mertens, 1997), and the cow requires time, space, and energy to break it down. In a previous study, Smith et al. (2018) demonstrated that a coarse chop of a feed with the same NDF value as a finer chop limited dry matter intake in dairy cows. This can be explained by the concept that the overall gut fill of the animal was higher, which allowed less room for further intake than would be available in the same amount of time with a finer chop where the particle size was pre-reduced before feeding. Additionally, the uniformity of NDF chemical composition cannot be assumed throughout the particle size of the diet (Mertens, 1997), which leads to the need to evaluate on a chemical level for degradability. It is, therefore, crucial to consider both the physical and chemical composition of feed when designing diets for dairy cows.

Forage quality is an essential aspect of dairy cow nutrition, and dietary fiber content is a crucial factor in determining feed quality (Kertz, 2006). Physical effectiveness of the fiber, as well as the chemical composition, play significant roles in determining its nutritional value (Grant, 2005). Physical analysis of fiber evaluates the size of the particle and the degree to which it is degraded by the cow (Mertens, 1997; Mertens, 2002). The peNDF fraction has been shown to be an essential component of dairy cow diets, and studies have shown a correlation between peNDF intake and milk production (Grant and Albright, 2001; Zebeli et al., 2012). The main function of the peNDF fraction is to stimulate rumen function, which helps maintain rumen pH and prevent rumen acidosis (Tafaj et al., 2018; Zebeli et al., 2008). Various studies have shown that high peNDF levels in dairy cow diets have positive effects on ruminal pH and microbial activity (Nozière and Michalet-Doreau, 2010; Zebeli and Taraj, 2014; Huhtanen and Hetta, 2013). Increasing the peNDF content of the diet has been shown to improve rumen pH, increase milk production, and reduce the incidence of subacute rumen acidosis (SARA) (Oba and Allen, 2003; Zebeli et al., 2012; Beauchemin et al., 1999). Furthermore, additional literature reported cows fed high peNDF diets had elevated rumen pH levels and fiber digestion, along with depressed VFA production (McCarthy et al., 2016; Khol-Parisini et al., 2014).

In addition to its effect on rumen health, peNDF also plays a significant role in the performance of dairy cows. Research has shown that higher peNDF intake has positive associative effects on improving milk yield, fat, and protein content (Grainger et al., 2013; Hristov et al., 2015). For example, increasing peNDF intake resulted in a 5.5% increase in milk yield and a 0.18% increase in milk fat content in one study (Chen et al., 2019). The size and degradation rate of the particle are key factors in determining the physical effectiveness of the fiber. Feeding diets with high peNDF content has positive effects on rumen health and performance in dairy cows. PeNDF intake is essential in maintaining rumen pH and preventing subacute rumen acidosis. High peNDF levels in the diet can lead to improved milk yield, fat and protein content, and overall cow performance. In conclusion, physical analysis of forage fiber, specifically the measurement of peNDF, is a critical tool in evaluating forage quality and cow nutrition. Feeding dairy cows with diets containing high peNDF levels has been shown to have positive effects on rumen health, milk production, and overall cow performance.

Chemical Analysis of Forage Fiber (uNDF)

The degree of NDF digestion in the rumen is frequently assessed as undegradable NDF at 240 hours of *in vitro* fermentation (uNDF240), which represents the portion of NDF that can only exit the rumen through digesta passage. Because NDF undergoes slow turnover, it contributes to rumen gut fill, and when it accumulates, it poses a challenge to the animal because it leaves less room for additional feed intake. Dry matter intake (DMI) is a critical factor influencing a cow's performance (Mertens, 2015), including the energy and nutrients required for milk and milk component production (Goeser, 2020). Van Soest first introduced the fractionation system in 1967, which allowed for the separation of fiber into indigestible NDF (iNDF) and potentially digestible NDF (pdNDF) fractions, creating a reasonable chemical method to analyze forages (Van Soest, 1967; Waldo et al., 1972). Because it is not feasible to measure iNDF directly, uNDF is used as an approximation (Mertens, 2016; Raffrenato et al., 2018). Dietary NDF occurs in slow and fast fermenting forms (Cotanch et al., 2014), which are usually associated with DMI estimates. It is generally assumed that uNDF240 and perhaps the slow-NDF fractions are mainly responsible for limiting DMI (Cotanch et al., 2014).

Forage is a critical source of nutrients for ruminants, with its fiber content serving as an essential energy source for microbial fermentation in the rumen. Measuring the fiber content of forage, particularly the undigestible fiber, is critical to evaluate its nutritional quality for ruminants. One of the most used measurements of fiber content is the neutral detergent fiber (NDF), which consists of cellulose, hemicellulose, and lignin (Van Soest et al., 1991). However, not all NDF fractions are indigestible, and recent research has focused on identifying more precise measurements of fiber to better evaluate forage quality (Huhtanen et al., 2015). One of the more recent developments is the use of the undegradable NDF (uNDF) fraction as a more accurate measurement of forage fiber content (Casler et al., 2015). uNDF is measured with long-term incubations by using neutral detergent solution with heat-stable amylase and sodium sulfite (Raffrenato et al., 2018). Several studies have examined the relationship between uNDF and animal performance in dairy cows (Oba and Allen, 1999).

However, the use of uNDF as a sole measurement of fiber content is limited, as it does not take into account the physical characteristics of the fiber such as particle size and chewing time (Zebeli and Ametaj, 2010). Therefore, researchers have proposed combining uNDF with a physical measure of fiber, such as physically effective NDF (peNDF), to provide a more accurate evaluation of forage quality (Mertens, 1997). The combination of uNDF and peNDF into one measurement, known as peuNDF240, has been shown to improve the prediction of dry matter intake and milk production in dairy cows (Miller et al., 2020). Overall, the use of uNDF as a measurement of fiber content in forage has shown promise in providing a more accurate evaluation of forage quality for ruminants (Casler et al., 2015). However, combining uNDF with a physical measure of fiber, such as peNDF, provides an even more precise evaluation of forage quality and its potential impact on animal performance (Zebeli et al., 2012).

Relationship Between peNDF and uNDF (peuNDF240)

Fiber content is a critical factor in dairy cattle nutrition and health, and physically effective neutral detergent fiber (peNDF) is an important parameter used to describe fiber. PeNDF however also contains varying amounts of undigested and indigestible NDF, which may not accurately reflect its true composition (Dann et al., 2017). To address this limitation, peuNDF240 was proposed, which represents physically effective and undigested NDF at 240 hours and has been suggested as a better predictor of forage digestibility and its potential effects on dairy cow performance (Grant, 2019; Miller et al., 2020).

The work of Smith (2018) indicated that uNDF was related to ECM and DMI. When Smith included the physical component of fiber in with the chemical component through simple calculation, pef * uNDF240 = peuNDF240, the relationship became significantly tighter. However, an even more recent study found uNDF to be non-uniformly distributed (Smith et al., 2021) within an overall linear regression developed using 5 previous studies conducted at the W. H. Miner Agricultural Research Institute that accurately predicted average DMI and ECM values via peuNDF240 (Miller et al., 2020). The data points reported by Smith (2021) were clustered around 4% peuNDF240 while the data from the other studies ranged between 5 and 8%. In comparing the relationships of uNDF240 and peuNDF240 to DMI and ECM within the 5 corn silage based studies, the addition of a physical component of analysis to uNDF240 significantly tightened the relationship with both DMI and ECM. DMI’s relationship changed from 32.7 to 60.4% when comparing uNDF240 and peuNDF240, respectively. For ECM, the relationship with peuNDF240 is 20% stronger than it was with uNDF240, increasing from 58.5 to 77.8%. The linear relationship between ECM and peuNDF240 suggested that for every 1% increase in peuNDF240, there was a predicted 2.229 kg/d drop in ECM.

With the greater use of peuNDF240 the non-uniformity recently reported (Smith et al, 2021) poses a highly interesting question for members of the dairy industry. Therefore, this research project will seek to determine if non-uniformity of uNDF240 across particle size fractions is unique to Smith's study or if it is a more common issue. This information will aim to clarify if there is still value in the simple mathematical calculation approach or if the chemical analysis needs to become the prominent method of evaluating peuNDF240. Previously, peuNDF240 was calculated by multiplying the pef fraction of a fiber with the uNDFom analysis. However, in the case of corn silage based TMR, we assume that diets will have roughly equivalent NDF values for the whole sample and top particles, and therefore aim to conduct a second method of evaluating peuNDF240 by directly measuring the uNDF240 organic matter (uNDFom) of only the pef fraction of each TMR for comparison. However, peuNDF240 is affected by both the physical and chemical properties of the feed, and the relationship between peuNDF240 and animal performance can vary depending on the feed composition (Miller et al., 2020; Smith et al., 2021). Some studies have reported different peuNDF240 values for different feedstuffs, indicating that it may not be a universal measure of fiber effectiveness.

In conclusion, peuNDF240 can serve as an accurate predictor of DMI and ECM in dairy cows and can provide a better indication of fiber effectiveness than either peNDF or uNDF alone. Thus, it is important to recognize that peuNDF240 may vary depending on the composition of the feed, and more research is needed to fully understand its implications on animal performance (Miller et al., 2020; Smith et al., 2021; Zebeli et al., 2012).

Traditional Methods: Calculated peuNDF240

PeuNDF240, a measure of physically effective NDF particles greater than 1.18mm in length after 240 h *in vitro* fermentation, is an important factor in dairy cow nutrition and health.

Traditional methods for measuring peuNDF240 involved calculating it from the proportion of particles retained on a 1.18 mm screen and using uNDF240om analysis (Van Soest et al., 1991). This calculation method is widely used because it is relatively simple and cost-effective. However, it has some limitations that should be considered. The calculation method assumes that all particles that pass through the 1.18 mm screen are digestible, and all particles retained on the screen are indigestible (Grant, 2019). However, beyond particle size, factors such as plant species, maturity, and storage conditions can also affect peuNDF240 and may not be fully accounted for in the traditional calculation method (Zebeli et al., 2012).

Despite these limitations, the traditional method of calculating peuNDF240 remains widely used and is still a useful tool for dairy farmers and nutritionists (Grant, 2019). However, researchers are continuing to develop and refine methods for measuring peuNDF240 to account for nonuniform distribution of uNDF fractions within the physically effective fiber fractions. In conclusion, while the traditional method of calculating peuNDF240 has some limitations, it remains a useful tool for measuring this important parameter in dairy cow nutrition (Van Soest et al., 1991). Continued research in this area will help to further refine methods for measuring peuNDF240 and improve dairy cow nutrition and health (Kammes et al., 2019).

New Methods: Measured peuNDF240

Forage quality is a critical factor in dairy cow feeding and management, and the measurement of peuNDF240 is a vital component in determining the quality of forages. Traditional methods for calculating peuNDF240 involve the use of pef fraction and uNDF240om analysis. However, these methods have limitations, including the potential for errors due to variable conditions and labor-intensive procedures. Recently, new methods have been developed to directly measure uNDF240om in the pef fraction, which eliminates the need for calculation and provides more accurate results. Direct measurement of uNDF240om in the pef fraction involves the use of a centrifuge to separate the pef fraction from the forage sample. The uNDF240om is then measured in the pef fraction using wet chemistry analysis. This method has several advantages, including the elimination of the need for calculation, increased accuracy, and reduced labor and time requirements. The development of the direct measurement method has significant implications for dairy cow feeding and management. The more accurate and consistent results provided by the direct measurement method can help farmers make more informed decisions about the quality of forages, which can have a significant impact on animal health and performance. Additionally, the reduced labor and time requirements of the direct measurement method can save farmers valuable resources and improve efficiency. In conclusion, the direct measurement of uNDF240om in the pef fraction is a promising new method for measuring peuNDF240 in forages. It offers several advantages over traditional methods, including increased accuracy, precision, and efficiency. Further research is needed to validate the effectiveness of the direct measurement method in different environments and conditions, but it is clear that this method has significant implications for dairy cow feeding and management.

Forage Quality Assessment

Forage quality is a critical factor that affects the productivity and health of dairy cows (Ball, 1998). It is defined as the nutritional value of forage, which is dependent on the type of forage, the stage of maturity at harvest, and the weather conditions during the growing season (Mertens, 2002). Digestibility (the extent to which forage is absorbed as it passes through an animal’s digestive tract) varies greatly. Immature, leafy plant tissues may be 80 to 90% digested, while less than 50% of mature, stemmy material is digested (Ball et al., 2001). The higher the palatability and forage quality, the higher the intake. (Ball et al., 2001). Intake potential decreases and NDF concentration increases as plants age. This is because NDF is more difficult to digest than the non-fiber components of forage. Also, the rate at which fiber is digested slows as plants mature. Therefore, digestion slows dramatically as forage becomes more mature (Ball et al., 2001).

Extensive research has been conducted on forage quality and its influences on animal health and performance, which has provided valuable insights into the optimal feeding practices for dairy cows (Collins et al., 1999). One of the primary focuses of research on forage quality has been on the impact of fiber on forage quality (Jung et al., 2015). In a study published in the Journal of Dairy Science, it was found that fiber content in forage had a significant effect on dry matter intake and milk production (Mertens and Martin, 1983). Increasing the fiber content from 30 to 40% in alfalfa hay reduced the digestibility of the fiber, leading to a decrease in dry matter intake and milk production (Weiss et al., 2002). This finding highlights the importance of balancing the fiber content in dairy cow diets to maximize nutrient utilization and milk production (Davis and Madsen, 2012; Ogunade et al., 2018).

Another key factor that affects forage quality is the stage of maturity at harvest. Researchers studied the influence of plant maturity on the nutritive value of alfalfa hay and found that the fiber content increased as the plant matured, resulting in a decrease in digestibility and energy content (Ball and Collins, 1980; **Figure 1.4**). The researchers also reported that the protein content decreased, which could impact milk production and overall animal health (Jung et al., 2015). This research suggests that it is critical to harvest forage at the optimal stage of maturity to maintain the nutrient content and maximize the productivity of dairy cows (Kung and Muck, 2012; Lauer, 2015). In addition to the fiber and maturity content of forage, the weather conditions during the growing season also affect forage quality (Sinclair et al., 2014).

Researchers examined the effect of weather conditions on the nutritive value of corn silage and found that drought conditions during the growing season resulted in a lower yield and higher fiber content, reducing the digestibility and energy content of the forage (Liu et al., 2019; Martin and Mertens, 1988). This study emphasizes the importance of considering weather conditions when planning forage harvest to ensure optimal forage quality for dairy cow feeding (Licitra et al., 1996).

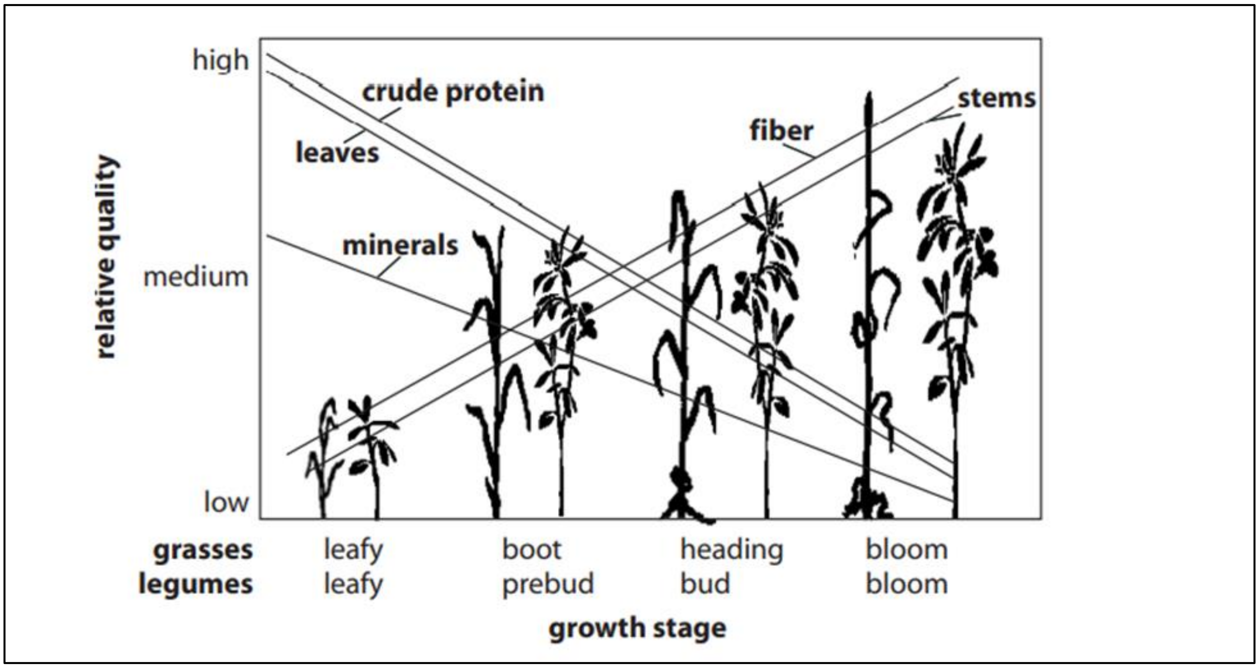


Figure 1.4. Inverse Relationship between Forage Crop Maturity and Quality (Blaser et al., 1986 via Ball et al., 2001).

Environmental conditions such as climate and weather may change the growth and development of forage crops. Ball et al. (2001) via Blasser et al. (1986) presented an excellent graphic description (**Figure 1.3**) of the relationship between plant maturity and forage quality. The longer a plant is allowed to grow, the more lignified the stalk of the plant becomes. The quality of the forage is improved with the more fibrous plants because they promote chewing behaviors associated with rumination and maintaining a healthy gut environment. However, as lignin is the component of the cell wall most associated with indigestibility (Van Soest, 1967;

Oba and Allen, 1999), more mature plants may exhibit higher readings of uNDF. Therefore, the growth stage at harvest may carry a significant impact on dietary intake and digestibility. How we can utilize the prediction model for peuNDF240 to benefit us here is looking for an ideal peuNDF240 % and choose chop size of forage particles accordingly. If there is a dry spell that causes less growth of crops are forced to be harvested early due to an influx of rain or extreme weather, and the forage is less mature, feeding larger particle sizes may replicate the peuNDF240 value considered necessary to stimulate sufficient chewing behavior and encourage a healthy gut and efficient production.

Furthermore, research explored the impact of forage quality on animal health and performance (Ball, 1998). It was found that high-quality forage is essential for optimal rumen function and digestive health in dairy cows (NRC, 2001; Bach, 2016). Additionally, feeding lower-quality forage led to a decrease in milk production, reproductive performance, and immune function (McCarthy et al., 2015; Burgos et al., 2011). This research highlights the critical role of forage quality in maintaining the health and productivity of dairy cows (Burgos et al., 2011; Firkins et al., 2001). Thus, it is important for dairy producers to consider these factors when formulating dairy cattle diets to optimize peuNDF240 content for better cow health, nutrition, and productivity.

Influence on Animal Performance

Forage quality is crucial for dairy cow nutrition, and its impact on animal performance has been extensively studied. The physical properties of forage, such as particle size, can affect dry matter intake (DMI) and milk production in dairy cows. Studies have also suggested that feeding a diet high in peuNDF240 can enhance rumen motility and reduce the risk of rumen acidosis (Grant et al., 2018). Additionally, maintaining optimal levels of peuNDF240 can enhance DMI, milk yield, and promote rumen health. The appropriate level of peuNDF240 varies depending on factors such as feed type, animal stage, and production level. The correlation between peuNDF240 and animal performance is complex and affected by several other factors, including diet composition, particle size, and feed management practices (Miller and Grant, 2018). Future research is needed to identify optimal peuNDF240 levels for different stages of lactation and production levels and investigate the underlying physiological mechanisms of its effects on animal performance.

Farmer, Nutritionist, and Industry Scale Impact

As a final remark, peuNDF240 has been extensively studied in the last five years and is an important measure of forage quality in dairy cow nutrition. Analyzed peuNDF240 was found to be a more reliable measure than calculated peuNDF240, however further research is needed to determine the most accurate and practical method for analyzing peuNDF240. A range of factors such as plant species, maturity, particle size and processing, storage and preservation, and environmental conditions influence peuNDF240, and understanding these factors could help optimize forage quality and improve animal performance. Correlations between peuNDF240 and dry matter intake, milk yield and composition, and animal health have been established, but further investigation is needed to understand the effects of peuNDF240 on specific milk components and digestive function.

The current research highlights the importance of accurately measuring peuNDF240 to optimize dairy cow nutrition and management. By accurately measuring peuNDF240, farmers and nutritionists can make more informed feeding decisions, leading to improved animal performance and health. Understanding the differences between traditional and newer direct measurement methods of peuNDF240 can aid in making informed decisions about how to feed cows and maximize their performance. Ultimately, the ability to accurately measure and analyze peuNDF240 can have important implications for milk production, milk composition, and overall cow health, and can lead to a more efficient and sustainable dairy industry.

