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Effects of NDF digestibility on Lactating Jersey Cows: Observed and Modeled Performance

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by

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Improving the digestibility of fiber and our understanding of how to feed it will optimize the ruminants' niche in our society, which is to convert human inedible products to high quality protein in the form of meat and milk. Neutral detergent fiber (NDF) digestibility has been studied and modeled for decades, but it is increasingly important as livestock production is scrutinized for resource use and sustainability. Increasing the amount of NDF fed and improving models of NDF digestibility will improve sustainability by increasing the precision at which we feed livestock which results in less nutrient and greenhouse gas (GHG) emissions. Profitability for farmers will also improve because feeds with greater NDF concentrations are generally cheaper than those with fat, starch, or other energy dense nutrients.

We evaluated how in vitro estimates of NDF digestibility (IVNDFD) affected animal performance predictions from ration software. Forages and fibrous byproducts from 8 energy balance studies were evaluated for IVNDFD, and the IVNDFD estimates were used in place of feed library NDF digestibility (NDFD) values from the Cornell Net Carbohydrate and Protein System (CNCPS) to evaluate if using IVNDFD of feeds improved ration formulation predictions of milk and CH_4 production. The CNCPS predictions demonstrated that using IVNDFD improved predictions of CH_4 production, but not of milk production. Our results suggest that using IVNDFD may aid in predicting

CH_4 production which is of increasing importance as GHG emissions from livestock are scrutinized, but that other strategies, like estimating the indigestible NDF (iNDF) fraction of feeds, should be explored as a way of improving model predictions of milk production.

A second experiment evaluated feeding NDF from different sources and processing methods as techniques to optimize NDFD. Seven rumen cannulated Jersey cows were fed in a crossover design with a 2 × 2 factorial treatment arrangement; the factors were forage concentration and DDGS form. Treatment combinations were low forage with meal DDGS (LF-mDDGS), low forage with pelleted DDGS (LF-pDDGS), high forage with meal DDGS (HF-mDDGS) and high forage with pelleted DDGS (HF-pDDGS). Increasing forage concentration slowed rumen passage rate, increased rumen pH and increased rumen NH_3, but did not change NDF digestibility or energy corrected milk yield, as we hypothesized. Interestingly, pelleting DDGS appeared to increase the NDF and energy digestibility of the rations, which mirrored results from in vitro evaluations of meal and pelleted DDGS. Further investigation of the effects pelleting has on fibrous feeds is warranted because it may be an effective procedure to improve the feeding value of DDGS or other fibrous feeds by improving their NDFD.

"The two most important days in your life are the day you are born and the day you find out why"

Mark Twain

"To leave the world a bit better, whether by a healthy child, a garden patch, or a redeemed social condition; to know even on life breathed easier because you lived. This is to have succeeded."

Ralph Waldo Emerson

"If our expectations – if our fondest prayers and dreams are not realized – then we should all bear in mind that the greatest glory of living lies not in never falling, but in rising every time you fall."

Nelson Mandela

"Agriculture is the most healthful, most useful, and most noble employment of man."

George Washington

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

GENERAL INTRODUCTION

Corn dried distillers grains with solubles (DDGS), a co-product of the dry milling ethanol process, has been heavily researched in regards to dairy cattle nutrition (Kleinschmit et al., 2007; Anderson et al., 2015a,b,c). Much of the research has evaluated maximum levels of inclusion that maintain animal performance of lactating cattle while other research with DDGS has compared it to alternative protein sources such as canola and soybean meal (Owens and Larson, 1991; Gaillard et al., 2017). Some research has observed that DDGS can comprise a sizable portion of the diet DM, 30% for lactating cows and 50% for heifers (Shingoethe et al., 2009; Manthey et al., 2016). Additionally, Foth et al. (2015) has investigated the energy value of DDGS and CH_4 production when cattle were fed DDGS; they found that dairy cattle fed DDGS as 28.8% of the diet DM produced 6.3% less total CH_4 and 9.6% less on a per kg of milk basis. Feeding DDGS, in combination with other byproducts, also increases the net protein production of dairy cattle; Karlsson et al. (2018) demonstrated feeding byproducts to dairy cattle increases human edible feed conversion efficiency of energy and protein by 200% and 250%, respectively.

Dried distillers grains is a well understood feedstuff from a chemical analysis standpoint. Extensive research has been conducted to evaluate the nutrient composition of DDGS, particularly protein. This research has demonstrated that DDGS is a source of rumen undegradable protein (RUP). The research also suggests that protein content, as well as the different protein fractions, are affected by manufacturing site and processing method. The RUP varied by as much as 38 units and the various fractions of the protein (digestible, potentially digestible, undigestible) differed as well (Spiehs et al., 2002; Mjoun et al., 2010a,b). Beyond protein, DDGS is also a source of digestible neutral

detergent fiber (NDF), nearly one-third of DDGS is comprised of NDF. Understanding NDF has supported understanding the valuation of DDGS but fiber characterization has changed and improved over the recent decades (Hall and Mertens, 2017). With that evolution, the strategy to measure and predict NDF's contribution to the animal performance and behavior has also changed (Raffrenato et. al., 2010; Cotanch, 2014).

The Cornell Net Carbohydrate and Protein System in particular (**CNCPS**; Sniffen et al., 1992a,b; Higgs et al., 2015; Van Amburgh et al., 2015), uses a 3 pool model of fiber digestion which partitions fiber into fast, slow, and undigested fractions (Raffrenato et al., 2019). These fractions affect the predicted output of the ration model which means that accurate characterization of NDF is vital for extracting the greatest possible value from our models and feeds. An additional justification for this research is that models have under predicted average daily gain (ADG) by as much as 20% in heifers when diets with high concentrations (≥20% Diet DM) of co-products were fed (Anderson et al., 2015a, Manthey et al., 2016). Even though this was not a lactating cow study, it is a reflection of the possible misrepresentation of energy availability to the animal when high levels of co-products are fed. This may be due to the fact that when large amounts of DDGS are included, the animal received a greater proportion of energy from NDF rather than starch or fat. In addition to improving the accuracy of our models, we must determine if there are ways to improve the availability of the nutrient fractions that exist within DDGS. One way to improve the use of nutrient fractions in DDGS is to increase retention time in the rumen. Welch (1986) conducted research showing that greater specific gravity and smaller particle size led to decreased retention time in the rumen. Decreases in retention time allow for less exposure to microbial digestion of the NDF and

result in less digestion of the feed. In the field, one common strategy to mitigate this is the inclusion of roughages like grass hay and straw (Shaver and Hoffman, 2010). Other past research has investigated this and shown that particle size of the diet can affect retention time and rumination activity (Ramirez-Ramirez et al., 2016). Improving fiber digestibility may result in extracting more value from feeding DDGS. To do so, we must determine if increasing retention time will improve nutrient utilization of the TMR when DDGS are fed. If we can determine ways to increase the digestibility of the NDF component of co-product feeds, like DDGS, we can add value to producers by decreasing their ration costs. This is valuable because most of the costs associated with dairy production are feed costs (Buza, 2014).

Since NDF is a major component of energy in DDGS, our goal is to evaluate if altering the NDF digestibility (NDFD) will impact the model's prediction of metabolizable energy (ME) and metabolizable protein (MP) allowable milk production. We will also evaluate whether the inclusion of NDFD within the model improved predictions of ration software Predicting NDFD will follow the in vitro method laid out by Goering and Van Soest (1970). Incorporating in vitro measurements may improve the accuracy of our models which may reduce nutrient excretion and feed waste. Additionally, improving our estimates of co-product contributions to livestock diets could lead to their increased inclusion which may aid in greater net food and protein production for human consumption.

