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Relationships Between Undigested And Physically Effective Fiber In Lactating Dairy Cow Diets

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RELATIONSHIPS BETWEEN UNDIGESTED AND PHYSICALLY EFFECTIVE FIBER IN LACTATING DAIRY COW DIETS

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ABSTRACT

In recent years, economic, social, and environmental factors have encouraged higher forage diets to be fed to dairy cows. Consequently, a better understanding of both the chemical and physical properties of dietary forage fiber is needed. Undigested neutral detergent fiber after 240 hours of fermentation (uNDF240) is the fiber residue remaining after 240 hours of in vitro fermentation and has only recently been defined. Physically effective neutral detergent fiber (peNDF) was defined about two decades ago and is the fraction of dietary fiber with a particle size (i.e., ≥1.18-mm screen) that stimulates chewing behavior, forms the rumen digesta mat, and is resistant to passage from the rumen. To-date, the relationship between these two dietary fiber measurements has not been evaluated. The overall goal of this thesis research was to quantitate the relationship between dietary uNDF240 and peNDF on feed intake, lactational performance, chewing behavior, and the ruminal environment of lactating Holstein dairy cows.

The focal study (Chapter 2) investigated the effects of dietary uNDF240 (low or high) and peNDF (low or high) on lactating dairy cows. The four treatments were: 1) low uNDF240, low peNDF (8.8%, 20.1%; LULP; 2) low uNDF240, high peNDF (8.9%, 21.8%; LUHP); 3) high uNDF240, low peNDF (11.4%, 18.6%; HULP); and 4) high uNDF240, high peNDF (11.6%, 22.0%; HUHP). Additionally, a new descriptive term, physically effective uNDF240 (peuNDF240) was calculated as the product of the dietary physical effectiveness factor (pef; % of particles retained on ≥1.18-mm screen with dry sieving) and uNDF240 as a percentage of dry matter (DM). This new descriptive term aimed to integrate the effects of dietary particle size and NDF (in)digestibility. The dietary peuNDF240 concentrations were 5.4% (LULP), 5.8% (LUHP), 5.9% (HULP), and 7.1% (HUHP). The LULP treatment resulted in greater dry matter intake (DMI) and energy corrected milk (ECM), as well as more favorable chewing behavior (i.e., no effect on rumination but less time spent eating) in comparison to the HUHP diet. When comparing the same two treatments, total volatile fatty acid concentration was greater, mean ruminal pH was lower, and NDF turnover rate tended to be greater for the LULP treatment. Milk fat percentage was influenced by dietary uNDF240 with the high uNDF240 diets having an elevated percentage. The LUHP and HULP treatments often did not differ in animal response variables, such as DMI, ECM, mean ruminal pH, and chewing behavior, reflecting their similar dietary peuNDF240 concentration. Importantly, by reducing peNDF of the high uNDF240 treatments, DMI increased to an amount similar to the low uNDF240 treatments.

Animal responses were consistently different between the LULP and HUHP treatments as expected: the low uNDF240 diet, chopped more finely, encouraged greater DMI than the high uNDF240 diet chopped coarsely. However, the LUHP and HULP diets with similar peuNDF240 often resulted in similar cow responses, even though the peuNDF240 was obtained differently for each diet. With these diets fed to high-producing cows, it appears that the integration of particle size and indigestibility of fiber using a peuNDF240 measurement is highly related to DMI, ECM yield, chewing behavior, and ruminal environment. In the future, this relationship may prove useful in predicting DMI of lactating dairy cows fed a range of diets differing in uNDF240 and particle size.

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CHAPTER 1: LITERATURE REVIEW

1.1. Introduction

Fiber has been a focal point of ruminant nutrition research for decades. One essential goal of this research has been to optimize dietary fiber with a wide range of forage and non-forage ingredients that vary in their inclusion rates in diets. In recent years, there has been a large push within the US to feed higher forage diets due to economic, social, and environmental reasons (Martin et al., 2017). At times, forage availability may be restricted due to unfavorable weather conditions or other reasons prompting greater use of non-forage fiber sources. Regardless of whether the ration contains lower or higher amounts of forage, there is a continuing need to better understand how dietary fiber – both its chemical and physical properties - influences feed intake and chewing behavior, rumen dynamics, and lactational performance of dairy cattle.

1.2. Characterizing Neutral Detergent Fiber

Prevailing perspectives of fiber nutrition to-date have been largely influenced by the initial breakthroughs by Peter Van Soest revolving around measuring carbohydrates in the 1960s and 1970s. Van Soest (1967) developed a chemical fractionation system of forage or feed dry matter that, for the first time, had nutritional relevance. This system transformed the field, providing the ability to chemically analyze feeds and formulate diets that would elicit predictable animal responses in dry matter intake (DMI) and milk production.

In the analytical method of Van Soest (1967), forage or feed dry matter is separated into two fractions based on solubility in neutral detergent solution. The first dry matter fraction of the feedstuff is primarily the cellular contents of the forage, or neutral detergent solubles (NDS): lipids, soluble carbohydrates, most proteins, and other water soluble constituents. This fraction is considered to be essentially 98% digestible for many common forages and feeds (Van Soest, 1994). The second fraction, assayed as neutral detergent fiber (NDF), contained primarily cell wall constituents: cellulose, hemicellulose, and lignin. Unlike the soluble fraction, NDF was found to have variable rumen digestibility – but, it was predictable and(or) directly measurable (Van Soest, 1967; Van Soest, 1994). Using a summative approach, the digestible dry matter content of a forage or feed could be estimated by adding together the digestible NDS and the digestible NDF with a correction for metabolic losses (Goering and Van Soest, 1970).

Neutral detergent fiber became the most common measure of dietary fiber because of its relationship with the slowly fermenting and bulky portion of the plant cell wall that has the potential to fill the rumen and limit feed intake. David Mertens at the USDA-ARS Dairy Forage Research Center in Madison, WI developed a NDF-intake feeding system for ration formulation in the 1980's that is widely used today (summarized in Mertens, 2009). This system rests on the observation that there is an optimal concentration of ration NDF where forage intake is maximized without hindering 4% fat-corrected milk yield.

Figure 1.1 illustrates the relationship between NDF intake and 4% fat-corrected

milk yield (adapted from Mertens, 2009). There is a linear response in NDF intake as ration NDF content increases, but with a curvilinear response of fat-corrected milk yield to ration NDF content.

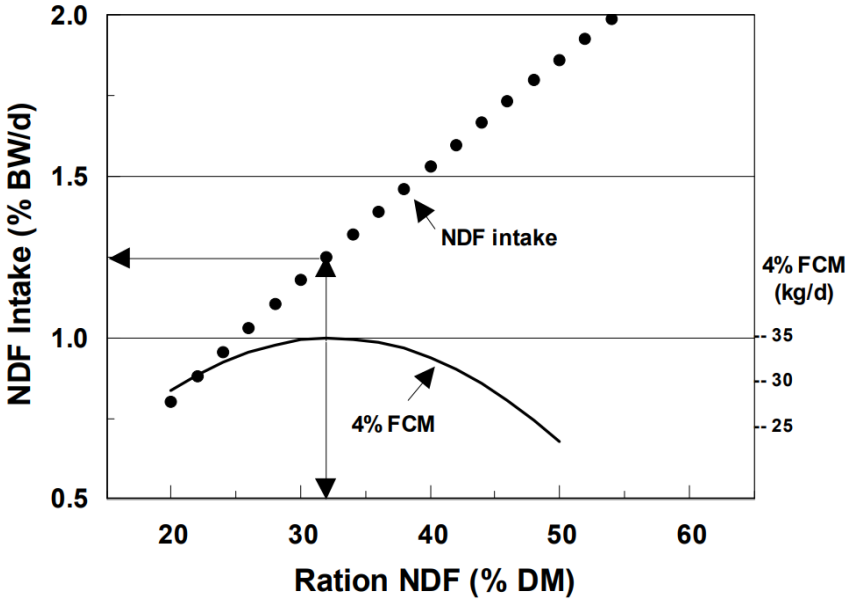


Figure 1.1. Relationship between production of 4% fat-corrected milk (FCM) and neutral detergent fiber (NDF) intake (illustration adapted from Mertens, 2009). Ration NDF is expressed as % of dry matter (DM) and NDF intake as a % of body weight (BW) per day (d).

For a high producing dairy cow, about 32% of the ration dry matter, or 1.2% of the animal's body weight, is the target concentration of NDF to maximize both milk output and forage inclusion in the diet (Mertens, 2009). Below the optimal dietary NDF inclusion level, lower milk yield reflects a diet lacking in fiber where rumen health and chewing behavior may be compromised. Above this optimal point, 4% fat-corrected milk declines due to a reduction in dry matter intake associated with the rumen filling effect of NDF (Mertens, 2009). This system provides flexibility in ration formulation

because it predicts animal performance when diets contain either an optimal, high, or low concentration of NDF relative to intake and milk production. This flexibility is important because the desired dietary NDF for any dairy herd will be influenced by several factors including regional feed ingredient availability and cost as well as on-farm forage availability and inventory.

Although the NDF-intake system has been widely used in the US, NDF alone does not account for all of the variability observed in dry matter intake and milk yield. Mertens (2009) recognized this and acknowledged that particle size and digestibility of the NDF in the diet have the potential to substantially influence animal response.

1.3. Physically Effective Neutral Detergent Fiber

While the development of the detergent analysis system was transformational for dairy cattle nutrition, a significant limitation of the NDF measurement and its use in ration formulation is that the simple summative approach (i.e., digestible NDF + digestible NDS) does not account for the physical characteristics of the fiber (Van Soest, 1994).

Mertens (1997) incorporated particle size of fiber into the NDF system to account for some of the variability observed in animal performance that was not explained by dietary or forage NDF content alone. The physically effective NDF (peNDF) system allowed for both the total chemical fiber (i.e., NDF) and particle size of fiber to be characterized and integrated into one number (Mertens, 1997). With this measurement the understanding of fiber in dairy cow diets expanded beyond simple chemical fractionation of the plant cell wall.

Physically effective NDF is calculated specifically by multiplying NDF content by a particle size measurement. The NDF concentration of the feedstuff or diet is expressed as percentage of the dry matter (theoretical scale from 0 to 100). The physical effectiveness factor (pef) refers to the fraction of particles that are retained on the 1.18-mm screen or greater when dry sieved (theoretical scale from 0 to 1). This fraction of forage particles was determined to be resistant to passage from the rumen, requiring rumination for passage, and are the longer and buoyant particles that form the rumen digesta mat (Poppi et al., 1985; Mertens, 1997). An as-fed, on-farm pef value can also be determined using the Penn State Particle Separator (PSPS) adapted with a 4-mm sieve that provides pef values similar to the standard dry sieving method (Cotanch et al., 2010). The Penn State Particle Separator is the primary tool used to evaluate silage and total mixed ration particle size distributions in North America and throughout the world (Kononoff et al., 2003).

In Mertens' original peNDF publication, a summary of all of the relevant particle size research was compiled into one data set. From these data, Mertens (1997) developed correlations between peNDF and the chewing behavior of dairy cows. Using regression analysis, a $r^2 = 0.76$ was found between peNDF and total chewing behavior (i.e., rumination and eating). Building on this concept, Mertens (1997) theorized that rumen pH was reflective of the animal's chewing behavior and tied to salivary buffer secretion. Mertens (1997) established that dietary peNDF content and rumen pH had a positive relationship with $r^2 = 0.71$. It was also determined that, in order to maintain a rumen pH of 6.0, the dietary peNDF concentration needed to be approximately 22% of

the ration dry matter. Finally, it was determined that milk fat percentage was related to dietary peNDF concentration with a $r^2 = 0.63$ and that, in order to maintain a 3.4% milk fat, a peNDF concentration of approximately 20% of the dietary dry matter was required.

Since Mertens (1997), several meta-analyses have been published that assessed the effect of peNDF on various cow responses. Most notably, Zebeli and co-workers conducted a series of meta-analyses that support the importance of peNDF in maintaining an optimal rumen environment and production of fat-corrected milk (e.g., Zebeli et al., 2006; Zebeli et al., 2008). However, within their data base several different methods were used to characterize peNDF, some of which do not agree with the dry sieving method that underpins the original peNDF system, and this confounded their conclusions relative to recommended peNDF percentages for ration formulation. Specifically, methods such as a 2-sieve Penn State Particle Separator (19- and 8-mm sieves), a 3-sieve Penn State Particle Separator (19-, 8-, and 1.18-mm sieves), and a wet oscillating sieve system with a 1.18-mm sieve were used to determine the pef of diets and were all included in the data base on an equal basis. Due to the inconsistent measuring of pef, a recommended peNDF value of 30 to 33% of the ration dry matter was determined by Zebeli et al. (2008). Although this value is reflective of the data summarized, it is much inflated over the original value of 20 to 22% determined by Mertens (1997). The primary reason for this discrepancy is that wet or as-fed forage particles do not pass through a 1.18-mm sieve as dry particles would, and so the pef value becomes inflated and biologically meaningless (Grant and Cotanch, 2005).

Although these recent meta-analyses have limitations, the authors were able to extract critical information relating dietary peNDF with subacute rumen acidosis (SARA) and immune status (Gozho et al., 2005; Khafipour et al., 2006; Zebeli et al., 2006; Zebeli et al., 2008).

1.3.1. Effect of Dietary peNDF on Chewing Behavior

Since the development of the peNDF system, subsequent research has evaluated how peNDF affects chewing behavior in dairy cows. A key realization has been that altering the particle size of the diet dramatically affects the time required by the dairy cow to consume forage and feed particles (Allen and Grant, 2000; Yansari et al., 2004; Jiang et al., 2017). These studies found that greater dietary peNDF (>21% of dry matter) increased eating time, on average, by 13 minutes for each percentage increase, whether resulting from more forage, longer chop length, or substitution of forage for non-forage NDF. When increasing peNDF, the amount of larger particles in the diet increased which required more time for the animal to consume them.