Introduction

The dairy industry needs a more suitable method to predict feed intake and milk production for lactating cows. Economic, environmental, and social factors of sustainability contribute to profitability of a dairy farm. There is a need for high quality fiber, better nutrient management, and pressure from the public to feed more forage fiber than grain. The ability of neutral detergent fiber (NDF) components that are highly fermentable in the rumen to explain variation in dry matter intake (DMI) can be improved with measures of digestibility and particle size. Undigested neutral detergent fiber after 240 h of *in vitro* fermentation (uNDF240) is a laboratory measure of indigestible NDF that is related to rumen fill and NDF turnover. Physical effectiveness factor (pef) is the fraction of feed particles retained on ≥ 1.18 mm screen with dry, horizontal sieving. Combining these measures of indigestibility (uNDF240) and particle size (pef) may allow us a better prediction of DMI and ECM than either measure alone. Dietary uNDF240 of a Total Mixed Ration (TMR) can be multiplied by the pef fraction to calculate physically effective uNDF240 (peuNDF240). By analyzing the uNDF240 of only the pef fraction we can directly measure peuNDF240.

The first objective of this project was to expand an existing database (**Figures C.1-C.4** via Miller et al., 2020) of relationships between uNDF240 and peuNDF240 with DMI and ECM in high-producing Holstein cows. We predicted that the Miller et al. (2020) relationships would get tighter with the addition of three newly analyzed studies (Dann et al., 2021, Smith et a., 2018, and Smith et al., 2021). The second objective was to assess the relationship between calculated peuNDF240 and the directly measured uNDF240 content of the pef fraction of the three newly analyzed studies. We hypothesized that there would be no significant difference between the calculated and measured values of peuNDF240.

Materials and Methods

Experimental Design and Treatments

This study was conducted at the William H. Miner Agricultural Research Institute (Chazy, NY). Experimental samples come from 6 TMR samples from 2 previous studies at the Miner Institute (Smith et al., 2018, Dann et al., 2021) and 4 previously analyzed TMR samples (Smith et al., 2021). Each TMR was fractionated by particle size into three groups: whole contents, pef fraction (top), and non-pef fraction (bottom) according to retainment on a Ro-Tap dry, vertical sieve (**Table A.1**). Samples of each TMR were analyzed in duplicate. Additionally, a standard composite sample and a blank flask with only inoculum was used as a control for any existing fiber particles in the collected rumen fluid used for the inoculation.

The diets utilized to meet these objectives are reported in **Table 1.1.** Diets were composed of corn silage, hay crop silage, chopped wheat straw, and two diets containing substantial beet pulp. The total database compiled for Objective 1 consisted of 22 treatment means from seven experiments conducted at the William H. Miner Agricultural Research Institute in Chazy, New York. The 3 studies (10 treatment means) focal to Objective 2 are of only the pef fraction of the TMR was directly measured *in vitro* for comparison with the calculated peuNDF240 value.

Table 1.1. Database of seven studies conducted at Miner Institute, Chazy, NY with high producing lactating Holstein cattle fed primarily corn silage diets.

Study	Treatments	Forage, % of DM	Forages
Cotanch et al., 2014	High and low forage diets with brown midrib 3 (bm3) or conventional corn silage	65 vs. 50%	bm3 or conventional corn silage and hay crop silage
Miller et al., 2017	High forage diets with bm3 corn silage at varying amounts and coarse or fine straw	42 vs. 60%	bm3 corn silage and wheat straw
Smith et al., 2018	High forage and by-product diets with fine or coarse-chopped hay	61 vs. 47%	Conventional corn silage, timothy hay, and wheat straw
Coons et al., 2019	High forage diets with conventional, bm3 or bm3-floury corn silage	55.30%	Conventional, bm3, bm3-floury corn silage and hay crop silage
Miller et al., 2019	High forage diets with bm3 or conventional corn silage	56.80%	bm3 or conventional corn silage and wheat straw
Smith et al., 2021	High and low forage diets with bm3 or conventional corn silage	57.90%	bm3 or conventional corn silage and chopped wheat straw
Dann et al., 2021	Dual-purpose conventional corn silage hybrid or full floury leafy corn silage hybrid	69.00%	dual-purpose conventional corn silage hybrid or full floury leafy corn silage hybrid

Data Collection, Sampling Procedures, and Analytical Methods

Frozen samples of TMR from Dann et al., 2021 were thawed and equally composited by trial period. A 600 mL sample was dried at 55°C for 36-48 h and then ground through a 1 mm screen (Wiley mill; Arthur H. 39 Thomas, Philadelphia, PA) for the whole TMR. A second portion was separated into two 300 mL samples to be dried at the same temperature and time points. Both were vertically sieved (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering, Inc., Mentor, OH) to separate particle sizes above and below the pef gradient of 1.18 mm (**Table A.1**). Top (Ro-Tap≥1.18mm) and bottom (Ro-Tap<1.18mm) samples were then composited and ground separately through a 1 mm Wiley mill screen. Smith (2018) samples were already dried and ground prior to the start date of this study. Sample dry matters were determined by weighing 1-1.5 g of the respective sample and drying in a forced-air oven at 105°C for 24 h.

A 0.5 g sample in duplicate was analyzed for uNDF240 using the *in vitro* fermentation system (Raffrenato et al., 2018) as modified by the Miner Institute. In total, one run of the experiment contained 108 samples. Forty mL of the chemically prepared buffering reagent was added to each 125 mL Erlenmeyer flask then evacuated with CO_2 to create an anaerobic environment. Rumen fluid was manually collected from below the digesta mat of two cannulated, high-producing lactating Holstein cows fed a corn-silage based diet (approximately 500 mL each). Animal handling and maintenance were approved by the W.H. Miner IACUC. The digesta was strained through 4 layers of cheesecloth and nylon hosiery before inoculation of 10 mL into each of the sample flasks. All flasks were then closed with stoppers connected to a CO2 Nalgene Vacuum Manifold (Thermo Scientific 09-752-5) and placed in a 40°C water bath for 240h. Each flask was swirled gently once a day.

Samples were removed from the water bath after 240 h and placed on ice to stop fermentation. Glass microfiber filters (VWR 28297-289) were individually weighed and labeled prior to the filtration. Each sample was filtered using the same methodology (Raffrenato et al. 2018) that employed detergent, amylase, boiling water, and suction in order to retain all of the particles left in each flask after 240 hours. At the end of each filtration, the filter was folded around the sample residue and dried in a foil tin at 105°C for 24 h before it was weighed to calculate uNDF240. The final step was to place the dried filter in a ceramic crucible and bake at 500°C for 3 h in a muffle furnace. The filter was weighed again to calculate the uNDF240om.

Energy corrected milk and DMI from all three studies were retrieved from the Miner Institute database to analyze against the TMR uNDF240 and TMR calculated peuNDF240 values determined during this project. Milk samples were collected and electronically recorded (Provantage Information Management system; Bou-Matic, Madison, WI) daily over a nine-day period (Smith et al., 2018). The milk data were automatically summarized with mathematical equations to provide individual milk yields at each milking. The same method was used to collect milk yield data from Dann et al., (2021). DMI was also determined in each study from a net calculation of daily feed offered and refused. For the sake of quality control, all data sets within the database were determined using the same chemistry.

Statistical Analysis

All data collected throughout the study from Dann et al., 2021 and Smith et al., 2018 were analyzed individually and in conjunction with data from Smith et al. 2021 and the previously published 5 study database (Miller et al., 2020; Supplementary **Figures C.1-C.4**) using linear regression procedures in R (version 3.6.3). Additionally, all dietary treatments from the Smith et al., 2018 study were reanalyzed for quality control and consistent chemistry across all studies so the physical and chemical fiber measurements for that study were updated in the database.

Results and Discussion

All three measurements, uNDF240, calculated peuNDF240, and directly measured peuNDF240, were considered for comparison between the three studies (Dann et al., 2021, Smith et a., 2018, and Smith et al., 2021) before addition to the total database (**Table A.1**). Animal response values used to evaluate the three measurements are reported in detail for all seven studies in **Table C.1**. It was found that calculated peuNDF240 tightened the relationship with DMI but did not change as the TMR uNDF when evaluating measured peuNDF240 of the three studies. All three measurements were strongly related to DMI, agreeing with related previous studies (Miller et al., 2020) allowing them to be used as a prediction model for DMI. As a cautionary note, only 3 studies were evaluated and compared in this exercise; hence this relationship may change as more data is included in the prediction model. When evaluating ECM, calculated peuNDF240 accounted for half of the variability of both TMR uNDF and measured peuNDF240. As dry matter intake is the very first event associated with the fiber fractions and diet composition (Rosler, 1994; Grant, 2004), it makes sense that it would hold a stronger relationship with the chemical and physical components of the diet when compared with ECM.

The main objective of this study was to add the three novel studies to the existing (Miller et al., 2020) database. All data for the Smith et al., 2018 study were recalculated to evaluate measured peuNDF240 values for the diets as well (**Table A.1**). The linear regressions between uNDF240 and calculated peuNDF240 and ECM or DMI from the previous database (Miller et al., 2020) were corrected with the fiber analysis for Smith et al. (2018) completed during this project (**Figures A.2-A.5**). After including data from Dann et al. (2021) and Smith et al. (2021), the relationship of calculated peuNDF240 with DMI improved (R = -.85, P<.001), increasing the R squared value by 8% from the previously established database (**Table A.1; Figures A.3** and **C.2**), therefore supporting the hypothesis for Objective 2. This relationship may be interpreted by stating that calculated peuNDF240 accounts for 71% of the variability in DMI. The combined analysis of physical and chemical components provided a stronger relationship for predicting animal performance than uNDF240 alone (R=-.69, P<.001) which only accounted for 47% of the variance.

In contrast to the DMI data, as more studies were added to the database, the relationship between calculated peuNDF240 and ECM (**Figure A.5**) decreased the R-squared value by 22% from the original database (**Figure C.4**) to now only account for 43% of the variance. There is still a moderate relationship (R = -.66, P<.001) that explains a 1.7 kg/d decrease in ECM for every 1% of peuNDF240. It is important to consider the limited sample size when making prediction statements. When evaluating ECM, the relationship with uNDF240 alone was determined to be stronger (R = -.73, P<.001) within the 7-study database (**Figure A.3**); however, the relationship only accounted for 53% of the variance (5-study database in **Figure C.3**). Nevertheless, neither relationship with ECM was as effective as the relationship of calculated peuNDF240 with DMI.

The greater correlation with DMI as compared with ECM may be best explained by the fact that dietary composition, forage quality, chop or particle size, or another factor such as environmental conditions all may contribute to the feed’s digestibility (Ball et al., 2001). In contrast, ECM not only accounts for multiple factors of milk production but is impacted by animal physiology and body processes (Miller et al., 2020; Antanaitis et al., 2015).

Consequently, ECM will likely continue to be harder to predict than DMI as more studies are added to this database. The decreased R-square value included in the calculations was in fact foreseeable due to the ranging chemical and nutritional compositions among the seven study diets within the growing database. Notwithstanding the limitations, it is encouraging that the chemical and physical measurements of fiber may be useful parameters to predict DMI.

As for our second objective, it must be acknowledged that this relationship displays a limited amount of data from 10 treatment means of the 3 highlighted studies. We foresee the addition of more studies to analyze this relationship as one important to continue because there was a strong linear relationship (r^2=.76) between the two measures. The results of this project established that, for the case of the three corn silage studies that evaluated true peuNDF240, the hypothesis for Objective 2 was not supported. Upon examination of results, there was a distinct difference between the calculated and measured values of peuNDF240. The slope of 1.79 (**Figure A.1**) may relate to a potential relationship between the two methods of evaluating peuNDF240. Theoretically, the measurement should be equal to the calculated value of peuNDF240, so we expected to see a 1:1 ratio. However, an observed ratio of 1.8 in turn led us to hypothesize a linear relationship nearing y = 2x as a future consideration once more corn silage-based studies are conducted.

Conclusions

With the expanded database, calculated peuNDF240 predicted DMI better than uNDF240, confirming and expanding upon the previous work of Miller et al., 2020. Both calculated and measured peuNDF240 hold promise for predicting DMI and ECM and there is a need for continued research to understand these relationships with a wider range of NDF sources. A major takeaway from this project was that calculated and directly measured peuNDF240 are not equivalent measurements. However, the ratio of their linear relationship indicated measured peuNDF240 may be reliably predicted from the calculated value. For farmers working with a TMR where the uNDF is not evenly distributed, this may mean that a straightforward ratio of 1.8 between the two measures of peuNDF240 may hold value and be quickly utilized to adjust ration contents for improved predicted performance. It was seen that measured peuNDF240 held a tighter relationship with DMI than calculated peuNDF240, however, both were very strong and may be used for prediction. Calculated peuNDF240 held a tighter relationship with ECM than measured peuNDF240, which was a moderate goodness of fit with negative relationship; however, the relationship between peuNDF240 and ECM became weaker after adding additional studies.

Limitations should be considered after reviewing the work of this project. There are few total studies included in the peuNDF240 database thus far so the prediction models for ECM and DMI should not be used as a standard calculation for industry usage yet. Additionally, more research with alternative NDF sources, such as high legume diets and their production responses, need to be evaluated. There may be a potential 2:1 relationship between true and calculated peuNDF240 that can be explored further with future study diets high in corn-silage. It would also be interesting and beneficial to the dairy industry to evaluate peuNDF240's relationship with feed efficiency and milk composition in future work. Lastly, after finding nonuniformity within studies that overall fit the linear regression of the completed database, future research may need to consider the effect of study when evaluating peuNDF240.

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CHAPTER 2: Use of natural organic acid as an alternative to feed antibiotics to alleviate subacute ruminal acidosis (SARA) in lactating cows.

Abstract

Occurrence of subacute ruminal acidosis (SARA) in dairy cows may eventually lead to decreased performance factors that affect profitability. Production of short-chain fatty acids (SCFA) inevitably causes depressed rumen pH due to acid accumulation, while SARA is defined by a drop between 5.2 and 5.5 or prolonged periods under 5.8. Our objective was to determine the effect of malic acid on rumen fermentation in mixed cultures of microbes fed diets varying in the forage to concentrate ratio. Three diets were formulated with corn silage and ground corn (DM basis) in the following ratios: 100% corn silage (CS), 70:30 corn silage: ground corn (LC), and 50:50 corn silage: ground corn (HC). The HC diet was prepared with the intent to induce a drop in pH that simulated SARA. The effect of malic acid (MA) was tested at four levels: 0, 4, 8 and 16 g/kg of diet DM. The 12 experimental diets were inoculated with mixed microbial cultures in an *in vitro* batch system for 6 and 24 h to assess the effect of treatment on rumen fermentation. Each diet was weighed in triplicate and the entire experiment was replicated (n = 2). Data were analyzed as a completely randomized block design using the PROC MIXED procedure of SAS version 9.4 (SAS Institute. 2013. Cary, NC). The main effects included the effect of diet, effect of malic acid and the interaction between diet x malic acid. Orthogonal contrasts were used to determine linear and quadratic effects of malic acid levels. Statistical significance was reported at $P \leq 0.05$ and tendencies were reported at $P < 0.10$. Increasing ground corn in the diet induced a drop in pH ($P = 0.01$ and 0.01, respectively) at both 6 and 24 h. Increasing levels of MA inclusion raised the culture pH at 6 h in the LC diet (P=0.02) as well as in the LC and HC diets at 24 h (P=0.03). Diet also significantly impacted SCFA accumulation, as the CS diet recorded the highest mol % of Acetate and Propionate and lowest Butyrate. Additional ground corn increased the DM % but reduced the ADF and NDF % digestibility.

Results from the study indicate that MA shows potential for stabilizing ruminal pH in diets with increasing quantities of concentrate and may in fact be a potential alleviant of SARA.

Literature Review

Starch versus Fiber: Differences in digestibility and passage potential

The dairy cow's digestive system is designed to optimize the digestion of forages, and to an extent, concentrate feeds such as grains, by means of microbial fermentation in the rumen. Both forages and concentrates have different rates of digestibility and passage potential in the rumen. The digestibility and passage potential of feed in the rumen influence the amount and quality of nutrients that are absorbed by the cow.

Starch and sugar, the primary components of concentrate feeds, are more easily digested in the rumen than forage fiber. Starch is rapidly fermented and produces propionate, which is a precursor for glucose synthesis. It is known that when the amount of propionate produced increases, the overall energy production of the cow is enhanced (Krause and Oetzel, 2005). Sugars are also rapidly fermented in the rumen, producing both propionate and lactate. However, excessive lactate production can lead to a decrease in ruminal pH, causing the onset of subacute ruminal acidosis (SARA).

In contrast, forage fiber has a slower rate of digestion in the rumen due to its structural and chemical complexity, which requires greater microbial action to break it down. The digestion of forage fiber leads to the production of acetate, which is a precursor for fatty acid synthesis (Hungate, 1966). Furthermore, fiber has a lower passage rate in the rumen, which allows for a greater time of exposure to rumen microbes, leading to greater microbial activity (Huhtanen and Hristov, 2009). The fermentation of fiber results in the production of volatile fatty acids, including acetic, butyric, and propionic acids, which are absorbed across the rumen wall and used as a source of energy for the cow (Van Soest, 1994). Although forages have lower digestibility and passage potential than concentrates, they play a critical role in maintaining the health of the rumen and the cow. The physical structure of forages stimulates rumination and salivation, which in turn aids in the maintenance of optimal rumen pH and function. Furthermore, forage has been shown to decrease the incidence and severity of subacute ruminal acidosis and laminitis in lactating dairy cows (Zebeli et al., 2012).

In summary, the digestibility and passage potential of feed in the rumen have a significant impact on the health and performance of dairy cows. Starch and sugar are rapidly fermented in the rumen and can enhance energy production, while forage fiber is more slowly fermented but provides essential structural and functional benefits to the rumen and the cow (Russell and Wilson, 1996). Therefore, a balance between the intake of concentrate and forage is crucial for the maintenance of optimal rumen health and the overall productivity of the cow.

Organismic Demand for High Energy Diets

Feeding high-grain diets to dairy cows is common in the industry, as it supplies a highly concentrated source of energy that supports high milk production. The starch in grains is rapidly fermented in the rumen, resulting in the production of volatile fatty acids (VFAs), which are the primary source of energy for the cow. Earlier studies have demonstrated that high-grain diets can significantly increase milk production and milk protein yield in lactating dairy cows (Drackley, 1999; Oba and Allen, 1999). Furthermore, high-grain diets can also result in a reduction in the amount of dietary fiber available for fermentation in the rumen. This leads to a decrease in the production of potentially detrimental fermentation products such as lactate and ammonia, which are known to contribute to the development of subacute ruminal acidosis (SARA) in dairy cows (Plaizier et al., 2008). However, it should be noted that high-grain diets can also increase the risk of SARA, especially if the diet is not properly balanced or if the cow is unable to adapt to the high-grain diet (Krause and Oetzel, 2006). In addition to supporting high milk production, high-grain diets can also result in a increase in the cow's body condition score (BCS) during early lactation, which is associated with improved reproductive performance. Research has proven that cows with a lower BCS at the time of breeding have a shorter interval from calving to conception and a higher probability of pregnancy than cows with a higher BCS (Roche et al., 2000; Macdonald et al., 2005). Overall, high-grain diets can offer benefits to the dairy industry including increased milk production and improved reproductive performance. However, it is important to balance the dietary rations appropriately and monitor cows for signs of SARA to ensure optimal animal health and productivity.

Transition and peak lactation cows have specific dietary requirements to support their increased energy and nutrient needs. During the transition period, cows experience a negative energy balance, which can lead to metabolic disorders and reduced milk production. The diet for transition cows should be high in fiber and low in non-fiber carbohydrates to maintain rumen health and prevent acidosis (Coppock et al., 2010; Grummer, 1995). For peak lactation cows, the diet should be high in energy, protein, and minerals to support increased milk production (Grummer, 1995). These changes in dietary requirements are essential for maintaining the health and productivity of dairy cows during different stages of lactation.

During early lactation, dairy cows partition more nutrients toward milk production and less toward body maintenance and growth, resulting in negative energy balance and mobilization of body fat reserves. As lactation progresses toward mid-lactation, cows become more efficient at utilizing nutrients for milk production and maintaining body weight, resulting in positive energy balance. This shift in nutrient partitioning is critical for maximizing milk production while minimizing negative health consequences, such as metabolic disorders (Bell, 1995; Bauman and Currie, 1980; Grummer, 1995).

Industry Pressures Driving the Need for High Energy Diets

The dairy industry has seen a shift toward feeding higher grain diets over the past several decades, driven by a variety of factors including advancements in feed processing technology, increased demand for milk and milk products, the desire to increase milk production per cow, and the effects of global warming on forage availability and quality (Bauman and Currie, 2013; West et al., 2013). However, there are concerns about the environmental and animal welfare implications of this shift (Capper, 2013).

One of the primary factors driving the shift toward higher grain diets is the development of improved feed processing technology. This technology allows for the efficient processing of grains, which can improve their digestibility and reduce the risk of digestive disorders in cows (Bauman and Currie, 2013). For example, the use of steam flaking or rolling can increase the digestibility of corn and other grains, allowing cows to better utilize the energy and nutrients in their feed.

In addition, the dairy industry has experienced increased demand for milk and milk products, which has led to a desire to increase milk production per cow. Higher grain diets can help achieve this goal by providing cows with more energy and nutrients, which can translate to increased milk production. Additionally, by increasing milk production per cow, dairy farmers can reduce the number of cows needed to produce a given amount of milk, which can help reduce feed and labor costs (Bauman and Currie, 2013).