Knowledge gaps remain that need to be addressed in regards to extracting the most value from DDGS. We need to improve our understanding of how to quantify the nutritive contribution of the fiber component, better understand how to characterize it,

and incorporate the information into our nutrition models. Finally, methods need to be investigated, in vivo, for ways to maximize nutrient use from feed ingredients. Given these needs, the objectives of this thesis is to 1) measure in vitro NDFD of feed co-products and forages used in dairy nutrition studies and determine if the characterization of this digestibility improves the prediction of milk yield and 2) evaluate ration formulation strategies for increasing total tract NDF digestion of diets fed to lactating dairy cattle.

CHAPTER 1

LITERATURE REVIEW

Ethanol

Ethanol Production. The United States is the largest producer of ethanol in the world and produces almost twice as much ethanol as Brazil, which is the world's second largest producer (Annual Fuel Ethanol Production, 2019). In 1981, the United States produced 314,155,000 L of ethanol (U.S. EIA, 2018) today this volume has grown to 59.8 billion L (U.S. EIA, 2018; Figure 1.1). Ethanol production now equates to 10% of the total fuel consumed by volume in the United States (U.S. EIA, 2019)

As ethanol production has grown over the last 30 years, it has created a strong and compared to historical trends, an alternative market for corn, so much that it is now the primary end-use for corn grain grown in the United States (USDA ERS, 2019). Use of corn for alcohol has grown from 53,822,600 metric tons in 2006 to 142,367,000 metric tons in 2017, nearly a threefold increase. Corn for ethanol represents 37.9% of the US corn supply end use in the United States (USDA ERS, 2019: Figure 1.1). The increase in the supply of corn used for ethanol came from increased hectares planted in corn and improved genetics and technology (Riley, 2015). Ethanol has influenced the agriculture and energy industry landscape in the United States. Unfortunately, at the time of this writing we are in the COVID-19 pandemic which has had dramatic impacts on ethanol production. Due to "social distancing" policies and stay at home orders, fuel consumption has declined to its lowest level in 30 years (Voegele, 2020b). Weekly ethanol production

has decreased 33% (Figure 1.2) and ethanol biorefinery closures are occurring throughout the corn belt (Voegele, 2020a).

Ethanol Policy. Ethanol has long been used in vehicles in the U.S. ; Henry Ford's first vehicles were designed to use either ethanol or gas, or a mixture of the two. Growth in ethanol production was, in part, a result of government regulations. A timeline of significant legislative actions that spurred ethanol growth is listed in Table 1.1. Ethanol production has been the benefactor of various tax breaks and production incentives (Solomon et al., 2007). Most notably, the energy shortages in the 1970's led to the first ethanol tax credit in 1978. This tax credit exempted blends of at least 10% ethanol from the excise tax on gasoline, creating a $0.14/L subsidy. Additional policies created during the 1980's, such as the Alternative Motor Vehicles Act of 1988, increased the demand for ethanol by awarding credits toward fuel efficiency requirements to car manufacturers by producing so called "flex fuel" vehicles which run on renewable fuels (Duffield and Xiarchos, 2015).

Ethanol production also grew as environmental and energy regulations incentivized its use. The Clean Air Act of 1990 was the first major environmental policy to spur growth in ethanol demand by mandating the addition of oxygen containing compounds to gasoline. In 1992, the Energy Policy Act extended the fuel tax exemption to blend rates containing less than 10% ethanol to incentivize its use in more fuel blends (Duffield and Xiarchos, 2015). The next event that led to growth in ethanol demand came from California where methyl tertiary butyl ether (MTBE), a competing fuel oxygenate, was banned due to environmental concerns. Twenty-four other states followed California with similar policies, which led to increased ethanol demand as a fuel oxygenate

(Duffield and Xiarchos, 2015). The Renewable Fuel Standard (RFS), first outlined in 2005, increased ethanol production by requiring U.S. fuel to include a minimum amount of renewable fuel each year. The RFS awarded tax credits to businesses to build facilities that produced and offered alternative fuels (Duffield and Xiarchos, 2015). The RFS was updated in 2007 to the "RFS 2" which placed fuels into 4 categories based on their reduction of GHG emissions compared to petroleum-based fuels. The categories are as follows, renewable fuel (20% reduction of GHG), advance biofuel (50% reduction of GHG), biomass-based diesel (50% reduction GHG) and cellulosic biofuel (60% reduction of GHG; Duffield and Xiarchos, 2015). Current production targets for alternative fuels are set by the federal government. Renewable fuels, such as corn ethanol, is set at 56.8 billion L and this target was capped in 2015 (Duffield and Xiarchos, 2015).

State legislative actions also led to increased demand for ethanol. For example, Minnesota was the first state to implement an ethanol mandate in 1997which required all fuel sold in the state to have a minimum ethanol concentration of 10% (Duffield and Xiarchos, 2015). Hawaii, Montana, Missouri, and Washington have similar 10% ethanol blend mandates. These policies contributed to the doubling of ethanol production from 1999 to 2003, and ethanol production has continued to grow until 2016 (Administration, 2018).

Impact on Corn and Livestock Production. The growth of ethanol has not only affected the fuel sector, but also the agriculture sector. Over 36.1 million hectares of corn were planted in 2018, and 12.96 million hectares of that were used for ethanol production (USDA ERS, 2019). This is just one example of the impact the ethanol industry has had on corn grain production. It has also affected livestock production.

Prior to 1996 the United States agriculture industry was a supply-controlled system and the aim of this system was to support commodity prices and in turn, increase farm incomes. The 1996 Farm Bill allowed more hectares to be used for the production of corn in response to increased domestic consumption as ethanol. This increase in area planted in corn came at the expense of land deemed for conservation programs and other crops, such as hay, wheat, barley, and sorghum (Riley, 2015). Reductions in hay crop hectares led to reductions in hay supplies for livestock producers. With less corn grain and less hay available for livestock feed, DDGS rose as an important feedstuff for livestock. This feedstuff has become of particular importance to the beef and dairy industries; but DDGS is also used for swine and poultry, albeit to a lesser extent. The use of DDGS when feeding swine and poultry is limited because of feeding challenges related to the high concentration of fiber in DDGS (Noll et al., 2001).

Ethanol Production. Corn ethanol can be produced from wet milling or dry grind processes. Dry grind production will be covered in depth because currently 90% of U.S. ethanol production comes from dry grind manufacturing (Figure 1.3), however I will highlight principal differences between wet milling and dry grind ethanol production. Both processes are able to manufacture ethanol, but the process by which the corn kernel is broken down results in different co-products and these are associated with different nutrient composition. To begin, the corn kernel is composed of the germ, bran, and endosperm. Germ represents 12% of the seed mass and contains of 35% fat, 19% protein, and 8% starch. The endosperm contains most of the starch, which is interlocked in a starch-protein matrix. The endosperm is approximately 86% starch and 9% protein. The bran is 6% of the dry mass of a corn kernel, 88% of which is fiber (Anderson and Lamsal,

2011). Wet milling ethanol production employs a steeping process to designed to separate the fiber and germ from the endosperm prior to fermentation (Anderson and Lamsal, 2011). Each component is then processed separately resulting in corn bran feed, corn gluten meal, corn oil, and steep. In comparison to wet grind processing, dry grind does not fractionate the kernel prior to fermentation and results in corn oil, condensed distillers solubes (CDS), and dried distillers grains with solubles (DDGS). It is important to note that CDS and steep are not the same feed. Steep is 4.51% fat (NASEM, 2016) while CDS from dry grind plants will contain as much as 20% DM as fat (Klopfenstein et al., 2007) if fat has not been extracted. This is because during wet milling the germ is separated from the kernel for corn oil while dry milling does not separate this portion prior to processing the grain.