Along with chewing during eating, peNDF also affects ruminative chewing and ruminating time (Allen and Grant, 2000; Beauchemin et al., 2003; Yansari et al., 2004; Jiang et al., 2017). Increasing peNDF in the diet resulted in an increase in total rumination minutes or rumination minutes per kilogram consumed, although often to a lesser extent than ingestive chewing (Jiang et al., 2017). For the four studies (Allen and Grant, 2000; Beauchemin et al., 2003; Yansari et al., 2004; Jiang et al., 2017), total rumination minutes increased by 26 minutes for each percentage increase in peNDF. This elevation in rumination time is presumably driven by swallowed particles that are

still sufficiently long to require particle size reduction prior to passage from the rumen. However, research (Schadt et al., 2012) suggested that chewing during eating controls the size of particles delivered to the rumen, at least for silage-based diets, and the impact of peNDF on eating time should not be over looked.

To understand why peNDF influences eating time, it is important to differentiate eating (strictly initial mastication) and rumination activity. Schadt et al. (2012) delved deeper into initial mastication and measured the size of the particles entering the rumen from the esophagus. They found that although the beginning particle size of dry forages, silages, or total mixed rations may be different in particle length, the particle size of the swallowed bolus was rather consistent. A mean particle size of approximately 10 mm was observed for dry forages, silages, and total mixed rations fed to dairy cattle.

With this information, the difference in eating time among forages or diets of varying peNDF can be explained as the difference in time it takes the dairy cow to reduce the particle size in order to form an ensalivated bolus and swallow it. This concept is rather important because altering dietary particle size may influence eating time without necessarily influencing rumination time. For example, Tayyab et al. (2018) observed a difference in eating time expressed as minutes per kilogram of DMI but identified no difference in rumination minutes per kilogram of DMI when comparing forages of different chop lengths. This research supports the concept that dietary particle size alters eating time, but may have little or no effect on rumination since the particles delivered to the rumen are of a relatively consistent particle size.

1.3.2. Effect of Dietary peNDF on Rumen pH and Fermentation

Several studies have shown that dietary peNDF content is related to chewing behavior and consequently rumen pH (Mertens, 1997; Allen and Grant, 2000; Beauchemin et al., 2003; Yansari et al., 2004) although reductions in peNDF do not always reduce rumen pH (Farmer et al., 2014). It is clear that peNDF has the ability to alter rumen pH, but the degree of change is not well understood. At times, feeding below the recommended peNDF content as originally defined by Mertens (1997) will result in a rumen pH lower than 6, but in other cases it may not lower rumen pH to that extent. Stone (2004) investigated the relationship between dietary peNDF and rumen pH with a focus on subacute ruminal acidosis (SARA), defined as the time ruminal pH is less than 5.8. Stone (2004) described the situation of adjusting dietary NDF and peNDF to avoid harmful SARA conditions in the rumen as a "balancing act." The table below, adapted from Stone (2004), depicts the balance between high, low, and marginal risk of SARA in relation to dietary peNDF based on his summary of published research.

Table 1.1. Nutritional relationships associated with subacute ruminal acidosis (adapted from Stone, 2004).

Risk of SARA	Increased	Marginal	Low
NDF, % of DM	25	28 to 32	35
Forage NDF, % of DM	16	20 to 25	27
peNDF, % of DM	18	21 to 23	25
Mean ruminal pH	< 5.6	5.8 to 6.1	> 6.4

Zebeli et al. (2008) concluded that 3 to 5 h/d below pH of 5.8 resulted in an increased concern for negative rumen health consequences associated with SARA. It is

critical to understand that the duration and severity of low rumen pH needs to be taken into consideration. Subacute rumen acidosis conditions can be harmful to cellulolytic bacteria which in turn can negatively influence fiber digestion, DMI, milk production, and milk composition (Khafipour et al., 2009). Reducing the risk of SARA can be achieved by feeding at least 21% peNDF in the diet (Stone, 2004).

Similar to changes in rumen pH, researchers have measured responses in rumen volatile fatty acid (VFA) concentrations and ratios as dietary peNDF varied. Yansari et al. (2004) found that increasing the physically effective portion of the diet resulted in a 6.8% decrease in total VFA and 2.4% decrease in propionate concentration, whereas acetate concentration increased by 2.9% leading to a higher acetate-to-propionate ratio. Farmer et al. (2014) observed that reducing peNDF tended to increase total VFA concentration in the rumen reflecting an increase in fermentability of the carbohydrates in the lower peNDF diets that included non-forage sources of NDF in place of forage NDF.

1.3.3. Effect of Dietary peNDF on Rumen Fiber Dynamics and Total Tract Digestibility

Using cannulated cows, rumen volume, mass of organic matter and NDF, and digesta density have been measured in response to varying dietary peNDF (Allen and Grant, 2000; Yang et al., 2002). In these studies, shorter forage particle size reduced the NDF pool size in the rumen reflecting greater turnover. Farmer et al. (2014) found no effect of dietary peNDF on rumen NDF pool size but ruminal NDF turnover rate increased when reducing peNDF amounts. When greater rumen turnover of NDF is

observed in response to lower peNDF, it reflects greater passage of NDF out of the rumen and(or) greater NDF fermentability presumably associated with a larger difference in surface area for microbial attachment.

Total tract NDF digestibility is typically correlated positively with rumen NDF digestibility since the majority of NDF digestion occurs within the rumen and not post-ruminally (Yansari et al., 2004; Farmer et al., 2014). Reducing dietary peNDF resulted in a reduction in total tract digestibility of dry matter, organic matter, and NDF (Farmer et al., 2014). These observed changes in digestibility coincide with what is expected because a reduction in particle size allows for a shortened rumen retention time and less NDF fermentation.

1.3.4. Effect of Dietary peNDF on Dry Matter Intake and Lactation Performance

Variations in rumen retention time and total tract NDF digestion directly influence dry matter intake (Allen and Grant, 2000; Yansari et al., 2004; Farmer et al., 2014). When dietary peNDF decreases by 2 to 5%, DMI increases on average by 12% (Allen and Grant, 2000; Yansari et al., 2004; Farmer et al., 2014). This increase in DMI can be attributed to shorter rumen fiber retention times as previously discussed.

While DMI often changes, milk yield remains unaffected by the change in peNDF in many studies (Beauchemin et al., 2003; Yansari et al., 2004; Farmer et al., 2014). By decreasing peNDF, intake increases, but fiber utilization decreases due to the reduction in fiber total tract digestibility yielding similar milk production. Combining the change in intake with the lack of milk yield response, a reduction in production efficiency is often observed (i.e., milk/DMI).

One of the most consistent effects of dietary peNDF is on milk fat content and output. Mertens (1997) related peNDF and chewing behavior to maintaining milk fat percentages and determined that a ration peNDF concentration of at least 20% was required to maintain milk fat percentage above 3.4%. Similarly, Yansari et al. (2004) found that decreasing dietary peNDF decreased milk fat percentage. In these studies, the decreases in milk fat output reflected the reductions in chewing behavior and ruminal pH.

Building upon this, Woolpert et al. (2017) identified nutrition and on-farm management variables that most significantly influenced the fatty acid profile of milk. Herds with higher *de novo* milk fatty acid content, and overall greater milk fat output, were fed rations with more dietary peNDF compared with those herds that had lower *de novo* fatty acids and milk fat production. In addition, the cows in these herds were fed less dietary ether extract, had lower feed bunk and free stall stocking density, and were fed twice versus once per day (Woolpert et al., 2017). *De novo* fatty acids are associated with milk fat percentage and reflect the rumen environment since the building blocks of these short-chain fatty acids are acetate and butyrate produced in the rumen from fiber digestion (Barbano et al., 2014). Elevated *de novo* fatty acid concentrations and greater milk fat output can be achieved by feeding greater dietary peNDF (Woolpert et al., 2017).

1.3.5. Summary of peNDF and Its Limitations

It is clear that altering peNDF concentration within the diet will impact the dairy cow. It is important to understand that there are several assumptions made when using

peNDF. Mertens (1997) explained this and highlighted that it is assumed that NDF is uniformly distributed across all particles. It is also assumed that the chewing activity is equal for all particles that are retained on a 1.18-mm sieve. Another important assumption is that particle fragility does not differ among sources of NDF. This means that all fiber particles will break down upon mastication in a similar manner and at a similar rate. Although these assumptions may be true at times, there are important feeding situations where they are not true.

The peNDF system does not explain all of the variation observed in chewing behavior, rumen pH, and dry matter intake attributable to the fiber fraction in dairy cow diets. It is focused mainly on the physical aspect of NDF and does not take into account the digestibility of the fiber.

1.4. Undigested Neutral Detergent Fiber Background

Understanding the physical aspects of NDF is crucial, but understanding the digestibility characteristics of NDF is also necessary in order to accurately predict cow response to NDF. Waldo et al. (1972) first recognized that NDF could be fractionated into potentially digestible and indigestible fractions. Lignin concentration in fiber influenced the extent of *in vivo* ruminant digestion (i.e., an indigestible NDF residue), with variable effects on the rate of digestion of the potentially digestible fraction (Van Soest, 1994). The recognition that an indigestible NDF fraction existed and could be measured *in vitro* with long-term fermentations was a major breakthrough. The resulting potentially digestible fraction followed first-order digestion kinetics, and rates of NDF digestion could be calculated for the first time, and identifying this relationship

set the stage for development of dynamic fiber digestion models (Mertens and Ely, 1979).

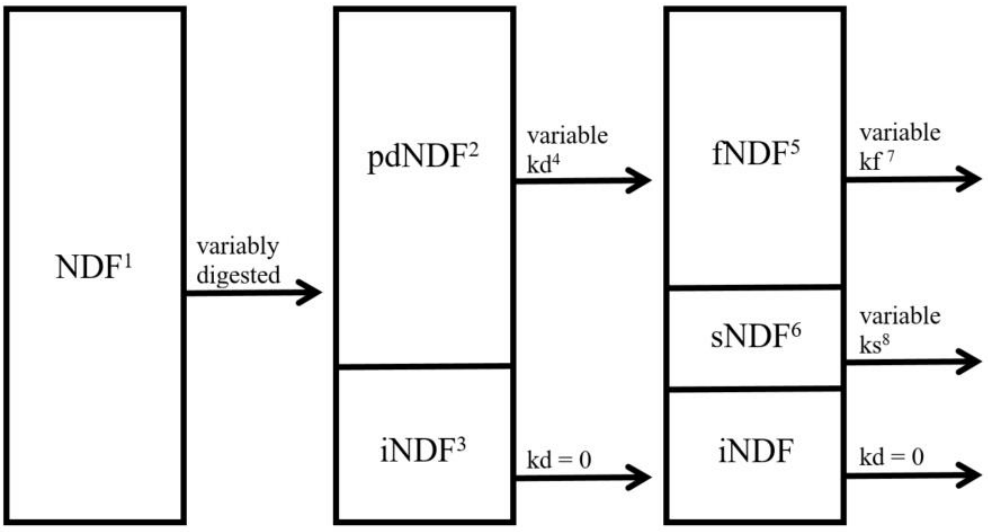
The indigestible fraction of NDF comprises cellulose and the hemicellulose that is cross-linked with lignin that will not digest in the rumen, theoretically even with infinite fermentation time (Van Soest, 1994). Indigestible NDF (iNDF) as described in the published literature refers to an endpoint of fermentation (such as 72, 96, 120, or 240 hours) although it is truly a theoretical value that has meaning only within the context of a specific model of rumen fiber digestion (Mertens, 1977). Consequently, Mertens (2013) coined the term undigested NDF (uNDF) when referring to the laboratory measure of the remaining NDF residue at a specified fermentation length, as opposed to iNDF which is a more theoretical number related to infinite fermentation time.

Recently, several fermentation time points have been explored as the potential time required to obtain a NDF residue amount representative of iNDF. The objective of this research has been to identify a fermentation time point where the potentially digestible fraction is depleted and the remaining NDF residue does not change significantly with additional hours of fermentation (Raffrenato et al., 2018). Raffrenato and Van Amburgh (2010) and Raffrenato et al. (2018) determined that 240 hours of fermentation in an *in vitro* system consistently yielded a value representative of iNDF. Similarly, European researchers have found that 288 hours of *in situ* fermentation results in similar values for uNDF (Krizsan et al., 2012). Utilizing the *in vitro* 240-hour method, Mertens (2016) theorized that the remaining NDF residue was truly

indigestible in the rumen environment and could be fractionated from a potentially digestible NDF (pdNDF). Current nutrition models such as the Cornell Net Carbohydrate Protein System Model (CNCPS, Van Amburgh et al., 2015) utilize this measure of uNDF240 and potentially digestible NDF to predict ruminal NDF digestion.

Focusing on the potentially digestible NDF fraction, Raffrenato and Van Amburgh (2010) and Raffrenato et al. (2018) divided the potentially digestible pool into two components. Using multiple time points of fermentation, it was determined that within the pdNDF portion there are fast and slow digesting pools. Mertens (1977) and Mertens and Ely (1979) had originally hypothesized that the pdNDF consisted of a fast and slow digesting pool, and this work by Raffrenato and Van Amburgh (2010) confirmed it. The fast and slow pools are determined and modeled by measuring the undigested NDF residue remaining after 30, 120, and 240 hours of fermentation for forages. Due to differences in digestibility characteristics of non-forage fiber sources (NFFS), uNDF values are measured after 12, 72, and 120 hours of fermentation (Zontini et al., 2015). Very immature forages, such as pasture grasses, as well as the NFFS have neither the maturity or the capacity to develop crosslinking between hemicellulose and lignin, and consequently NDF digestion proceeds more rapidly and to a much greater extent (Raffrenato et al., 2018).

Figure 1.2 illustrates the evolution of the NDF digestion model moving from initial NDF fractionation of Van Soest (1967) to the 2-pool model of Waldo et al. (1972), and finally to a 3-pool NDF digestion model that represents the current state of modeling rumen fiber turnover.



[1] Neutral detergent fiber.
[2] Potentially digestible neutral detergent fiber.
[3] Indigestible neutral detergent fiber.
[4] Rate of digestion.
[5] Fast-pool neutral detergent fiber.
[6] Slow-pool neutral detergent fiber.
[7] Digestion rate of fast-pool neutral detergent fiber.
[8] Digestion rate of slow-pool neutral detergent fiber.