However, the shift toward higher grain diets has also been driven by the effects of global warming on forage availability and quality. Rising temperatures and changing weather patterns have led to reduced forage availability and quality, as well as decreased crop yields in certain regions (West et al., 2013). This creates a shortage of high-quality forage, which is a key component of a balanced diet for dairy cows. As a result, dairy farmers have turned to higher grain diets, which can provide a more concentrated source of energy and nutrients compared to forage. Grains such as corn, barley, and wheat are typically higher in energy and lower in fiber compared to forage, making them an attractive alternative when forage is scarce or of lower quality (Bauman and Currie, 2013; West et al., 2013).

Despite the potential benefits of feeding higher grain diets, there are concerns about the environmental and animal welfare implications of this shift. One of the primary environmental concerns is the increased use of resources, such as water, land, and energy, needed to produce the grain used in these diets (Capper, 2013). In addition, the use of high-grain diets has been linked to increased greenhouse gas emissions, primarily due to the production and transportation of the grain (Capper, 2013).

There are also concerns about the potential negative impact on animal welfare associated with feeding higher grain diets, namely the risk of digestive disorders such as SARA. An excess of fermentable carbohydrates in the diet can induce rumen pH drops indicative of SARA, which can culminate in inflammation and damage to the rumen epithelial lining (Kleen and Cannizzo, 2012). This can result in reduced feed intake, decreased milk production, and other health problems in cows.

To mitigate the risk of SARA and other digestive disorders, it is important to carefully balance the forage to concentrate ratio in the diet, ensuring that cows receive enough fiber to maintain rumen health and function (NRC, 2001). Additionally, other factors such as particle size and degradability of the diet, cow comfort, and on-farm management practices can also play a role in maintaining gut health and productivity (Kleen and Cannizzo, 2012; **Figure 2.1**).

Risk of SARA[1]	Increased	Marginal	Low
NDF[2]	25	28 – 32	35
Forage NDF	16	20 – 25	27
peNDF[3]	18	21 – 23	25
Ruminal pH[4]	< 5.6	5.8 – 32	> 6.4
NFC[5]	45	42 – 35	30
NSC[6]	35	32 – 28	25

Forage considerations

longer	←	Forage particle size	→	shorter
slower	←	Rate of fiber digestion	→	faster

Concentrate considerations

slower	←	Ruminal starch digestiblity	→	faster
more	←	High fiber byproducts[7]	→	less
more	←	Buffers	→	less

Management and environmental considerations

minimal	←	Heat stress, stall and bunk over-crowding	→	severe
excellent	←	stall comfort	→	poor
TMR fed	←	feeding system	→	grain fed
consistently accurate	←	forage DM	→	infrequently variable accuracy
entire height or face of silo	←	silage obtained for a load of feed	→	regions within the silo

[1]SARA = subacute ruminal acidosis
[2]NDF = neutral detergent fiber
[3]peNDF = physically effective NDF
[4]Ruminal pH
[5]NFC = non-fermentable carbohydrates
[6]NSC = non-soluble carbohydrates

Figure 2.1. Forage Fiber Component Levels and Farm System Considerations Associated with Risk of Sara (via Stone 2004). *Note:* Relative impact factors of dietary components (%DM), forage to concentrate ratio, and husbandry practices on rumen pH and thus the occurrence of SARA in dairy cows. Footnotes are explained in depth within the original article. Sourced from "Nutritional Approaches to Minimize Subacute Ruminal Acidosis and Laminitis in Dairy Cattle" by Stone, 2004, p. E17, Fig. 1. Copyright (2004) by *American Dairy Science Association*.

Overall, the shift toward higher grain diets in the dairy industry is a complex issue with multiple drivers, including global warming, changing dietary preferences, and the need to increase efficiency and productivity. While high-grain diets have conditionally shown promise, there is a need for ongoing research and development of alternative feeding strategies to ensure the long-term health and welfare of dairy cows, as well as the sustainability of the dairy industry (Beauchemin and Kebreab, 2019). This is particularly important as the environmental impact of the dairy industry comes under increasing scrutiny, with concerns around greenhouse gas emissions, land use, and water pollution (Capper, 2013). As such, it is important for the industry to consider the environmental impact of its production practices, including feed production and utilization, and to explore alternative strategies that can improve efficiency and reduce waste. For example, the use of precision feeding technologies, such as real-time monitoring and adjustment of feed intake, can help reduce overfeeding and waste, while improving animal health and productivity.

Similarly, the use of alternative feed sources, such as by-products from the food industry and non-food crops, can reduce the reliance on traditional feed sources and provide additional environmental benefits, such as reduced greenhouse gas emissions and land use (Lie et al., 2019). For instance, research has shown that incorporating by-products from the ethanol and biodiesel industries, such as distillers grains and soybean meal, into dairy cow diets can reduce the carbon footprint of milk production (Capper et al., 2009). In addition, non-food crops such as switchgrass and miscanthus can be grown on marginal lands not suitable for traditional crops and thus sustainably source forage to reduce the environmental impact of dairy production (Beck et al., 2014). The dairy industry has a responsibility to consider the environmental impact of its operations and to implement sustainable practices that can help reduce its carbon footprint and improve the long-term health and welfare of dairy cows. By adopting alternative feeding strategies, implementing precision feeding technologies, and improving on-farm management practices, the industry can reduce waste, improve efficiency, and help mitigate the environmental impact of dairy production.

Impact of Subacute Ruminal Acidosis (SARA) on the Dairy Industry

Subacute ruminal acidosis (SARA) is a common digestive disorder in dairy cattle that results from feeding high-grain diets. SARA has been shown to have significant financial and health impacts on the dairy industry. From a financial perspective, SARA can result in decreased milk production, increased feed costs, and increased veterinary and treatment expenses. Numerous studies estimated that SARA costs the North American dairy industry approximately $500 million to upwards of $1 billion per year (Plaizier et al., 2008; Oetzel and Nordlund, 2010; Cook, 2018). This is due to reduced milk yield, decreased milk quality, and increased incidence of metabolic and digestive disorders such as laminitis, displaced abomasum, and liver abscesses. In addition, SARA can increase the risk of culling, which results in further financial losses for dairy farmers. From an animal health perspective, SARA can cause significant discomfort and lead to a decreased quality of life for dairy cows. The symptoms of SARA include decreased feed intake, decreased rumination, and diarrhea (Plaizier et al., 2012). SARA can also cause changes in the composition of the rumen microbiome, leading to a higher incidence of pathogenic bacteria and decreased production of beneficial bacteria. This can lead to a weakened immune system, increasing the risk of secondary infections and further health complications. Furthermore, SARA can result in the development of laminitis, a painful and debilitating condition that affects the hooves of dairy cattle (Cook et al., 2010). Laminitis can lead to lameness and decreased milk production, as well as necessitating costly treatment and increased risk of culling (Garrett et al., 1999). When net cost of SARA was further broken down, studies estimated the annual monetary loss per cow to range between $200 to $500 (Krause et al., 2019; Stone, 1999). Overall, the financial and health impacts of SARA are significant for both the dairy industry and individual farmers. The prevention and treatment of SARA are crucial for maintaining the welfare and productivity of dairy cattle, as well as the economic viability of the dairy industry.

SARA is considered the most prevalent metabolic disease in the dairy industry worldwide. The incidence of SARA in dairy cattle has been reported to range from 19% to 58%, which may vary based on the feeding and management practices of the farm (Zebeli and Metzler-Zebeli, 2012, Donovan, 1997; Abdela, 2016). Within the US system specifically, SARA is estimated within the range of 19% to 26% (Plaizier et al., 2008; Hall and Averhoff, 2000). The primary reason for the prevalence of SARA in the dairy industry is the shift toward feeding high concentrate diets to cows to maximize milk production. The high concentration of readily fermentable carbohydrates in these diets lowers the ruminal pH, which can lead to SARA (Zebeli et al., 2008). The fermentation of excess dietary starch produces lactic acid, which, when not efficiently removed or neutralized by the buffering system, accumulates in the rumen, further decreasing the pH. This acidic environment can lead to the death of the fiber-digesting bacteria in the rumen, reducing the digestion of fiber and thus limiting the cow's ability to meet her nutrient requirements (Enemark, 2008). Other factors which may contribute to the development of SARA in dairy cattle include a lack of physically effective fiber, inadequate buffering capacity of the diet, poor feeding management, and cow comfort (Nocek, 1997). In addition to its prevalence, SARA is a challenging disease to detect early. The symptoms of SARA are often subclinical, making it difficult to diagnose in its preliminary stages (Khafipour et al., 2009). Though data is representative of acidosis rather than SARA, **Figure 2.2** (Antanaitis, et al., 2015) demonstrates that significant reductions in milk production may occur multiple days prior to emergence of symptoms. This makes it challenging to manage and treat effectively, leading to long-term negative impacts on the cow's health and productivity. Overall, the prevalence of

SARA in the dairy industry highlights the importance of balancing the forage to concentrate ratio in dairy cow diets, maintaining adequate physically effective fiber, and managing the feeding and management practices on the farm. It also highlights the need for ongoing research to identify new preventative measures and diagnostic tools to effectively manage and prevent SARA in dairy cattle (Zebeli et al., 2012).

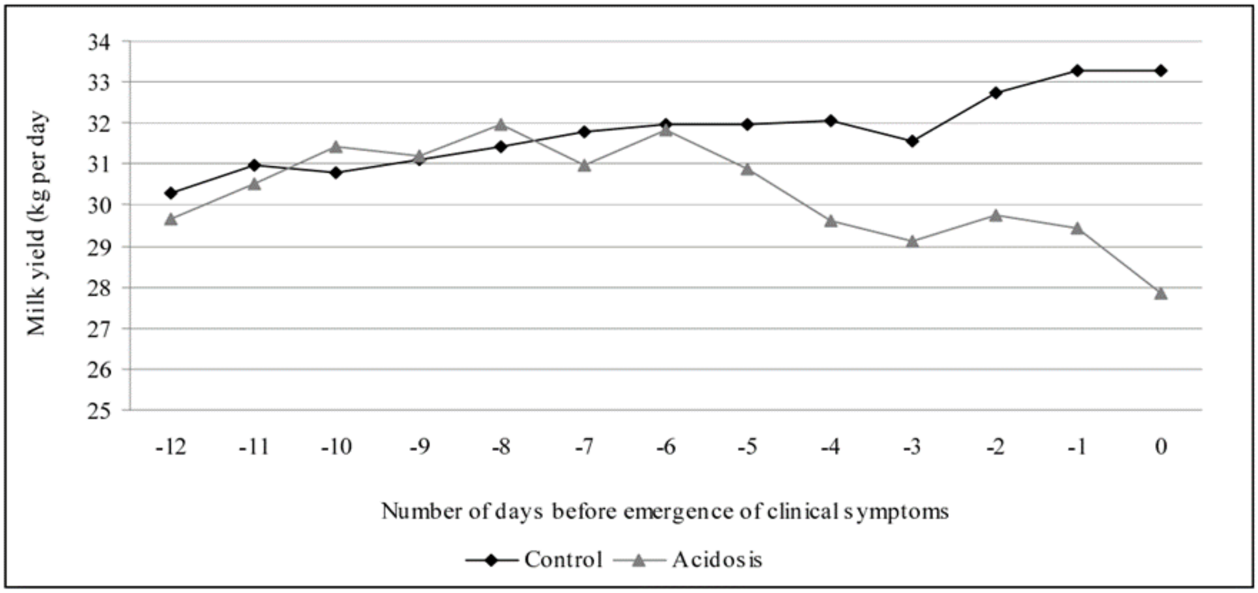

Figure 2.2. Early-onset Deficits to Milk Yield Preceding Symptomatic Response of Acidosis (via Antanaitis, et al., 2015). *Note:* In this research, subjects were 60 (n= 30 cows with acidosis and n=30 healthy cows i.e., control group) Lithuanian Black and White breed cattle with the average milk production of 7500 kg per lactation. Sourced from "Changes in Cow Activity, Milk Yield, and Milk Conductivity Before Clinical Diagnosis of Ketosis, And Acidosis" by Antanaitis et al., 2015, p. 6, Fig. 4. Copyright (2015) by *Veterinarija ir Zootechnika*.

Physiological Effects of SARA.

The physiological explanation for why pH drops associated with SARA cause elevated methane, ammonia, and VFA production in the rumen of a dairy cow is complex and involves the interaction between various microorganisms, feed components, and the host animal's physiology. The decrease in rumen pH associated with SARA causes a shift in the microbial population, with a decrease in fiber-digesting bacteria and an increase in lactate-producing bacteria (Krause and Oetzel, 2006). The lactate produced is not readily metabolized by the cow, leading to a buildup of lactic acid in the rumen, further lowering the pH. This low pH environment favors the growth of certain species of bacteria that produce more methane, ammonia, and VFA (Russell and Rychlik, 2001). Methane production is increased in cows with SARA because of the shift in microbial populations (Beauchemin and McGinn, 2006). Lactate-producing bacteria consume hydrogen and produce lactate (Hristov et al., 2015). As the lactate-producing bacteria increases, hydrogen concentration increases, which allows more methanogens to utilize the hydrogen and produce methane (Russell and Rychlik, 2001).

The increase in ammonia production associated with SARA is due to an increase in protein degradation by proteolytic bacteria. These bacteria break down protein into peptides and amino acids, which can be further degraded to ammonia (Hungate, 1966). The low pH in the rumen favors the growth of these bacteria, and the excess ammonia is absorbed into the bloodstream and excreted by the cow in urine (Mackie and Gilchrist, 1979). The increase in VFA production associated with SARA is due to an increase in fermentation of nonstructural carbohydrates, such as starch, in the rumen (McGuffy and Richardson, 2001). The shift in microbial populations leads to an increase in amylolytic bacteria, which are better adapted to ferment nonstructural carbohydrates. The excess VFA produced in the rumen is absorbed into the bloodstream and used as an energy source by the cow. However, the increased production of VFA can also contribute to the development of acidosis and SARA (Nozière et al., 2013). In summary, the decrease in rumen pH associated with SARA leads to a shift in microbial populations, which can lower methane, ammonia, and VFA production in the rumen of a dairy cow. The increase in these byproducts can have negative effects on cow health and productivity.

Significance of Rumen Microbial Populations

The rumen is a complex microbial ecosystem, with diverse populations of microorganisms that play a vital role in the digestion of feed in ruminants. The rumen microbial population can be divided into four groups, including gram-positive bacteria, gram-negative bacteria, archaea, and protozoa (Hungate, 1966). Each of these groups has specific roles in the rumen fermentation process and contributes to the end products of fermentation.

Gram-positive bacteria are one of the most dominant microbial groups in the rumen, accounting for up to 70% of the total microbial population. These bacteria are responsible for the breakdown of plant cell walls, which is an essential step in the digestion of fiber (Kung, 2018). They ferment carbohydrates to produce lactic acid, which can be further metabolized to produce volatile fatty acids (VFAs), such as acetate, propionate, and butyrate, as the main end products. The most prevalent species of gram-positive bacteria in the rumen include *Streptococcus bovis*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* (Krause et al., 2003).

Gram-negative bacteria are less abundant in the rumen than gram-positive bacteria, accounting for approximately 20% of the total microbial population (Hobson and Stewart, 2012). These bacteria play a crucial role in the fermentation of protein, producing ammonia as an end product, which can be used by other microbes in the rumen to synthesize amino acids. The most prevalent species of gram-negative bacteria in the rumen include *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, and *Succinivibrio dextrinosolvens* (Krause et al., 2003).

Archaea are another group of microorganisms found in the rumen, and they account for up to 5% of the total microbial population (Henderson et al., 2015). They are involved in the production of methane gas, which is a significant greenhouse gas and a byproduct of the rumen enteric fermentation process. Methanogens, a type of archaea, convert hydrogen and carbon dioxide into methane through a process called methanogenesis (Rivkina et al., 2000). Protozoa are the least abundant group of microorganisms in the rumen, accounting for less than 1% of the total microbial population. They play a crucial role in the digestion of fiber, as they help break down plant cell walls, and are also involved in the fermentation of starch and sugars. Protozoa can produce acetate, propionate, and butyrate as end products of fermentation.

Micro and Macro Symptoms of SARA

SARA occurs when the pH of the rumen drops below normal range for an extended period of time, leading to the proliferation of lactic acid-producing bacteria and the disruption of the rumen microbiota (Plaizier et al., 2008). More specifically, occurrence is defined as recurring daily episodes where the rumen pH falls below 5.8 for 3 to 5 hours (Zebeli et al., 2008; Kleen et al., 2003). The disease is often considered the most prevalent digestive disorder in the dairy industry, with a reported prevalence rate of up to 50% in lactating dairy cows. SARA can manifest as both micro and macro symptoms.

Micro symptoms of SARA are caused by the changes in the rumen microbiota and fermentation patterns. These include a decrease in fiber digestion, increased lactic acid production, and changes in volatile fatty acid (VFA) profiles (Penner et al., 2009). The microbial changes can lead to decreased dry matter intake, decreased milk production, and decreased feed efficiency, which ultimately results in decreased profitability for dairy farmers.

Macro symptoms of SARA observed in dairy cows may include changes in behavior such as reduced feed intake, reduced milk production, and reduced body condition score. Other macro symptoms can include changes in diarrhea and manure production. Cows with SARA may also exhibit a higher incidence of lameness, which may be related to reduced feed intake and altered metabolic profiles (Plaizier et al., 2017). In severe cases, SARA can lead to metabolic and digestive disorders, such as displaced abomasum, liver abscesses, and laminitis, which can have significant economic and animal welfare implications.

The clinical diagnosis of SARA is challenging, and the symptoms can be subtle and nonspecific. However, early detection and intervention are crucial to minimize the negative impact of SARA on animal health and production (Garrett et al., 2018). Regular monitoring of the rumen pH, VFA profiles, and dry matter intake can aid in the early detection of SARA. Additionally, feeding strategies that maintain a healthy rumen environment and optimize rumen function can help to prevent the development of SARA.

Existing Technological Solutions in Dairy Industry

The dairy industry has witnessed tremendous advancements in technology and management practices that have significantly improved dairy cow health, productivity, and welfare. One major advancement is the use of precision farming technologies such as automated milking systems (AMS) and robotic feeding systems, which have been shown to improve milk yield, decrease labor costs, and reduce stress on animals (Pinedo et al., 2010). Another management strategy that has improved dairy cow welfare and productivity is the use of improved housing and ventilation systems. The implementation of herd health programs and proper feed management practices, including the use of feed additives, have also contributed to improved dairy cow health and productivity. Moreover, advances in genetics and breeding have led to the development of high-producing dairy cow breeds (Pryce et al., 2012).

Individualized cow monitoring technology is an additional development that has gained popularity in dairy cow farming. This technology involves the use of sensors and data analytics to track various parameters such as milk production, body temperature, rumination activity, and behavior patterns. By monitoring these factors in real-time, farmers can quickly detect any signs of health problems or distress in individual cows, allowing them to intervene promptly and provide appropriate care (Mayer and Fiechter, 2018). Studies have shown that the implementation of individualized cow monitoring technology has significant benefits for animal welfare and productivity, including the reduction of lameness, an increase in milk production, and an improvement in reproductive performance (Maltz et al., 2016; Bewley et al., 2008). In addition to direct benefits, individualized cow monitoring technology can also have economic benefits for farmers by reducing production losses and the need for costly veterinary interventions. While technologies such as precision feeding and real-time monitoring can help farmers identify and manage individual cases of SARA, the condition is often caused by broader management and feeding practices that cannot be fully addressed through technology alone.

Previous Pharmacological Approaches for SARA in Dairy Industry

Antibiotics have been used in the treatment of SARA due to their ability to reduce the number of acid-producing bacteria in the rumen. Studies have found that administering antibiotics, such as monensin, can improve rumen pH and reduce the incidence of SARA in dairy cattle (Gozho et al., 2005). However, concerns have been raised about the overuse of antibiotics and the development of antibiotic resistance, which has led to a decrease in the use of antibiotics in dairy cattle production.

Ionophores, such as monensin and lasalocid, have also been explored as a potential treatment for SARA in dairy cattle. Ionophores work by altering the rumen microbial population, which can reduce the production of lactic acid and improve rumen pH. Studies have found that ionophore treatments can improve rumen pH and reduce the incidence of SARA in dairy cattle (Kleen et al., 2003). However, ionophore use in dairy cattle is regulated and may be restricted in some regions due to concerns about potential adverse effects on animal health and the environment.

Hormone treatments, such as melatonin and somatotropin, have been explored as potential interventions for managing and preventing subacute ruminal acidosis (SARA) in dairy cattle. Melatonin is a hormone involved in regulating the circadian rhythm and has been shown to improve rumen function and reduce lactic acid production in some studies (Abdelatti et al., 2020; Khiaosa-ard et al., 2016). Somatotropin, a peptide hormone that regulates growth and metabolism, has also been investigated for SARA and has been found to improve rumen pH and fermentation in some studies (Südekum et al., 2015). However, not all studies have found a significant benefit of hormone treatments for SARA in dairy cattle (Gozho et al., 2010; Ghavi Hossein-Zadeh et al., 2016).

