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The impact of plant cell wall lignin on energy utilization in lactating Jersey cows.

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by

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Fiber from forages comprises a significant proportion of dairy cattle rations and by extension, it contributes largely to the energy concentration of the diet. While the proportion of fiber in the diet is important, the composition of fiber is probably more important as the different constituents of this fraction vary in their contributions to the nutritive value of the feed. Lignin has been described as an important factor limiting the digestion of NDF, reducing intake, and compromising milk production. Although lignin's effects on these responses have been well characterized, the literature lacks data on the use of indirect calorimetry to evaluate the dietary lignin concentration. Therefore, there is a need to evaluate the energetics of lignin, and its relationship with the energy concentration of the entire NDF fraction.

The first experiment used 16 NDF residues from individual feeds or mixed rations to analytically determine the GE concentration of feed NDF. This value was compared to that of fecal NDF, which was analytically determined from 34 fecal NDF residues. The GE concentration of feed NDF was found to be lower than that used in the Dairy NASEM (2021) model's equations used to calculate dietary gross and digestible energy concentrations. If the observed NDF GE concentration is representative of the true GE concentration of NDF, this result suggests that the Dairy NASEM (2021) model is overpredicting the energetic contribution of NDF. Additionally, this study reports that feed NDF is of a greater energy concentration relative to fecal NDF. This result suggests that nutritional models likely do not capture the full scope of NDF digestibility in their predictions of energy utilization.

Lignin's impact on utilization of energy and nitrogen was examined using twelve multiparous lactating Jersey cows in a two period crossover design. Diets were formulated so to be equal in NDF concentration but differing in their NDF profiles. The LoLig diet contained 32.5% NDF (% DM) and 9.59% lignin (% NDF) while the HiLig diet contained 31.0% NDF (% DM) and 13.3% lignin (% NDF). Interestingly, increasing the concentration of lignin not only decreased the digestibility of NDF, but also CP and starch, likely due to decreased fermentability by ruminal microbes. The effects of reduced digestibility carried through to metabolizable energy concentration but not net energy concentration, likely due to an underpowered experiment or cumulative error associated with calculating net energy. Increasing the concentration of dietary lignin shifted nitrogen excretion from the urine to the feces, which is considered to be better for the environment. Feeding the HiLig diet resulted in lower yields of milk, fat, and protein, suggesting that the impacts of increasing dietary lignin concentration might impact more factors than NDF digestibility.

“But I keep cruising, can’t stop, won’t stop moving. It’s like I got this music in my mind saying ‘It’s gonna be alright.’” – Taylor Swift

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TABLE OF CONTENTS

Page

CHAPTER 3

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Due to a unique symbiotic relationship with ruminal microbes, ruminants have the ability to utilize mammalian enzyme-resistant fiber to synthesize milk. These microbes convert fiber into a variety of fermentation end-products that can be metabolized by the cow to support the energy demands of lactation. The complete oxidation of fiber from either fermentation or bomb calorimetry liberates the same amount of energy (Alberts et al., 2002). Consequently, bomb calorimetry can provide nutritional insights into the supply of energy from fiber. The digestion of fiber in ruminant is complex, and under the influence of both dietary and animal factors. Dietary factors that influence the digestion of fiber includes the concentration of NDF (Miller et al., 2021), starch (Mertens and Loften, 1980), protein (Lee et al., 2012), and lipids (Pantoja et al., 1994) in the ration, as well as the sources of these nutrients (Weld and Armentano, 2017). Dry matter intake and ruminal passage rate (Jung and Allen, 1995) are also important animal factors that influence the extent of fiber digestion, and therefore affects the yield of energy yield from fiber. Jung (1989) reported that of all dietary and animal factors, the concentration of lignin within the NDF fraction to be the most limiting factor of NDF digestibility. Although lignin's effects on the digestibility of NDF have been well characterized, less literature has been published on the broader impact of lignin on the energy balance of lactating dairy cattle. Uddin et al. (2020) increased the lignin content of rations similar in NDF content by 3.7% (% NDF) by replacing corn silage with alfalfa silage and decreased the metabolizable energy concentration of the diet by 4.6%. In this study heat production was not measured so investigators could not report observed impacts of lignin on the net energy for lactation (**NEL**). Gislon et al. (2020) observed a 20% decrease in NEL concentration when increasing lignin concentration from 7.01 to 9.29% (% NDF) by varying the proportions of corn silage, alfalfa hay, and Italian ryegrass

hay. The difference in lignin concentrations in these diets was small (2.28% % NDF) and therefore might not capture the entirety of lignin's impacts on energy balance of lactating dairy cattle and the supplementation of Italian ryegrass hay might make this study less applicable to U. S. dairy production.

The current Dairy NASEM (2021) assumes a gross energy (**GE**) coefficient of 4.20 Mcal/kg for NDF in its estimation of the dietary GE and digestible energy (**DE**) concentrations. However, the use of this GE coefficient to describe the NDF fraction might be imprecise and thus contribute to additional variance in point estimates of GE and DE. This is because the NDF fraction is composed of several chemically unique and distinct entities. The NDF fraction consists of namely cellulose, hemicellulose, and lignin (Van Soest et al., 1991). The major constituents of the NDF fraction are known to vary in their proportions within the NDF fraction (Van Soest, 1994a). Additionally, the energy concentration of a substance is a function of its chemical structure, and so the respective energy concentrations of cellulose, hemicellulose, and lignin are different because each entity varies in chemical structure and GE concentration. The GE concentration of cellulose, a simple polymer of linear glucose units bound by 1, 4-beta linkages, is 4.15 Mcal/kg (Colbert et al., 1981). This contrasts with the GE concentration of lignin of softwood trees, an amorphous polyphenolic entity, has been experimentally determined to be 6.00 Mcal/kg by Voitkevich et al. (2012). The literature lacks a universal GE concentration of hemicellulose, another complex polymer with a xylan backbone with several different branching sugars. The reported GE concentration for hemicellulose range from 3.04 Mcal/kg (Dorez et al., 2014) to 3.25 Mcal/kg (Gorensek et al., 2019). Additionally, the ash content of the NDF fraction can also influence the overall GE concentration. Changes in the chemical composition of the NDF fraction, most importantly lignin, are realized over the entirety of the

fiber's GE concentration. This suggests the use of a GE coefficient may be imprecise and impair the estimation of NDF energy to the animal. Thus, the relationship between NDF content and energy supplied should be further examined because it could be a contributing factor to variation in estimating DE. For these reasons, the objectives of this study were 1) analytically measure the energy concentration of NDF and 2) evaluate the impact of lignin on fiber digestibility and energy supply in vivo and to compare it to that estimated by Dairy NASEM (2021).

CHAPTER 1

LITERATURE REVIEW

Common forages in dairy diets

Corn silage as a forage in dairy rations

Corn silage is commonly used in dairy cattle rations because of its high concentration of energy (Johnson et al., 1999). The typical corn silage contains 40.9 ± 4.75% NDF and 32.9 ± 6.42% starch (NASEM, 2021) on a dry matter basis, this makes corn silage an excellent source of fiber without comprising energy density. It is important to note that the lignin content of typical conventional corn silage is 3.05 ± 0.564% on a DM basis or 7.46% on an NDF basis (NASEM, 2021). This makes the proportion of lignin in the NDF fraction relatively low compared to other forages, such as alfalfa hay. Corn silage is also relatively low in protein. According to the NASEM (NASEM, 2021), the average corn silage only contains 7.7 ± 0.94% CP on a DM basis. This is well below the Erickson and Kalscheur (2020) recommendation of 16% CP for high-producing dairy cows and thus there are other forage options for formulating dairy cattle rations. The DM content of a typical mid-maturity corn silage averages 35.4 ± 5.38%, which is an important factor in the stability of corn silage (Borreani et al., 2018).

Corn silage is a lactic acid-based fermented feed, which is known to increase storage stability of the feed. This is because the predominant microbes of silage fermentation produce a fermentation profile that selectively inhibits microbes involved in the spoiling process of silage (Avila and Carvalho, 2019). Fermented feeds, like corn silage, are also known to increase in their ruminal digestibility of starch due to the degradation of the starch-protein matrix while ensiling (Der Bedrosian et al., 2012). The effects of ensiling time on NDF digestibility remain unclear. Ferraretto et al. (2015) reported no differences in NDF digestibility for corn silage ensiled for lengths of time, where Hristov et al. (2020) reported a linear decrease in in situ 48-hour NDF

digestibility for silages ensiled from 0 to 150 days. The 9.2% decrease in ruminal NDF digestibility observed in Hristov et al. (2020) is thought to be a function of degrading hemicellulose during the storage phase in the production of lactic acid. Decreasing the proportion of hemicellulose during ensiling would increase the relative proportion of indigestible lignin in the NDF fraction during feed out, decreasing NDF digestibility. However, Kang et al. (2009) reported that the use of an enzymatic inoculant used at the time of ensiling increased in vitro 48-hour NDF digestibility by 5.7% after 110 days of ensiling. The use of silage inoculants to increase NDF digestibility yields inconsistent results, but the use of inoculants to decrease spoilage of silage upon feed out has yielded more consistently positive results (Muck et al., 2018). Although corn silage is easily produced and fed on U. S. dairies, lack of adequate rainfall or irrigation technologies can limit corn production for ensiling in arid or semi-arid environments (M. Simsek et al., 2011). To overcome this, producers in areas with restricted access to water often supplement rations with alternative forages, such as triticale (Santana et al., 2019) and sorghum (McCary et al., 2020), that require less water to produce.

Alfalfa hay as a forage in dairy rations

Alfalfa hay is another commonly utilized forage in the U.S. dairy industry. Typical mid-maturity alfalfa hay has an NDF content of 41.1 ± 4.84% and a starch content of 1.5 ± 0.85% on a DM basis (NASEM, 2021). Therefore, most of its energy associated with alfalfa is in the form of fermentable NDF. Although alfalfa hay is relatively similar in NDF content compared to corn silage, the lignin content of alfalfa on average is 6.64 ± 1.15% on a DM basis or 16.2% on an NDF basis (NASEM, 2021). With regard to chemical composition, alfalfa hay is relatively similar to corn silage in NDF content but differs in starch and lignin contents. The potential detrimental nutritional effects of increasing dietary lignin concentration have been extensively covered in the literature and will be discussed later in this review. The low starch content of

alfalfa hay is compensated for by its relatively high CP content of 20.7 ± 2.37% of DM (NASEM, 2021). Alfalfa hay can be fed as an alternative to corn silage in terms of meeting protein requirements of the lactating dairy cow. The DM content of alfalfa hay is 88.1 ± 2.95%. The high DM content of alfalfa hay is beneficial in its storage capacity.

Logistically, Coblentz et al. (1996) observed that long-term storage of alfalfa hay of low moisture (10-15% moisture) content generally does not have an effect on NDF content. Alfalfa hay is typically stored at 85% or greater dry matter for greatest nutritive value and minimal nutritional losses from microbial spoilage (Killerby et al., 2021). Achieving this dry matter content can be difficult due to the nature of harvesting alfalfa and the drying process. The alfalfa crop is chopped, swathed, and left in the field to wilt until the desired dry matter content is reached. The drying process can take several days but during this time the drying process may be negatively impacted by precipitation, which can increase the moisture content of the feed and subject it to microbial degradation upon storage (Tomes et al., 1990). The drying process can be expedited via different drying methods such as swath turning and oven drying, which potentially reduces the probability of swathed alfalfa getting rained on (Neres et al., 2010). Yang et al. (1993) demonstrate that heat-treating alfalfa hay increased the acid detergent insoluble nitrogen (**ADIN**) fraction of alfalfa. This treatment is not perfect, but it makes protein resistant to microbial degradation in the rumen and allows it to be partially digested in the abomasum and metabolized directly by the cow (Yang et al., 1993).

Utilization of corn silage and alfalfa in tandem

There is evidence that supports using both corn silage and alfalfa as forage sources. Hristov and Broderick (1996) reported that cows fed an all-corn silage diet had greater digestibility of NDF and CP compared to cows fed an all-alfalfa hay and all-alfalfa silage diets.

Brito and Broderick (2006) reported a 5% increase in DMI and a 12% increase in milk fat concentration when feeding rations formulated with alfalfa silage compared to corn silage as the sole forage source. The complimentary nutrient profiles of corn silage and alfalfa lend these ingredients to be used in tandem when formulating rations for dairy cattle. Arndt et al. (2015) observed that using equal amounts of corn and alfalfa silages yielded the greatest fat- and protein-corrected milk yield relative to diets formulated with solely corn or alfalfa silage. Hassanat et al. (2013) observed an environmentally-advantageous shift in nitrogen metabolism from urinary nitrogen to fecal nitrogen (covered more extensively later in this review) when feeding equal inclusions of corn silage and alfalfa silage. The incorporation of corn silage and alfalfa into dairy rations not only has nutritional benefits, but also agronomic benefits. Grasses like corn silage are known to deplete soil organic matter, specifically nitrogen, whereas legumes like alfalfa foster beneficial relationships with soil microbes that help to replenish soil nitrogen concentrations (Havlin et al., 1990; Carlsson and Huss-Danell, 2003).

The NDF fraction

The NDF fraction and its contribution to nutrition and rumen health

The concentration of NDF within a feed is an estimate of its cell wall concentration and is representative of forage quality (Jung and Lamb, 2004). The NDF fraction is heterogenous in nature, with each component differing in chemical, physical, and nutritional properties (Van Soest et al., 1991). Cellulose, hemicellulose, and lignin are the main constituents of the NDF fraction, but nitrogen and ash can also be bound within this fraction. The proportions of cellulose, hemicellulose, and lignin within the NDF fraction are dependent upon forage species, but cellulose is typically the most abundant, followed by hemicellulose, and then lignin (Van Soest, 1967a). Smith et al. (1972) compared legumes and grasses of different species and maturities and reported the average relative concentrations of cellulose, hemicellulose, and lignin

to be approximately 50, 35, and 15% on an NDF basis, respectively. The insoluble protein bound within the NDF fraction (**NDICP**) is thought to be largely insoluble with a ruminal digestibility of only 6-32% depending on the feed source (Mustafa et al., 2001). These proteins are in the form of extensin proteins, which function to maintain structural integrity among plant cells (Mnich et al., 2020). Crocker et al. (1998) compared NDF residues from multiple forages before and after ashing and reported no more than a 1% difference between the samples on a DM basis.

Ruminants have a unique digestive tract that allows them to form symbiotic relationships with microbes. The host cow provides an anaerobic environment with a relatively steady flow of substrates and in return the microbes convert otherwise indigestible forages into volatile fatty acids (**VFA**) which can be metabolized by the animal for energy. Dietary forages not only contributes an average of 1.35 Mcal NE_L/kg of energy to cows during lactation (Weiss, 1993), but are also crucial for reducing incidences of ruminal acidosis.

The concentration of acids, and therefore pH, in the rumen is primarily a function of VFA and lactic acid production and assimilation. The rate of digestion for starch is inherently faster than that of fiber, and therefore the rate of acid production is much faster for starch relative to fiber. Acid accumulation in the rumen can cause systemic metabolic disease and needs to be assimilated (Plaizier et al., 2008). Volatile fatty acids can be assimilated via absorption through the rumen epithelium, passage to the lower gut, and neutralization from bicarbonate. Bicarbonate can enter the rumen via saliva from rechewing and reswallowing or from antiport of VFA across the rumen wall, the latter being the more prominent pH-balancing mechanism (Ash and Dobson, 1963). Although the proportion of buffering capacity of saliva is of lesser significance, the influx of bicarbonate from saliva is under dietary control. Yang and Beauchemin (2007) observed feeding alfalfa silage of greater particle size increased the mean pH and chewing time per kg DM

of lactating dairy cows. Longer feed particles are regurgitated and rechewed to break the protective layers of plants and expose new nutrients as well as increases the surface area for microbial fermentation (Beauchemin, 1991). The remastication of feed particles inoculates them with bicarbonate from saliva and aids in the neutralization of VFA in the rumen.

Cellulose

Cellulose typically accounts for the majority of the structural components responsible for maintaining the structural integrity of plants. Colburn and Evans (1967) completed fiber analysis on 21 forage samples, including grasses and legumes, and reported cellulose concentrations ranged from 24.2 to 35.0% on a DM basis or 40.7 to 59.8% on an NDF basis. Fibrils of cellulose are bound together by mostly linear 1,4-beta bond between glucose monomers. The stereochemistry of the cellulose fibril is unique because mammalian enzymes are unable to cleave beta bonds. The beta bond linkage between glucose units allows for a highly uniform, crystalline structure that allows for hydrogen-bonding between and among fibrils (Parthasarathi et al., 2011). However, microbial enzymes can degrade the glycosidic bonds found in cellulose. Using bomb calorimetry, Colbert et al. (1981) observed the average gross energy (**GE**) concentration of cellulose to be 4.15 Mcal/kg.

Hemicellulose

The structure of hemicellulose is that of a branched, heteropolymer of pentoses and hexoses (Deng et al., 2016). Colburn and Evans (1967) report hemicellulose accounts for 23.3% of DM and 38.5% of the NDF fraction. Hemicellulose is important for crosslinking cellulose with lignin through xylose and glucomannan complexes. It is important to note that hemicellulose covalently binds lignin whereas hemicellulose forms hydrogen bonds with cellulose (Morrison, 1979). A study by Buxton and Brasche (1991) testing NDF component digestibility from four forage types across two cuttings reports the average digestibility of

cellulose was 34.9% than that of xylose, the principal constituent of hemicellulose. In addition, they also observed the digestibility of secondary sugars in hemicellulose, arabinose, galactose, mannose, and rhamnose were 19.6, 26.0, 26.3, and 18.7%, respectively, more digestible than cellulose. This ultimately makes hemicellulose, on average, 24.4% more digestible relative to cellulose in the forages tested (Buxton and Brasche, 1991). Dorez et al. (2014) reports the GE concentration of xylan (two bound xylose sugars) to be 3.04 Mcal/kg.

Lignin

Plants deposit lignin into their cell walls to aid in structural integrity and defense against pathogens (Brown and Chang, 2014). Colburn and Evans (1967) report the average concentration of lignin to be 2.8% on a DM basis and 4.8% on an NDF basis in the forages they analyzed. The lignin found in forages can either be categorized as core lignin or non-core lignin (Hartley, 1972). Feofilova and Mysyakina (2016) defines core lignin as a heteropolymers of aromatic phenyl propane units covalently bound together. Non-core lignin is then defined as extensions of the core lignin, usually as single aromatic subunits that form covalent bonds with hemicellulose (Jung, 1989). Due to its unique physical and chemical structure, both core and non-core lignins are considered completely undegradable by mammalian and bacterial enzymes (Dong et al., 2011). Microbial enzymes often use free radicals to oxidize cell wall components, but the aromatic structure of lignin monomers make lignin resistant to this degradation (Dizhbite, 2004). Some fungi encode enzymes, such as magnesium peroxidase, that are successful in oxidizing lignin, but these fungi are only found in aerobic conditions and do not apply to ruminal conditions (Hofrichter, 2002). Due to its ability to evade microbial degradation in the rumen, the lignin content of forages is the primary factor affecting NDF digestibility (Van Soest, 1994a). Because the chemical structure of lignin is more reduced relative to that of cellulose and hemicellulose, the GE concentration of lignin is greater at 6.0 Mcal/kg (Voitkevich et al., 2012).

Laboratory assays to measure NDF and lignin

The standard assay for measuring NDF in forages is according to Van Soest et al. (1991). In this assay, samples are boiled in detergent of pH 7 because cellulose, hemicellulose, and lignin are least soluble at this pH, meaning they will be retained on a filter as other soluble carbohydrates will not. This procedure includes the addition of 100 microliters of heat stable alpha amylase and 0.5 grams of sodium sulfite to remove starch and protein, respectively, found within the cytoplasm of the plant cell. It is important to note that if one wishes to do further analysis on the NDF residue produced (lignin analysis), sodium sulfite should not be used as it solubilizes a small portion of lignin.

There are multiple methods for determining the lignin concentration of a feed sample, but the most common used in nutrition are the acid detergent lignin (**ADL**) and Klason lignin (**KL**) methods (Jung et al., 1997). The ADL method was derived from Van Soest (1963) and starts with an NDF residue. The sample is then refluxed in acid detergent solution for an hour before being filtered. This process yields an acid detergent fiber (**ADF**) residue of primarily lignin and cellulose with some potential ash and nitrogen contamination. The ADF residue is then soaked in concentrated sulfuric acid for 3 hours to remove cellulose and ash, yielding a pure lignin sample. The KL procedure uses the same methodology as the ADL procedure, but uses spectrometry to account for lignin that has been solubilized by the strong sulfuric acid (Van Soest et al., 2018). It is for this reason that the KL method will always yield a greater lignin content than ADL method.

Neutral detergent fiber and the cell wall

The plant cell wall consists of cellulose, hemicellulose, lignin, and structural proteins embedded in a matrix of gelatinous pectin (Cosgrove, 2016). Because the cell wall contains pectin, which is solubilized in neutral detergent solution, the NDF fraction is differentiated from

the cell wall by definition (Cassida et al., 2007). The recoverable portion of pectin in forages fed to dairy cattle is relatively low (< 1%) (Borshch et al., 2020), but the structural role of pectin in the cell wall is important nonetheless. Pectin is composed primarily of a galacturonic acid backbone with branching of arabinose, fructose, glucose, and xylose (Udén, 2018). Pectin is not only present in the primary cell wall, but also the middle lamella. The middle lamella serves to connect the growing primary cell walls of various plant cells together, aiding in the structural integrity of the plant (Zamil and Geitmann, 2017). The plant's primary cell wall is initially synthesized from plasma membrane-bound proteins, but as the structural polymers elongate, the plant will start to secrete synthetic enzymes into the primary cell wall to further these biosynthetic processes (Zhang et al., 2021). The lumen of the plant is surrounded by the plasma membrane and contains the proteins and organelles required for the plant's metabolism. Directly adjacent to the plasma membrane is the primary cell wall, which contains the structural components discussed previously.

Digestion of NDF

Factors of NDF digestibility

Ruminal digestion of fiber is a function of the competing rates of degradation and passage out of the rumen (Allen and Mertens, 1987). A more intensive rate of degradation of fiber would result in greater VFA production and therefore increase the energy supply to the cow, but this might come at the cost of passage rate and limit new DM from entering the rumen. Fiber either leaves the rumen as fermentation end products, is absorbed through the rumen wall or is left intact where it might be further degraded in the abomasum or large intestine. Using omasal and duodenal cannulas, Ahvenjärvi et al. (2000) observed an average of 6.1% difference between ruminal and total-tract NDF digestibility, suggesting postruminal fiber digestion is relatively minimal. Increasing fiber digestibility is of utmost importance because for every one

percent increase in NDF digestibility, cows can produce 0.25 kg of fat-corrected milk (Oba and Allen, 1999a). This is supported by the NRC (2001) calculation of one kg of digested NDF supplying 2.50 Mcal of NE_L, which supports 3.57 kg of milk. The rate of ruminal NDF digestion is complex and is influenced by physical, chemical, environmental, agronomic, and genetic factors.

The effect of ruminal passage rate and particle size on NDF digestibility

A predictive equation developed by Allen and Mertens (1987) for NDF digestibility demonstrates the relationship between the rate of digestion and the rate of passage from the rumen. The simplified version of this model is as follows:

$$\text{Extent of Digestibility} = k_d / k_d + k_p \quad [1]$$

k_d = rate of digestion (proportion/h)

k_p = rate of passage (proportion/h)

Theoretically, because k_p is in the denominator, there is an inverse relationship between the extent of fiber digestion and the rate at which fiber leaves the rumen. Many experiments evaluating particle size and specific gravity have been conducted to describe the relationship between the rates of digestion and passage (Campling and Freer, 1962; Welch, 1986; Kaske et al., 1992). Particle size is an important factor contributing to the rates of digestion and passage of fiber. Forage particles that are too small to be ruminated can be fermented too quickly and this minimizes their role in pH buffering and also increases passage rate. Teimouri Yansari et al. (2004) demonstrates this by chopping alfalfa silage at three different lengths (2, 10, and 19 mm sieves) at the time of harvest to somewhat mimic the screens of the modified Penn State Particle Separator (Kononoff et al., 2003). Their study reports a significant linear decrease in pH with

fine (1.66 mm geometric mean) alfalfa silage compared to long (3.34 mm geometric mean) alfalfa as well as decreasing forage particle size increased rate of passage from the rumen. Larger forage particles require rumination to reduce particle size and also are more likely to become trapped within the fiber mat in the rumen compared to smaller particles. Thus, larger particles are retained in the rumen longer, slowing passage rate and limiting DMI (Allen and Mertens, 1987). Yang et al. (2001) reports digestibility of NDF was greater for larger forage particles as they were retained in the rumen for longer periods of time. The particle size of a feedstuff has direct impacts on its specific gravity. Williamson and Wiemann (2010) defines specific gravity of plants as the density of plant material relative to another medium. Specific gravity is a ratio, where a linear relationship exists with increasing cell wall structural components and elevated specific gravity. Wattiaux et al. (1991) reported an increase in forage particle digestibility as specific gravity increased and predicted that these particles lose their buoyancy in the gaseous phase and are allowed to sink to the fluid and solid phases where microbial digestion of fiber is more complete.

The effect of pH and readily-fermentable carbohydrates on NDF digestibility

Low pH has been well-established as a factor negatively affecting the degradation of NDF. Work by Burroughs et al. (1949) and Stewart (1977) suggest that fiber digestibility is negatively correlated with dietary starch inclusion. There are many proposed mechanisms for this inhibition, one of which proposed by Smith et al. (1973) is that of the "carbohydrate effect." The "carbohydrate effect" implies that if a microbe has the enzymatic capability to metabolize easily-degradable sugars like maltose-glucose, they will preferentially degrade them over complex sugars like cellulose. Hoover (1986) suggests that this phenomenon occurs first before more complex mechanisms of fibrolytic inhibition take place. Mould et al. (1983) report a pH below 6.0 - 6.1 severely inhibits cellulolytic functions in the rumen. Cellulolytic enzyme

performance is greatest at a pH of 6.5-6.8 (Colombatto et al. 2007) because cows consuming rations formulated with sufficient forage are continuously regurgitating fiber particles and thus buffering the rumen quite well. Miwa et al. (1997) quantified gene expression involved in microbial acid tolerance and observed common fibrolytic bacteria do not express as many proton pumps as saccharolytic bacteria, even at the same pH points (6 to 8, at 0.5 increments). This indicates that fibrolytic bacteria do not have the genetic potential to maintain homeostasis under low pH environments like saccharolytic bacteria and therefore fiber digestion is compromised. In the case of Colombatto et al. (2007), NDF digestion decreased by 37% when comparing pH values of 5.72 and 6.72.

