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The Effect Of Physically Effective Undegradable Neutral Detergent Fiber And Rumen Fermentable Starch On Lactating Holstein Cows

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THE EFFECT OF PHYSICALLY EFFECTIVE UNDEGRADABLE NEUTRAL DETERGENT FIBER AND RUMEN FERMENTABLE STARCH ON LACTATING HOLSTEIN COWS

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ABSTRACT

In the dairy industry, a common way of adding energy to the diet is in the form of fermentable starch. However, an overabundance of fermentable carbohydrates can cause a buildup of volatile fatty acids that exceed the buffering capacity of the rumen. These changes can lead to negative health consequences such as subacute rumen acidosis and milk fat depression. Not only does a cow need readily fermentable sources of energy in her diet, but she also needs physically effective structural carbohydrates.

Fiber has been a difficult portion of the diet to characterize as both the chemical and physical properties are important for the maintenance of animal health and to support requirements for proper production. The physical nature of fiber can be described using the measurement physically effective neutral detergent fiber (peNDF). This describes the fraction of dietary fiber that stimulates chewing and forms the rumen digesta mat. The chemical nature of fiber can be described in multiple terms, but to describe the undegradable fraction, a measure named undegradable neutral detergent fiber after 240-h of fermentation (uNDF240) was developed. This fraction is important to characterize because of its effect on gut fill, degradation, and passage dynamics in the rumen. Research has observed a relationship between uNDF240 and peNDF. Combining these two characteristics into one measurement could give nutritionists a more useful measurement when balancing diets for dairy cattle. This measurement is the physically effective uNDF240 (peuNDF240). But, in order to fully implement this measurement in ration balancing, there must be more research on how peuNDF240 affects dry matter intake (DMI) and milk responses.

The primary goal of this thesis research was to observe the interactions between rumen fermentable starch (RFS) and peuNDF240 in the rumen. The focal study (Chapter 2) investigated the effects of RFS and peuNDF240 on DMI, lactation performance, behavior, and the rumen environment of lactating Holstein dairy cows. The four diets were: 1) low peuNDF240 (6.4% of DM), low RFS (16.7% of DM); 2) low peuNDF240 (6.1% of DM), high RFS (19.2% of DM); 3) high peuNDF240 (8.6% of DM), low RFS (16.9% of DM); and 4) high peuNDF240 (8.0% of DM), high RFS (19.0% of DM). Cows fed higher peuNDF240 diets consumed less DMI as a percentage of body weight than cows fed lower peuNDF240 diets. Cows fed diets containing higher RFS produced less milk fat and 3.5% fat-corrected milk (FCM) than cows fed lower RFS. Lower peuNDF240 diets resulted in lower *de novo* and mixed origin fatty acids in milk fat and a greater degree of unsaturation. Overall, higher RFS diets tended to reduce the efficiency of FCM production (FCM/DMI) compared with lower RFS diets. There was no effect of peuNDF240 or RFS on eating or ruminating time per day, but higher RFS diets reduced meal length and increased daily meal bouts. The lower RFS diets reduced acetate:propionate ratios, but there was no effect of treatment on measures of rumen pH. Diets with higher RFS tended to increase rumen pool size of starch while higher peuNDF240 diets increased pool size of uNDF240 although there were few dietary effects on rumen turnover of starch or uNDF240. Lower peuNDF240 diets resulted in greater total tract NDF digestibility than higher peuNDF240 diets. Even low to moderate RFS can elicit milk fat depression when dietary peuNDF240 is low and ranging between 6.0 and 8.6% of DM.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADF: acid detergent fiber
aNDFom: amylase- and sodium sulfite-treated neutral detergent fiber, organic matter basis
BMR: brown midrib
BW: body weight
BCS: body condition score
CLA: conjugated linoleic acid
CON: conventional corn silage
CP: crude protein
D: day
DGC: dry-ground corn
DIM: days in milk
dL: decileter
DMI: dry matter intake
ESC: ethanol soluble carbohydrates
FCM: 3.5% fat-corrected milk
H: hour
HMC: high-moisture corn
iNDF: indigestible neutral detergent fiber
kg: kilgram
LPS: lipopolysaccharide
MFD: milk fat depression
Mg: miligram
Min: minute
mM: milimoles
NDF: neutral detergent fiber
NDS: neutral detergent solubles
NFC: non-forage carbohydrates
NSC: nonstructural carbohydrates
OM: organic matter
pdNDF: potentially degradable neutral detergent fiber
pef: physical effectiveness factor
peNDF: physically effective neutral detergent fiber
peNDF4.0mm: physically effective neutral detergent fiber measured using the adapted 4.0-mm sieve on the Penn State Particle Separator
peuNDF240: physically effective undegradable neutral detergent fiber after 240-h of fermentation
RFS: rumen fermentable starch
RR: reticulorumen
SARA: subacute rumen acidosis
SAS: statistical analysis system
SCM: solids-corrected milk

TTD: total tract digestibility
uNDF: undegradable neutral detergent fiber
uNDF30: undegradable neutral detergent fiber after 30-h of fermentation
uNDF72: undegradable neutral detergent fiber after 72-h of fermentation
uNDF120: undegradable neutral detergent fiber after 120-h of fermentation
uNDF240: undegradable neutral detergent fiber after 240-h of fermentation
VFA: volatile fatty acid
Wk: week

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW

1.1. Introduction

The interaction between fiber and starch in the rumen has been well documented in the scientific literature (el-Shazly et al., 1961; Mertens and Loften, 1980; Sarwar et al., 1992; Van Soest, 1994; Beauchemin, 2007). However, there is still needed information on how fiber of differing degradability and particle size interacts with fermentable starch in the rumen, and what combinations elicit the best response from cows fed starchy, highly digestible diets. As we have learned previously from Smith (2019), the physical nature of fiber influences its rumen degradability. A measure that combined ration particle size and fiber undegradability predicted dry matter intake (DMI) better than either measure alone (Smith, 2019). We need to understand how a combined measurement interacts with different concentrations of rumen fermentable starch (RFS), and how they together influence DMI, chewing response, lactation performance, and rumen dynamics. This information will allow the industry to better formulate diets for high producing lactating dairy cows using today's dynamic nutrition models.

1.2. Characterizing physically effective undegradable fiber

1.2.1. What is fiber?

Fiber varies widely in bioavailability to the microbial population within the rumen and can be defined as the slowly degrading or undegradable fraction of feeds that occupies space in the animal's gastrointestinal tract (Van Soest, 1994; Mertens, 1997). Fiber is often classified chemically by its covalent linkages, but this simple approach fails to capture the complex physical and chemical makeup of the plant cell wall. To better understand fiber, it should be considered a macromolecule with large-scale

organization instead of an entity made of chemical linkages (Van Soest, 1994). The polysaccharides that are associated with the plant cell wall may be ether-linked or not linked to the lignified core (Van Soest, 1994). The differentiation of linkages to the lignified core is based on both biological composition of the plant and nutritional availability for the animal (Van Soest, 1994). The structural carbohydrates that are linked to the core via covalent linkages are partially or incompletely fermented in the rumen and are the structures that make up the primary cell wall matrix (Van Soest, 1994).

Hemicellulose, cellulose, and lignin comprise the different polysaccharides and polyphenolics in the cell wall matrix, respectively. Hemicellulose content varies widely among different plant species and is a heterogeneous mixture of polysaccharides with β 1-4 linkages in the core polymer with a variety of glycosidic bonds that link sugars to their backbone (Van Soest, 1994). Hemicellulose has a close relationship with lignin, and its degradability correlates directly to cellulose and to lignin (Van Soest, 1994). Hemicellulose is held in place by cross-links with lignin, making up the secondary cell wall, generally making it insoluble unless it is delignified (Van Soest, 1994).

Cellulose, like hemicellulose, contains β 1-4 glucan linkages, which combine, to some degree, with hemicellulose and lignin in the plant cell wall structure (Van Soest, 1994). Cellulose is the most abundant carbohydrate in the world. However, while it constitutes a major proportion of the cell wall's structure, its concentration is not a reliable measurement of cell wall degradability or undegradability (Van Soest, 1994).

Lignin, unlike hemicellulose and cellulose, is not as simple to define. There are varying definitions based on scientific discipline and nomenclature (Van Soest, 1994). In simplest terms, lignin is a major plant phenolic polymer that gives rigidity to the plant,

and creates a hydrophobic barrier to degradation (Van Soest, 1994; Jung et al., 2012). This rigidity provides resistance to abiotic and biotic stressors like disease, insects, cold temperatures, and drought conditions (Buxton and Redfearn, 1997). Lignin is a necessary evolutionary adaption for the plant, and so there are limitations to how little lignin a plant can contain (Buxton and Redfearn, 1997).

Concentration of a given polymer in the cell wall is not always a useful measurement of fiber or its degradability. It is here that the problem with measuring meaningful attributes of fiber in the plant and estimating how they will affect degradability in the cow begins. The amount of lignin present, even though it is the major component affecting degradability, is not always an acceptable measurement of undegradability. The amount of hemicellulose present is not an acceptable measurement of potentially degradable fiber. In addition, separately measuring hemicellulose and cellulose does not provide a meaningful interpretation of fiber degradability either. The attempt to measure fiber using chemical methods has been a topic of discussion for dairy nutritionists for the last 100 years (Hall and Mertens, 2017). Fiber is difficult to categorize as both the chemical and physical characteristics affect rumen function and fermentation, metabolism, and therefore production of the cow (Hall and Mertens, 2017). The degree of lignification in the plant cell wall dictates the degree of degradability in the rumen, but its physical structure is just as important to consider when measuring degradability (Van Soest, 1994). Finding a measurement that considers both the fiber content and its degradability is important for ration formulation and improving predictions of animal response to a specific diet.

1.2.2 What are the chemical characteristics of fiber?

The early 1960s and late 70s were a break-through time for fiber nutrition. Van Soest first published a comprehensive chemical fractionation system for forages in 1967. This gave nutritionists a way to chemically analyze forage samples and utilize these measurements in a nutritionally relevant way. In the Van Soest system, the forage dry matter is separated into two categories based on its solubility in neutral detergent solution. This approach separates the cell wall of the plant from the plant cellular contents (Van Soest, 1967).

Insoluble fiber, or the cell wall constituents, forms the rumen digesta mat and therefore stimulates rumen function. This portion is measured as neutral detergent fiber (NDF), named after the solution it is boiled in (neutral detergent solution), and it primarily measures the amount of hemicellulose, cellulose, and lignin in the plant cell wall (Van Soest et al., 1991). The NDF usually accounts for around 30-80% of the organic matter in most forages (Buxton and Redfearn, 1997). Unlike NDF, the second fraction containing the plant cell's cellular contents, otherwise known as neutral detergent solubles (NDS), is almost 98% digestible for most forages (Van Soest, 1994). In the plant, NDS is primarily water-soluble carbohydrates, protein, and fat (Van Soest, 1994). The NDF of feed is highly related to degradability because it is less degradable than the cell contents and its degradability varies in relation to lignification. This is an important concept because NDF content and the large difference in degradability between NDF and NDS is the most important influencer of how a feed or diet will affect animal performance, specifically DMI (Mertens, 2015).

Generally, when NDF content in the diet increases, DMI decreases because of limits to rumen distension (Allen, 2000). This limitation results from the decrease in the

rate of digesta removal, passage, and breakdown of forage with higher NDF content (Allen, 2000). The reticulorumen (RR) is the main site at which distension regulates DMI in ruminants. Distension in this area stimulates mechanoreceptors in the muscle layer of the RR, concentrated in the reticulum and the cranial sac of the rumen (Allen 2000, Van Soest 1994). These stretch and tension receptors send feedback to the brain satiety center to trigger meal cessation. The extent that distension affects the DMI of lactating cows depends on the animal's stage in lactation and energy requirements (Allen, 2000). Cows that are in a lipolytic state during early lactation are more likely to be affected by the chemical regulation of feed intake instead of distension (Allen and Piatonni, et al., 2013).

This association between NDF and DMI was explored in a study that fed four diets with different roughage concentrations that linearly increased in 10% increments from 40 to 70% roughage (Jiang et al., 2017). As roughage increased in the diet, so did NDF content while DM of the diet was kept constant. The NDF ranged from 35.4% of DM in the diet with 40% roughage to 43.4% of DM in the diet with 70% roughage. As a result, DMI linear decreased by 4 kg/d as NDF content increased from 35.4 to 43.4% (Jiang et al., 2017).

Even though the NDF fraction has consistent effects on DMI, its degradability can vary substantially. The degradability of the NDF fraction is highly variable because different growing conditions can have an influence on degradability (Van Soest, 1994). During maturation, several grass cell types become more lignified and cross-linked, and environmental stress can have a significant effect on maturation (Buxton and Redfearn, 1997). In general, the degradability of a forage is influenced by its maturity so any environmental or agronomic factors that impact the maturity of the forage will impact

the degree of lignification (Buxton, 1996). Specifically, environmental factors such as increases in temperature are what cause year-to-year and seasonal variations in forage quality (Buxton, 1996). A rise in ambient temperature will increase the maturation of the plant and will lower forage quality when compared to the same forage grown at normal temperatures and harvested at the same morphological stage (Buxton, 1996). On average, a 1° C increase in temperature will decrease degradability of cool season forages by 3-7 g/kg, and this is attributed to an increase in NDF concentrations and lignification (Buxton, 1996).

The NDF system quickly became popular within the dairy industry because of its ability to measure the cell wall constituents that stimulate rumination and fill in the rumen. However, NDF alone does not account for all of the variability observed in intake and milk production of dairy cattle (Mertens, 2015). The NDF fraction also does not represent a class of chemical components that is homogenous (Raffrenato and Van Amburgh, 2010). However, the variability within NDF is much less than its difference from NDS (Mertens, 2015). Mertens (1997) stated that formulating rations solely based on NDF will not account for differences in digestion and passage of fiber, but it is still incredibly valuable when separating feed into two categories of digestible (NDS) and potentially degradable (highly variable) feed (NDF; Mertens, 2015). To accurately predict how a cow will react to a diet, the degradability of the NDF present is also necessary because the degradability of NDF dictates animal performance independent of the dietary NDF concentration (Mertens, 2009; Raffrenato and Van Amburgh, 2010). However, the non-uniform characteristics of NDF degradability dictate that NDF has fractions with different degradation properties (Mertens, 2015).

1.2.3 NDF fractionation

Neutral detergent fiber can be fractioned into two main categories: potentially degradable (pdNDF) and indigestible (iNDF). Waldo et al. (1972) first described this theoretical distinction between digestible and indigestible cell wall fractions in 1972. As previously stated, the degree of cross-linking with lignin is the greatest deterrent to rumen NDF degradability, and therefore, it has the greatest influence on *in vivo* degradation in the rumen (Van Soest, 1994). The iNDF is comprised of hemicellulose and cellulose that are highly associated with lignin and are therefore unable to break down in the rumen regardless of the amount of time it ferments (Van Soest, 1994). This transformative discovery allowed for the development of fiber digestion models because the pdNDF fraction (i.e., NDF – iNDF) follows first-order degradation kinetics and rates and extents of NDF degradation can be easily calculated. Consequently, the concept of iNDF and pdNDF has been used in fiber research and model development for the last 45 years (Mertens, 2015). The problem is that iNDF is a theoretical term and cannot actually be measured because it requires an infinite amount of time, and so an approximation of iNDF, termed undegradable NDF (uNDF), was created (Mertens, 2016; Raffrenato et al., 2018). Undegradable NDF is a laboratory estimation of iNDF based on specific fermentation lengths. Research has been done to evaluate what time point should be used to represent the residue remaining after complete fermentation in the rumen. Recently, Raffrenato et al. (2018) have specified that a 240-h in vitro fermentation appears to be adequate. To determine this, Raffrenato et al. (2018) analyzed 102 forages of different species for amylase- and sodium sulfite-treated NDF reported on an organic matter basis (aNDFom) and uNDF using different time points between 12- and 504-h. Their goal was

to reach a point in fermentation where the weight of the residue did not significantly change with additional fermentation time (Cotanch et al., 2014). The difference in aNDFom disappearance was not detected in any fermentation time past the 240-h time point. Furthermore, to test if degradable aNDFom was depleted at the 240-h time point, a Student's *t*-distribution was used to make a paired comparison between the means and showed that there was no difference between the 240- and 504-h time points. This allowed for the practical fractionation of NDF into uNDF and pdNDF by commercial feed testing laboratories (Raffrenato and Van Amburgh, 2010).

Mertens has hypothesized that NDF degradation is more accurately predicted when pdNDF is assumed to be the sum of two degradable pools, fast and slow fermenting NDF (Mertens, 1977). Raffrenato and Van Amburgh (2010) have since confirmed this theory. They modeled the disappearance rate of 35 different forage samples from 0- to 240-h of fermentation (Raffrenato and Van Amburgh, 2010). They observed that, by 30-h the fast pool of NDF was mostly exhausted, and by 120-h the slow pool was exhausted, leaving the undigested pool after 240-h (uNDF240; Raffrenato and Van Amburgh, 2010). Making this distinction between the two pools is important because the degradation rate of pdNDF appears to affect meal patterns and DMI together with uNDF240 (Cotanch et al., 2014).

The uNDF240 content is highly correlated to the DMI limitations of the cow (Mertens, 2016). The uNDF240 fraction influences DMI because of its effect on gut fill, digestion dynamics, and passage dynamics in the rumen (Cotanch et al., 2014). According to Cotanch et al. (2014), rumen fill is a function of dietary uNDF240, slowly fermenting NDF, and undegraded fast-pool NDF, and the total mass of uNDF240 within

the rumen establishes a baseline of fill. This helps improve estimations of DMI because, in a diet, there is a maximum, or how much uNDF240 a cow can consume before filling her rumen, and a minimum, or how much uNDF240 is required to maintain rumen fill and digestive efficiency (Cotanch et al., 2014). There can be greater DMI when there is greater turnover since rumen space results from turnover of the fast, slow, and uNDF240 pools (Cotanch et al, 2014). However, in a diet with greater uNDF240, rumen turnover rate decreases, retention time in the rumen increases, and this restricts DMI. This effect was observed in a study that assessed feeding diets with either lower (50%) or higher (65%) forage content and differing forage NDF source (conventional corn silage vs. brown midrib (BMR) corn silage; Miller et al., 2021). The difference in forage NDF source caused a difference in uNDF240 between the diets, where the conventional corn silage was around 9% of ration DM and the BMR corn silage was around 7% of ration DM. The BMR corn silage, or the low uNDF240 diet, increased DMI when fed at a higher forage content (67%) compared to the conventional corn silage diet fed at the same forage content (Miller et al., 2021). In addition, the DMI as a percentage of body weight increased for cows fed the low uNDF240 diet in a higher forage diet compared to the high uNDF240 diet. This response was due to less uNDF240, hence higher degradable NDF, which allowed for greater turnover in the rumen, and therefore greater intake. The diets with BMR corn silage, or lower uNDF240, also caused a reduction in rumen digesta volume and mass across forage inclusion levels.

1.2.4 What are the physical characteristics of fiber?

The degradability of NDF is a necessary component for predicting the DMI and milk production of the cow. However, degradability of a forage is not the only variable

accounted for when predicting the production potential of a cow when fed a specific diet (Mertens, 1997). The physical characteristics of fiber, such as particle size and density, are also important as they also contribute to the wide variation observed in lactation performance that is not accounted for simply by NDF content (Van Soest, 1994; Mertens 1997). Physical characteristics of fiber can influence rumen fermentation, milk fat production, and animal health independently of chemical composition (Mertens, 1997). Consequently, a measurement for particle size was needed that could be determined quantitatively using repeatable laboratory methods (Mertens, 1997).