Overall, past research has shown that antibiotics and ionophores can be effective treatments for SARA in dairy cattle but concerns about antibiotic resistance and environmental impacts have led to a decrease in their use. Dairy producers should collaborate with their veterinarian to develop a comprehensive management plan for SARA that includes proper treatment and prevention strategies. It is important to note that while melatonin and somatotropin have been investigated for their potential to manage and prevent SARA in dairy cattle, the effectiveness of hormone treatments in dairy cattle is variable and may depend on the specific hormone used and the diet and management practices of the herd (Mujahid et al., 2019). Further research should be conducted to better understand the mechanisms by which hormones impact rumen function and pH and to identify the most effective hormone treatment strategies for SARA in dairy cattle.

The Push for Alternative Solutions for SARA: Feed Additives

The increased prevalence of subacute ruminal acidosis (SARA) in dairy cows has led to the need for antibiotic alternatives to treat this condition. Antibiotic resistance has become a growing concern in both animal and human health, leading to restrictions on the use of antibiotics and increased interest in alternative treatments for animal diseases. Subacute ruminal acidosis is a metabolic disorder that affects dairy cows when they consume excessive amounts of fermentable carbohydrates that are rapidly fermented in the rumen (Plaizier et al., 2017). This leads to a decrease in rumen pH, which causes changes in the microbial populations in the rumen, leading to a variety of health problems in dairy cows. Traditional treatment of SARA involves the use of antibiotics, but with increasing concerns about antibiotic resistance, alternative treatments have become more important (Khafipour et al., 2012). Antibiotic resistance is the ability of bacteria to resist the effects of antibiotics, making it difficult to treat bacterial infections.

Overuse and misuse of antibiotics have contributed to the emergence of antibiotic-resistant bacteria, making infections more difficult and costly to treat. Antibiotic resistance is a growing concern in both animal and human health, as it can result in longer hospital stays, higher healthcare costs, and increased mortality rates (O'Hara et al., 2019). The use of antibiotics in animal agriculture is a contributing factor to the development of antibiotic resistance in both animals and humans. Antibiotics are commonly used in livestock production to treat and prevent bacterial infections, but their use can contribute to the emergence of antibiotic-resistant bacteria in animals (Cao et al., 2018). In response to the concerns about antibiotic resistance, there has been a growing interest in alternative treatments for animal diseases. Feed additives are commonly used in dairy cattle nutrition to improve feed efficiency, milk production, and animal health by modifying rumen microbe fermentation and were therefore turned to as potential alleviates to SARA and other digestive diseases in dairy cows.

Antibiotics employed in the treatment of SARA act by inhibiting lactate-producing bacteria in the rumen as a mechanism to prevent lactate accumulation during carbohydrate fermentation, which is a major cause of SARA (Zebeli et al., 2012). Propionic acid has been shown to mimic the action of antibiotics by improving the conversion of lactate to propionic acid by the propionibacteria in the rumen. This is accomplished through the Wood-Ljungdahl pathway, which is used by the propionibacteria to convert lactic acid to propionic acid through a series of biochemical reactions that produce ATP and reduce CO_2 to form acetate (Nagaraja and Titgemeyer, 2007). The improved conversion of lactate to propionic acid by the propionibacteria can also help to increase the availability of glucose for milk production by elevating energy available for gluconeogenesis in the liver, which is important for the health and productivity of dairy cattle (Kleen et al., 2003).

Direct-fed microbials (DFMs) and probiotics have been explored as potential interventions to manage and prevent SARA in dairy cattle. DFMs and probiotics are live microorganisms that are added to the diet to improve rumen health and function. DFMs can promote rumen microbial diversity, increase rumen pH, and reduce the incidence of SARA in dairy cattle (Kim et al., 2019). Probiotics, which contain specific strains of beneficial bacteria, have also been found to be effective at managing and preventing SARA in dairy cattle (Plaizier et al., 2009). Several studies have investigated the effectiveness of DFMs and probiotics for SARA in dairy cattle. One study found that feeding a probiotic containing *Lactobacillus acidophilus* and *Streptococcus faecium* reduced the incidence and severity of SARA in dairy cows fed a high-grain diet (Plaizier et al., 2009). Another study found that feeding a DFM containing a combination of lactic acid bacteria, yeasts, and cellulase reduced the severity of SARA in dairy cows fed a high-starch diet. However, not all studies have found a significant benefit of DFMs and probiotics for SARA in dairy cattle. One study found that feeding a probiotic containing *Lactobacillus acidophilus* did not improve rumen pH or reduce the incidence of SARA in dairy cows fed a high-grain diet (Krause et al., 2003). Another study found that feeding a DFM containing a combination of lactic acid bacteria and yeasts did not improve rumen pH or reduce the incidence of SARA in dairy cows fed a high-starch diet (Bekele et al., 2010). Overall, the effectiveness of DFMs and probiotics for SARA in dairy cattle is variable and may depend on the specific strains and combinations of microorganisms used, as well as the diet and management practices of the herd.

In recent years, plant oils, fat, and tannin dietary supplementation have been explored as potential treatments for SARA in dairy cattle. Plant oils and fat are high-energy supplements that can be added to the diet to reduce the risk of SARA in dairy cattle. One study found that feeding a high-concentrate diet supplemented with linseed oil decreased the incidence of SARA and improved milk production in dairy cows (Li et al., 2012). Another study found that feeding a high-concentrate diet supplemented with soybean oil reduced the severity of SARA and improved rumen fermentation in dairy cows. Other studies have also found a positive effect of plant oils and fat supplementation on rumen pH and fermentation in dairy cattle. Tannins are plant compounds that have been shown to have antimicrobial properties and can be used as a dietary supplement to manage and prevent SARA in dairy cattle. One study found that feeding a high-concentrate diet supplemented with tannin reduced the incidence and severity of SARA and improved feed efficiency in dairy cows (Cardozo et al., 2005). Another study found that feeding a high-grain diet supplemented with quebracho tannin reduced the concentration of lactic acid in the rumen and improved rumen pH in dairy cows with SARA (Yang et al., 2010). However, not all studies have found a significant benefit of plant oils, fat, and tannin dietary supplementation for SARA in dairy cattle. One study found that feeding a high-concentrate diet supplemented with a combination of plant oils and tannin did not improve rumen pH or reduce the incidence of SARA in dairy cows (Huhtanen et al., 2015). Overall, the effectiveness of plant oils, fat, and tannin dietary supplementation for SARA in dairy cattle is variable and may depend on the specific supplement used and the diet and management practices of the herd. Further research is needed to better understand the mechanisms by which these dietary supplements impact rumen function and pH and to identify the most effective dietary supplementation strategies for SARA in dairy cattle. In conclusion, plant oils, fat, and tannin dietary supplementation have been investigated for their effectiveness in managing and preventing SARA in dairy cattle. While some studies have found a benefit of these dietary supplements for SARA, others have not, indicating that further research is needed to fully understand the effectiveness of these interventions.

Organic acids have been investigated as a potential treatment for SARA in dairy cattle due to their ability to improve rumen fermentation, increase microbial protein synthesis, and maintain rumen pH. Fumarate and aspartate are two organic acids that have been studied for their potential to prevent or treat SARA in dairy cattle. Fumarate is a Krebs cycle intermediate that can be utilized by rumen microorganisms as a substrate for the production of propionate, which is an important end-product of rumen fermentation. In a study by Klevenhusen et al. (2012), feeding fumarate to dairy cows with SARA increased ruminal pH and propionate production, while decreasing lactate and acetate production. This suggests that fumarate may be a useful treatment for SARA in dairy cattle. Aspartate is another organic acid that has been studied as a potential treatment for SARA. Aspartate can be converted to oxaloacetate, which is an important intermediate in the Krebs cycle and a precursor for glucose synthesis. In a study by Zhang et al. (2018), feeding aspartate to dairy cows with SARA increased rumen pH, propionate production, and microbial protein synthesis, while decreasing lactate production. These results suggest that aspartate may be an effective treatment for SARA in dairy cattle. Other organic acids, such as malate and citrate, have also been investigated for their potential to prevent or treat SARA in dairy cattle. Malate can be utilized by rumen microorganisms to produce propionate, while citrate can be utilized to produce acetate and propionate. In a study by Kleen et al. (2003), feeding a combination of malate and citrate to dairy cows increased ruminal pH and propionate production, while decreasing lactate production. This suggests that a combination of organic acids may be more effective at preventing or treating SARA than a single organic acid alone. In conclusion, organic acids such as fumarate, aspartate, malate, and citrate have been studied for their potential to prevent or treat subacute ruminal acidosis in dairy cattle. These organic acids can improve rumen fermentation, increase microbial protein synthesis, and maintain rumen pH, which may help to prevent or alleviate the symptoms of SARA (Kleen et al., 2003; Klevenhusen et al., 2012; Zhang et al., 2018). While more research is needed to fully understand the potential benefits and limitations of using organic acids as a treatment for SARA in dairy cattle, the available evidence suggests that they may be a promising avenue for future research.

Promise of Malic Acid as a Preventative Feed Additive

Malic acid is a naturally occurring organic acid in fruits and vegetables such as apples, grapes, and carrots. It is also present in some forages fed to dairy cows (Zhang et al., 2019; El-Sherbiny et al., 2019). Despite the natural presence of malic acid in forages, research has shown that malic acid supplementation enhances the growth of beneficial rumen bacteria, such as

Fibrobacter succinogenes and *Ruminococcus flavefaciens*, while inhibiting the growth of acid-producing bacteria, such as *Streptococcus bovis* and *Lactobacilli* (Plaizier et al., 2017).

Malic acid is a dicarboxylic acid that plays an important role in the metabolic pathways of rumen microorganisms. In particular, malic acid can serve as a metabolic pathway intermediate between succinate and propionate in the rumen, helping to maintain a healthy microbial balance and promoting efficient fermentation of dietary carbohydrates. Succinate is an important intermediate in the rumen fermentation of carbohydrates, but its accumulation can lead to a decrease in rumen pH and the development of subacute ruminal acidosis (SARA). One way to prevent the accumulation of succinate is to convert it to propionate, which is a major end-product of rumen fermentation and an important source of energy for the host animal. Malic acid can serve as a key intermediate in this process by acting as a substrate for the conversion of succinate to propionate (illustrated in **Figure 2.3** via Macy et al., 1978). Malic acid in the form of malate is converted to oxaloacetate by malate dehydrogenase, which can then be converted to phosphoenolpyruvate (PEP) by phosphoenolpyruvate carboxykinase (PEPCK) during the citric acid cycle. PEP can then be used in the reductive pathway of succinate to produce propionate (Kim et al., 2018).

Research has shown that dietary supplementation of malic acid can enhance the production of propionate in the rumen, leading to improved feed efficiency and increased milk production in dairy cows (Chen et al., 2017; Zhang et al., 2017). In addition, malic acid has been shown to have antimicrobial properties and can help to inhibit the growth of pathogenic bacteria in the rumen, further promoting a healthy microbial balance (Schnürer et al., 1999). To conclude, malic acid serves as a metabolic pathway intermediate between succinate and propionate in the rumen, which can mimic the antimicrobial action of propionibacteria, and thus help to maintain a healthy microbial balance and promote efficient fermentation of dietary carbohydrates.

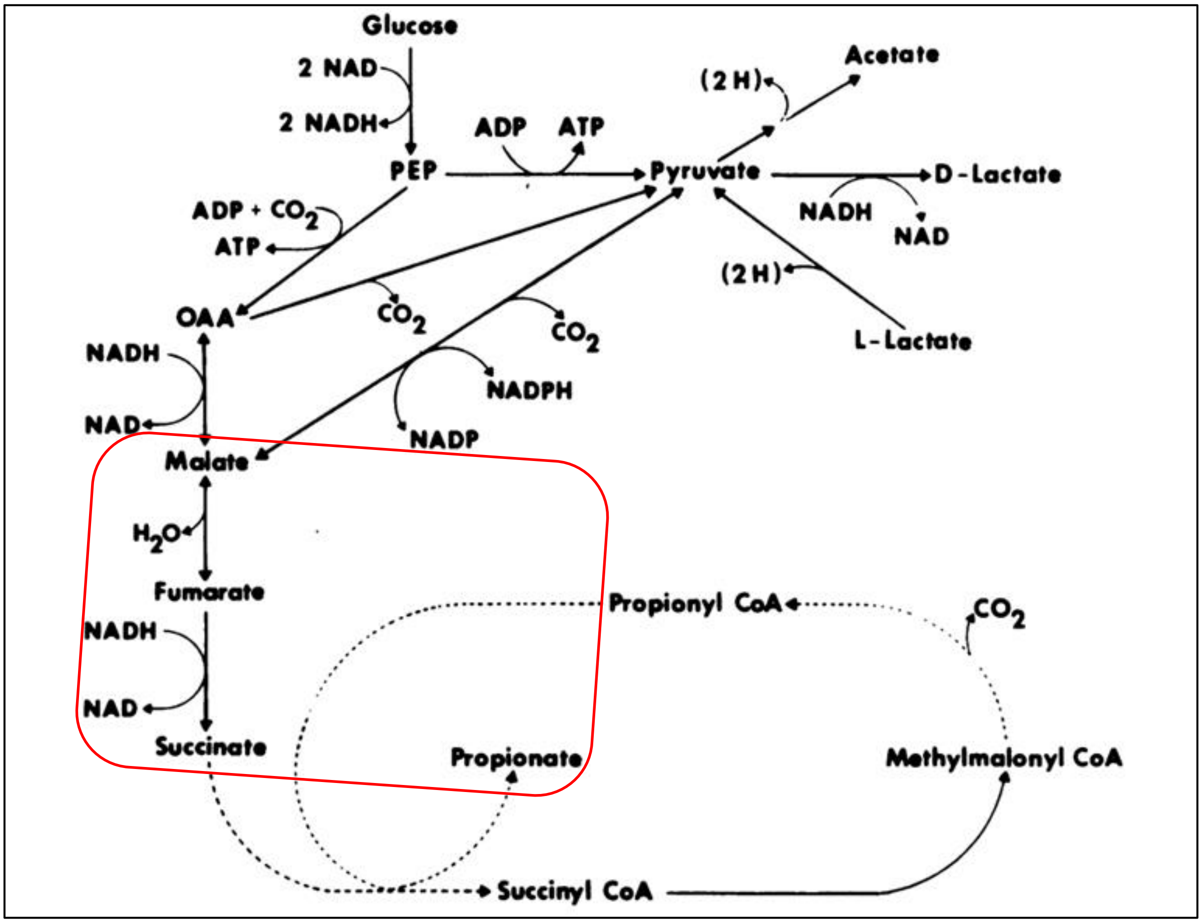

Figure 2.3. Conceptualization of the Role of Malate in the Succinate to Propionate Pathway (Adapted from Macy et al., 1978). *Note:* This figure illustrates a research hypothesis that *B. fragilis* ferments glucose via a pathway similar to that of Embden-Meyerhof and was unsupported by the results of the study. Adapted from "Pathway of Succinate and Propionate Formation in Bacteroides fragilis" by Macy et al., 1978, p. 88, Fig. 1. Copyright (1978) by *American Society for Microbiology*.

As a result of its inherent role in biochemical pathways of metabolism, the use of malic acid as a feed additive has been found to be effective in preventing and treating SARA in dairy cows. Previous studies have investigated the effect of malic acid supplementation on ruminal fermentation and microbiota in dairy cows fed a high-concentrate diet and found that malic acid supplementation increased rumen pH, reduced lactate concentration, and increased the production of propionate in the rumen (Cao et al., 2021, Zhang et al., 2018; Liu et al., 2018; Owens et al., 1998). It was also found that malic acid supplementation altered the composition of the rumen microbiota, increasing the population of cellulolytic fiber-degrading bacteria and reducing the population of lactate-producing bacteria (Cao et al., 2021; Zhang et al., 2018). On a larger organismic scale, research study by Vyas et al. (2020) reported that feeding malic acid to dairy cows with SARA has resulted in improved dry matter intake, feed efficiency, milk yield, and milk fat content (Vyas et al., 2020; Owens et al., 1998; Yang et al., 2018).

In conclusion, previous research has consistently demonstrated the potential of malic acid supplementation to prevent subacute ruminal acidosis in dairy cattle fed high-concentrate diets. Malic acid supplementation has been shown to improve ruminal fermentation, increase rumen pH, and increase the production of propionate, a critical energy source for dairy cattle. Additionally, malic acid supplementation has been associated with increased feed intake and milk production. While further research is necessary to fully understand the mechanism of action of malic acid in the rumen, existing research suggests that malic acid may be a useful preventative strategy for SARA in dairy cattle.

Limitations of Research on Malic Acid and SARA

Despite the positive results from some previous studies on the use of malic acid as a preventative feed additive for subacute ruminal acidosis (SARA) in lactating dairy cattle fed high concentrate diets, there are still several research gaps and needs that should be addressed in order to determine its full significance and efficacy. One major research gap is the need for more repeated measures over time. Most previous studies on the use of malic acid for SARA prevention have only used a single measurement or a small number of measurements taken over a short period of time. However, repeated measures over longer periods are necessary to establish the full effects of malic acid on rumen health and productivity. This is because the rumen environment is dynamic and can change over time, and the effects of malic acid may differ based on the timing of administration and the conditions of the rumen. Another research need is to evaluate the effects of different dosages of malic acid. Previous studies have used varying dosages of malic acid, but the optimal dosage has not been established. It is also unclear whether there is a potential for negative effects on rumen health or animal performance at higher dosages. Therefore, future studies should evaluate the dose-response relationship of malic acid to identify the optimal dosage for SARA prevention.

Furthermore, the mechanisms underlying the effects of malic acid on rumen health and productivity are not fully understood. Some studies have suggested that malic acid may improve rumen buffering capacity and microbial fermentation, while others have proposed that it may enhance the utilization of dietary fiber. More studies are needed to elucidate the specific mechanisms and metabolic pathways involved in the beneficial effects of malic acid. It is also important to determine the effects of malic acid on other aspects of animal health and productivity. Some studies have reported improvements in milk production, milk composition, and feed efficiency with malic acid supplementation, while others have not observed significant effects. However, these studies were often conducted under different conditions, such as different diets and feeding regimens, making it difficult to compare results. Future studies should investigate the effects of malic acid on a wider range of animal health and productivity parameters to determine its overall impact on dairy cow performance.

Finally, the economic viability of malic acid as a preventative for SARA should be evaluated. Malic acid is not commonly used in dairy cow diets and may be more expensive than other preventative measures such as dietary fiber or ionophores. Therefore, it is important to determine the potential return on investment of malic acid supplementation in terms of improved animal health and productivity, as well as reduced treatment costs for SARA. In conclusion, while the existing literature suggests that malic acid has potential as a preventative feed additive for SARA in lactating dairy cattle fed high concentrate diets, there are still several research gaps and needs that should be addressed to determine its full significance and efficacy. Repeated measures over time, evaluation of different dosages, clarification of the mechanisms underlying its effects, investigation of its impact on other animal health and productivity parameters, and economic viability studies are all necessary to further elucidate the potential of malic acid as a preventative for SARA in lactating dairy cattle.

In vitro batch culture fermentation is a useful tool for evaluating the effects of feed additives on rumen fermentation in a controlled environment. It allows for the manipulation of feedstuffs and additives in a way that is not possible *in vivo*, making it possible to isolate the effects of specific feed additives on rumen function (Jayanegara et al., 2017). However, *in vitro* systems lack the complexity of the rumen environment and host animal interactions present *in vivo*, which can limit their ability to accurately predict the productive response of a dairy cow to feed additives. To improve the accuracy of *in vitro* fermentation studies, more advanced models have been developed that better simulate the rumen environment and host animal interactions. Nonetheless, *in vitro* batch culture fermentation remains a valuable tool for screening potential feed additives and gaining insight into rumen fermentation processes.

While malic acid has been shown to be effective in preventing subacute ruminal acidosis (SARA) in dairy cows fed high concentrate diets, its use is still limited due to various reasons. One of the major limitations is its cost. Malic acid is more expensive than other organic acids, such as fumaric and citric acids. The higher cost of malic acid can be a barrier to its widespread use in the dairy industry. Additionally, the logistics of feeding and administering malic acid can also pose a challenge, particularly on smaller farms with limited resources. Another limitation is the accessibility of malic acid. While it is widely available, it may not be easily accessible in some regions. Farmers may need to go to specialty feed stores or order it online, which can add to the cost and logistical challenges. Moreover, while malic acid has been shown to be safe for dairy cows at the recommended doses, its long-term effects on animal welfare have not been fully studied. In addition, its use may mask other underlying issues with cow nutrition and rumen health, leading to missed opportunities to address larger problems. Furthermore, using malic acid as a preventative measure for SARA is just one part of a comprehensive approach to dairy cow nutrition and health. Other factors, such as the forage to concentrate ratio, particle size, and degradability of the feed, cow comfort, and on-farm management practices, also play a crucial role in maintaining healthy rumen function and preventing SARA.

Despite these limitations, malic acid remains a promising tool for preventing SARA in dairy cows fed high concentrate diets. As research continues to demonstrate its effectiveness and safety, farmers may be more willing to invest in its use as part of a comprehensive approach to improving cow health and productivity. Additionally, innovations in delivery methods and more widespread availability may help to address some of the logistical challenges associated with malic acid use. In summary, while malic acid is effective in preventing SARA in dairy cows fed high concentrate diets, its use is limited by factors such as cost, accessibility, and potential animal welfare concerns. Continued research and innovation in delivery methods and availability may help to address some of these limitations and increase its practical application in the dairy industry.