Cellulase enzymes are part of a coordinated scheme used by fibrolytic bacteria to degrade complex oligomers of carbohydrates into monomer subunits. Therefore, enzymatic activity of cellulases is subject to end product regulation by concentrations of monomeric glucose. Hsieh et al. (2014) demonstrated the glucose-binding activity and active site catalysis of cellulases diminish when free glucose concentrations in solution are high. High glucose concentration is thought to decrease cellulase activity because glucose is the final product of cellulose hydrolysis and therefore represents an environment where substrate is being produced faster than it can be utilized by the microbe (Andrić et al., 2010). If cellulolytic activity was not inhibited, maintaining high enzymatic activity would be energetically wasteful to cellulolytic microbes as well as potentially giving competing microbes a substrate to utilize (Solden et al., 2018).

The effect of nitrogen and lipids on NDF digestibility

In addition to readily-fermentable carbohydrates, dietary concentrations of rumen degradable protein (**RDP**) and tallows and oils are known to influence the efficacy of fibrolytic bacteria. Cellulolytic microbes utilize nitrogen from free ammonia to synthesis the enzymes they

use to maintain metabolism, produce fermentative enzymes, and replicate. Lee et al. (2012) reports that diets formulated to contain adequate RDP increased NDF digestibility by 4% compared to diets formulated to be inadequate in RDP as free ammonia was not limiting for synthesis of cellulolytic enzymes. While increasing supplemental protein in cattle rations is known to increase the extent of fiber digestion, results of increasing dietary lipid concentration have been variable. de Souza and Lock (2019) observed a 2.8% increase in NDF digestibility when supplementing cows with palmitic acid specifically. The increase in fiber digestibility could have been a function of palmitic acid serving as a growth factor as fibrolytic bacteria conserve energy by intaking palmitic acid from the environment opposed to synthesizing it de novo (Hackmann and Firkins, 2015a). A recent meta-analysis on the effects of fat supplementation on fiber digestion by Weld and Armentano (2017) found that in 27 studies using tallow, the average increase in fiber digestibility as 0.4% while in 16 studies using plant oils, the average decrease in fiber digestibility was 2.3%. The exact inhibitory mechanism for lipids impeded fiber digestion is unknown, but Henderson (1973) speculates it could be a function of decreasing ruminal pH by shifting rumen fermentation towards more rapid propionate production or due to inhibited microbial attachment to fiber particles. Pantoja et al. (1994) even observed the saturation of dietary fats plays an important role in fiber digestion, with unsaturated lipids being more potent against ruminal fiber digestion. The double carbon-carbon bonds in unsaturated fatty acids are thought to be at least somewhat responsible for their toxicity towards rumen bacteria. The double bond forms a ‘kink’ in the fatty acid, which may interfere with the structural integrity of the phospholipid bilayer (Maia et al., 2010).

The effect of plant species on NDF digestibility

Forage type is also an influential factor in the digestibility of the fiber component. The major forage classifications are legumes and grasses, with grasses being subcategorized into C3

and C4 plants. C3 plants grow in cooler climates where heat stress is generally not as prominent. Plants in warmer climates, such as C4 plants, synthesize lignin in order to give the plant the structural integrity it needs in order to stay upright and dissipate heat (Akin, 1989). Grasses and legumes differ primarily in their lignin content, which has adverse consequences for NDF digestibility (Jung, 1989). The NASEM (NASEM, 2021) compiled and averaged samples of oat grass hay (N = 12,949) and pea hay (N = 80) for lignin content on an NDF-basis and reported grasses to contain 5.3% less lignin (8.0% vs 13.3%) when reported as a percent of NDF compared to legumes. Although grasses generally contain less lignin relative to legumes, the composition and distribution of grass lignin accounts for more of the variation in NDF digestibility compared to legumes (Smith et al., 1972). Moore and Jung (2001) observed that at low concentrations of lignin (5% of NDF), NDF digestibility of grasses can be as high as 90% while legume NDF is approximately 70% digestible, suggesting there is an interaction between lignin concentration and species of plant. They (Moore and Jung, 2001) report that at high concentrations of lignin (15% of NDF), legume NDF averages 60% digestible while grass NDF averages 40% digestible. Legumes naturally contain more leafy material compared to grasses and leaves, which are composed of more non-core lignin than core lignin (Jung, 1989), and are the most digestible part of the plant. Therefore, total lignin content is generally greater for legumes, but a greater proportion of this total lignin is non-core lignin, which Jung (1989) suggests is potentially less limiting in terms of fiber digestibility. Work by Burritt et al. (1982) even suggests that the degree and composition of phenolic acids that connect lignin to hemicellulose in the plant cell have implications on NDF digestibility. For these reasons, Jung (1989) suggests that the composition of lignin, and not necessarily the quantity of lignin, is the most important contributing factor to fiber digestibility.

The effect of environmental and agronomic factors on NDF digestibility

Plants upregulate the epigenetic expression of lignin biosynthetic enzymes in response to agronomic and environmental stress (Lange et al., 1995). Grasses grown in warmer and drier climates need to effectively transport water from their roots to leaves to overcome metabolic stress (Giordano et al., 2014). These plants are able to transport a large capacity of water due to the increased lignin deposition in their cell walls. The warmer growth conditions of the plant ultimately have negative consequences on rumen fiber digestion. Galloway et al. (1991) reports the lignin content of Bermudagrass (warm season grass) was 5.8% on a DM basis compared to ryegrass (cool season grass) at 4.4% lignin on a DM basis. The same study (Galloway et al., 1991) reports the NDF digestibility of ryegrass was nearly 12.3% greater compared to that of Bermudagrass. In addition to where forages are grown, the density in which forages are planted within a plot influences NDF content and in vitro NDF digestibility. An agronomic study by Zheng et al. (2017) reports increasing plant density linearly increased NDF digestibility in wheat plants, citing an induced physiological response due to spatial and resource stresses. Jung (1989) suggests that the most influential factor of forage NDF digestibility is plant maturity. Multiple studies have reported more mature forages have greater lignin contents and are subsequently less digestible (Coblentz et al., 1996; Grev et al., 2020).

The effect of chemical and genetic treatments on NDF digestibility

Chemical treatments such as ammonia, sodium hydroxide, and oxidizing agents have been applied to forages in attempts to increase fiber digestibility (Hartley, 1983; Bals et al., 2010). Compounds such as ammonia are thought to have physio-chemical effects on the fiber constituents. Important work by Zorrilla-Rios et al. (1985) supports this notion by demonstrating that ammoniated wheat straw had similar concentrations of cell wall components but a greater fragility value compared to untreated wheat straw. The fragility of forage particles is defined as

the rate at which fiber particles are reduced in size during chewing or grinding (Prinsloo, 2014). Fragility coefficients are usually determined by the amount of energy a grinding mill uses to reduce the initial particle size of forages (Anelich, 2017). The lower the energy demand by the grinding mill, the greater the fragility of the forage. The fragility value of a forage is highly positively correlated with NDF digestibility, potentially due to lignin's role in maintaining structural support within the plant coupled with its indigestibility (Grant, 2010). Kendall et al. (2009) conducted a similar experiment with ammoniated wheat straw and observed a 20% increase in vitro NDF digestibility as well as a 4.35% increase in fat-corrected milk while maintaining NDF contents. These studies indicate that chemical treatments of fiber disrupt the bonds between fiber components, without altering the quantities of said cell wall entities. While these studies did not report any adverse pathological consequences of treating forages with ammonia, Lewis (1960) and Kertz (2010) suggest coupling ammonia treatments with urea supplementation can result in ammonia toxicity. The potential of ammonia toxicity in the herd and required equipment associated with applying chemical treatments to forages might allow for other means of increasing NDF digestibility, such as genetics. The brown midrib (**BMR**) genes in forage plants are an excellent example of characterized genetic factors that influences cell wall digestibility (Barrière and Argillier, 1993). The mutant alleles for the genes are known to have advantageous effects on lignin and total NDF content, but are also known to come at an agronomic cost because of the reduced crop yield associated with harvesting BMR forages (Rook et al., 1977). Oba and Allen (1999b) and Keith et al. (1979) replaced conventional isogenic corn silage with BMR corn silage and observed increases in milk yield due to increased fiber digestibility. In theory these responses are to be expected when feeding diets with greater NDF digestibility, but a later study by Tine et al. (2001) reports no difference in milk yield or

components when replacing isogenic corn silage with BMR corn silage. The discrepancies between these studies highlight the need for further investigation into the energetic contributions of NDF and the factors that influence its digestibility.

Microbial assimilation of NDF

Microbial attachment to fiber

Ruminal microbes have evolved many different mechanisms to survive in the rumen. Some microbes have established niches like adhering to the rumen wall to perform ureolysis from urea re-entering the rumen (Cheng and Wallace, 1979). Microbes associated with the rumen wall usually forego their motility and cannot move with feed particles in the solid and liquid phases of the rumen where more feed substrates are likely to be (Cheng and Costerton, 1980). Rumen microbes in the liquid and solid phases must first translocate to close proximity to forage particles if they are not already attached to start the process of enzymatic degradation of forages. Once close enough to act upon forage particles, bacteria will either secrete enzymes to degrade fiber while remaining unattached, loosely associated with the feed particles while degrading them, or firmly attach to feed particles while degrading them (Cheng and McAllister, 1997). Microbes that loosely associate with feed particles use non-covalent forces from weak dipole moments like van der Waals forces (Pell and Schofield, 1993). Fibrolytic bacteria that firmly attach to forage particles will initially partake in similar mechanisms, but then form iconic, hydrogen, or covalent bonds between adhesin proteins or binding sites on the cellulosome complex expressed by the bacteria (Pell and Schofield, 1993).

Microbial degradation of cellulose

Once fibrolytic bacteria are proximate or secured to fiber particles, they can begin to enzymatically degrade large polymers of cellulose and hemicellulose into smaller oligomers and eventually into monomer subunits of their respective sugars. The cellulosome is a complex of

active and structural proteins expressed on the surface of bacterial cells that aids in the attachment and cleavage of cellulose polymers (Koike and Kobayashi, 2009). The catalytic and scaffold protein composition of cellulosome complexes is known to vary between different species of bacteria depending on the specific degradation pattern used by the species (Morrison and Miron, 2000). Ohara et al. (2000) reported that *Ruminococcus albus*, a common fiber digesting bacterial species in the rumen, encodes 15 different proteins that comprise its cellulosome complex. The catalytic proteins found within the cellulosome are typically endoglucanases, exoglucanases, and beta-glucosidases (Weimer, 1992). The primary catalytic function of endoglucanases is to cleave long oligopolymers of cellulose into smaller polymers (cellodextrin) to increase the number of attack sites for downstream enzymes (Brown and Jurasek, 1979). Secondarily, these enzymes are also known to disrupt hydrogen bonding between highly condensed linear polymers of cellulose, furthering the degradation process (Wood and McCrae, 1979). Cellodextrin polymers produced via endoglucanase hydrolysis are then loaded into the active sites of exoglucanase proteins. Exoglucanases cleave the modified strands of cellulose into cellobiose, a disaccharide of glucose molecules connected via a beta bond (Wu and Wu, 2020). Cellobiases, also known as beta-glucosidases, then cleaves the glycosidic bond between the glucose sugars, yielding two molecules of free glucose (Krause et al., 2003). While some fibrolytic bacteria strictly metabolize free glucose of cellulose-origins, other bacteria can transport and metabolize cellobiose directly (Kawahara et al., 2012).

Microbial degradation of hemicellulose

Enzymatic degradation of hemicellulose is more involved than that of cellulose due to its more complex branching structure of various sugars (Houfani et al., 2020). Hemicellulolytic bacteria contain enzymes that degrade long oligopolymers of hemicellulose into shorter xylodextrin (hemicellulose-equivalent of cellodextrin) polymers, called endoxylanases (Warner

et al., 2012). Xylodextrins are cleaved by beta-xylosidases and other sugar debranching enzymes such as glucuronidases, arabinases, and mannases that release individual xylose sugars as well as the other sugars associated with the accessory hydrolytic enzymes (Juturu and Wu, 2013). Once complex oligopolymers of fiber have been broken down to single sugar monomers, bacteria use a variety of uptake mechanisms for sugar uptake. Different species of rumen microbes are known to use conserved and differentiated mechanisms, such as facilitated diffusion, symport/antiport channels, and osmotic pressure gradient differentials (Dills et al., 1980). Upon uptake of monomer sugars from complex structural carbohydrates, microbes metabolize these sugars into three main energetically-important end products: acetate, propionate, and butyrate (Carroll and Hungate, 1954).

Microbial fermentation end product synthesis

Volatile fatty acid production is not only a function of the rumen microbes present, but also the interactions between different groups of microbes and the rumen environment. As mentioned previously in this review, increasing the dietary forage concentration is associated with higher pH (lower H+ concentrations) and slower passage rate of digesta. A higher ruminal pH would be more thermodynamically favorable for H+ formation by microbes (Janssen, 2010). Microbial pathways associated with acetate and butyrate formation are known to produce H+ as a byproduct, which explains the positive correlation between acetate and butyrate production and dietary fiber content (Ungerfeld, 2020). Slower ruminal passage rate also allows for methanogens to resist washout from the rumen, which allots them more time to utilize H+ for methanogenesis, increasing ruminal pH and therefore favoring acetogenic and butyrogenic pathways (Ungerfeld, 2020). While VFA production is energetically favorable to the host animal, some microbes will specialize in the production of end products that contain energy

unavailable to the host but help to maintain a healthy rumen environment. Ruminal methanogenesis is a key example of this phenomenon.

Microbial acetogenesis

The first step in sugar metabolism of cellulolytic bacteria is glycolysis, the conversion of a single glucose molecule to two pyruvate molecules via ten enzymatic reactions which release CO_2. From this point, there are two types of acetogenesis pathways: acetate synthesis from pyruvate or CO_2. le Van et al. (1998) found the latter to be nearly negligible as the affinity for hydrogen used by CO_2-acetogenic bacteria is much lower than that of methanogenic archaea and therefore the most significant acetogenic pathway in the rumen is pyruvate-acetogenesis. Microbes decarboxylate two molecules of pyruvate to two molecules of acetyl-CoA using pyruvate-ferredoxin oxidoreductase which yields the hydrogen associated with acetate production (Schuchmann and Müller, 2016). The two molecules of acetyl-CoA are then converted to two molecules of acetylphosphate by phosphotransacetylase (Drake, 2013). Acetate kinase then converts acetylphosphate to acetate by dephosphorylating acetylphosphate, yielding two ATP for the microbe as well as acetate for the ruminant (Drake, 2013).

Microbial propiogenesis

The two major propionate-producing pathways employed by ruminal bacteria are the succinate decarboxylase pathway and the acrylate pathway (van Houtert, 1993). The succinate decarboxylase pathway converts succinate produced within or by other rumen bacteria to propionate. For bacteria that have to produce their own succinate to metabolize, glucose metabolism proceeds as it normally would under aerobic conditions until oxaloacetate is formed in the Krebs cycle. At this point, oxaloacetate is reduced to succinate when succinate decarboxylase removes a carboxyl group from succinate, yielding propionate. An experiment by Whiteley (1953) reports anaerobic decarboxylation of succinate yields equal parts CO_2 and

propionate, indicating this chemical reaction mechanism is a simple hydrogen shift. The acrylate pathway is responsible for the conversion of lactate into propionate (Ladd and Walker, 1959). In this pathway, lactate is protonated twice to yield propionate and assimilate hydrogen (Gonzalez-Garcia et al., 2017). It is important to note that the acrylate pathway acts as a hydrogen assimilation mechanism when ruminal pH falls below 5.5 - 6, which is too low for methanogenic archaea to energetically compete for hydrogen assimilation into methane (Ungerfeld, 2020).

Microbial butyrogenesis

Microbial synthesis of butyrate is unique in that its formation is more involved compared to other VFA due to the stoichiometry of synthesizing butyrate from glucose (Hackmann and Firkins, 2015a). To overcome this inefficiency, butyrate-synthesizing microbes have evolved mechanisms to convert acetate to butyrate. A study using heavy carbon isotopes by Bergman et al. (1965) demonstrated that 61% of all butyrate synthesized so via the conversion of acetate to butyrate. Butyrogenesis derived from acetate starts with the conversion of acetate to acetyl-CoA where a thiolase enzyme catalyzes the formation of a covalent bond between two, two-carbon acetyl-CoA molecules, forming the characteristic four-carbon butyrate precursor, acetoacetyl-CoA (Stim-Herndon et al., 1995). This intermediate is modified three more by hydroxybutyryl-CoA dehydrogenase, crotonase, and butyryl-CoA dehydrogenase before forming butyryl-CoA (Bennett and Rudolph, 1995). Synergistic catalysis of phosphotransbutyrylase and butyrate kinase substitute coenzyme A from butyryl-CoA with a phosphate group, which is subsequently removed to generate butyrate (Wiesenborn et al., 1989). Although the majority of butyrate is synthesized directly from acetate, acetyl-CoA produced from glucose-origins can be metabolized as well (van Houtert, 1993). This pathway utilizes the same enzymes from point of acetyl-CoA formation, differing only in the origins of the acetyl-CoA molecule.

Microbial methanogenesis

Excessive H+ in the rumen is detrimental to both microbes and the host. Low pH alters microbial metabolic pathways and can be fatal to microbes that are intolerant to low pH. From the host's perspective, a high acid concentration can compromise the rumen epithelium, allowing pathogenic organisms or their antigens to potentially cause systemic health incidences (Owens et al., 1998). Krause and Oetzel (2005) demonstrated that dairy cows challenged with subacute acidosis experienced a significant decrease in milk production for up to three days following onset of subacute acidosis. Bacteria and protozoa are primarily responsible for the production of VFA and therefore H+ in the rumen, while archaea are responsible for producing CH_4 and H_2O from pools of H_2 and CO_2 in the rumen (Van Soest, 1994b). Under normal metabolic conditions and rumen parameters, protozoa produce substantial amounts of acetate and butyrate (Russell and Hespell, 1981). As mentioned previously, the production of these acids is associated with H+ production and this has led to a symbiotic relationship between protozoa and archaea. Excessive H+ buildup in protozoa inhibits their metabolism by blocking the dissociation of hydrogen from electron carriers in the transfer of electrons used in metabolic pathways (Hegarty and Gerdes, 1999). In return, methanogens are able to use hydrogen from protozoa to establish a proton gradient in an effort to conserve energy input in the production of ATP (Buan, 2018). Thus transfer of protozoal hydrogen to methanogens is essential for maintaining microbial metabolism and the partial pressure of hydrogen in the rumen (Hungate, 1966). Upon hydrogen transfer, methanogens use a series of reactions known as the Wolfe Cycle to reduce one molecule of CO_2 to one molecule of CH_4 (Thauer, 2012). The complete reaction is reported below:

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O \qquad [2]$$

Ruminant metabolism of VFA

Ruminant metabolism of VFA

The lactating dairy cow is highly dependent upon VFA for meeting the energetic demands of maintenance and lactation. It is estimated that about 70% of the animal's energy comes come the metabolism of VFA produced by microbes (Bergman, 1990). As mentioned previously, dietary manipulations can be made to influence the fermentation profile produced by rumen microbes. This is important as microbial VFA are processed differently in the host and are associated with different concentrations of energy yield upon metabolism. For example, one propionate molecule can be modified to produce one molecule of glucose via hepatic gluconeogenesis. Complete aerobic oxidation of glucose theoretically yields approximately 30 ATP molecules per molecule of glucose (and therefore one molecule of propionate) (Rich, 2003). This is contrasted with the compete metabolism of one acetate molecule, which theoretically only yields 10 molecules of ATP (van Houtert, 1993).

Ruminant acetate metabolism

Acetate is the primary VFA produced by microbes in the rumen and is the favored end product of cellulolytic bacteria. Acetate is first transported across the rumen wall and into the blood pool of the host. Once acetate has been absorbed, it is converted to acetyl-CoA. Acetyl-CoA can either be used for energy production via oxidative phosphorylation or lipogenesis in adipose tissue or the mammary gland. During glycolysis, glucose is converted to pyruvate in the cytoplasm, which is then taken up by the mitochondria to be converted to acetyl-CoA before entering the TCA cycle. Acetyl-CoA synthetase uses coenzyme A and ATP to oxidize acetate to acetyl-CoA, conserving glucose for other metabolic processes (Schug et al., 2015). Acetyl-CoA for lipogenesis in ruminant adipose tissue is the primary pathway for acetate metabolism as the ruminant liver is almost obligately reserved for gluconeogenesis (Ingle et al., 1972). The first

specific step in fatty acid synthesis in ruminant adipocytes is the formation of malonyl-CoA by acetyl-CoA carboxylase (Burns, 2011). Fatty acid synthase is a large, multi-unit protein that carries out a series of condensation reactions to connect acetate molecules to the malonyl-CoA. In ruminant adipocytes, this process occurs until the fatty acid is 16 carbons long and is then bound to glycerol to form a triglyceride. Triglycerides can undergo beta-oxidation in the mitochondria to produce seven acetyl-CoA molecules, which enter the TCA cycle to reduce electron carriers for the electron transport chain and produce other intermediates used for amphibolic metabolic functions ((Han) van der Kolk et al., 2017). In the mammary gland, acetate is the primary carbon source used for the production of membrane-bound lipids (Ingle et al., 1972). The membrane surrounding these lipids fuse with the cell membrane of the alveolar cell during the secretory process to release triglycerides into the lumen of the mammary (Mather and Keenan, 1998). The site of lipogenesis is important to the fate of lipids formed. Somatic adipocytes synthesize lipids to contribute to the energy balance of the whole animal while mammary gland cells specialize in synthesizing lipids that will contribute to the nutritive value of milk.

Ruminant propionate metabolism

The predominant fate of propionate absorbed from the rumen is gluconeogenesis in the liver. van Houtert (1993) outlines the mechanism of converting propionate to succinyl-CoA, which enters the TCA cycle to be converted into oxaloacetate. Oxaloacetate can then either be directly converted to phosphoenolpyruvate or to malate by mitochondrial malate dehydrogenase before being exported to the cytoplasm (Wiltrout and Satter, 1972). Cytoplasmic malate is then modified to glyceraldehyde-3-P which forms hexoses capable of being converted to glucose. Glucose from the liver is transported to other tissues where it undergoes complete oxidative phosphorylation to yield maximal aerobic ATP yield. This makes propionate the most important

VFA in ruminant energy metabolism as it is the most predominant glucogenic fermentation end product produced by rumen microbes. Though propionate yields the most ATP per unit of VFA, the oxidation of glucose produced from propionate requires reducing agents produced during the metabolism of acetate and butyrate. Alternatively, metabolism of glucose can also yield glycerol, used in binding fatty acids formed from acetate together in lipogenesis.

Ruminant butyrate metabolism

The rumen epithelium is responsible for metabolizing butyrate into ketone bodies, namely beta-hydroxybutyrate by butyryl-CoA synthetase (Kristensen et al., 1998). These ketone bodies serve as energy-containing molecules that can be metabolized in different bodily tissues in the ruminant. Beta-hydroxybutyrate is oxidized further to acetyl-CoA and enters the TCA cycle. Once in the TCA cycle, acetyl-CoA of butyrate-origins can be metabolized similarly to that of acetate-origins. All VFA pass through the rumen epithelium before entering the bloodstream to get to their respective tissues for further metabolism. Butyrate is of great importance in rumen epithelium cells because the K_m of ketogenic enzymes used in the metabolism of butyrate is the most favorable of all VFA (Baldwin and McLeod, 2000). NADH is limited in rumen epithelial cell and therefore a more energetically-efficient K_m allows the metabolism of butyrate to ketone bodies to occur over metabolism of other VFA. This is of importance because butyrate has detrimental effects on other bodily tissues outside the rumen epithelium as well as sparing propionate for gluconeogenesis in the liver.

Energy balance

Gross energy

Hall et al. (2013) defines GE as the sum of the total amount of energy contained within the chemical bonds in the nutrients animals consume. The GE content of feedstuffs can be observed via bomb calorimetry or calculated using chemical composition and the GE

concentration values associated with said nutrients (NASEM, 2021). The GE concentration for a given substrate can be determined through bomb calorimetry. Following ignition of sample, the carbons and hydrogens in the sample are completely oxidized into CO_2 and H_2O, releasing heat as a byproduct of these reactions. The amount of heat released is representative of the energy contained within all the bonds within the sample. This magnitude of change of initial and peak water temperature within the bomb calorimeter is converted the energy content using the specific heat of water (Melville, 2014).

For feeds fed to lactating dairy cattle, the energy-containing nutrients are primarily protein, lipids, and carbohydrates in the form of starch and NDF. These nutrients differ in molecular composition and therefore differ in the energy contained within them. Lipids and carbohydrates are the main energy sources in dairy cattle diets, but lipids are much more energy-dense compared to carbohydrates. This is because carbohydrates are generally richer in carbon-oxygen bonds relative to lipids, which in turn decreases the ratio of hydrogen-to-oxygen. A greater ratio of hydrogen-to-oxygen ratio in a compound is indicative of a greater oxidation potential and therefore a greater GE concentration (Merten, 1970). For example, glucose and hexanoic acid both have six carbons and 12 hydrogens, but glucose has six oxygens where hexanoic acid only has two oxygens. The GE concentration of hexanoic acid is 7.19 Mcal/kg (NLM, 1981) while the GE concentration of glucose is 3.77 Mcal/kg (Dorez et al., 2014).

Gross energy intake (**GEI**) is a function of the concentration of GE of the diet and the mass of DM consumed. Increasing GEI provides more potentially-available energy to the cow, which may be of interest to overcome negative energy for animals in early lactation (Collard et al., 2000). There are many strategies used to increase the GEI of cows, but some of these strategies are not sustainable across the entire energy cascade. Lignin has an GE concentration of

6.0 (Voitkevich et al., 2012) Mcal/kg whereas cellulose and hemicellulose have approximate GE concentrations of 4.15 (Colbert et al., 1981) and 3.05 Mcal/kg (Dorez et al., 2014), respectively. Increasing the proportion of lignin within the NDF fraction should increase the overall GE concentration of this fraction, but this comes at an expense to digestible energy (**DE**) as lignin is not degraded in the rumen.