Dry Grind Process. Ninety percent of corn-ethanol produced in the United States originates from dry grind ethanol processing (Figure 1.1). The dry grind process begins by grinding the corn grain through a hammer mill. Next, water is added to create a *mash*. The mash is heated to approximately 100°C and treated with heat stable alpha-amylase to break down the starch which is a branched molecule composed of both amylose and amylopectin. After heat is applied, additional alpha-amylase is added. Next, the mash is cooled and glucoamylase is added to help convert the starch to glucose (Bothast and Schlicher, 2005). There are also low heat processes that depend upon enzymes to aide in degrading starch prior to fermentation. Reducing the temperature during liquefaction by 17°C (88°C vs 65C°) reduced the NDF and ADF concentration by 26% and 18% respectively (Nkomba et al., 2016). After the mash is moved to fermenters, yeast is added to hydrolyze the glucose and convert it to ethyl alcohol while carbon dioxide is produced

as a byproduct of this fermentation. Nitrogen sources, or proteases to degrade corn protein to amino acids, may also be added as a nitrogen source for the yeast. Fermentation lasts for 48-72 hours. At this point, the carbon dioxide produced from fermentation may be captured so that it can be used in other industrial and manufacturing processes. After fermentation is complete, the solution remaining is called *beer*. The beer is distilled through a column to concentrate the alcohol. The remaining solution is referred to as *whole stillage*. Whole stillage is processed into the animal feeds. After distillation, the ethanol containing solution still contains 5% water. This residual water is removed through molecular sieves to produce 100% ethanol. Before transport and sale of ethanol, the pure ethanol is denatured, usually through the addition of gasoline as 5% of the mixture (Bothast and Schlicher, 2005). Denaturing the ethanol renders it unsuitable for human consumption.

The feedstuffs are processed from the whole stillage. The whole stillage includes the non-starch fractions of the corn kernel. Whole stillage is centrifuged resulting in *thin stillage* and wet distillers grains (WDG). Thin stillage is then evaporated to CDS or "syrup" (Bothast and Schlicher, 2005). The CDS may be further processed through a centrifugation step to remove more fat. Centrifuging separates the CDS into corn oil and low-fat CDS. Low fat CDS is then added back to the WDG to create wet distillers grains with solubles (WDGS) while the corn oil is used in biodiesel production or as an animal feed. Further drying of WDGS, in a drum or ring drier, removes moisture and results modified distillers grains with solubles (MDGS) or dried distillers grains (DDGS). These products only differ in DM concentration with WDGS being 31%, MDGS being 48%, and DDGS being 90% (NASEM, 2016) Modifications to the dry grind processes are also

becoming commercialized. These processes, some described by Singh et al. (2005), result in protein concentration ranging from 36%-58%, compared to 30.8% for conventional DDGS (Schingoethe et al., 2009).

Feeding Dried Distillers Grains with Solubles

Current use. Increases in ethanol production across the corn belt have made corn ethanol co-products a staple in livestock diets. In 2014 the dairy industry used 10,269,331 metric tons of DDGS, beef cattle consumed 16, 229,535 metric tons of DDGS, and swine and poultry combined to use 3,782,960 metric tons (Wisner, 2015). In comparison, livestock industries feed 123,558,663 metric tons of corn annually (USDA 2020). Dried distillers grains has also become an important export as 9,307,715 metric tons were exported in 2014 (Wisner, 2015).

Nutrient Characterization. Dried distillers grains with solubles is recognized as a good source of protein, fiber, and energy for dairy cattle (Schingoethe et al., 2009, Foth et al., 2015). Crude protein (CP) and neutral detergent fiber (NDF) constitute most of the DDGS mass. Table 1.2 lists the range of CP and NDF, which range from 28-35% and 26-43% DM, respectively. Fat concentration has changed over time; in 2006, 4 ethanol biorefineries performed fat extraction, by 2012, 90 biorefineries had fat extraction capabilities (Harangody, 2012). Fat extraction has decreased fat concentration of DDGS to 3-8% depending on the fat extraction method. Processes using centrifugation of the CDS leads to fat concentration of DDGS between 5% and 8% while using hexane extraction can result in the production of DDGS with < 5% fat (Mjoun et al., 2010a, Mjoun et al., 2010c). Singh et al. (2005) outlined other processes that results in 4-12% crude fat.

Dried distillers grains with solubles are also a good source of rumen undegradable protein (RUP). Table 1.3 lists the protein characterization of DDGS using the mobile bag technique or the modified three step procedure (Gargallo et al., 2006, Paz et al., 2014). Dried distillers grains are 55.9% ± 12.5% (Mean ± SD) of the CP as RUP. The RUP concentration of DDGS can be affected by many variables, one is the amount of solubles added back to the product. Cao et al. (2009) suggested that increasing the proportion of solubles in DDGS decreases the RUP concentration of the DDGS. Even though the addition of solubles decreased the RUP concentration, the total digestible protein (TDP) of DDGS did not change as the proportion of CDS was increased. Additionally, rumen degradable dry matter (RDDM) increased with the increasing level of CDS, likely driven by increased rumen degradable protein (RDP; Cao et al., 2009).

Understanding the RDP and RUP concentrations of a feed is valuable for feed characterization, but some ration formulation models use multiple pool systems to describe protein (Sniffen et al., 1992, Van Amburgh et al., 2015). Protein is partitioned into A (soluble), B (potentially digestible), and C (indigestible) fractions and each fraction possesses different rumen rates of digestion (k_d). The A fraction is assigned a rumen degradation rate of 10-200%/hr, the B fraction is assumed to degrade in the rumen at 1 – 20 %/hr, and the C fraction is assumed to have a degradation rate of 0 %/hr (Van Amburgh et al., 2015). Dried distillers grains ranges from 7.42% to 19.7% of the CP the A fraction (Kleinschmit et al., 2007, Kelzer et al., 2010). The B fraction of DDGS ranges from 53% up to 90% of the CP, and the C fraction ranges from <1% to 27.9% of CP. These observations suggest that the rate and extent digestion of DDGS differs from source to source. The variability of the C fraction also indicated that protein damage may

vary. The amount of CDS also influences these fractions; as CDS increased the A fraction increased, while the B fraction decreased as a proportion of total CP. Effects on the indigestible fraction were inconsistent (Cao et al., 2009).

As knowledge surrounding protein nutrition has improved, the dairy industry has moved towards consideration of specific amino acids. The individual amino acids of DDGS vary in digestibility from source to source. In general when considering the feeding values of DDGS concern surrounds the low concentration of Lys. Research has observed that heat damage decreases the digestibility of Lys (Newkirk et al., 2003, Boucher et al., 2009). Lysine digestibility of DDGS was 10.8% when excessively heated while the Lys digestibility in other literature ranges from 42.2% to 96.5% (Boucher et al., 2009, Kelzer et al., 2010, Paz et al., 2014). Manufacturing ethanol requires heat to aid in breaking down starch before fermentation, but there are processes that use lower temperatures (Nkomba et al., 2016) and research should be conducted to determine if lower temperatures used during ethanol production improve Lys digestibility in DDGS. Milk has a Lys concentration of 7.9 % (Lapierre et al., 2012), while DDGS has a Lys concentration of 3.2% (Mjoun et al., 2010c). When considered together, the susceptibility of Lys to heat damage and the low concentration of Lys in DDGS may result in concerns when including DDGS in dairy rations.