Introduction

Maintaining a healthy rumen is essential to supply a relatively constant pattern of nutrient supply to dairy cows and optimize efficiency of animal performance. Ruminal fermentation naturally induces a more acidic rumen environment that is buffered and kept stable with the saliva from chewing and rumination. Lower feed intake is commonly associated with decreased lactation performance, however other dietary parameters can also affect production. The physical and chemical breakdown of a feed may lead to variation in rumination and digestibility (Bargo et al., 2002). A common strategy by dairy producers to improve milk yield is to increase the quantity of grain in a dietary ration (Krause and Oetzel, 2006) to provide the cow more energy. However, altering dietary composition has a major impact on rumen fermentation and overall gut health. Fiber with greater particle size induces greater rumination activity and chewing behavior that generates more saliva to buffer ruminal pH. In contrast, diets with increased proportions of concentrate, and therefore less physically effective fiber, often lead to an extended reduction in ruminal pH (Mertens, 1997). Sub-acute ruminal acidosis (SARA) is a prominent ruminant digestive disorder prevalent in high-producing dairy cattle (Abdela, 2016). In the US, 26% of dairy cows are reported to have SARA (Plaizier et al., 2008, Abdela, 2016) resulting in an average cost of $400 to $475/cow/yr. (Stone, 1999). Unmistakably, SARA is a multimillion-dollar economic cost to the dairy industry. SARA is associated with a significantly decreased dry matter intake (DMI) and milk production (McCann et al., 2016, Vyas et al., 2015). Kitkas et al., (2013) have shown that decreased gut health from SARA negatively affects animal production and profitability. With the high prevalence of SARA in the U.S. it holds economic ramifications for producers across the dairy industry. However, practical attempts to improve milk yield in dairy cows often result in digestive health problems that, in turn, decrease animal performance (Vyas et al., 2015). Dairy producers face the challenge of minimizing the risk of under-detected SARA within their herd while maximizing production (Abdela, 2016).

One traditional dietary strategy to prevent SARA has been the use of antimicrobial ionophores (Liu et al., 2009). Although ionophores are still approved in diets of lactating cows in the U.S., there is growing public concern over their non-judicious use and the potential of antibiotic resistant pathogens (Castillo-Gonzalez et al., 2014). Consequently, it is with high demand and peak interest that scientists seek effective alternatives. Malic acid (MA) has been widely used as a natural additive with beneficial effects on rumen function and animal performance (Chen et al., 2017; Zhang et al., 2017). However, the results involving MA as a feed additive have been inconsistent. Direct supplementation of MA as a feed additive is perhaps not the most cost-efficient option at $0.11/cow/d (Sniffen et al., 2005) as a result producers are encouraged to feed forages high in organic acid content as an alternative method of MA inclusion in dairy cow diets. Much of the variation in response to malic acid may be due to the lack of information on an 'ideal' dosage of MA for lactating cows and differences in dietary ingredients. The primary objective of this study was to determine the effect of increasing levels of malic acid when included in corn silage-based diets with low and high ground corn. We hypothesized that the high corn diet would exhibit a drop in pH, a primary symptom of SARA, and the addition of MA would alleviate rumen pH and other fermentation patterns associated with SARA.

Materials and Methods

Dietary Treatments

This study was conducted at North Carolina State University (Raleigh, NC). Corn silage that had been frozen (-20°C) was thawed, and then processed in a food chopper (~1 cm length;

model FC 19, G. S. Blakeslee & Co., Cicero, IL). Chopped corn silage was mixed with ground corn to prepare experimental diets that included three ratios of corn silage to ground corn as follows: Control (CS), 100% Corn silage; Low Corn (LC), 70% CS + 30% ground corn and, High corn (HC), 50% CS + 50% ground corn. Each diet was prepared with four levels of supplemental malic acid: 0, 4, 8, and 16 g/kg DM. Corn silage and ground corn were weighed and mixed in separate containers to avoid cross contamination. Following thorough mixing, diets were placed in Ziplock bags and stored at 4°C.

In Vitro Batch Cultures and Experimental Design

In vitro batch cultures were used to determine the effect of dietary treatments on rumen fermentation. Experimental treatments (~1 g DM basis) were added, in triplicate, to individual batch culture vessels as described in earlier studies (Pisarčíková et al., 2016). The experiment was replicated (n = 2) for statistical analysis. Glass culture bottles (100 mL nominal volume) were equipped with rubber lined screw caps to allow for gas sampling. Each culture vessel was purged with a continuous stream of CO_2 before and during the addition of the inoculum to displace residual oxygen and maintain anaerobic conditions within the vessels (Raffrenato et al., 2018).

Rumen contents were obtained from a Hereford steer surgically fitted with a re-entrant rumen cannula that was fed a forage diet. The animal was housed at the Metabolism Education Unit at NC State University. Animal care and housing was approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC). On the day of inoculation, fresh rumen contents were manually obtained using a hand-held vacuum. Contents were sampled from various sites within the rumen and the sampling probe was maintained below the digesta mat (Fellner *et al.*, 1995). Approximately 4.0 L of whole rumen contents were collected and immediately transported to the laboratory in preheated thermal vacuum containers. Rumen inoculum was prepared by straining whole ruminal contents through four layers of cheesecloth and then mixed with chemically prepared bicarbonate buffer as artificial saliva (Slyter et al., 1966) in a 1:2 rumen fluid: saliva ratio. Forty milliliters of the inoculum were delivered to each culture vessel via a calibrated repeater dispenser. All manipulations with the rumen inoculum were performed under a continuous stream of CO_2 to maintain anaerobic conditions. Following the addition of the rumen inoculum, culture bottles were immediately capped and placed in a water bath set at 39˚C (Liu et al., 2008) to mimic and maintain the anaerobic culture conditions of the rumen in each fermenter.

Each experimental run contained 84 samples and was replicated (n = 2). Within each run, dietary treatments were weighed in triplicate with rumen blanks in duplicate. Diets were fermented for 0, 6 and 24 h. The 0 h bottles received the dietary substrate and inoculum and were then immediately placed in ice to inhibit microbial fermentation. The 6 and 24 h bottles were placed in water baths set at 39°C. Bottles were very gently swirled every few hours to loosen particulates from the sides of the bottles and keep them hydrated.

Sample Collection and Analytical Procedures

At the end of 6 and 24h, respective culture bottles were removed from the water bath and immediately placed on ice to inhibit further fermentation. All bottles were placed in a walk-in cooler until subsequent analysis. Analytical measurements included culture pH, methane, volatile fatty acids, ammonia-N, and digestibility of dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF). Prior to opening the culture bottles, gas samples were taken and analyzed for methane (Eun et al., 2004). Gas samples (10 μL) were withdrawn directly from the headspace with the aid of a gastight syringe (Hamilton Co., Reno, NV) and immediately analyzed for methane concentration by glass liquid chromatography (GLC CP-3800, Varian, Walnut Creek, CA) equipped with a Molsieve 5A 45/60 mesh stainless steel column (Supelco Inc., Bellefonte, PA).

Following all methane analysis, culture bottles were opened and analyzed for pH using a pH probe (VWR SympHony – model AR25; Accumet Research, Dual Channel pH/Ion Meter Fisher Scientific). A pH buffer 4.01 and 7.00 (Orion Application Services) was used to calibrate the probe before data collection and between each sample. Two milliliter aliquots from each fermenter bottle were obtained, in duplicate, for VFA and NH_3-N analysis. The remaining culture contents were transferred into a 50 mL conical tube for subsequent fiber analysis (Falcon® 25 mL High Clarity PP Centrifuge Tube, Conical Bottom, Sterile). All samples were stored in the freezer (-20°C) until analysis (Ivan, 2006; Jiang, 2017). Samples for VFA analysis were processed by mixing 1 mL of culture contents with 0.2 mL of metaphosphoric acid and internal standard (2-ethylbutyric acid), then centrifuged (model Micromax; International Equipment Co., Needham Heights, MA) at 21,000 × g for 10 min at 4°C. Supernatant was stored at −70°C until subsequent analysis. VFA were analyzed by gas liquid chromatography (GLC model CP-3380; Varian) using a fused-silica capillary column, 30 m × 0.25 mm with 0.25-μm film thickness (Nukol; Supelco Inc., Bellefonte, PA) according to the method of Fellner et al. (1997). Ammonia-N samples were thawed, centrifuged (Sorvall RC-3) at ~1050 x g for 10 minutes, and pipetted (10μl) into prelabeled test tubes and diluted 1:10 with Di H_2O. Phenol reagent (0.5 mL) and sodium hypochlorite (0.5 mL) were added, vortexed, and incubated at room temperature for 30 minutes. Following incubation, Di H_2O (4 mL) was added, vortexed, and samples were transferred to cuvettes. Ammonia-N was analyzed according to the procedure outlined by

Beecher and Whitten (1970) using a PASCO spectrophotometer (PS-2600) calibrated to 620 nanometers. A standard curve was prepared to determine the concentration of unknown samples.

Neutral and acid detergent fiber concentrations were determined according to the procedure described by Van Soest et al., (1991, 1994). Filter bags were labeled with a Secureline Superfrost Marker. Culture samples were thawed, centrifuged and the supernatant was removed and "pellet" dried (~72 hours) in an oven (60°C). Next, contents were quantitatively weighed (Balance Mettler Toledo AG-245) directly into an Ankom filter bag (0.5g) and sealed within 1 cm from the open edge using a heat sealer (NYCLAVE Impulse Heat Sealer (110v, 310 watt)). Following sealing, the sample was spread uniformly in the bag by shaking to avoid clumping. Samples were placed in the digestion vessel of an Ankom 200 Fiber Analyzer (Ankom Technologies, Fairport, NY) with neutral detergent solution. After each run, bags were rinsed with hot water, then acetone, prior to drying in the oven (100 °C). Dried samples were transferred to a desiccator to cool before final weights for NDF residue were measured. Experimental diets were submitted to Cumberland Valley Analytical Services for chemical analyses (**Table B.1**).

Statistical Analysis

All data were analyzed according to a randomized complete block design using PROC MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC) with replicate within run as the random term. Main effects were diets, level, and diet x level interaction. Pre-planned linear and quadratic orthogonal contrasts were included to determine the effect of level of malic acid inclusion. We considered P-values ≤ 0.05 to be statistically significant and $0.05 > P$-values < 0.1 as a statistical tendency. Tables and figures were generated from statistical data using Microsoft Excel.

Results

The ingredient and chemical composition of the experimental diets are reported in Table B.1. The diets differed in the amount of corn silage and ground corn. Decreasing the amount of corn silage increased the diet % DM and decreased dietary ADF and NDF % (*P*=0.01 and 0.01 and 0.01, respectively). Increasing ground corn resulted in an increase in dietary crude fat, nonstructural carbohydrates as well as the NE and ME content of the diets. Crude protein content was low but similar and averaged 8% across all diets.

The effect of treatments on short chain fatty acids (SCFA) after 6 h of *in vitro* fermentation is shown in **Table B.2**. Accumulation of total SCFA (mM) was significantly affected by diet (P = 0.01). Malic acid level and the interaction of level x treatment were not significant (P > 0.10). Total SCFA (mM) increased with the LC diet compared with CS and HC diets (88.6 versus 77.4 and 78.5 mM, respectively). Molar % of acetate and propionate were significantly greater (P = 0.01 and 0.02 respectively) with the CS compared with the LC and HC diets. The A:P ratio tended to be similar in the CS and LC diets (3.6 and 3.6, respectively) with the HC averaging 3.9. In contrast, CS resulted in significantly lower butyrate (P = 0.01) compared with the LC and HC diets. Isovalerate was greatest in the CS diet (1.2 mol%) and decreased (P <0.01) in the LC (1.0 mol%) and HC (1.0 mol%) diets (**Table B.2**). The effect of treatments on SCFA after 24 h of *in vitro* fermentation is shown in **Table B.3, Figures B.1-6**. Total SCFA concentrations (mM) averaged 90, 99 and 96 mM across CS, LC, and HC diets, respectively and were not different (P > 0.10). The molar % of acetate was greater in the CS diet (61%) compared with LC and HC diets (58 and 59%, respectively). Diets did not alter (P > 0.10) molar % of propionate, which also resulted in similar A:P ratios across treatments (Table B.3). However, molar % of butyrate was significantly affected by diet with CS resulting in a lower butyrate (11 mol%) when compared with LC and HC diets (13 and 13 mol%, respectively). The CS diet resulted in reduced valerate (*P* = 0.01) and increased isovalerate (*P* < 0.01) concentrations compared with the LC and HC diets.

Data on the effect of treatments on CH_4, pH, and NH_3 after 6 and 24 h of *in vitro* fermentation are summarized in **Table B.4**. At 6 h, there was a significant effect of diet on CH_4 (*P* = 0.02). Methane concentration was increased with the CS diet (1.4 mmol/L) compared with LC and HC diets (1.3 and 1.2 mmol/L, respectively). There was a diet by level interaction for rumen NH_3. Cultures receiving the CS diet resulted in a decrease in NH_3 with increasing levels of malic acid while cultures receiving the HC diet showed an increase in NH_3 with an increase in the level of malic acid; LC diets were not affected by level of malic acid which averaged 5.3 mg/100 mL). At 6h, there was a significant effect of diet and diet x level on rumen culture pH (Table B.4). Culture pH averaged 6.0, 5.9 and 6.1 for CS, LC, and HC diets, respectively. With the CS diet, pH decreased with an increase in level of malic acid addition whereas with the LC and HC diets pH either remained the same or increased with an increase in the level of malic acid. There was also a significant effect of diet on DM and fiber digestibility at 6 h (Table B.4). Dry matter digestibility was lowest for CS (57 %) and increased with an increase in the proportion of corn in the diet with LC and HC diets averaging 67 and 70%, respectively. Neutral detergent (NDF) and acid detergent (ADF) fiber digestibility averaged 48 and 26 %, respectively for the CS diet and were significantly greater when compared to corresponding values for the LC and HC diets (40 and 20%, and 38 and 18%, respectively).

At 24 h, diets had no effect (*P* >0.10) on methane and ruminal ammonia (Table B.4). Methane averaged 2.7 mmol/L and NH_3 averaged 5.0 mg/100mL across all dietary treatments.

Culture pH was affected by diet and diet x level interaction (Table B.4). As expected, increasing the proportion of corn in the diet decreased culture pH ($P < 0.01$). The pH in the CS diet averaged 5.6 and the LC and HC diets averaged 5.5 and 5.5, respectively. In the CS diet, increasing the level of MA did not alter culture pH; however, in the LC and HC diets, increasing MA was associated with an increase in pH. At 24h, both LC and HC diets had a greater DM digestibility compared with CS (75 and 76 versus 69%, respectively). In contrast, NDF digestibility was greater in the CS diet (53%) compared with LC (42%) and HC (45%) diets (Table B.4). Similarly, ADF digestibility was greater in the CS diet (26%) compared with LC and HC diets (18 and 23 %, respectively). There was an interaction between diet and level for ADF digestibility. Both the CS and LC reduced ADF digestibility with an increase in level of MA; with the HC diet increasing malic acid resulted in an increase in ADF digestibility.

Discussion

The experimental diets used in this study were composed solely with corn silage and ground corn, common ingredients present in lactating cow diets. However, they did not resemble diets recommended by the NRC (2001) particularly with respect to crude protein content that averaged 8% across all treatments. Our intent was to keep corn as the main source of starch in the diet and minimize confounding effects with a protein source as we hoped to elicit an extreme response in the ruminal culture to corn inclusion to evaluate the potential of malic acid in alleviating symptoms of SARA. It must also be noted with *in vitro* studies that there is no additional dietary substrate over time. This leads to a limiting quantity of forage and concentrate that may leave an excess of malic acid and promote its ability to influence pH as well as other concentrations at 24 h. Furthermore, Martin (2003) found that malate is completely fermented *in* *vitro* between 10 to 24 h, so our analytical time point of 6 h may not accurately assess microbial activity and effect of MA.

The increased energy observed with additional concentrate was anticipated because high dietary starch content is typically associated with an increase in overall energy and milk production. Each increment of ground corn resulted in greater dietary NSC content as well as higher ME and energy partitioned for maintenance and lactation, NE_M and NE_L. Furthermore, additional ground corn supplementation in the diets resulted in a greater DMD because the increase of starch concentrates allowed a higher overall substrate digestion and therefore a greater total absorption of feedstuffs into the body throughout digestive passage. However, NDFD was lower in both corn diets, LC and HC, compared with CS. Similarly, ADFD was reduced with the inclusion of ground corn and the decrease was greater in the HC compared with the LC diets. Fiber content, both NDF and ADF, was greater in the CS diet and decreased with the proportion of corn added in the diet due to the decreased proportion of fibrous cell wall components in the diet with the addition of concentrate. Increasing ground corn therefore increased the risk of SARA, as is typical with high grain diets (Abdela, 2016, Krause and Oetzel, 2006). The HC diet in this study recorded a 21.4% NDF (**Table B.1**), below the association of dietary NDF < 25 with increased risk of SARA according to Stone (2004) (**Figure 2.1**). Previous studies have shown that inclusion of malate in high grain diets may decrease the risk of acidotic behaviors and symptoms including lameness, decreased intake and rumination time, as well as decreased milk production (Martin, 1988, Jalc 2002, Callaway and Martin, 1997, Khampa 2006).

Various forage fiber components influence the occurrence of SARA and Stone (2004) determined an increased risk when pH < 5.6. Previous *in vitro* studies using alfalfa and coastal bermudagrass hay found that malate supplementation at all levels increased ruminal culture pH values (Martin, 2003). As hypothesized, increasing the proportion of ground corn clearly decreased *in vitro* culture pH, irrespective of dietary treatment, after 24 h of fermentation. This drop in pH is typically associated with substrate fermentation and consequent production of SCFA. However, increased dietary concentrations of malate have successfully ameliorated depressed ruminal pH values observed in cases of SARA (Martin and Streeter, 1995; Callaway et al., 1997; Martin et al., 1999). In the LC and HC diets, malic acid supplementation increased pH within diets. The HC diet meant to elicit the largest acidotic response reported the highest ruminal pH values, strongly indicative that the impact of MA was significantly positive on improving ruminal pH. Ruminal pH values in this study ranged from 5.39-5.64, agreeing with the usual pH range of 5.5 to 5.6 in dairy cattle with SARA (Mishra et al., 1970; Oetzel et al., 1999; Keunen et al., 2002).

Plaizier et al. (2008) estimated that SARA impacts more than 20% of US dairy cows, so while the pH values recorded in the experimental diets far exceed this proportion, it should be noted that this was a controlled *in vitro* study designed to induce conditions of SARA to determine the ability of MA to mitigate it. With the CS diet, pH values declined irrespective of additional MA but in the two diets where ground corn was supplemented, pH remained the same or increased with MA. For both LC and HC, MA inclusion elevated ruminal pH when compared with the control diet (CS). At both 6 h and 24 h, 16 g/kg MA inclusion resulted in the highest rumen culture pH in both LC and HC diets. While pH did not rise above 5.6, MA still proved effective at raising pH. We expected a decrease in pH with an increase in the concentration of ground corn due to decreased dietary fiber content and our data were consistent with the experimental hypothesis and suggest MA ameliorated the pH decline. Based on the dietary chemical factors associated with SARA, the HC diet registered an increased risk for SARA (Stone, 2004). Our data are consistent with an earlier study that also elicited a similar beneficial effect of MA on rumen pH of lactating dairy cows fed diets designed to induce SARA (Sniffen et al., 2005).

It is important to note however that our study did not measure the length or duration of time associated with reduced pH readings, a main criterion to determine SARA. While an animal's daily average pH may read at or above 6, it may spend multiple h/d below a stable pH. A prime example of how analytical technique may influence interpretation of data is seen in study by Krause et al. (2002b) where the mean ruminal pH values across four treatments were ~6 yet however recorded significant lengths of time below a pH of 5.8, between 5.5 and 14.3 h/d, indicating the occurrence of SARA despite the assumption the mean values present. Persistent daily episodes of low ruminal pH levels contribute to inflammation and injury within the digestive system (Zebeli et al., 2012) associated with detrimental reduction in animal performance (Vyas et al., 2015). While we cannot assess duration of time at a given pH for this study, the low values across all dietary treatments in this study agreed with high-risk SARA conditions (Mishra et al., 1970, Oetzel et al., 1999, Keunen et al., 2002, Stone, 2004). Furthermore, the significant amount of time registered at low ruminal pH in cows that otherwise demonstrated stable mean pH by Krause et al. (2002b) emphasizes the criticalness of early intervention to minimize the incidence of SARA. Significant economic loss is associated with SARA due to decreased cow production and performance with an estimated annual total by Stone (1999) to be between $500 million and $1 billion within the US dairy industry alone.