One definition of the effectiveness of fiber is the ability of the fiber to maintain rumen and animal health (Mertens, 1997). Physically effective NDF (peNDF) is a measurement that relates to the physical characteristics, specifically particle size, that influences the chewing activity and rumen digesta mat development of the animal (Mertens, 1997). This means that the peNDF relates to a feed's ability to form the rumen mat and the pattern of rumination that maintains rumen pH (Mertens, 1997). This measurement provides consistency in the measurement of physically effectiveness because it takes into account the two variables that affect chewing the most: the NDF content and the particle size, and minimizes the animal variation (Mertens, 1997). The peNDF was developed as a metric to indicate the chewing activity based on known associations with both the NDF content of the diet and the physical effectiveness factor (pef). The pef is a scale that defines physical effectiveness as a reference for which all feeds are comparable against a hypothetical standard (Mertens, 1997). This hypothetical standard would elicit the maximum amount of chewing per kg of NDF, specifically a long grass hay with 100% NDF and a pef of 1.0 (Mertens, 1997). The pef is on a

theoretical scale of 0 to 1 where 0 means the NDF does not stimulate chewing, and 1 elicits the maximum chewing response (Mertens, 1997). Mertens (1997) suggested that only the fiber particles that were large enough to stay in the rumen and require chewing should be related to peNDF. The hypothesis was that particles that do not require or stimulate chewing would appear in the feces because they have escaped the rumen (Poppi et al., 1985). It was concluded that particles that fall below the 1.18-mm sieve with dry vertical sieving have the potential to escape from the rumen and do not stimulate the cow to chew, and so the particles that are retained on and above the 1.18-mm sieve are considered physically effective (Poppi et al., 1985). An on-farm pef value for silages and chopped dry forages can be determined on as-fed basis using the Penn State Particle Separator, but it must be adapted with a 4-mm sieve that provides pef values similar to the standard dry sieving method (Poppi et al., 1985; Cotanch et al., 2010). When the 4-mm sieve is used, the peNDF is commonly referred to as $peNDF_{4.0mm}$ to differentiate between when the 4-mm sieve for the Penn State Particle Separator is used and when a dry vertical sieve is used (1.18-mm sieve).

In 1997, Mertens gathered information from 45 studies that had applicable particle size data in a meta-analysis. His goal was to develop a system that evaluates the effectiveness of fiber in feeds and define the effective fiber requirements of dairy cattle (Mertens, 1997). Mertens (1997) developed correlations between chewing activity and the amount of peNDF in a forage using a regression analysis. An r^2 of 0.76 to 0.81 was calculated between total chewing activities, including rumination, and peNDF once outliers and experiments were corrected for in the regression (Mertens, 1997).

Mertens further hypothesized a link between rumination and rumen pH, based on salivary buffer secretion, and this link aided in establishing a set requirement of peNDF for dairy cattle (Mertens, 1997). He found that there was a strong positive relationship ($r^2 = 0.71$) between dietary peNDF and rumen pH (Mertens, 1997). Based on regression analysis, a peNDF intake of 4.40 kg/d or a concentration of 22.3% of ration DM was required to maintain a rumen pH of 6.0 (Mertens, 1997). However, to maintain 3.5% fat-corrected milk (FCM) during mid-lactation for Holstein cows, the concentration of peNDF should be closer to 19% of ration DM (Mertens, 1997). These differences in requirement represent the difficulty in defining a set requirement of fiber characteristics in the diet of lactating cows. There may be an optimal peNDF depending on the situation and the goal. The cows in the data set were in early to mid-lactation, so the author hypothesized that maintaining FCM during this stage of lactation may be a more accurate indicator of the minimum requirements for peNDF for dairy cows compared to rumen pH (Mertens, 1997). Although the peNDF system mostly relates to the particle size of the diet, it explains a large amount of the variation in chewing activity, rumen pH, and milk fat depending on the forage used (Grant et al., 2018). Both the amount and the effectiveness of the fiber has an effect on the metabolism and chewing behavior of the animal (Mertens, 1997).

It is key to remember that the physical characteristics of the diet drastically affect the amount of the time the cow will spend consuming feed (Jiang et al., 2017). For example, to evaluate the ability of a forage to mitigate subacute rumen acidosis by promoting chewing activity and saliva secretion, a study used four treatment diets with increasing forage inclusions ranging from 40, 50, 60 and 70% roughage (Jiang et al.,

2017). Consequently, the $peNDF_{4.0mm}$ increased with increasing forage inclusion, ranging from 14.9, 19.2, 23.9, and 29.0 for the 40, 50, 60, and 70% roughage diets, respectively. As $peNDF_{4.0mm}$ in the diets increased, there was a linear increase in daily chewing time as an effect of increased eating time rather than ruminating (Jiang et al., 2017). As dietary roughage inclusion increased, the contribution of rumination to daily chewing decreased from 60 to 42%. The increase in daily chewing time was mainly a result of prolonged time spent eating per meal. There was also an increase in total chewing time per kilogram of NDF intake as the dietary roughage inclusion increased. Eating rate (g of DM/min) decreased with increased roughage inclusion, but ensalivation of ingested feed (mL/g of DM) increased causing saliva secretion rate to be unaffected by diet (Jiang et al., 2017).

Although peNDF has a strong effect on the DMI and chewing response of the cow, commonly, there is no response in milk yield (Allen and Grant, 2000; Farmer et al., 2014; Smith, 2019). As seen in Allen and Grant (2000), as peNDF increases, fiber utilization increases in the rumen, resulting in similar milk yields compared with a diet with lower peNDF. A similar effect was observed in Farmer et al. (2014) where the treatment diets involved feeding lower starch diets (21%) with decreasing forage content (52, 47, 43, and 39% of ration DM). As corn silage and haycrop silage was removed from the diet, chopped wheat straw was added to maintain chewing activity, but the peNDF was still reduced as forage in the diet decreased. As expected, the DMI increased as forage content and peNDF decreased, but there was no statistical difference in milk production (Farmer et al., 2014). There was also no statistical difference in milk composition in this study. Different peNDF content often affects the milk fat content

because of its effect on eating and rumination. According to Mertens (1997), both eating and ruminating increases saliva production, and both the rumen pH and the pattern of fermentation is a function of the production of buffer from the saliva. This effect was observed in a study that fed TMR with three different sizes of alfalfa measured as geometric mean of particle size: long (7.83 mm), medium (4.04 mm), or fine (1.14 mm) (Yansari et al., 2004). The peNDF of the TMR decreased as the length of the alfalfa decreased. The diet with fine alfalfa had a significant reduction in rumen mean retention time and a significant increase in rumen passage rate compared to the diets with long and medium length alfalfa (Yansari et al., 2004). This resulted in significantly greater DMI, lower pH, and lower total chewing activity for the diet with fine alfalfa. This corresponded to a significant difference in milk fat yield (Yansari et al., 2004). The diet with fine alfalfa had a significantly lower milk fat produced compared to the diets that used long and medium length alfalfa (Yansari et al., 2004).

1.2.5 Utilizing both the physical and chemical nature of fiber

Within the peNDF system, there is an assumption that all fiber elicits the same chewing response no matter the forage type (Mertens, 1997). However, this is not always the case. Grant (2010) characterized that different forages can have different fragilities (Grant, 2010). Fragility was defined as the rate of reduction in particle size when a feed is ball milled (Grant, 2010). A study evaluating four forages was designed to observe how fragility affected the pef values to give a more accurate prediction of the cow's chewing response (Grant, 2010). The first comparison examined two hays with either low NDF degradability and low fragility or high NDF degradability and high fragility. The second comparison assessed two forages with either high or low fragilities that had

similar NDF degradability. The basis behind this research was the assumption that different forages of similar particle size would stimulate different chewing responses from the cow based on the forage's anatomical and chemical differences such as cell wall thickness or lignin content and cell wall degradability (Grant, 2010; Van Soest, 1994). When comparing hays lower in NDF degradability and fragility to the hay with higher NDF degradability and fragility, the hay with lower NDF degradability and fragility resulted in a greater chewing response. When comparing the two hays with similar NDF degradability but different fragilities, there was little difference in chewing response. These data show that the degradability of fiber can influence its physical effectiveness (Grant, 2010).

Smith (2019) proposed a way of combining the physical as well as the chemical nature of fiber into a single measurement. This new term was calculated by multiplying the pef of a feed or diet by the uNDF240 content of the feed or diet and was named physically effective undegradable NDF after 240-h of in vitro fermentation (peuNDF240). This simple calculation quantitatively predicted the uNDF240 content of the physically effective fraction, or the portion of undegradable fiber that stays in the rumen for chewing and particle size reduction. (Smith, 2019). Using a five-study database, Miller et al. (2020) conducted a regression analysis that further looked into the relationships between uNDF240, particle size, and their combination. This database showed that when combining the pef and the uNDF240, there was a stronger r^2 compared to either measurement individually when predicting DMI and energy-corrected milk in high producing Holstein cows fed primarily silage-based diets.

1.2.6 Conclusions for fiber

Fiber is sibylline. It is difficult to measure and conceptualize, yet it has extreme importance to the health and productivity of the cow. The degree of degradability is an evolutionary adaptation for plant survival that increases rigidity and impenetrability, and this has negative effects on the ruminant ingesting it. The chemical as well as the physical nature of fiber have effects on the cow and understanding how these two characteristics work together will give needed information to nutritionists who are formulating diets. However, the relationship between fiber and other major components of the diet is also important to understand, specifically starch and its rumen fermentability.

1.3 Rumen fermentable starch: moderation and balance

1.3.1. What is starch and what makes it fermentable?

An important goal in dairy cattle nutrition is to maximize the energy intake of the cow to enhance the amount of milk she can produce. A common approach in achieving this goal is to increase the energy density of the diet by adding energy in the form of starch (Oba and Allen, 2003a).

Starch is the most essential carbohydrate reserve in plants. However, not all starch is the same and its conformational characteristics relate to varying levels of fermentability, or substrate breakdown by microorganisms, in the rumen (Huntington, 1997). The fermentability of grain in the rumen is affected by two main factors: the physical characteristics of the plant, and the degree of processing prior to feeding (Giuberti et al., 2014).

Natural variation in fermentability exists among different grain types. For example, wheat is more fermentable than barley, barley is more fermentable than corn, and corn is more fermentable than sorghum (Van Soest, 1994; Huntington, 1997). To

further support this, a meta-analysis evaluating the effect of cereal grain type noted that, compared to corn, the starch provided by barley and wheat were 17 and 25% more digestible, respectively (Ferraretto et al., 2013).

These differences in fermentability are associated with the anatomy and composition of the plant. In the seed, starch is stored within the endosperm and is used as an energy source to aid in germination and early growth of the young plant. Within the endosperm, there are also lipids found both on the surface and inside the starch granule ranging from 15-55% of the fraction (Giuberti et al., 2014). Along with starch and fat, proteins, known as prolamins, are located within and surrounding the endosperm. Prolamins are storage proteins and have the ability to decrease starch fermentability due to the hydrophobic protein matrix encapsulating the endosperm that acts as a physical barrier to rumen bacteria (Giuberti et al., 2014). The type and location of prolamins are responsible for large differences in starch digestion (Giuberti et al., 2014). For example, corn contains a larger percentage of zeins, a type of storage protein. The rumen environment results in a very slow zein degradation rate, therefore higher zein levels in the diet relate negatively to rumen starch digestibility (Giuberti et al., 2014).

Within the endosperm, there are two polymers of starch: amylose and amylopectin. Conformational characteristics of these polymers influence solubility. Amylose is made of long linear chains of glucose residues that have strong intermolecular association. This makes them less soluble and reduces digestion potential when amylose content in grains is high. Amylose can also join with lipids to form amylose-lipid complexes that make the starch granule denser and less accessible to microbial fermentation (Giuberti et al., 2014). Alternatively, amylopectin consists of highly

branched glucan chains that increase solubility; therefore, it has more surface area for bacterial attachment (Martin and Smith, 1995; Van Soest, 1994). Characterizing kernel vitreousness is a tool used to assess the type of corn endosperm by characterizing the ratio of floury to vitreous endosperm (Giuberti et al., 2014). The greater vitreousness of the kernel corresponds with less floury endosperm, reduced starch fermentability, and, in corn, it is visually identifiable (Lopes et al., 2009; Giuberti et al., 2014). There is a higher concentration of prolamin proteins in corn types with more vitreous endosperm, which is the reason increased vitreousness decreases rumen starch fermentability and total tract digestibility (Lopes et al., 2009). In a study evaluating the effect of type of corn endosperm on nutrient digestibility, starch digestibility was 6.3% greater for cows fed diets containing 0% vitreous endosperm and on average 5.2% less prolamin as a percentage of DM (Lopes et al., 2009). Vitreousness also corresponds to maturity at harvest with more mature grains having a more vitreous kernel (Giuberti et al., 2014). The potential for enzymatic hydrolysis decreases as vitreousness in the endosperm increases due to an increase in secondary intermolecular bonding (Giuberti et al., 2014).

The pericarp surrounds the grain kernel, which surrounds the endosperm; it acts as a protective barrier and is resistant to bacterial attachment and fermentation (Huntington, 1997). Because of this structure, the outer layer of the seed must be broken to make the grain more readily available to the microbes to increase fermentability through chemical or physical processing. Decreasing the particle size of grains increases the surface area and allows for bacterial attachment and enzymatic breakdown, increasing rumen fermentability (Huntington, 1997). A 2013 meta-analysis found that coarser processing in dry or ensiled corn would decrease total tract starch digestibility

due to the increased passage rate of denser, coarser particles into the lower gastrointestinal tract (Ferraretto et al., 2013). For dry ground corn (DGC), coarser processing decreased total tract starch digestibility from 93.3 to 77.7% and for ensiled corn, coarser processing decreased total tract starch digestibility from 95.2 to 89.5% (Ferraretto et al., 2013).

When the whole grain is intact, it is almost completely indigestible to ruminants because the pericarp protects it from bacterial attachment in the rumen (Yang et al., 2000). This is the basis behind the different processing and conservation methods. Processing methods make the grain more fermentable by exposing the soft endosperm within the seed to the microorganisms within the rumen (Van Soest, 1994). Processing methods include cold processing, adding dry heat, and hydrothermal processes. These processes work by breaking open the protein matrix, making the endosperm more available, and inevitably, reducing the particle size. The potential for starch digestion is inversely related to particle size because of the increase in surface area for enzymatic hydrolysis (Giuberti et al., 2014). Other methods change the structure of the starch granule including methods like high-moisture ensiling that gelatinize and rupture the starch granule. There are different advantages and disadvantages to using different methods, and some elicit higher animal performance when using one method instead of the other (Mathison, 1996). A meta-analysis compared the effect of corn grain harvesting and processing methods using 102 different trials. For corn, it was observed that compared to dry grinding, starch became more fermentable with ensiling and steam treatments (Ferraretto et al., 2013). This is because gelatinization during steam treatments increases fermentability by breaking the hydrogen bonds in the polymers using moist

heat, disrupting the protein matrix (Van Soest, 1994), and proteolysis during ensiling breaks down the protein matrix (Firkins et al., 2001; Oba and Allen 2003b). For example, Oba and Allen (2003b) observed that there was a 13.6%/h greater rate of starch digestion when substituting high-moisture corn (HMC) for DGC in high starch diets (Oba and Allen, 2003b).

1.3.2. Effect of rumen fermentable starch on rumen dynamics and total tract digestibility

As rumen starch digestibility increases, so does total tract starch digestibility (TTD). Specifically, according to a meta-analysis of different corn grain types and harvesting methods, for every 3.36% increase in rumen starch digestibility, there was a 1% increase in starch TTD measured as a percentage of intake (Ferraretto et al., 2013). Rumen fermentable starch digestibility can range from 50-90% of starch intake, while post rumen digestion can range from 6-44% (Allen, 2000; Firkins, 2001). Although it is more efficient for fermentation of starch to occur in the rumen, post-rumen absorption and fermentation will compensate for the differences in rumen digestion for different grain types. This causes less variability in TTD between grain types and their conservation methods (Allen, 2000; Ferraretto et al., 2013). A majority of this variability within the rumen has to do with the grain type and the degree of processing the grain undergoes.

In the rumen, amylolytic bacteria can either loosely or tightly attach to the grain particle. These bacteria work together to produce enzymes that hydrolyze the α 1-4 bonds of amylose and the α 1-6 bonds of amylopectin (Huntington, 1997). Increasing the opportunity for bacterial attachment by utilizing different processing methods allows for

greater rumen digestion of the starch granules. Cereal grain source affects the degree of rumen digestibility of starch. Starch in corn is 54.1% rumen digestible, while barley is 70.6%, and wheat is 78.9%. Specifically, when looking at corn grain harvesting and processing methods, starch is numerically more rumen digestible when it is ensiled (64.1%) compared to when it is steam-treated (58.5%) or dry rolled, ground or cracked (53.5%; Ferraretto et al., 2013). Greater rumen digestibility also corresponded to greater starch TTD for the ensiled and steam-treated corn grain compared to dry ground corn because of the breakdown of the protein matrix prior to ingestion (Ferrareto et al., 2013). However, when looking at the TTD of the earlier mentioned cereal grain types, they had significantly different rumen starch digestibility, but there was no statistical difference in TTD of starch (Ferraretto et al., 2013).

Starch digestion is not only related to grain type and processing method, but also to passage rate and intake. Increasing levels of intake correspond to an increase in rumen turnover rate of both the liquid and solid form of the digesta (Ferraretto et al., 2013; Van Soest, 1994). A study using two processing methods with two starch concentrations reported a greater passage rate of DGC compared to the HMC for both high (32%) and low (21%) starch concentrations (Oba and Allen, 2003b). However, there was a tendency for this relationship to be greater in the higher starch diets compared to low starch diets. The authors hypothesized that this was due to greater DMI in the high starch diets and because of the differences in physical characteristics between DGC and HMC.

This increase in passage rate due to an increase in intake is thought to decrease starch TTD because of the reduced time for starch hydrolysis in the rumen (Ferraretto et al., 2013). However, depending on the grain type and processing method, the reduction

in rumen digestion of starch may be compensated for in the lower gastrointestinal tract, although there will be an inefficient use of microbial protein as it will pass into the feces (Allen, 2000).

1.3.3 Effect of rumen fermentable starch on pH and fermentation

Different grain types and conservation methods can affect rumen pH. A study using mid-lactation Holstein cows observed that when ground HMC was replaced with dry-cracked corn, there was a decrease in mean rumen pH from 5.82 to 5.67 and an increase in the hours per day when pH was <5.8 from 4.4 to 6.4 h (Krause and Combs, 2003). Another study observed that when feeding steam-rolled barley to Holstein cows, there was a linear drop in pH as the processing of the barley increased (Yang et al., 2000). These declines in rumen pH are associated with greater production of volatile fatty acids (VFA) from the microorganisms fermenting starch in the rumen. Greater content of starch and RFS will decrease the acetate:propionate ratio because of the increase in the molar proportion of propionate (Firkins et al., 2006). A study that increased fermentability of diets by substituting refined corn for dry cracked shelled corn, while maintaining a similar starch content, altered the pattern of VFA. The increased fermentability resulted in a decrease in acetate molar percentage and an increase in propionate molar percentage, ultimately decreasing the acetate:propionate ratio (Krause et al., 2003).

Highly fermentable diets have a greater risk of causing subacute rumen acidosis (SARA) in the cow. Rapid production of VFA will disturb the intrarumen acid-base balance causing a subsequent drop in rumen pH (Penner et al., 2011). If the pH nadir is sufficiently severe and prolonged, SARA will ensue (Humer et al., 2017). Currently,

there is a controversy surrounding the definition of SARA due to the lack of clinical symptoms, but one accepted guideline states that there is a higher risk of SARA if the rumen pH is below 5.8 for more than four to six hours per day (Humer et al., 2017).