Digestible energy

Digestible energy is the energy absorbed from ingested feedstuffs following digestion. The ruminant's ability to digest different nutrients can vary tremendously. The digestibility of dietary starch is fairly controlled, but can range from 70-100% depending on grain type and processing methods (Fredin et al., 2014). Dietary NDF digestibility is considerably more variable and can range from 40 % in older, highly lignified legumes to 90 % in young, fresh grasses (Goeser and Combs, 2009). Converting GE to DE is highly dependent upon the nutrient profile of the diet and is almost never completely efficient. Due to the inefficiencies of ruminant digestion, some nutrients escape the animal in the form of feces and do not contribute to energy for metabolism or production. Digestible energy is therefore calculated by subtracting fecal energy (**FE**) output from GEI. These partial efficiencies of digestion, host cell sloughing, and microbial protein synthesis in the hindgut all contribute to the energy content observed in feces. This means it is impossible to definitely determine if the nutrients in feces are of dietary-, host-, or microbial-origins (Weiss, 1993). It is important to note measures of DE are estimates and not directly observed and thus contain a small degree of error (NASEM, 2021).

Naturally, the most important factors that influence DE are GEI and nutrient digestibility. Increasing the initial energy concentration of rations increases the concentration of energy available for downstream fractions of the energy cascade. Increasing nutrient digestibility

decreases the amount of dietary energy-containing substrates that escape the ruminant's digestive tract and contribute to the energy content of feces. This is consistent with Tine et al. (2001) who fed nonlactating cows diets almost entirely of BMR corn silage or isogenic corn silage. They (Tine et al., 2001) observed greater digestibility across all nutrients (except protein) and significantly greater conversion of daily GE to DE intake for cows feed BMR corn silage. The first experiment in Kellaway (1969) also observed an important relationship between *in vivo* organic matter digestibility and observed daily DE content. The second experiment in Kellaway (1969) reports lignin concentration of forages accounts for nearly 70% of the variation associated with DE concentration of those forages. This study supports the notion that the DE concentration of a forage is inherent to its digestibility, which lignin influences.

Metabolizable energy

Metabolizable energy (**ME**) is the energy contained within various molecules that can be partitioned towards maintenance or production. Nutrient absorption, locomotion, and cardiac function are just a few examples of the processes that ME can be partitioned towards. For ruminants, ME is calculated by subtracting urinary energy (**UE**) and methane energy (**CH_4E**) from DE. This is done to account for energy that lost before it can be productively utilized by the animal. The conversion of DE to ME is 85% efficient on average, but is subject to variation depending on dietary and metabolic factors (Morris et al., 2021). Although the conversion of conversion from DE to ME is relatively high, dietary manipulations can be made in regard to limiting energy lost in urine and as methane. The energy content of urine is representative of the carbon- and nitrogen-containing substrates excreted by the host as waste. Elliot and Loosli (1959) reported a high correlation between urinary nitrogen and urinary energy loss, suggesting that a large degree of energy loss associated with urine is in the form of nitrogen-containing compounds. Because of this, dietary factors affecting urinary nitrogen, and therefore energy,

excretion will be covered more extensively later in this review. Methane, however, does not contain nitrogen and therefore its excretion is purely associated with an energetic loss. Methane emissions can account for 2 to 12% of GEI, and is subject to dietary manipulation (Johnson and Johnson, 1995). Ruminal methane production not only comes at an energetic cost to dairy cows, but also at an environmental cost to the planet and therefore reducing methane emissions from dairy cattle will have mutually beneficial effects (Saunois et al., 2016). Manipulating the ratio of dietary forage and concentrate, utilization of alternative forages, supplementation of dietary fats, and chemical feed additives have been studied in methane emission trials (Knapp et al., 2014). Reducing energetic losses in CH_4E and UE makes the conversion of DE to ME more efficient and reduce the environmental impacts of dairy production. Judy et al. (2019) partially replaced ground corn with distillers' grains and calcium sulfate to significantly reduce methane emissions while simultaneously increasing the ME content of the diet. As mentioned previously, the substitution of rapidly-fermentable carbohydrates with forage carbohydrates is known to result in a chemical environment more conducive to productive propionogenesis relative to wasteful methanogenesis. Aguerre et al. (2011) titrated concentrates into the diet at 7% increments from 32% to 53% and observed a linear decrease in daily methane emissions from 648 to 538 g/d coupled with a linear decrease in pH from 6.59 to 6.38. The authors report this is the classical response one would expect when increasing the proportion of rapidly-fermentable carbohydrates in the diet as propiogenesis becomes the favored H+ assimilation mechanism as ruminal pH decreases.

The opportunity of incorporating seaweeds as alternative forages sources in dairy rations has received a lot of attention in recent years. Seaweeds, such as *Asparagopsis taxiformis*, are known to contain vesicle-bound halogens which have antimicrobial effects, especially towards

protozoa (Genovese et al., 2012). Work by Stefenoni et al. (2021) demonstrated that the inclusion of the red seaweed *Asparagopsis taxiformis* into lactating dairy diets decreased methane emissions by 120 g/d compared to the control diet. These investigators (Stefenoni et al., 2021) observed dietary inclusion of the red seaweed shifted microbial fermentation away from acetatogenesis and towards propiogenesis. While the methane mitigation potential of *Asparagopsis taxiformis* appears to be strong, the harvesting and processing of red seaweed remains difficult and expensive (Bharathiraja et al., 2015). A more feasible 'alternative' forage to reduce methane emissions from dairy cows might be corn silage. The starch content of corn silage is substantially greater than that of barley silage while maintaining a relatively equal NDF content (NASEM, 2021). Benchaar et al. (2014) observed replacement of barley silage with corn silage tended to reduced methane production, likely due to the observed increase in starch digestion associated with corn silage.

Fatty acids are known to be toxic to protozoa, and therefore by association decreases the production of acetate and butyrate in the rumen (Girard and Hawke, 1978). Since the production of these VFA results in net output of hydrogen that has to be assimilated through methanogenesis, disrupting these pathways should yield less methane. This implication is realized by Alvarez-Hess et al. (2019) whom observed that increasing the amount of canola oil from 0 kg to 0.80 kg significantly decreased methane production (546 vs 581 g/d) while having potentially positive effects on energy-corrected milk yield.

Upon approval from governmental health and safety agencies, chemical additives can be incorporated into ruminant diets to aid in methane mitigation, and therefore the conversion of DE to ME. Currently, ionophores are legally approved to feed to lactating dairy cattle in the United States per the FDA. Ionophores are known antibiotics that target Gram-positive bacteria

associated with production of acetate and butyrate (Russell and Strobel, 1989). Russell (1986) indicates ionophores selectively target Gram-positive bacteria due to the lack of a protective outer membrane. Without a selective outer membrane, monensin disrupts the inner membrane, allowing H+ ions to flow into the cell. The translocation of these protons back across the membrane requires energy and eventually depletes the cell of ATP to pump protons out, acidifying the cell. It is theorized that the reduction in these bacteria allows for propiogenic bacteria to assimilate hydrogen into propionate over methane. To explore this, Grainger et al. (2010) compared the ruminal acetate-to-propionate ratio in cows supplemented with monensin compared to control cows and found a tendency for a lower ratio for cows supplemented with monensin, but no change in methane emissions. Odongo et al. (2007) reported a 7% decrease in methane production from cows supplemented with monensin, but this came at the cost of a slight decrease in milk fat concentration. This was speculated to be a function of monensin's antimicrobial effects on acetogenic and butyrogenic bacteria, which produce the primary precursor molecules used to synthesize fatty acids at the mammary gland. While monensin is legal and has had inconclusive results, 3-nitrooxypropanol (**3-NOP**) is still in process of certification from the FDA but has proven to be effective in methane mitigation. 3-nitrooxypropanol targets methyl-CoM reducatse, the final enzyme in the methanogenesis pathway in archaea (Jayanegara et al., 2017). Melgar et al. (2021) reports 3-NOP dietary inclusion drastically reduces methane emissions from 411 to 301 g/d, while maintaining milk production and DMI. Methane emissions decreased by 26% and diatomic hydrogen gas emissions were nearly seven times greater for cows fed 3-NOP. Hydrogen gas is recognized as an indirect greenhouse gas as it contributes to atmospheric methane later in its lifecycle and therefore still contributes to climate change (Prather, 2003).

Net energy for lactation

Until the point of ME, all major energy losses have been represented by FE, CH_4E, and UE. The last major unproductive energy loss to the animal is in the form of heat production (**HP**), and therefore the difference between ME and net energy (**NE**) is HP. No chemical reaction is completely efficient in the transfer of energy between reactant and products, and according to the first law of thermodynamics, the lost in the reaction process needs to be accounted for (Kleiber, 1975). Heat emitted by the animal represents the cumulative energy that is not transferred from reactants to products (Baldwin, 1995). The heat released from the animal can be quantified using calorimetry methods, which will be discussed later in this review. Heat production is a cumulative term that accounts for all reactants and products, regardless of the origins of the reactants. Reactants used to synthesize milk components can come from the diet and host tissues or synthesized from more elementary molecules. All of which release heat in their conversion to milk components. For example, Baldwin et al. (1985) reports milk fat synthesized from dietary or tissue fats differs by about 3% in efficiency. This difference in efficiency is known as the heat increment (Weiss, 2019).

Once heat energy has been subtracted, the remaining energy to be accounted for is productive energy in the form of milk components and tissue energy (**TE**). Analysis by Moraes et al. (2015) demonstrates the conversion of dietary nutrients to milk nutrients is 0.63, which was much less efficient relative to using bodily tissues for milk components at 0.89. The same study reports greater efficiency in converting dietary nutrients to bodily tissues relative to milk at 0.70. Although it is more efficient for animals to synthesize bodily tissues over milk components, the allocation of productive energy is under the influence of stage of lactation and energy demands (Moe et al., 1971). Cows in peak lactation can mobilize up to 90 kg of body fat to meet the

energetic needs of lactation, which demonstrates the energy ‘sink and faucet’ relationships involved in energy utilization (Komaragiri et al., 1998).

Nitrogen utilization

Nitrogen utilization

Metabolizable protein (**MP**) is the amino acid profile that reaches the small intestine and is biologically available to the ruminant. The two prominent sources of MP are microbial cell protein (**MCP**) and rumen by-pass protein. Protein absorbed by the ruminant can be used for maintenance, growing additional tissue, and milk protein synthesis (NASEM, 2021). Nitrogen-containing compounds that are not used incorporated into meat or milk are excreted and therefore balancing dairy rations properly for protein is energetically, economically, and environmentally important.

In the rumen, proteolytic microbes rapidly deaminate dietary amino acids to release carbon skeletons and ammonia (Lewis and Emery, 1962). Free ammonia is then utilized by other microbes to synthesize proteins and replicate. When microbes are washed out of the rumen, they are subject to true digestion in the abomasum and small intestine, contributing to the MP pool. Microbial cell protein can account for up to 85% of the amino acids reaching the small intestine (Storm et al., 1983). Microbes can effectively convert feedstuffs of low protein content to high-quality MCP to meet the protein requirements of the host. For example, corn silage is only about 8% CP (NASEM, 2021), but microbes can convert this feed to MCP, which contains approximately around 80% CP (Hackmann and Firkins, 2015). Nitrogen not incorporated into MCP is converted to urea , which is either excreted or recycled to the rumen for assimilation as ammonia when energy and carbon are more available (Lapierre and Lobley, 2001). Metabolism of rumen by-pass protein is more straightforward as it escapes the rumen without being

converted into MCP, directly contributing to the MP supply after chemical digestion in the abomasum.

Productive nitrogen

In productive ruminant nitrogen metabolism, amino acids absorbed in the small intestine are incorporated into milk and tissue protein, with minimal conversion to urea. The value of dairy products to human nutrition is in milk amino acids and proteins as these are forms of nitrogen-containing molecules that are biologically useful to humans. Therefore, nutritional strategies have been developed to maximize the proportion of nitrogen in these forms in products from the dairy cow. Multiple studies report increasing the inclusion of concentrates in rations results in an increased milk protein response. Aguerre et al. (2011) manipulated the forage-to-concentrate ratio of diets and observed a linear increase in milk protein concentration and yield with increasing inclusions of rapidly-fermentable ingredients, such as corn grain, high-moisture corn, soybean meal, and roasted soybeans. Morris et al. (2020) formulated iso-energetic diets with either starch or fat as the primary energy sources and observed that the high starch diet resulted in a tendency to increase daily milk protein yield. This was speculated to be a function of starch promoting a healthier rumen environment with greater microbial protein synthesis, an increase in plasma insulin concentration, and/or amino acid sparing for energy. There is also a potential for increasing efficiency of conversion from dietary protein to milk protein through the use of rumen-protected protein products that allow high-quality protein to escape microbial degradation. Mikolayunas et al. (2011) observed an increase in milk protein from lactating ewes fed rumen-protected protein in both a pasture and intensive production systems. This was likely due to a more targeted approach of delivering essential amino acids directly to the animal without unwanted proteolysis from rumen microbes. Lastly, increasing the CP content of the diet is also a known strategy for increasing milk and tissue protein, but this is typically also

accompanied by increased nitrogen excretions as nitrogen efficiency decreases as dietary nitrogen content increases (Alstrup et al, 2014).

Environmental nitrogen

Environmental nitrogen refers to nitrogen that is excreted by the animal in in feces and urine. Gaseous nitrogen emissions in the form of ammonia directly from the rumen are minimal, and therefore the most important nitrogen losses to the environment are in the form of urea in the urine and reactive nitrogen in the feces (Hristov et al., 2011). Ammonia-nitrogen is readily absorbed through the rumen wall and intestines, where it is converted to urea to cycle in the host's bloodstream (Firkins and Reynolds, 2005). Also, when amino acid supply overwhelms amino acid demand, they are deaminated in the liver to yield ammonia and carbon skeletons (Bergen, 2021). Carbon skeletons can then be used for energy metabolism while the ammonia is converted to urea. Urea from both sources is excreted in urine where it is the principle nitrogen-containing molecule present (Schuba et al., 2017). In addition to excreting nitrogen that could have been used for productive metabolism, McBride and Kelly (1990) report the energetic cost of converting ammonia to urea in the ruminant liver is four ATP per molecule of urea synthesized. Reed et al. (2017) suggests imbalances in the amino acid profile at the mammary gland as a result of including protein in dairy rations above requirements and is associated with negative impacts on synthesis of milk components. This indicates a more targeted approach to feeding nitrogen in dairy rations is not only environmentally-conscious, but economical as well depending on dairy markets.

Urinary urea is rapidly converted to ammonia due to the presence of microbial urease in the feces (Muck, 1982). James et al. (1999) reports that over 50% of the ammonia in manure can be released into the air through volatilization, depending on dietary CP concentration and manure

management procedures. (Hou et al., 2015) reports reducing the pH of manure slurry pits from 6.5 to 4.5 reduced ammonia emissions by 83% compared to applying a layer of oil over the manure slurry, which reduced ammonia emissions by nearly 100%. Phuong et al. (2013) reviewed many nitrogen efficiency trials and reported that the most effective way to reduce urinary nitrogen excretions is to monitor CP inclusions in the diet as well as dietary CP digestibility. Reducing the urinary nitrogen output of dairy cattle increases their nitrogen and energetic efficiency, while also having beneficial implications for the environment. Ammonia is a known precursor to particulates that harm the health of animals and humans as well as eutrophication of waterways (Behera et al., 2013).

While urinary nitrogen is a metabolic marker for nitrogen metabolism in dairy cows, fecal nitrogen is a metric of nitrogen digestibility, host-derived nitrogen, and hindgut microbial protein synthesis (Firkins and Reynolds, 2005). Powell et al. (2006) reports a correlation between fecal nitrogen concentration and dietary NDIN concentration, indicating that fecal nitrogen content is largely a function of CP digestibility. Conversely, numerous studies have observed increases in CP digestibility potentially increase microbial protein synthesis, and therefore contribute to greater fecal nitrogen excretions (Broderick, 2003; Groff and Wu, 2005). Host-derived nitrogen, or metabolic nitrogen, is nitrogen from host cells and is largely dependent upon the energy balance of the animal. Animals in negative energy balance mobilize tissue nitrogen, up to 24 kg (Komaragiri et al., 1998), to meet the demands for milk production. As a result, a miniscule amount of this liberated nitrogen (less than 1%) is excreted in the feces while the rest that is not used for productive responses is excreted in the urine (Strozinski and Chandler, 1972). Reactive fecal nitrogen is of great importance because nitrogen in this form can be converted to nitrates by soil microbes, contributing to eutrophication in marine ecosystems (Arriaga et al.,

2009). Additionally, reactive nitrogen from feces can be converted to N_2O by soil microbes, which acts as a GHG in the atmosphere (Luo et al., 2008). Broderick et al. (2007) indicates shifting nitrogen from urinary excretions to fecal excretions would be of interest as urinary nitrogen is more reactive and can lead to greater environmental damage compared to fecal nitrogen.

Milk urea nitrogen

Nitrogen in the form of milk urea (**MUN**) does not fit directly into either category of productive and environmental nitrogen and so it will be discussed as a separate entity. Urea secreted into the milk can be a result of either conversion from ruminal ammonia or amino acid deamination (Patton et al., 2014). Although nitrogen excretion in milk does not directly result in environmental damage, MUN is highly correlated with urinary nitrogen excretion (Kauffman and St-Pierre, 2001), which has environmental consequences. Greater MUN concentrations can also serve as an indicator for poor nitrogen utilization efficiency as MUN does not provide nitrogen in a biologically-available form for human nutrition (Kies and Fox, 1978).

Calorimetry methods

General calorimetry

Calorimetry in regard to ruminants refers to the measure of the release of heat due to inefficiencies of chemical reactions used for maintenance, gain, and lactation to the environment (Blaxter, 1963). Nienaber et al. (2009) suggests there are two common ways of measuring the heat transfer from animals to their environments: direct calorimetry, which accounts directly for heat loss by the animal and transferred to a controlled environment, and indirect calorimetry, which is used to calculate the theoretical heat produced by the animal.

Direct calorimetry

The first direct calorimetry experiment was conducted by Lavoisier and Laplace in the 1700s where they placed a guinea pig in an ice calorimeter (Lodwig and Smeaton, 1974). The guinea pig was placed inside a chamber that was surrounded by ice, and as the guinea pig oxidized substrates, heat was released as a product of this reaction. The heat released from the animal melted the ice and the subsequent water was collected for temperature change analysis. Under the assumption that all heat lost by the animal is directly transferred to the ice, heat production by the animal was calculated using the mass of water collected and the specific heat of water. In modern direct calorimetry metabolism chambers, heat excreted from the animal is captured directly by heat sinks within the chamber, usually in the form of water baths or water coils lining the chambers walls (Kenny et al., 2017). In regard to the Lavoisier and Laplace experiment, guinea pigs are monogastric organisms, meaning the nutritional contribution of fermentation end products from gastrointestinal microbes is minimal. It is important to note that fermentation is not completely efficient and therefore heat is produced (Luong and Volesky, 1982). This heat radiates from the animal and is detected as metabolic heat generated by the host in direct calorimetry chambers, which can confound measures of heat production (Waversveld et al., 1988). Direct calorimetry chambers are also expensive and can come with logistical and spatial concerns. For these reasons, indirect calorimetry might be more feasible for some facilities and is potentially more accurate for determining metabolic heat production in ruminants.

Indirect calorimetry

While direct calorimetry is conducted using whole-animal metabolic chambers that directly measure heat lost from the animal, indirect calorimetry uses respiratory devices that measure O_2 consumption and CO_2, CH_4, and urea production to calculate heat production. There

are multiple types of indirect calorimetry methods, such as open-circuit and closed-circuit respiration chambers and respiratory facemasks (Li et al., 2019). The premise of these respiratory apparatuses is the determination of gas exchange between the animal and the environment. Upon determination of environmental gas exchange, the volumes of said gases can be applied to the Brouwer (Blaxter, 1965) equation to determine heat production. Extensive research has been conducted in order to determine the relationship between O_2 consumption and CO_2, CH_4, and urea production associated with the metabolism of different nutrients and the respective heat energy generated from these reactions (Mtaweh et al., 2018). Measuring gaseous consumption and production allows for the calculation of the respiratory quotient (**RQ**), which is a ratio of CO_2 produced over O_2 consumed. The RQ gives a basic idea of the metabolic state of the animal. For example, an RQ above 1.0 indicates a greater proportion of CO_2 production compared to O_2 consumption which is associated with the formation of fatty acids in adipose tissue while an RQ closer to 0.80 is indicative of protein and lipid oxidation (Van Soest, 1994c). Fatty acid synthesis in adipose tissue indicates positive energy balance while greater rates of oxidation of protein and lipid represents negative energy balance. Below is the Brouwer equation (Blaxter, 1965) used to calculate heat production from gas consumption and production data.

$$HP = 16.18\ O_2 + 5.02\ CO_2 - 2.17\ CH_4 - 5.99\ N \quad [3]$$

HP = metabolic heat production rate, MJ

O_2 = Oxygen consumption rate, mL/s, STPD

CO_2 = Carbon Dioxide production rate, mL/s, STPD

CH_4 = Methane production rate, mL/s, STPD

N = nitrogen excretion rate, g/s

STPD = standard pressure (760 mm Hg or 101.325 kPa), temperature (0 Degrees C) and dry air.

Of the different indirect calorimetry methods, open-circuit headbox-style chambers are regarded as the most versatile in their utilization in metabolism facilities. Headboxes allow the animal to consume feed and water normally without obstruction to either process while simultaneously collecting inspired and expired gases. The headbox-style chambers are also portable and conducive to the milking of dairy cows.

SUMMARY OF LITERATURE

Corn silage and alfalfa hay are commonly utilized forages in dairy production systems due to their nutritive and logistical qualities. Forages typically account for half of a dairy ration and are essential in meeting a variety of nutrient requirements of dairy nutrition due to their chemical compositions. The NDF fraction is essential in providing dairy cows with energy while maintaining rumen health. Ruminants have the unique ability to digest fiber due to their symbiotic relationship with anaerobic microbes lining their gut. There are many factors that influence the microbial population's ability digest fiber, including physical and chemical factors. Lignin is regarded as the primary plant limiting factor in NDF digestion, suggesting that the chemical composition of NDF fraction, and not just the dietary NDF content, is important for formulation of diary rations to optimize the ruminant's ability to extract energy from the ration.

The rumen environment is complex but is essential for meeting the energy and nitrogen requirements of the ruminant. The chemical environment of the rumen not only affects the rate at which fiber is digested, but also the fermentation profile associated with different nutrients. Manipulating the conversion of feed to VFA is of importance because each VFA is metabolized

differently by the host and therefore contributes different concentrations of energy per unit of VFA. Understanding rumen environment is not only important in terms of energetics, but also from an environmental point of view. Methanogenesis and propiogenesis are examples of competing ruminal biochemical pathways with very different outcomes using the same substrates. Decreasing methanogenesis would decrease contributions of greenhouse gas emissions from animal agriculture as well as increase the energy available to animal for milk production. Increasing propiogenesis is a net positive as propionate is glucogenic in the ruminant liver and has the best ATP yield of all VFA as well as not contributing to environmental damage. In conclusion, increasing fiber digestion is highly dependent on the dynamics of the rumen and increasing its digestion results in a greater energy status of the animal.

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TABLES AND FIGURES

Table 1.1 Chemical composition of typical corn silage and alfalfa hay according to the feed library of the Dairy NASEM (2021).

	Alfalfa hay			Corn silage		
Item, % DM	Mean	Standard deviation	n	Mean	Standard deviation	n
DM	88.1	2.95	100,858	35.4	5.38	535,422
Ash	10.8	1.44	101,438	3.8	0.91	535,923
CP	20.7	2.37	102,002	7.7	0.94	536,303
ADIP	0.74	0.160	45,707	0.82	0.141	288,591
NDIP	1.85	0.596	45,773	1.23	0.293	288,614
ADF	32.1	3.96	101,978	24.3	3.27	537,131
NDF	41.1	4.84	101,963	40.9	4.75	536,939
48h NDF in vitro digestibility	52.4	9.10	49,252	52.0	6.25	130,789
Lignin	6.64	1.148	101,932	3.05	0.564	537,082
Starch	1.5	0.85	25,588	32.9	6.42	536,519
Sugar	9.0	1.84	26,447	3.0	1.22	70,737
TFA[1]	1.50	0.466	27,726	2.35	0.394	370,294

[1]Total fatty acid.

Figure 1.1 2-dimensional visualization of the NDF fraction. This figure shows how the main structural constituents (cellulose, hemicellulose, and lignin) of the cell wall are associated with each other in a planar view.

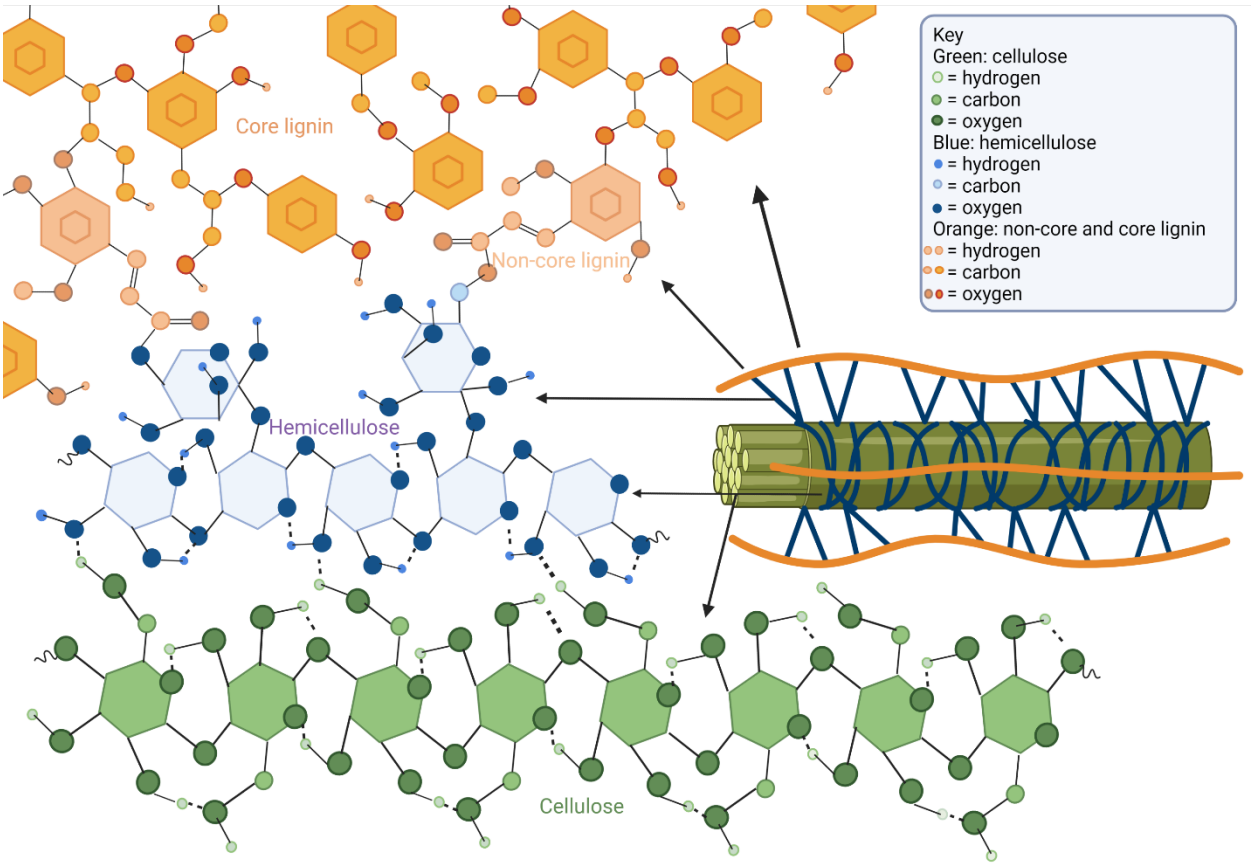


Figure 1.2 Anatomy and chemistry of the plant cell, plasma membrane, cell wall, and middle lamella.