Accumulation of SCFA during fermentation in the rumen is a primary cause of pH decline and onset of SARA (Krause and Oetzel, 2006). As one would expect, the total SCFA increased over time. Also, we would expect diets with greater amounts of corn to have increased fermentability which would be reflected in a greater total SCFA concentration. We found the following atypical results in our present study. Both LC and HC diets were associated with a greater total SCFA concentration when compared with CS however Total SCFA decreased linearly with an increase in the MA level in the LC diet but not the HC diet. Allen et al. (1997) reported a negative correlation between pH and SCFA across 99 treatment means, which associated low pH with higher SCFA. In comparison, the total SCFA concentrations in this study ranged between 83.2 and 123.6 mM/L. According to Allen et al.'s (1997) regression, our SCFA values would predict a ruminal pH near or above 6. Ruminal culture pH values in this study however were not consistent with the regression model as the depression of pH observed at 24 h was resemblant of high-risk SARA conditions (Stone, 2004). Previous research (Liu et al., 2008) indicated a decreased effectiveness of MA on dairy cows fed high concentrate diets, and while pH increase was most effective in the HC diet, the SCFA (mol %) concentration across treatments in this study did in fact prove to be relatively constant at 24 h.

The VFA production at 6 h *in vitro* fermentation of the CS diet aligned with data from Erdman (1988) that predicted a 3.875 A:P by a 22.5% ADF, similar to the average of all 4 MA levels (3.573) and equal to LC4 (3.87). Acetate was greater in the CS diet, reflecting the greater concentration of dietary fiber as fibrolytic bacteria extract glucose from cellulose for conversion to acetate. In contrast, propionate did not reflect the increase in dietary ground corn and was similar across all diets. This was inconsistent compared to a study by Martin (2003), in which malate inclusion increased propionate, as well as literature describing glucose conversion to propionate by amylolytic bacteria in high starch diets. As a result, the A:P ratio was also greater in our study when compared with the decreased A:P reported by Martin (2003). In the study by

Martin (2003) malate increased CH4 however we did not see any effect of MA inclusion on methane production which was similar across all diets at 24 h. There was, however, a significant effect of MA on ruminal ammonia which was lowest in the LC diet and greater but similar for the CS and HC diets. Furthermore, rumen ammonia increased linearly in the HC diet but remained unaffected with MA levels in the CS and LC diets. Firkins et al. (2007) also reported a decrease in rumen ammonia with increased dietary starch and sugar content.

Conclusions

Although organic acids have been extensively researched as potential alternative to antimicrobial feed additives for ruminants, the data have been very inconsistent across studies (Martin and Nisbet, 1992). There is, nevertheless, evidence for the potential of organic acids to impart similar alterations of rumen fermentation as ionophoric compounds that warrant further research for a better understanding of their mode of action (Castillo-Gonzalez et al., 2014, Lynch, 1997, Martin et al., 1999, Sniffen et al., 2005). Our preliminary results support the use of malic acid as a potential alleviation to depressed ruminal pH for diets with increasing quantities of ground corn. To determine the true potential of malic acid to reduce symptoms of SARA however, we need to extend our *in vitro* knowledge to *in vivo* conditions. Additionally, the observed malic acid induced response to rumen pH may likely be altered when crude protein content is higher and more resemblant of practical diets that contain soybean meal.

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PERSPECTIVES AND CONCLUSIONS

The two chapters of this thesis - comparing measures of peuNDF240 and exploring malic acid as an alternative to antibiotics to prevent subacute ruminal acidosis (SARA) - are connected by their common theme of improving dairy cow health and productivity. Both topics address issues related to dairy cow nutrition and the challenges that arise from feeding high-concentrate diets.

Chapter one compares two methods of measuring peuNDF240, a measure of physically effective fiber in the diet that is crucial for maintaining proper rumen function in dairy cows. The study aims to determine which of the two methods is more dependable for accurately measuring peuNDF240 and ensuring that dairy cows are receiving the appropriate level of fiber in their diet. This is important because a lack of fiber can lead to issues like SARA, which can have negative impacts on cow health and milk production.

Chapter two explores the use of malic acid as an alternative to antibiotics for preventing SARA, a common digestive disorder in dairy cows that is caused by feeding high-concentrate diets. SARA can lead to decreased milk production, poor feed efficiency, and other health issues in dairy cows. Antibiotics have traditionally been used to prevent SARA but concerns over antibiotic resistance and consumer demand for antibiotic-free products have led to a need for alternative solutions. Malic acid has shown promise as an effective preventative measure for SARA, and the chapter explores the potential benefits and limitations of its use.

While the two chapters focus on distinct aspects of dairy cow nutrition, they are connected by their goal of improving cow health and productivity. Accurately measuring and providing adequate levels of fiber in the diet is essential for maintaining proper rumen function and preventing issues like SARA. The use of malic acid as an alternative to antibiotics for preventing SARA is also a crucial step in improving cow health and productivity, as it addresses the need for antibiotic-free solutions to common dairy cow health issues.

In conclusion, the two chapters of this thesis highlight the importance of proper nutrition and preventative measures in maintaining the health and productivity of dairy cows. By addressing the challenges associated with feeding high-concentrate diets and providing alternative solutions to traditional treatments, these chapters contribute to the ongoing efforts to improve the sustainability and welfare of the dairy industry.

APPENDICES

Appendix A: Chapter 1

Table A.1. Determination of calculated and measured physically effective neutral detergent fiber after 240 h *in vitro* fermentation (peuNDF240) [via physical effectiveness of DM with a Ro-Tap and laboratory analysis of undegradable NDF240 (uNDF240)] of 6 dietary rations from 2 studies conducted at the W. H. Miner Institute (Smith et al., 2018; Dann et al., 2021).

Measure			Study Description					
			Smith et al., 2018				Dann et al., 2021	
			TMR G	TMR H	TMR J	TMR K	TMR D	TMR F
	[1]pef >1.18, % of whole		0.58	0.60	0.50	0.60	0.70	0.73
Rotap, % Retained	pef	19mm	0.00	0.04	0.01	0.10	0.16	0.17
		13.2mm	0.18	0.13	0.11	0.58	0.20	0.31
		9.5mm	2.32	1.90	0.90	1.56	2.01	1.93
		6.7mm	11.87	12.39	6.06	7.31	7.29	10.50
		4.75mm	9.43	9.56	7.87	8.61	10.63	13.30
		3.35mm	9.26	9.32	8.89	10.01	14.54	15.21
		2.36mm	7.67	8.32	7.48	9.78	13.47	12.35
		1.18mm	16.93	18.05	19.05	21.76	21.28	19.70
	bottom	0.6mm	18.97	18.38	22.93	18.65	18.52	16.75
		0.3mm	14.32	13.34	16.09	12.80	8.52	6.95
		Pan	9.05	8.57	10.62	8.83	3.39	2.83
[2]uNDF240, % DM avg	whole	uNDF240	10.41	9.99	11.94	13.08	9.13	9.74
		CV	0.0288	0.0643	0.0198	0.0607	0.0325	0.028
		StdDev	0.299	0.643	0.236	0.794	0.297	0.273
		[3]uNDF240om	8.94	8.77	11.12	12.33	8.50	9.33
		CV	0.0077	0.016	0.0173	0.0359	0.0046	0.0356
		StdDev	0.069	0.14	0.192	0.443	0.039	0.333
	pef	uNDF240	10.82	11.19	12.63	13.67	10.02	11.30
		CV	0.0602	0.0796	0.0326	0.0093	0.0121	0.0656
		StdDev	0.652	0.891	0.412	0.127	0.121	0.742
		uNDF240om	9.47	10.00	12.08	13.28	9.65	10.92
		CV	0.0234	0.0262	0.001	0.0229	0.0597	0.0204
		StdDev	0.221	0.262	0.012	0.304	0.576	0.223
[4]peuNDF240	[5]calculated		5.19	5.26	5.56	7.40	5.95	6.81
	[6]measured		9.47	10.00	12.08	13.28	9.65	10.92

[1]pef = physical effectiveness fraction; particles >1.18 mm retained on dry, vertical sieve
[2]uNDF240 = undegradable NDF over 240 h *in vitro* fermentation
[3]uNDF240om = uNDF240 organic matter; corrected for ash
[4]peuNDF240 = physically effective uNDF240
[5]calculated = total pef % retained * whole uNDF240om
[6]measured = pef uNDF240om

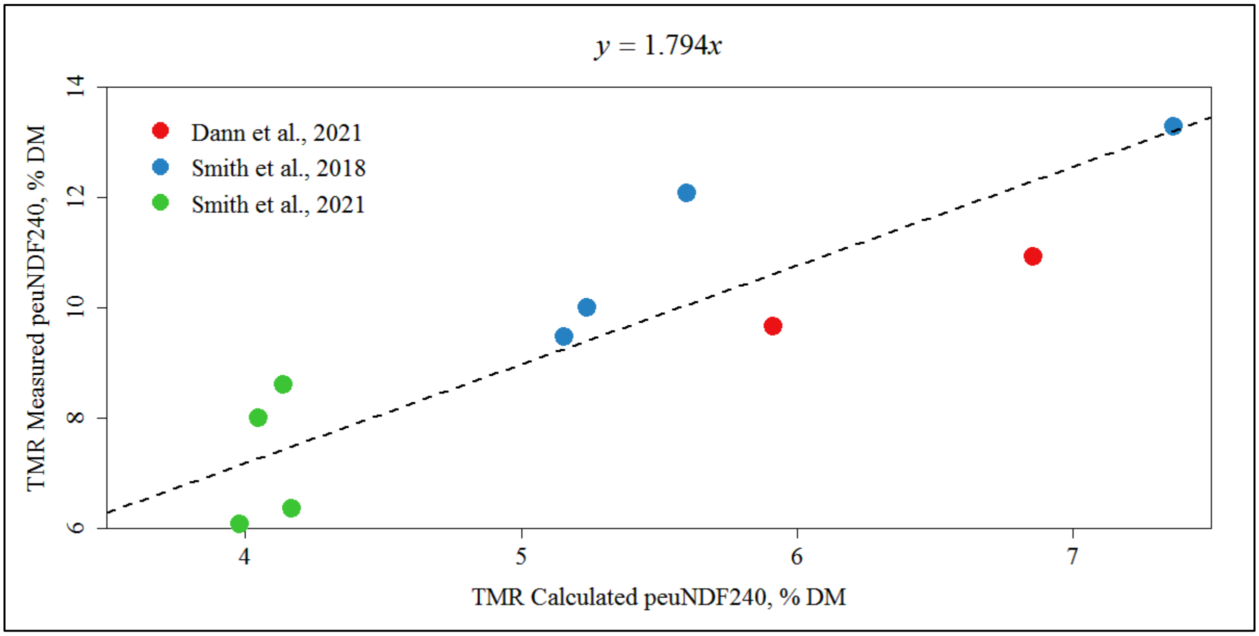

Figure A.1 Linear relationship of calculated and measured peuNDF240 values from 3 corn silage studies conducted at the W.H. Miner Agricultural Research Institute. (Dann, 2021, Smith, 2021, Smith, 2019).

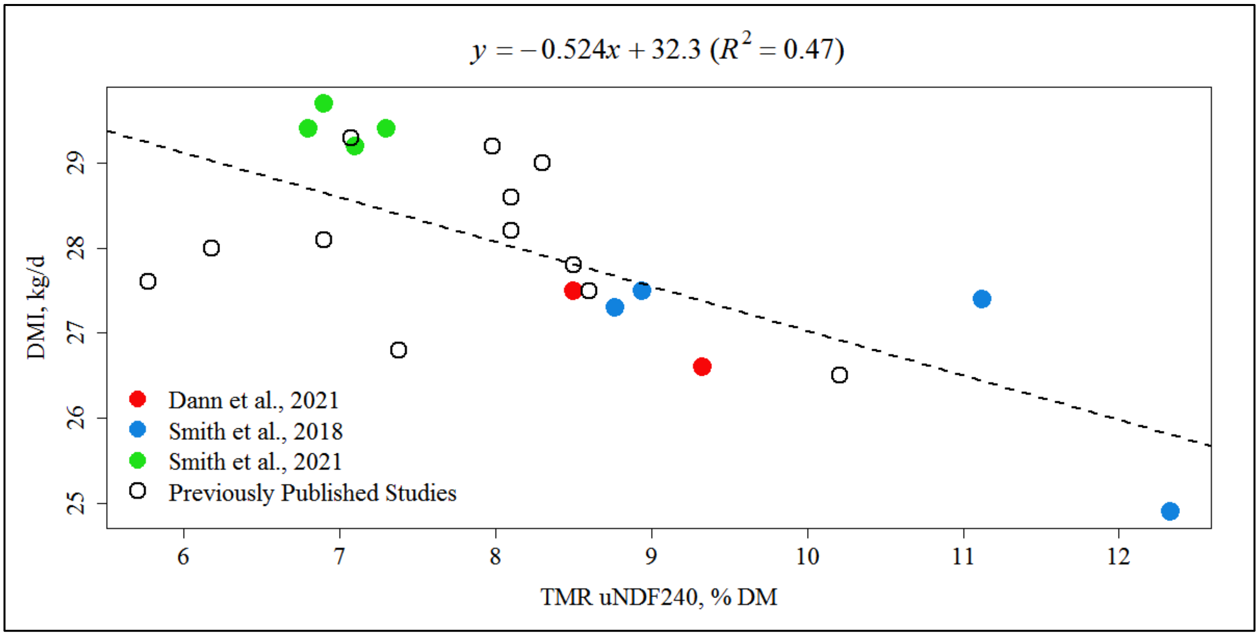

Figure A.2. Linear relationship of TMR uNDF240 and DMI values from 7 corn silage diet studies conducted at the W.H. Miner Agricultural Research Institute, new data highlighted in color to contrast the Miller et al. 2020 database. (Coons, 2019, Cotanch, 2014, Dann, 2021, Miller, 2017, Miller, 2020, Smith, 2021, Smith, 2019).

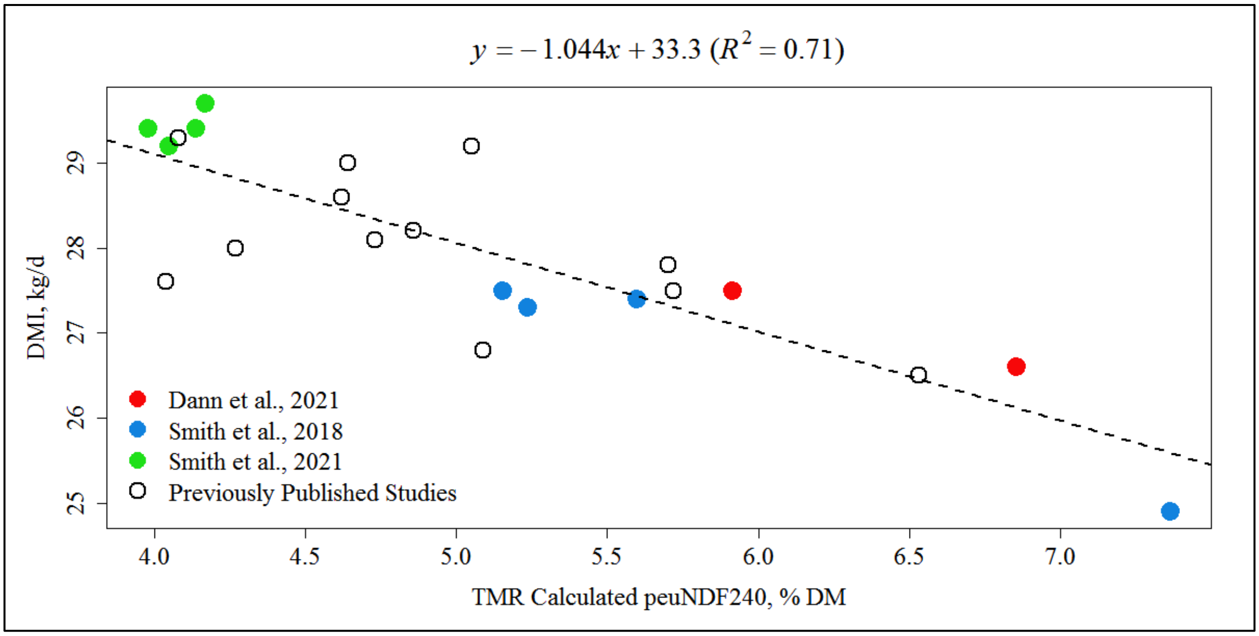

Figure A.3. Linear relationship of TMR calculated peuNDF240 and DMI values of 7 corn silage diet studies conducted at the W.H. Miner Agricultural Research Institute, new data highlighted in color to contrast the Miller et al. 2020 database. (Coons, 2019, Cotanch, 2014, Dann, 2021, Miller, 2017, Miller, 2020, Smith, 2021, Smith, 2019).

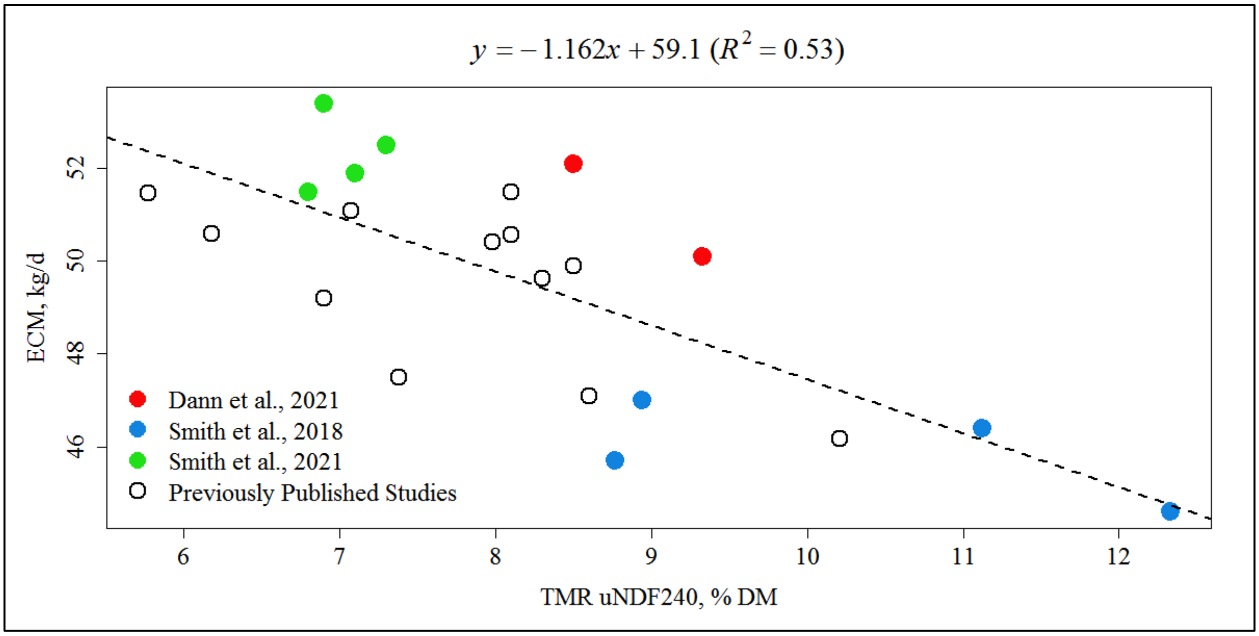

Figure A.4. Linear relationship of TMR uNDF240 and ECM values from 7 corn silage diet studies conducted at the W.H. Miner Agricultural Research Institute, new data highlighted in color to contrast the Miller et al. 2020 database. (Coons, 2019, Cotanch, 2014, Dann, 2021, Miller, 2017, Miller, 2020, Smith, 2021, Smith, 2019).

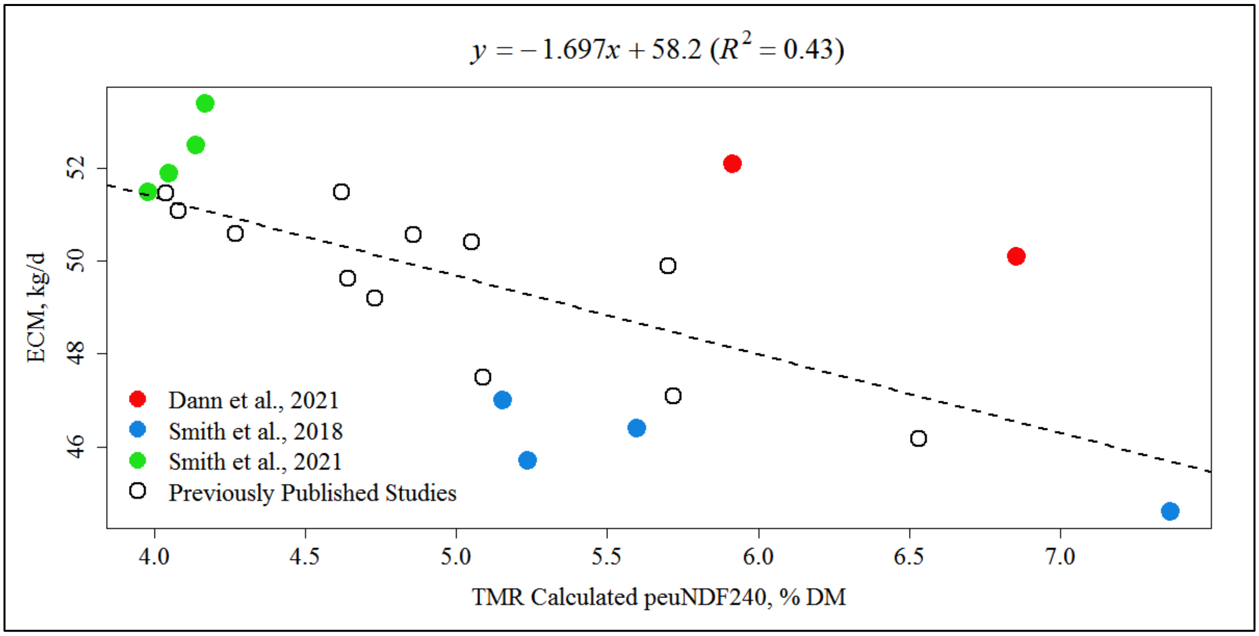

Figure A.5. Linear relationship of TMR calculated peuNDF240 and ECM values of 7 corn silage diet studies conducted at the W.H. Miner Agricultural Research Institute, new data highlighted in color to contrast the Miller et al. 2020 database. (Coons, 2019, Cotanch, 2014, Dann, 2021, Miller, 2017, Miller, 2020, Smith, 2021, Smith, 2019).