There has been extensive research on the consequences of SARA on host health due to the damage that occurs within the rumen epithelium resulting from increased acidity. A healthy rumen epithelium is the key to the stability of the animal's energy balance through the metabolism and transport of rumen synthesized VFA (Steele et al., 2009). Steele et al. (2009) observed substantial negative effects of the rumen stratified squamous epithelium (SSE) during SARA. They used light microscopy on the rumen papillae of a non-lactating dairy cow transitioning from a high forage (100% of DM) to a high grain (75% of DM) diet to categorize the morphological alterations in the rumen epithelium during the initial stages of rumen acidosis. They observed that the stratum corneum had evidence of sloughing, weakening its protective barrier function. They also observed that cellular adhesion in the stratum corneum and stratum spinosum were weakened (Steele et al., 2009). The sloughing of protective barrier cells and the weakening of cellular junctions shows that there may be rapid structural change to the rumen epithelium during SARA that compromises the integrity of the SSE, increasing its permeability (Steele et al., 2009).

An increase in permeability can be detrimental for the ruminant because the degradation of cell junctions allows for microbes and lipopolysaccharide (LPS), a bacterial endotoxin, to bypass the protective barrier of the SSE to enter portal circulation (Steele et al., 2009). Lipopolysaccharide is a constituent of the outer membrane of gram-negative bacteria, which are the major bacterial type of fiber-digesting microbes (Van

Soest, 1994). Although LPS are bound to the outer membrane, they are released when the gram-negative bacteria lyse due to the drop in pH (Plaizier et al., 2012). Once LPS is in its free form, it is toxic and capable of evoking an immune response once it enters the blood circulation (Plaizier et al., 2012). This breach makes dairy cattle with SARA more susceptible to secondary disturbances such as liver abscesses and laminitis due to the translocation of free LPS (Beauchemin, 2007; Humer et al., 2017; Plaizier et al., 2012).

1.3.4 Effect of rumen fermentable starch on intake and meal bouts

The first conceptualization of a link between feed intake and the oxidation of fuels in the liver was in 1963 by Russek (Russek, 1963). He proposed that glucoreceptors in the liver were the influencing factors in the feeding behavior of dogs (cited by Allen et al., 2009). Since then, this theory, now known as the hepatic oxidation theory, has evolved into the idea that a signal stimulated by the oxidation of fuels in the liver reaches the brain and causes satiety (Allen et al., 2009). This process has been documented extensively in nonruminant animals such as rats and guinea pigs (Forbes, 1988). However, this theory proves challenging to apply to ruminant animals because of the way rumen fermentation changes the substrates that the liver oxidizes, as well as the timing of delivery for those substrates (Allen et al., 2009).

The ruminant is different from a monogastric in many ways. Most monogastrics have steady meals throughout the day, causing a consistent pattern of substrate breakdown, glycogen breakdown, and gluconeogenesis regulation. Ruminants have a chronic upregulation of gluconeogenesis because the rapid breakdown of glucose in the rumen impedes the amount of glucose flowing to the small intestine. Metabolically, ruminants rely heavily on the end products of fermentation, specifically VFA, from the

rumen that provide precursors for glucose, amino acids, and fatty acids. Different diets result in differing patterns of VFA that reflect substrate composition and fermentability. Specifically, when diets have a large amount of RFS, there is an increase in the proportion of propionate relative to acetate (Firkins et al., 2006).

Propionate is the primary glucose precursor for ruminants and appears to be more hypophagic than lactate or absorbed glucose (Allen, 2000). Propionate is thought to be the signal that stimulates satiety for ruminants since it is rapidly metabolizable, can stimulate hepatic oxidation in the liver, and is the primary end product of starch fermentation. However, the mechanism is not fully understood and infusion of propionate has resulted in inconsistent hypophagic responses due to hypothesized threshold responses (Allen, 2000; Oba and Allen, 2003a; Allen et al., 2009). In a study by Oba and Allen (2003a), DMI was not affected by lower rates of propionate infusion, but infusion did increase plasma glucose concentration. They hypothesized that when a considerable amount of incoming propionate is utilized for glucose synthesis, it does not have a hypophagic effect. However, as propionate infusion rates were increased, the effect on plasma glucose concentration decreased, suggesting that once the glucose demand from the body is met, then the use of propionate for gluconeogenesis decreases, causing a reduction in DMI (Oba and Allen, 2003a).

Diets that are more fermentable have inconsistent effects on DMI in the literature. In a review by Allen (2000) comparing studies that used grains varying in starch digestibility, three out of the ten comparisons saw, on average, a 3 kg/d reduction in DMI when substituting more fermentable starch sources in the diets. The author hypothesized that this was due to increased propionate production and a shorter meal length and size

(Allen, 2000). However, seven out of the ten experiments saw no effect on DMI. The author hypothesized that the differences observed across the ten studies might be from the pattern of absorbed metabolic fuels, their clearance from the blood, or the animal's own threshold to the diets. The hypophagic effects of highly fermentable diets is also more likely to affect cows in a lipolytic state (Allen, 2000). Albornoz and Allen (2018) evaluated the combined effects of dietary starch concentration and starch fermentability in the early postpartum cows. The starch treatments used DGC or HMC to manipulate fermentability and concentration (22 vs. 28%) by altering concentration of corn grain and soyhulls. They saw that there was a decrease in DMI when the higher fermentable starch source (HMC) was fed no matter the starch concentration but saw a greater reduction in DMI when HMC was fed at a higher concentration in the diet (Albornoz and Allen, 2018). They saw no effect of starch concentration on DMI but hypothesized that this was due to the same forage NDF in the treatment diets that added a filling effect to the lower starch (22%) diets (Albornoz and Allen, 2018). It is likely that as lactation progresses and the lipolytic state diminished, distension in the rumen rather than hepatic oxidation in the liver would take over as the principal intake inhibitor (Allen and Piantoni, 2013).

The pattern of fuel oxidation is a main regulator of feeding behavior (Allen et al., 2009). As previously stated, the pattern of VFA traveling to the liver for oxidation is at a constant state because of the retention of digesta in the rumen and the grazing meal patterns of cattle. After meals and ingestion of new substrate, microorganisms rapidly produce VFA and the VFA are then absorbed through the rumen wall and into portal blood circulation (Allen et al., 2009). For example, a more extensively processed grain, like HMC, can reduce meal size and therefore decrease intake (Oba and Allen 2003a).

Specifically, when HMC was fed in a high starch diet (32% of DM), there was a 1.7 kg/d decrease in DMI. This reduction in DMI was due to a reduction in meal size for cows fed the high starch, HMC treatment by 0.4 kg per meal. They hypothesized that this effect was due to greater rumen fermentation and propionate production. The type of grain used has also shown an effect. For example, starch from wheat and barley reduce intake more than diets that use corn and this relates to the availability of starch (Allen et al., 2009). Starch from wheat and barley are more readily fermentable in the rumen and therefore should produce more propionate at a faster rate compared to corn. McCarthy et al. (1989) evaluated the effects of different energy and protein sources using either ground-shelled corn (around 45% of diet) or steam-rolled barley (around 50% of diet) found a similar association. They found that diets that used ground-shelled corn as its energy source had a greater DMI response compared to diets with steam-rolled barley by almost 4 kg per day (McCarthy et al., 1989). They also observed greater production of VFA in diets with barley, and a trend for diets with barley to have a greater molar proportion of propionate. The authors hypothesized that these differences were due to barley's increased levels of RFS compared to corn-based diets.

1.3.6 Effect of rumen fermentable starch on lactation performance

A high producing dairy cow requires up to three to six times as much energy as a cow at maintenance because of the energy demands from lactation (Church, 1988). An easy way to meet these energy demands is to add highly fermentable carbohydrates or starch sources to the diet to produce VFA. Propionate is the main VFA produced by the fermentation of starch (Van Soest, 1994). However, adding highly fermentable starch sources can cause milk fat content to decrease. In a meta-analysis looking at the effect of

different grain type and corn grain processing methods on lactation performance in dairy cows, for every 1% increase in RFS concentration there was a 0.02% reduction in milk fat (Ferraretto et al., 2013).

Low milk fat in ruminants has been thoroughly investigated since its first mention in 1885 (cited by Van Soest, 1994), and has been a challenging syndrome to characterize because of its interrelationship with digestive processes and tissue metabolism (Bauman and Griinari, 2003). There are a number of theories to explain the phenomenon, but they all begin with the same basis of alteration in rumen microbial processes.

There is a clear relationship between milk fat yield and the production of VFA in the rumen, and the proportion of VFA produced correlates directly with the microbial populations present within the rumen (Van Soest, 1994). When there is more readily available starch for the amylolytic bacteria to breakdown, the population of amylolytic bacteria will increase, causing greater production of propionate, which decreases the rumen pH, inevitably decreasing activity of fibrolytic bacteria present in the rumen. Fiber fermentation produces acetate and butyrate, but simply producing less of these important VFA is not the cause of the depression in milk fat. Besides a decrease in fat content of milk, there is also a notable increase in unsaturation (Bauman and Griinari, 2003). This is peculiar because under normal circumstances, fatty acids are biohydrogenated to a more saturated form (Van Soest, 1994).

Biohydrogenation is a process performed in the rumen by the microbes to dispose of hydrogen from the reducing environment of the rumen. Through this process, the microbes add hydrogen to free fatty acid double bonds, and this hydrogenation occurs rapidly after ingestion. If fully saturated, all double bonds will convert to single bonds.

However, saturation is normally not fully complete in the rumen and a variety of fatty acid intermediates are a consequence (Church, 1988; Van Soest, 1994). *Trans*-10 18:1 is an intermediate in the biohydrogenation of linoleic and linolenic acid. This major *trans*-octadecenoic acid is a potent inhibitor of milk fat synthesis and has been shown to increase in milk fat during milk fat depression (MFD). An increase in *trans*-10 C18:1 is the result of a change in microbial processes that change the pathways of biohydrogenation and requires a dietary supply of unsaturated fatty acids (Bauman and Griinari, 2003). In a study that used either DGC or HMC, when feeding HMC, a more fermentable source, there was a reduction in milk fat percentage (Bradford and Allen, 2004). They also saw that the HMC treatment had increased concentration of unsaturated fatty acids in the milk, specifically *trans*-10 18:1 (Bradford and Allen, 2004).

In the case of diets with high fermentable starch, a depression in pH and a shift in VFA production is often observed with the change in microbial processes described above. The results from Griinari et al. (1999) showed that a lower pH results in incomplete biohydrogenation of fatty acids and an increase in *trans*-10 isomers. Therefore, it has been assumed that a consequence of SARA is MFD. In a study, replacing HMC for DGC in diets with either high or low starch (21% v. 32% starch), observed that the addition of HMC to high starch diets, results in decreased fat yield changes in rumen pH (Oba and Allen, 2003a). The study did not measure RFS in these diets, but the diets with HMC would be expected to have more RFS than diets with DGC because of likely increased fermentability. The results from these studies suggest that due to higher fermentability, there was less completion of rumen biohydrogenation,

increasing the production of *trans*-10 18:1 fatty acid isomer in the rumen, leading to MFD without any change in rumen pH.

Milk fat is not the only component in milk composition affected by an increase in RFS. There has also been an association with increased propionate and microbial protein production allowing for an increased amount of amino acids available for milk protein production in the mammary gland (Firkins et al., 2006). Microbial protein supplies the ruminant with their essential amino acids, which the dairy cow utilizes for milk protein synthesis (DePeters and Cant, 1990; Firkins et al., 2006). In a meta-analysis comparing corn grain harvesting and processing methods effect on milk components, they observed an increase in milk protein when dietary starch concentrations were increased (Ferrareto et al., 2013). Firkins et al. (2001) observed a similar pattern in a previous review.

1.3.7 Conclusions for starch

Rumen fermentable starch is an important component of any dairy cattle ration. It provides energy to the high producing animal in the form of propionate, but the fermentability of these rations requires consideration. Negative effects on the animal's health and production will occur if the diet is too fermentable. Understanding how the fiber component of the diet can work with or against RFS to have either positive or negative effects on the animal is an important concept in ruminant nutrition to understand to formulate diets for the high producing dairy cow.

1.4. Interactions between fiber and starch: associative effects

We often focus on specific measurements of the diet and this has helped to fine tune our understanding and ability to predict and understand responses to changing

metrics in the diet, but the diet is composed of multiple chemical and physical factors that interact. Interactions among feeds is a common occurrence in nutrition and are known as associative effects (Van Soest, 1994). By definition an associative effect is a feed interaction that can cause a positive or a negative effect on the animal's productivity that often leads to an under- or overvaluation of the animal's performance on the diet (Van Soest, 1994). More simply, an associative effect occurs when digestion or intake of one feed is not independent of another, showing a nonlinear result (Niderkorn and Baumont, 2009). Associative effects can occur to varying degrees depending on the combination of feed types in the diet, and so the composition and physical characteristics of the diet as a whole should be considered when determining animal response (Van Soest, 1994). For example, a figure in Van Soest's Nutritional Ecology of the Ruminant (1994) illustrates the associative effects that occur when poor quality feed is substituted for high quality feed as shown in Figure 1. He explains that in different situations substituting poor quality for high quality feed can have either a positive or a negative effect. When substituting wheat straw with protein, there will be a positive effect because of the increase in nitrogen availability of the microbial population in the rumen. This allows for better utilization of the straw. However, when supplementing a high grain diet with a pelleted or finely chopped forage, there will be a negative effect, especially for rumen pH. Either way, the expected digestibility (the dashed line) will be different from the actual outcome.

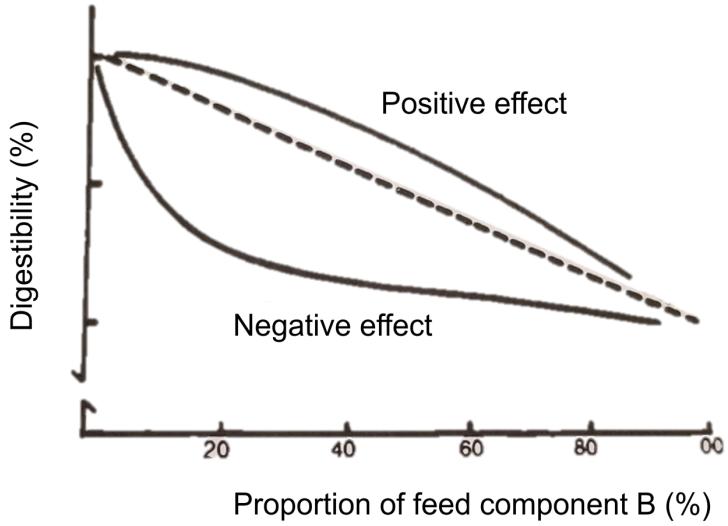

Figure 1.1 Associative effects occur when poor quality feed (B) is substituted for high quality feed (A). The dashed line represented the expected digestibility, while the positive and negative effects are represented by the upper and lower curved lines, respectively. (Van Soest, 1994).

Formulating rations is a balancing act between the different fractions of the diet that can cause associative effects. As previously discussed, high producing dairy cows require enough energy in their diets to reach their full production potential, but fiber is also essential for these animals to function at a high level. Inadequate levels of fiber, specifically peNDF, coupled with high levels of energy in the form of RFS is associated with SARA and MFD, while too much fiber and low RFS is associated with low feed intake and low milk production.

This balancing act between RFS and fiber can also be thought of as a balance between the production of VFA and the neutralization and removal of VFA (Beauchemin, 2007). In other words, a balance between high feed intake, rapidly fermentable carbohydrates, increased acid production, and neutralization through

buffering, absorption of VFA through the rumen wall, and passage of digesta from the rumen (Beauchemin, 2007). All of these factors will affect the pH of the rumen (Beauchemin, 2007). Early research in this field has focused on *in vitro* work. This technique is used to study rate and extent of digestion and provides a lower cost option that is rapid and repeatable compared to live animal trials (Church, 1988).

To understand the depression in fiber degradability when starch is added to forage-based diets, an *in vitro* study was designed to look at forage degradation kinetics (Mertens and Loften, 1980). The authors hypothesized that measuring NDF degradation kinetics would reveal the factors in forage fiber fermentation that were most affected by starch addition (Mertens and Loften, 1980). The treatments consisted of four forages: alfalfa, bermudagrass, fescue, and orchardgrass; and two starches: corn and wheat grain. There were four different inclusions of starch ranging from 0 to 80% as fed, while the acid detergent fiber and crude protein contents were held constant across diets. This study suggested that starch inclusion increased lag time prior to NDF degradation *in vitro* (Mertens and Loften, 1980). However, they found discrepancies between *in vitro* and *in vivo* fiber degradability because this effect cannot explain the extent of the depression in fiber degradability seen in *in vivo* studies. The authors hypothesized that this was due to differences in pH between the *in vitro* and *in vivo* systems (Mertens and Loften, 1980). The pH never dropped below 6.8 in the continuous culture system throughout the study, while a wide range of pH is observed in live animals. Rumen degradation of fibrous components is dependent on the pH of the rumen environment. Degradation is severely reduced at a pH that is below 6.2 and is considered insignificant below 6.0 (Stewart, 1977; Dixon and Stockdale, 1999). Knowing this, the authors hypothesized that the acidic

conditions in the rumen when adding fermentable starch causes a reduction in fiber degradability because of the reduction in cellulolytic activity (Mertens and Loften, 1980). Later work building on this topic concluded that not only does low pH reduce degradation and digestion rates in the rumen, but the addition of starch will intensify these negative effects (Grant and Mertens, 1992).

It is thought that adding fermentable starch to the rumen causes a cascade of effects that decreases the degradability of fibrous material (Dixon and Stockdale, 1999). Fermentation of substrates produces VFA. This decreases the pH when VFA production and concentration are often increased due to the rapid fermentation of starch coupled with a reduction in VFA transfer across the rumen wall. The decrease in rumen pH decreases the growth of cellulolytic rumen bacteria, therefore decreasing fiber degradation (Dixon and Stockdale, 1999).

A reduction in rumen pH is not the only deterrent to fiber degradation. When forage and starch are fed together in a diet, the rumen's fibrolytic bacteria are forced to compete for substrates that microbes utilize for growth such as nitrogen (Dixon and Stockdale, 1999). Rate of availability of substrates for energy and amino acids limits the rate of microbial growth in the rumen (Dixon and Stockdale, 1999). These growth substrates are going to be preferentially used by microorganisms that ferment starch, so fiber-degrading microorganisms are the most negatively affected (Dixon and Stockdale, 1999). In an *in vitro* suspension of rumen fluid, the degradation of cellulose was decreased with the addition of starch in the form of corn (el-Shazly et al., 1961). When urea was added to the suspension, there was less of decrease in degradation of cellulose (el-Shazly et al., 1961). This information shows that nitrogen is a limiting growth

substrate for fibroyltic bacteria. Fiber is degraded much slower compared to starch by the microbial ecosystem (Dixon and Stockdale, 1999). Following the ingestion of a diet with both forages and starch, substrates used for microbial growth are more likely to be utilized by starch digesting bacteria first. This leaves the fiber degrading bacteria to deteriorate and deplete (Dixon and Stockdale, 1999).

Even though *in vitro* studies have their benefits, they restrict observations and do not allow for the observation of the animal's response on digesta outflow, intake, or performance (Niderkorn and Baumont, 2009). An *in vivo* approach allows for a whole animal look at the interaction, and this approach is required to validate any observation that is observed in an *in vitro* experiment (Niderkorn and Baumont, 2009). A study designed to examine the potential of replacing soy hulls for forage fiber and to evaluate replacing soy hulls for corn grain, looked at lactation performance, rumen characteristics, and total tract digestibility (Sarwar et al., 1992). As NDF from forage decreased (replacement of forage for soy hulls), there was a linear increase in total VFA concentration, linear reduction in acetate to propionate ratio, and a reduction in pH. This corresponded to a decrease in organic matter total tract digestibility because the decrease in pH decreased the proportion of potentially digestible material in the rumen (Sarwar et al., 1992). However, there was no effect on lactation performance when replacing forage NDF for soy hulls.