Figure 1.2. Progressive development of models of rumen fiber digestion (illustration adapted from Mertens, 2016).

Although current fiber nutrition models do not include a fast and slow pool of NDF, future models such as CNCPS version 7.0 will presumably account for these fiber fractions (Higgs and Van Amburgh, 2016). While fiber models are becoming more dynamic and ration formulation is incorporating additional digestion measurements such as uNDF240 and fast or slow NDF digestion rates, uNDF240 as a stand-alone measure remains a critical quality descriptor of NDF in dairy cow diets. As a measure of fiber indigestibility, uNDF240 is correlated to the rumen filling effect of NDF and limitations on dry matter intake (Mertens, 2016). Due to uNDF240 as a routine measure still being in its infancy, knowledge and research are limited. Further research is needed to fully understand uNDF240 in ration formulation and as an on-farm benchmark.

1.4.1. Effect of uNDF240 on Rumen Fill and Dry Matter Intake

Grant and Cotanch (2017) described uNDF240 as the functional fraction of fiber that influences gut fill, digestion and passage dynamics, and the physical effectiveness of forages. Gut fill references rumen fill of fiber and the ruminal uNDF240 load. Grant and Cotanch (2017) explained that rumen fiber fill is the result of fast-pool NDF, slowly fermenting NDF, and uNDF240. Due to the limited volume of the rumen, increasing uNDF240 reduces the rumen volume available for potentially digestible NDF. With this in mind, a maximum load of uNDF240 within the rumen is possible. Weakley (2011) theorized that there may be an optimal mass of digesting NDF within the rumen. Exceeding that amount may reduce intake via gut fill, but feeding below the amount may increase dry matter intake at the expense of feed efficiency. Cotanch et al. (2014) found the rumen mass of uNDF240 to range from 0.48 to 0.62% of body weight across a range of diets based primarily on corn silage and haycrop silage. As ruminal uNDF240 mass increases, the rumen fiber dynamics adjust reflecting this. Specifically, ruminal turnover rate decreases, time in the rumen increases, and dry matter intake is restricted.

Grant and Cotanch (2012) found that feeding a lower uNDF240 diet reduced the ruminal digesta volume by 15 L and mass by 14 kg, and simultaneously decreased the NDF pool size of the rumen by 0.8 kg. Additionally, turnover rate and mean retention time of the NDF pool was lower and longer for the higher uNDF240 conventional corn silage, high forage treatment. Ruminal turnover of the indigestible fraction occurs by passage only and it will be retained in the rumen for an extended length of time in

comparison to the potentially digestible fraction (Harper and McNeill, 2015). Decreasing turnover rate and increasing retention time hinders DMI (Harper and McNeill, 2015). Grant and Cotanch (2017) elaborated on the shift in DMI, explaining that the rumen space is a result of the potentially digestible fiber fraction digesting and escaping the rumen. By increasing dietary uNDF240, the potentially digestible fraction decreases, reducing DMI (Grant and Cotanch, 2012; Fustini et al., 2017). Intake of uNDF240 has ranged from 0.3 to 0.4% of body weight in lactating Holstein cows fed primarily silage-based rations (Cotanch et al., 2014). For corn silage-based diets, it appears that uNDF240 intake of approximately 0.4% of body weight is the maximum as it reflects 0.62% uNDF240 in the rumen. In general, Cotanch et al. (2014) found a consistent ratio between rumen and intake uNDF240 of approximately 1.6 kg/kg. Fustini et al. (2017) found a maximum uNDF240 intake for alfalfa-based diets to be slightly higher at 0.48% of body weight. Source of dietary uNDF240 is important. Cows fed the alfalfa-based diets consumed a greater amount of uNDF240, reflecting legume plant structure and passage characteristics that differ markedly from grasses such as corn silage and haycrop silages.

1.4.2. Effect of uNDF240 on Total Tract Digestibility

Reflecting the impact on DMI, total tract digestibility of the dietary fiber fractions shifts with the change in dietary uNDF240. Higher uNDF240 reflects a more highly lignified plant cell wall and so it is expected that increasing uNDF240 of the diet would decrease total tract digestibility of the fiber. Recently, Fustini et al. (2017) found that decreasing the uNDF240 concentration in alfalfa-based diets allowed for greater

digestion of the NDF and pdNDF fractions.

1.4.3. Effect of uNDF240 on Chewing Behavior and Rumen pH

Corresponding with the shift in fiber turnover, chewing behavior changes in response to varying dietary uNDF240. Increasing forage content and uNDF240 elicited greater eating time (Grant and Cotanch, 2017). Similarly, total rumination minutes increased with higher forage and uNDF240 diets (Grant and Cotanch, 2017). Fustini et al. (2017) found no difference in total rumination minutes but an increase in rumination minutes per kilogram of uNDF240 consumed as dietary uNDF240 content increased. The increase in rumination behavior is reflective of the reduction in fiber capable of escaping the rumen from both digestion and size reduction. As uNDF240 increases, a greater proportion of the fiber is more reliant upon chewing behavior for passage.

Rumen pH varied with changing dietary uNDF240, reflecting the shift in chewing behavior. Fustini et al. (2017) observed a tendency for mean pH to increase with greater dietary uNDF240 concentration. The elevated rumen pH reflected the increase in chewing behavior and presumably secretion of salivary buffer.

1.4.4. Effect of uNDF240 on Milk Yield and Composition

As the result of the changes in DMI and ruminal environment, uNDF240 influences milk yield and composition. Although Fustini et al. (2017) observed no difference in milk yield to uNDF240, Kokko et al. (2012) found milk yield to increase by 4.1 kg with lower dietary uNDF240 content of high forage brown midrib corn silage treatment in comparison to a high forage conventional corn silage treatment. Additionally, milk fat percentage increased when feeding higher uNDF240

concentrations using high forage diets and conventional corn silage (0.26% increase; Kokko et al., 2012) and high uNDF240 treatments (0.08% increase; Fustini et al., 2017). Greater milk fat percentage reflected the elevated rumen pH and fermentation of fiber (Allen, 1997). While milk fat percentage decreased when feeding lower uNDF240, milk protein percentage increased (0.18%, Kokko et al., 2012; 0.02%, Fustini et al., 2017). The increase in milk protein percentage was likely a result of greater microbial protein yield linked to more fermentable fiber (Van Soest, 1994). Reflecting the changes in milk yield and composition, fat-corrected milk yield tended to increase with decreasing dietary uNDF240 (Kokko et al., 2012; Fustini et al., 2017).

1.5. Potential Relationships Between uNDF240 and peNDF

Undoubtedly, altering the dietary uNDF240 (and associated changes in fast and slow digesting NDF) concentration impacts intake, digestibility, and performance of the lactating dairy cow. But the indigestibility, or digestibility, of the fiber appears to also influence how a cow will respond to forage of a given particle size. In other words, the digestibility of the fiber also influences the characteristics of the physical effectiveness of the fiber. Fiber fragility reflects the impact that digestibility has on particle size and the corresponding chewing responses. It has commonly been measured in the laboratory as grinding energy or time to reduce particle size with a specific mill, such as ball milling (Grant, 2010). Within the peNDF system, it is assumed that all fiber particles elicit identical chewing responses and break down during mastication in a similar fashion (Mertens, 1997). While this is true at times, it is not always the case. Chenost (1966), Winter and Collins (1987), and Grant (2010) determined that chewing

responses may vary and that digestibility of the fiber is correlated with fiber fragility. As fiber digestibility decreased, particle fragility declined.

One new attempt at incorporating fragility/digestibility with peNDF has been the development of physically adjusted neutral detergent fiber (paNDF) (White et al., 2017). This approach aims to more accurately model physical and chemical characteristics of fiber that influence rumen pH. In the paNDF system, significant factors when predicting rumen pH include NDF and its digestibility, starch and its digestibility, particle size as assessed using the Penn State Particle Separator, and fragility. The acid detergent fiber (ADF) to NDF ratio is used as a simple proxy for fragility because it reflects the grass-to-legume ratio and therefore is sensitive to differences between grasses and legumes and anatomy and chemical composition that affect fragility. Although incorporation of paNDF into nutrition models will aid in the understanding of NDF and rumen pH, it is not a value that can be assigned to a feedstuff or diet and consequently of little value for ration formulation directly. Going forward, peNDF and uNDF240 are likely to remain the prevailing measures of fiber physical form and indigestibility at least in the short-term.

1.6. Research Hypotheses and Objectives

When evaluating peNDF and uNDF240 it becomes apparent that they share response variables. A significant amount of data have been collected on peNDF with a minimal amount focused on uNDF240. Additionally, virtually no research has concentrated on evaluating peNDF and uNDF240 together. As a result, the possible interaction of the two has become an important question for the dairy nutrition

community arising from increased forage diets and strained economic circumstances.

From the industry perspective there are several questions stemming from this relationship that are important. Some key questions include: how important is physical form if the digestibility pools are understood? Can the diet be adjusted for a lack of peNDF by supplementing uNDF240 in the diet? Are there optimal peNDF concentrations with varying uNDF240?

We hypothesize that low uNDF240 and low peNDF concentrations in the ration will result in greater amount of time when rumen pH < 5.8, less chewing per kilogram of NDF, and greater dry matter intake. We further hypothesize that high uNDF240 and high peNDF concentrations will result in less time when rumen pH < 5.8, more chewing per kilogram of NDF, and less dry matter intake. Finally, we hypothesized that treatments containing similar peuNDF240 concentrations will result in similar rumen pH, chewing behavior, and dry matter intake.

In order to address these questions the objective of this thesis was to evaluate the effect of feeding different dietary amounts of uNDF240 and physically effective NDF on ruminal fermentation, chewing behavior, and performance of lactating Holstein cows.

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CHAPTER 2: RELATIONSHIP BETWEEN UNDIGESTED AND PHYSICALLY EFFECTIVE FIBER IN LACTATING DAIRY COW DIETS

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2.1. ABSTRACT

The objective of this study was to evaluate the effect of feeding different dietary concentrations of 240-h undigested neutral detergent fiber (uNDF240) and physically effective NDF (peNDF) on dry matter intake (DMI), milk yield and composition, chewing behavior, ruminal pH, volatile fatty acid (VFA) concentrations, ruminal digesta turnover, and total tract digestibility. Sixteen Holstein cows, eight ruminally cannulated, averaging 123 (SD = 9) days in milk (DIM) were used in a replicated 4 x 4 Latin square design with 4-wk periods. Cows were fed diets formulated to differ only in uNDF240 and peNDF content by changing forage-to-concentrate ratio and particle length of timothy hay. Treatments were: 1) 8.8% uNDF240 and 20.1% peNDF (LULP), 2) 8.9% uNDF240 and 21.8% peNDF (LUHP), 3) 11.4% uNDF240 and 18.6% peNDF (HULP), and 4) 11.4% uNDF240 and 22.0% peNDF (HUHP). A new descriptive term, physically effective uNDF240 (peuNDF240), was calculated as the product of the dietary physical effectiveness factor (pef) and uNDF240 as a percentage of dry matter (DM) with the purpose of integrating particle size and digestibility of fiber. Dietary peuNDF240 concentrations were 5.4% (LULP), 5.8% (LUHP), 5.9% (HULP), and 7.1% (HUHP) of ration DM. Cows were housed in individual tie stalls, fed TMR once daily, and milked 3x/d. All data were analyzed as a replicated Latin square design using ANOVA and the MIXED procedure of SAS (version 9.4). Models included the fixed effects of diet, period, replicate, and time (as appropriate) and the random effect of cow within replicate. The DMI was greater for LULP (27.5 kg/d) compared to HUHP (24.9 kg/d) with similar DMI between LUHP (27.3 kg/d) and HULP (27.4 kg/d). Energy-

corrected milk (ECM) yield was similar for the LUHP (45.7 kg/d) and HULP (46.4 kg/d) treatments with greater yield for LULP (47.0 kg/d) compared to HUHP (44.6 kg/d). High uNDF240 treatments resulted in greater milk fat percentage than the low uNDF240 treatments. No treatment differences were observed for total rumination time, but total eating time was greater for HUHP (300 min/d) compared to the LULP (255 min/d) treatment. Daily mean ruminal pH was greater for the HUHP (6.24) treatment in comparison to the LULP (6.11) treatment, while the LUHP (6.17) and HULP (6.22) treatments were similar. Total VFA concentration was greater for LULP (122.8 m*M*) compared to HUHP (112.3 m*M*). Finally, total tract digestibility of DM was greater for HULP (69.5 %) compared to LUHP (65.6 %) with similar responses between LULP and HUHP. Overall, DMI, ECM yield, chewing behavior, and ruminal fermentation reflected changes in dietary peuNDF240 concentration.

Key words: Digestibility, ruminal digesta, particle size, undigested fiber

2.2. INTRODUCTION

Dairy cattle are highly dependent on microbial fermentation of fibrous plant material in the rumen for a large portion of their energy and nutrient supply. In recent years, feeding higher forage diets has been of interest due to economic, social, and environmental reasons (Martin et al., 2017). Due to the increased interest, recent research has focused on optimizing fiber in dairy cow diets and understanding how both the chemical and physical properties of fiber influence intake and lactation performance.

Detergent analysis of NDF was transformational for dairy cattle nutrition, but it does not account for the physical or digestibility characteristics of fiber (Van Soest, 1994). Mertens (1997) incorporated particle size of fiber into the NDF system with the development of physically effective NDF (peNDF). Physically effective fiber is defined as the fraction of dietary fiber with sufficient particle length to stimulate chewing (ruminative and eating) and create a well-formed rumen digesta mat (Mertens, 1997). Physically effective NDF is commonly measured as the portion of NDF that has a particle size greater than 1.18-mm (dry vertical sieve; Mertens, 1997) or 4-mm (Penn State Particle Separator; Cotanch et al., 2010). This measurement accounts for both the physical and chemical properties of the feedstuff. Optimizing the peNDF content and the digestible carbohydrates in the diet are critical for maintaining efficient rumen metabolism (Plaizier et al., 2008), supporting rumen and metabolic health of the cow (Zebeli et al., 2012), and promoting efficient milk and milk component production.