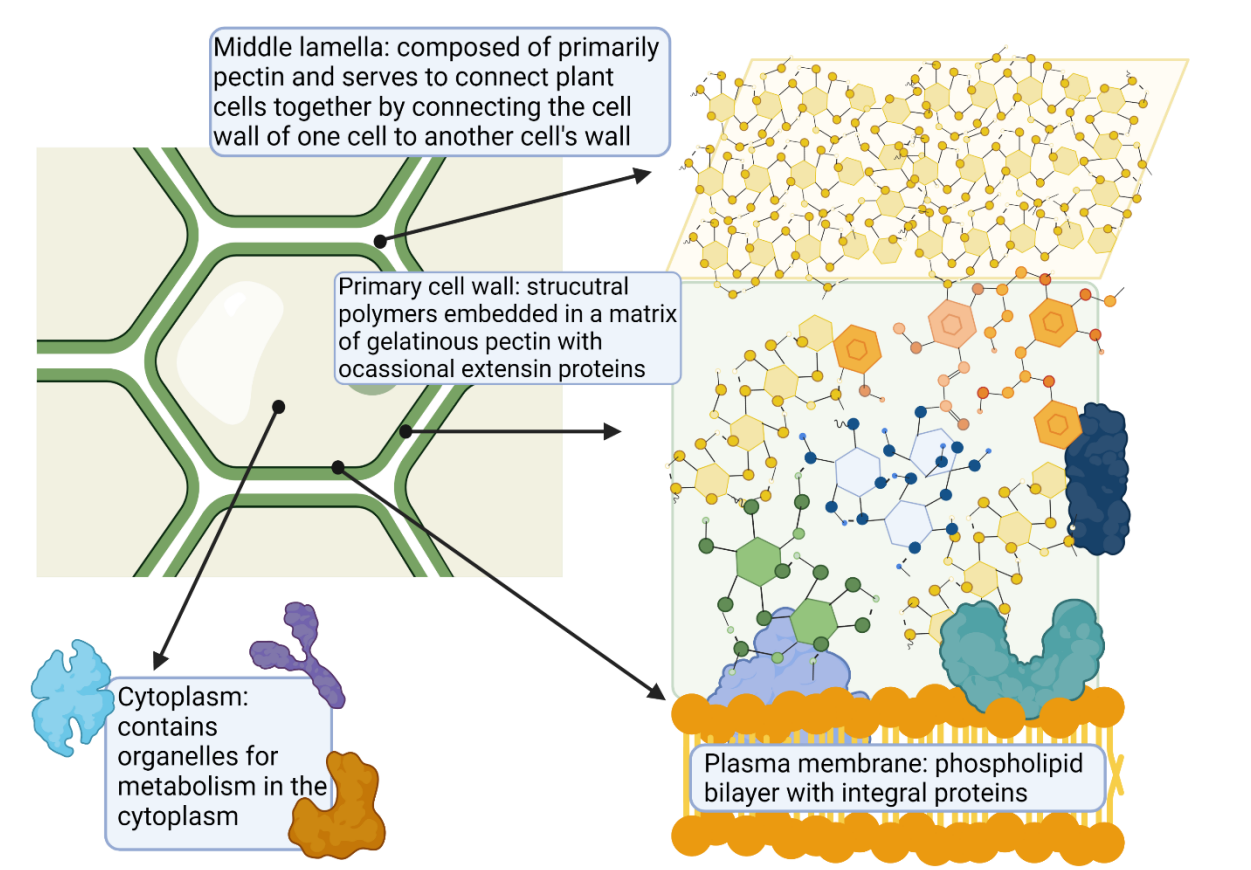


Figure 1.3 Dietary, plant, animal, and management factors that influence the digestion of NDF.

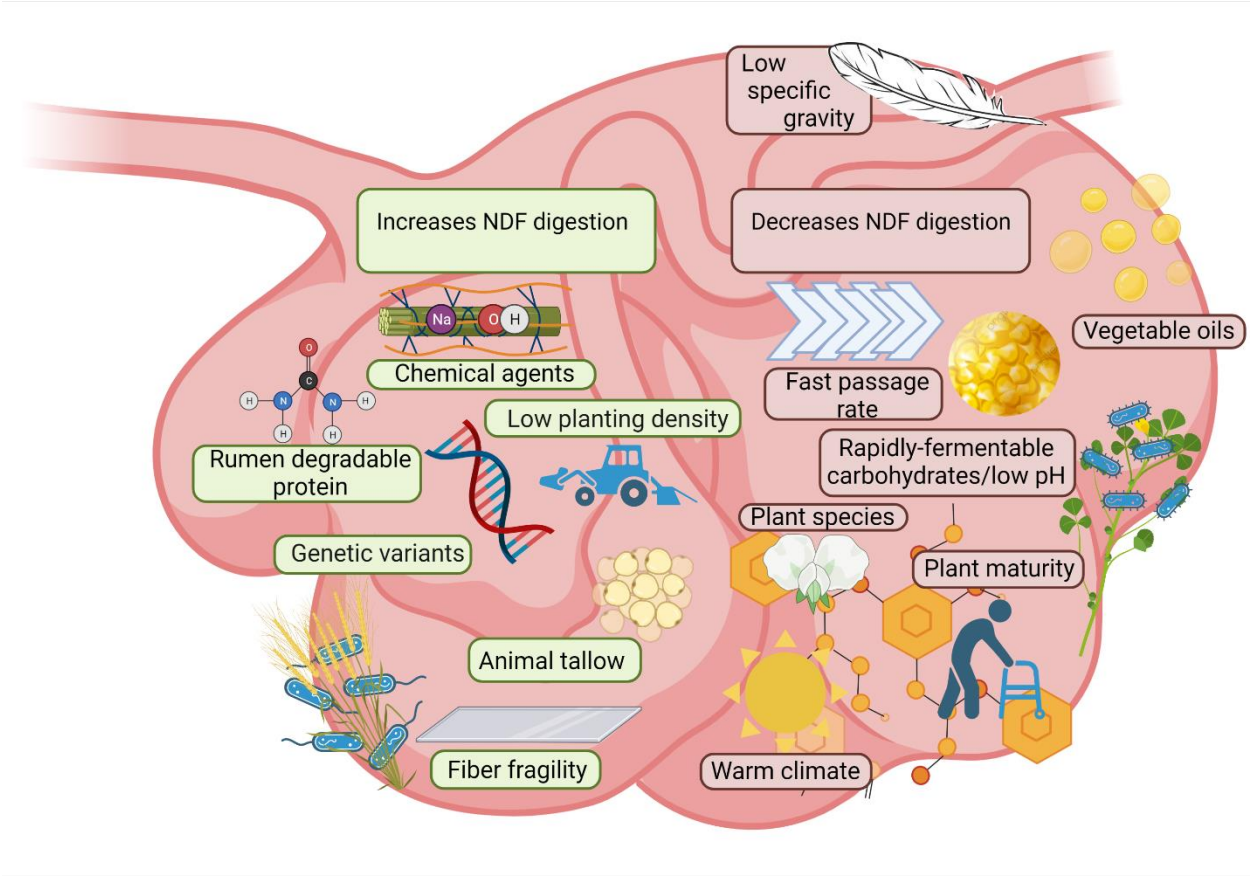


Figure 1.4 The interactions between forages, rumination, ruminal pH, and microbial fermentation profile.

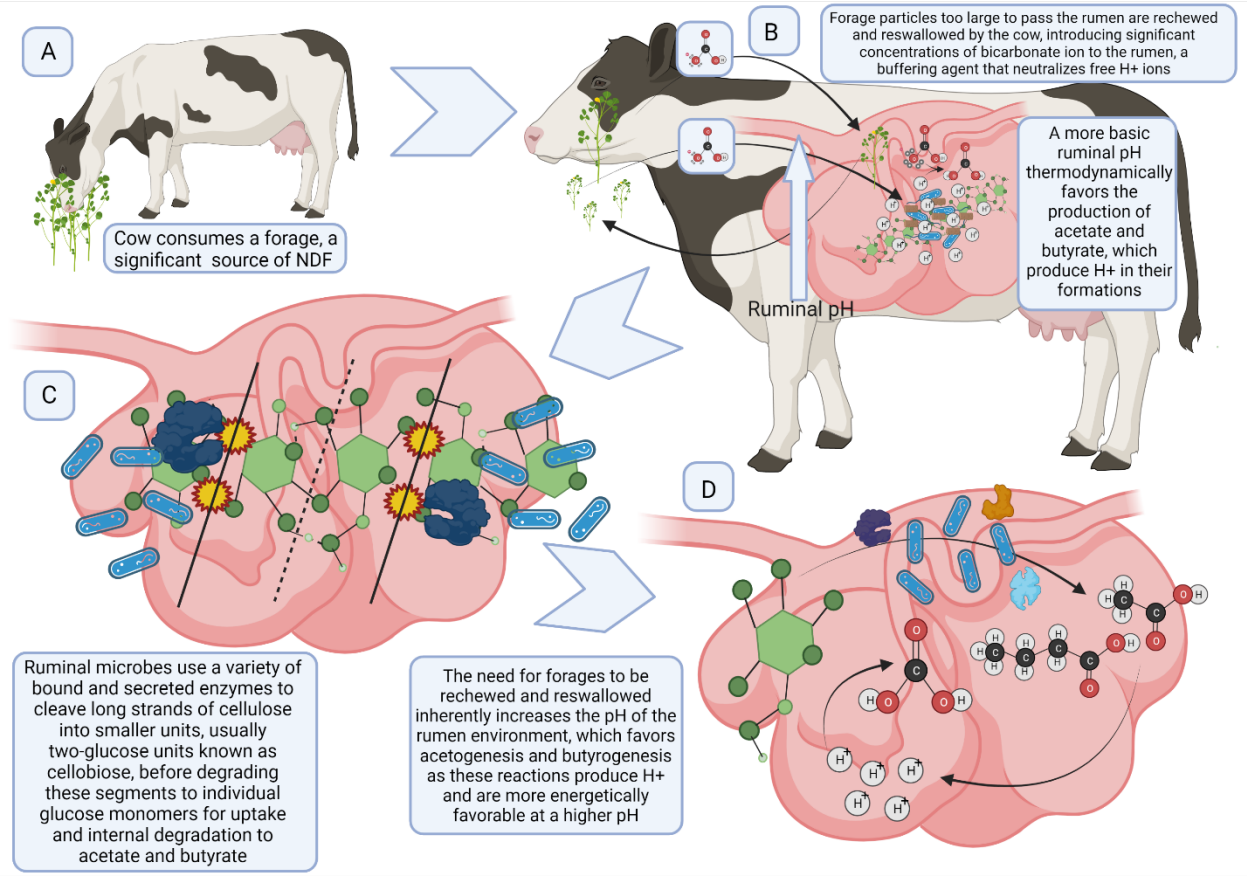


Figure 1.5 The interactions between concentrates, rumination, ruminal pH, and microbial fermentation profile.

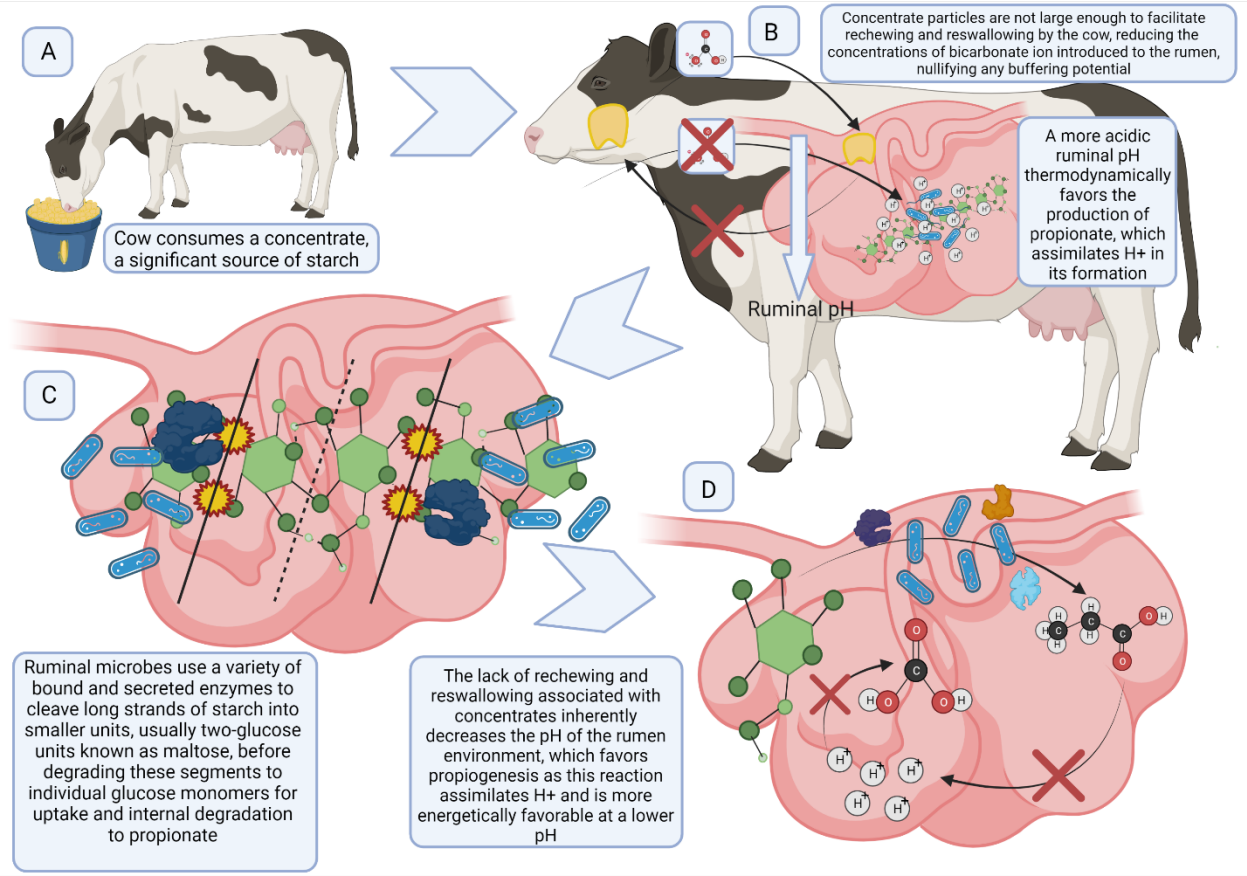


Figure 1.6 Ruminant metabolism of acetate, propionate, and butyrate.

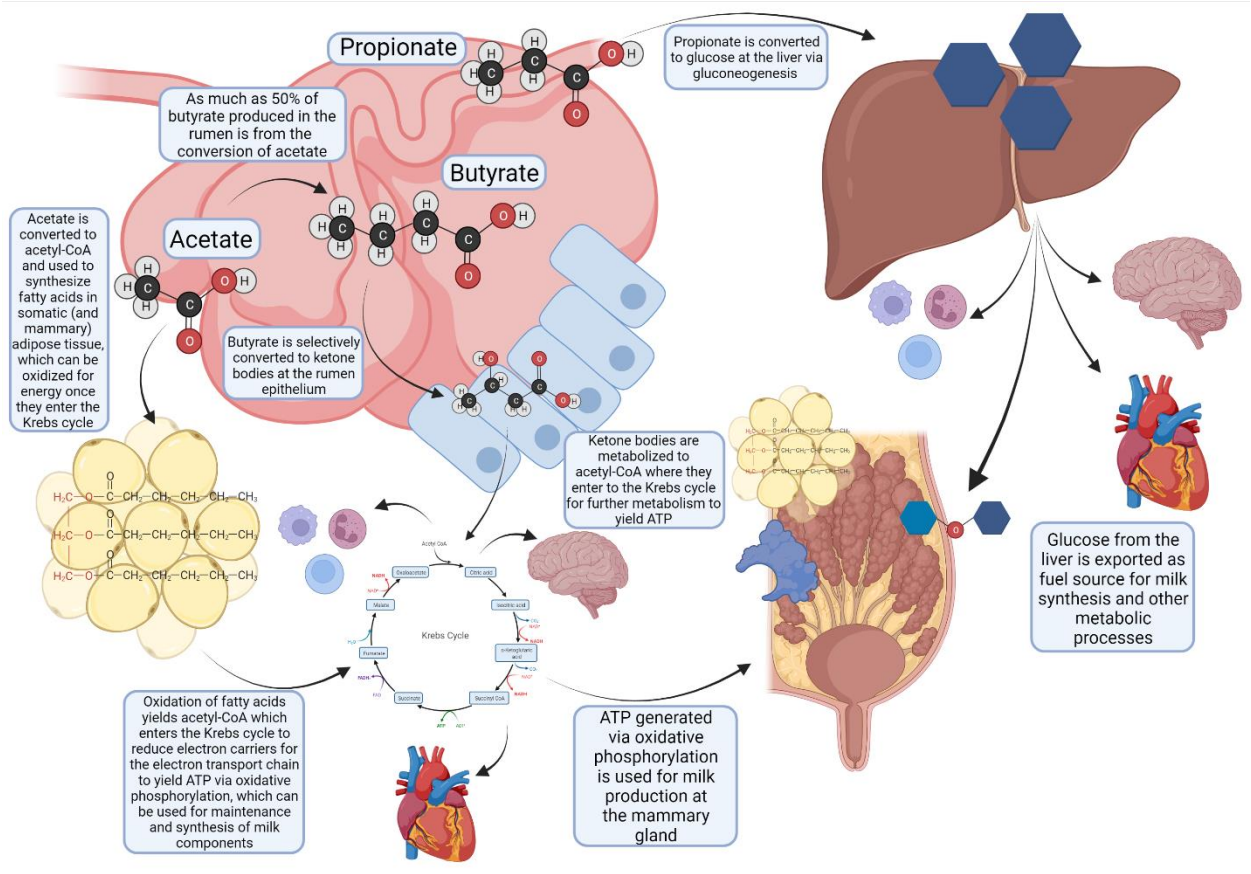


Figure 1.7 Visualization of the California Net Energy System: how energy enters the system and how it leaves.

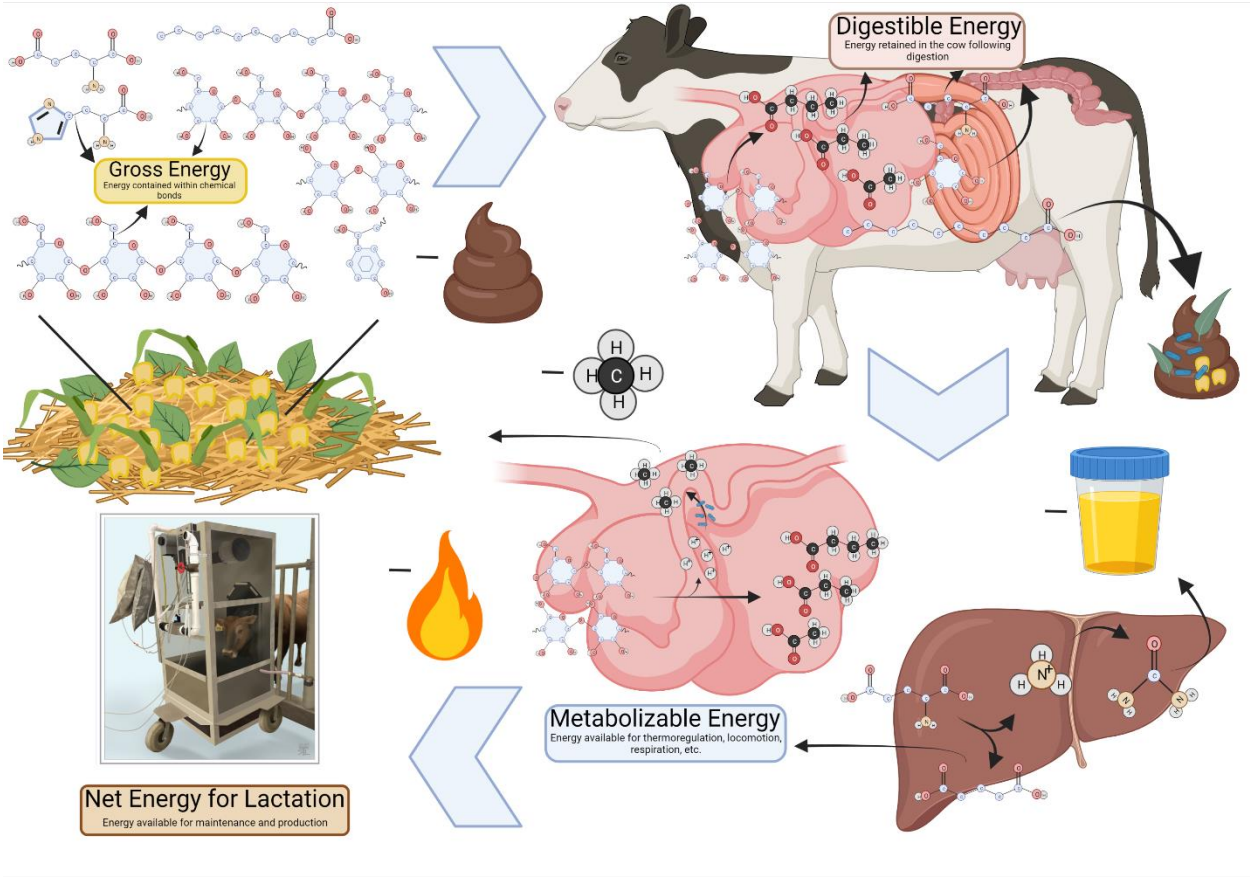


Figure 1.8 Feeding strategies and mechanisms for methane mitigation.

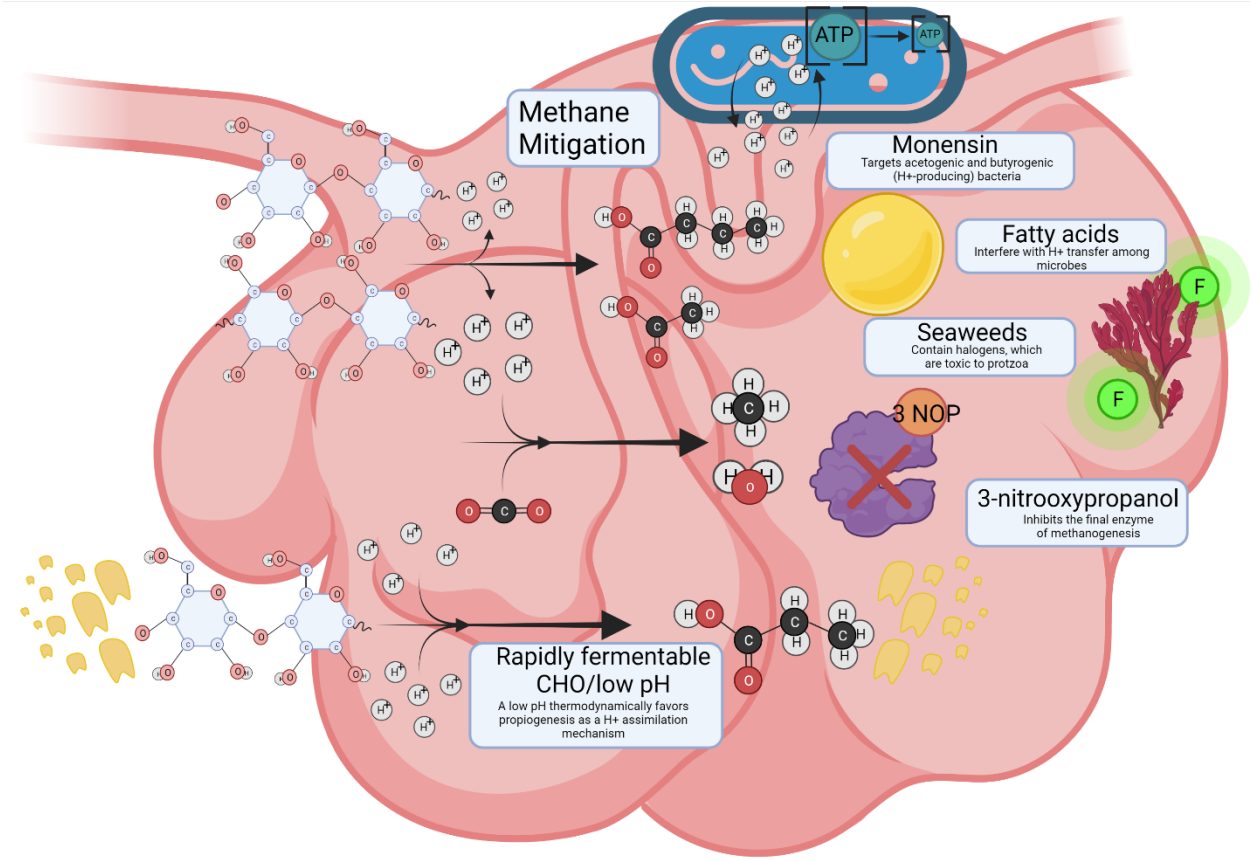


Figure 1.9 Productive and environmentally-favorable utilization of feed nitrogen by lactating dairy cows.