On the other hand, when replacing soy hulls for corn grain (decreasing the nonstructural carbohydrate component in the diets); there was a higher acetate:propionate that the authors related to a higher rumen pH. This corresponded to a linear increase in NDF digestibility. The authors hypothesize this is due to a decrease in negative

associative effects in the rumen (Sarwar et al., 1992). There was also a tendency for milk production to increase when feeding less nonstructural carbohydrates, and there was a linear increase in milk fat production and feed efficiency (FCM/net energy for lactation).

1.5 Objectives and hypothesis

It is important to understand how fiber will interact with RFS when formulating diets for peuNDF240. When a diet is low in peuNDF240, how will the fermentability of the diet affect the productivity of the animal? Will negative associative effects occur when the fermentability of the diet is too high? The objective of this study was to evaluate the effect of two dietary concentrations of peuNDF240 and two dietary concentrations of RFS on DMI, lactation performance, chewing behavior, rumen fermentation, rumen turnover, and total tract nutrient digestibility of high-producing Holstein cows. We hypothesized that diets that have lower peuNDF240 will have negative associative effects from starch and fiber interactions like milk fat depression, subacute rumen acidosis, and a decrease in NDF degradability. However, when diets have higher peuNDF240, these negative associative effects will be mitigated at moderate rumen fermentable starch content.

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CHAPTER 2: EFFECTS OF PHYSICALLY EFFECTIVE UNDEGRADABLE NEUTRAL DETERGENT FIBER AND RUMEN FERMENTABLE STARCH ON INTAKE, LACTATION PERFORMANCE, RUMEN FERMENTATION, RUMEN DIGESTA CHARACTERISTICS, AND NUTRIENT DIGESTIBILITY OF LACTATING HOLSTEIN COWS

2.1. Abstract

Sixteen multiparous Holstein cows were used in a 4 x 4 replicated Latin square design with a 2 x 2 factorial arrangement of treatments to evaluate the effect of feeding different dietary concentrations of physically effective undegradable neutral detergent fiber after 240-h of fermentation (peuNDF240) and rumen fermentable starch (RFS; 7-h in vitro starch digestibility) on dry matter intake (DMI), milk yield and composition, chewing behavior, rumen pH, volatile fatty acids, rumen digesta turnover, and total tract nutrient digestibility. Within period, the adaptation period was d 1 to 18 while the collection period was d 19 to 28. Diets differed in peuNDF240 and RFS by inclusion of either brown midrib or conventional corn silage hybrids and varying the concentration of corn meal and beet pulp. The peuNDF240 was measured as the uNDF240 content of the physically effective fraction (retained on 1.18-mm screen with dry vertical sieving) of the ration. Analyzed treatment composition was: 1) low peuNDF240 (6.4% of DM), low RFS (16.7% of DM); 2) low peuNDF240 (6.1% of DM), high RFS (19.2% of DM); 3) high peuNDF240 (8.6% of DM), low RFS (16.9% of DM); and 4) high peuNDF240 (8.0% of DM), high RFS (19.0% of DM). Data were summarized by collection period and analyzed with fixed effects of RFS, peuNDF240, and the interaction of peuNDF240 and RFS within a square, and period using the MIXED procedure of Statistical Analysis

System. Significance was declared at $P \leq 0.05$ and a trend was declared at $0.05 < P \leq 0.10$. Cows fed higher peuNDF240 diets consumed less DMI as a percentage of body weight than cows fed lower peuNDF240 ($P = 0.04$). Additionally, the higher peuNDF240 diets resulted in greater uNDF240 intake while the higher RFS diets increased starch intake as expected. Cows fed diets containing higher RFS produced less milk fat and 3.5% fat-corrected milk (FCM) than cows fed lower RFS ($P = 0.01$). Lower peuNDF240 diets resulted in lower mixed origin fatty acids in milk fat ($P = 0.008$) and a greater degree of unsaturation ($P = 0.005$). Overall, higher RFS diets tended to reduce the efficiency of FCM production (FCM/DMI) compared with lower RFS diets ($P = 0.06$). There was no effect of peuNDF240 or RFS on eating or ruminating time per day, but higher RFS diets reduced meal length ($P = 0.05$) and increased daily meal bouts ($P = 0.04$). Reflecting changes in milk fat, the lower RFS diets reduced acetate:propionate ratios ($P = 0.05$) whereas the higher peuNDF240 diets increased isovalerate molar proportion ($P = <0.001$). There was no effect of treatment on measures of rumen pH. Diets with higher RFS tended to increase rumen pool size of starch ($P = 0.06$) while higher peuNDF240 diets increased pool size of uNDF240 ($P = 0.06$) although there were few dietary effects on rumen turnover of starch or uNDF240. Lower peuNDF240 diets resulted in greater total tract NDF digestibility than higher peuNDF240 diets ($P = 0.002$). Feeding moderately high levels of RFS (19% of ration DM) depresses FCM production when diets contain relatively low concentrations of uNDF240 and peuNDF240.

2.2 Introduction

Carbohydrates make up approximately 70% of diets fed to lactating dairy cows and are the major source of energy for both the rumen microbiota and the cow (NRC,

2001). Carbohydrates can be broadly classified as either structural or nonstructural based on their location in the plant, and the more common of these two fractions used in diet formulation are fiber and starch.

The NDF content of forages and TMR quantifies, but does not explain all, of the variation in DMI as source and content of NDF in the ration varies (Van Soest, 1994). The chemical composition, rumen degradability, and particle size characteristics of NDF all affect cow responses to forage (Mertens, 1997). The NDF is comprised of an undegradable and potentially degradable fraction. The potentially degradable fraction can be separated into a fast and slow fermenting pool (Raffrenato et al., 2018). The indigestible component of NDF is measured as undegradable NDF (ash-corrected) after a 240-h in vitro fermentation (uNDF240om) and represents the remaining NDF residue after complete fermentation (Mertens, 2016; Raffrenato et al., 2018). The amount of uNDF240om is highly negatively correlated to DMI in the cow because of the effect on gut fill, as well as degradation and passage dynamics in the rumen (Mertens, 2016; Miller et al., 2021). Greater rumen turnover of the fast fermenting, slow fermenting, and uNDF240om pools allows for greater DMI, but greater uNDF240om in the diet decreases turnover rate of NDF within the rumen because the retention time of digesta is increased (Miller et al., 2021).

Although too much dietary aNDFom and uNDF240om can limit DMI, too little may compromise rumen health and digestive efficiency. Diets with uNDF240om greater than 10.0% of DM are more likely to limit DMI due to gut fill limitations (Miller et al., 2020). But there is little information about the lower end of this range. Previous research with cows fed diets containing less than 30% NDF (DM basis) indicates an increased risk

of subacute rumen acidosis (SARA) because of the increase in turnover rate and DMI, but research is needed to understand how lower uNDF240om affects rumen pH, fermentation, and digesta turnover (Raffrenato et al., 2018).

In addition to NDF degradability, forage particle size can also influence rumen fermentation and animal health (Mertens, 1997). The dairy cow requires physically effective fiber which influences chewing activity and rumen digesta mat development (Mertens, 1997). Diets that are higher in physically effective NDF (peNDF) increase the amount of eating and ruminating time, or total chewing time because of a larger particle size or physical effectiveness factor (pef; Jiang et al., 2017; Smith, 2019). In diets comprised of dry forages and silages, particle size has a greater effect on eating time compared to ruminating time when it is long (i.e, 50 mm) because the cow must break down the diet to a swallowable size of 10 to 11 mm (Schadt et al., 2012). Once the diet reaches the rumen it is a relatively uniform size. However, shorter feed particle sizes (i.e., 13 mm) have a greater effect on rumination because they are not long enough to stimulate regurgitation (Schadt et al., 2012). This can negatively impact rumen pH because there will be a decrease in saliva production associated with rumination (Zebeli et al., 2012).

A five-study database that consisted of corn silage and haycrop silage-based diets was developed to understand the relationship that uNDF240 and pef have with DMI and energy-corrected milk (Miller et al., 2020). This database consisted of a range in uNDF240 of 5.5 to 11.5% and a peuNDF240 of 4.0 to 7.3% (Miller et al., 2020). By combining both the physical and chemical aspects of the diet by multiplying the pef by the uNDF240om, a new measurement of physically effective uNDF240om (peuNDF240) was created to better describe the fiber characteristics of a forage or TMR (Smith, 2019;

Miller et al., 2020). The authors noted that peuNDF240 was better able to predict DMI compared to using uNDF240 alone (R^2 = 0.60 vs. 0.32, respectively; Miller et al., 2020). A similar pattern was observed for energy-corrected milk (ECM) as peuNDF240 increased predictability of ECM compared to uNDF240 alone (R^2=0.78 vs. 0.58, respectively Miller et al., 2020).

Interactions among feed ingredients, known as associative effects, are well known occurrences in nutrition (Van Soest, 1994). An associative effect can cause either a positive or negative effect on animal productivity meaning one feed is not independent of another, showing a nonlinear result (Niderkorn and Baumont, 2009). Specifically, interactions between starch on rumen fiber degradation and peNDF requirements are well known. An *in vitro* study observed an increase in lag times prior to NDF degradation with an increased inclusion of starch (Mertens and Loften, 1980). Subsequent research *in vivo* has shown that as the fermentability of starch increased, SARA can occur, milk fat can decrease, and NDF degradation can decrease (Bradford and Allen, 2004; Agle et al., 2010; Albornoz et al., 2019). A better understanding of how starch and fiber interact in the rumen is needed so nutritionists can improve the delivery of energy to the animal.

Diets formulated to contain more physically effective fiber can be used to mitigate lower rumen pH in diets that are highly fermentable. Diets that have a peuNDF240 of 4.0% of DM are more likely to be lower in forage, or at least have less undegradable forage like straw, hay, or haycrop silage. In past research on the physical and/or chemical characteristics or fiber, starch has been held constant so there is little information on how negative associate effects would affect the cow. There is needed information on how different rumen fermentable starch contents influence rumen

dynamics in diets that differ in peuNDF240 content. Specifically, when peuNDF240 content is on the lower end of what would be fed in the industry (~4.0%), will moderate rumen fermentable starch have negative effects on the animal?

The objective of this study was to evaluate the effect of two dietary concentrations of peuNDF240 and two dietary concentrations of rumen fermentable starch (RFS) on DMI, lactation performance, feeding behavior, rumen fermentation, rumen turnover, and total tract digestibility of high-producing Holstein cows. We hypothesized that diets that have lower peuNDF240 will have negative associative effects like milk fat depression, subacute rumen acidosis, and a decrease in NDF degradability when diets have only moderate rumen fermentable starch contents. However, when diets have higher peuNDF240, these negative associative effects will be mitigated at moderate rumen fermentable starch contents.

2.3. Material and Methods

This study was conducted at the William H. Miner Agricultural Research Institute in Chazy, NY. All experimental procedures involving cows were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee (ACUC# 2017AUR02).

2.3.1 Experimental design and management of cows

Sixteen lactating Holstein cows (8 rumen cannulated) that were 85 ± 15 DIM were enrolled, blocked by parity, DIM, and milk production and were used in a replicated 4 × 4 Latin Square design with a 2 × 2 factorial arrangement of treatments. The study had four 28 d periods. Each square was conducted concurrently with the first 18 d of the period serving as an adaptation period and the last 10-d serving as the collection period.

The objective was to evaluate the effect of lower or higher concentrations of peuNDF240 and lower or higher concentrations of RFS using 2 x 2 factors (Table 2.1). The four diets were formulated with either lower or higher concentration of peuNDF240 by using either brown midrib-3 (BMR) or conventional corn silages (CON), and the RFS concentration varied with lower or higher corn meal inclusion (Table 2.2). Prior to the start of the study, the corn silages and the timothy hay were spot sampled and analyzed using wet chemistry analysis [CPM Plus; Cumberland Valley Analytical Services (CVAS), Inc., Waynesboro, PA] to provide initial nutrient composition values. Feed library values were used for nutrient composition of grain mix ingredients, beet pulp, corn meal, and wheat straw for ration formulation (AMTS.Cattle.Professional, Agricultural Modeling & Training systems, LLC, Groton, NY; version 4.8; Table 2.3).

The four dietary treatments were: (1) lower RFS and lower peuNDF240: (2) higher RFS and lower peuNDF240: (3) lower RFS and higher peuNDF240: and (4) higher RFS and higher peuNDF240. Diets (Table 2.1) were formulated for high producing lactating Holstein cows producing 61 kg of milk/d, consuming 29 kg of DM/d, and weighing 795 kg using a commercial ration formulation platform with Cornell Net Carbohydrate Protein System biology (AMTS).

Cows were fed a TMR for ad libitum intake (approximately 1.10 x expected intake) once daily at 1400 h. Diets were mixed and delivered daily with a Super Data Ranger (American Calan, Inc., Northwood, NH). Cows were housed in a tie-stall barn equipped with individual feed boxes. Cows were removed from the stalls three times daily (0430, 1230, and 2030 h) for milking in a double-twelve parallel milking parlor (Xpressway Parallel Stall System; Bou-Matic, Madison, WI). Cows were moved through

an animal handling area at the beginning and end of each period after the 1230 h milking for body weight determination.

2.3.2. Data collection, sampling procedures, and analytical methods

Feed Ingredients and Diets. During the adaptation period, forages, diets, and orts were collected three times per week. Grain mixes were collected once per week. A portion of each sample was dried in a forced-air oven at 105°C for 18 to 24 h for DM determination. Diets were adjusted for changes in DM content of the feed ingredients when a feed ingredient DM value was outside the normal range (mean ± 1.2 standard deviations used, in general, as the normal range).

During the collection period, feed ingredients, diets, and orts were collected daily, stored frozen at -20°C, and then composited by collection period by combining equal volumes from each portion of the daily as-fed samples. A portion of each sample was dried in a forced-air oven at 105°C for 18 to 24 h. The composites of feed ingredients were analyzed for chemical composition using wet chemistry analysis [CPM Plus; Cumberland Valley Analytical Services (CVAS), Inc., Waynesboro, PA]. Analyses included DM, ash (method 942.05; AOAC International, 2012), OM (method 942.05; AOAC International, 2012), CP (method 990.03; AOAC International, 2012), soluble protein according to Krishnamoorthy et al. (1982), ether extract (method 2003.05; AOAC International, 2012), ADF (method 973.18; AOAC International, 2012), NDF using α-amylase on OM basis (Van Soest et al., 1991), ADL (Goering and Van Soest, 1970), starch according to Hall (2009), sugar as ethanol soluble carbohydrates according to DuBois et al. (1956), and minerals (method 985.01; AOAC International, 2012).

A portion of the forage and diet composite samples was used to determine particle size distribution on an as-fed basis using a Penn State Particle Separator (Lammers et al., 1996) modified to include a 4-mm screen. A portion of the period composite samples for forages and grain mixes was also used to determine particle size distribution on a DM basis (55°C) by dry vertical sieving (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering, Inc., Mentor, OH) for 10 min.

Undegraded NDF, on an OM basis, for 30-, 120-, and 240-h time points (uNDF30om, uNDF120om, uNDF240om) for forages and 12-, 72-, and 120-h time points (uNDF12om, uNDF72om, uNDF120om) for the corn meal, grain mixes, and beet pulp were assessed using an in vitro rumen fermentation system (Raffrenato et al., 2019). Fermentation analysis was determined on the forage composite samples (CVAS). Starch digestibility at 7-h (Starch D; CVAS) was determined on the composite samples of corn silage, TMR, and corn meal. The RFS of the diet was calculated by multiplying the Starch D by the starch content in the composited TMR samples. The calculated peuNDF240 was achieved by multiplying pef by the uNDF240om content of the diet (Smith, 2019) which assumes an equal distribution of uNDF240om across all sizes of particles in the diet.

A study composite was made of all diets where all four periods within diet were combined into one sample on an AF basis. A portion of the study composite for each diet was used to determine analyzed peuNDF240 by directly measuring the uNDF240om content in the portion of the diet that was retained on the ≥1.18 mm sieve. This approach does not assume an equal distribution of uNDF240 across all particle size fractions of the diet unlike the calculated peuNDF240. Each composite sample was separated using dry

vertical sieving (Ro-Tap), and then separated at the 1.18-mm sieve. This gave two separate samples, the physically effective portion of the diet and the non-physically effective portion of the diet. The physically effective samples were used to conduct fermentation analysis using an in vitro rumen fermentation system for 240-h (Raffrenato et al., 2019).

Dry Matter Intake. Individual DMI was determined by recording feed offered and refused daily. Samples of diets and orts were collected on d 19 to 28, composited by period and cow, and a portion of each composited sample was dried in a forced-air oven at 105°C for 18 to 24 h for DM determination.

Milk Yield and Composition. Milk yield was recorded electronically at each milking (ProVantage Information Management System; Bou-Matic, Madison, WI). Milk samples from six consecutive milkings for each cow were collected on d 25 and 26 of each period. The milk samples were analyzed for fat, true protein, lactose (anhydrous), solids nonfat, urea nitrogen, and *de novo*, mixed, and preformed fatty acids by mid-infrared procedures (CombiScope FTIR 300 Hp; Delta Instruments, Drachten, The Netherlands; Wojciechowski and Barbano, 2016; Wojciechowski et al., 2016; Woolpert et al., 2016). Somatic cell count was analyzed by flow cytometry (CombiScope FTIR 300 Hp, Delta Instruments, Drachten, The Netherlands). Milk samples were composited mathematically by day, after analysis, in proportion to milk yield at each sampling within a day. Somatic cell count was transformed and analyzed as SCS according to Shook et al. (1993) using the equation: $SCS = \log_2(SCC/100) + 3$ where SCC is in units of 1,000 cells/mL. Fat-corrected (3.5%) milk was calculated as 0.4324 × kg of milk + 16.216 × kg of fat (Hutjens, 2005). Solids-corrected milk (SCM) was calculated according to

Tyrrell and Reid (1965): [(12.3 × kg of fat) + (6.56 × kg of solids non-fat) – (0.0752 × kg of milk)]. Energy-corrected milk was calculated using a formula modified to account for use of true protein instead of total protein (Tyrrell and Reid, 1965; as cited by Mark Stephenson, University of Wisconsin; https://dairymarkets.org/PubPod/Reference/Library/Energy%20Corrected%20Milk): 0.327 × kg of milk + 12.95 × kg of fat + 7.65 × kg of true protein.

Feed Efficiency. On d 20 through 28 of each period, DMI and milk production data was averaged over the period and used to calculate feed efficiency. Feed efficiency (kg/kg) was expressed as milk/DMI, FCM/DMI, ECM/DMI, and SCM/DMI.

Body Weight and Body Condition Score. Body weight was measured (Allweigh computerized scale; Allweigh Scale System Inc., Red Deer, AB, Canada) and BCS was assigned in 0.25-unit increments on a 1 to 5 scale (Ferguson et al., 1994) at the end of each period. Three individuals assigned BCS independently at each time of scoring throughout the study.

Total Tract Nutrient Digestibility. Total tract digestibility was determined on d 19 to 22 of each period by collecting diet, ort, and fecal samples. Representative samples of the diets were taken and composited by treatment and period (Harvatine and Allen, 2006). Representative samples of the orts were collected for each cow and composited by cow by period. Fecal grab samples were collected on d 20 to 22 for each period so that every 3 h in a 24-h period was represented (eight samples total). Fecal samples from each cow were composited by combining approximately 112 mL of wet feces from each time point and for each period.