Development of undigested neutral detergent fiber after 240-hours of *in vitro*

fermentation (uNDF240om) has allowed for accurate laboratory measurement of indigestible NDF (iNDF; Raffrenato et al., 2018) for individual feedstuffs and total mixed rations. Using uNDF240om, the potentially digestible NDF (pdNDF) fraction of NDF can be calculated and ultimately the fractional rate of digestion for pdNDF (Waldo et al., 1972). Aside from its use in accurately determining pdNDF, uNDF240om has been shown to influence gut fill, digestion and passage dynamics, and the physical effectiveness of forages (Grant and Cotanch, 2017). Diets with greater uNDF240om content lead to a reduction in DMI associated with slower ruminal turnover of NDF (Cotanch et al., 2014) and lower output of milk and milk components (Kokko et al., 2012).

A better understanding of the relationship between uNDF240om and peNDF in lactating dairy cow diets will provide the information needed for more optimal diet formulation. Both dietary measurements are directly related to the passage of fiber and have been individually researched. But, virtually no research has been conducted evaluating both uNDF240om and peNDF in rations for lactating dairy cows and the effects on ruminal digesta turnover, chewing behavior, and DMI. It is possible that combining a measure of particle size such as physical effectiveness factor (% of particles ≥1.18-mm) with uNDF240om would allow better prediction of DMI.

The objective of this study was to evaluate the effect of feeding two different dietary concentrations of uNDF240om and peNDF on feed intake, lactational performance, chewing behavior, and the ruminal environment of lactating Holstein dairy cows. It was hypothesized that low uNDF240om and low peNDF concentrations

in the diet would result in a greater amount of time when ruminal pH < 5.8, less chewing per kg of DMI, and greater DMI compared to high uNDF240 and high peNDF diets. Additionally, it was hypothesized that treatments containing similar peuNDF240 concentrations would result in similar rumen pH, chewing behavior, and DMI.

2.3. MATERIALS AND METHODS

2.3.1. Experimental Design and Treatments

Design. The study was conducted at the William H. Miner Agricultural Research Institute (Chazy, NY) in the Charles J. Sniffen Dairy Research and Education Complex. All experimental procedures involving animals were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee (ACUC# 2017AUR02). Sixteen multiparous Holstein cows (8 ruminally cannulated) were used in a replicated 4 × 4 Latin square design study with 28-d periods (squares were conducted concurrently). The first 19 d served as the adaptation period and the last 9 d served as the collection period. At the start of the study, animals were blocked by fistulation status, days in milk (mean ± SD; 123 ± 9), milk production (53.0 ± 4.5 kg), and parity (2.4 ± 0.7).

Diets. Four diets were formulated to contain either a low or high concentration of uNDF240om and either a low or high concentration of physically effective NDF (peNDF). The different amounts of uNDF240om were achieved by varying the forage to concentrate ratio of the diet (Table 2.3). A hammer mill bale processor (Haybuster; DuraTech Industries International, Inc., Jamestown, ND) with two different sieve sets (7.62 and 5.08 cm; 1.27 and 0.95 cm) were used to achieve two particle sizes of dry

timothy hay. In addition, for the lower forage diets, timothy hay was replaced with 12.9% pelleted beet pulp to balance the fiber fractions. The four dietary treatments were: 1) low uNDF240om and low peNDF concentrations (**LULP**), 2) low uNDF240om and high peNDF concentrations (**LUHP**), 3) high uNDF240om and low peNDF concentrations (**HULP**), and 4) high uNDF240om and high peNDF concentrations (**HUHP**). Diets were formulated for high producing lactating Holstein cows using a commercial ration formulation platform (AMTS.Cattle.Professional, Agricultural Modeling & Training systems, LLC, Groton, NY; version 4.8) with CNCPS biology (v 6.5.5 Cornell University, Ithaca, NY). Inputs used for dietary formulation included 28.1 kg DMI, 52.2 kg milk with 3.60% fat and 3.05% true protein, and a 726 kg body weight (BW). The lower uNDF240 diets contained 46.8% forage and the higher uNDF240 diets contained 60.5% forage on a dry matter (DM) basis (Table 2.1).

Management. Cows were fed treatment total mixed rations for ad libitum intake (approximately 1.05 × expected intake) at 14:00 h once daily (Calan Data Ranger; American Calan, Inc., Northwood, NH). Cows were housed in tie-stalls equipped with individual feed boxes and water troughs. Cows were milked three times daily (04:30, 12:30, and 20:30 h) in a double-twelve parallel milking parlor (Xpressway Parallel Stall System; Bou-Matic, Madison, WI). Using Hobo data loggers (Onset, Bourne, MA), temperature and relative humidity was recorded at 15-min intervals during the study.

2.3.2. Data Collection, Sampling Procedures, and Analytical Methods

Body Weight and Body Condition Score. Body weight was measured (Allweigh

computerized scale; Allweigh Scale System Inc., Red Deer, AB, Canada) and body condition score (BCS) was assigned in 0.25-unit increments on a 1 to 5 scale (Ferguson et al., 1994) for each cow the day before the start of the study period and then on d 28 of each period by two individuals and the average was used for BCS.

Dry Matter Intake. Dry matter intake was determined by recording feed offered and refused on d 20 to 28 for each cow during each period. Samples of diets and corresponding orts were collected daily from d 20 to 28. Dry matter was determined by drying a portion of each sample in a forced-air oven at 105°C for 18 to 24 h.

Milk Yield and Composition. Milk yields were recorded electronically on d 20 to 26 of each period (ProVantage Information Management System; Bou-Matic, Madison, WI). Each period, milk samples for each cow were collected from six consecutive milkings on d 25 and 26. Fat, true protein, lactose, solids non-fat, urea nitrogen, and de novo, mixed, preformed fatty acids, and unsaturated double bonds were determined by mid-infrared procedures (CombiScope FTIR 300 Hp, Delta Instruments, Drachten, The Netherlands) for the milk samples. Somatic cell count was analyzed by flow cytometry (CombiScope FTIR 300 Hp, Delta Instruments, Drachten, The Netherlands). Milk samples were mathematically composited in proportion to milk yield at each sampling by day and averaged for the period after analysis. Somatic cell count was transformed and analyzed as somatic cell score (SCS) according to Shook (1993) using the equation: $SCS = \log_2(SCC/100) + 3$ where SCC is in units of 1,000 cells/mL. The 3.5% fat-corrected milk was calculated as 0.4324 × kg of milk + 16.216 × kg of fat (Hutjens, 2005). Solids-corrected milk 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); 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. Feed efficiency (kg/kg) was calculated and expressed as milk yield/dry matter intake, 3.5% fat-corrected milk/dry matter intake, solids-corrected milk/dry matter intake, and energy-corrected milk/dry matter intake for d 20 through d 26 of each test period.

Chewing Behavior. Chewing activity (eating, ruminating, or no chewing activity) and posture (standing, perching, or lying) were recorded every 5 min for three consecutive 24-h periods (d 23 at 14:00 h through d 25 at 13:59 h) for each period. While cows were out of the tie stall for milking, behavior and posture recording continued. By multiplying the total number of observations for each activity by 5 min, the total time in minutes for each activity for each day was calculated. Eating and ruminating bouts were determined with a 20-min inter-bout criterion, with new bouts established if the cow spent greater than 20 min performing another behavior before performing the same behavior (Black et al., 2016). Number of bouts and bout length were recorded.

Feed Ingredients and Diets. The DM of individual feed ingredients was determined by collecting samples on Monday, Wednesday and Friday weekly, and

drying in a forced-air oven at 105°C for 18 to 24 h. Diets were adjusted accordingly to the change in dry matter when a feed ingredient dry matter value was ± 1.2 standard deviations outside the range of the dry matter.

During the collection period, feed ingredients, diets, and corresponding orts were collected d 20 through d 28 and a portion of each sample was dried in a forced-air oven at 105°C for 18 to 24 h for dry matter determination. Equal volumes of each sample from collection day were frozen at -20°C and then composited by period. Diets and corresponding orts for d 20 through 22 were composited for total tract digestibility measurements. Diets were composited by treatment by period and orts were composited by cow by period. Feed ingredients for d 23 through 28 were composited for dietary composition. Period composites were stored at -20°C prior to analyses. From each period composite, a portion of each sample was analyzed for chemical composition (CPM Plus; Cumberland Valley Analytical Services, Inc., Waynesboro, PA). Particle size distribution on an as-fed basis, using the Penn State Particle Separator (modified with a 4-mm screen; Cotanch et al. 2010), was determined for each feed ingredient and diet using a portion of the period composite samples. Particle size distribution on a dry matter basis (55°C) was determined for each period composite sample by dry vertical sieving (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering, Inc., Mentor, OH). Physically effective NDF of the treatment diets was calculated as the product of its NDF content and its physically effective factor (pef; Mertens, 1997).

Amylase- and sodium sulfite-treated and ash-corrected neutral detergent fiber (aNDFom) was determined according to the procedure of Van Soest et al. (1991).

Using the Tilley-Terry rumen fermentation system (Raffrenato et al., 2018) undigested NDF for 30-, 120- and 240-h time points (uNDF30om, uNDF120om, uNDF240om) for composite samples was determined. The undigested NDF for 12-, 72-, and 120-h time points (uNDF12om, uNDF72om, uNDF120om) for grains and non-forage fiber sources was determined according to Zontini (2016). Fermentation analyses were performed on the ensiled forage composite samples (Cumberland Valley Analytical Services, Inc., Waynesboro, PA).

Rumen Evacuations and Analysis of Pool Size. Ruminal contents of the cannulated cows were evacuated manually through the ruminal cannula after daily feeding on d 27 and prior to the end of d 28. To ensure that cows experienced the same interval of time between rumen evacuations, cows were divided into two groups of four cows each. The first group was evacuated 3.5 h after feeding on d 27 (17:30 h) and 3.5 h prior to feeding of d 1 of next treatment period (10:30 h). The second group of cows were evacuated 4.5 h after feeding on d 27 (18:30 h) and 4.5 h prior to feeding of d 1 of next treatment (9:30 h). Rumen content mass and volume were determined. While evacuating, hand grab samples, representing 10% of the contents were subsampled into a separate container. Subsample solid and liquid phases were separated using a nylon screen (1-mm pore size) and each weighed. Aliquots (approximately 300 g) from both the solid and liquid phases were collected and stored frozen at -20°C until processing. Within 30 min of initiating the evacuation, the remaining ruminal contents were returned through the ruminal cannula. Solid phase aliquots were dried using a forced-air oven at 55℃ for 18 to 24 h and ground through a 1-mm screen (Wiley mill; Arthur H.

Thomas, Philadelphia, PA). Liquid phase aliquots were dried using a forced-air oven at 55°C for 36 to 48 h and ground through a 2-mm screen (UDY Cyclone Sample Mill; UDY Corp., Fort Collins, CO). Corresponding solid and liquid phases were recombined based on the proportion of dry matter. Recombined ruminal contents were analyzed for ash (modified method 942.05; AOAC, 1990; 4 h at 600°C), neutral detergent fiber (as described previously), uNDF240om (as described previously), and starch (Cumberland Valley Analytical Services, Inc., Waynesboro, PA).

Ruminal pool size of organic matter, neutral detergent fiber, uNDF240om, and starch were calculated as the product of the dry matter mass of the ruminal contents and the nutrient content of the ruminal contents. Ruminal turnover rate (%/h) of organic matter, neutral detergent fiber, uNDF240om, and starch were calculated as [100 × (intake of nutrient/ruminal pool of nutrient)/24], according to Oba and Allen (2000). Nutrient intake was calculated using dry matter intake from d 27 and 28 and the nutrient content of the diets from d 23 to 28. Ruminal turnover time (h) was calculated as 1/(ruminal turnover rate (%/h)/100).

Ruminal Fermentation. Indwelling ruminal pH/ORP/REDOX units (Penner et al., 2006; LRCpH; Dascor, Escondido, CA) were used to measure ruminal pH and redox potential at 30-s intervals for 96 consecutive h on d 23 to 26 in the 8 ruminally fistulated cows. Within d 23 to 26, ruminal pH and redox measurements were averaged over a 10-min period. Mean pH, minimum pH, maximum pH, the area below a pH of 5.8 in the pH curve, and hours per day that pH is below 5.5 or 5.8 as an indicator of sub-acute ruminal acidosis were calculated using 10-min period ruminal pH values.

Time to one and two standard deviations of ruminal pH drop from mean ruminal pH was calculated using a 20-min calculated mean (13:50-14:10 h) and previous day standard deviation at the start of study day for d 24 to 26.

Ruminal fluid samples (approximately 500 mL), hand grabbed from below the ruminal digesta mat, were collected each period on d 26 every 4-h (14:00, 18:00, 22:00, 02:00, 06:00, 10:00 h) for 24 h. After straining through 4 layers of cheesecloth, a portion (approximately 40 mL) of the ruminal fluid was separated for analysis for volatile fatty acid (VFA) concentration (Bulletin 856B; Supelco, Inc., Bellefonte, PA) 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). Additionally, 10 mL of ruminal fluid was added to 100 μL of concentrated HCl for analysis of ruminal NH3-N concentration. NH_3-N concentration was determined according to the procedure of Chaney and Marback (1962). Both ruminal fluid aliquots were stored at -20°C prior to analysis.