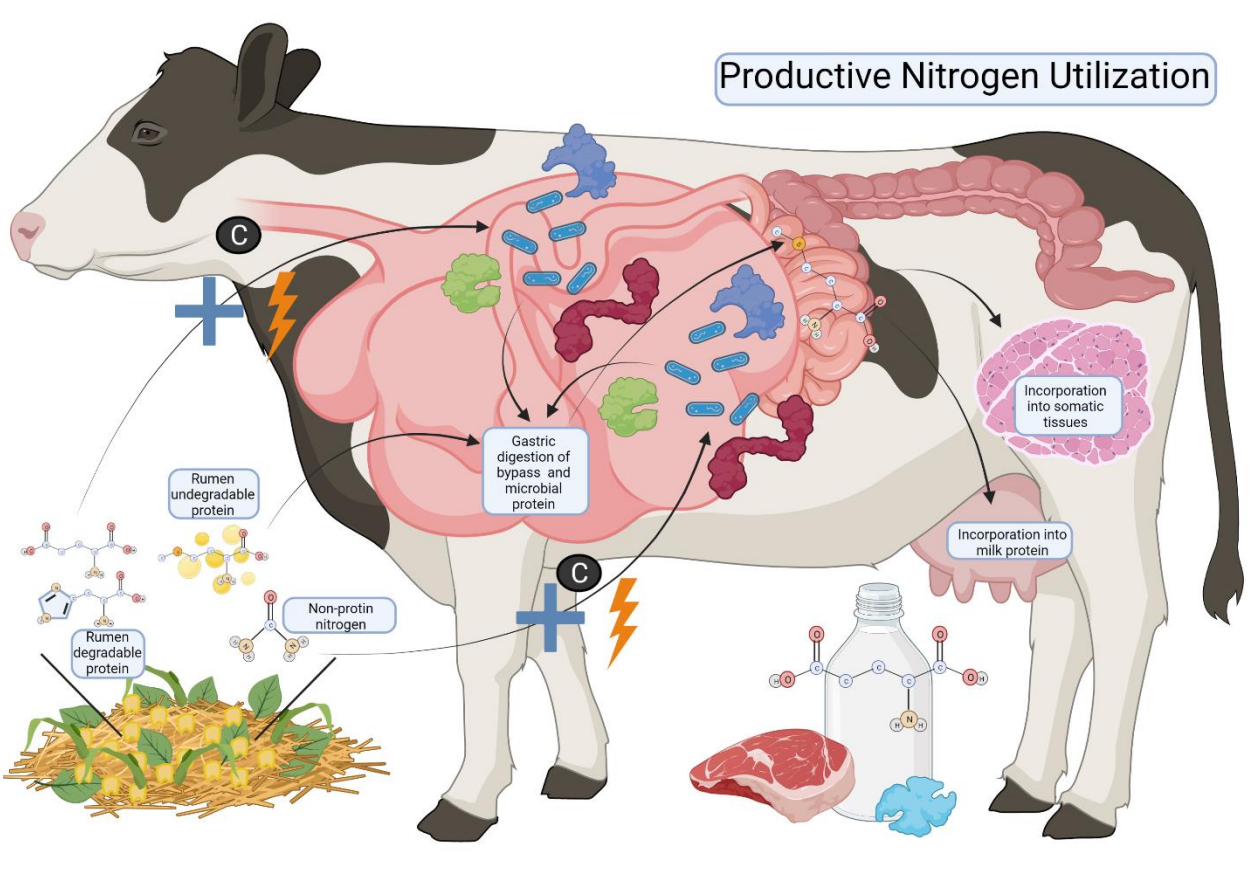
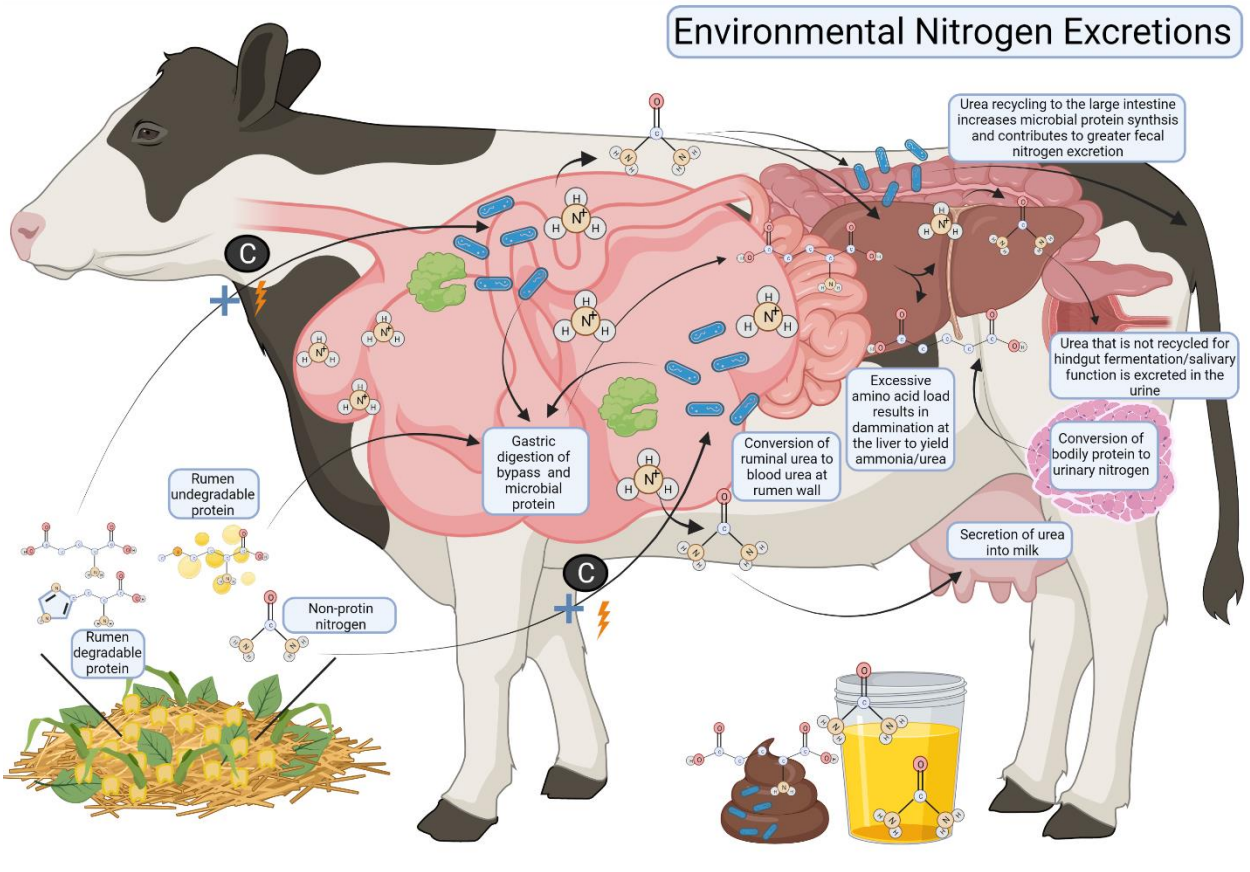


Figure 1.10 Unproductive and environmentally-unfavorable utilization of feed nitrogen by lactating dairy cows.



CHAPTER 2

RUNNING HEAD: FIBER GROSS ENERGY CONCENTRATION

Evaluation of gross energy concentration of fiber contained in feed and fecal samples

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ABSTRACT

Although believed to be similar in gross energy concentration, compared to starch, the of NDF is considerably more variable in chemical composition and extent of digestibility. The proportion of lignin within the NDF fraction is deemed as a primary factor limiting NDF digestion. It has been well-established that lignin is indigestible by rumen bacteria and therefore should escape the rumen undegraded and of the same gross energy value. The literature lacks information on whether this phenomenon is consistent for cellulose and hemicellulose with regard to its gross energy concentration following digestion. Therefore, this study sought to compare the gross energy content of feed and fecal NDF residues to determine if fecal cellulose and hemicellulose were partially degraded and supplying additional energy to the animal. The results of this study show that feed and fecal NDF residues were similar in gross energy concentration ($P = 0.23$) and suggest that cellulose and hemicellulose escaping the rumen are partially degraded. The potential digestion, and additional energy availability to the animal, is currently not accounted for in our nutritional models to predict the digestible energy contribution from fiber.

INTRODUCTION

Cellulose, hemicellulose, and lignin account for the majority of the NDF fraction, but this nutritive entity can also contain varying concentrations of ash, protein, silica, and pectin (Van Soest, 1967b). The NDF fraction can be fermented by rumen microbes to yield VFAs, which account for up to 70% of the cows' total energy supply (Bergman, 1990). More specifically, a typical, multiparous Jersey cow producing 37 kg of milk (4.8% fat and 3.5% protein) and consuming 24 kg of DM containing 32% NDF would receive approximately 16 Mcal/d of

digestible energy (**DE**), or 22% of her total DE from NDF (NASEM, 2021). Although the NDF fraction contributes substantially to the energy balance of the animal, the proportion of lignin within the NDF fraction can limit the digestibility of cellulose and hemicellulose, and therefore affect the energy available to the animal.

The energy concentration of a nutrient is a function of the atoms present and the bonds that connect them (Hall et al., 2013). For homogenous and uniform nutrients such as starch, variance around the true mean gross energy (**GE**) concentration is small. Kabo et al. (2013) reported the GE concentration of starch in feed is approximately 4.20 Mcal/kg with a coefficient of variance (**CV**) of less than 0.1%. The GE concentration of NDF is generally assumed to be similar to starch (4.20 Mcal/kg) but in practice it is likely subject to greater variation. This is because the variation surrounding the true mean GE concentration of NDF is a function of the entities that comprise NDF, and these vary. For example, the arrangement of carbon atoms in lignin are more reduced compared to other NDF components and therefore lignin is associated with a greater GE concentration of 6.0 Mcal/kg (Voitkevich et al., 2012). In turn, the proportions of NDF constituents contribute variation to and have direct impacts on the overall GE concentration of the NDF fraction. Because lignin has a significantly greater GE concentration than cellulose (4.15 Mcal/kg, Colbert et al. (1981)) and xylan (the principle dimer of hemicellulose) (3.25 Mcal/kg, Gorensek et al. (2019)), the GE concentration of an NDF profile should increase when the centration of lignin increases. This notion holds true in the case of comparing feed and fecal NDF residues as fecal NDF profiles are enriched in lignin as it is indigestible, while digestible cellulose and hemicellulose decrease in relative proportions in fecal NDF. Hindrichsen et al. (2006) reported the lignin content of six forages to be 5.2% (% DM) and the lignin content of 35 fecal samples 13.3% (% DM). Comparison of the GE concentration of

the NDF residues from the feed and fecal samples used for lignin analysis most likely would have resulted in an increase in GE concentration for fecal NDF. This response would be a function of the increased energy contribution from fecal lignin.

The Dairy NASEM (2021) uses a summative equation to predict the GE concentration of diet. This is based on proportions of major nutrients and their respective GE concentrations. Due to the heterogeneity in the chemical composition of NDF as a nutrient, the accuracy of the current GE coefficient utilized in the Dairy NASEM (2021) model might not accurately represent the true GE concentration of NDF. The current GE concentration utilized by the model for predicting GE from NDF is 4.20 Mcal/kg. As mentioned previously, a greater proportion of lignin within the NDF fraction will inflate the overall GE concentration of the NDF fraction, resulting in the Dairy NASEM (2021) model underestimating the true GE contribution from the NDF fraction. The lignin content within the NDF fraction has also been identified as a critical factor in the extent of NDF digestion. Lignin's impacts on NDF digestion are represented in the calculation for NDF digestibility in the summative equation utilized by the Dairy NASEM (2021) to predict the DE concentration of a ration. The equation used by the Dairy NASEM (2021) to predict DE from NDF also assumes that NDF escaping digestion in the rumen, abomasum, and hindgut is completely undegraded. This assumption may not be accurate as digestion of NDF is complex and under the influence of many plant and animal factors. Because lignin contributes more energy to fecal NDF than feed, we hypothesize that fecal NDF would be greater in energy concentration relative to feed NDF. The objects of this study were to evaluate the GE coefficient for calculation of dietary GE concentration from NDF and describe lignin's impact on estimates of DE.

MATERIALS AND METHODS

Sample type and source

To evaluate the GE concentration of feed and fecal samples, approximately 0.20 g of NDF residues from 16 feeds (corn silage N = 2, grass hay N = 2, alfalfa hay N = 2, wheat straw N = 1, cottonseed hulls N=1, soyhulls N = 1, DDGS N = 1, and TMR N = 6) and 34 fecal samples were collected. All samples originated from dairy nutrition studies conducted at Ohio Agricultural Research and Development Center of The Ohio State University (Wooster, OH). The fecal samples provided were from experiments where dairy cows were fed various TMRs from multiple IACUC-approved experiments. Fecal and feed samples were not paired because they did not all originate from the same study.

To isolate NDF residues, feed and fecal samples were dried at 60° C for 48 hours and ground through a 1-mm sieve (Wiley Mill; Arthur A. Thomas Co., Philadelphia, PA). Once ground, NDF residues were isolated using the Ankom technique (Ankom200 Fiber Analyzer, Ankom Technology Corp., Fairport, NY). This assay was conducted in quadruplicate and included 0.5 g sodium sulfite and one mL alpha-amylase (Sigma A3306; Sigma-Aldrich, St. Louis, MO). Neutral detergent fiber residues were then further ground manually using a mortar and pestle and once again dried again at 60° C for 24 h. A bomb calorimeter (Parr 6400 Calorimeter, Moline, IL) was used to determine concentration of GE. The bomb calorimeter was calibrated after two benzoic acid standards were within the range of 6318 ± 18 Mcal/kg. Then 0.2 grams of the ground NDF residue was placed in a tared metal cap, followed by 0.4 grams of mineral oil. Samples were set to rest overnight so that the mineral oil could completely soak the sample prior to being placed in the bomb calorimeter. Regrettably, because the amount of NDF residue was limiting (~ 2 g of residue sample) we were unable to perform additionally assays on the residues.

Statistical analysis

Differences in GE energy content between feed and fecal samples were tested using the TTEST procedure of SAS (9.4). Prior to analysis outliers were determined using the UNIVARIATE procedure method within SAS, where outliers were determined at ± 2.5 standard deviations from the treatment means.

RESULTS AND DISCUSSION

The aim of this study was to evaluate the impact of lignin on the analytical estimates of energy contained in fiber. Practically, this is of interest to the field of dairy nutrition because in estimating DE, the current Dairy NASEM (2021) employs a summative equation that uses an assumed a GE concentration of nutritive entities that supply energy. In the case of NDF, lignin is used to derive the digestibility, but we speculated that because of its greater inherent GE concentration, the appearance of lignin in NDF could also influence analytical estimates of energy in this fraction. The GE concentration of NDF used in the Dairy NASEM (2021) is assumed to be 4.2 Mcal/kg while in the current study, we observed this to be 4.03 ± 0.245 Mcal/kg. It is possible that the lower estimate of the GE concentration was at least in part due to contamination of ash in the NDF residue (Higgs et al., 2015); because this is inorganic in nature, the ash fraction would contribute mass but not possess a GE concentration (Weiss and Tebbe, 2019). Together these factors could lead to underestimating true energy from fiber in a given sample. If indicative of practical conditions, this observation serves as support of the practice of correcting for ash contamination when determining the NDF content of feeds. Van Soest et al. (1991) suggests either correcting NDF values for ash contamination or reporting dietary ash

content when studying forages or other feeds because ask from soil contamination during harvesting methods may vary. If sample had not been limiting, lignin and ash contents of all residues would have been measured to elucidate nutrients responsible for the difference observed in GE concentrations. We also hypothesized that because lignin is indigestible and has a greater GE concentration than carbohydrate, that GE concentration would be greater for NDF in fecal residue than for NDF in feed residue. If this hypothesis should hold, we believed this could be a contributing factor to the variation in DE which has been reported (Tebbe et al., 2017). Surprisingly, the GE concentration between feed (4.03 ± 0.245 Mcal/kg) and fecal samples (3.94 ± 0.245 Mcal/kg) was not observed to be different ($P = 0.23$). This result suggests that observed variation in DE is more likely to be a function of the digestibility of the NDF fraction, which lignin is known to negatively impact.

The current study suggests that the GE concentration of fecal cellulose and hemicellulose may be lower than that of feed cellulose and hemicellulose. This is because feed and fecal NDF GE concentration were observed to be similar, despite lignin being in an assumed higher proportion in fecal NDF residues compared to feed NDF residues coupled with a greater GE concentration than carbohydrates. In order for both types of NDF residues to be equivalent in GE concentration, fecal cellulose and hemicellulose must be partially degraded in order to reflect a decrease in their energetic contribution to the NDF fraction as a whole and offset the inflated energetic contribution from lignin. If fecal cellulose and hemicellulose truly are partially degraded, additional DE is supplied to the animal that is not accounted for using current Dairy NASEM (2021) models or bomb calorimetry in energy balance studies.

While the current study primarily focuses on lignin and its contribution to GE and DE of the NDF fraction, varying proportions of hemicellulose could also be a contributing factor to

differences in these energetic fractions. GE coefficients for xylan in the literature range from 3.04 (Dorez et al., 2014) – 3.25 Mcal/kg (Gorensek et al., 2019). This range in GE values is similar to the difference in the observed NDF GE concentration value from the current study (4.03 Mcal/kg) and the GE concentration used by the Dairy NASEM (4.20 Mcal/kg). Xylose can account for 30-90% of the sugars present in hemicellulose depending on analytical methodology and type of hemicellulose, with the remaining sugars consisting of glucose, galactose, arabinose, and fructose (Peng et al., 2019). Xylose being a pentose sugar is relatively more oxidized compared to the other hexose sugars that compose, and therefore xylose should have a lower GE concentration compared to the other sugars of hemicellulose. If the true GE concentration of hemicellulose is approximately 3.04 – 3.25 Mcal/kg, the energetic contribution from feeds with NDF profiles rich in hemicellulose, like distillers' grains, will be greatly overestimated by nutritional models employing the current NDF GE coefficient. Additionally, the GE concentration values of Dorez et al. (2014) and Gorensek et al. (2019) were derived using hemicellulose from softwood trees as the extraction of hemicellulose from forages lacks a sound analytical procedure. The application of tree hemicellulose in feed energetics might be inaccurate. The heterogeneity and lack of laboratory methods to accurately precipitate feed hemicellulose hinder our understanding of how hemicellulose contributes to the energy concentration of NDF.

Although narrow in scope, this study provides information on important assumptions used to estimate energy by the Dairy NASEM (2021) model. Because the variation (CV = 6.21%) in the GE concentration of NDF was high (> 5%), future research should seek to identify major sources of variation. Additionally, the current study only evaluated the GE concentration of NDF and that of its individual components. That the GE concentration was different than

starch, a more uniform carbohydrate, should probably not come as a surprise because in addition to lignin, cellulose, and hemicellulose, NDF residues could also include some interfering protein and ash. In conclusion, being less than what is assumed, the energy supplied by fiber may be lower than what is used by the Dairy NASEM (2021) model and sources of variation in NDF's GE concentration should be further evaluated.

ACKNOWLEDGEMENTS

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TABLES AND FIGURES

Table 1. Comparison of the gross energy concentration for feed and fecal NDF residues[1].

	Sample Type			
	Feed	Fecal	SEM[2]	P-value
Gross Energy (Mcal/kg)	4.03	3.94	0.034	0.23

[1]Residues collected using the Ankom technique (Ankom200 Fiber Analyzer, Ankom Technology Corp., Fairport, NY).

[2]Least squares means; largest standard error of treatment mean is shown.

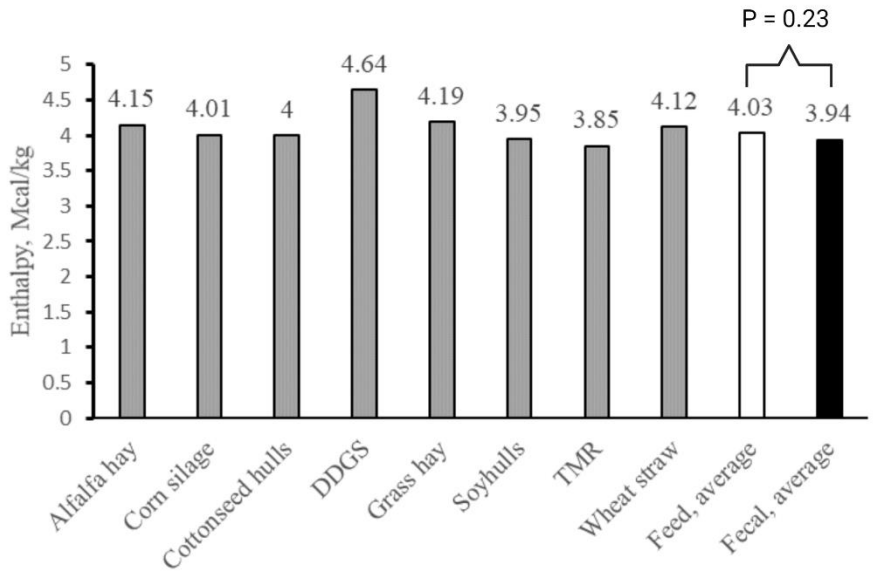

Figure 2.1 Gross energy concentration of NDF residue isolated from various feed and fecal NDF residues. Samples include: Alfalfa hay (N = 4.15, SD = 0.078), Corn silage (N = 2, SD = 0.078), Cottonseed hulls (N = 1), DDGS (N = 1), Grass hay (N = 2, SD = 0.078), Soyhulls (N = 1), TMR (N = 6, SD = 0.212), Wheat straw (N = 1), Feed average (feed and TMR) (N = 16, SD = 0.245) and fecal samples (N = 34, SD = 0.245); Difference between the mean feed and fecal samples were not different.

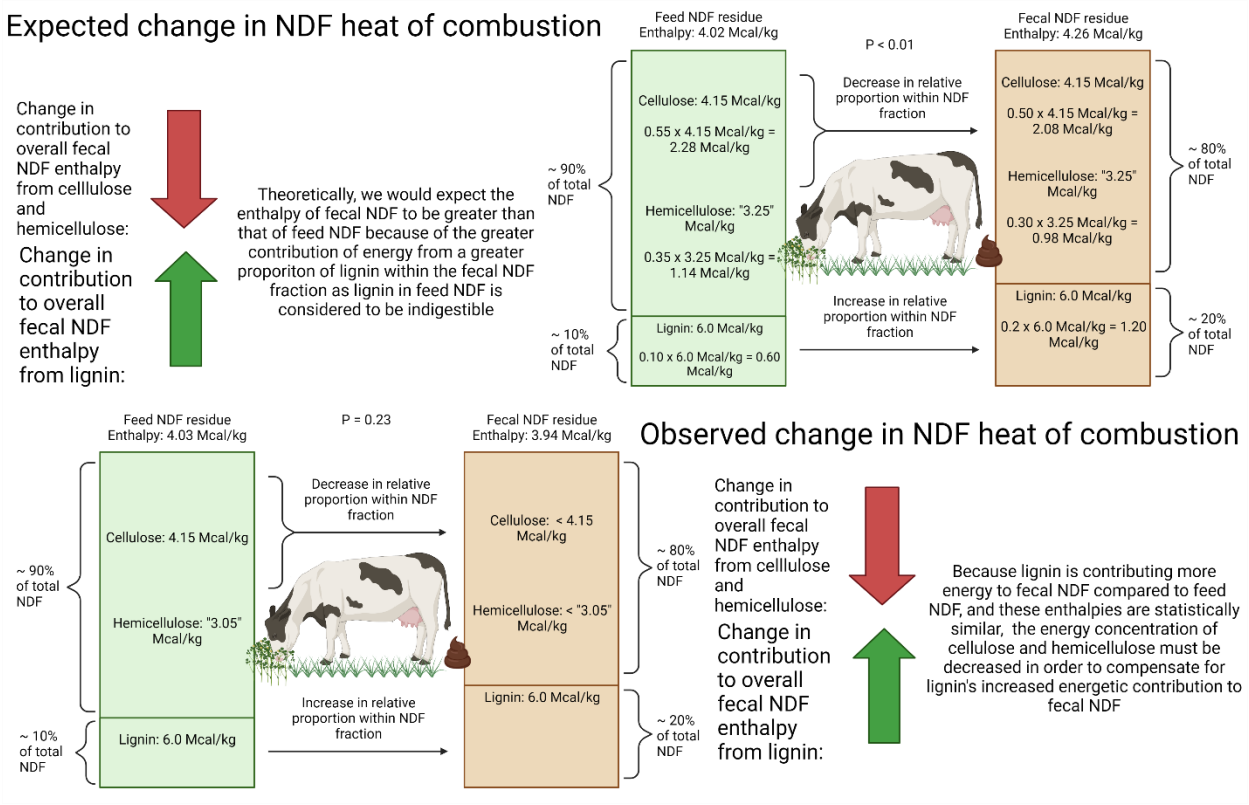

Figure 2.2 Visualization of theoretical and observed changes in feed and fecal NDF GE concentration.

CHAPTER 3

INTERPRETIVE STUDY. Stypinski et al. (2022). “Effect of lignin concentration for diets formulated to be similar in NDF content on energy and N utilization in lactating Jersey cows.” The current experiment observed increasing dietary lignin concentration reduced DM digestibility and intake which ultimate decreased milk yield and component yields. The environmental impacts of increasing lignin concentration of the diet are minimal while the impact of lignin on the energy contribution from NDF is more profound.”