Samples of diets, orts, and feces were frozen at -20°C until further analysis. Composited samples were dried in a forced-air oven at 55°C for 48 h, ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA), and submitted for chemical analysis (CVAS). Composite samples of diets (by period), orts (by cow and period), and feces (by cow and period) were analyzed for DM, ash, aNDFom, starch, and uNDF240 (Van Soest et al., 1991; Hall, 2009; method 942.05, AOAC International, 2012; method 973.18, AOAC International, 2012). Undegraded NDF residue in diets, orts, and feces was quantified as NDF content of samples following an in vitro rumen fermentation (Raffrenato et al., 2017) in buffered rumen media for 240-h. The uNDF240 value was used as an internal marker. Total tract digestibility was calculated by the ratio technique using the concentrations of the nutrients and uNDF240 in the diet and feces. The nutrient content of the diet used in the digestibility calculation was adjusted for each cow based on the nutrient composition of the diet offered and refused.

Rumen Fermentation. Rumen pH was measured in the rumen cannulated cows (n = 8) with an indwelling rumen pH/ORP/REDOX measurement system (Penner et al., 2006; LRCpH; Dascor, Escondido, CA) at 1-min intervals for a 96-h period from d 23 to 26 of each period. Rumen pH measurements were averaged over a 10-min period within day and summarized as mean pH, minimum pH, maximum pH, pH range, the area that the pH curve was below a pH of 5.8 and 6.0, and minutes per day that pH was below 5.8 and 6.0.

Samples of rumen fluid (approximately 500 mL) were collected by hand grab method from beneath the rumen digesta mat at 4 h intervals for 24-h starting at feeding time on d 26 of each period. Feeding and the associated sampling times were staggered

by 10-min between squares of cows to allow for collection of samples at desired times. Samples were strained though 4 layers of cheesecloth. A portion of each sample of rumen fluid (approximately 40 mL) was frozen and stored at -20°C until analysis for VFA molar proportions (Bulletin 856B; Supelco, Inc., Bellefonte, PA). Volatile fatty acid molar proportions were determined by gas chromatography with use of a Varian CP-3800 gas chromatograph (Varian, Inc., Palo Alto, CA) equipped with a flame-ionization detector and an 80/120 Carbopack B-DA/4% Carbowax 20M column (Supelco, Inc., Bellefonte, PA). Ten mL of rumen fluid was added to 100 μL of concentrated HCl and frozen at -20°C until analysis of rumen NH_3-N concentration using a colorimeter (Chaney and Marback, 1962).

Chewing Behavior. Cows were monitored for chewing activity (i.e., eating and ruminating) and posture (i.e., standing and lying) every 5 min for 72-h (d 23 to 25). Total time, in minutes, spent on each activity for each day was quantified by multiplying the total number of observations for that activity by 5 min. Number of bouts and the length of bout of eating and ruminating were recorded. A bout was defined as at least two consecutive observations of eating or ruminating behavior not interrupted by more than four observations, or 20-min, of a different behavior (Black et al., 2016).

Rumen Evacuations and Analysis of Pool Size and Turnover. Rumen contents of rumen cannulated cows (n = 8) were evacuated manually through the ruminal cannula. To ensure that cows experienced the same interval of time between rumen evacuations, feeding and the associated sampling times were staggered between squares of cows. The first group was evacuated 3.5 h after feeding on d 27 and 20.5 h after feeding on d 28. The second group of cows were evacuated 4.5 h after feeding on d 27 and 19.5 h after

feeding on d 28. Rumen content mass and volume were determined. During the evacuation, approximately 10% of the contents was subsampled and squeezed through a nylon screen (1-mm pore size) to separate solid and liquid phases and each phase as weighed.

Aliquots (~300 g) from both the solid and liquid phases were collected. Remaining rumen contents were returned to the cow within 60 min of initiating the evacuation. Subsamples of each phase were frozen at -20°C, dried at 55°C, ground (solids: 1-mm screen; Wiley mill; liquid: 2-mm screen, UDY Cyclone Sample Mill; UDY Corp., Fort Collins, CO), and recombined based on the proportion of DM of each phase. The recombined ruminal contents were analyzed for ash (modified method 942.05; AOAC International, 2012; 4-h at 600°C), aNDFom (as described previously), uNDF240om, and starch (Hall, 2009; Raffrenato et al., 2018; CVAS).

Rumen pool size of OM, aNDFom, uNDF240om, and starch were calculated as the product of the DM mass of the rumen contents and the nutrient content of the rumen contents. Rumen turnover rate (%/h) of OM, aNDFom, uNDF240om, and starch were calculated as [100 × (intake of nutrient/rumen pool of nutrient)/24] (Voelker and Allen, 2008). Nutrient intake was calculated using DMI from d 27 and 28 and the nutrient content of the diets from d 20 to 28. Rumen turnover time (h) was calculated as 1/[rumen turnover rate (%/h)/100].

Statistical Analysis. Statistical computations were performed using the Statistical Analysis System (SAS; version 9.4; SAS Institute Inc., Cary, NC). Data from the analysis of feed ingredients and diets were analyzed using the MEANS procedure of SAS and are reported as descriptive statistics (mean ± standard deviation).

Data were checked for homogeneity of variance and normality assumptions using Shapiro-Wilk and Levene's test. All available data collected during the last 10-d of each period (DMI, milk yield and composition, feed efficiency, BW, BCS, and rumen pH) were analyzed as a replicated Latin square design with a 2 x 2 factorial arrangement of treatments. The model included fixed effects of RFS, peuNDF240, and RFS × peuNDF240 interaction, period, and square using the MIXED procedure of SAS. Cow within square was a random effect. Repeated measurements of performance data from collection period (i.e., DMI, milk yield and composition, BW, BCS, and rumen pH) were reduced to period means for each cow before statistical analysis. The data for ammonia and VFA were analyzed using repeated measures of time using the MIXED procedure of SAS. This model included the effect of RFS, peuNDF240, and RFS × peuNDF240 interaction, period, hour, and also the interaction of RFS × peuNDF240 and hour. Cow within replicated square was included as a random effect. Least squares means were separated using the Tukey's procedure when a significant F-test ($P \leq 0.05$) was detected. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Data from 14 of 16 cows enrolled in the study were used for statistical analysis. One non-cannulated cow did not finish the study due to severe mastitis in the third period that was caused by *Klebsiella* (Quality Milk Production Services, Ithaca, NY) and her data was not included in final dataset. One cannulated cow was removed from the dataset due to two mastitis incidences during two of the sampling periods caused by *Klebsiella* in which she was not sampled but at the time was not removed from the study.

2.4 Results and Discussion

2.4.1 Dietary and ingredient nutrient composition

Table 2.1 presents the formulated ingredient composition of the four treatment diets. All diets contained 47.6% of DM as corn silage and included either BMR corn silage (low peuNDF240 diets) or CON corn silage (high peuNDF240 diets). All diets contained 7.90% of DM as timothy hay and 1.59% of DM as chopped wheat straw. The ratio of beet pulp and corn meal varied among diets to achieve the targeted concentration of dietary starch and RFS for each diet. The two grain mixes (lower RFS grain mix and higher RFS grain mix; Table 2.1) were formulated to keep other dietary nutrient fractions similar across the four diets.

The goal of the study was to have the higher peuNDF240om diets have uNDF240 content within the range of 8.8 to 11.5% of DM as shown in Table 2.2 and the lower peuNDF240om diets were targeted to have approximately 7.0% uNDF240. Table 2.3 contains the analyzed chemical composition of the ingredients fed to cows throughout the study. The uNDF240om content of the CON corn silage during the study was lower than what was estimated when the diets were formulated. Therefore, the resulting uNDF240om for all four experimental diets averaged 7.03 ± 0.96% of DM.

Across diets, we aimed to keep aNDFom, pef, and peNDF similar which is in Table 2.4 for the dietary ingredients and Table 2.5 for the diets. The aNDFom for the diets was 32.9 ± 0.5% of DM approximately. The pef was 0.58 ± 0.02 and the peNDF were 19.1 ± 0.5% of DM approximately. Results from Smith (2019) showed that calculated peuNDF240 value could be a useful measurement in integrating the effects of particle size and NDF undegradability into one measurement to more accurately predict DMI and ECM.

Calculated peuNDF240 values for the treatment diets are in Table 2.5 and averaged 4.09 ± 0.11% of DM. Although they are similar, the calculated peuNDF240 is an accurate depiction of these diets. When comparing the diets to the Miller et al. (2020) database, the diets were at the lower calculated peuNDF240 range. The diets fit the pattern of increased DMI and ECM with lower peuNDF240. However, to separate these diets based on their fiber characteristics, an analyzed peuNDF240 was used. We hypothesized that these diets did not have a uNDF240 content that was uniformly distributed across the different particle sizes. This would explain why there were treatment differences based on the fiber characteristics of the diet, discussed later. Analyzing the peuNDF240 directly aided in distinguishing the uniformity of the uNDF240 content that the calculated peuNDF240 was unable to detect.

There is little information about differences in uniformity of uNDF240 across a diet or a forage and more research is needed in this area. In previous work on uNDF240, the distribution of uNDF240 was assumed to be similar across particle sizes within diets. In this study, this appeared to not be a correct assumption, and it was assessed whether uNDF240om was uniformly distributed above the 1.18-mm sieve by directly measuring the uNDF240 of the physically effective fraction (Poppi et al., 1985). Since uNDF240om was not uniformly distributed in this present study, a calculation of peuNDF240 (i.e., pef × uNDF240) did not capture the true undegradability of the physically effective fraction to the degree that was necessary to differentiate between the diets. The analyzed peuNDF240 is reported in Table 2.6. For the lower analyzed peuNDF240 diets, the peuNDF240 averaged 6.21 ± 0.14% of DM, and for the higher peuNDF240 diets, the peuNDF240 averaged 8.30 ± 0.30% of DM. This approach is a more accurate

measurement for these treatment diets compared to a calculation that assumes equal distribution of uNDF240om across all particle sizes because it detects more specific differences in the chemical and physical characteristics of these diets.

During the study, the CON (36.8 ± 0.4% of DM) and BMR (37.9 ± 2.7% of DM) corn silages were lower in starch than what was used for initial formulation (40 and 41% of DM, respectively). This resulted in a reduction in starch content and RFS in all of the diets. The calculated chemical composition of starch in the diets averaged 20.8 ± 0.1 and 24.7 ± 0.1% of DM for the low and high RFS diets, respectively. This resulted in an RFS of 16.8 ± 0.1% of DM for the lower RFS diets and 19.1 ± 0.1% of DM for the higher RFS diets. Although the starch content of the treatment diets was not as high as we had formulated for, there was still a 4 percentage-unit difference between the higher and lower RFS diets for starch content and a 2.5%-unit difference between RFS levels.

2.4.2 Dry matter intake

There were no interactions between peuNDF240 and RFS for DMI, represented as either kilograms per day or as a percentage of BW per day, among the four diets as shown in Table 2.7 ($P > 0.10$). However, there was a significant effect of peuNDF240 on DMI as a percentage of BW with cows fed the lower peuNDF240 diets consuming more than the cows on the higher peuNDF240 diets (4.22 ± 0.02 vs. 4.30 ± 0.02 % of BW/d; $P = 0.04$). This was expected as the higher peuNDF240 diets would have a greater amount of undegradable feed in the pef which could limit intake and passage rate (Allen, 2000). A cow is limited by how much physical space she has available in her rumen and is affected by the particle size and the undegradable fiber portion of the diet (Van Soest, 1994; Allen, 2000).

Research evaluating the effect of fiber degradability on intake has consistently shown that a more degradable diet will increase DMI because of a reduction in rumen and digestive tract fill (Block et al., 1981; Mertens, 1997; Oba and Allen 1999; Tine et al., 2001). Recent research has compared BMR, a corn silage hybrid bred to have lower lignin content, and conventional corn silage to study how diets with greater NDF degradability affects DMI (Gencoglu et al., 2008). A review found that, on average, there was a 1.2 kg/d increase in DMI when more degradable diets were fed (Gencoglu et al., 2008). In contrast, other studies (Frenchick et al., 1976; Sommerfeldt et al., 1979) have not observed increases in DMI, most likely due to differences in stage of lactation, diet composition, or similar degradabilities of the corn silages, which may be the case with the present study. There was a small numerically but significant effect of peuNDF240 on BW where the cows fed the higher peuNDF240 diets weighed, on average, 8 kg more. There was no effect on BCS.

Starch fermentability can also influence DMI. In a meta-analysis that evaluated the effect of diet on short-term feed intake regulation, daily DMI of lactating cows in 3 out of 10 comparisons observed a significant increase in DMI when diets with greater rumen starch fermentability was fed (Allen, 2000). This effect was speculated to be caused by increased VFA production, specifically propionate (Allen, 2000). The 10 studies had a range of 19 to 45% of DM in starch content. The diets that resulted in a decrease in DMI had a starch content of 29 to 45% which would be considered high for typical formulations for high producing dairy cows in the US. The diets in the current study had moderate starch content, less than 25% of DM, and RFS was less than 20% and had no effect ($P > 0.10$) of RFS level on DMI. Potentially, there was not a high

enough inclusion of starch for RFS to cause a difference in DMI. However, a study that replaced dry cracked shelled corn with refined corn starch to increase the fermentability of the diets also saw no significant difference in DMI with increased fermentability and the starch content of their diets were much higher, around 29 to 32% of DM (Krause et al., 2003). We hypothesize that with little restrictions from the fiber portion of the diet and a relatively low starch and RFS content, it would be expected that all four of the treatment diets would not impact DMI.

For NDF, starch, and uNDF240 intake (kg/d and % of BW) there were no significant interactions between peuNDF240 and RFS. However, cows fed the higher RFS diet had lower NDF intake as a percentage of BW ($P = 0.03$) but greater starch intake ($P < 0.0001$). There was an effect of peuNDF240 on uNDF240 intake with cows fed the higher peuNDF240 diets having greater uNDF240 intake ($P < 0.0001$). Based on the dietary composition of the diets in this study, these results were expected.

2.4.3 Milk yield, composition, and feed efficiency

There were no significant interactions between peuNDF240 and RFS on milk yield or composition. Milk yield was greater for cows fed the lower peuNDF240 diets by only 0.8 kg (52.6 ± 0.6 vs. 51.4 ± 0 kg/d; $P = 0.01$; Table 2.8). A review of 11 different studies that compared diets with differing fiber degradability noted that, on average, milk yield was 1.7 kg/d greater for cows fed diets that had 11.5% greater NDF degradability (Gencoglu et al., 2008). The authors attributed the increase in milk yield to the increase in DMI. In the present study there were no differences in DMI so the difference in milk yield was a result of a different factor. It is a possibility that the slight decrease in milk yield was due to greater uNDF in the diets with higher peuNDF240 because the greater

uNDF240 in the larger particles took longer to leave the rumen leading to overall less fermentable material in the rumen.

There was a significant effect of peuNDF240 on milk fat percentage ($P = 0.05$; Table 2.8). The cows fed diets with higher peuNDF240 had a greater fat percentage compared to the cows fed diets with lower peuNDF240 (3.67 ± 0.07 vs. 3.54 ± 0.05%). Previous observed has found that, in diets with higher uNDF240, cows had greater milk fat percentage was attributed to elevated rumen pH and the fermentation of fiber (Fustini et al., 2017). The peNDF of a diet has been related to the maintenance of milk fat percentages, and research that decreased peNDF observed a decrease in milk fat percentage (Mertens, 1997; Yansari et al., 2004). This decreased milk fat percentage is related to the reduction of chewing behavior and rumen pH with less peNDF content (Mertens, 1997). With this information, diets that have greater uNDF240 in the physically effective fraction would be expected to have a significantly greater milk fat percentage compared to diets with less peuNDF240.

There was a main effect of RFS on fat yield ($P = 0.01$), FCM ($P = 0.01$), SCM ($P = 0.03$), ECM ($P = 0.02$), and a trend for an effect of RFS on percent ($P = 0.06$). On average, there was less FCM, SCM, ECM, and fat as a percent produced by the cows fed the diets with higher RFS (Table 2.8). Typically, feeding diets higher in fermentable carbohydrates like corn grain reduces milk fat by reducing the amount of fatty acids that have 6- to 16-carbons and increasing the amount of 18-carbon unsaturated fatty acids, otherwise known as milk fat depression (MFD; Jenkins and McGuire, 2006). The diets in the present study had relatively moderate to low starch and RFS content across diets. Nonetheless, this concentration of RFS was sufficient to reduce milk fat yield. This

indicates that lactating dairy cows are sensitive to the fermentability of starch in the diet, especially when the uNDF240 content of the diet is low (~7% of DM). In a meta-analysis that evaluated the effects of corn grain type and corn grain-processing methods on lactation performance in dairy cows, it was observed that for every 1% increase in RFS concentration in the diet there was a 0.02% reduction in milk fat percentage (Ferraretto et al., 2013). Using this calculation, there would be an average difference of 0.05% between the milk fat percentage of the diets with lower and higher RFS because there is a 2.5% difference between the RFS contents in these diets (Ferraretto et al., 2013). When comparing diets, there was a trend for RFS to affect milk fat percentage when cows fed the diets with greater RFS tended to have less milk fat percentage compared to diets with less RFS (3.54 ± 0.06% vs. 3.67 ± 0.08%). The 2.5% increase in RFS resulted in a 0.13% difference in milk fat percent in the present study, so for every 1% increase in RFS there was an increase of 0.05%. This reduction of milk fat percentage with the increase in RFS is almost double the average response that was observed by Ferraretto et al. (2013). This adds greater evidence that when diets are as low in uNDF240 and peuNDF240, the cow is exceedingly sensitive to the fermentability of the diet.

Changes in milk fat in response to varying concentrations of fermentability of starch have been observed across a range of diets. Cows fed diets with 32% starch and either dry-ground corn or high-moisture corn experienced a 15% reduction in milk fat (3.59 vs. 3.05%) when the high-moisture corn diet was fed because of the increased fermentability (Oba and Allen, 2003a). The results from Oba and Allen (2003b) suggest that due to higher fermentability, there was less complete rumen biohydrogenation, increasing the production of *trans*-10 C 18:1 fatty acid isomer in the rumen, leading to

MFD (Oba and Allen, 2003a). In the current study, a similar occurrence of reduced biohydrogenation may have occurred, but we did not measure biohydrogenation intermediates. In the future, there may be value in monitoring the ratio of uNDF240 and(or) peuNDF240 relative to RFS as a useful indicator for MFD. Based on the data (Table 2.8), diets could be associated with lower milk fat percent (<3.75%) when the ratio of uNDF240:RFS is at or below 0.43 and when the ratio of peuNDf240:RFS is at or below 0.51. These ratios were obtained from the nutrient composition of the treatment diets. When the uNDF240 and peuNDF240 content, 7.3 and 8.6 respectively, is divided by the RFS (16.9) of the high peuNDF240 and low RFS diet, you get a baseline. The high peuNDF240 and low RFS diet has a milk fat percent of 3.74. So, it gives a general idea of where the increase in risk of MFD is when looking at the uNDF240:RFS or peuNDF240:RFS

Changes in mixed and preformed milk fatty acids mirrored the observed reductions in milk fat percentage. There was a significant (P = 0.008) effect of peuNDF240 on mixed origin fatty acid concentration (g/100 g milk) with cows fed the lower peuNDF240 diets having lower mixed origin fatty acid concentration. When cows begin to experience MFD, mixed origin fatty acids are often the first group of fatty acids to decrease (Barbano et al., 2018). The shift in the fatty acid profile of milk has a two-phase response (Harvatine and Bauman, 2011). During the first phase, the decrease in milk fat synthesis involves an equal depression in *de novo* synthesis and preformed uptake in the mammary gland, and the second involves only a decrease in *de novo* synthesis (Harvatine and Bauman, 2011).