Another major limiting amino acid is Met. Dried distillers grains with solubles has 1.1% to 2.2% Met as a % of CP (Boucher et al., 2009, Kelzer et al., 2010). In two studies supplementing a mix of rumen protected Lys and Met there were no effects on DMI or milk yield, but in one instance milk protein yield increased by 5% (Nichols et al., 1998, Liu et al., 2000b). Animal responses when supplementing rumen protected AA

(RPAA) to cows fed DDGS are listed in Table 1.4; supplementing AA to cows fed DDGS does not consistently improve animal performance.

Histidine is often considered as the third limiting amino acid in dairy cattle. It has been observed to be a limiting AA in diets that are deficient in MP when supplemented with Lys and Met (Lee et al., 2012). Dried distillers grains with solubles is a good source of His; DDGS contain 3.0% His which is similar to the His concentration of milk which is 2.89%. It should be noted that that the RUP fraction of the original feed and the feed that reaches the small intestine may have different AA compositions. After rumen incubation DDGS had 30% lower Lys and 23% lower His concentrations when compared to the intact feed (Boucher et al., 2009).

Dried distillers grains with solubles range from 3.5% to 13.2% crude fat (Table 1.2), most of which are C16:0, C18:1, and C18:2 fatty acids (Anderson et al., 2006, Ranathunga et al., 2010, Díaz-Royón et al., 2012). Palmitic acid, or C16:0, has increased DMI, milk yield, and ECM of lactating dairy cattle (Mathews et al., 2016, de Souza and Lock, 2018). Oleic acid, C18:1, has increased FA digestibility, milk yield, and ECM of cows producing > 60 kg of milk/d (de Souza et al., 2019). Oleic acid has been evaluated as a factor causing milk fat depression (MFD), but results are in consistent. Lock et al. (2007) infused *trans*-10 C18:1 into the abomasum and observed that it did not impact milk fat, suggesting that *trans*-10 C18:1 is not an inhibitor of fat synthesis. Another study (Shingfield et al., 2009) using a similar method delivered a mixture of *cis*-9 C18:1, *trans*-10 C18:1, and *trans*-11 C18:1 and observed a decrease in milk fat synthesis. These studies did differ in the amount of C18:1 supplemented; Lock et al. (2007) provided 42.6 g/day of *trans*-10 C18:1 while Shingfield and others (2009) provided 92.1 g/day of *trans*-

10 C18:1 along with 155 g of *cis*-9, *cis*-10 and *trans*-11 C18:1. Linoleic acid, C18:2, is the fatty acid with the greatest concentration in DDGS. Linoleic acid is also of greater concern because during the biohydrogenation of linoleic acid *trans*-10, *cis*-12 C18:2 may be formed (Bauman and Griinari, 2003). *Trans*-10, *cis*-12 C18:2 is a potent milk fat inhibitor; infusing 6 g/d or less of *trans*-10, *cis*-12 C18:2 has reduced milk fat yield by up to 40% (Lock et al., 2007, Shingfield et al., 2009).

Nearly one third of DDGS are NDF and this fraction is highly fermentable. Varga and Hoover (1983) performed an in situ and observed the NDF in DDGS to be 76% digestible after 24 hours compared with 32% for hay and corn silage. Inclusion of DDGS when feeding BMR corn silage increased total tract NDF digestibility (TTNDFD) by 13%, but in this instance it also reduced fat corrected milk yield (Ramirez et al., 2012). In other studies, increasing TTNDFD also increased ECM (Ramirez-Ramirez et al., 2016). These studies both included DDGS at 30% of the diet DM, but one study included conventional corn silage and the other included BMR silage which increased TTNDFD and decreased rumen pH (Ramirez et al., 2012). The increase in fermentability when feeding BMR silage led to decreased pH which may decrease the rate of biohydrogenation within the rumen (Troegeler-Meynadier et al., 2003). Poorer rumen function likely reduced milk yield.

In summary, DDGS are a good source of protein and energy. They contain soluble protein that can be digested by rumen microbes while also providing RUP. Unfortunately, the amino acid profile of DDGS is not ideal for milk production so RPAA may be beneficial, but results from studies supplementing RPAA are inconsistent (Nichols et al., 1998, Paz and Kononoff, 2014). Additionally, when feeding considerable

amounts of DDGS, the fatty acid concentration of the TMR must be monitored. Although DDGS do not contain any known fatty acid isomers that inhibit milk fat synthesis, the incomplete biohydrogenation of unsaturated fatty acids may lead to potent bioactive intermediates that may reduce the milk fat concentration and milk yield from lactating dairy cows.

Types of DDGS. The Association of American Feed Control Officials (AAFCO) defines DDGS as "… the product obtained after the removal of ethyl alcohol by distillation from the yeast fermentation of a grain or a grain mixture by condensing and drying at least ¾ of the solids of the resultant whole stillage by methods employed in the distilling industry (AAFCO, 2016)." This definition allows ethanol producers to manufacture different types of DDGS that meet the needs of livestock producers.

Traditional DDGS. As ethanol production expanded, livestock producers needed information on how to feed this new and readily available feedstock. Due to its growing supplies and promise, numerous animal feeding studies were conducted evaluating the feeding value of DDGS (Owen and Larson, 1991, Nichols et al., 1998, Liu et al., 2000a). The first generation of DDGS was higher in crude fat than most available today. The early research conducted evaluated maximum inclusions that could be fed while also maintaining animal performance. Table 1.5 summarizes many studies of animal performance from research using DDGS of varying types and sources.

Dried distillers grains have been included as a large proportion of the diet and maintained or improved animal performance. In a study evaluating DDGS, inclusions of 10 and 20% of the diet DM maintained DMI and increased milk production (Anderson et al., 2006). Fat and protein yield also increased with the inclusion of DDGS. Similar DMI

responses were seen during a study that evaluated DDGS from three different sources when fed to lactating dairy cattle (Kleinschmit et al., 2006). Kleinschmit et al. (2006) also observed similar improvements in milk and fat yield with the inclusion of DDGS. In both studies, the improvements in performance and similar DMI led to 5-15% increase in feed efficiency. This research clearly demonstrates that DDGS can be included at up to 20% of the diet DM without decreasing DMI, milk, fat, and protein yield. It is also frequently observed that feeding DDGS results in a reduction in DMI while maintaining milk yield; this leads to increased feed efficiency (FE) of lactating dairy cows. In a study designed to test the use of fiber from DDGS and soybean hulls (SBH) to replace starch from corn grain, cows were observed to consume less and maintain performance (Ranathunga et al., 2010). Feed efficiency increased in a linear manner as the concentration of starch decreased. The authors suggested 2 possible reasons that resulted in a reduction in DMI. First, increasing concentration of NDF in the diet may have added bulk and induced gut distention to limit DMI (Dado and Allen, 1995). The other possibility was that increasing the inclusion of co-products resulted in an increase in the concentration of fat in the diet by 1.35 units which may have decreased intake by triggering gut hormone mechanisms, like increased cholecystokinin which is a signal of satiety (Allen, 2000). The results from Ranathunga et al. (2010) are consistent with others (Anderson et al., 2006, Kleinschmit et al., 2006, Mjoun et al., 2010a) that FE is improved when DDGS are fed. The only study I reviewed where FE decreased due to DDGS supplementation was Owen and Larson (1991). The DDGS were included as 19% or 36% of the diet DM and diets were balanced to be 14.5% and 18% CP, respectively. When the lower CP treatment was fed both soybean meal (SBM) and DDGS led to similar milk, fat and protein yield. At 18% CP,

feeding DDGS decreased milk, milk fat, and protein yield. They do not report the fat concentration of the DDGS in this study, but such a large inclusion of DDGS would have likely increased total and unsaturated fatty acid concentration have depress intake through gut hormone mechanisms (Allen, 2000) and increase risk of MFD (Bauman and Griinari, 2001).