Appendix B: Chapter 2

Table B.1. Ingredient and chemical composition [dry matter (DM) basis] of experimental diets[1] fed to rumen microbes in an *in vitro* batch culture system.

	[1]Treatments		
	CS	LC	HC
Ingredient composition, % of DM			
Corn silage	100	70	50
Ground corn	0	30	50
Chemical composition, % of DM			
DM	39.1	44.7	53.3
Crude protein	7.3	8.1	8.4
Acid detergent fiber	22.5	16.3	11.7
Neutral detergent fiber	37.6	28.6	21.4
Lignin	3.0	2.2	1.5
Crude Fat	3.2	2.4	3.7
Nonstructural carbohydrates	49.8	59.9	65.6
Ash	2.6	1.48	0.1
NE_L, Mcal/lb.	0.77	0.81	0.87
NE_M, Mcal/lb.	0.87	0.93	1.02
ME (Mcal/lb.)	1.29	1.38	1.48
Calcium	0.3	0.13	0.10
Phosphorus	0.18	0.2	0.23
Magnesium	0.17	0.14	0.13
Potassium	0.62	0.52	0.48
Sodium	0.02	0.01	0.02
Iron, ppm	123	41	43
Manganese, ppm	33	21	17
Zinc, ppm	24	20	21
Copper, ppm	7	5	4

[1]CS = 100% Corn silage; LC = Low corn, and HC = High corn. Each experimental diet was prepared at four levels of malic acid inclusion i.e., 0, 4, 8 and 16 g/kg DM.

Table B.2. Short chain fatty acid concentrations of corn silage-based diets supplemented with different levels of malic acid and fermented by rumen microbes for 6 h in *in vitro* batch cultures.

	Treatments[1]													Prob <		Level Effect	
	CS				LC				HC								
	Malic acid, g/kg DM																
VFA	0	4	8	16	0	4	8	16	0	4	8	16	SE	Diet	D x L	L	Q
Total, mM	67.3	79.0	78.8	82.5	86.4	85.8	90.3	92.0	82.7	79.8	72.0	79.7	4.53	0.01	0.17	0.14	0.08
Individual, mM																	
Ac	43.1	52.9	51.5	52.8	53.3	54.3	54.2	56.9	51.4	50.8	45.0	51.2	3.39	0.04	0.32	0.26	0.05
Pr	12.7	13.9	14.1	16.1	15.6	15.4	15.3	14.3	12.7	12.2	12.6	13.8	0.86	0.00	0.13	0.12	0.20
IsoBu	0.4	0.4	0.5	0.4	0.3	0.4	0.4	0.4	0.5	0.4	0.3	0.3	0.05	0.88	0.27	0.81	0.51
Bu	10.1	10.1	11.3	13.6	15.6	14.3	18.9	18.6	16.7	15.1	13.0	13.3	1.42	0.01	0.02	0.15	0.74
IsoVa	0.8	1.0	1.1	1.0	0.9	0.8	0.9	1.0	0.9	0.8	0.7	0.7	0.08	0.00	0.06	0.41	0.80
Va	0.4	0.5	0.6	0.6	0.6	0.7	0.7	0.7	0.5	0.6	0.4	0.5	0.07	0.00	0.14	0.33	0.07
A:P	3.5	3.9	3.6	3.3	3.4	3.6	3.5	4.0	4.1	4.2	3.6	3.7	0.22	0.10	0.17	0.48	0.47
Individual, mol %																	
Ac	63.8	67.3	65.0	62.4	61.6	63.0	59.9	61.9	61.9	63.5	62.6	64.1	1.57	0.01	0.42	0.57	0.23
Pr	19.1	17.8	18.0	19.0	18.3	17.9	17.0	15.6	15.7	15.2	17.7	17.4	0.94	0.02	0.14	0.97	0.47
IsoBu	0.6	0.5	0.6	0.4	0.4	0.4	0.5	0.5	0.6	0.5	0.5	0.4	0.06	0.34	0.69	0.63	0.14
Bu	14.8	12.4	14.3	16.2	18.2	16.9	21.0	20.2	20.3	19.1	17.8	16.7	1.50	0.01	0.03	0.47	0.40
IsoVa	1.2	1.2	1.3	1.1	1.0	1.0	1.0	1.1	1.1	1.0	1.0	0.84	0.11	0.01	0.66	0.70	0.29
Va	0.5	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.6	0.7	0.5	0.7	0.07	0.09	0.12	0.52	0.09

[1]CS = 100% corn silage; LC = Low Corn (70% CS + 30% ground corn) and HC = 50% CS and 50% ground corn.

L = Linear; Q = Quadratic

NS = Not Significant (*P* > 0.10).

Table B.3. Short chain fatty acid concentrations of corn silage-based diets supplemented with different levels of malic acid and fermented by rumen microbes for 24 h in *in vitro* batch cultures.

	Treatments[1]													Prob <		Level Effect	
	CS				LC				HC								
	Malic acid, g/kg DM																
VFA	0	4	8	16	0	4	8	16	0	4	8	16	SE	Diet	D x L	L	
Total, mM	87.0	95.6	84.7	4.0	123.6	96.2	95.3	3.2	97.4	95.0	95.7	7.6	63.9	0.12	0.01	0.03	.1
Individual, mM																	
Ac	53.8	58.3	52.2	7.0	73.7	57.9	57.0	9.6	55.6	55.7	56.7	6.9	4.07	0.31	0.02	0.04	.4
Pr	22.1	24.5	21.5	4.4	30.8	23.8	23.9	1.3	25.6	24.5	4.4	5.4	1.56	0.12	0.01	0.03	.4
IsoBu	0.6	0.5	0.4	.4	0.8	0.6	0.5	0.4	0.4	0.4	0.6	.5	0.09	0.27	0.02	0.03	6
Bu	8.9	1.6	9.2	0.4	15.9	12.2	12.1	0.5	14.0	12.6	12.3	3.1	0.89	0.01	0.02	0.02	.2
IsoVa	0.9	1.0	0.9	.0	1.2	0.9	0.9	0.7	0.8	0.8	0.8	.8	0.7	0.7	0.01	0.01	9
Va	0.6	0.8	0.6	.7	1.2	0.8	0.8	0.7	0.9	0.8	0.8	.9	0.08	0.01	0.00	0.02	1
A:P	2.4	2.4	2.1	.3	2.4	2.4	2.4	2.3	2.2	2.3	2.3	.2	0.13	0.37	0.67	0.4	9
Individual, mol %																	
Ac	61.6	60.9	61.5	0.0	59.6	60.2	59.8	9.6	57.3	58.7	59.2	8.3	0.79	0.01	0.71	0.9	2
Pr	25.7	25.7	25.4	6.3	24.9	24.9	24.7	5.2	25.6	26.3	25.9	5.6	26.0	0.73	0.20	0.97	4
IsoBu	0.7	0.5	0.5	.6	0.6	0.7	0.5	0.5	0.4	0.5	0.6	.5	0.08	0.22	0.05	0.14	.6
Bu	10.3	11.1	10.8	0.9	13.0	12.5	12.7	2.7	12.7	14.2	13.3	2.9	13.5	0.54	0.01	0.18	8
IsoVa	1.1	1.1	1.0	.1	1.0	1.0	0.9	0.9	0.9	0.9	0.9	.9	0.06	0.00	0.88	0.24	8
Va	0.7	0.8	0.7	.7	0.9	0.9	0.9	0.8	0.9	0.9	0.9	.9	0.04	0.01	0.07	0.3	5

[1]CS = 100% corn silage; LC = Low Corn (70% CS + 30% ground corn) and HC = 50% CS and 50% ground corn.

L = Linear; Q = Quadratic

NS = Not Significant ($P > 0.10$).

Table B.4. Methane, pH, ammonia, DM and fiber digestibility of corn silage-based diets supplemented with different levels of malic acid and fermented by rumen microbes for 6 and 24 h in in vitro batch cultures.

	Treatments[1]													Prob <		Level Effect	
	CS				LC				HC								
	Malic acid, g/kg DM																
Item	0	4	8	16	0	4	8	16	0	4	8	16	SE	Diet	D x L	L	Q
---------- 6 h ----------																	
CH_4	1.37	1.43	1.48	.37	1.18	1.10	1.42	.37	1.3	1.23	1.16	.06	0.15	0.02	0.33	0.78	.49
pH	6.07	6.04	5.99	.99	5.94	5.92	5.92	.94	6.0	6.05	6.04	.07	0.04	0.01	0.02	0.79	.71
NH_3	5.46	5.44	5.4	.4	5.34	5.33	5.32	.35	5.4	5.44	5.43	.46	0.03	0.01	0.02	0.84	.74
Digestibility, %																	
DM	61.6	54.5	54.3	8.5	71.5	67.3	63.8	4.8	71.6	69.4	70.6	9.6	2.81	0.01	0.51	0.03	.08
NDF	47.4	49.2	48.3	7.8	37.2	40.0	40.8	1.3	33.8	40.3	38.7	1.0	2.54	0.01	0.76	0.06	.03
ADF	25.2	25.8	26.2	4.9	17.1	19.2	21.6	1.7	15.6	19.2	18.3	0.3	2.51	0.01	0.56	0.02	.06
---------- 24 h ----------																	
CH_4	2.87	2.78	2.59	.68	2.4	2.68	2.53	.64	2.91	2.47	2.66	.59	0.15	0.23	0.17	0.37	.61
pH	5.64	5.55	5.56	.56	5.46	5.51	5.49	.49	5.39	5.46	5.48	.47	0.03	0.01	0.03	0.54	0.7
NH_3	5.09	5.01	5.0	.01	4.91	4.96	4.94	.94	4.85	4.91	4.93	.52	0.12	0.35	0.03	0.06	.06
Digestibility, %																	
DM	66.8	70.1	69.3	9.1	74.9	73.7	77.6	7.5	74.6	75.3	75.7	5.7	1.29	0.01	0.47	0.17	.24
NDF	55.0	50.7	53.2	1.3	44.0	44.4	38.9	0.0	43.0	44.7	46.0	5.6	2.8	0.01	0.1	0.25	.43
ADF	29.2	24.6	26.2	5.5	19.4	20.4	15.9	6.8	20.1	23.1	24.5	3.4	2.42	0.01	0.01	0.28	.52

[1]CS = 100% corn silage; LC = Low Corn (70% CS + 30% ground corn) and HC = 50% CS and 50% ground corn.

L = Linear; Q = Quadratic

NS = Not Significant ($P > 0.10$).

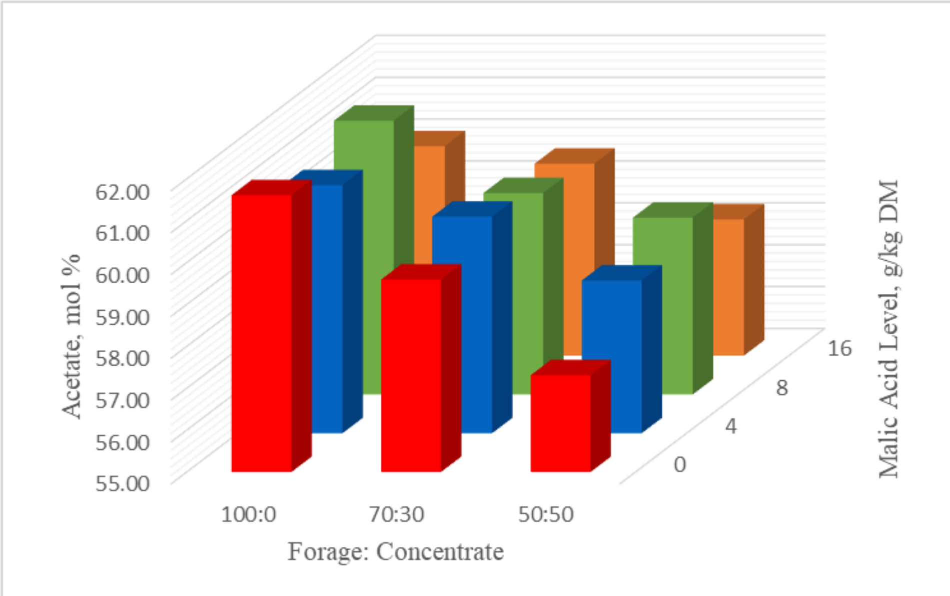

Figure B.1. Molar ratio of Acetate in total ruminal SCFA at 24 h *in vitro* fermentation

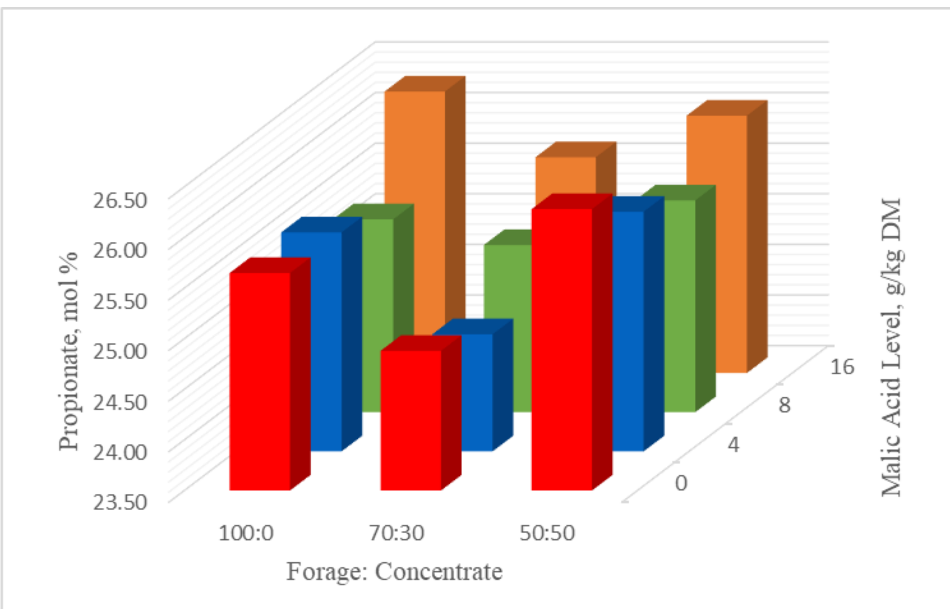

Figure B.2. Molar ratio of Propionate in total ruminal SCFA at 24 h *in vitro* fermentation

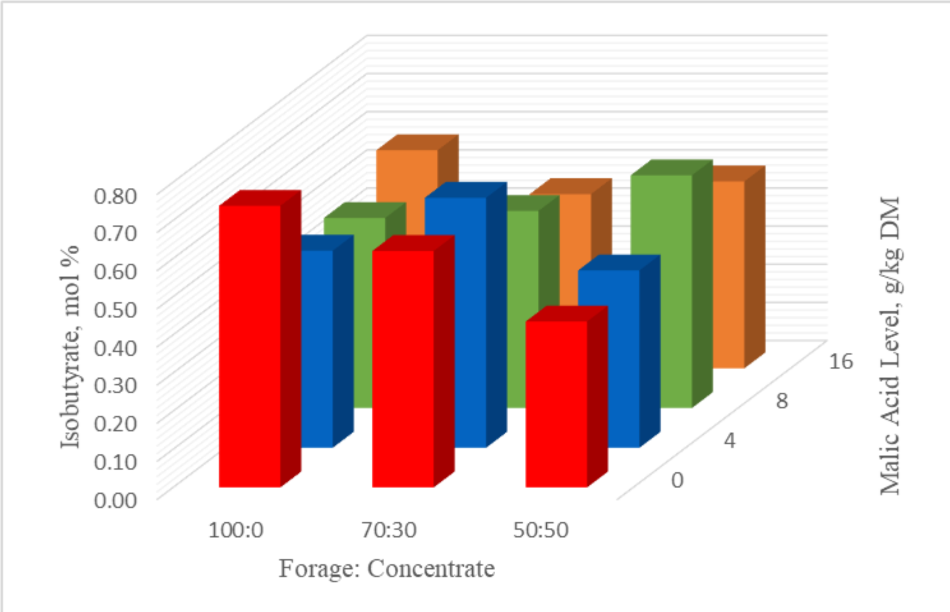

Figure B.3. Molar ratio of Isobutyrate in total ruminal SCFA at 24 h *in vitro* fermentation

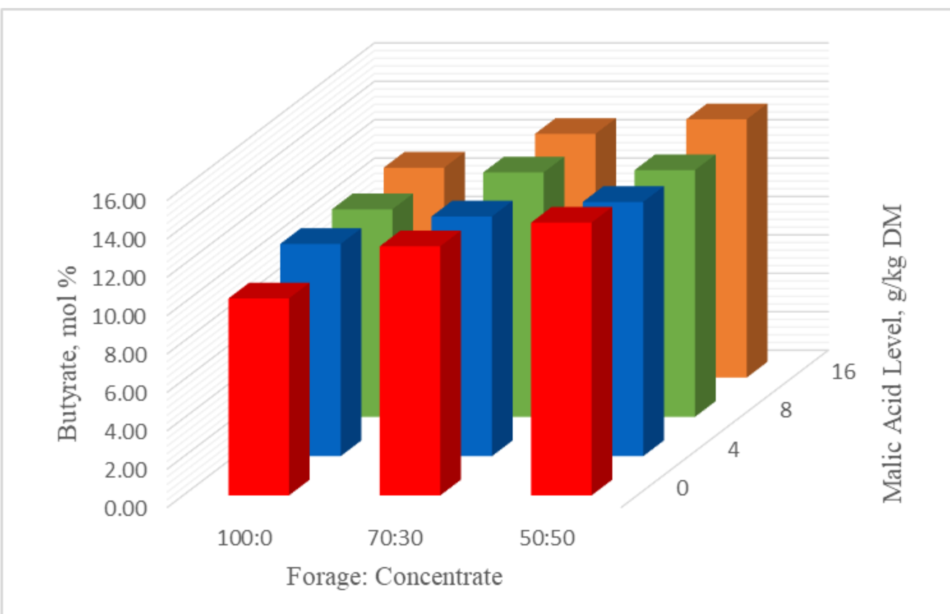

Figure B.4. Molar ratio of Butyrate in total ruminal SCFA at 24 h *in vitro* fermentation

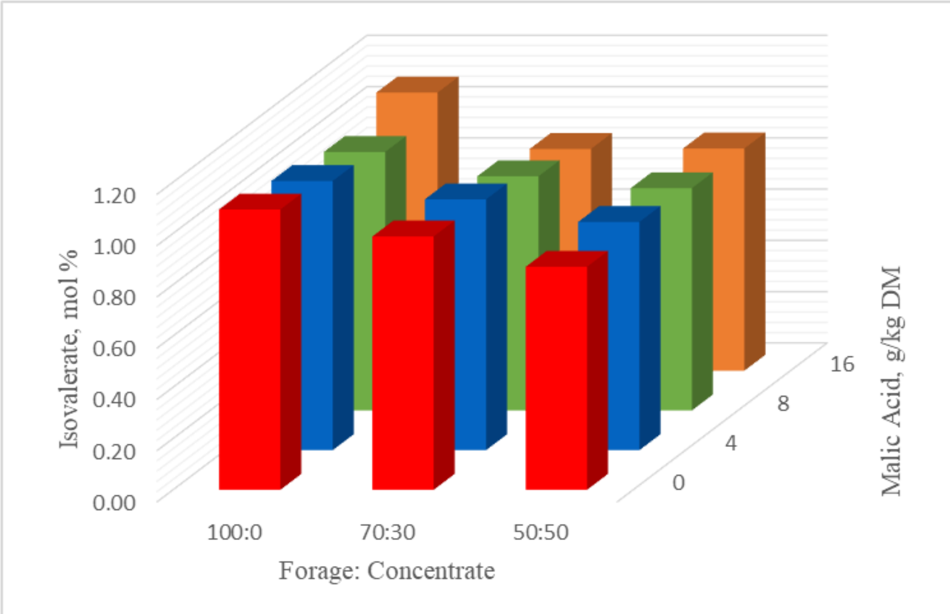

Figure B.5. Molar ratio of Isovalerate in total ruminal SCFA at 24 h *in vitro* fermentation