The unsaturated fatty acid index followed a similar pattern to milk fat percentage (i.e., more unsaturation with lower milk fat percentage), where there was a significant effect of peuNDF240 (P = 0.005). Barbano et al. (2018) indicated that increases in unsaturation is an early sign of the onset of CLA-mediated milk fat depression and suggested that to achieve a 3.75% milk fat the degree of unsaturation should stay below 0.31 double bonds per fatty acid when looking at bulk tank samples. In the current study, none of the diets supported a milk fat percentage above 3.75%, but the unsaturation for all diets were between 0.280 and 0.295 double bonds/fatty acid. An additional consideration is the concentration of mixed origin fatty acids in a bulk tank sample, which has been suggested to be above 1.40 g/100 g milk to achieve a milk fat percentage of above 3.75% (Barbano et al., 2018). In the current study, only one diet had mixed origin fatty acids at or above 1.40 g/100 g milk. The high peuNDF240 and low RFS diet had 1.43 g/100 g milk of mixed origin fatty acid, but the high peuNDF240 diets as a whole averaged 1.41 ± 0.03 g/100 g milk. It's important to acknowledge that this isn't a perfect comparison as these are averages from specific cows compared to bulk tank samples of multiple cows in different stages of lactation. When the mixed origin + *de novo* fatty acid groups were combined, there was a difference in peuNDF240 where the cows fed the higher peuNDF240 diets had greater *de novo* and mixed origin fatty acids compared to cows fed the lower peuNDF240 diets (2.11 ± 0.04 g/100 g milk vs. 2.21 ± 0.03 g/100 g milk; P = 0.03). This shows that the difference in mixed origin fatty acids is driven by *de novo* synthesis. Ruminants utilize acetate as a carbon source for fatty acid synthesis in the mammary gland (Bauman and Griinari, 2003). Diets with higher peuNDF240 have more undegradable material in the physically effective fraction meaning that the longer

particles need to be broken down to a specific size to leave the rumen. The longer particles will stay in the rumen longer and retain potentially degradable fiber longer, allowing for more microbial degradation (Allen, 2000). This allows for more time for potentially degradable NDF to ferment, more acetate to be produced within the rumen, and therefore produce a greater concentration of *de novo* and mixed origin fatty acids within the mammary gland. This adds to the theory from Cotanch et al. (2014) that dairy cows have a minimum amount of uNDF240 required in the diet, and that the uNDF240 must be physically effective to have positive effects on the animal's rumen fermentation because this allows development of the rumen digesta mat. The fatty acid profile of the milk shows that when diets are low in uNDF240 (~7.0%), cows are better able to handle fermentable starch when there is more undegradable physically effective fiber.

While peuNDF240 affected the mixed origin and *de novo* fatty acids, the preformed fatty acids were affected by RFS ($P = 0.02$). Cows fed diets with less RFS had a greater concentration of preformed fatty acid compared to cows fed diets with more RFS (1.33 ± 0.02 g/100 g milk vs. 1.28 ± 0.02 g/100 g milk). Preformed fatty acids are fatty acids are derived from circulating lipoproteins and non-esterified fatty acids that derive from lipids absorbed in the digestive tract and mobilized body reserves, respectively (Bauman and Griinari, 2003). It can be hypothesized that the cows fed the lower RFS diets had to utilize more fat from body tissue compared to higher RFS diets because of higher energy content. However, this effect was not great enough to cause a difference in body weight so it is unlikely the cause of the difference. It is more likely caused by a difference in passage rate of incomplete biohydrogenated fatty acids in the

rumen. Diets with higher starch content have been observed to have an increased passage rate out of the rumen (Oba and Allen, 2003b).

Overall, there was no effect of peuNDF240 or RFS on efficiency of milk, ECM, or SCM production (Table 2.8; $P > 0.10$) This was surprising since there were main effects on milk production, SCM, and ECM, but DMI (kg/d) was not different among treatments and could have compensated for any differences observed in milk production (Table 2.8). However, there was a tendency ($P = 0.06$) for an effect of RFS on FCM/DMI where cows fed the diets with higher RFS produced less FCM from the same DMI, showing a decrease in efficiency (1.77 ± 0.02 vs. 1.81 ± 0.00 kg/kg). This could be a factor of a difference in fiber degradability in the rumen from associative effects.

2.4.4 Eating and ruminating behaviors

Chewing behavior is most effected by the distension within the rumen caused by the undegradability of the diet and by the time required to chew the diet to the correct particle size for swallowing (Allen, 2000). All treatment diets had similar uNDF240om (7.0% of DM) and peNDF (19.1% of DM) content. As a result, there were no differences in eating time as minutes per day or minutes per kg of DMI (Table 2.9). However, there were differences in rumination time and, therefore, chewing time because chewing time is a summation of eating and ruminating. When peuNDF240 was lower, rumination increased when RFS was increased, and decreased when RFS was decreased (526 vs. 503 min/d $P = 0.03$). When peuNDF240 was higher, rumination decreased when RFS was increased, and increased when RFS was lower (513 vs. 523 min/d; $P = 0.03$). This was an unexpected result (Oba and Allen, 2003a; Salfer et al., 2018). As starch increases in the diet, rumination will decrease, as seen in the diets with higher peuNDF240, because

there is generally a decrease in particle size of the diet with the addition of starch. It is unusual to see rumination increase with greater RFS content in the diet with lower peuNDF240. A study looking at fermentable carbohydrates and forage particle size observed a similar occurrence of a significant increase in rumination due to the use of a more fermentable starch source (Maulfair and Heinrichs, 2013). The authors stated that the increase in rumination may seem counterintuitive, but it was likely caused by adaptive responses of the animal to decrease the severity of a low rumen pH (Maulfair and Heinrichs, 2013).

Meal length is known to be smaller when rapidly fermentable carbohydrates are added to a diet because of propionate production (Allen, 2000). Shorter meal sizes are affected by the stimulation of hepatic receptors by propionate which transmit signals to the brain satiety center (Allen, 2000). The cows fed diets with greater RFS had shorter meals compared to the cows fed diets with lower RFS (31.14 ±0.12 vs. 33.34 ± 0.87 min/meal; P = 0.05). This response was also observed in a study that increased fermentability of the diet by substituting refined corn starch for dry cracked shelled corn which resulted in a decrease in meal length (Krause et al., 2003). Interestingly, this effect was observed with the low to moderate starch content diets fed in the current study, yet the difference in meal length between the lower and higher RFS diets did not facilitate a difference in DMI. There was a main effect of RFS on meal bouts, so as RFS increased there was a greater number of meals (11.23 ± 0.18 vs. 10.52 ± 0.19 bouts/d; P = 0.04). The increase in meal bouts in the diets with higher RFS most likely compensated for the decrease in meal length which explains why starch did not affect DMI. Similarly, Krause

et al. (2003) also observed a trend for the number of meals to increase with an increased fermentability of starch with no changes in DMI and decreased meal length.

2.4.5 Rumen fermentation and rumen pH

There were no significant interactions of the main effects on total VFA (m*M*; $P > 0.10$; Table 2.10). Diets with higher RFS had a tendency for less acetate (62.1 ± 0.0 vs. 62.9 ± 0.0 m*M*; $P = 0.09$), greater propionate (23.1 ± 0.3 vs. 21.9 ± 0.3 m*M*; $P = 0.10$), and a significantly lower acetate:propionate ratio (A:P; 2.73 ± 0.04 vs. 2.94 ± 0.05; $P = 0.05$) and acetate + butyrate:propionate ratio (A+B:P) (3.26 ± 0.05 vs. 3.53 ± 0.07; $P = 0.05$). These reductions follow the reduction in FCM yield. As fermentable starch content increased, a shift in molar proportions of VFA from acetate, generally produced during structural carbohydrate fermentation, to propionate would be expected (Krause and Combs, 2003; Firkins et al., 2006). Krause et al. (2003) observed a significant reduction in acetate and an increase in propionate in the rumen when a more fermentable diet was fed. This also corresponded with a decrease in A:P. These changes in acetate, propionate, and A:P are consistent with a shift in rumen fermentation that would be expected with MFD (Bauman and Griinari, 2003). Isovalerate concentration was significantly ($P < 0.0001$) increased for cows fed diets with higher peuNDF240 compared to diets with lower peuNDF240. Isovalerate is produced during fiber fermentation so a greater molar proportion in the rumen of cows fed diets with greater peuNDF240 would be expected (Van Soest, 1994; Mangwe et al., 2020).

There were no significant effects of peuNDF240 or RFS on pH (Table 2.11). With the changes observed in the milk fat and fatty acid content of cows in this study, changes in rumen pH due to higher RFS was expected; however, this was not the case. Declines

in pH have been noted in the literature when cows are fed higher concentrations of starch in their diet (Plazier et al., 2007). A study using mid-lactation Holstein cows observed that when ground high-moisture corn was replaced with dry-cracked corn, there was a decrease in mean rumen pH from 5.82 to 5.67 and an increase in the hours per day when pH was <5.8 from 4.4 to 6.4 h (Krause and Combs, 2003). Krause and Combs (2003) fed a diet that had a starch content above 28% of DM and an aNDFom of less than 26% of DM. The current study had a starch content between 20 to 24% of DM and an aNDFom content of 33% of DM. The starch and RFS content in the current study may not have been high enough to elicit the same response, but the NDF content of the diet could have also caused an increase in rumination, therefore increasing the amount of salivary buffer entering the rumen and overall increasing rumen pH (Van Soest, 1994). However, a study that replaced refined-corn starch for dry-cracked shelled corn had diets with a starch content of 29 to 32% of DM and an aNDFom of 26 to 28% of DM, and also saw no significant difference in pH measurements similar to the current study (Krause et al., 2003). There was an increase in rumination in the lower peuNDF240 and higher RFS diet that may have compensated for any lower pH. This could have caused the pH measurements to not be significantly different from each other. A study that looked at the effect of forage particle size and fermentable carbohydrates saw a similar pattern where there was an increase in rumination activity when the cows were on a fermentable diet, but this also corresponded no difference in rumen pH metrics similar to the current study (Maulfair and Heinrich, 2013).

In the case of diets with high fermentable starch, a depression in pH and a shift in the VFA profile is often observed with the change in microbial processes described

during incomplete biohydrogenation and increases in *trans*-10 isomers that has been assumed to result in MFD (Bauman and Griinari, 2003). There were no interaction or main effects in the current study pH observations statistically, but the cows fed the diet with lower peuNDF240 and higher RFS had 347 min/d that the rumen pH was < 5.8, or approximately 5.8 h/d, although it was not statistically significant. Using guidelines from Humer et al. (2017), there is a higher risk of SARA when the rumen pH is < 5.8 for more than 5 h/d. Therefore, the cows fed the lower peuNDF240 and higher RFS would be considered to have a higher risk of SARA, unlike the other three treatment diets where cows averaged around 4 h/d when the rumen pH was < 5.8. This shows that even when starch (24% of DM) and RFS (19% of starch) content are moderately low, there can be negative effects on the animal's rumen environment, when the peuNDF240 of the diet is at approximately 6% of ration DM.

2.4.6. Rumen pool size and turnover

Table 2.12 details the digesta characteristics of cows fed the four treatment diets. There was not a significant interaction of main effects, but there were significant effects of peuNDF240 and RFS on rumen digesta volume ($P < 0.05$). The cows fed the diets with lower peuNDF240 had less rumen digesta volume compared to the cows fed the diets with higher peuNDF240 (109.5 ± 2.5 vs. 114.5 ± 1.5 L), and the cows fed the diets with lower RFS had less rumen digesta volume compared to cows fed the diets with higher RFS (110 ± 3 vs. 115 ± 2 L). There was a similar pattern for rumen digesta mass where there was an effect of peuNDF240 ($P = 0.04$) and a trend for RFS ($P = 0.06$) with cows fed the diets with higher peuNDF240 likely required more time to reduce the

particle size of the fiber particle so that it could pass through the omasal orifice and out of the rumen (Allen, 2000).

There were no interactions ($P > 0.10$) between peuNDF240 or RFS on rumen pool size for aNDFom, starch, uNDF240om, or OM, as shown in Table 2.12. However, there was a trend ($P = 0.06$) for RFS to have an effect on rumen pool size of starch (0.3 ± 0.0 vs. 0.35 ± 0.05 kg). There was also a significant effect of peuNDF240 on uNDF240 rumen pool ($P = 0.06$; 3.0 ± 0.0 vs. 3.2 ± 0.0 kg). This would be an expected pattern when considering the nutrient content of the diet. There were no effects ($P > 0.10$) of dietary peuNDF240 or RFS on rumen turnover rate or retention time for aNDFom, starch, or uNDF240. There was a trend ($P = 0.10$) for RFS to have an effect on rumen turnover time of starch, and a trend for peuNDF240 ($P = 0.09$) to have an effect on uNDF240 rumen turnover rate. Additionally, there was an effect of peuNDF240 on OM rumen turnover rate ($P = 0.04$). The cows fed the diets with lower peuNDF240 had a lower OM turnover rate compared to the higher peuNDF240 diets (11.0 ± 0.2 vs. 11.8 ± 0.1 %/h). This follows the same pattern observed in unsaturation and fatty acid composition of the milk. Ingested material will leave the rumen either through fermentation of carbohydrates or proteolysis by microorganisms, through absorption, or through passage, and they offer competition to each other for the removal of digesta (Van Soest, 1994). A study using two starch processing methods with two starch concentrations observed a greater passage rate of dry-ground corn compared to the high-moisture corn for both high (32% of DM) and low (21% of DM) starch concentrations (Oba and Allen, 2003b). However, Oba and Allen (2003b) observed a tendency for passage rate to be greater in the higher starch diets compared to the low starch diets. The authors hypothesized that this was due to greater

DMI in the high starch diets and because of the differences in physical characteristics between dry ground corn and high moisture corn. In contrast, the present study had no differences in DMI for the four diets, and there was no difference in source of starch used and overall particle size of the diet was the same.

There was an interaction between peuNDF240 and RFS for starch rumen turnover rate. As RFS increased in diets with higher peuNDF240, starch turnover rate decreased. As RFS increased in diets with lower peuNDF240, the rumen turnover rate of starch increased.

2.4.7. Total tract nutrient digestibility

There were no interactions or main effects on total tract DM, OM, or potentially degradable NDF (pdNDF) digestibility (Table 2.13). Potentially degradable NDF is the fraction of NDF that is available for microbial fermentation and disappears in the rumen (Harper and McNeill, 2015). It is a measurement that is associated with extended rumen retention, and this fraction has the greatest impact on energy supply to the ruminant in forage-based diets (Harper and McNeill, 2015). Considering that the retention time in the rumen was not different (Table 2.12), the total tract digestibility results for DM, OM, and pdNDF were expected. The amount of degradable material that can escape to the abomasum and the overall rate of fermentation is determined by the rate of digestion and the rate of passage from the rumen (Van Soest, 1994).

There was an effect of peuND240 ($P = 0.002$) on aNDFom digestibility. The cows fed the diets with higher peuNDF240 had lower aNDFom digestibility compared to the cows fed the low peuNDF240 diets (58.1 ± 0.5 vs. 61.1 ± 0.95 % of DM). This is not surprising because the diets with higher peuNDF240 had more uNDF240om in the

physically effective fraction compared to the lower peuNDF240 diets. This means there was more aNDFom that was undegradable in the rumen and therefore passed through the gastrointestinal tract and into the feces. There was also a trend for an effect for RFS, with the cows fed diets higher RFS diets tending to have a lower aNDFom digestibility (58.9 ± 1.3 vs. 60.3 ± 1.7; $P = 0.10$ % of DM). In a meta-analysis looking at effect of cereal grain type and processing found that as dietary starch concentration increased there was a significant reduction in rumen NDF degradability and NDF TTD (Ferraretto et al., 2013). Specifically, they observed a decrease in rumen NDF degradability of 0.61 and by 0.48 percentage units per percentage unit increase in dietary starch content (Ferraretto et al., 2013). This suggests that the amount of starch in the diet should be considered antagonistic to aNDFom digestibility (Ferraretto et al., 2013) and the decrease in aNDFom digestibility in the present study agrees with their findings. A meta-analysis that used 147 different studies to evaluate the current NRC dairy model (2001) observed an interaction between starch and DMI that suggests that an increase in starch intake will cause a reduction in aNDFom digestibility (White et al., 2015). This is thought to be caused by a reduction in rumen pH when there is an increased amount of fermentable carbohydrates in the diet. This causes the rumen environment to become too acidic and therefore not suitable to fiber digesting bacteria (Van Soest, 1994). This decreases the capabilities of the rumen to ferment and utilize aNDFom, increasing the amount that passes through the gastrointestinal tract and into the feces.

In the rumen, starch fermentability can range from 50 to 90% of starch intake, while post-rumen digestion can range from 6 to 44% (Allen, 2000; Firkins, 2001). Post-rumen absorption will compensate for the differences in rumen digestion so there is less

variability in total tract starch digestibility between grain types and their conservation methods (Allen, 2000; Ferraretto et al., 2013). There was an effect of peuNDF240m on starch total tract digestibility, with the cows fed the high peuNDF240 diets having greater total tract starch digestibility (98.8 ± 0.1%), while the cows fed the lower peuNDF240 diets had less (98.4 ± 0.2%; P = <0.001). However, these values are very similar and it is unlikely to have biological significance. The average starch digestibility for the meta-analysis that evaluated the NRC dairy model was ranged from 68.5 to 99.7% with an average of 92.1% (White et al., 2015) which is much lower compared to the current starch digestiblities and less than a 0.6% difference across all diets. A majority of the variability in rumen starch digestibility is related to grain type and the degree of processing the grain undergoes (Ferraretto et al., 2013). These diets contained the same ground-corn meal with varied inclusion rates. So, it is not surprising that these diets had total tract starch digestibilities that were less than 1% different from each other.

2.5 Conclusions

Undegradable NDF after 240 hours of fermentation is becoming a common measurement that the industry uses to formulate dairy cattle rations and predict production indicators such as DMI and ECM. While uNDF240 does a reasonable job predicting DMI and ECM, it does not consider the particle size of the diet. Therefore, a measurement that takes both the undegradability as well as the particle size into account would be useful if it were able to improve predicted responses of the cow to a formulated diet. By analyzing peuNDF240, both uNDF240 and particle size are accounted for and can better allow nutritionists to formulate rations and predict the cow response.

Diets with lower peuNDF240 (~6% of DM) and higher RFS (19% of starch) elicited negative associative effects. Specifically, milk fat and mixed origin fatty acids were reduced for the cows when lower dietary peuNDF240 and higher RFS was fed. These negative associative effects were also reflected in the A:P and the total tract digestibility of aNDFom, where there was less aNDFom digested in the lower peuNDF240 diets. At moderate dietary content of RFS, when the peuNDF240 was at 6% of DM, there was likely a greater risk of SARA based on the decrease in mixed origin fatty acid, an increase in time rumen pH was <5.8, and lower aNDFom total tract digestibility. These negative associative effects were not as apparent when the peuNDF240 was above the 6% level at the same RFS concentration, however there did not appear to be an interaction between peuNDF240 and RFS, which was originally hypothesized. Likely, these effects will be amplified to a greater extent with higher inclusion of starch and need to be considered during ration formulation. Future research may find value in examining at the ratio between uNDF240 and RFS or peuNDF240 and RFS when predicting MFD in diets. Specifically, these data showed that when the uNDF240:RFS is at 0.43 or lower, or the peuNDF249:RFS is at 0.51 or lower, it can be associated with lower milk fat.

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2.7 Tables

Table 2.1. Ingredient composition of diets (% of DM) with varying concentrations of physically effective undegraded neutral detergent fiber at 240 h (peuNDF240) and rumen fermentable starch (RFS) fed to lactating Holstein cows.