Digestibility, especially Lys digestibility, has been affected by excessive heat. Boucher et al. (2009) heated DDGS to 140°C for 60 min and observed Lys digestibility decreased by 81%. When feeding DDGS plasma Lys concentrations have consistently decreased (Kleinschmit et al., 2006, Mjoun et al., 2010a, Paz et al., 2013b), but milk protein yield does not necessarily follow suit. Mjoun et al. (2010a) and Kleinschmit et al. (2006) both fed diets that were assumed to be deficient in Lys based upon NRC (2001), but in both cases the milk protein yield increased when DDGS were fed. Further, Paz et al (2013a) observed that as DDGS increased, Lys outflow decreased. Although Lys flow was affected by DDGS inclusion, milk protein concentration was unaffected. Researchers concluded that Lys should only be a concern if metabolizable protein supply is very close to requirements (Paz et al., 2013a). In a study evaluating rumen protected Lys supplementation when feeding DDGS, investigators observed that Lys supplementation improved the Lys concentration in the plasma, but milk, fat, and protein yield were not affected (Paz et al., 2013b). Compiling milk yield, protein yield, and protein concentration responses to RPAA when feeding DDGS (Liu et al., 2000a) Nichols et al., 1998; Paz et al., 2013a; and Paz and Kononoff, 2014) shows that the response in inconsistent (Table 1.4). Milk yield and protein yield changed by -0.24 % and 0.44% respectively. These studies may not have provided Lys deficient diets as designed, but

they do demonstrate that Lys supplementation is not required when feeding DDGS to lactating dairy cows.

As mentioned previously, earlier ethanol production was unlikely to remove additional fat in the production of DDGS. In a review, Paz et al (2013a) observed that cows producing milk with low milk fat concentration (<3.45%) did not have a negative relationship between DDGS and milk fat production. When fat concentration of the milk was greater than 3.45% there was a negative relationship between DDGS inclusion and milk fat production. This relationship is similar to one explored by Holloman et al. (2011) where cows with greater than 3.58% milk fat on the control diet had a decreased milk fat concentration when fed DDGS, cows with less than 3.58% milk fat had a positive response to DDGS supplementation.

Research also demonstrated that the composition of milk fatty acids shifts with DDGS inclusion (Anderson et al., 2006, Abdelqader et al., 2009, Ramirez et al., 2012). In these studies, a reduction in C10:0, C12:0, C14:0, and C16:0 were observed in cows consuming DDGS and this response is consistent with the risk of MFD (Baumgard et al., 2000). To the contrary, Ranathunga et al. (2010) fed diets with increasing concentrations of DDGS, up to 21% of the diet DM and observed no changes in the milk fat yield or fatty acid concentration. This may be due to the reduction in starch or increase physically effective NDF (peNDF) as DDGS increased (Ranathunga et al., 2010).

Bauman and Griinari (2003) state that two conditions must be met for MFD to occur; unsaturated fatty acids must be present and there must be an alteration of the biohydrogenation pathway creating potent milk fat inhibiting intermediates. If DDGS is fed, unsaturated fatty acids are present but that does not mean MFD will occur. A meta-

analysis demonstrated that when feeding DDGS, MFD only occurred when the diet contained less than 50% forage (Kalscheur, 2005). When feeding DDGS, nutritionists must also account for diet fermentability and starch concentrations; when dietary starch was >32% of diet DM, DDGS inclusion had a negative relationship with milk yield (Hollmann et al., 2011).

Dried distillers grains is also affective in promoting growth and reproductive development for growing dairy heifers (Anderson et al., 2015a, Anderson et al., 2015b, c, Manthey and Anderson, 2018) The authors fed as much as 33% of diet DM as DDGS replacing SBM, expellers SMB, and corn grain. They did not observe differences in DMI, growth, and FE across treatments Interestingly, feeding DDGS led to a 45 unit increase in the proportion of heifers cycling by 300 kg of BW. The authors hypothesized that improved reproductive measures were a result of increased FA and cholesterol intake which led to increases in steroid hormones such as progesterone (Anderson et al., 2015a). They also followed the heifers into their first lactation to measure reproductive performance and observed that the heifers fed DDGS had a 15% decrease in the number of AI services (Anderson et al., 2015c).

In conclusion, when feeding DDGS, care must be taken to ensure that adequate forage NDF (fNDF) and peNDF is included as well as limiting diet fermentability which will result increased rumen pH and complete biohydrogenation of unsaturated fatty acids. It also may be valuable to monitor the milk fatty acid profile for depressed de novo fatty acid synthesis, which is an indicator of MFD (Baumgard et al., 2000). Although there may be risks in feeding significant inclusions of DDGS, multiple examples of research show it can be effective in both cow and heifer rations (Ranathunga et al., 2010,

Anderson et al., 2015b). Additionally, Ranathunga et al. (2010) observed that increasing DDGS in the diet increased income over feed cost by 20%.

Reduced fat DDGS. As the ethanol industry advanced, corn oil extraction became more common; between 2006 and 2012 the number of ethanol plants performing corn oil extraction increased 20 fold (Harangody, 2012). The corn oil extracted from DDGS is often used as livestock feed or in the biofuel industry (Kalscheur, 2013). Most corn oil extraction from DDGS is conducted through centrifugation of the CDS to produce de-oiled CDS. De-oiled CDS are then added back to the WDGS before drying. Hexane extraction of DDGS is an additional method of fat removal used in the production of reduced-fat DDGS (RFDDGS; Mjoun et al., 2010c).

Reduced fat DDGS are useful in dairy cattle diets; feeding RFDDGS at 20 – 30 % inclusions maintains or improves DMI, milk yield, and FE (Mjoun et al., 2010a, Mjoun et al., 2010c, Foth et al., 2015), milk composition responses were inconsistent. Mjoun et al. (2010c) observed a linear increase in milk fat concentration from 3.18% to 3.72% and milk fat yield from 1.08 to 1.32 kg/d with increasing concentrations of RFDDGS up to 30% diet DM. Protein concentration exhibited a quadratic response, maximum milk protein concentration was achieved when including RFDDGS at 20% of diet DM (Mjoun et al., 2010c). Contrary to those results, Mjoun et al. (2010a) did not observe an increase in milk fat yield or concentration but did observe 3% and 7% increases milk protein concentration and yield, respectively. These studies did differ in what they took out of the diet as RFDDGS was added. Mjoun et al. (2010a) did not remove corn grain with the addition of RFDDGS, in comparison Mjoun et al. (2010c) removed corn grain from the treatment as RFDDGS was increased. This is important because starch can be a risk

factor for MFD when feeding corn co-products (Ramirez Ramirez et al., 2015). Once again, maintaining high rumen pH through adequate peNDF, fNDF, and monitoring diet fermentability can aid in preventing MFD when feeding RFDDGS (Kalscheur, 2005, Hollmann et al., 2011). In summary, animal performance and milk composition when RFDDGS are fed is comparable to other protein and energy sources.