RUNNING HEAD: LOW-LIGNIN OR HIGH-LIGNIN IN DAIRY DIETS

Effect of lignin concentration for diets formulated to be equal in NDF content on energy and nitrogen metabolism in lactating Jersey cows

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ABSTRACT

Lignin is a polyphenolic polymer that has been established to be an important factor in limiting fiber digestibility ruminants. The objective of the current study was to evaluate lignin's impacts on whole animal energy utilization in diets similar in fiber content. A low lignin **(LoLig)** treatment diet was formulated to contain 32.5% NDF (DM basis) and 9.59% lignin (NDF basis) and the high lignin **(HiLig)** diet was formulated to contain 31.0% NDF (DM basis) and 13.3% lignin (NDF basis). These diets were fed to 12 late-lactation (200 ± 14.9 DIM) multiparous Jersey cows (435 ± 13.9 kg) in a two period crossover design. Cows consuming the LoLig treatment consumed more DM ($P < 0.01$) than cows on the HiLig diet (19.9 vs. 18.7 ± 0.645 kg/d) while the LoLig diet was concurrently of a greater ($P < 0.01$) gross energy concentration (4.27 vs. 4.23 ± 0.03 Mcal/kg). As expected, increasing the concentration of lignin resulted in a reduction ($P < 0.01$) in total tract NDF digestibility (45.5 vs 40.4 ± 0.742%). Increasing lignin resulted in a reduction ($P < 0.01$) in the digestibility of starch (97.7 vs. 96.3 ± 0.420) and CP (65.0 vs. 60.0 ± 0.829). Lignin also decreased ($P < 0.01$) the concentration of digestible energy (2.83 vs. 2.63 ± 0.04 Mcal/kg) and metabolizable energy (2.52 vs. 2.36 ± 0.05 Mcal/kg) but the concentration of NE_L was similar ($P = 0.44$) averaging 1.67 ± 0.05 Mcal/kg. Increasing the concentration of lignin also reduced ($P < 0.02$) yields of energy-corrected milk (33.7 vs. 30.0 ± 1.08 kg/d), milk protein (1.00 vs 0.843 ± 0.052 kg/d), milk fat (1.03 vs. 1.19 ± 0.058 kg/d). Decreasing the dietary lignin concentration did not affect ($P = 0.73$) daily methane emissions, averaging 391 ± 29.6 L/d. Results of this study indicate feeding a diet greater in lignin decreases the digestibility of nutrients and provides less energy for production responses while methane emissions were not affected.

Keywords: NDF, lignin, indirect calorimetry

INTRODUCTION

Neutral detergent fiber accounts for 25-33% of typical dairy rations; this variation is to factors such as chemical composition of the feed and formulation decisions (NASEM, 2021). Neutral detergent fiber provides a significant proportion of the energy in dairy rations, but digestibility is a limiting factor in how much of this energy is available to the cow (Dado and Allen, 1996). The lignin concentration of the NDF fraction is a major plant-based factor limiting ruminal digestion of NDF (Jung et al., 1997). Lignin is a polyphenolic compound found in the cell wall of plants which primarily maintains structural integrity, but it also provides secondary functions such as nutrient transport (Liu et al., 2018). Therefore, reducing the lignin content of dairy diets is of interest because this may result in positive benefits of increasing digestibility, feed intake, and overall energy supply (Jung et al., 2011). Many studies have described lignin's impacts on NDF digestibility (Jung et al., 2011; Raffrenato et al., 2017; Van Soest et al., 2018), but few studies have utilized indirect calorimetry to quantify lignin's effects on NDF digestion to whole animal energy utilization. The current Dairy NASEM (2021) model uses dietary lignin content to estimate NDF digestibility, but the assumed relationship between lignin and whole animal energy utilization has not been completely studied.

Lignin and some of the other bound polymers are assumed to be completely indigestible and therefore the energy contained within these nutrients are not available to the animal (Dong et al., 2011). The gross energy (**GE**) concentration of lignin is 6.0 Mcal/kg (Voitkevich et al., 2012) while the assumed GE concentration of the entire NDF fraction is 4.2 Mcal/kg (NASEM, 2021). Therefore, increasing the proportion of lignin within the NDF fraction should result in an increase in the GE concentration of the entire NDF fraction, while also limiting the digestibility

of the NDF fraction and in turn, supply of digestible energy (**DE**). Additionally, data are lacking on the comparison of feed and fecal NDF residues for energy concentration. The comparison of feed and fecal NDF residue GE concentration could provide additional insight into the partitioning of energy from fiber. Stypinski et al. (2021) observed the GE concentration of NDF to be 4.03 Mcal/kg, which is lower than the GE concentration used in the Dairy NASEM (2021) equations (4.20 Mcal/kg) used for predicting GE and DE. As a consequence, if the true GE concentration of NDF is lower than assumed by the Dairy NASEM (2021) model, the resulting estimates of energy supply could result in an over prediction by the model. The fermentation of fiber is multifaceted, and its full scope is likely not captured in nutritional models used to predict the energetic contributions from fiber. The objective of this study is to evaluate energy and N metabolism of lactating cows fed diets with different lignin concentrations and to investigate broader effects on production and nutrient excretion. We hypothesized cows consuming a diet higher in lignin concentration will convert GE to DE at lower efficiency, and this will result in less energy partitioned towards milk production.

MATERIALS AND METHODS

Animals and Treatments

The University of Nebraska- Lincoln Animal Care and Use Committee approved animal care and experimental procedures. Twelve multiparous Jersey cows 200 ± 14.9 DIM and weighing 424 ± 46.5 kg were housed in individual tie stalls in a climate-controlled environment (20º C) at the University of Nebraska-Lincoln Dairy Metabolism Facility in the Animal Science Complex. Stalls were surfaced with rubber mats and cows were milked at 0700 and 1800 h. All

cows were less than 184 d pregnant at the end of the last experimental period thus fetal energy was assumed to be zero (NRC, 2001).

The experimental design was a two period cross-over with periods of 28 d each. In period 1, cows were randomly assigned to 1 of 2 treatment diets (6 cows per treatment per period): 1) a low-lignin diet (**LoLig**) or 2) high-lignin diet (**HiLig**). For period 2, the alternative diet was fed. Dietary treatments were formulated to be similar in protein and NDF but ingredients were manipulated so that they differed in the concentration of lignin. This was primarily achieved by including more alfalfa hay and cottonseed hulls in the HiLig treatment. Concentrate mixes for each treatment included all dietary ingredients except for forages (Table 1) and were mixed at the University of Nebraska-Lincoln feed mill. Corn silage, alfalfa hay, and concentrate were added to a Calan Data Ranger (American Calan, Inc., Northwood, NH), mixed, and fed as a TMR once daily at 0930 h with a target refusal rate of 5%. Each period included 24 d of ab libitum diet adaptation, followed by 4 d of collection where diets were fed at 100% of the prior week's intake to limit refusals.

Sample Collection and Analysis

Individual feed ingredients were sampled daily during collection periods and frozen at -20º C. All feed ingredients were dried at 60º C and were ground through a 1 mm screen. (Wiley Mill; Aurthur A. Thomas Co., Philadelphia, PA). A subsample of ground feed was sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis of DM (method 930.15, AOAC, 2000), CP (method 990.03, AOAC, 2000), Nitrogen (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085), soluble CP (Krishnamoorthy et al. 1982) ADICP and NDICP (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085), ADF (method 973.18, AOAC, 2000), NDF with sodium sulfite and α amylase corrected for ash contamination (aNDFom) (Van

Soest et al., 1991), lignin (Goering and Van Soest 1970), ether extract (method 920.29 AOAC, 2000) sugar (Hall, 2009), starch (Hall 2009), ash (method 942.05, AOAC, 2000), minerals (method 985.01, AOAC, 2000), total fatty acids (Sukhija and Palmquist, 1988). Feed samples were also analyzed for GE content using a bomb calorimeter (Parr 6400 Calorimeter, Moline, IL). Total mixed rations were sampled on d 1 of each collection period and used to determine particle size using the Penn State particle separator (Kononoff and Heinrichs, 2002) on an as is and DM basis (60ºC for 48 h). During each collection period refusals were sampled and composited on a weight basis. Refusals were analyzed for DM, CP, NDF, aNDFom, starch, ash, fatty acids, and GE according the same methods as feeds.

Total fecal and urine output was collected from each individual cow during the collection period for 4 consecutive d as described by McLain et al. (2021). After collections, approximately 600 g feces were dried at 60ºC for 48 h and ground to pass through a 1 mm screen (Wiley Mill; Aurthur A. Thomas Co., Philadelphia, PA). The ground feces were analyzed for chemical composition using the same methods as described for refusals. Milk production was measured daily, and milk samples were collected during the morning and evening milking of collection periods as described by McLain et al. (2021). Milk from individual milking events was preserved with 2-bromo-2nitropropane-1,3 diol and sent to Heart of America DHIA (Kansas City, MO). Milk samples were analyzed for fat, protein, lactose, SNF, MUN, and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). Additionally, milk from each milking event was composited on a weight basis. Cows were weighed, before feeding on the first and last day of each collection period.

Heat Production and Energy Utilization and Calculations

Heat production was determined indirectly through the headbox-type indirect calorimeters as described previously (McLain et al., 2021). However, total volume of gas flow

through the headbox was measured using mass flow meters (MCW-1000SLPM-D Whisper, Alicat Scientific) and corrected to standard temperature and pressure (0ºC, 101.3 kPa) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). System efficiency (head box and gas analyzer) was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O_2 and CO_2 were (average ± SD) 100 ± 2.9 and 99 ± 2.5 %, respectively. All energy calculations were performed according to McLain et al. (2021).

To isolate NDF residues from feed and fecal samples for GE analysis, feed and fecal samples were dried at 60° C for 48 hours and ground through a 1-mm sieve (Wiley Mill; Arthur A. Thomas Co., Philadelphia, PA). Once ground, NDF residues were obtained from feed and fecal samples using the Ankom technique (Ankom200 Fiber Analyzer, Ankom Technology Corp., Fairport, NY) in quadruplicate with sodium sulfite and α amylase (Sigma A3306; Sigma-Aldrich, St. Louis, MO). Neutral detergent fiber residues were then ground further using a pestle and mortar and dried again at 60 ° C for 24 hours. Once completely dried, 0.2 grams of ground NDF residue and 0.4 grams of mineral oil were weighed for bomb calorimetry (Parr 6400 Calorimeter, Moline, IL). Weighed samples were soaked in mineral oil for 24 h prior to being placed in the bomb calorimeter for determination of GE.

Pre-trial headbox training

Prior to the start of this experiment, cows were trained to place their heads inside headboxes to ensure they would eat and drink comfortably for the duration of their time inside the headbox during the study. On the first day of training, cows would place their heads inside headboxes with the circulation motors off for ten hours with the door left open. Staff would check to ensure these animals were eating and drinking regularly. If the cow ate and drank during the first training period, she would go back into the headbox for 24 hours with the door closed and motor running. Staff would check regularly to ensure she ate and drank and remove

her from the headbox if she had gone more than six hours without eating or drinking. Animals that did not eat or drink were encouraged to do so by a staff member holding the feed or water to her face.

Statistical Analysis

A type III analysis of variance with Kenward-Rodger's denominator degrees of freedom was complete using the PROC GLIMMIX function of SAS. This statistical model considered effects of cow and period as random and effects of treatment as fixed. All data are presented as least-squares means ± largest standard error. Significance and trends were declared with a *P*-value ≤ 0.05 and *P*-value ≤ 0.10.

RESULTS

Twenty-two out of the 24 planned observations were obtained for gas related calculations. During training one cow refused to drink water while in the headbox during training periods thus gas related measures were not collected on this cow. However, all other measures were collected and used from this cow in both periods.

Chemical composition

Diet composition of the two diet treatments is listed in Table 3.1 and particle size distributions of diets are listed in Table 3.2. The lignin content of the HiLig diet was about 1% greater than that of the LoLig diet, averaging 4.38 ± 0.129 and 3.29 ± 0.208% for the HiLig and LoLig diets, respectively. The NDF content of the LoLig diet (32.5 ± 0.655%) was similar to that of the HiLig diet (31.0 ± 0.404%), but lignin as a percent of NDF was lower for the LoLig treatment (9.59 ± 0.388%) than the HiLig treatment (13.3 ± 0.02%). The ADF content of LoLig (21.1 ± 1.28%) and HiLig (21.7 ± 0.744%) diets were also similar. The TFA content of the LoLig diet was 3.30 ± 0.371 % DM while the TFA content of the HiLig diet was 2.842 ± 0.118

% DM. The starch contents of the LoLig and HiLig diets were 27.3 ± 0.295% and 24.4 ± 0.332% for LoLig and HiLig, respectively. The CP content was similar across both diets with 16.3 ± 0.078% for the LoLig and 16.6 ± 0.149% for the HiLig diet. Chemical composition of corn silage, alfalfa hay, and concentrate mixes are listed in table 3.3. The primary forages utilized in these diets were corn silage (42.0 % DM, and 8.45 % CP, 37.7 % NDF, 3.84% ADL, and 39.5 % starch on a DM basis) and alfalfa hay (89.9% DM, and 19.7 % CP, 40.3 % NDF, 7.13 % ADL, and 2.10 % starch on a DM basis).

Intake energy

Cows consuming the HiLig diet consumed less dry matter of a lower GE concentration compared to the LoLig cows, averaging 19.9 vs. 18.7 ± 0.645 kg/d and 4.27 vs. 4.23 ± 0.03 Mcal/kg for the LoLig and HiLig diets, respectively. Feeding the HiLig diet reduced GE intake by 5.8 Mcal/d, averaging 85.0 vs. 79.2 ± 2.74 Mcal/d for the LoLig and HiLig diets, respectively.

Nutrient digestibility

Feeding the HiLig diet decreased ($P < 0.01$) total tract NDF digestibility from 45.5 to 40.4 ± 0.742%. Feeding the HiLig diet decreased ($P < 0.01$) starch digestibility from 97.7 to 96.3 ± 0.420%. Crude protein digestibility was lower ($P < 0.01$) for cows fed the HiLig treatment compared to the LoLig, averaging 60.0 and 65.0 ± 0.829%, respectively. Feeding the HiLig diet decreased ($P < 0.01$) digestibility of OM and energy, which averaged 69.0 vs. 65.9 ± 0.489% and 66.2 vs. 62.6 ± 0.516%, respectively. Increasing the lignin content of the diet had no effect ($P = 0.32$) on the digestibility of TFA, which averaged 76.7% vs. 78.5% ± 1.54% for LoLig and HiLig, respectively.

Energy utilization

The HiLig diet contained a lower ($P < 0.01$) concentration of DE and metabolizable energy (**ME**) per kg, averaging 2.83 vs 2.63 ± 0.04 Mcal/kg, 2.52 vs. 2.36 ± 0.03 Mcal/kg, respectively. However, increasing the concentration of lignin in the diet did not have an effect on net energy for lactation (**NEL**) concentration, averaging 1.70 vs 1.64 ± 0.05 Mcal/kg for LoLig and HiLig, respectively. Fecal, methane, and heat energy were not affected by treatment ($P \geq 0.12$) and averaged 28.7 vs. 29.6 ± 1.12 Mcal/d, 3.74 vs 3.64 ± 0.28 Mcal/d, and 23.3 vs 20.3 ± 1.21 Mcal/d for LoLig and HiLig, respectively. However, urinary energy tended to decrease ($P = 0.06$) with increasing lignin content (1.90 vs. 1.47 ± 0.16 Mcal/d).

Energy contribution from NDF

Gross energy concentrations for NDF residues from individual feed ingredients and feces from cows fed both diets were determined using bomb calorimetry. The GE concentration of HiLig NDF residues (feed and fecal) were greater ($P < 0.01$) compared to LoLig (feed and fecal), averaging 4.09 vs 3.99 ± 0.034 Mcal/kg, respectively. Feed NDF (LoLig and HiLig) residues were significantly greater ($P = 0.03$) in GE concentration compared to fecal NDF residues (LoLig and HiLig), averaging 4.12 vs. 3.96 ± 0.034 Mcal/kg, respectively.

Nitrogen metabolism

Nitrogen intake was greater ($P = 0.01$) for cows consuming the LoLig diet compared to cows consuming the HiLig diet (525 vs. 497 ± 16.9 g/d). Nitrogen balance as a function of N intake was not affected ($P = 0.45$) by increasing the lignin content of the diet and averaged 1.91 and 3.86 ± 2.17% for LoLig and HiLig, respectively. Increasing the lignin content of the diet increased fecal N as a percent of total N from 35.0 to 40.0 ± 0.829%. Feeding the HiLig diet decreased ($P < 0.01$) and tended to decrease ($P = 0.06$) milk and urinary N as a percent of total N, averaging 35.6 vs. 33.0 ± 0.616% and 27.4 vs. 23.1 ± 1.73%, respectively.

Environmental impacts

Feeding the HiLig diet had no effect ($P = 0.87$) on methane production per unit of DMI (methane yield), averaging 20.3 vs. 20.5 ± 1.60 L/kg for LoLig and HiLig, respectively. Increasing the lignin concentration of the diet had no effect ($P = 0.51$) on methane energy as a proportion of GEI, averaging 4.40 vs. 4.63 ± 0.171 for LoLig and HiLig, respectively. Methane produced per kg of ECM (methane intensity) was not affected ($P = 0.25$) by diet, averaging 11.8 vs. 12.9 ± 0.981 L/kg for LoLig and HiLig, respectively. Manure and productive N g/d were not affected ($P \geq 0.36$) by diet, averaging 327 vs. 314 ± 14.3 g/d and 198 vs. 183 ± 21.4 g/d, for LoLig and HiLig, respectively. Feeding the HiLig diet had no effect ($P = 0.83$) on manure and productive N as proportions of total N intake, averaging 62.5 vs. 63.1 ± 1.40 and 37.5 vs. 36.9 ± 1.40 for LoLig and HiLig, respectively. Methane produced per kg milk protein was increased ($P = 0.05$) in cows fed the HiLig diet, averaging 398 vs. 465 ± 28.1 L/kg. Feeding the HiLig diet increased ($P = 0.02$) methane produced per kg of NDF digested, averaging 138 vs. 165 ± 13.0 L/kg.

Milk production and composition

Feeding the HiLig diet decreased ($P < 0.01$) milk production and ECM, averaging 28.2 vs. 25.1 ± 1.21 kg/d, and 33.7 vs. 30.0 ± 1.08 kg/d, respectively. Milk protein and fat concentrations were not affected ($P \geq 0.11$) by treatment (3.55 vs. 3.38 ± 0.155% and 4.65 vs. 4.82 ± 0.264% for LoLig and HiLig, respectively), but yields of milk protein and fat were lower ($P \leq 0.02$) for the HiLig diet (1.00 vs. 0.843 ± 0.308 kg/d and 1.30 vs. 1.19 ± 0.058, for LoLig and HiLig, respectively). Energy-corrected milk produced per kg of DMI was lower ($P = 0.03$) for the HiLig diet, averaging 1.69 vs. 1.61 ± 0.027.

DISCUSSION

The objective of this study was to characterize NDF digestibility, DMI, energy and N utilization and environmental responses of lactating Jersey cows consuming diets with different concentrations of lignin. The HiLig diet was formulated to contain a higher lignin concentration by substituting corn silage for alfalfa hay as the primary forage, which had subsequent impacts on the associated concentrate mixes associated with each diet. Lignin has an GE concentration of 6.0 Mcal/kg (Voitkevich et al., 2012) which is relatively high compared to other nutrients. However, lignin is also considered to be entirely indigestible and therefore should not supply any energy to the animal. It is for this reason that we hypothesized that diets containing greater proportions of lignin will decrease the conversion of GE to DE, and therefore each subsequent fraction of the energy cascade.

Nutrient composition

According to the Dairy NASEM (2021) feed library, the corn silage used in the current study was representative of a mature corn silage as all nutrients (DM, CP, NDF, and starch) were within one standard deviation of the mean reported, with the exception of lignin, which was greater than the reported average, but within two standard deviations. The alfalfa hay utilized in the current study was also representative of a typical mid-maturity legume hay as DM, CP, NDF, ADL, and starch were all within one standard deviation of the average reported in the Dairy NASEM (2021) feed library. The alfalfa hay used in the current study contained a greater numerical ADL content (7.13 vs. 6.64 % DM) compared to the typical mid-maturity legume hay in the Dairy NASEM (2021) feed library.

Intake Energy

Feeding the HiLig diet reduced DMI by 1.2 kg and such a response is a common observation among other studies feeding reduced-lignin diets (Benchaar et al., 2014; Colombini et al., 2015). The Dairy NASEM (2021) notes that cows that are later in their lactation cycle

consume feed until the energetic demand of milk production and that gut fill is not the limiting mechanism for intake. The current study suggests that even intake by cows in late lactation could potentially still be limited by gut fill. In addition to reduced DMI, the HiLig diet also contained less GE (0.04 Mcal/kg). Gross energy of a diet is representative of the total energy contained within its chemical bonds (Hall et al., 2013). Using the Dairy NASEM (2021) summative equation to calculate dietary GE concentration, the NDF, starch, and TFA inclusions of the HiLig diet contributed 0.07, 0.12, and 0.043 less Mcal/kg of GE relative to those of the LoLig diet, respectively. The ash and iron content of the HiLig diet was 1.18% (% DM) and 95.4 mg/kg greater than that of the LoLig diet. While the ash fraction contains essential minerals for host and microbial metabolism, minerals are inorganic in nature and therefore have a GE of 0 Mcal/kg (NASEM, 2021) and dilute the energy density of a ration (Tebbe et al., 2017). Dry matter intake and GE concentration concurrently decreased the total amount of energy consumed by cows on the HiLig diet.

Nutrient digestibility

The corn silage and alfalfa used in this study were similar in NDF content but differed in the relative proportion of lignin within the NDF fraction (9.59 vs. 13.3% for LoLig and HiLig, respectively). The NDF profile of the HiLig diet contained a lower proportion of cellulose and hemicellulose and a greater proportion of indigestible lignin. In accordance with our hypothesis and observations by others (Chow et al., 2008; Brown et al., 2018; Lyons et al., 2019), NDF digestibility decreased by 5.1% with increasing concentration of dietary lignin. Both treatment diets were evaluated using the Dairy NASEM (2021) model for nutrient requirements of dairy cattle. In these simulations and using the lignin based equation (Equation 3.3a; pg. 24) total tract digestibility of NDF was predicted to be 7.55 % lower for the HiLig treatment (53.55 and 46.0 % for LoLig and HiLig respectively). We speculate that increasing the concentration of lignin

within the NDF fraction not only limited the digestibility of NDF, but also limited the overall fermentability of other key nutrients such as starch and CP. In support of this, greater fibrolytic microbial activity has been reported to enhance proteolytic and saccharolytic microbial activity through the production and release of intermediates and end products that can be utilized by other microbes to proliferate and further digest other nutrients (Millen et al., 2016). The Dairy NASEM (2021) equation 3-5a attempts to discount NDF digestibility by considering the generally negative impacts of dietary starch on fiber digestion. The current study, however, suggests that modeling associate effects in nutrient digestibility is complex and should potentially consider other factors.

Energy metabolism

Increasing the concentration of lignin has a negative effect on the conversion of GE to DE as the GE concentration of lignin is 6.0 Mcal/kg (Voitkevich et al., 2012) but has a DE concentration of 0.0 Mcal/kg (Morris, 2020). This is because lignin is considered completely indigestible and therefore none of the energy present in lignin is converted to energy available to the cow. This concept of a reduced efficiency of converting GE to DE with a greater lignin content was observed when feeding the HiLig diet, which reduced this efficiency from 66.2 to 62.6%. The Dairy NASEM (2021) model predicted the concentration of DE would only decrease by 0.04 Mcal/kg (2.96 vs. 2.92 Mcal/kg) when increasing the lignin concentration of the ration, while the observed effect was a decrease of 0.20 Mcal/kg (2.83 vs. 2.63 Mcal/kg). The Dairy NASEM (2021) model underpredicted lignin's impacts on DE concentration, likely due to not accounting for lignin's impacts on overall diet fermentability, while overpredicting overall DE concentration, namely overestimating NDF and CP digestibility. Similar to DE, the concentration of ME of the HiLig diet was lower than that of the LoLig diet and this is despite the fact that methane energy was similar and a trend for lower urinary energy excretions. We speculate that

the lack of a difference in methane production, despite differences in NDF digestibility and DMI, could have been at least in part due to a greater rumen pH induced by the HiLig diet. A greater rumen pH is known to favor methanogenesis over propiogenesis as a hydrogen assimilation mechanism (Ungerfeld, 2020). Hassanat et al. (2013) reported a linear decrease in ruminal pH with increasing proportions of corn silage at the expense of alfalfa silage. The decrease in nutrient digestibility associated with the HiLig diet may have also resulted in a decrease in microbial protein synthesis in the rumen, which could explain the lower urinary energy excretion. Greater metabolism of microbial protein is associated with assimilation of N and consequently excretion of purine derivatives in the urine (Dórea et al., 2017), and therefore greater urinary energy excretion. Oba and Allen (2000) compared isogenic corn silage to BMR corn silage and found an increase in overall DM digestibility coupled with an increase in microbial N flow to the small intestine with a similar difference in dietary lignin content to the current study. Thus, we speculate that the reduced ME concentration of the HiLig diet was a function of reduced nutrient digestibility and a less desirable rumen environment for productive fermentation. Uddin et al. (2020) also reported a decrease in DE and ME concentration with increasing dietary lignin concentration by replacing corn silage with alfalfa silage. Lastly, the current study observed NEL as a proportion of ME was similar across diets, suggesting the effects of reduced digestibility of the HiLig diet should have carried through to NEL.

Energy contribution from NDF

As previously noted, the Dairy NASEM (2021) assumes the GE concentration of feed NDF to be 4.20 Mcal/kg and this is used in the calculation of GE and DE. In the current study, we also directly measured the GE concentration of feed NDF residue and observed it to be 4.12 Mcal/kg. Admittedly, the sample size of feed NDF residues from corn silage, alfalfa hay, and grain mixes used to formulate the LoLig and HiLig diets was relatively small (N = 8).

Nonetheless, we speculate this discrepancy could be at least be one reason why nutrition model over-predict the concentration in rations, especially for rations with greater proportions of NDF. Using a larger sample size (N = 16) Stypinski et al. (2021) (Chapter 2 of this thesis) reported the GE concentration of feed NDF to be 4.03 Mcal/kg, which would result in an even larger overestimation of GE and DE from NDF by the Dairy NASEM (2021) model. The model over-predicted DE for the LoLig and HiLig diets by 2.7 and 4.8 Mcal/d, respectively. If the true digestibility and 4.12 Mcal/kg NDF GE coefficient were used in place of the calculated NDF digestibility and the standard NDF GE coefficient, the model would have only over-predicted DE by 0.1 and 3.3 Mcal/d for LoLig and HiLig, respectively. Feed NDF GE concentration was observed to be greater compared to fecal NDF GE concentration by a margin of 0.15 Mcal/kg. This is likely representative of partial degradation of cellulose and hemicellulose from the digestive process. The difference in energy concentration between feed and fecal NDF is not currently accounted for in the Dairy NASEM (2021) calculation of DE based on chemical composition. We suggest that the equation could be updated to account for the additional DE from partial degradation of cellulose and hemicellulose. We have previously reported that feed and fecal NDF were similar in energy concentration. However, in that study the GE concentration of fecal cellulose and hemicellulose is actually numerically decreased despite a lack of statistical significance. This is because lignin is assumed to be completely indigestible compared to cellulose and hemicellulose, which would increase its relative proportion within fecal NDF residues. Additionally, the GE concentration of lignin is 6.0 Mcal/kg (Voitkevich et al., 2012), while the cellobiose and xylan have GE concentrations of 4.15 and 3.04 Mcal/kg (Colbert et al., 1981; Dorez et al., 2014), respectively. Stypinski et al. (2021) suggests that in order for feed and fecal NDF to be of similar energy concentration, the GE concentration of

cellulose and hemicellulose must be decreased in order to offset the inflated proportion and greater GE concentration of lignin in fecal samples compared to feed samples. The greater GE concentration and proportion of lignin within the NDF residue is also likely why the GE concentration of HiLig samples was greater than that of LoLig samples.