Total Tract Nutrient Digestibility. Total tract digestibility of dry matter, organic matter, crude protein, aNDFom, starch, and uNDF240om was determined on d 20 to 22 of each test period. As previously described, diets and corresponding orts were composited accordingly for d 20 to 22. Fecal grab samples were collected every 9 h during d 20 to 22 for each period for a total of eight samples to represent every 3 h in a 24-h period. Approximately 100 g of feces from each time point were combined by cow for each period composite. Diet, orts, and fecal samples were dried in a forced-air oven

at 55°C for 48 h prior to being ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA). Total tract composite samples of diets (by period), orts (by cow), and feces (by cow) were submitted for wet chemistry analysis (Cumberland Valley Analytical Services, Inc., Waynesboro, PA) for dry matter, crude protein (method 990.03; AOAC International 2012), acid detergent fiber (ADF; method 973.18; AOAC International, 2012), acid detergent lignin (ADL; Goering and Van Soest, 1970), and starch according to Hall (2009). Ash (method 942.05, Modifications: 1.0 g sample weight, 4 h ash time, cold weigh; AOAC International 2012), aNDFom (previously described), and uNDF240om (previously described) were analyzed at the William H. Miner Agricultural Research Institute (Chazy, NY). Sample uNDF240om was used as an internal marker. Total tract digestibility was calculated by the ratio technique using the concentrations of the nutrients and uNDF240om 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.

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

One cow did not finish the study due to a severe case of mastitis (Klebsiella). A second cow was removed from the study due to severe mastitis (Staph species). The second animal responded to treatment and finished the study but was excluded from the data set due to milk yield being less than 50% of production prior to the occurrence of

mastitis. All data from the two cows were completely removed from the data set.

Data were checked for homogeneity of variance and normality assumptions using Shapiro-Wilk and Levene's tests using the GLM procedure of SAS. Data for DMI (d 20 to 26), milk yield and composition, feed efficiency, total tract nutrient digestibility, passage rates, body weight, and body condition score were analyzed as a replicated Latin square design with model effects of diet, period, and replicate using the MIXED procedure of SAS. Cow within replicate was a random effect. Repeated measurements on performance data (i.e., DMI, milk yield, and milk composition) were reduced to period means for each cow before statistical analysis. Data for ruminal pH, NH_3-N, and volatile fatty acids were analyzed with repeated measures using the MIXED procedure of SAS. The model included the effects of diet, period, time, and the interaction of diet and time. Cow was a random effect. Ruminal starch turnover failed normality assumptions and was log transformed. Least square means and 95% confidence limits are presented as non-transformed values and *P*-values correspond to the model effects corresponding to the transformed data. 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 tendencies were discussed at $0.05 < P \leq 0.10$.

2.4. RESULTS AND DISCUSSION

2.4.1. Environmental Conditions

The study began May 18, 2017 and ended on September 7, 2017. The average temperature during the study was 19.0°C (SE = 0.04) and relative humidity was 79.4%

(SE = 0.13). These measurements are below the heat stress threshold and within the thermoneutral zone of lactating dairy cattle (Atrian and Shahryar, 2012). Consequently, it was concluded that cow responses to diet observed in our study were unaffected by environment.

2.4.2. Experimental Diets

Increasing the forage-to-concentrate ratio from 46.8:53.2 for the low uNDF240om treatments to 60.5:39.5 for the high uNDF240om treatments resulted in an increase in dietary uNDF240om content (% of DM) from 8.8% (LULP) and 8.9% (LUHP) to 11.4% (HULP) and 11.6% (HUHP; Table 2.3). This range in dietary uNDF240om encompasses the reported range previously reported by Cotanch et al. (2015) to represent the lower and upper uNDF240om expected to be consumed by lactating dairy cattle, respectively. Although there has been little published research examining the interaction between uNDF240om and DMI, it was expected that dietary uNDF240om content of 8.9% of DM would pose little constraint to DMI, whereas a dietary uNDF240om content of 11.5% of DM would possibly limit DMI (Cotanch et al., 2015; Fustini et al., 2018).

Dietary peNDF concentrations were 20.1% (LULP), 21.8% (LUHP), 18.6% (HULP), and 22.0% (HUHP; Table 2.4) reflecting the product of the dietary physical effectiveness factor (pef) and aNDFom concentration. The dietary pef value varied slightly depending on whether it was measured using the Penn State Particle Separator (0.57, 0.61, 0.55, 0.62 for LULP, LUHP, HULP, and HUHP, respectively) or the dry sieving, Ro-Tap method (0.61, 0.66, 0.52, 0.61, respectively; Table 2.4). Nonetheless,

the dietary peNDF content spanned the commonly cited requirement of 21% for lactating cows within each level of uNDF240om (Mertens, 1997). Consequently, it was concluded that there was sufficient spread in dietary particle size, within a level of dietary uNDF240om, to influence cow response.

To describe the physically effect portion of uNDF240om, a new descriptive term was created: physically effective uNDF240 (peuNDF240). This new term was calculated for each treatment diet (Table 2.3). Physically effective uNDF240 was calculated as the product of the dietary pef and uNDF240om as a percentage of DM and was intended to integrate the effects of particle size and NDF indigestibility into one number. The treatment peuNDF240 concentrations were 5.4% (LULP), 5.8% (LUHP), 5.9% (HULP), and 7.1% (HUHP; Table 2.3). This treatment structure was expected to assess the effects of different dietary peuNDF240 concentrations on cow responses, and especially to be able to determine if the two intermediate diets (LUHP and HULP) resulted in a similar response to diet.

Aside from uNDF240om, peNDF, and peuNDF240, the treatment diets contained similar nutrient profiles that were within recommended ranges (NRC, 2001; Table 2.3).

2.4.3. Dry Matter Intake

Dry matter intake did not differ among the two low uNDF240 (LULP and LUHP) and the high uNDF240, low peNDF (HULP) treatments (Table 2.5). The DMI for these three treatments was 2.5 kg/d greater ($P < 0.001$) compared with the high uNDF240, high peNDF diet (HUHP). Of greatest interest is the 2.5 kg/d increase in

DMI when peNDF was reduced for cows fed the high uNDF240 diet. Previous studies have shown either no effect of peNDF on DMI (Beauchemin et al., 2003) or an increase in DMI as observed in our study (Allen and Grant, 2000; Yansari et al., 2004; and Farmer et al., 2014). From the data in Table 2.5, it appears that a reduction in particle size has a greater effect on DMI when diets contain more uNDF240om.

Reflecting the greater DMI, the aNDFom intake (1.42% of BW) for cows fed the HULP diet was greater ($P = 0.017$) than cows fed the low uNDF240 and HUHP treatments (1.33%, LULP; 1.34%, LUHP; and 1.34% of BW, HUHP). The amount of NDF consumed as a percent of BW was substantially greater than the commonly cited maximal level of 1.20% of BW associated with maximal milk response (Mertens, 2009). Within a level of dietary uNDF240om, the peNDF intake reflected DMI and the peNDF content of the diets. The peNDF intake was highest for the LUHP treatment, intermediate for the LULP and HUHP diets, and least for the HULP diet. Cows fed the low uNDF240 diets consumed less uNDF240om than cows fed the high uNDF240 diets ($P < 0.001$), as expected, with cows fed the HULP diet consuming the most uNDF240om (0.45% of BW). The 0.45% of BW intake of uNDF240om observed for cows fed the HULP treatment appears to be near the maximum uNDF240om intake for lactating dairy cows and is comparable to observations by Fustini et al. (2017) for cows fed a highly digestible alfalfa hay with a high uNDF240om.

The intake of peuNDF240 directly reflects dietary peuNDF240 concentration (Tables 2.3 and 2.5). Although the HUHP treatment had the lowest DMI, the elevated dietary peuNDF240 resulted in this treatment group having the highest peuNDF240

intake. The LUHP and HULP treatments had differing uNDF240om and peNDF concentrations, but both treatments contained similar concentrations of peuNDF240. Consequently, peuNDF240 intake was not different between these two diets. Finally, the lowest dietary peuNDF240 concentration of the LULP treatment yielded the lowest peuNDF240 intake. Given the magnitude of the difference in peuNDF240 intake between the LULP and HUHP diets (0.22 versus 0.26% of BW), substantial differences in lactational, chewing, and ruminal responses was anticipated. For diets LUHP and HULP, although differing markedly in both particle size and uNDF240om content, given the similar peuNDF240 intake, similar cow responses were expected.

Across the four treatments, BW and body condition score change were not different (Table 2.5). Consequently, it was assumed that observed differences in gross feed efficiency (ECM/DMI; discussed in subsequent section) were not confounded by mobilization or accretion of body tissue.

2.4.4. Milk Yield, Composition, and Efficiency of Production

For measures of milk production, cows fed the LULP and HUHP diets differed consistently, whereas cows fed the LUHP and HULP diets were similar (Table 2.6). This pattern of milk yield response tracked with dietary peuNDF240 content. Milk yield, 3.5% FCM, solids-corrected milk, and energy-corrected milk all followed this pattern (Table 2.6). Cows fed the HUHP diet produced less ECM than those fed the LULP diet, whereas the LUHP and HULP diets were intermediate (Table 2.6). This relationship does not hold for milk fat percentage, however, which appeared to be influenced primarily by dietary uNDF240 concentration, agreeing with previous

research (Kokko et al., 2012; Fustini et al., 2017). Mixed origin milk fatty acids (C16) were also depressed for cows fed the low versus high uNDF240 diets (Table 2.6). Palmitic acid is the major component of this fraction, and it is also the longest fatty acid produced in the chain elongation process of *de novo* fatty acid synthesis. Consequently, it is the first *de novo* fatty acid to decrease when trans fatty acid-induced milk fat depression occurs (Barbano et al., 2018). The actual reduction in mixed fatty acids was small, reflecting the modest although significant depression in milk fat percentage for cows fed the low uNDF240 diets. *De novo* milk fatty acid content was unaffected by diet ($P = 0.18$), and preformed fatty acids (C17 and longer) were greater for cows fed the high uNDF240 diets, although the relationship between preformed fatty acid content of milk and milk fat percentage is very weak ($R^2 = 0.07$; Barbano et al., 2017). Fatty acid unsaturation, which is the number of double bonds per fatty acid, was not altered by the four treatment diets. Although milk fat percentage was influenced by diet, output (kg/d) of milk fat was unaffected.

True protein percentage and yield were similar for cows fed the LUHP and HULP diets. In general, milk protein appeared to be more related with dietary peNDF than uNDF240om. Previous research has found that smaller forage particle size is associated with greater efficiency of microbial protein synthesis as a result of increase passage rate of digesta (Rode et al., 1985). The results support this relationship since greater milk protein percentage was observed for cows fed the LULP, LUHP, and HULP diets versus the HUHP diet that coincided with a higher ruminal aNDFom turnover rate (discussed in subsequent section). The difference in milk protein

percentage and output between the LULP and HUHP diets also may have been related to potentially greater microbial yield linked with increased fermentable fiber (Van Soest, 1994).

Milk urea nitrogen increased as dietary peuNDF240 increased, with the greatest difference between the LULP and HUHP treatments (Table 2.6). Hristov and Ropp (2003) reported that diets providing more ruminally fermentable fiber enhanced the transfer of ruminal ammonia milk protein thereby reducing MUN. Consequently, it makes sense that MUN would decrease in the study for cows fed lower uNDF240 diets and generally with smaller particle size within a level of uNDF240om.

Gross milk production efficiency was evaluated several different ways (Table 2.6). Milk yield per unit of dry matter intake (kg/kg) was not different among the low uNDF240 treatments. However, increasing dietary peNDF in the high uNDF240 treatments tended to increase efficiency of milk production ($P = 0.09$). When comparing the other methods of determining efficiency (3.5% FCM/DMI, SCM/DMI, and ECM/DMI) the low uNDF240 treatments did not differ in efficiency measurements and the HUHP diet resulted in the greatest efficiency. However, the fact that the HUHP diet resulted in the least amount of milk production implies that simple efficiency may not be the best metric for comparing diets from a profitability perspective.

2.4.5. Chewing Behavior

Eating and ruminating behaviors were markedly influenced by dietary peuNDF240 (Table 2.7). Total eating time was not different between the LUHP and HULP treatments. Total eating time was greatest for HUHP at 300 min/d reflecting the

less digestible NDF and greater particle size of the diet. In contrast, a 44.9 min/d decrease in total eating time, while consuming 2.6 kg more of DMI, was observed for cows fed the LULP treatment. When eating time was expressed as minute per kilogram of DMI, dietary fiber differences become more apparent. Eating time, expressed as minute per kilogram of DMI, was lowest for the LULP treatment at 9.1 min/kg of DMI, with LUHP (9.6) and HULP (10.1) not differing, and the HUHP diet eliciting the greatest eating time at 11.9 min/kg of DMI ($P < 0.01$). These differences in eating behavior are related to the relatively uniform particle size of feed boli entering the rumen as a result of initial mastication (Schadt et al., 2012).

Although total eating time was affected by dietary peNDF and uNDF240om, total rumination time (min/d) was not. Across the four treatments, total rumination time averaged 531 min/d. However, when expressed as min/kg of DMI, rumination reflected the dietary peuNDF240 content. Rumination time (min/kg of DMI) increased from the low uNDF240 and HULP treatments to HUHP (Table 2.7). The difference in rumination activity between the high uNDF240 treatments agrees with previous research (Allen and Grant, 2000; Beauchemin et al., 2003; Yansari et al., 2004) where decreasing peNDF reduced rumination (min/kg of DMI). Within the high uNDF240 treatments, less rumination (min/kg of DMI) associated with the decrease in dietary peNDF is reflective of the shortened fiber particles that are closer to the critical size to exit the rumen.

Meal length was influenced by dietary peuNDF240 concentration (Table 2.7). The LULP and HUHP diets differed in meal length by 10 min. The LUHP and HULP

treatments did not differ, reflecting similar peuNDF240 concentration, but were different from the LULP and HUHP treatments (Table 2.7). Similar to meal length, meal bouts were influenced by the peuNDF240 content of the diets. The LULP treatment (11.3 bouts/d) was different from HUHP (10.0 bouts/d, $P = 0.03$) but not differ from the LUHP and HULP treatments. By decreasing dietary uNDF240om and peNDF, ultimately decreasing peuNDF240, meal length decreased and frequency increased. These changes in meal patterns should result in a more stable ruminal pH for fiber fermentation (Pitt and Pell, 1997).