Feeding RFDDGS compared to feeding DDGS has reduced the risk of MFD. In a study directly comparing DDGS and RFDDGS, the corn co-products were fed at 29.9% of the diet DM. Fat corrected milk yield, milk fat concentration, and milk fat yield all increased when RFDDGS were fed compared to DDGS (Ramirez-Ramirez et al., 2016). Protein yield and concentration were unaffected by the change in co-product. When comparing the milk fatty acid profile of these two treatments, RFDDGS had greater de novo fatty acid concentration and lower C18:1 concentration when compared to DDGS, indicating reduced risk of MFD.

Even though research has provided evidence that feeding RFDDGS does not lead to MFD (Mjoun et al., 2010c, Foth et al., 2015, Ranathunga et al., 2018), there are some cases where research has demonstrated the challenges when feeding RFDDGS. In a project designed to test the additive risk factors of MFD when feeding RFDDGS they fed RFDDGS with and without added starch and fat (Ramirez Ramirez et al., 2015). In this study the investigator fed RFDDGS as 20% of the diet DM in all treatments. The authors observed fat yield and percentage decreases with the addition of starch, fat, and a combination of both. The addition of fat and starch also decreased fat corrected milk yield. The observations of milk fatty acid profile was consistent with MFD risk, as de novo fatty acid concentrations decreased and C18:1 isomers increased (Baumgard et al.,

2000). Ramirez-Ramirez et al., (2016) also confirmed that added fat, in the form of corn oil, reduced milk fat concentration and yield. Ramirez-Ramirez et al. (2015) evaluated the effects particle size, fat intake, and their interaction on MFD. They observed that coarse particle size of the TMR was more effective at maintaining milk fat composition and yield than fine particle size. These observations supported Kalsheur's (2005) assertion that effective fiber is important when feeding corn co-products. Coarser particle size may also reduce risk of MFD by reducing rate of passage of digesta out of the rumen. Greater rumen retention time of unsaturated fat may increase the likelihood of complete biohydrogenated. This would avoid the passage of potent milk fat inhibiting intermediates to the mammary gland (Ramirez Ramirez et al., 2016).

Another study that highlighting the risk of feeding RFDDGS fed 28.8% of the diet DM as RFDDGS with and without monensin (Morris et al., 2018). This study was unique because it was long term, which is different from previous studies that were mostly short term crossover designs (Castillo-Lopez et al., 2014, Foth et al., 2015, Reynolds et al., 2019). The authors contended that short term studies may not be adequate to evaluate dietary factors' effects on MFD. In this study, RFDDGS displaced SBM, SBH, and fat. They observed a 22% decrease in milk fat concentration and a similar decrease in milk fat yield. Protein yield and concentration also decreased with the inclusion of RFDDGS (Morris et al., 2018). Although forage was included at > 50% of diet DM, the fNDF was below the recommendation of 22% from Kalscheur (2005). Inadequate effective NDF and fNDF concentration may have resulted in a more rapid passage rate (k_p) and incomplete biohydrogenation leading to MFD. Additionally, there was a reduction in the dietary cation anion difference (DCAD) when including DDGS. Reducing the DCAD of

diets fed to lactating dairy cattle is correlated with lower milk fat concentrations (Erdman and Iwanuik, 2017). Feeding DDGS reduces DCAD due to the large sulfur concentration in DDGS.

Morris et al. (2018) also observed reductions in plasma Lys concentration when feeding RFDDGS which is similar to observations of other experiments (Mjoun et al., 2010a, Paz et al., 2013b, Paz and Kononoff, 2014). Unlike those studies, Morris et al. (2018) observed decreased milk protein concentration and yield which may have been related to reductions in dietary Lys. Although it appears Lys may have limited milk protein production in this instance, supplementation with RPAA when feeding DDGS has been inconsistent in increasing milk production (Liu et al., 2000a, Paz et al., 2013b, Paz and Kononoff, 2014).

In summary, although 40-60% of the fat is removed, RFDDGS is still a suitable source of energy for dairy cattle (Foth et al., 2015). Compared to DDGS, research has indicated that feeding RFDDGS is less likely to cause MFD (Mjoun et al., 2010a, Ramirez-Ramirez et al., 2016). However, the risks of MFD are not completely removed when feeding RFDDGS, increased peNDF (>20%) and fNDF (>22%) are still required and monitoring concentrations of fermentable carbohydrates and unsaturated fats in the diet (Kalscheur, 2005, Ramirez Ramirez et al., 2016, Morris et al., 2018) is prudent.

High Protein DDGS. High protein DDGS (HPDDGS) have become more commercialized and available in recent years. One reason for the increase in popularity of HPDDGS is that one method of producing HPDDGS through a pre-fermentation fractionation method increases ethanol yield (Singh et al., 2005). There is less literature evaluating HPDDGS and that which does exist uses HPDDGS that differ in

manufacturing process and nutrient composition. High protein DDGS ranged from 35% to 45.4% CP (Table 1.2). The 35% CP product originated from processes which utilize hexane extraction of the fat from DDGS, and this process concentrates both CP and NDF components of the feeds (Mjoun et al., 2010a, b, Mjoun et al., 2010c). The pre-fermentation fractionation technique, described by Singh et al. (2005) results in HPDDGS by first separating the germ, endosperm, and bran of the corn kernel prior to fermentation. The germ is then used for corn oil production, while the bran is used as a fibrous feed for ruminants, and lastly the endosperm is fermented for ethanol. Singh et al. (2005) summarized three procedures that increased the CP of the DDGS. Each process had a pre-fermentation fractionation that removes the germ, the germ and bran, or removed the germ, bran, and endosperm fiber before fermentation. As more corn kernel components were removed before fermentation, CP of the HPDDGS was 35.9, 49.3 and 58.5%, respectively (Singh et al., 2005).

In a study evaluating the AA digestibility of HPDDGS, it was observed that that total-tract Lys digestibility was increased by 49.5% above that of conventional DDGS (Kelzer et al., 2010). Mjoun et al. (2010b) also investigated AA digestibility and observed similar intestinal digestibility of AA between DDGS, RFDDGS, and HPDDGS. The digestible RUP of HPDDGS was similar to other DDGS products (Kelzer et al., 2010). Although more processing steps are required to produce HPDDGS the digestibility is similar, or slightly improved, when compared to DDGS and RFDDGS.

Three studies directly comparing HPDDGS and another protein source exist (Hubbard et al., 2009, Kelzer et al., 2009, Christen et al., 2010). These studies compared HPDDGS to different protein sources and at different inclusion amounts, up to 20% of

the diet DM (Hubbard et al., 2009). In general, including up to 20% of the diet DM as HPDDGS in diets fed to lactating dairy cows been observed to maintain or improve performance when compared to SBM and CM supplementation (Hubbard et al., 2009, Kelzer et al., 2009, Christen et al., 2010). Milk yield, milk fat yield, protein yield and FE were all improved when HPDDGS was fed at 20% of the diet DM in replace of SBM (Hubbard et al., 2009). Contrary to those results Kelzer et al. (2009) and Christen et al. (2010) observed no differences in milk, milk fat, or protein yield. Taken together this evidence suggests that HPDDGS is an effective protein source for dairy cattle. In an additional study using HPDDGS, Swanepoel et al. (2014) observed that mixing protein sources may be beneficial. Canola meal, HPDDGS, or mixtures of the two were fed to lactating dairy cattle as 20% of the diet DM and they observed that performance was optimized with a mix of 1/3 HPDDGS and 2/3 CM. These results echo those of Mulrooney et al. (2009) who evaluated a mixture of DDGS and CM as 10% of diet DM and observed ECM output was the greatest when feeding 1/3 DDGS and 2/3 CM. Plasma Lys, Met, and His concentrations were all equivalent or improved in the higher CM diets compared to DDGS, which may in part explain why production was enhanced when feeding a mix of protein supplements. The authors hypothesized that the improved performance when increasing CM was a response of the increased RDP concentration and improved digestibility of CM compared to HPDDGS (Swanepoel et al., 2014). Improving the concentration of RDP by increasing CM may enhance the microbial crude protein (MCP) production; the investigators evaluated the diets and observed that the HPDDGS treatment was deficient in RDP while the CM treatment was deficient in RUP

according to NRC (2001) recommendations. This is more evidence that blending the protein supplements may be beneficial (Mulrooney et al., 2009, Swanepoel et al., 2014).