Nitrogen utilization

Feeding the HiLig diet decrease DMI, and subsequently reduced total N intake. Increasing the lignin concentration of the diet shifted N excretion from the urine to the feces. This shift might represent reduced fermentability caused by feeding the HiLig diet, allowing less feed N to be incorporated into microbial protein that will be metabolized by the cow instead of being excreted in the feces. Wattiaux and Karg (2004) also observed a shift from urinary N to fecal N excretion when replacing corn silage with alfalfa silage. Urinary N has more potent environmental consequences compared to fecal N as urinary N is more rapidly converted to ammonia by microbial ureases present in the environment and feces upon mixing of manure (Muck, 1982). Cows on the HiLig diet also partitioned a lower proportion of intake N towards milk N, which is also likely driven by reduced efficiency in converting feed into microbial N and energy that can be used to support secretion of milk protein.

Environmental impacts

Methane emitted for ruminants not only represents an energetic loss to the animal but can also exacerbate the effects of climate change (Boadi et al., 2004). In changing the proportion of lignin with in the NDF fraction, the proportion of cellulose remained relatively constant across both diets while the proportion of hemicellulose was greater for the LoLig diet compared to the HiLig diet. Moe and Tyrrell (1979) report the fermentation of hemicellulose produces significantly less methane than that of cellulose and could be a viable dietary manipulation strategy aimed at methane mitigation. In the current study, dietary treatment had no impact on

methane yield. These results are interesting because methane production has been positively correlated with DMI and NDF digestibility (NASEM, 2021), which in the current study were both reduced when increasing the proportion of lignin in the diet. Drehmel et al. (2018) replaced hemicellulose with lignin in a similar proportion as the current study and observed an increase in NDF digestibility with a greater proportion of hemicellulose, but no difference in starch and CP digestibility, DMI, or methane emissions. Results of these studies suggest that methane emissions might be better predicted when considering the digestibility of starch and CP in addition to NDF digestibility and DMI. Feeding the HiLig diet increased methane emitted per kg of milk protein, often termed “methane intensity”. We speculate this to be a function of reduced nutrient digestibility resulting in a greater ruminal pH associated with the HiLig diet, which is known to thermodynamically favor the production of methane as a hydrogen assimilation mechanism (Ungerfeld, 2020). Hassanat et al. (2013) also reported decreased methane output per kg of milk protein when replacing alfalfa silage with corn silage. Collectively, these results indicate that feeding a diet lower in lignin concentration might prove to be a reliable way to target milk components while also reducing GHG emissions from dairy cows. Nitrogen excretion from manure is of great importance because of its role on environmental impact of dairy production, namely eutrophication in waterways and pollution of the air (Arriaga et al., 2009). The current study observed increasing the dietary concentration of lignin had negative effects on nutrient digestibility but observed no shifts from manure N partitioning to productive N partitioning. This is because the increase in urinary N by cows of the LoLig diet is in a similar magnitude as the increase in fecal N by cows on the HiLig diet. The same pattern is observed for milk and retained N for the LoLig and HiLig diets, respectively. Weiss and Wyatt (2006) also reported no difference in manure and productive N partitioning associated with dietary lignin

content when replacing conventional corn silage with a BMR mutant. Together these results suggest the lignin concentration of the diet impacts the partitioning of N towards urine or feces, but not total manure N excretion.

Milk production and composition

Feeding the HiLig diet decreased energy-corrected milk yield, which was a function of reduced intake and nutrient digestibility. Uddin et al. (2020) reported similar results with respect to energy-corrected milk yield coupled with enhanced NDF and CP digestibility when increasing the proportion of corn silage at the expense of alfalfa silage in the ration. Yields, but not concentrations, of milk fat and protein were lower for the HiLig diet compared to the LoLig. The digestion of NDF primarily yields lipogenic VFAs (Souza et al., 2022), which are utilized by the cow for energy production and milk fat synthesis. Reduced intake and nutrient digestibility, especially NDF digestibility, limited milk fat synthesis for cows on the HiLig diet. The reduction in milk protein secretion was likely an extension of reduced microbial protein synthesis coupled with less energy available from reduced intake and nutrient digestibility. Erdman et al. (2011) and Oelker et al. (2009) also observed increase in milk fat and protein yields, respectively, when replacing alfalfa hay and silage with corn silage. The Dairy NASEM (2021) calculates feed efficiency according to equation 3-21 (page 36) to account for milk and tissue energy. Feed efficiency defined according to the Dairy NASEM (2021) was 31.2 and 29.2% for the LoLig and HiLig diets, respectively. We speculate this decrease in efficiency associated with the HiLig diet is mostly driven by reduced nutrient digestibility. This study demonstrates that the composition of the NDF fraction, and not solely the proportion of NDF in the diet, is an important consideration for maximizing nutrient digestibility and production responses.

CONCLUSIONS

Increasing the concentration of lignin resulting in reducing effects on intake and digestibly of NDF and other nutrients. The impacts of increasing the lignin content of the diet were likely realized as a more synchronous rumen environment better suited for assimilation of nutrients into fermentation end products and microbial cell protein. The effects of decreased nutrient digestibility from the HiLig diet carried through to total supply of DE, ME, and NEL when accounting for intake, but only through DE and ME on a concentration basis. The effects of reduced intake and nutrient digestibility also resulted in decreases in milk yield and components results while environmental impact results were less profound. Results of the current study could be interpreted to suggest that 4.20 Mcal/kg for the NDF GE concentration in the Dairy NASEM (2021) model calculations of GE and DE might be incorrect as these variables are more accurately predicted when using the observed GE concentration of feed NDF of 4.12. The true GE concentration of NDF could be lower than reported in the Dairy NASEM (2021) due to varying proportions of ash (0 Mcal/kg, (Weiss and Tebbe, 2019)), hemicellulose (3.25 Mcal/kg, (Gorensek et al., 2019)), and lignin (6.0 Mcal/kg, (Voitkevich et al., 2012)).

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TABLES AND FIGURES

Table 3.1. Ingredients and chemical composition of low-lignin and high-lignin diets (% of diet DM)[1]

Items	LoLig	HiLig
Ingredients		
Corn silage	42.1	13.8
Alfalfa hay	13.8	42.1
Corn grain, dry ground	13.9	24.5
Non-enzymatically brown soybean meal[2]	4.03	3.12
Dried distillers grains and solubles	8.68	2.23
Soybean meal	5.72	3.34
Molasses beet	1.78	1.78
Soybean hulls ground	7.57	3.34
Cottonseed hulls	-	3.34
Fat[3]	0.668	0.668
Salt	0.401	0.401
Sodium bicarbonate	0.445	0.445
Calcium phosphate	0.267	0.267
Magnesium oxide	0.111	0.111
Calcium carbonate	0.445	0.445
Trace mineral premix[4]	0.004	0.004
Vitamin premix[5]	0.004	0.004
Water	-	0.004
Chemical composition, % DM unless noted[6]		
DM	60.4 (2.52)	60.5 (1.94)
CP	16.3 (0.078)	16.6 (0.149)
Cellulose[7]	17.8 (1.07)	17.3 (0.615)
Hemicellulose[8]	11.4 (0.621)	9.35 (0.341)
aNDFom[9, 10]	32.0 (0.728)	30.4 (0.366)
NDF[9, 10]	32.5 (0.655)	31.0 (0.404)
Cellulose, % NDF	54.8 (2.18)	55.8 (1.26)
Hemicellulose, % NDF	35.1 (2.62)	30.1 (1.49)
Lignin, % NDF	9.59 (0.388)	13.3 (0.002)
ADF[11]	21.1 (1.28)	21.7 (0.744)
Cellulose, % ADF	84.4 (0.046)	79.8 (0.098)
Lignin, % ADF	15.6 (0.046)	20.2 (0.098)
ADL[12]	3.29 (0.208)	4.38 (0.129)
ADICP[13]	1.00 (0.332)	1.33 (0.526)
NDICP[14]	1.97 (0.337)	2.41 (0.245)
Starch	27.3 (0.295)	24.4 (0.332)
Sugar	4.13 (0.478)	5.82 (0.197)
TFA[15]	3.30 (0.378)	2.84 (0.118)

16 Carbon	0.737 (0.060)	0.730 (0.039)
18 Carbon	0.108 (0.005)	0.101 (0.00)
Ash	7.56 (0.719)	8.74 (0.587)
Ca	0.711 (0.016)	1.07 (0.014)
P	0.435 (0.014)	0.424 (0.00)
Mg	0.266 (0.006)	0.273 (0.016)
K	1.71 (0.043)	2.24 (0.147)
S	0.243 (0.009)	0.216 (0.004)
Na	0.336 (0.023)	0.348 (0.029)
Cl	0.429 (0.023)	0.412 (0.006)
Fe, mg/kg[16]	273 (55.2)	368 (108)
NEL, Mcal/kg[17]	1.70	1.68

[1]LoLig = low-lignin diet; HiLig = high-lignin diet; values in parenthesis indicate SD (n = 2).

[2]Soypass (LignoTech, Overland Park, KS).

[3]Megalac (Church and Dwight Co., Princeton, NJ)

[4]Formulated to supply approximately 1,133.79 KIU/d vitamin A, 181.41 KIU/d vitamin D and 53.51 IU/d vitamin E in total rations.

[5]Formulated to supply approximately 2,000 mg/kg Co, 20,000 mg/kg Cu, 2,000 mg/kg I, 5 mg/kg Fe, 100,000 mg/kg Mn, 625 mg/kg Se and 15 mg/kg Zn in total rations.

[6]Mean and SD (n=2) for corn silage, alfalfa hay, and concentrate based on samples of feedstuff collected during each period and analyzed by commercial feed laboratory (Cumberland Valley Analytical Services, Waynesboro, PA.).

[7]Cellulose calculated by difference: ADF – ADL.

[8]Hemicellulose calculated by difference: NDF – ADF.

[9]Amylase-treated NDF on organic matter basis.

[10]Van Soest et al. (1991) using α-amylase and sodium sulfite.

[11]Acid detergent fiber.

[12]Acid detergent lignin.

[13]Acid detergent insoluble crude protein.

[14]Neutral detergent insoluble crude protein.

[15]Total fatty acid.

[16]Parts per million.

[17]Dairy NASEM (2021) prediction of NEL concentration.

Table 3.2. Particle size distributions (n=4) for experimental diets (% DM retained).

Item	LoLig [1]	HiLig [1]
Particle size (%DM retained)		
>19.0 mm	3.07 (0.696)	3.87 (1.60)
19.0 to 8.0 mm	24.7 (1.54)	18.8 (0.385)
8.0 to 1.8 mm	45.4 (0.619)	53.2 (0.313)
<1.8 mm	26.8 (1.62)	24.2 (2.30)

[1] LoLig = low-lignin diet; HiLig = high-lignin diet; values in parenthesis indicate SD (n = 2).

Table 3.3. Chemical composition of corn silage, alfalfa hay, and concentrate mixes used to formulate the low-lignin and high-lignin diets fed to lactating Jersey cattle.[1]

	Corn silage		Alfalfa Hay		LoLig [2] grain mix		HiLig [2] grain mix	
Items	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, % as-is	42.0	0.941	89.9	1.92	91.6	0.451	91.2	0.316
CP	8.45	0.495	19.7	0.636	22.7	0.495	16.3	0.424
ADF	24.7	1.84	32.3	1.26	14.2	1.63	10.7	0.778
NDF	37.7	1.63	40.3	1.34	25.2	0.354	20.2	0.141
aNDFom[3]	37.2	1.48	39.2	1.06	24.9	0.566	19.9	0.283
ADICP[4]	0.885	0.276	2.24	1.18	0.725	0.120	0.595	0.021
NDICP[4]	1.13	0.580	3.39	0.156	2.33	0.163	1.87	0.226
Lignin	3.84	0.728	7.13	0.530	1.56	0.057	1.94	0.014
Sugar	1.45	1.20	5.70	1.56	6.20	0.424	7.30	1.56
Starch	39.5	1.41	2.10	0.141	23.5	0.636	41.1	1.06
Ash	5.47	1.10	11.8	0.537	8.24	0.410	6.89	0.474
Ca	0.190	0.014	1.32	0.021	1.02	0.057	1.10	0.057
P	0.265	0.021	0.360	0.028	0.620	0.42	0.535	0.021
Mg	0.410	0.00	0.220	0.00	0.400	0.014	0.365	0.035
K	1.25	0.057	3.68	0.368	1.54	0.035	1.17	0.00
S	0.115	0.007	0.230	0.00	0.370	0.014	0.235	0.007
Fe, mg/kg[5]	136	41.7	584	246	236	2.12	307	8.49
30h NDFD[6]	48.6	3.96	45.6	0.849	-	-	-	-
48h NDFD[7]	51.3	10.3	52.3	8.89	-	-	-	-
120h NDFD[6]	64.4	1.27	52.2	0.141	-	-	-	-
240h NDFD[6]	66.1	0.212	53.2	3.11	-	-	-	-

[1] Mean and SD (n=2) for corn silage, alfalfa hay, and concentrate based on samples of feedstuff collected during each period and analyzed by commercial feed laboratory (Cumberland Valley Analytical Services, Waynesboro, PA.).

[2] LoLig = low-lignin diet; HiLig = high-lignin diet (n = 2).

[3]Van Soest et al. (1991) using α-amylase and sodium sulfite.

[4]$aNDF_{OM}$= NDF corrected for organic matter, ADICP = acid detergent insoluble crude protein, NDICP= neutral detergent insoluble crude protein.

[5]Parts per million.

[6]30, 120, and 240h NDF digestibility calculated using NIR technology (Cumberland Valley Analytical Services, Waynesboro, PA.). N = 2. Data not shown for concentrate mixes.

[7]48h NDF digestibility according to Van Soest et al. (1991), using α-amylase and sodium sulfite. N = 2, with three samples of each individual forage sample for each period. Data not shown for concentrate mixes.

Table 3.4. Dry matter intake, gross energy concentration, and intake energy of low-lignin and high-lignin diets fed to lactating Jersey cattle.

	Treatment[1, 2]		SEM	P-value
Items	LoLig	HiLig		
DMI, kg/d	19.9	18.7	0.645	< 0.01
GE, Mcal/kg	4.27	4.23	0.03	< 0.01
GEI, Mcal/d	85.0	79.2	2.74	< 0.01

[1] LoLig = low-lignin diet; HiLig = high-lignin diet.

[2]Least squares means; largest standard error of treatment mean is shown.

Table 3.5. Total-tract digestibility of low-lignin and high-lignin diets fed to lactating Jersey cattle.

Items	Treatment[1, 2]		SEM	P-value
	LoLig	HiLig		
DM	67.3	64.1	0.546	< 0.01
OM	69.0	65.9	0.489	< 0.01
NDF	45.5	40.4	0.742	< 0.01
NDFom	47.5	43.1	0.906	< 0.01
CP	65.0	60.0	0.829	< 0.01
Starch	97.7	96.3	0.420	< 0.01
TFA	76.7	78.5	1.54	0.32
16 carbon	76.5	74.1	1.85	0.23
18 carbon	76.9	79.6	2.80	0.17
Energy	66.2	62.6	0.516	< 0.01

[1] LoLig = low-lignin diet; HiLig = high-lignin diet.

[2]Least squares means; largest standard error of treatment mean is shown.

Table 3.6. Gas and energy measures of low-lignin and high-lignin diets fed to lactating Jersey cattle.

Items	Treatment[1, 2]		SEM	P-value
	LoLig	HiLig		
Gases				
O_2 consumption, L/d	4599	4044	237	0.12
CO_2 production, L/d	4915	4315	255	0.12
CH_4 production, L/d	396	385	29.6	0.73
RQ[3]	1.07	1.07	< 0.01	0.79
Components, Mcal/d				
Feces	28.7	29.6	1.12	0.15
CH_4	3.74	3.64	0.28	0.73
Urine	1.90	1.47	0.16	0.06
HP[4]	23.3	20.4	1.21	0.12
Milk	23.0	20.3	0.76	< 0.01
TE	3.50	2.86	1.52	0.68
Fractions, Mcal/d				
GE	85.0	79.2	2.74	< 0.01
DE	56.3	49.6	1.75	< 0.01
ME	50.2	44.2	1.80	< 0.01
NEL[5]	35.9	32.8	1.64	0.06
Fractions, Mcal/kg of DM				
GE	4.27	4.23	0.03	< 0.01
DE	2.83	2.63	0.04	< 0.01
ME	2.52	2.36	0.03	< 0.01
NEL	1.81	1.75	0.06	0.50
Efficiencies, %				
DE/GE	66.2	62.6	0.517	< 0.01
ME/DE	89.8	89.6	0.384	0.80
NEL/ME	71.5	74.2	1.45	0.36

[1] LoLig = low-lignin diet; HiLig = high-lignin diet.

[2]Least squares means; largest standard error of treatment mean is shown.

[3]RQ = respiratory quotient, CO_2 production/O_2 consumption, L/L.

[4]HP = heat production (Mcal/d); $3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times N$ (Brouwer, 1965), where O_2, CO_2, and CH_4 are in mL/s at STPD, and N is urinary N excretion in g/s.

[5]NEL = $0.10 \times BW^{0.75}$ + milk energy + tissue energy corrected for efficiency of conversion to milk energy (NASEM, 2021).

Table 3.7. Gross energy concentrations of feed and fecal NDF residues of low-lignin and high-lignin diets fed to lactating Jersey cattle.

Items	Treatment[1, 2]				SEM	P-value		
	LoLig FD[3]	LoLig FL[3]	HiLig FD[3]	HiLig FL[3]		D[6]	T[7]	D × T[8]
GE of NDF residue, Mcal/kg[4]	4.05	3.93	4.19	3.98	0.034	0.03	< 0.01	0.40

[1] LoLig = low-lignin diet; HiLig = high-lignin diet.

[2]Least squares means; largest standard error of treatment mean is shown.

[3] LoLig FD = LoLig feed NDF residue; LoLig FL = LoLig fecal NDF residue; HiLig FD = HiLig feed NDF residue; HiLig FL = HiLig fecal NDF residue.

[6]D = the main effects of diet.

[7]T = the main effects of type of sample.

[8]D × T = the interaction of diet and type of sample.

Table 3.8. Fecal and urinary output and nitrogen excretions for low-lignin and high-lignin diets fed to lactating Jersey cattle.

	Treatment[1, 2]		SEM	P-value
Items	LoLig	HiLig		
Output, kg/d				
Feces, wet weight	40.6	41.9	1.19	0.58
Feces, dry weight	6.51	6.72	0.262	0.25
Urine, wet weight	20.8	27.8	1.32	< 0.01
Mass, g/d				
N intake	525	497	16.9	0.01
Fecal N	184	199	8.23	0.08
Urinary N	143	115	8.87	0.02
Milk N	187	164	6.60	< 0.01
N balance	10.9	18.5	10.5	0.59
As percent of N intake, %				
Fecal N	35.0	40.0	0.829	< 0.01
Urinary N	27.4	23.1	1.73	0.06
Milk N	35.6	33.0	0.616	< 0.01
N balance	1.91	3.86	2.17	0.45

[1] LoLig = low-lignin diet; HiLig = high-lignin diet

[2]Least squares means; largest standard error of treatment mean is shown.

Table 3.9. Environmental impacts for low-lignin and high-lignin diets fed to lactating Jersey cattle.

Items	Treatment[1, 2]		SEM	P-value
	LoLig	HiLig		
CH_4 production				
CH_4 L/kg DMI	20.3	20.5	1.60	0.87
CH_4 E/GE, %	4.40	4.63	0.171	0.51
CH_4 L/kg milk	14.3	15.9	0.785	0.32
CH_4 L/kg ECM	11.8	12.9	0.981	0.25
CH_4 L/kg milk fat	308	325	25.7	0.52
CH_4 L/kg milk protein	398	465	28.1	0.05
CH_4 L/kg NDF digested	138	165	13.0	0.02
N utilization[3]				
Manure N g/d	327	314	14.3	0.43
Productive N g/d	198	183	21.4	0.36
Manure N/ Intake N, %	62.5	63.1	1.40	0.83
Productive N/ Intake N, %	37.5	36.9	1.40	0.83

[1] LoLig = low-lignin diet; HiLig = high-lignin diet.

[2]Least squares means; largest standard error of treatment mean is shown.

[3]Manure N = urinary + fecal N, productive N = milk + retained N.

Table 3.10. Dry matter intake, milk production and components (concentrations and yields), water intake, body weight, and body condition score for low-lignin and high-lignin diets fed to lactating Jersey cattle.

Items	Treatment[1, 2]		SEM	P-value
	LoLig	HiLig		
DMI, kg/d	19.9	18.7	0.645	< 0.01
Milk yield, kg/d	28.2	25.1	1.21	< 0.01
ECM, kg/d[3]	33.7	30.0	1.08	< 0.01
ECM/DMI	1.69	1.61	0.027	0.03
Fat, %	4.65	4.82	0.264	0.45
Fat kg/d	1.30	1.19	0.058	0.02
Protein, %	3.55	3.38	0.155	0.11
Protein, kg/d	1.00	0.843	0.308	< 0.01
Lactose, %	4.76	4.57	0.083	0.13
Lactose, kg/d	1.34	1.15	0.052	< 0.01
MUN, mg/dL	11.2	11.9	1.22	0.26
SCC	60.8	91.6	43.2	0.53
Free water intake, L/d	90.9	91.0	5.96	0.99
BW, kg	437	433	13.9	0.05
BCS	3.21	3.15	0.084	0.20
Feed efficiency, %[4]	31.2	29.2	-	-

[1] LoLig = low-lignin diet; HiLig = high-lignin diet.

[2]Least squares means; largest standard error of treatment mean is shown.

[3]ECM= 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × true protein (kg) (Tyrrell and Reid, 1965).

[4]Feed efficiency calculated according to the Dairy NASEM (2021), equation 3-21 on page 36.

GENERAL SUMMARY AND CONCLUSIONS

The lignin content of forages is almost considered to be an important factor in fiber digestibility. Although the lignin content of fiber is of great importance, there are many other dietary and animal factors that influence the digestibility, and therefore energetic density of forages. A clear understanding of the energy availability of forages is essential in diet formulation as imprecise estimation detrimental impacts on performance and animal health. Because the energy contained within fiber and it's availability to the animal are multifaceted, and these elements are likely not represented fully in the models used to balance rations. Therefore, the objective of the first experiment was to quantify differences in feed and fecal NDF GE concentration to describe potential variation in the digestible energy content of NDF. This experiment was followed by a second in vivo experiment determine lignin's effects on intake, digestibility, milk production, nutrient utilization, and environmental impacts.

Experiment 1. The gross energy concentration of neutral detergent fiber and potential impacts on digestible energy. This experiment aimed to evaluate the GE concentration of NDF used by the NASEM and other nutritional models, as well as describe the factors that contribute to the variance around the true mean GE concentration. Using a variety of individual feeds and mixed rations, NDF residues were obtained and using bomb calorimetry analyzed for gross energy concentration. A similar process was used to obtain fecal NDF residues from feces collected from lactating dairy cattle fed mixed rations. The GE concentration of feed and fecal NDF was similar, averaging 4.03 and 3.94 ± 0.245 Mcal/kg, respectively. The NDF fraction primarily consists of cellulose, hemicellulose, and lignin (Van Soest et al., 1991), and small concentrations of ash, silica, and insoluble nitrogen are recovered in the NDF procedure (Dairy NASEM, 2021). While ash, silica, and insoluble nitrogen are recovered in the

NDF assay, they do not possess the same nutritional qualities as the primary components. The coefficient of variance across all samples used in this experiment was 6.21%, likely due to the anticipatedly high variability in ash and lignin contents of the NDF residues. Ash is inorganic and has an GE concentration of 0.00 Mcal/kg and therefore dilutes the overall GE concentration of the NDF fraction (Weiss and Tebbe, 2019), which could have been why the observed GE concentration of feed NDF was lower than that used in the Dairy NASEM model. Feed and fecal NDF residues being similar in GE concentration is an interesting finding because lignin contributes more energy to fecal NDF residues than it does in feed NDF residues. This indicates that fecal cellulose and hemicellulose must be partially degraded, suggesting fiber contributes more digestible energy to the animal than previously thought. This is an important finding that should be further evaluated for possible incorporation into nutritional models. The lack of a reliable GE concentration for hemicellulose in the literature and a lack of analytical laboratory assays that yield pure hemicellulose residues in reasonable quantities exasperates the complexity surrounding the GE concentration of the total NDF fraction. One method to estimate an GE concentration coefficient for hemicellulose could be to compare GE concentration values of NDF and ADF and solve for the difference using the percent hemicellulose of the sample. The percent hemicellulose of the sample would be calculated by the difference between ADF and NDF.

Experiment 2. Lignin's impact on energy and nitrogen partitioning. Numerous studies have reported the impacts of lignin on DMI, nutrient digestibility, and milk production responses, but data is lacking on lignin's direct impact on the energy balance of the cow. The current study observed just a 3.7% increase in lignin as a percent of NDF decreased the digestible energy concentration of the HiLig by 0.2 Mcal/kg. Increasing the concentration of lignin in the diet not only increased the digestibility of NDF, but also that of starch and CP.

These effects of increased nutrient digestibility carried through to ME but not NEL. Upon comparison of feed and fecal NDF residues from animals enrolled in the current experiment, feed NDF residues were statistically greater than fecal NDF residues. These results were interpreted in a similar fashion as the results from the previous experiment, suggesting that cellulose and hemicellulose escaping digestion must be partially degraded to an extent. Cows consuming the LoLig diet emitted similar volumes of methane but consumed greater DM and digested NDF to a greater extent. This suggests that reducing the lignin content of NDF fed to dairy cattle could aid in maintaining DMI and NDF digestibility without the production of additional methane. Nitrogen utilization shifting towards urinary from fecal nitrogen excretion when feeding the LoLig diet, which potentially has greater environmental consequences relative to the HiLig diet. The greater intake and digestibility of the LoLig diet fostered greater milk production and yields of component relative to the HiLig diet.