2.4.6. Ruminal pH and Fermentation

Daily mean ruminal pH was influenced by dietary changes in uNDF240om and peNDF following the peuNDF240 pattern. The LULP treatment had a lower daily mean pH in comparison to the HUHP treatment (Table 2.8; $P = 0.03$). There were no differences between the LUHP and HULP treatments for daily mean ruminal pH. Unlike daily mean pH, differences in minimum pH were observed between the two low peNDF treatments, where the HULP treatment tended to have a higher minimum ruminal pH in comparison to the LULP treatment. Maximum ruminal pH reflected peuNDF240 concentration with LULP lower than HUHP and no differences between LUHP and HULP (Table 2.8).

Another variable examined to assess the impact of altering dietary uNDF240om and peNDF was the time, in minutes, observed until ruminal pH decreased by 1 or 2 standard deviations from the mean ruminal pH of the start of the study day. The HULP treatment required less time than the two high peNDF treatments for 1 SD shift and

tended to be shorter in time compared to the LUHP treatment for 2 SD change in pH. The differences in the rate that ruminal pH declined reflected differences among diets in how rapidly fermentation was occurring. The reduction in pH associated with the HULP diet in comparison to the high peNDF diets reflects the reduced particle size of the diet. Similarly, Allen (1997) observed that forage particle size was negatively related with ruminal pH. The increased fiber particle surface area with finer chopping presumably aided in microbial attachment and ultimately fermentation (Van Soest, 1994). Another factor contributing to the difference in ruminal pH change is the higher inclusion rate of beet pulp in the low uNDF240 treatments. McBurney et al. (1983) determined that beet pulp had a greater cation exchange capacity (i.e., buffering capacity) because of the elevated pectin content which serves to mitigate ruminal pH decline. Finally, when examining ruminal pH more closely, time when ruminal pH was lower than 5.8, time when ruminal pH was lower than 5.5, and area when ruminal pH was below 5.8 by hour, although numerically in the same direction as mean ruminal pH, were not different among treatment diets ($P > 0.10$, Table 2.8).

Total VFA concentration shifted in agreement with the dietary peuNDF240 content (Table 2.9). The LULP total VFA concentration was 10.5 m*M* greater ($P = 0.05$) than the HUHP treatment with no differences between the LUHP and HULP treatments (Table 2.9). The increase in total VFA concentration for the LULP versus the HUHP diet is associated with the greater fermentability for the finer, low uNDF240 diet (Yansari et al., 2004). Acetate, expressed as % of total VFA, did not differ among treatments ($P = 0.18$). Molar proportion of propionate was influenced by dietary

uNDF240om, with the low uNDF240 treatments containing more fermentable fiber resulting in a greater proportion of propionate which agrees with the results of Yansari et al. (2004). Butyrate differed among diets, (11.0 to 11.5; % of total VFA; Table 2.9), although the magnitude was very small and of doubtful biological importance. Similar to propionate, rumen isobutyrate and isovalerate concentrations were greater for cows fed the high uNDF240 versus the lower uNDF240 diets (Table 2.9). In line with this observation, Zhang et al. (2013) reported greater rumen isobutyrate and isovalerate molar percentages with increased NDF fermentability. As expected, the acetate-to-propionate (A:P) and acetate plus butyrate-to-propionate (A+B:P) ratios shifted, reflecting the changes in propionate and butyrate. The HULP treatment ratios for both A:P and A+B:P were greater than the two low uNDF240 treatments. Similarly, the HUHP treatment ratios were roughly 0.2 units greater than the LULP treatment. These differences in VFA ratios aid in explaining the observed milk fat percentage differences. The increase in milk fat percentage of the high uNDF240 diets was associated with greater A:P ratios as previously discussed by Kokko et al. (2012).

2.4.7. Ruminal Digesta Characteristics

Across the four treatment diets there were no differences in ruminal digesta volume or mass. As a result, ruminal density was similar for the diets (Table 2.10). Differences in ruminal digesta characteristics became apparent when examining the ruminal pool of uNDF240om. The differences among the treatments reflect dietary uNDF240om, with a 19% increase in pool size when comparing the high uNDF240 treatments to the low uNDF240 diets ($P < 0.01$). Starch, aNDFom, and organic matter

ruminal pool sizes did not differ among the four treatments. Although, the ruminal pool size of aNDFom did not differ, ruminal turnover rate (%/h) followed the peuNDF240 relationship previously described. The LULP tended ($P = 0.04$) to have a greater turnover rate (4.4%/h) in comparison to the HUHP treatment (3.9%/h), with no difference between the LUHP and HULP treatment. Coinciding with the change in ruminal turnover rates, ruminal turnover time of aNDFom tended to be 2.9 h less for the LULP treatment in comparison to HUHP. Turnover rate and time were both lower than previously reported (4.76 to 5.52%/h; 19.0 to 21.4 h; Grant and Cotanch, 2012) likely as a result of the greater uNDF240om content of our diets versus that of Cotanch and Grant (2012). The change in ruminal turnover rate and time is reflective of the shift in digestibility and particle size of the fiber in the diets.

Ruminal turnover rate and turnover time of starch was not different among the four treatment diets (Table 2.10). In general, the values are similar, or higher, in magnitude to previous research with corn silage-based diets (92.2 to 103.1, Ivan et al., 2005; 69.6 to 99.4, Farmer et al., 2014) and reflect the high fermentability of the starch sources within our diets such as high moisture corn (from silage), steam-flaked corn grain, and finely ground corn meal.

2.4.8. Total Tract Digestibility

One of the few variables where the LUHP and HULP diets differed was DM digestibility (Table 2.11). A 3.9% increase in DM digestibility was observed for HULP in comparison to the LUHP treatment ($P = 0.05$). No differences were observed for organic matter and aNDFom digestibility among the four treatment diets. Potentially

digestible NDF (pdNDF) was influenced by dietary uNDF240om when comparing the low peNDF treatments. The HULP treatment had a 3.2% increase in pdNDF digestibility in comparison to the LULP treatment. Previous research indicated the change in pdNDF digestibility was a result of extended ruminal retention time (Harper and McNeill, 2015). Finally, starch total tract digestibility followed the peuNDF240 relationship with a difference among the LULP and HUHP treatments and no difference among the LUHP and HULP treatment (Table 2.11). Although biologically similar, starch digestibility was greater for the HUHP treatment in comparison to the LULP treatment.

2.5. CONCLUSIONS

The objective of this study was to evaluate the relationship between dietary uNDF240om and peNDF. Agreeing with the hypotheses, feeding low dietary uNDF240 and low peNDF resulted in greater intake, milk yield, and less eating time in comparison to the high uNDF240 and high peNDF diet. With the high uNDF240 diets, reducing dietary peNDF increased DMI. Interestingly, the low uNDF240, high peNDF and high uNDF240, low peNDF treatments often elicited the same animal response, reflective of similar dietary peuNDF240 concentrations. If future research confirms this relationship, it suggests that the integration of pef and uNDF240om could be a useful metric in ration formulation.

2.6. ACKNOWLEDGMENTS

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Table 2.1. Ingredient composition (% of DM) of treatment diets with unmixed concentrate.

	Diets			
	Low uNDF240		High uNDF240	
Ingredient	Low peNDF	High peNDF	Low peNDF	High peNDF
Corn silage	34.68	34.68	34.68	34.68
Long timothy hay	-	10.48	-	24.19
Short timothy hay	10.48	-	24.19	-
Wheat straw, chopped	1.61	1.61	1.61	1.61
Concentrate mix				
Steam flaked corn	9.68	9.68	8.32	8.32
Fine corn meal	5.68	5.68	7.10	7.10
Aminomax Pro[1]	7.72	7.72	9.12	9.12
Soybean meal	5.71	5.71	5.71	5.71
Soy hulls	3.23	3.23	-	-
Wheat middlings	-	-	1.61	1.61
Beet pulp pellets	12.90	12.90	0.36	0.36
Canola meal	1.42	1.42	-	-
Rumen inert fat[2]	2.01	2.01	2.01	2.01
PGI amino enhancer 1[1]	0.73	0.73	0.73	0.73
99% sugar	-	-	0.45	0.45
Calcium carbonate	0.89	0.89	1.14	1.14
Sodium sesquicarbonate	0.74	0.74	0.74	0.74
E Gold[3]	0.79	0.79	0.94	0.94
Salt	0.42	0.42	0.42	0.42
Magnesium oxide	0.32	0.32	0.32	0.32
Urea	0.25	0.25	-	-
Omnigen-AF[4]	0.18	0.18	0.18	0.18
Chromium propionate	0.04	0.04	0.04	0.04
Trace mineral and vitamin premix[5]	0.11	0.11	0.11	0.11
Meta Smart[6]	0.05	0.05	0.05	0.05
Smartamine[6]	0.04	0.04	0.04	0.04
AjiPro-L Gen 2[7]	0.03	0.03	0.03	0.03
XPC yeast culture[8]	0.05	0.05	0.05	0.05
Avail-Zn 120[9]	0.03	0.03	0.03	0.03
Mono dicalcium phosphate	0.19	0.19	-	-
Rumensin[10]	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00

[1] Poulin Grain; Newport, VT.
[2] BergaFat; Berg + Schmidt America, LLC; Libertyville, IL.
[3] Commercial fat, Poulin Grain; Newport, VT.
[4] Phibro Animal Health Corp., Teaneck, NJ
[5] 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.
[6] Adisseo USA, Inc.; Alpharetta, GA.
[7] Ajinomoto Heartland, Inc., Chicago, IL, USA.
[8] Diamond V Mills, Inc; Cedar Rapids, IA.
[9] Zinpro Corp., Eden Prairie, MN
[10] Elanco Animal Health; Greenfield, IN.

Table 2.2. Analyzed (dry matter basis) chemical composition, in vitro digestibility, and fermentation analysis of ingredients[1] used in diets fed to lactating Holstein cows during the study.

Item	Corn silage	Long hay	Short hay	Wheat straw	Beet pulp pellets	Low uNDF240 Grain Mix	High uNDF240 Grain Mix
Dry matter (DM), %	34.6±1.0	91.7±0.6	91.4±0.6	91.1±0.7	94.0±2.0	91.3±0.1	91.2±0.1
Crude protein (CP), %	7.6±0.2	11.2±0.3	11.9±0.3	3.2±0.3	8.1±0.3	25.6±0.6	26.0±0.4
Soluble protein, % CP	4.8±0.1	4.1±0.2	4.1±0.2	1.2±0.1	1.1±0.2	6.1±0.7	4.2±0.3
Ammonia, % of CP	2.2±1.1	-	-	-	-	-	-
ADF[2], %	24.3±0.7	40.9±0.7	40.7±0.1	54.0±0.6	32.4±2.4	10.6±0.2	7.5±0.3
aNDFom[3], %	39.2±1.5	66.2±1.0	64.6±0.8	81.9±0.5	38.4±1.1	16.1±0.4	13.0±0.7
NFC[4], %	47.2±0.7	17.1±0.4	16.2±0.5	11.6±0.3	40.1±2.3	45.0±0.9	46.0±0.5
Lignin, % of DM	3.2±0.1	6.6±0.1	6.4±0.1	9.0±0.4	4.5±1.2	3.0±0.0	2.8±0.1
NSC[5], %	35.9±1.0	7.9±0.1	8.0±0.2	2.0±0.2	10.7±1.9	35.1±0.9	34.0±0.5
Starch, %	35.1±0.9	0.7±0.1	0.5±0.1	1.0±0.2	0.7±0.2	30.4±0.8	28.8±0.5
Sugar (ESC[6]), %	0.9±0.2	7.2±0.1	7.5±0.1	1.0±0.1	10.0±1.3	4.7±0.2	5.3±0.1
Ether extract, %	3.3±0.1	1.9±0.2	2.1±0.0	1.4±0.1	1.0±0.1	4.6±0.3	4.8±0.4
NE_L[7], Mcal/kg	0.3±0.0	0.3±0.0	0.3±0.0	0.2±0.0	0.3±0.0	0.4±0.0	0.4±0.0
Ash, %	3.3±0.1	7.5±0.2	8.2±0.3	4.7±0.3	12.5±1.5	9.8±0.5	11.1±0.1
Calcium, %	0.26±0.02	0.34±0.02	0.39±0.01	0.28±0.02	1.53±0.10	1.78±0.09	1.98±0.03
Phosphorus, %	0.22±0.00	0.24±0.01	0.26±0.01	0.07±0.03	0.09±0.01	0.61±0.01	0.59±0.01
Magnesium, %	0.19±0.01	0.20±0.01	0.21±0.00	0.10±0.01	0.39±0.02	0.77±0.02	0.78±0.02
Potassium, %	0.96±0.05	2.15±0.04	2.23±0.04	1.03±0.04	0.38±0.03	1.12±0.02	1.22±0.00

Sulfur, %	0.14±0.00	0.19±0.01	0.20±0.00	0.09±0.01	0.38±0.05	0.39±0.01	0.44±0.02
Sodium, %	0.01±0.00	0.04±0.00	0.04±0.00	0.01±0.00	0.12±0.02	1.16±0.09	1.20±0.04
Chloride ion, %	0.22±0.01	0.82±0.01	0.82±0.01	0.16±0.03	0.04±0.01	0.72±0.05	0.81±0.01
Iron, mg/kg	197±10	220±25	286±56	117±18	1877±173	453±14	344±20
Copper, mg/kg	7±0	8±0	8±0	4±0	9±0	24±1	24±1
Manganese, mg/kg	22±1	36±1	38±2	38±9	120±10	84±1	86±3
Zinc, mg/kg	26±0	34±1	35±1	9±3	28±2	199±7	205±2
Lactic acid, %	4.6±0.3	-	-	-	-	-	-
Acetic acid, %	4.3±0.3	-	-	-	-	-	-
Propionic acid, %	0.6±0.1	-	-	-	-	-	-
Isobutyric acid, %	-	-	-	-	-	-	-
Butyric acid, %	-	-	-	-	-	-	-
Total VFA[8], %	8.9±0.5	-	-	-	-	-	-
pH	4.0±0.0	-	-	-	-	-	-
uNDF12om[9], %	-	-	-	-	16.7±2.9	12.4±0.3	11.1±0.3
uNDF30om[10], %	20.6±1.2	39.9±2.2	39.7±1.0	57.1±1.2	-	-	-
uNDF72om[11], %	-	-	-	-	8.4±1.5	3.5±0.5	4.4±0.8
uNDF120om[12], %	10.8±0.5	25.1±0.4	22.8±0.5	32.9±1.2	7.0±1.9	3.5±0.4	4.7±0.4
uNDF240om[13], %	10.7±0.3	22.9±1.0	21.8±0.4	31.0±1.0	-	-	-

[1] Mean ± standard error. Sample n = 4/ingredient.