	Diets			
	Low peuNDF240		High peuNDF240	
Ingredients	Low RFS	High RFS	Low RFS	High RFS
Conventional corn silage	-	-	47.60	47.60
Brown midrib corn silage	47.60	47.60	-	-
Timothy hay, chopped	7.94	7.94	7.94	7.94
Wheat straw, chopped	1.59	1.59	1.59	1.59
Corn meal	2.78	7.94	3.57	8.73
Beet pulp, pelleted	7.14	5.16	6.35	4.37
Concentrate mix				
Aminomax Pro[1]	6.99	6.99	6.99	6.99
Canola meal	6.98	6.98	6.98	6.99
Soybean meal	5.56	5.56	5.56	5.56
Dried distillers grains	4.76	1.59	4.76	1.59
99% Sugar[2]	1.41	1.41	1.41	1.41
Soybean hulls	1.58	1.58	1.58	1.58
BergaFat[3]	1.16	1.16	1.16	1.16
Energizer Gold[4]	1.09	1.09	1.09	1.09
Calcium carbonate	0.78	0.78	0.78	0.78
Sodium sesquicarbonate	0.74	0.74	0.74	0.74
Cane molasses	0.46	0.46	0.46	0.46
Salt	0.39	0.39	0.39	0.39
Magnesium oxide	0.31	0.31	0.31	0.31
Urea	0.28	0.28	0.28	0.28
Omnigen[5]	0.17	0.17	0.17	0.17
Trace vitamin premix[6]	0.10	0.10	0.10	0.10
Yeast culture[7]	0.04	0.04	0.04	0.04
Clarifly[8]	0.04	0.04	0.04	0.04
MetaSmart[9]	0.03	0.03	0.03	0.03
Smartamine-L[9]	0.03	0.03	0.03	0.03
Mintrex zinc[10]	0.02	0.02	0.02	0.02
Vitamin E[11]	0.02	0.02	0.02	0.02
Selenium yeast	0.01	0.01	0.01	0.01
Total	100.0	100.0	100.0	100.0

[1] Afgritech, LLC. Watertown, NY.
[2] Pure Sugar.
[3] BergaFat; Berg + Schmidt America, LLC; Libertyville, IL.
[4] IFFCO (Malaysia) Sdn.Bhd., Sharjah, United Arab Emirates.
[5] Phibro Animal Health Corp., Teaneck, NJ.

[6] Contained 21.66 % Ca, 0.91% Cl, 0.72% Mg, 0.17% P, 0.16% S, 0.01% K, 25,438 mg/kg Zn, 21,802 mg/kg Mn, 6,427 mg/kg Cu, 500 mg/kg Fe, 428 mg/kg I, 269 mg/kg Se, 154 mg/kg Co, 5,732 kIU/kg Vitamin A, 1,589 kIU/kg Vitamin D, and 29,762 kIU/kg Vitamin E.
[7] Diamond V, Cedar Rapids, IA.
[8] Central Garden and Pet Company, Schaumburg, IL.
[9] Adisseo USA, Inc.; Alpharetta, GA.
[10] Novus International Inc., St. Charles, MO.
[11] Contained 8,800 IU vitamin E/kg.

Table 2.2. Formulated composition of diets with varying concentrations of physically effective undegraded neutral detergent fiber after 240-h fermentation (peuNDF240) and rumen fermentable starch (RFS) fed to lactating Holstein cows. Formulation was based on estimated chemical composition of ingredients. The corn silages and timothy hay were based on wet chemistry and in vitro digestibility analysis from samples taken before the study began. All other ingredients were based on AMTS (version 4.8; Groton, NY) feed library values.

	Diets			
	Low peuNDF240		High peuNDF240	
Item	Low RFS	High RFS	Low RFS	High RFS
DM, %	57.2	57.1	54.0	53.9
CP, % of DM	15.4	15.3	15.5	15.4
Soluble protein, % of CP	30.3	30.2	31.2	27.6
ADF, % of DM	20.6	18.7	21.5	19.6
aNDFom[1], % of DM	33.3	30.9	33.6	31.1
uNDF240om[2], % of DM	7.24	6.92	10.14	9.81
NFC[3], % of DM	39.0	41.7	38.3	41.0
Lignin, % of DM	2.7	2.6	3.1	3.0
Starch, % of DM	23.5	27.2	23.5	27.2
RFS[4] % of DM	16.5	19.9	15.9	19.3
Sugar (ESC[5]), % of DM	5.9	5.7	5.4	5.3
Ether extract, % of DM	5.5	5.6	5.4	5.5
Calcium, % of DM	0.93	0.90	0.95	0.91
Phosphorus, % of DM	0.33	0.34	0.33	0.34
Magnesium, % of DM	0.40	0.40	0.43	0.42
Potassium, % of DM	1.02	0.98	1.02	0.98
Sulfur, % of DM	0.24	0.24	0.23	0.23
Sodium, % of DM	0.45	0.45	0.45	0.45
Chloride ion, % of DM	0.52	0.52	0.52	0.52
Iron, mg/kg of DM	288	262	283	256
Copper, mg/kg of DM	15	15	15	15
Manganese, mg/kg of DM	64	63	64	63
Zinc, mg/kg of DM	93	93	93	93

[1] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[2] Undegraded neutral detergent fiber after 240 h of in vitro fermentation, ash corrected.
[3] Nonfiber carbohydrates.
[4] Rumen fermentable starch was calculated as the starch content of the diets multiplied by a 7h degradability, 70% of starch, which has been previously measured in prior research the Miner Institute (Farmer et al., 2014)
[5] Ethanol soluble carbohydrates.

Table 2.3. Analyzed chemical composition (mean ± SD), in vitro digestibility, and fermentation analysis (for silages) of ingredients used in treatment diets varying in physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS) fed to lactating dairy cows.

Item	CON[1] corn silage	BMR[2] corn silage	Timothy hay	Wheat straw	Beet pulp pellets	Low RFS grain mix	High RFS grain mix	Corn meal
DM, %	38.0±0.3[3]	39.4±0.5	88.9±0.2	88.0±0.1	87.9±0.1	88.1±0.1	88.0±0.0	85.4±0.1
CP, % of DM	7.4±0.1	7.5±0.1	5.5±0.2	3.9±0.1	9.3±0.1	33.8±0.7	33.8±0.2	8.2±0.1
Soluble protein, % DM	4.5±0.1	4.1±0.1	2.0±0.2	1.8±0.1	1.6±0.3	9.5±0.7	8.7±0.5	1.7±0.3
ADF, % of DM	21.1±0.3	21.6±0.6	43.5±0.2	55.0±0.8	28.4±0.5	16.1±1.1	16.3±0.8	2.65±0.4
aNDFom[4], % of DM	36.2±0.7	35.4±1.8	65.9±0.2	79.9±1.0	42.6±0.2	21.9±1.6	23.2±0.8	9.8±0.4
Lignin, % of DM	2.26±0.2	1.6±0.1	6.3±0.0	8.9±0.3	2.34±0.1	5.0±0.1	5.2±0.3	0.6±0.1
NFC[5], % of DM	51.0±0.9	51.6±2.0	23.9±0.6	11.5±0.8	47.2±0.4	28.1±0.7	27.4±0.5	77.9±0.3
NSC, % of DM	37.5±0.1	38.5±2.6	11.7±0.3	1.3±0.3	8.7±0.3	10.3±0.4	10.8±0.9	76.8±0.3
Starch, % of DM	36.8±0.4	37.9±2.7	0.6±0.1	0.5±0.3	0.4±0.1	1.4±0.1	1.7±0.4	75.1±0.5
Starch D, % of DM	66.0±1.9	72.4±2.1	-	-	-	-	-	53.8±2.5
Sugar (ESC[6]), % of DM	0.7±0.2	0.6±0.1	11.1±0.3	0.8±0.2	8.3±0.3	8.9±0.3	9.2±0.8	1.7±0.2
Ether extract, % of DM	3.3±0.1	3.4±0.0	1.1±0.0	1.2±0.1	0.9±0.1	5.9±0.2	5.7±0.1	3.8±0.0
Ash, % of DM	3.2±0.1	3.3±0.1	5.3±0.1	5.9±0.40	9.1±0.20	14.1±0.30	13.8±0.30	1.5±0.10
Calcium, % of DM	0.27±0.01	0.22±0.02	0.18±0.01	0.22±0.01	2.05±0.03	2.64±0.12	2.68±0.12	0.02±0.00
Phosphorus, % of DM	0.24±0.00	0.25±0.01	0.15±0.00	0.06±0.00	0.10±0.00	0.68±0.01	0.68±0.01	0.28±0.01
Magnesium, % of DM	0.19±0.01	0.18±0.01	0.07±0.00	0.07±0.00	0.21±0.01	1.01±0.03	1.16±0.05	0.11±0.00
Potassium, % of DM	0.90±0.03	0.92±0.03	1.46±0.02	1.56±0.04	0.39±0.01	1.57±0.02	1.56±0.03	0.38±0.00
Sulfur, % of DM	0.11±0.00	0.16±0.01	0.09±0.00	0.09±0.00	0.14±0.00	0.54±0.01	0.58±0.03	0.12±0.00
Sodium, % of DM	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.05±0.00	1.57±0.11	1.54±0.05	0.01±0.00

Chloride ion, % of DM	0.29±0.03	0.24±0.01	0.2±0.004	0.14±0.02	1.17±0.04	0.93±0.07	0.97±0.01	0.06±0.00
Iron, mg/kg of DM	146±11	120±6	117±6	73±5	556±28	441±16	475±11	31±2
Copper, mg/kg of DM	6±0	6±0	6±0	5±0	6±0	34±2	35.5±3	2.25±0
Manganese, mg/kg of DM	23±1	15±2	31±1	19±2	45±0.5	111±7	108±4	5±0
Zinc, mg/kg of DM	25±0	27±0.7	16±0	8±1	32±2	246±1	256±9	20±0
Lactic acid, % of DM	4.7±0.1	3.6±0.4	-	-	-	-	-	-
Acetic acid, % of DM	3.3±0.2	3.2±0.2	-	-	-	-	-	-
Propionic acid, % of DM	0.1±0.0	0.1±0.0	-	-	-	-	-	-
Total VFA[7], % of DM	8.0±0.2	6.8±0.4	-	-	-	-	-	-
pH	3.8±0.0	4.0±0.1	-	-	-	-	-	-
uNDF12om[8], % of DM	-	-	-	-	10.8±0.4	10.8±1.1	11.3±0.6	6.4±0.1
uNDF30om[9], % of DM	14.5±0.3	11.6±0.4	33.0±0.3	50.0±0.2	-	-	-	-
uNDF72om[10], % of DM	-	-	-	-	5.5±0.1	6.0±0.2	6.3±0.1	2.5±0.1
uNDF120om[11], % of DM	9.1±0.3	7.5±0.3	21.0±0.2	32.8±0.3	5.0±0.1	5.4±0.2	5.5±0.2	2.0±0.1
uNDF240om[12], % of DM	8.6±0.2	6.7±0.3	19.9±0.2	33.7±2.4	-	-	-	-

[1]Conventional.
[2]Brown midrib.
[3] Mean ± standard deviation. Sample n = 4/ingredient.
[4] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[5] Nonfibrous carbohydrates.
[6] Ethanol soluble carbohydrates.
[7] Volatile fatty acids.
[8] Undegraded neutral detergent fiber after 12 hours of in vitro fermentation, ash corrected.

[9] Undegraded neutral detergent fiber after 30 hours of in vitro fermentation, ash corrected.
[10] Undegraded neutral detergent fiber after 72 hours of in vitro fermentation, ash corrected.
[11] Undegraded neutral detergent fiber after 120 hours of in vitro fermentation, ash corrected.
[12] Undegraded neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

Table 2.4. Particle size distribution, physical effectiveness factor (pef), and physically effective neutral detergent fiber (peNDF) of ingredients used in treatment diets varying in physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS) fed to lactating dairy cows.

Item	COV[1] corn silage	BMR[2] corn silage	Timothy hay	Wheat straw	Beet pulp pellets	Low RFS grain mix	High RFS grain mix	Corn meal
Particle size, % as-fed[3]								
>19.0 mm	4.9±0.2[4]	3.4±0.2	22.8±1.5	28.7±1.6	-	-	-	-
8.0 to 19.0 mm	71.7±0.8	76.9±1.2	30.5±0.6	35.7±0.9	-	-	-	-
4.0 to 8.0 mm	12.9±0.3	11.5±0.5	15.7±0.2	17.5±0.2	-	-	-	-
<4.0 mm	10.5±0.4	8.3±0.9	31.1±1.8	18.2±0.8	-	-	-	-
pef[5]	0.89±0.00	0.92±0.01	0.69±0.01	0.82±0.01	-	-	-	-
peNDF[6], %	32.2±0.79	32.3±1.8	45.0±1.03	64.5±0.38	-	-	-	-
Particle size, % DM[7]								
>19.00 mm	0.5±0.1	0.1±0.0	0.2±0.0	0.7±0.3	0.00±0	0.00±0	0.00±0	0.00±0
13.20 to 19.00 mm	0.8±0.3	0.7±0.3	0.6±0.0	1.4±0.3	0.00±0	0.00±0	0.00±0	0.00±0
9.50 to 13.20 mm	4.0±0.2	5.3±1.0	1.4±0.1	2.7±0.3	0.00±0	0.00±0	0.00±0	0.00±0
6.70 to 9.50 mm	15.1±1.1	18.9±1.0	4.3±0.2	7.1±0.3	51.8±2.5	0.2±0.2	0.7±0.1	0.00±0
4.75 to 6.70 mm	20.0±0.6	22.7±0.6	5.4±0.1	7.0±0.6	3.0±0.2	0.2±0.1	0.5±0.3	0.00±0
3.35 to 4.75 mm	20.8±0.2	21.4±0.7	7.0±0.4	11.9±0.3	4.0±0.3	0.6±0.1	0.7±0.1	0.00±0
2.36 to 3.35 mm	14.0±0.4	11.5±0.8	9.6±0.4	14.9±0.2	6.4±0.4	1.6±0.2	1.5±0.1	0.1±0.1
1.18 to 2.36 mm	13.0±0.2	10.5±0.4	28.2±0.3	33.7±0.9	17.1±0.9	18.2±1	17.9±0.6	10.1±1.9
0.60 to 1.18 mm	6.2±0.1	4.8±0.3	20.6±0.4	14.3±0.5	8.9±0.7	32.8±0.3	32.3±0.4	27.9±1.9
0.30 to 0.60 mm	3.1±0.2	2.4±0.2	13.5±0.2	4.7±0.4	5.3±0.5	28.3±1.2	28±0.6	31.3±0.6
< 0.30 mm	2.5±0.1	1.7±0.2	9.3±0.4	1.7±0.2	3.5±0.4	18.2±0.3	18.4±0.8	30.5±4.5
pef[8]	0.88±0.00	0.91±0.01	0.57±0.01	0.79±0.01	0.82±0.01	0.21±0.01	0.21±0.01	0.10±0.01
peNDF[9], %	31.8±0.72	32.3±1.8	37.0±0.4	64.5±0.38	31.2±0.60	4.4±0.45	4.6±0.25	0.9±0.2

[1] Conventional.
[2] Brown midrib.
[3] Measurements made with the Penn State Particle Separator.
[4] Mean ± standard deviation. Sample n = 4/ingredient.
[5] pef = physical effectiveness factor with the Penn State Particle Separator, % of DM ≥ 4.0 mm. (Lammers et al., 1996).
[6] peNDF = physically effective neutral detergent fiber with the Penn State Particle Separator, % DM ≥ 4.0 mm.
[7] Measurements made with the Ro-Tap sieve.

[8] pef = physical effectiveness factor, % DM ≥1.18 mm.
[9] peNDF = physically effective neutral detergent fiber, % DM ≥1.18 mm.

Table 2.5. Particle size distribution (mean ± SD) of treatment diets varying in physically effective undegraded neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS) fed to lactating dairy cows.

	Diets			
	Low peuNDF240		High peuNDF240	
Item	Low RFS	High RFS	Low RFS	High RFS
Particle size, % as-fed[1]				
>19.0 mm	4.7±0.2[2]	4.9±0.3	5.6±0.3	6.1±0.5
8.0 to 19.0 mm	45.4±0.6	43.5±1.1	40.8±0.6	41.1±1
4.0 to 8.0 mm	10.3±0.2	10.2±0.4	10.4±0.1	9.8±0.4
<4.0 mm	39.6±0.5	41.4±1.2	43.3±0.6	43.1±0.7
pef[4]	0.60±0.01	0.57±0.01	0.57±0.01	0.57±0.01
peNDF[4]	19.9±1.12	19.0±0.80	18.9±0.61	18.6±0.31
Calculated peuNDF240[5], % of uNDF240	4.17±0.04	3.98±0.17	4.14±0.07	4.05±0.17

[1]Measurements made with the Penn State Particle Separator.
[2]Mean ± standard deviation. Sample n = 4/ingredient.
[3] pef = physical effectiveness factor with the Penn State Particle Separator, % of DM ≥ 4.0 mm. (Lammers et al., 1996)
[4] peNDF = physically effective neutral detergent fiber with the Penn State Particle Separator, % of DM ≥ 4.0 mm.
[5] Physically effective undegraded neutral detergent fiber after 240 hours of in vitro fermentation. The pef multiplied by undegraded neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected (uNDF240om) from composited diets. This value assumes a normal distribution of uNDF240om across all particle sizes in the diet.

Table 2.6. Calculated diet composition based on wet chemistry analysis of individual feed ingredients. The diets were different in physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

	Diets			
	Low peuNDF240		High peuNDF240	
Item	Low RFS	High RFS	Low RFS	High RFS
DM, %	55.3±0.4[1]	55.3±0.4	54.4±0.3	54.2±0.2
CP, % of DM	16.1±0.2	15.3±0.1	16.0±0.3	15.2±0.1
Soluble protein, % of CP	40.6±0.9	39.8±1.0	43.4±0.8	42.5±1.1
ADF, % of DM	22.0±0.6	21.2±0.4	21.6±0.5	20.7±0.2
aNDFom[2], % of DM	33.1±1.3	32.4±0.8	33.3±0.8	32.6±0.2
NFC[3], % of DM	41.4±1.0	43.4±0.9	41.4±0.6	43.4±0.4
Lignin, % of DM	3.21±0.1	3.1±0.1	3.5±0.1	3.42±0.1
NSC, % of DM	25.4±1.3	29.0±1.2	25.5±0.3	29.1±0.2
Starch, % of DM	20.7±1.3	24.6±1.3	20.8±0.2	24.7±0.2
Starch D[4,5], % of DM	80.5±1.7	78.1±1.6	81.4±1.4	77.0±2.3
RFS[6]	16.7±1.0	19.2±1.0	16.9±0.3	19.0±0.7
Sugar, % of DM	3.9±0.1	4.5±0.2	4.7±0.2	4.5±0.2
Ether Extract, % of DM	3.83±0.08	3.76±0.06	3.81±0.06	3.75±0.08
Ash, % of DM	7.44±0.12	6.77±0.12	7.29±0.13	6.67±0.09
Calcium, % of DM	1.14±0.03	1.02±0.04	1.15±0.04	1.04±0.04
Phosphorus, % of DM	0.36±0.00	0.36±0.00	0.37±0.00	0.36±0.00
Magnesium, % of DM	0.44±0.01	0.46±0.02	0.45±0.02	0.46±0.01
Potassium, % of DM	1.14±0.01	1.09±0.01	1.12±0.02	1.08±0.02
Sulfur, % of DM	0.27±0.00	0.27±0.01	0.25±0.00	0.25±0.01
Sodium, % of DM	0.53±0.04	0.47±0.02	0.53±0.04	0.47±0.02
Chloride ion, % of DM	0.52±0.02	0.48±0.01	0.54±0.03	0.50±0.01
Iron, mg/kg of DM	250±5	240±6	262±8	248±7
Manganese, mg/kg of DM	49±2	45±2	53±3	48±2
Zinc, mg/kg of DM	97±4	93±3	97±4	92±3
Copper, mg/kg of DM	15±0.8	14±1	15±1	14±1
uNDF30om[5,7], % of DM	13.5±0.2	15.2±1.1	15.1±0.3	15.5±0.4
uNDF120om[5,8], % of DM	7.5±0.2	7.6±0.1	8.5±0.2	8.5±0.2
uNDF240om[5,9], % of DM	6.9±0.1	6.8±0.2	7.3±0.1	7.1±0.3
peuNDF240om[5,10], % of uNDF240	6.35	6.07	8.60	8.00

[1] Mean ± standard deviation. Sample n = 4/ingredient.
[2] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[3] Nonfibrous carbohydrates.
[4]Starch Digestibility
[5] The reported values were analyzed from the total mixed ration to reflect the associative effects of all ingredients and were not calculated from individual ingredient in vitro analysis.
[6] Rumen fermentable starch, starch multiplied by starch digestibility.
[7] Undegraded neutral detergent fiber after 30 hours of in vitro fermentation, ash corrected.