Overall technical observations and recommendations. In order to accurately describe the GE concentration of NDF, great effort was taken to minimize analytical error in the preparation of samples for bomb calorimetry. Due to the hydrophilic nature of NDF residues, samples were dried overnight and transported using a desiccator prior to analysis. Upon weigh up and oil application, samples were allowed to soak in the oil on the lab benchtop overnight in an attempt to minimize moisture in the sample. The oil layer is supposed to aid in the combustion of the sample as well as serving as a hydrophobic layer to stop moisture from accumulating in the sample. The oil is quite effective in blocking moisture accumulation in the sample, but the oil takes times to actually soak into the sample before having this protective function. The measures taken to reduce moisture contamination in samples were fairly effective but might have been more effective if the samples were allowed to sit overnight inside a desiccator. Additionally,

having more sample to do further analysis on some of the samples from the first experiment would have helped to link the change in chemical composition of the NDF residue to its respective GE concentration.

APPENDIX A: DAIRY NASEM (2021) PREDICTION OUTPUT FOR LOLIG AND HILIG DIETS

Diet summary for LoLig:

Report 2. Diet Summary (DM Basis)

2.1 Macronutrients

Nutrient	Content
Dry Matter, %	61.0
Forage, %	56.2
CP, %	15.2
ME, Mcal/kg	2.58
NEL, Mcal/kg	1.70
RUP, Base, %	5.7
RDP, %	9.4
Dig. RUP, %	4.3
ADF, %	23.6
NDF, %	35.4
Forage NDF, %	21.5
Starch, %	25.3
WSC, %	5.8
Ash, %	6.3
Total FA, %	2.30
Ca, %	0.64
P, %	0.39
Mg, %	0.27
K, %	1.33
Na, %	0.22
Cl, %	0.50
S, %	0.19
DCAD, mEq/kg	176
Cost, $/ton As Fed	0.00
Cost, $/day	0.00

2.2 Diet Ingredients

Ingredient	As Fed kg/d	% As Fed	DM kg/d	% of DM
01 *Corn silage, typical- 2101DA updated	20.047900	61.424475	8.420118	42.312151
02 *Legume hay, mature-2101DA updated	3.073805	9.417787	2.763351	13.886186
03 Calcium carbonate	0.089044	0.272821	0.089044	0.447457
04 Calcium phosphate (di)	0.053426	0.163691	0.053426	0.268472
05 Calcium soaps	0.145922	0.447088	0.133665	0.671683
06 Corn grain dry, medium grind	3.034235	9.296549	2.779359	13.966628
07 DDGS, high fat	1.896124	5.809507	1.736850	8.727889
08 Magnesium oxide	0.020470	0.062718	0.020470	0.102864
09 Molasses	0.388836	1.191349	0.356174	1.789819
10 Sodium chloride (salt)	0.073771	0.226026	0.073771	0.370709
11 Soybean hulls	1.676118	5.135434	1.514741	7.611764
12 Soybean meal, extruded	0.880343	2.697270	0.806394	4.052231
13 Soybean meal, solvent 48CP	1.249519	3.828383	1.144559	5.751553
14 VitTM Premix, generic	0.008780	0.026901	0.008078	0.040593
Totals	32.638	100.00	19.900	100.00

Report 4. Energy

4.1 Energy Supply

Energy	Mcal/d	Mcal/kg	% of GE	% of DE	% of ME
DE	58.99	2.96	69.6	100.0	
Urinary E	1.88	0.09	2.2	3.2	
Gaseous E	5.83	0.29	6.9	9.9	
ME	51.29	2.58	60.5	86.9	100.0
NEL	33.85	1.70	39.9	57.4	66.0

Diet summary for HiLig:

Report 2. Diet Summary (DM Basis)

2.1 Macronutrients

Nutrient	Content
Dry Matter, %	76.8
Forage, %	56.2
CP, %	16.1
ME, Mcal/kg	2.55
NEL, Mcal/kg	1.68
RUP, Base, %	5.5
RDP, %	10.6
Dig. RUP, %	4.0
ADF, %	22.7
NDF, %	31.3
Forage NDF, %	22.3
Starch, %	24.0
WSC, %	7.3
Ash, %	7.6
Total FA, %	3.28
Ca, %	0.92
P, %	0.36
Mg, %	0.29
K, %	1.60
Na, %	0.25
Cl, %	0.63
S, %	0.15
DCAD, mEq/kg	245
Cost, $/ton As Fed	0.00
Cost, $/day	0.00

2.2 Diet Ingredients

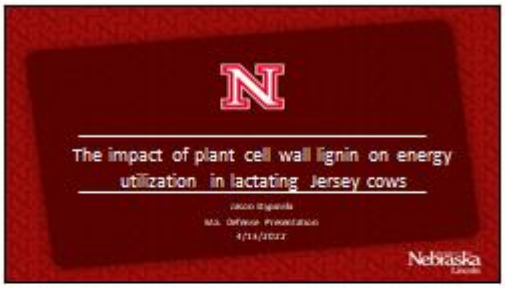
Ingredient	As Fed kg/d	% As Fed	DM kg/d	% of DM
01 *Corn silage, typical- 2101DA updated	6.149700	25.371304	2.582874	13.873740
02 *Legume hay, mature-2101DA updated	8.757900	36.131738	7.873352	42.291198
03 Calcium carbonate	0.083403	0.344089	0.083403	0.447994
04 Calcium phosphate (di)	0.049929	0.205988	0.049929	0.268190
05 Calcium soaps	0.131078	0.540778	0.124917	0.670984
06 Corn grain dry, medium grind	5.272649	21.752928	4.582301	24.613532
07 Cottonseed hulls	0.686770	2.833350	0.624961	3.356937
08 DDGS, high fat	0.467805	1.929984	0.416641	2.237960
09 Magnesium oxide	0.020757	0.085635	0.020757	0.111495
10 Molasses	0.509577	2.102319	0.333238	1.789966
11 Sodium chloride (salt)	0.074988	0.309372	0.074988	0.402793
12 Soybean hulls	0.691543	2.853041	0.624961	3.356937
13 Soybean meal, extruded	0.624509	2.576485	0.583073	3.131939
14 Soybean meal, solvent 48CP	0.700103	2.888357	0.624961	3.356937
15 VitTM Premix, generic	0.018090	0.074632	0.016643	0.089397
Totals	24.239	100.00	18.617	100.00

Report 4. Energy

4.1 Energy Supply

Energy	Mcal/d	Mcal/kg	% of GE	% of DE	% of ME
DE	54.44	2.92	68.6	100.0	
Urinary E	2.10	0.11	2.6	3.9	
Gaseous E	4.93	0.26	6.2	9.0	
ME	47.41	2.55	59.7	87.1	100.0
NEL	31.29	1.68	39.4	57.5	66.0

APPENDIX B: FINAL DEFENSE PRESENTATION



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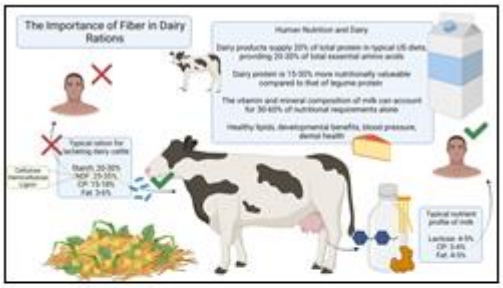

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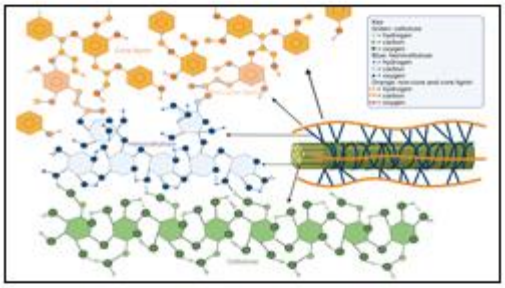

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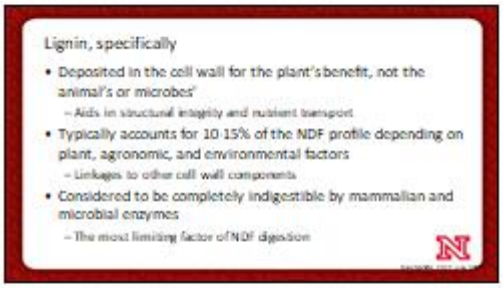

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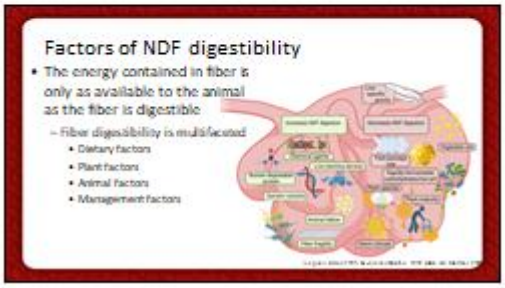

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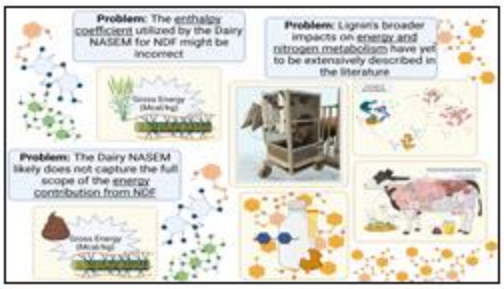
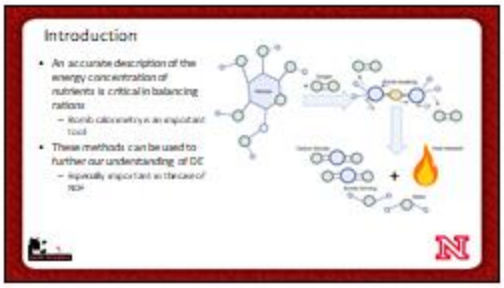

Problem: The enthalpy coefficient utilized by the Dairy NASEM for NDF might be incorrect
Problem: Lignin's broader impacts on energy and nitrogen metabolism have yet to be extensively described in the literature
Problem: The Dairy NASEM likely does not capture the full scope of the energy contribution from NDF

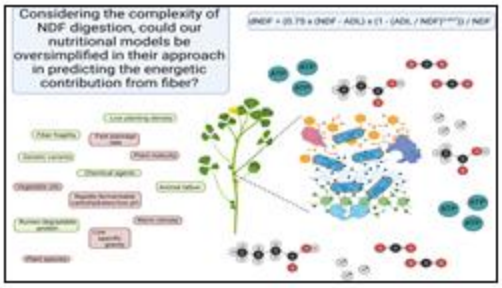
7


Evaluation of heats of combustion of fiber contained in feed and fecal samples
Nebraska

8


Introduction
An accurate description of the energy concentration of nutrients is critical in balancing rations
These methods can be used to further our understanding of DE

9


Considering the complexity of NDF digestion, could our nutritional models be oversimplified in their approach in predicting the energetic contribution from fiber?

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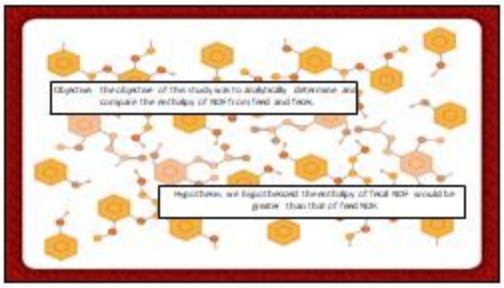
Objective: The objective of this study was to analytically determine and compare the enthalpy of NDF from feed and feces.
Hypothesis: we hypothesized the enthalpy of fecal NDF would be greater than that of feed NDF.

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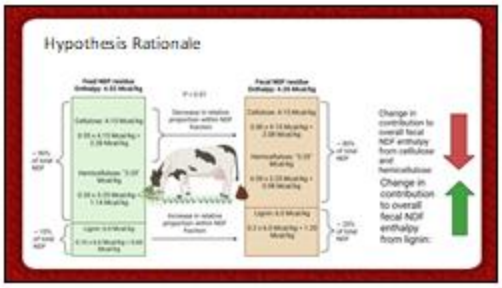
Hypothesis Rationale
Change in contribution to overall fecal NDF enthalpy from cellulose and hemicellulose:
Change in contribution to overall fecal NDF enthalpy from lignin:

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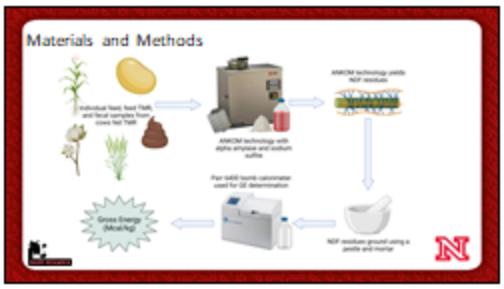

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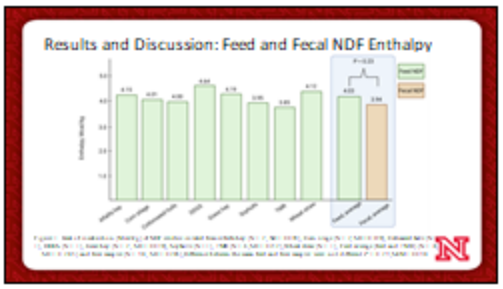

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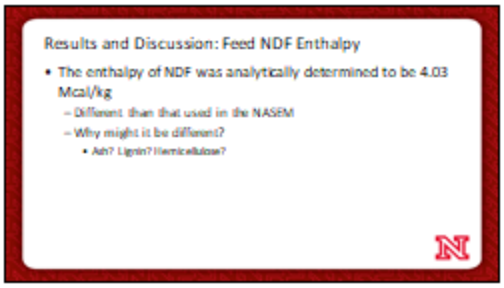

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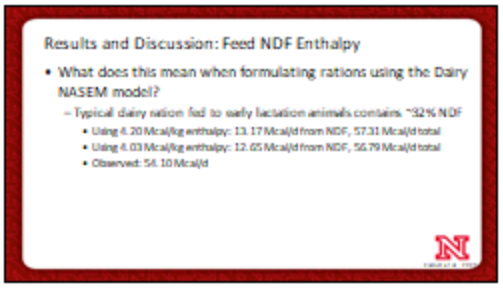

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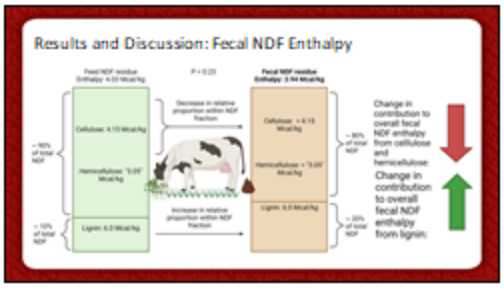

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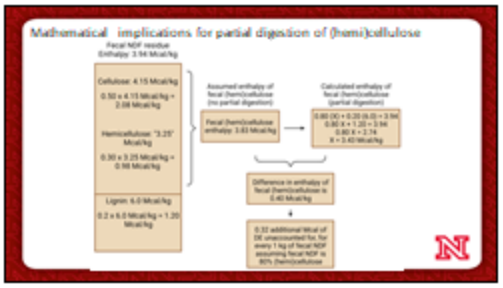

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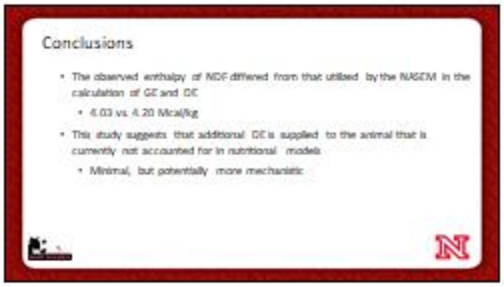

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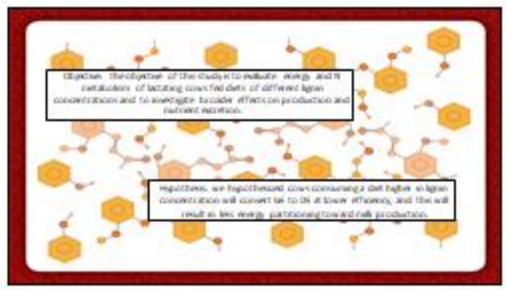

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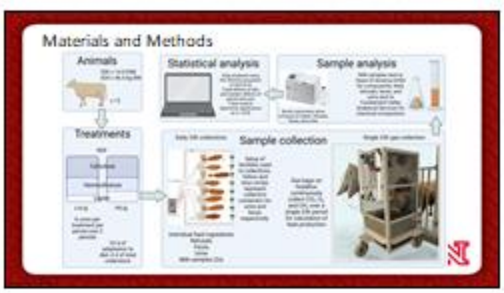

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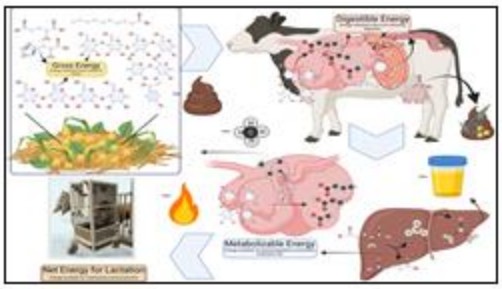

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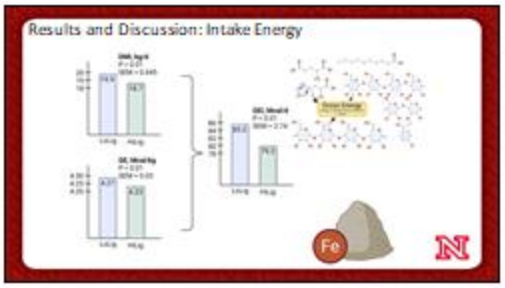

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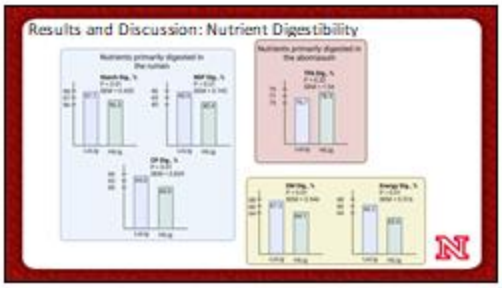

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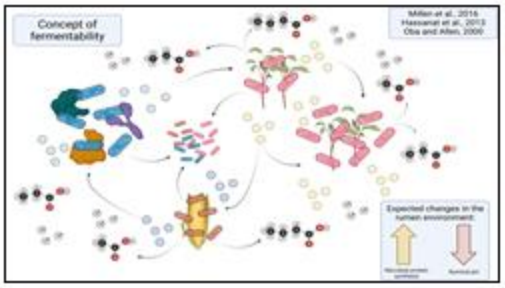

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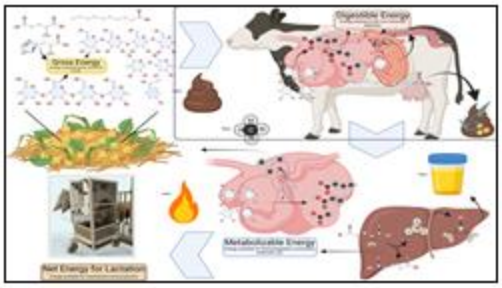

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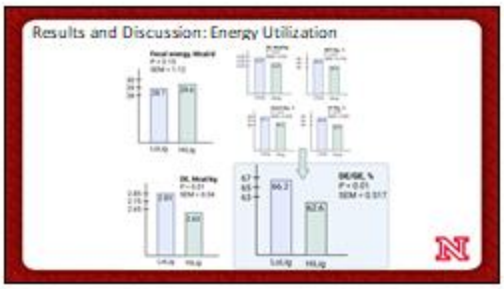

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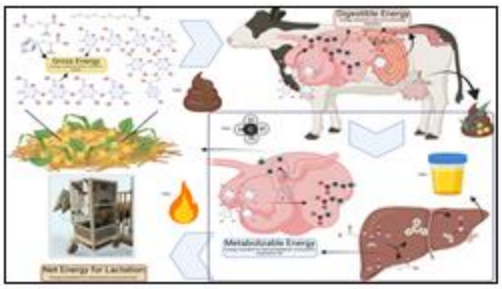

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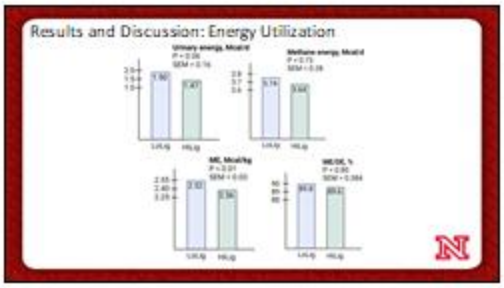

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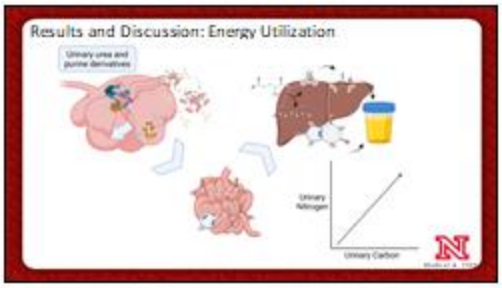

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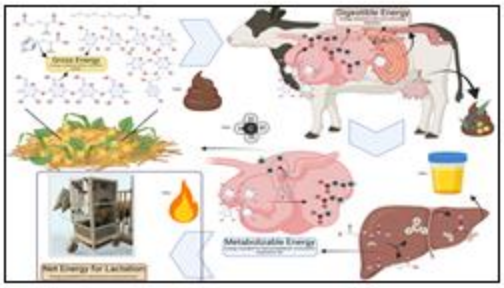

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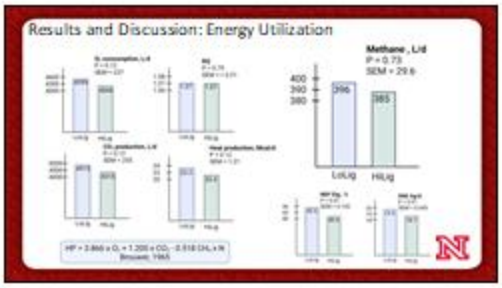

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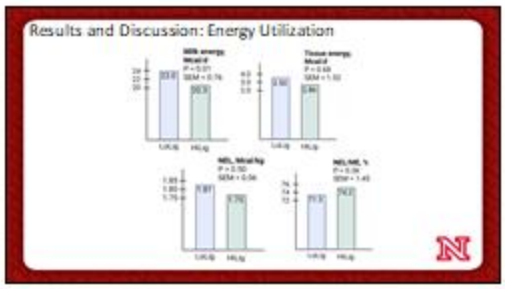

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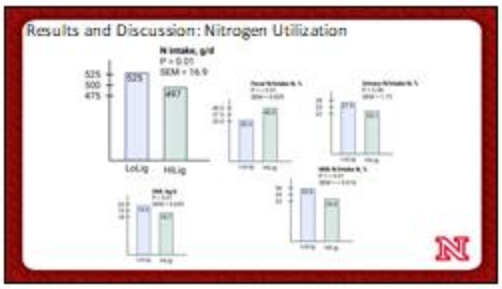

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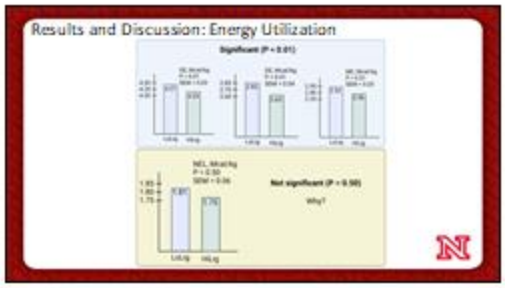

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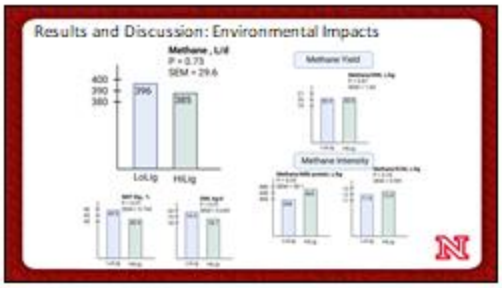

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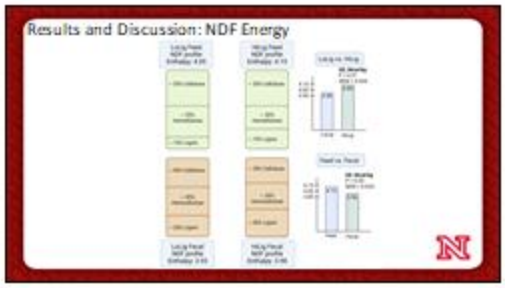
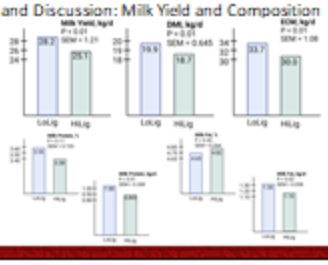


39

42

Results and Discussion: Milk Yield and Composition



43

Conclusions

- Feeding the HiLig diet reduced digestibility of not just NDF, but also starch and CP
- The NDF content of the diet is important, but the composition of the NDF profile is more important
- Reduced digestibility results in less efficient conversion of GE to DE, providing less energy for downstream energy fractions

44

Overall Conclusions

- The enthalpy of feed NDF was observed to be lower in both studies than that reported in the Dairy NASEM (2021)
  - The Dairy NASEM (2021) and other nutritional models should reconsider the use of a 4.20 Mcal/kg enthalpy coefficient
- Dietary lignin not only influences GE and DE concentrations, but also ME and potentially NEL
  - Nutritional models are likely oversimplified with respect to the digestion of NDF

45

Next Steps

- Evaluate factors that impact the variation in GE concentration of NDF
  - Ash, lignin, hemicellulose
    - Hemicellulose enthalpy
- Challenge nutritional models with an NDF enthalpy coefficient of 4.08 Mcal/kg to compare predicted and observed GE and DE concentrations

46

Next Steps

- Validate the suggestion of microbial cross feeding and fermentability through in vitro work
- Evaluate the impact of different lignin cross linkages on energy balance and environmental impacts

47

Thank you!

- **Advisor:** Dr. Paul Kononoff
- **Committee:** Dr. Andrea Watson and Dr. Samodha Fernando
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- **Undergraduate Students:** Jade, Tyson, Sarah, Abby, Annabelle

48

49

50

51