[2] Acid detergent fiber.

[3] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.

[4] Nonfibrous carbohydrates.
[5] Nonstructural carbohydrates.
[6] Ethanol soluble carbohydrates.
[7] Net energy for lactation.
[8] Volatile fatty acids.
[9] Undigested neutral detergent fiber after 12 hours of in vitro fermentation, ash corrected.
[10] Undigested neutral detergent fiber after 30 hours of in vitro fermentation, ash corrected.
[11] Undigested neutral detergent fiber after 72 hours of in vitro fermentation, ash corrected.
[12] Undigested neutral detergent fiber after 120 hours of in vitro fermentation, ash corrected.
[13] Undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

Table 2.3. Calculated diet composition based on wet chemistry analysis of ingredients fed to lactating Holstein cows.

	Diets[1]			
	Low uNDF240		High uNDF240	
Item	Low peNDF	High peNDF	Low peNDF	High peNDF
Dry matter (DM), %	59.3±1.1	59.0±1.1	59.5±1.1	59.1±1.5
Crude protein (CP), % of DM	15.3±0.3	15.2±0.3	15.7±0.1	15.5±0.1
Soluble protein, % of CP	37.2±1.1	37.4±1.2	36.9±0.5	37.4±0.9
ADF[2], % of DM	22.0±0.2	22.0±0.1	22.2±0.3	22.2±0.5
aNDFom[3], % of DM	33.1±0.8	33.3±0.9	35.7±0.9	36.1±1.0
NFC[4], % of DM	41.6±0.5	41.7±0.5	38.4±0.0	38.6±0.1
Lignin, % of DM	3.7±0.1	3.7±0.1	3.9±0.0	3.9±0.0
NSC[5], % of DM	28.8±0.4	28.8±0.4	28.1±0.5	28.1±0.5
Starch, % of DM	24.6±0.3	24.6±0.3	23.4±0.4	23.5±0.4
Sugar, % of DM	4.3±0.2	4.3±0.2	4.6±0.1	4.6±0.1
Ether Extract, % of DM	3.4±0.1	3.4±0.1	3.6±0.2	3.5±0.2
NE_L[6], Mcal/kg	1.6±0.0	1.6±0.0	1.6±0.0	1.6±0.0
Ash, % of DM	7.6±0.4	7.6±0.3	7.5±0.1	7.4±0.1
Calcium, % of DM	1.05±0.06	1.05±0.06	0.96±0.02	0.95±0.02
Phosphorus, % of DM	0.36±0.01	0.36±0.01	0.37±0.00	0.37±0.00
Magnesium, % of DM	0.45±0.01	0.45±0.01	0.42±0.01	0.42±0.01
Potassium, % of DM	0.36±0.01	0.36±0.01	0.37±0.00	0.37±0.00
Sulfur, % of DM	0.28±0.01	0.28±0.01	0.27±0.01	0.27±0.01
Sodium, % of DM	0.49±0.04	0.49±0.04	0.48±0.02	0.48±0.01
Chloride ion, % of DM	0.46±0.02	0.46±0.02	0.59±0.00	0.59±0.00
Iron, mg/kg	525±25	518±23	279±13	263±7
Copper, mg/kg	14±0	14±0	14±0	14±0
Manganese, mg/kg	61±2	61±1	51±1	51±1
Zinc, mg/kg	97±3	96±3	97±1	97±1
uNDF30om[7], % of DM	19.4±0.3	19.4±0.5	22.0±0.7	22.1±1.1
uNDF120om[8], % of DM	9.2±0.3	9.4±0.2	11.5±0.6	12.1±0.6

uNDF240om[9], % of DM	8.8±0.1	8.9±0.1	11.4±0.2	11.6±0.4
peuNDF240[10], % of DM	5.4±0.1	5.8±0.1	5.9±0.1	7.1±0.3

[1] Mean ± standard error. Sample n = 4/ingredient.
[2] Acid detergent fiber.
[3] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.
[4] Nonfibrous carbohydrates.
[5] Nonstructural carbohydrates.
[6] Net energy for lactation.
[7] Undigested neutral detergent fiber after 30 hours of in vitro fermentation, ash corrected.
[8] Undigested neutral detergent fiber after 120 hours of in vitro fermentation, ash corrected.
[9] Undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.
[10] Physically effective undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

Table 2.4. Particle size distribution of the diets[1].

	Diets			
	Low uNDF240		High uNDF240	
Item	Low peNDF	High peNDF	Low peNDF	High peNDF
Particle size distribution, % DM[2]				
>19.00 mm	0.2±0.2	0.1±0.0	0.0±0.0	0.1±0.1
13.20 to 19.00 mm	0.1±0.1	0.2±0.1	0.1±0.1	0.2±0.1
9.50 to 13.20 mm	1.0±0.1	1.3±0.2	1.3±0.2	2.2±0.4
6.70 to 9.50 mm	14.2±1.4	15.3±1.6	6.1±0.5	8.4±0.5
4.75 to 6.70 mm	10.2±0.5	10.3±0.3	8.2±0.1	9.6±0.3
3.35 to 4.75 mm	10.3±0.4	11.3±0.6	9.0±0.1	10.3±0.7
2.36 to 3.35 mm	8.1±0.3	9.3±0.4	7.6±0.4	9.9±0.4
1.18 to 2.36 mm	16.7±0.6	17.9±0.7	19.7±0.5	20.2±0.6
0.60 to 1.18 mm	17.0±0.5	15.3±0.8	21.6±0.6	18.1±0.9
0.30 to 0.60 mm	13.2±0.2	11.2±0.3	15.6±0.5	12.2±0.5
<0.30 mm	9.0±0.5	7.9±0.5	10.9±0.5	8.9±0.4
pef[3]	0.61±0.01	0.66±0.01	0.52±0.01	0.61±0.02
peNDF[4], %	20.1±0.6	21.8±0.8	18.6±0.7	22.0±1.0
Particle size distribution, % as-fed[5]				
>19.0 mm	1.6±0.1	5.2±0.3	1.6±0.2	10.0±1.2
8.0 to 19.0 mm	42.1±1.5	45.5±0.8	35.6±0.9	40.0±0.9
4.0 to 8.0 mm	12.8±0.2	10.4±0.2	18.3±0.4	12.2±0.2
<4.0 mm	43.5±1.6	39.0±0.9	44.5±1.1	37.7±0.9
pef [6]	0.57±0.02	0.61±0.01	0.55±0.01	0.62±0.01
peNDF[6]	18.8±0.9	20.3±0.8	19.8±0.9	22.5±0.9
pef[7]	0.60±0.02	0.60±0.03	0.57±0.02	0.66±0.01
peNDF[7]	19.9±0.8	19.9±0.8	20.2±0.5	23.6±0.9

[1] Mean ± standard error. Sample n = 4/ingredient.

[2] Measurements made with the Ro-Tap sieve.

[3] pef = physical effectiveness factor, % DM ≥1.18 mm.

[4] peNDF = physically effective neutral detergent fiber, % DM ≥1.18 mm.

[5] Measurements made with the Penn State Particle Separator.

[6] pef = physical effectiveness factor with the Penn State Particle Separator, % of DM ≥ 4.0 mm.

[7] pef = physical effectiveness factor with the Z-box, % of DM ≥ 3.18 mm.

Table 2.5. Least squares means of intake, body weight, and body condition score data of lactating Holstein cows (n = 14) fed treatment diets.

	Diets					
	Low uNDF240		High uNDF240			
Item	Low peNDF	High peNDF	Low peNDF	High peNDF	SEM	*P*-value
DMI[1], kg/d	27.5[a]	27.3[a]	27.4[a]	24.9[b]	0.6	<0.001
DMI, % of BW[2]/d	4.02[a]	4.04[a]	3.99[a]	3.73[b]	0.10	0.003
aNDFom[3] intake, kg/d	9.1[b]	9.1[b]	9.7[a]	9.0[b]	0.2	0.008
aNDFom intake, % of BW/d	1.33[b]	1.34[b]	1.42[a]	1.34[b]	0.03	0.017
peNDF[4] intake, kg/d	5.6[b]	5.9[a]	5.1[c]	5.4[b]	0.1	<0.001
peNDF intake, % of BW/d	0.81[b]	0.88[a]	0.74[c]	0.81[b]	0.02	<0.001
uNDF240om[5] intake, kg/d	2.4[c]	2.4[c]	3.1[a]	2.9[b]	0.1	<0.001
uNDF240om intake, % of BW/d	0.35[c]	0.36[c]	0.45[a]	0.43[b]	0.01	<0.001
peuNDF240[6] intake, kg/d	1.5[c]	1.6[b]	1.6[b]	1.7[a]	0.03	<0.001
peuNDF240 intake, % of BW/d	0.22[c]	0.24[b]	0.24[b]	0.26[a]	0.01	<0.001
Body weight change, kg	8.5	7.5	6.6	0.6	4.6	0.63
Body condition score change	0.01	-0.02	-0.06	-0.02	0.05	0.81

[1] Dry matter intake.

[2] Body weight.

[3] Amylase- and sodium sulfite-treated neutral detergent fiber, ash corrected.

[4] Physically effective neutral detergent fiber, % DM ≥1.18 mm.

[5] Undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

[6] Physically effective undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.

[abc] Least squares means within a row without a common superscript differ ($P \leq 0.05$).

Table 2.6. Least squares means of lactation performance data of lactating Holstein cows (n = 14) fed treatment diets.

	Diets					
	Low uNDF240		High uNDF240			
Item	Low peNDF	High peNDF	Low peNDF	High peNDF	SEM	*P*-value
Milk, kg/d	46.1^{a}	44.9^{ab}	44.0^{bc}	42.6^{c}	0.9	<0.001
3.5% FCM[1], kg/d	47.6^{a}	45.4^{ab}	46.8^{ab}	44.8^{b}	1.1	0.04
SCM[2], kg/d	43.6^{a}	41.4^{ab}	42.6^{ab}	40.3^{b}	1.0	<0.01
ECM[3], kg/d	47.0^{a}	45.7^{ab}	46.4^{ab}	44.6^{b}	0.9	0.03
Fat, %	3.68^{b}	3.66^{b}	3.93^{a}	3.92^{a}	0.10	<0.001
Fat, kg/d	1.70	1.62	1.71	1.64	0.05	0.12
True protein, %	2.93^{a}	2.88^{ab}	2.96^{a}	2.84^{b}	0.06	<0.01
True protein, kg/d	1.35^{a}	1.27^{b}	1.29^{ab}	1.19^{c}	0.03	<0.001
Lactose (anhydrous), %	4.64^{a}	4.61^{ab}	4.58^{b}	4.58^{b}	0.02	0.01
Lactose (anhydrous), kg/d	2.16^{a}	2.05^{ab}	2.02^{b}	1.93^{b}	0.05	<0.01
Solids nonfat, %	8.63^{a}	8.56^{ab}	8.61^{a}	8.49^{b}	0.06	<0.01
Solids nonfat, kg/d	4.01^{a}	3.80^{ab}	3.78^{bc}	3.56^{c}	0.08	<0.001
Urea nitrogen, mg/dL	8.5^{c}	9.4^{bc}	10.1^{ab}	11.0^{a}	0.6	<0.001
Somatic cell score	0.57	0.37	0.69	0.46	0.27	0.52
De novo FA[4], g/100 g milk	0.87	0.85	0.90	0.88	0.03	0.18
Mixed origin FA, g/100 g milk	1.41^{b}	1.40^{b}	1.51^{a}	1.51^{a}	0.04	<0.001
Preformed FA, g/100 g milk	1.24^{b}	1.24^{b}	1.34^{a}	1.38^{a}	0.04	<0.001
Unsaturation, double bonds/FA	0.27	0.27	0.26	0.26	0.01	0.30
Milk/DMI[5], kg/kg	1.68^{xy}	1.65^{xy}	1.61^{y}	1.71^{x}	0.04	0.09
3.5% FCM/DMI, kg/kg	1.71^{b}	1.69^{b}	1.71^{ab}	1.82^{a}	0.04	<0.01
SCM/DMI, kg/kg	1.57^{ab}	1.54^{b}	1.56^{ab}	1.64^{a}	0.03	0.03
ECM/DMI, kg/kg	1.71^{ab}	1.68^{b}	1.70^{ab}	1.79^{a}	0.04	0.02

[1] Fat corrected milk.
[2] Solids corrected milk.
[3] Energy corrected milk.
[4] Fatty acids.

[5] Dry matter intake.

[abc] Least squares means within a row without a common superscript differ ($P \leq 0.05$).

[xy] Least squares means within a row without a common superscript differ ($P \leq 0.10$).

Table 2.7. Least squares means of behavior and meal data of lactating Holstein cows (n = 14) fed the treatment diets.