Although research to date is limited in scope, HPDDGS has been observed to be an effective protein supplement (Hubbard et al., 2009, Kelzer et al., 2009, Christen et al., 2010). The AA profile of DDGS and HPDDGS are similar and , like when feeding DDGS, reduced blood plasma concentration of Lys has been observed when feeding HPDDGS (Swanepoel et al., 2014). No research was found that evaluated feeding RPAA to cows consuming HPDDGS, but the data do provide evidence that mixing protein supplements improves milk production (Mulrooney et al., 2009, Swanepoel et al., 2014). Another benefit of HPDDGS is the feed had reduced fat, similar to fat concentrations of RFDDGS, and may reduce the risks of MFD (Swanepoel et al., 2014). Taken together research has demonstrated that corn ethanol co-products are effective feeds, but they may be further enhanced through mixing of protein supplements. Also, when feeding dairy cattle balancing for AA and supplying adequate RDP should be done to optimize lactation performance (Patton et al., 2014).

Fiber

Defining fiber. Ruminants' great niche is their ability to take nutrients of little value to humans, like fiber, and convert it to protein. Fiber is defined as components that are unable to be digested by mammalian enzymes (Van Soest et al., 1991). The original analysis used to define fiber in animal nutrition was crude fiber, which was a component of the proximate analysis system. Crude fiber is measured through the reflux of fat extracted residue along with sulfuric acid and sodium hydroxide. Then the residue is ashed to determine the crude fiber concentration (Van Soest, 1994). The major challenges with crude fiber are the inconsistent extraction of carbohydrates such as cellulose which

leads to an inconsistent relationship between crude fiber and actual chemical compounds (Van Soest, 1994). At the time crude fiber was constructed, it was assumed to be a temporary measure and used until better lab procedures were developed (Hall and Mertens, 2017).

An improved analytical system came in the form of the detergent fiber system (Van Soest and Wine, 1963, Van Soest, 1963). This system rapidly became the accepted method for measuring fiber of feeds. The detergent system provides a rapid analysis that determines concentration of insoluble components of a feed sample and the remaining residue consists of cellulose, hemicellulose, and lignin (Van Soest, 1994). The detergent fiber system can be characterized by two segments of fiber, neutral (NDF) and acid detergent fiber (ADF). The detergent used to determine NDF solubilizes the intracellular components and pectin while hemicellulose, cellulose, lignin, bound proteins, and minerals are left behind in the residue. Neutral detergent fiber and ADF do not include other cell wall components such as pectin and β-glucans because these are water soluble non-starch polysaccharides (Van Soest, 1991). Although still undigested by mammalian enzymes, this fractionation scheme is useful because pectin and β-glucans are readily digested by rumen microbes. They are also nutritionally important because, like cellulose, their fermentation does not give rise to lactic acid (Van Soest, 1994). Analytically, isolation of NDF residue has been improved with the addition of heat stable amylases and sulfites to solubilize a greater portion of the non-cell wall components. The heat stable amylase removes starch which may interfere with the refluxing process (Van Soest, 1991). Sodium sulfite cleaves sulfide bonds and aides the process by removing bound N within the residue, but inclusion of it in the assay is considered optional and should not

be used if measuring neutral detergent insoluble crude protein (NDICP) because “the sulfite reaction is nonbiological”(Van Soest, 1991). Additionally, sulfite can interfere with lignin so if sequential analysis of NDF, ADF, and acid detergent lignin (ADL) is being done sulfite should be omitted (Van Soest, 1991).

Acid detergent fiber represents the portion of NDF that is insoluble in 1.0 N sulfuric acid (Van Soest, 1963), which is composed mostly of cellulose and lignin. This is useful because subtracting the ADF concentration from the NDF concentration is the hemicellulose concentration; hemicellulose is a highly fermentable nutrient (Herrick et al., 2012, Drehmel et al., 2018). Cellulose and lignin are closely linked, and the digestibility of cellulose is dependent upon the extent of lignification within the cell wall. Specifically, greater lignification reduced the digestibility of cellulose (Van Soest, 1994, Buxton and Redfearn, 1997). Lignin itself is a poorly defined and understood component of fiber. However, it is often considered the greatest factor limiting digestibility of cell wall components (Van Soest,1994). This is because lignin inhibits digestibility by acting as a physical barrier for microbes to access cellulose and hemicellulose and by bonding with cellulose and hemicellulose through ferulate bridges (Buxton and Redfearn 1997). Further difficulty lay in the relationship between lignin and digestibility; research conducted has indicated that the relationship between lignin and NDF digestibility (NDFD) is variable (Raffrenato al., 2017). Although the detergent system has been useful in defining the chemical constituents and partitioning the cell wall components, it does not wholly capture the physical and biological attributes of fiber.

Physical characteristics. Understanding the chemical composition of feeds is important for ration formulation, but the physical form of feeds is important as well. The

physical form of feed can impact animal metabolism and milk fat production (Mertens, 1997). To define and evaluate particle size, Mertens (1997) proposed the use of peNDF which is defined as the portion of fiber that influences chewing activity and contributes to rumen mat formation. Mertens (1997) also proposed a static recommendation of 22.3 % of the diet as peNDF. A popular method of measuring particle size is through use of the Penn State Particle Separator (PSPS; Kononoff et al., 2003a). This method requires the use of a tiered box enclosed with different sized sieves. The sieves measure 19 mm, 8 mm, 1.18 mm, and a pan to catch the remaining residue. A 4 mm sieve is often used in place of the 1.18 mm sieve (Kmicikewycz et al., 2015). Physically effective NDF is calculated by multiplying the proportion of material retained on the top three sieves, which is referred to as the physical effectiveness factor (pef), and the total NDF concentration of the diet. The calculation is as follows:

Equation 1.1. $peNDF, \%\, DM = \%\, NDF \times \%\, \geq\, 1.18\, mm$

This calculation allows for easy field application. Physically effective NDF has affected animal behavior measures. In an experiment evaluating corn silages differing in peNDF, Beauchemin and Yang (2005) observed that chewing activity increased linearly with increasing peNDF concentrations. They did not detect differences in rumen pH, but the treatments tested in this study were all low in peNDF (8.9% - 11.5%) and thus may have been too low to affect rumen pH.