[8] Undegraded neutral detergent fiber after 120 hours of in vitro fermentation, ash corrected.
[9] Undegraded neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.
[10] Physically effective undegraded neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected. The uNDF240om from composited diet that was retained above the 1.18 mm sieve. This value is sensitive to differences in uNDF240om across particle sizes in the diet

Table 2.7. Least square means of intake, body weight, and body condition change of lactating Holstein cows fed diets with varying concentrations of physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

	Diets							
	Low peuNDF240		High peuNDF240			*P*-value		
Variable	Low RFS	High RFS	Low RFS	High RFS	SE	peuNDF240	RFS	Interaction
DMI kg/d	29.7	29.4	29.4	29.2	0.7	0.27	0.40	0.72
DMI, % of BW/d	4.31	4.28	4.24	4.20	0.12	0.04	0.41	0.84
aNDFom[1] intake, kg/d	9.9	9.5	9.8	9.6	0.3	0.75	0.03	0.63
aNDFom intake, % of BW/d	1.44	1.39	1.42	1.37	0.05	0.37	0.03	0.86
Starch intake, kg/d	6.1	7.2	6.0	7.2	0.2	0.74	<0.001	0.98
Starch intake, % of BW/d	0.88	1.06	0.87	1.04	0.03	0.35	<0.001	0.76
uNDF240om[2] intake, kg/d	2.2	2.2	2.5	2.4	0.06	<0.001	0.008	0.80
uNDF240om intake, % of BW/d	0.32	0.32	0.35	0.35	0.01	<0.001	<0.001	0.76
BW, kg	691	690	695	700	14	0.04	0.51	0.39
BCS	2.87	2.87	2.89	2.91	0.09	0.17	0.68	0.72

[1] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[2] Undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

Table 2.8. Least square means of lactation performance of lactating Holstein cows fed diets with varying concentrations of physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

	Diets							
	Low peuNDF240		High peuNDF240			*P*-value		
Variable	Low RFS	High RFS	Low RFS	High RFS	SE	peuNDF240	RFS	Interaction
Milk, kg/d	53.1	52.0	51.2	51.5	1.3	0.01	0.35	0.19
3.5% FCM, kg/d	53.8	51.5	52.9	52.2	1.3	0.85	0.01	0.19
SCM[1], kg/d	49.0	47.2	48.1	47.7	1.2	0.77	0.03	0.17
ECM, kg/d	53.4	51.5	52.5	51.9	1.3	0.56	0.02	0.18
Fat, %	3.59	3.48	3.74	3.60	0.08	0.05	0.06	0.87
Fat, kg/d	1.90	1.79	1.90	1.84	0.06	0.41	0.01	0.34
True protein, %	2.83	2.87	2.85	2.86	0.05	0.61	0.12	0.42
True protein, kg/d	1.50	1.48	1.45	1.47	0.03	0.02	0.94	0.34
Lactose, %	4.57	4.57	4.59	4.61	0.05	0.04	0.58	0.33
Lactose, kg/d	2.43	2.38	2.35	2.37	0.06	0.09	0.60	0.14
Solids nonfat, %	8.50	8.54	8.55	8.58	0.09	0.03	0.04	0.85
Solids nonfat, kg/d	4.51	4.44	4.37	4.41	0.10	0.04	0.66	0.17
Urea nitrogen, mg/dL	12.0	10.1	12.4	10.5	0.44	0.08	<0.001	0.97
Somatic cell score	20.9	23.5	26.5	23.3	4.75	0.27	0.90	0.24
De novo FA[2], g/100 g milk	0.80	0.76	0.81	0.80	0.03	0.15	0.26	0.54
Mixed origin FA, g/100 g milk	1.34	1.31	1.43	1.38	0.03	0.008	0.13	0.69
Preformed FA, g/100 g milk	1.31	1.26	1.34	1.29	0.03	0.17	0.02	0.83
De novo and Mixed origin FA, g/100 g milk	2.14	2.07	2.24	2.18	0.06	0.03	0.17	0.98
Unsaturation, double bonds/FA	0.288	0.294	0.281	0.280	0.005	0.005	0.43	0.35
Milk/DMI, kg/kg	1.79	1.77	1.75	1.76	0.03	0.29	0.89	0.44
3.5% FCM/DMI, kg/kg	1.81	1.75	1.81	1.79	0.03	0.41	0.06	0.35
SCM/DMI, kg/kg	1.65	1.69	1.64	1.63	0.03	0.46	0.15	0.35
ECM/DMI, kg/kg	1.80	1.75	1.80	1.78	0.03	0.59	0.12	0.37

[1] Solids corrected milk.

[2] Fatty acids.

Table 2.9. Least squares means of feeding behavior data of lactating Holstein cows fed diets with varying concentrations of physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

Item	Diets				SE	*P*-value		
	Low peuNDF240		High peuNDF240					
	Low RFS	High RFS	Low RFS	High RFS		peuNDF240	RFS	Interaction
Eating time								
min/d	291	292	297	291	10	0.59	0.53	0.40
min/kg of DMI	10	10	10	10	1	0.33	0.73	0.42
min/kg of aNDFom[1]	30	31	31	31	2	0.89	0.61	0.69
min/kg of starch	48	41	50	41	3	0.60	<0.001	0.27
min/kg of uNDF240om[2]	133	137	123	122	8	<0.001	0.56	0.35
Rumination time								
min/d	503	526	523	513	11	0.65	0.43	0.03
min/kg of DMI	17	18	18	18	1	0.36	0.30	0.03
min/kg of aNDFom	52	56	54	54	2	0.76	0.07	0.08
min/kg of starch	83	74	87	72	3	0.74	<0.001	0.08
min/kg of uNDF240om	228	245	215	214	7	<0.001	0.04	0.03
Total chewing time								
min/d	795	818	820	804	14	0.50	0.69	0.02
min/kg of DMI	27	28	28	28	1	0.24	0.50	0.04
min/kg of aNDFom	82	87	85	85	3	0.78	0.13	0.15
min/kg of starch	132	115	137	112	5	0.65	<0.001	0.09
min/kg of uNDF240om	361	381	337	336	14	<0.001	0.07	0.04
Meal length, min/meal	33.45	32.01	33.22	30.27	1.68	0.37	0.05	0.50
Meal bout, bouts/d	10.33	11.05	10.70	11.40	0.47	0.31	0.04	1.00

[1] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[2] Undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

Table 2.10. Fermentation data of lactating Holstein cows fed diets with varying concentrations of physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

	Diets							
	Low peuNDF240		High peuNDF240			*P*-value		
Item	Low RFS	High RFS	Low RFS	High RFS	SE	peuNDF240	RFS	Interaction
Total VFA, m*M*	126.6	124.5	124.3	125.6	2.70	0.80	0.87	0.46
VFA, % of total VFA								
Acetate (A)	62.9	62.1	62.9	62.1	0.78	0.86	0.09	0.94
Propionate (P)	21.6	23.4	22.2	22.8	0.89	0.94	0.10	0.39
Butyrate (B)	13.0	12.1	12.3	12.5	0.32	0.66	0.32	0.09
Isobutyrate	0.49	0.49	0.54	0.51	0.04	0.05	0.41	0.31
Valerate	1.5	1.5	1.5	1.5	0.06	0.96	0.67	0.72
Isovalerate	0.50	0.53	0.60	0.59	0.04	<0.001	0.31	0.12
A:P	2.98	2.69	2.89	2.76	0.13	0.94	0.05	0.42
A+B:P	3.59	3.21	3.46	3.32	0.16	0.89	0.05	0.34
Ammonia-N, mg/dL	6.51	6.11	7.72	6.85	0.85	0.11	0.29	0.68

Table 2.11. Rumen pH data of lactating Holstein cows fed diets with varying concentrations of physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

	Diets							
	Low peuNDF240		High peuNDF240			*P*-value		
Item	Low RFS	High RFS	Low RFS	High RFS	SE	peuNDF240	RFS	Interaction
Daily mean pH	6.10	6.06	6.11	6.14	0.07	0.19	0.85	0.31
Minimum pH	5.48	5.46	5.52	5.52	0.09	0.40	0.83	0.78
Maximum pH	6.61	6.59	6.63	6.62	0.05	0.39	0.64	0.81
Range	1.13	1.13	1.11	1.11	0.07	0.69	0.98	0.90
Standard deviation pH	0.28	0.28	0.27	0.27	0.02	0.74	0.85	1.00
Time pH < 5.8, min/d	267	347	254	211	89	0.12	0.69	0.20
Time pH < 5.8, h/d	4.5	5.8	4.2	3.5	1.5	0.12	0.68	0.20
Time pH < 6.0, min/d	487	582	492	409	122	0.25	0.93	0.22
Time pH < 6.0, h/d	8.2	9.7	8.2	6.8	2.0	0.24	0.96	0.24
Area[1] < 5.8	56.8	77.3	53.6	43.8	22.2	0.16	0.67	0.24
Area[2] < 6.0	132	170	127	106	43	0.72	0.14	0.21

[1] Area < 5.8 = rumen pH units below pH 5.8 by hour.
[2] Area < 6.0 = rumen pH units below pH 6.0 by hour.

Table 2.12. Rumen digesta characteristics and digestion kinetics of lactating Holstein cows fed diets with varying concentrations of physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

	Diets							
	Low peuNDF240		High peuNDF240			*P*-value		
Item	Low RFS	High RFS	Low RFS	High RFS	SE	peuNDF240	RFS	Interaction
Rumen digesta volume, L	107	112	113	116	4	0.02	0.05	0.75
Rumen digesta mass, kg	92	96	96	98	3	0.04	0.06	0.29
Rumen density, kg/L	0.85	0.86	0.87	0.85	0.01	0.56	0.87	0.42
Rumen pool, kg								
aNDFom[1]	7.1	7.7	7.6	7.4	0.24	0.48	0.40	0.11
Starch	0.3	0.3	0.3	0.4	0.04	0.34	0.06	0.31
uNDF240om[2]	3.0	3.0	3.2	3.2	0.10	0.06	0.63	0.98
Organic matter	25.1	25.5	25.3	24.6	0.69	0.59	0.87	0.41
Rumen turnover rate, %/h								
aNDFom	5.5	4.9	4.9	5.1	0.2	0.26	0.33	0.07
Starch	111	137	145	81	20.1	0.60	0.36	0.05
uNDF240om	2.2	2.5	2.0	2.1	0.1	0.09	0.17	0.33
Organic matter	10.8	11.1	11.7	11.9	0.44	0.04	0.51	0.78
Rumen turnover time, h								
aNDFom	18.8	20.5	20.8	20.4	0.9	0.25	0.45	0.23
Starch	1.2	1.2	1.1	1.6	0.2	0.37	0.10	0.18
uNDF240om	49.4	42.0	50.4	49.5	2.8	0.16	0.17	0.28
Organic matter	19.5	14.2	11.5	12.3	3.4	0.16	0.52	0.38

[1] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[2] Undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

Table 2.13. Total tract digestibility data of lactating Holstein cows fed diets with varying concentrations of physically effective neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS).

Item	Diets					*P*-value		
	Low peuNDF240		High peuNDF240					
	Low RFS	High RFS	Low RFS	High RFS	SE	peuNDF240	RFS	Interaction
DM, %	76.1	76.7	76.0	75.7	0.52	0.22	0.76	0.29
Organic matter, % of DM	77.4	78.0	77.4	76.9	0.50	0.19	0.90	0.24
aNDFom[1], % of DM	62.0	60.1	58.6	57.6	0.59	0.002	0.10	0.63
pdNDF[2], % of DM	77.1	75.5	75.8	74.7	0.74	0.30	0.18	0.79
Starch, % of DM	98.2	98.5	98.9	98.7	0.14	<0.001	0.67	0.07

[1] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[2] potentially digestible neutral detergent fiber

CHAPTER 3: SUMMARY AND PERSPECTIVES

This thesis focused on the interaction of physically effective undegraded neutral detergent fiber after 240 h of fermentation (peuNDF240) and rumen fermentable starch (RFS). From the data, we concluded that as RFS increased in the diet, the cows exhibited negative associative effects such as milk fat depression (MFD), a drop in A:P ratio, and a decrease in NDF degradability even when the RFS content was moderate. These data showed the true sensitivity cows have to fermentability in their diets especially when the amount of physically effective undegraded material in the diet is lower (~6-8%). When the amount of peuNDF240 was greater in the diet, these negative associative effects were not as apparent, but there did not appear to be an interaction. There is still a need to characterize how different RFS contents and different starch sources would affect these results. In this study, the RFS was not as high as originally formulated and there also was not as large of a difference in the fiber portion of the diet as originally formulated. If the study was repeated and the diets were fed as formulated, it would be interesting to compare what responses were observed at moderate levels of fermentation compared to much higher. It may also be useful to perform an experiment with a set amount of peuNDF240 and have a range of differing RFS contents that reflects the range in what is commonly fed in the industry. This would cause a dilution effect where the severity of negative associative effects could be directly assessed.

It is known that different starch sources have different fermentabilities, and different conservation and processing methods can increase or decrease their fermentabilities. More research needs to be applied to the idea that peuNDF240 can help

mitigate negative effects from a fermentable diet. In this study, the starch and RFS content in the diets were low relative to what is normally fed on dairies in the U.S. Typical starch content in diets can range from as low as 20 to higher than 30% of ration DM. The content depends on the availability and price of grain as well as the forage sources and quality. The amount of peNDF in the diet has already been established as an important factor in maintain a normal rumen pH and milk fat, but more research is needed to understand if the amount of undegradable physically effective fiber gives a better measurement to help balance diets than peNDF alone.

Prior research suggests that not only is the amount of physically effective fiber important but also the amount of undegradable fiber. There is a minimum amount of undegradable fiber required in the rumen to help develop the rumen mat as well as stimulate rumination. These data addressed the theory that combining the physical and chemical nature of fiber would optimize how fiber is fed to dairy cows. In previous research, a multiplication of the pef and the uNDF240 of a TMR or forage was used to evaluate this theory (calculated peuNDF240), but the diets fed in the present study required a more direct measurement of the physically effective undegradable portion of the diet because of the lack of uniformity in uNDF240 distribution across particle size fractions. The reason for this lack of uniformity is not yet understood, but it is most likely caused by differences in source of NDF. There are other contributions like non-forage vs. forage fiber sources, concentrate types, as well as fragility of NDF. Although the calculated peuNDF240 was not specific enough to differentiate and accurately describe the treatment diets because of how low the uNDF240 content was, it still is a helpful

measurement and still aids in describing the fiber characteristics of these diets. There is an analogous problem in the peNDF system. In the 1997 paper by David Mertens, he mentions the shortcomings of assuming the uniformity of NDF percentage throughout all the particle sizes in a diet (Mertens, 1997). Although that is a shortcoming of the system, it seems to still provide valuable knowledge to nutritionists in describing diets. More testing of forages such as alfalfa and alfalfa-based TMR is needed to increase the robustness of the current data set that is based primarily on corn silage and haycrop diets. The ultimate difference is based on the uNDF characteristics of the various forage types. Legumes have higher uNDF240, but the rate of degradation of the pdNDF is much higher compared to corn silage. There is also a need to test diets that have a large inclusion of non-forage fiber sources such as beet pulp pellets and wheat middlings. Non-forage fiber sources are a way of providing degradable fiber, but for the most part, they are not sufficient in stimulating rumination or developing the rumen digesta mat because of their smaller pef. Plus, although they are lumped into one category, they are different from each other in nutrient makeup. For instance, beet pulp pellets are high in degradable fiber but also high in pectin, while cottonseed hulls have less degradable fiber and have been known to stimulate rumination to a substantial degree. Each non-forage fiber source would need to be individually analyzed to be give a better idea of how they affect peuNDF240.

Another interesting aspect of this study that requires greater consideration is the pH metrics. In this study, there were no significant differences in any pH measurement which was incredibly surprising when looking at the milk components produced by the

cows on these diets. Milk fat depression is usually associated with a drop in pH that usually results in subacute rumen acidosis (SARA). Although some research has shown that this is not always the case, more research is needed to understand why some cows drop in pH and why others do not. There is also needed information on the best way to diagnose SARA, as well as if some cows have different thresholds as to what pH they can endure and function properly. It is known that there is a large degree of variability when analyzing pH data of cows. Some cows are able to maintain their rumen pH relatively high compared to the fermentable diet they are ingesting, while other cows have the opposite effect of having more severe nadirs and time when rumen pH is low. It is possible that in this specific study the sample size was too small to distinguish any true significant differences, but the fact that it is not uncommon to see these unexpected rumen pH results gives traction to the idea that there needs to be a better way of looking at rumen pH data altogether. Other research groups have looked into this problem and have evaluated how to separate cows in to either high or low risk groups for SARA. In 2015, Gao and Oba wanted to evaluate a noninvasive measurement of identifying whether a cow was at high or low risk for SARA (Gao and Oba, 2015). They used milk urea nitrogen and milk fat content of cows of late lactation cows. If the cows had lower milk urea nitrogen (MUN) and milk fat content, they would be considered at higher risk for SARA compared to cows on the same diet with higher MUN and milk fat content. To test this hypothesis, they had 35 late lactation cows on a high grain diet for 21 days. From milk samples taken the last 3 days, the top 5 cows with the highest MUN and milk fat content were presumed to be low risk of SARA, while the bottom 5 cows with the lowest

MUN and milk fat content were presumed to be high risk of SARA. These 10 animals were cannulated and when they were in midlactation, they were fed the same high grain diet as they were fed during their previous late lactation, and their pH was evaluated. Interestingly, the low risk of SARA group had a significant less amount of time where the pH was below 5.8 (52.5 v 395; $P = 0.04$). This could be a useful tool to use when enrolling cows on different research studies. It would be good to have an balanced mix of both high and low risk cows to obtain a more accurate representation of the population.

Finally, this sensitivity that cows have to carbohydrate fermentability needs to be evaluated in different management systems. The cows on this study were in a non-competitive feeding environment with their own personal feed bunks, stalls, and waterers. Through previous research on management and behavior of dairy cows, it is known that a perfect diet cannot compensate for imperfect management strategies. Cows that are in an overstocked situation where there is greater competition at the feed bunk and stalls have been shown to have lower rumen pH, less recumbent rumination, and more slug feeding. Whatever negative associative effects were observed from this study would likely be exacerbated at a higher stocking density or if there was limited access to the feed bunk. Time in the holding area and amount of feed push-ups per feeding would also increase negative effects, and the extent of these increases is necessary to understand as most dairy farms are overstocked and understaffed. These cows would likely have more time when the pH is below 5.8 and a greater degree of milk fat depression. It would be interesting to research if higher peuNDF240:RFS ratios would be able to mitigate

these negative effects. Is there a specific cut off in a diet where milk fat depression will occur, and will raising the peuNDF240 or uNDF240 help mitigated this effect?

Characterizing fiber in dairy cow diets has been a focus of research for over 6 decades. The data from this study gives greater evidence that combining the physical and chemical nature of fiber will provide a measurement that is closely related to dry matter intake and energy-corrected milk, as well as its potential ability to mitigate negative consequences of a fermentable diet. Although more information is needed on this measurement, peuNDF240 is proving to be a useful way to characterize fiber and improve ration balancing for high producing lactating cows.

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