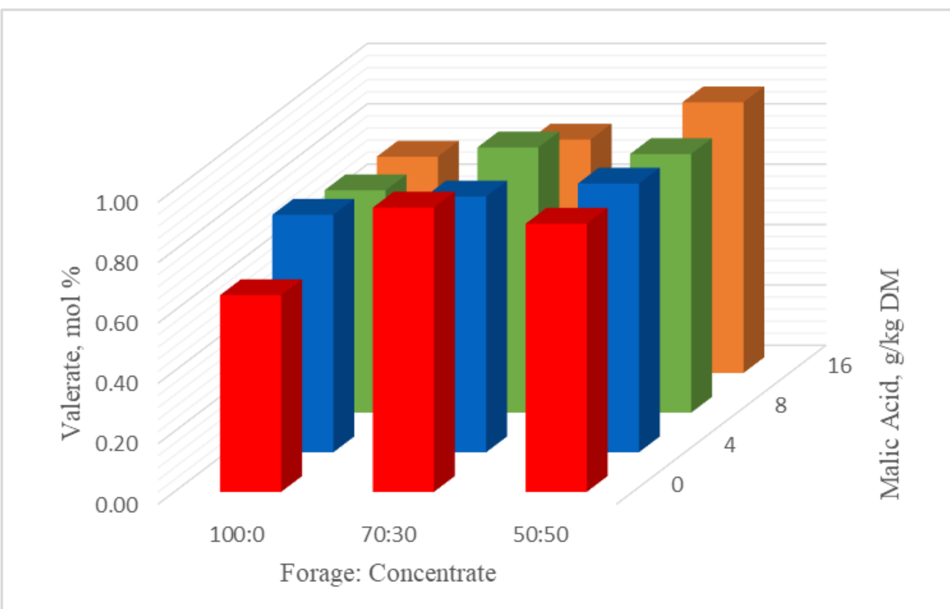

Figure B.6. Molar ratio of Valerate in total ruminal SCFA at 24 h *in vitro* fermentation

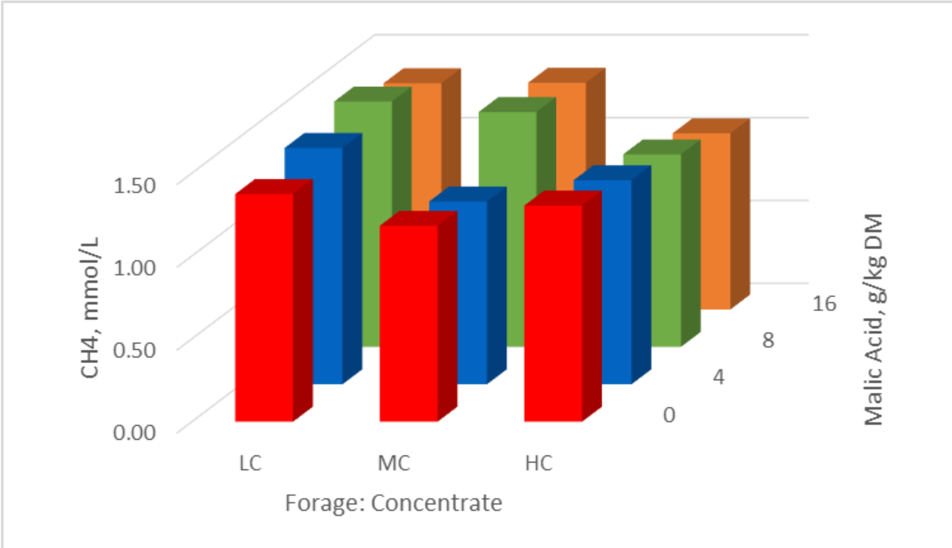

Figure B.7. Ruminal batch culture methane concentration (mmol/L) at 6 h *in vitro* fermentation

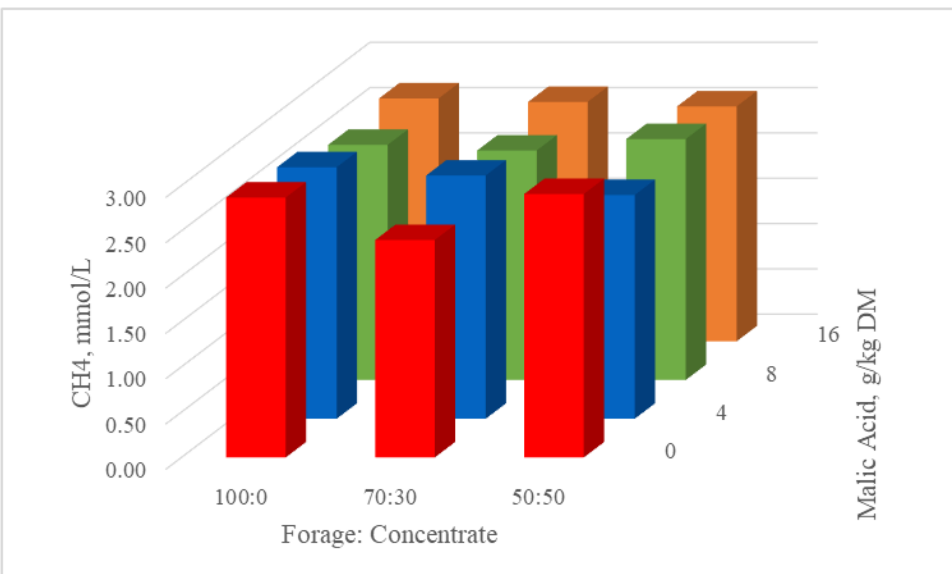

Figure B.8. Ruminal batch culture methane concentration (mmol/L) at 24 h *in vitro* fermentation

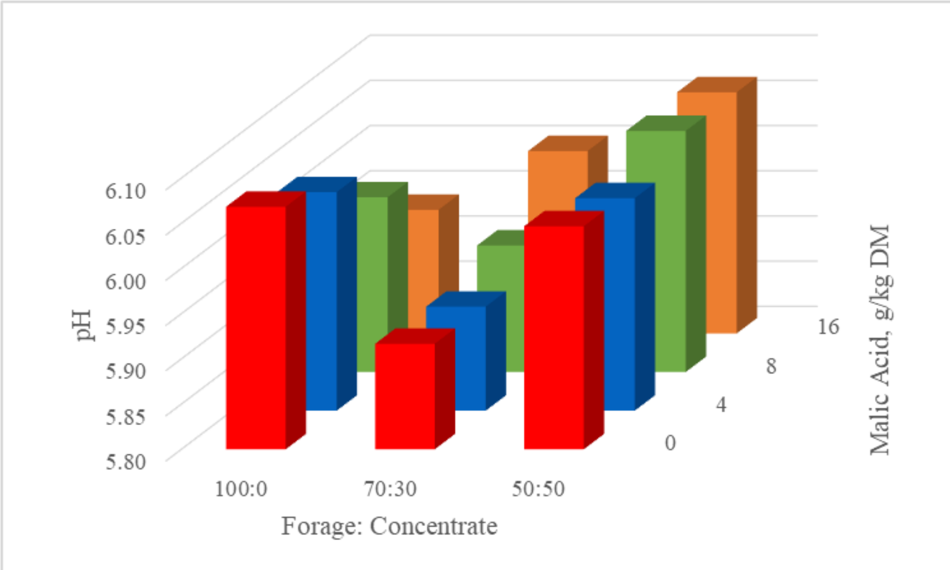

Figure B.9. Ruminal batch culture pH at 6 h *in vitro* fermentation

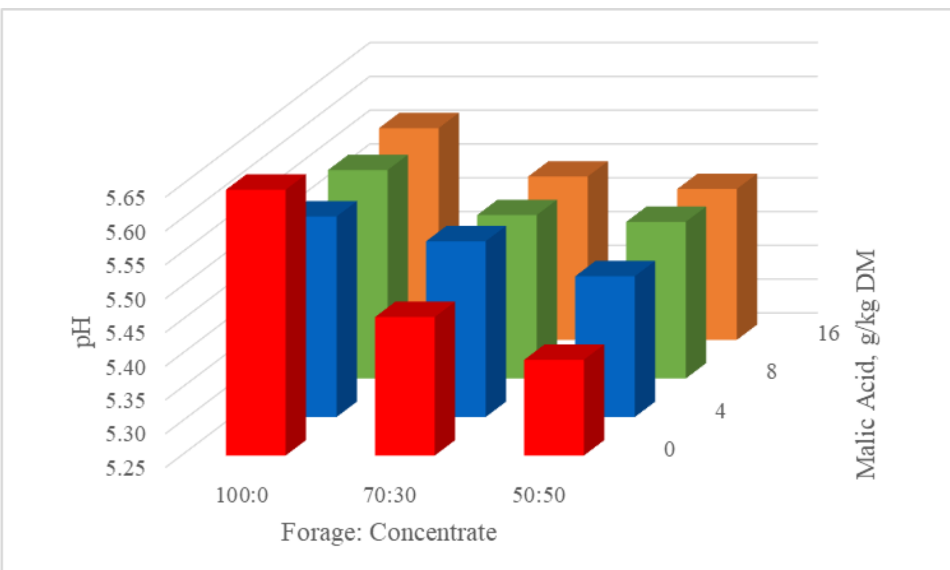

Figure B.10. Ruminal batch culture pH at 24 h *in vitro* fermentation

Appendix C: Supplemental Information

Table C.1. Expanded database of cow response to 7 different corn silage studies conducted at the William H. Miner Agricultural Research Institute in Chazy, NY

Study	DMI, kg/d	ECM, kg/d	uNDF240	Calculated peuNDF240	Measured peuNDF240
Miller et al., 2017	28.60	51.48	8.10	4.62	-
	28.20	50.56	8.10	4.86	-
	27.80	49.89	8.50	5.70	-
Cotanch et al., 2014	29.00	49.62	8.30	4.64	-
	26.50	46.16	10.21	6.53	-
	29.30	51.08	7.07	4.08	-
	29.20	50.42	7.98	5.05	-
Miller et al., 2019	27.50	47.08	8.60	5.72	-
	28.10	49.19	6.90	4.73	-
Coons et al., 2019	26.80	47.50	7.38	5.09	-
	28.00	50.59	6.18	4.27	-
	27.60	51.47	5.77	4.04	-
Smith et al., 2018	27.50	47.00	8.94	5.16	9.47
	27.30	45.70	8.77	5.24	10.00
	27.40	46.40	11.12	5.60	12.08
	24.90	44.60	12.33	7.36	13.28
Dann et al., 2021	27.50	52.10	8.50	5.91	9.65
	26.60	50.10	9.33	6.86	10.92
Smith et al., 2021	29.70	53.40	6.90	4.17	6.35
	29.40	51.50	6.80	3.98	6.07
	29.40	52.50	7.30	4.14	8.60
	29.20	51.90	7.10	4.05	8.00

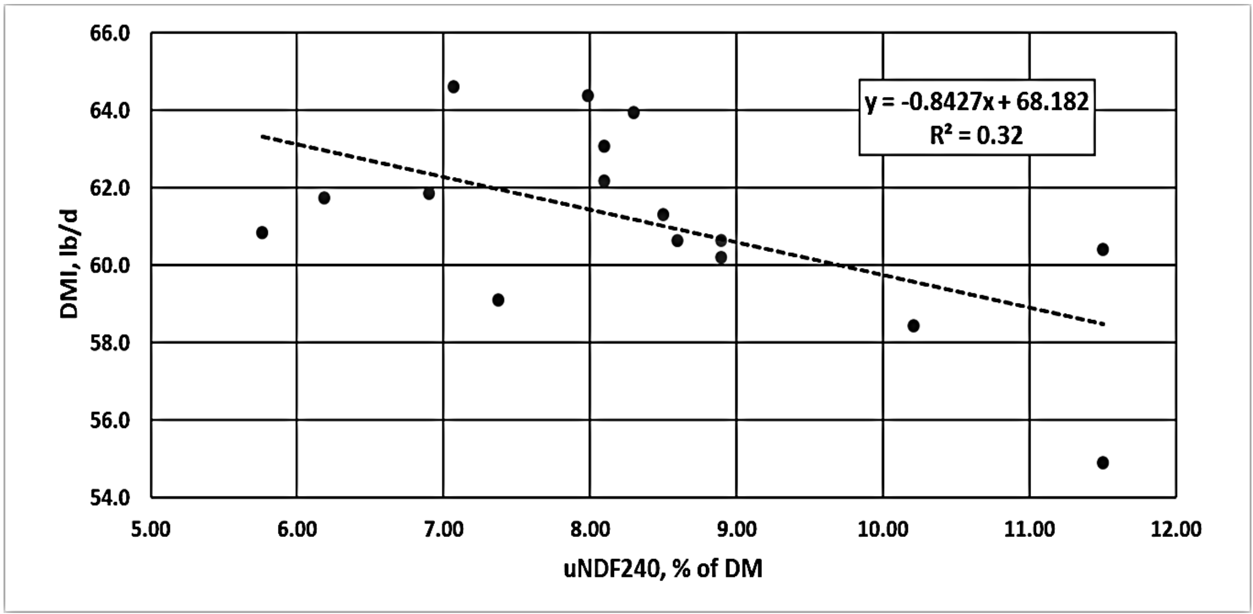

Figure C.1. Linear relationship between uNDF240 and DMI of 5 corn silage studies conducted at the W. H. Miner Agricultural Research Institute, as summarized in Miller et al., (2020) (Coons, 2019, Cotanch, 2014, Miller, 2017, Miller, 2020, Smith, 2019).

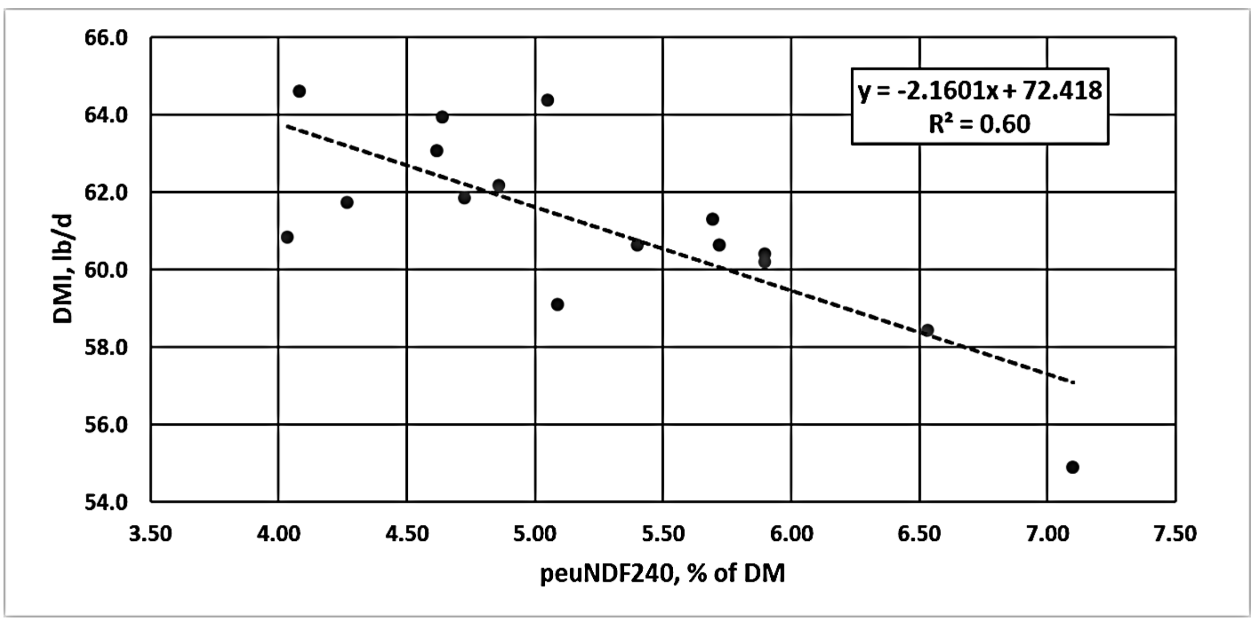

Figure C.2. Linear relationship between peuNDF240 and DMI of 5 corn silage studies conducted at the W. H. Miner Agricultural Research Institute, as summarized in Miller et al., (2020) (Coons, 2019, Cotanch, 2014, Miller, 2017, Miller, 2020, Smith, 2019).

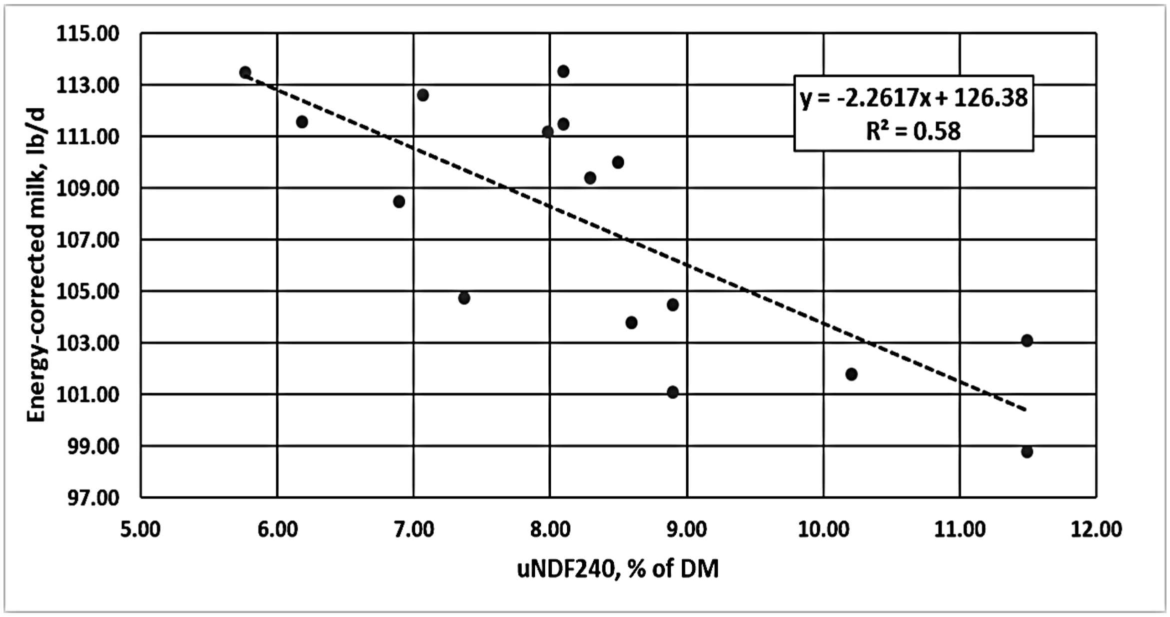

Figure C.3. Linear relationship between uNDF240 and ECM of 5 corn silage studies conducted at the W. H. Miner Agricultural Research Institute, as summarized in Miller et al., (2020) (Coons, 2019, Cotanch, 2014, Miller, 2017, Miller, 2020, Smith, 2019).

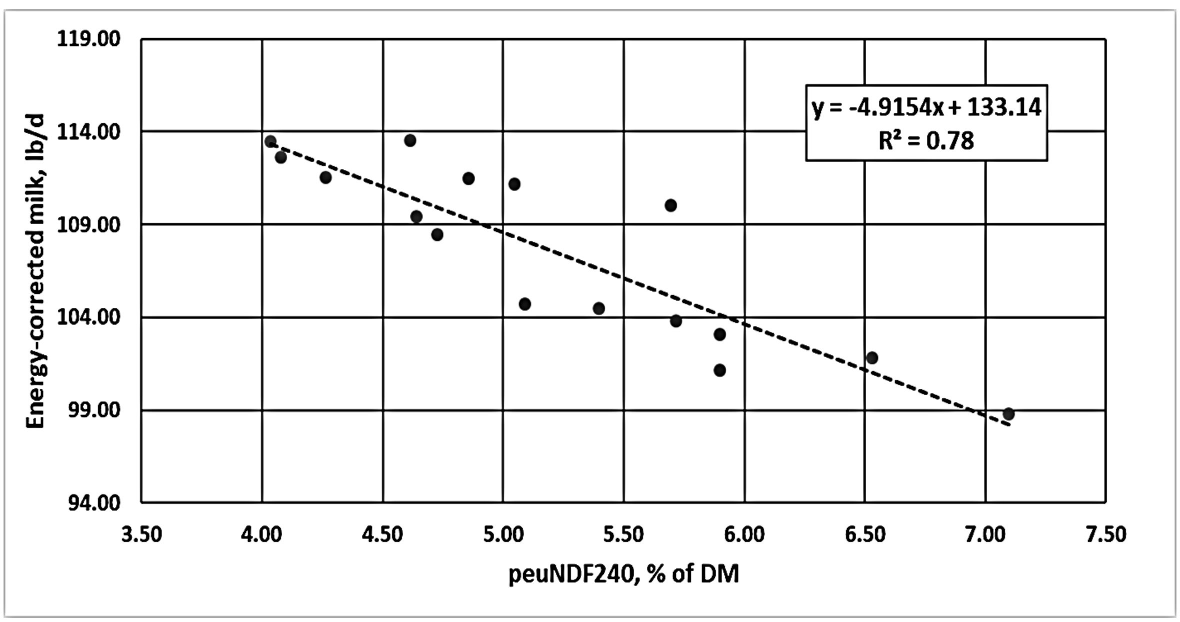

Figure C.4. Linear relationship between peuNDF240 and ECM of 5 corn silage studies conducted at the W. H. Miner Agricultural Research Institute, as summarized in Miller et al., (2020) (Coons, 2019, Cotanch, 2014, Miller, 2017, Miller, 2020, Smith, 2019).