	Diets					
	Low uNDF240		High uNDF240			
Item	Low peNDF	High peNDF	Low peNDF	High peNDF	SEM	*P*-value
Eating time						
min/d	255.4^{b}	262.5^{b}	279.1^{ab}	300.3^{a}	12.4	<0.001
min/kg of DMI[1]	9.1^{c}	9.6^{bc}	10.1^{b}	11.9^{a}	0.5	<0.01
min/kg of aNDFom[2]	28.1^{b}	29.3^{b}	28.9^{b}	33.6^{a}	1.5	<0.01
min/kg of peNDF[3]	46.2^{b}	44.8^{b}	55.8^{a}	55.6^{a}	2.6	<0.01
min/kg of uNDF240om[4]	106.2^{a}	108.8^{a}	90.5^{b}	105.0^{a}	5.2	<0.01
min/kg of peuNDF240om[5]	174.3	166.3	174.6	173.6	8.8	0.61
Rumination time						
min/d	523.2	526.5	531.8	544.5	16.4	0.36
min/kg of DMI	18.6^{b}	19.3^{b}	19.3^{b}	21.7^{a}	0.8	<0.01
min/kg of aNDFom	57.6^{ab}	58.4^{ab}	54.9^{b}	61.0^{a}	2.2	<0.01
min/kg of peNDF	94.8^{bc}	89.1^{c}	105.8^{a}	100.7^{ab}	3.8	<0.01
min/kg of uNDF240om	217.3^{a}	218.3^{a}	172.7^{c}	190.2^{b}	7.0	<0.01
min/kg of peuNDF240om	357.1^{a}	332.5^{b}	332.1^{b}	313.4^{b}	12.1	<0.01
Total chewing time						
min/d	778.6^{b}	789.1^{b}	810.9^{ab}	844.8^{a}	24.7	0.001
min/kg of DMI	27.7^{c}	28.9^{bc}	29.3^{b}	33.6^{a}	1.2	<0.01
min/kg of aNDFom	85.8^{b}	87.7^{b}	83.8^{b}	94.6^{a}	3.4	<0.01
min/kg of peNDF	141.0^{b}	134.0^{b}	161.6^{a}	156.2^{a}	5.8	<0.01
min/kg of uNDF240om	323.4^{a}	327.1^{a}	263.2^{c}	295.2^{b}	10.9	<0.01
min/kg of peuNDF240om	531.4^{a}	498.8^{b}	506.5^{ab}	487.0^{b}	18.8	<0.01
Meal length, min/meal	27.7^{c}	32.8^{b}	32.6^{b}	37.7^{a}	2.5	<0.001
Meal bout, bouts/d	11.3^{a}	10.5^{ab}	10.7^{ab}	10.0^{b}	0.5	0.03

[1] Dry matter intake.

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

[3] Physically effective neutral detergent fiber, % DM ≥1.18 mm.
[4] Undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.
[5] Physically effective undigested neutral detergent fiber after 240 hours of in vitro fermentation, ash corrected.
[abc] Least squares means within a row without a common superscript differ ($P \leq 0.05$).

Table 2.8. Ruminal pH data of lactating Holstein cows (n = 14) fed the treatment diets.

	Diets					
	Low uNDF240		High uNDF240			
Item	Low peNDF	High peNDF	Low peNDF	High peNDF	SEM	*P*-Value
Daily mean pH	6.11[b]	6.17[ab]	6.22[ab]	6.24[a]	0.05	0.03
Minimum pH	5.48[y]	5.51[xy]	5.64[x]	5.55[xy]	0.09	0.09
Maximum pH	6.66[b]	6.73[ab]	6.70[ab]	6.75[a]	0.04	0.03
Standard deviation pH	0.29	0.29	0.24	0.28	0.03	0.10
Time to 1 SD[1] drop (min)	80.8[ab]	98.1[a]	52.9[b]	89.0[a]	10.4	0.01
Time to 2 SD drop (min)	184.0[xy]	221.1[x]	155.0[y]	206.5[xy]	26.1	0.09
Time pH < 5.8, min/d	253.1	208.1	166.3	164.4	61.4	0.24
Time pH < 5.5, min/d	71.3	71.0	39.7	31.0	22.1	0.16
Area[2] < 5.8	52.0	49.6	33.5	30.0	15.0	0.29

[1] Standard deviation.

[2] Area < 5.8 = ruminal pH units below 5.8 by hour.

[ab] Means within same row without a common superscript differ ($P \leq 0.05$).

[xy] Within a row, different superscripts differ at ($P \leq 0.10$).

Table 2.9. Fermentation data of lactating Holstein cows (n = 14) fed the treatment diets.

	Diets					
	Low uNDF240		High uNDF240			
Item	Low peNDF	High peNDF	Low peNDF	High peNDF	SEM	*P*-Value
Total VFA[1], m*M*	122.8^{a}	120.6^{ab}	118.3^{ab}	112.3^{b}	4.1	0.05
VFA, % of total VFA						
Acetate (A)	63.4	63.8	63.9	64.1	0.9	0.18
Propionate (P)	22.7^{a}	22.5^{a}	21.5^{b}	21.6^{b}	0.8	<0.001
Butyrate (B)	11.2^{ab}	11.0^{b}	11.5^{a}	11.3^{ab}	0.4	0.01
Isobutyrate	0.57^{b}	0.59^{b}	0.68^{a}	0.71^{a}	0.03	<0.001
Valerate	1.68^{xy}	1.64^{y}	1.80^{x}	1.66^{xy}	0.12	0.08
Isovalerate	0.45^{b}	0.50^{b}	0.62^{a}	0.62^{a}	0.03	<0.001
A:P	2.83^{c}	2.89^{bc}	3.04^{a}	3.01^{ab}	0.15	<0.001
A+B:P	3.33^{c}	3.39^{bc}	3.58^{a}	3.54^{ab}	0.16	<0.001
Ammonia-N, mg/dL	4.38	5.04	4.72	4.93	0.61	0.45

[1] Volatile fatty acid.

^{abc} Means within same row without a common superscript differ ($P \leq 0.05$).

^{xy} Within a row, different superscripts differ at ($P \leq 0.10$).

Table 2.10. Ruminal digesta characteristics and digestion kinetics of lactating Holstein cows (n = 14) fed the treatment diets.

	Diets					
	Low uNDF240		High uNDF240			
Item	Low peNDF	High peNDF	Low peNDF	High peNDF	SEM	*P*-Value
Ruminal digesta volume, L	110	111	116	111	4	0.42
Ruminal digesta mass, kg	95	95	100	97	4	0.34
Ruminal density, kg/L	0.86	0.86	0.87	0.87	0.01	0.72
Ruminal pool, kg						
Starch	0.40	0.37	0.36	0.31	0.03	0.16
aNDFom[1]	8.19	7.93	8.72	8.38	0.36	0.06
uNDF240om[2]	3.83[b]	3.72[b]	4.51[a]	4.42[a]	0.16	<0.001
Organic matter	12.67	12.34	12.93	12.38	0.53	0.43
Ruminal turnover rate, %/h						
Starch	123.1 (91.3 to 166.1)[3]	154.6 (114.6 to 208.5)[3]	121.0 (89.7 to 163.2)[3]	154.3 (114.4 to 208.0)[3]	-	0.39
aNDFom	4.4[x]	4.4[x]	4.2[xy]	3.9[y]	0.2	0.04
uNDF240om	2.7	2.8	3.0	2.7	0.1	0.29
Organic matter	8.7	8.8	8.4	8.0	0.4	0.15
Ruminal turnover time, h						
Starch	1.4	1.3	1.3	1.3	0.1	0.84
aNDFom	23.2[y]	23.2[y]	24.4[xy]	26.1[x]	1.3	0.04
uNDF240om	37.4	37.1	34.6	37.0	1.8	0.34
Organic matter	11.8	11.8	12.1	12.9	0.6	0.13

[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.

[3] 95% confidence level.

[a,b] Means within same row without a common superscript differ ($P \leq 0.05$).

[x,y] Means within a row without a common superscript differ ($P \leq 0.10$).

Table 2.11. Total tract digestibility data of lactating Holstein cows (n = 14) fed the treatment diets.

	Diets					
	Low uNDF240		High uNDF240			
Item[1]	Low peNDF	High peNDF	Low peNDF	High peNDF	SEM	*P*-value
Dry matter, %	68.0^{ab}	65.6^{b}	69.5^{a}	68.1^{ab}	1.0	0.05
Organic matter, % of DM	70.2	68.4	71.0	69.8	0.9	0.25
aNDFom[2], % of DM	51.2	51.0	52.0	50.8	0.8	0.74
Potentially digestible NDF, % of DM	70.8^{b}	71.7^{ab}	74.0^{a}	73.7^{ab}	0.8	0.02
Starch, % of DM	98.3^{b}	98.5^{ab}	98.6^{ab}	98.8^{a}	0.2	0.07

[1] Values are ash-corrected.

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

^{ab} Means within same row without a common superscript differ ($P \leq 0.05$).

CHAPTER 3: PERSPECTIVES AND CONSIDERATIONS

This thesis focused on the relationships between dietary undigested neutral detergent fiber after 240 hours of fermentation, ash corrected (uNDF240om) and physically effective neutral detergent fiber (peNDF). It was found that cows fed rations formulated to contain low uNDF240om and low peNDF content exhibited greater intakes and milk yield and more favorable chewing behavior compared to high uNDF240om and high peNDF concentrations. A useful relationship between the low uNDF240om, high peNDF and high uNDF240om, low peNDF treatments was found, reflective of similar dietary physically effective uNDF240 (peuNDF240). Additionally, reducing dietary peNDF or the particle size of feedstuffs when uNDF240om was elevated resulted in greater intakes, milk yield, and less time spent eating.

If future research confirms this relationship between uNDF240om and peNDF, optimization of the physical and digestible components of fiber in dairy cow diets could be better achieved. For example, in situations where forage maturity is advanced due to weather conditions it could be assumed that uNDF240om concentration has become elevated as well. Adjusting the chop length at the time of harvest, thereby altering peuNDF240, to compensate for the elevated uNDF240om would allow for greater intake, milk yield, and less eating time. If future research confirms this concept, the dairy industry will benefit greatly, providing options at the time of harvest or forage feeding to proactively adjust forage chop length to aid in ration formulation.

3.1. Forages and Feeding Systems

The current study focused on corn silage- and grass-based total mixed rations (TMR) which are common in the northeastern US and upper Midwest. However, it is important to note that there are several other ingredients that need to be investigated to test the uNDF240om and peNDF relationship. The use of legumes in the place of grass is a common practice in the dairy industry. Comparing legumes and grasses with characteristic differences in uNDF240om and rate of NDF digestion would allow for a more complete understanding of the relationship between particle size and NDF indigestibility. Legumes typically contain greater uNDF240om concentrations compared to grasses, but the fractional rate of digestion of the potentially digestible NDF (i.e., aNDFom – uNDF240om) is much greater in legumes than grasses (Raffrenato et al., 2019). Furthermore, it is anticipated that legumes would elicit faster ruminal passage rates due to selective retention of grasses, related to structural differences (such as pattern of lignification) and resultant fragmenting into longer particles than legumes (Kammes and Allen, 2012).

In addition to comparing legumes and grasses, varying starch content of diets and possible interactions with peuNDF240 (i.e., particle size and indigestibility) needs to be better understood. The study presented in this thesis contained moderate starch (approximately 24 to 25% of DM), but rations are commonly formulated within a range of 20 to 30% starch in the US. Previous research has shown starch content/rumen starch fermentability and fiber fermentation to be negatively associated (Poore et al., 1993). A better understanding of how differing concentration and rumen fermentability of dietary

starch interacts with varying uNDF240om and peNDF is needed. The current study altered the forage-to-concentrate ratio within a reasonable range observed in the industry. Focusing on the extreme cases where the forage percentage exceeds 70% or is below 40% would aid in situations when forage availability dictates different feeding amounts. When the forage percentage is extremely low (<40% of ration DM), non-forage fiber source (NFFS) ingredients need to be examined. Generally, NFFS contain differing carbohydrate profiles depending on their source, have small particle size, and low uNDF240om concentrations, and the interaction with forage fiber in dairy cow rations needs to be explored. The current study focused on the use of beet pulp pellets, but the use of corn gluten feed, wheat middlings, or other NFFS is not well understood from a uNDF240 versus peNDF perspective.

Finally, other feeding systems aside from TMR need to be investigated. Dietary uNDF240om and peNDF in pasture-based systems and the use of partial mixed rations (PMR) in robotic milking systems need to be researched. Both of these systems deliver fiber in a different manner compared to the method used in the present study and varied animal response is anticipated because of this.

3.2. Incorporating Management Effects

Another critical aspect of this study was the minimization of management influences. The use of the tie-stall system allowed for unrestricted use of the feedbunk, stalls, and water bowls. The incorporation of variable stocking density when comparing dietary uNDF240om and peNDF would allow for a better understanding of a competitive feeding scenario. Additionally, during the current study, a minimal amount

of time (<1 hour per day) was spent away from the stalls for milking. While this can be achieved in the dairy industry with common free-stall systems, it is not common. Previous research indicated that elevated stocking density is more adverse to ruminal pH than increasing dietary peNDF or uNDF240om (Campbell and Grant, 2016). An understanding of how varying dietary uNDF240om and peNDF impacts animal responses when the animal's time budget is compromised is needed. Time budget influencers include extended times away from the pen for milking, the time spent locked up at head-locks, and feed availability. Presence of these influencers will elevate stress experienced by the cow and may impact how the animal responds to the dietary treatments.

Finally, feeding frequency and feed push-up strategy are two common management practices that will influence meal behavior and ultimately the response to differences in dietary fiber. Increasing feeding frequency and feed push-up and reducing dietary uNDF240om and peNDF will promote smaller and more frequent meals which should result in better ruminal pH for fiber fermentation (Pitt and Pell, 1997).

3.3. Animal Differences

The last area that will need to be investigated to fully understand differences in uNDF240 and peNDF is differences characteristic of the dairy cow herself. The present study used high producing Holstein dairy cows. To truly understand how uNDF240om and peNDF impact dairy cows, other breeds and cows at different stages of lactation will need to be tested. Implications of differing fiber and fermentable carbohydrate

requirements of early and late lactation as well as non-lactating mature and immature cattle needs to be understood. Due to the reduced energy requirements of the non-lactating animal, it is expected that dietary uNDF240om and peNDF concentrations would be elevated in comparison to the high producing lactating animal to maintain healthy ruminal function.

3.4. Final Perspectives

A better understanding of the previously discussed variables will aid in gaining a true nutritional understanding of dietary uNDF240om, peNDF, and ultimately peuNDF240. With the findings of the present study, it appears that peuNDF240 is closely related to several animal response variables, notably dry matter intake, energy-corrected milk yield, eating time, ruminal pH, and VFA concentrations. If future research confirms the usefulness of integrating pef and uNDF240om, then peuNDF240 could become a useful aid in characterizing fiber in dairy cow diets.

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