The impact of corn silage particle size is particularly important considering that corn silage is the most popular forage used on dairy farms in the United States (Kellogg et al., 2001). Kononoff et al. (2003b) observed that decreasing corn silage theoretical chop length (TCL) from 22.3 mm to 4.8 mm increased DMI, reduced sorting behavior, and had no effect on rumen pH. Total chewing time was unaffected but chewing time per

unit of DMI linearly increased as proportion of corn silage with 22.3 mm TCL increased from 0 to 57% of diet DM. This demonstrates that particle size can influence chewing activity but that differences in feed intake or diet fermentability may be of larger consequence when managing rumen pH (Penner, 2019). With the increased use of fibrous byproducts for livestock it is also important to understand the interaction of peNDF and nonforage fiber sources. Kononoff and Heinrichs (2003a) designed a study in which corn silage with 22.3 or 4.8 mm TCL was fed with and without cottonseed hulls. They observed minimal differences due to the forage TCL but diets including cottonseed hulls resulted in lower pH that diets without cottonseed hulls indicating the fiber source, not particle size, affect rumen pH.

Alfalfa haylage is another popular forage in the United States (Kellogg et al., 2001). Kononoff and Heinrichs (2003b) fed alfalfa with different TCL (22.3 or 4.8 mm). as 50% of diet DM in all treatments with differing proportions of short:long alfalfa. Increasing the TCL of alfalfa haylage resulted in a linear decrease in DMI of 16.1%, linear increase in total chewing per unit of DMI by 21%, and no differences in milk production. These observations indicate that alfalfa is an effective source of peNDF and can stimulate increased chewing and rumination activity in lactating dairy cows. These data also demonstrate that excessive particle size may limit DMI.

Straw is often fed because of its high NDF and peNDF concentrations. Altering the particle size of straw fed to fresh cows resulted in no differences in DMI, milk production, or rumen pH. Cows fed straw with a shorter chop length had more stable rumen pH and more stable milk production in early lactation (Coon et al., 2018).

We may reduce the peNDF through grinding or pelleting of forages. The previous studies (Kononoff and Heinrichs, 2003b, Kononoff et al., 2003b) changed particle size by changing TCL at harvest of a forage. Pelleting timothy grass hay fed to dairy cows reduced milk fat yield by 14%, reduced the yield of short chain fatty acids by 40-60%, and reduced chewing time by 19% (Ramirez Ramirez et al., 2016). Although there are risks when reducing particle size, it also reduces sorting behavior in lactating dairy cows (Leonardi and Armentano, 2003, Coon et al., 2018). In general, peNDF is informative and can be used to manipulate chewing and rumination activity of lactating dairy cattle.

The peNDF system is based upon several key assumptions, 1) that NDF is equally distributed across particle size, 2) chewing activity response is equal for all retained particles, and 3) there is no difference in fragility and particle size reduction is the same for all particles (Mertens, 1997). To eliminate some of these assumptions a new system has been proposed to evaluate the particle size of diets fed to lactating dairy cattle (White et al., 2017a, b). In a pair of research papers, the authors derive equations and propose a system of feeding recommendations regarding particle size based on chemical composition of the diet. The new system is referred to as physically adjusted NDF (paNDF). The paNDF system is different from peNDF because it separates NDF concentration from particle size measurements and includes other dietary and biological measures (White et al., 2017a). They observed that predicting rumen pH was improved by incorporating dNDF and digestible starch (dStarch; White et al., 2017a). Using the equations derived from this meta-analysis White et al. (2017b) generated an ensemble model to predict the required particle size distribution to maintain a desired rumen pH. The characteristics within the model are starch, NDF, dNDF, dStarch, fNDF, and

ADF:NDF. This allows for robust recommendations of particle size based on unique dietary situations.

Biological characteristics. In addition to chemical and physical qualities of fiber, the biological traits of fiber are also important. A one unit increase in NDF digestibility of forages leads to 0.17 kg and 0.25 kg increases in DMI and 4% fat corrected milk (Oba and Allen, 1999). Forage NDF digestibility is variable; it is affected by hybrid, plant maturity, and other agronomic conditions (Oba and Allen, 1999). Considering that forages represent a large proportion of a dairy rations, proper characterization of their digestible nutrients are vital in the precision feeding of dairy cattle.

Characterizing and modeling NDFD has been a topic of research within ruminants for decades (Allen and Mertens, 1988, Raffrenato and Van Amburgh, 2011, Raffrenato et al., 2019). Although, there are apparent relationships between the chemical constituents of NDF and digestibility, the digestibility of NDF cannot be estimated from the concentrations of cellulose, hemicellulose, or lignin alone. Currently, NDFD is understood to be a result of the physical matrix that exists within the plant cell wall and not just a relationship of different chemical characteristics like ADF or lignin (Hall and Mertens, 2017). Other plant anatomical factors such as waxes, plant cuticles, and vascular tissue all my affect NDFD (Buxton and Redfearn, 1997). Phenolic compounds, like ferulic and *para*-coumaric acids, in the cell wall are also limiting because they may be harmful to rumen bacteria (Buxton and Redfearn, 1997, Raffrenato et al., 2017)

To measure NDFD the use of in vitro and in situ methods are often employed. Most in vitro methods employed now are based upon the method outlined by Tilley and Terry (1963). Goering and Van Soest (1970) refined that method, and further evaluations

of in vitro systems was conducted by (Raffrenato et al., 2018). For proper digestion during in vitro fermentations a temperature of 39°C, pH > 6.0, nutrient availability, and anaerobicity must be maintained. For optimal results donor animals should match the target species, rumen fluid should be harvested from at least 2 donor animals due to interanimal variation , rumen fluid should be harvested 8-12 hours after feeding because that is when fibrolytic enzymes activity is maximized, and crude protein should be greater than 11% DM (Weiss, 1994). Adding N to the rumen fluid and buffer is also recommended to ensure N does not limit NDFD (Goering and Van Soest, 1970). Additionally, Raffrenato et al. (2018) observed that using a filter pore size of 1.5 µm when conducting the NDF assay after in vitro fermentations led to a more uniform recovery of small particles and improved repeatability. They also demonstrated that 240 in vitro fermentations were adequate in estimating the indigestible NDF (iNDF) of feeds. This is one advantage of in vitro systems, because there is evidence that in situ or the Daisy incubator (Ankom Technology Corp., Fairport, NY) methods require greater than 288 hours to estimate similar iNDF concentrations as in vitro systems (Raffrenato et al., 2018). In situ and Daisy methods also led to greater analytical error (Raffrenato et al., 2018). This is likely due to the use of bags with a porosity that is larger than the 1.5 µm filter paper used when filtering after in vitro fermentations. Large porosity bags may also increase variability by allowing undigested material to exit the bag. Based on published data it could be suggested that the use of in vitro systems be used to estimate NDFD because it reduces analytical variation, reduces the time needed to estimate iNDF, and has a standardized protocol (Goering and Van Soest, 1970) for use in commercial laboratories.

Characterizing NDFD is important for modeling of ruminal digestion. Allen and Mertens (1988) reviewed various equations and methods that suggested a simple 2 pool model for NDFD. Neutral detergent fiber is now characterized in 2 or 3 pool systems with 1 or 2 potentially digestible fractions and an iNDF (pdNDF; Raffrenato et al., 2019). The three-pool system partitions the pdNDF into fast and slow digesting segments. Rates of digestion for fast and slow digesting pools of pdNDF for typical forages fed to dairy cattle range from 4-13%/hr and 0.7-2.4%/hr (Raffrenato et al., 2019). These models assume these digestible fractions have uniform k_d and that the indigestible fraction has a degradation rate of 0. In vivo or in vitro methods may be used to determine k_d for pdNDF and the amount iNDF (Hall and Mertens, 2017) in feeds.

Another method to characterize NDFD has been outlined by (Lopes et al., 2015a). In this method an in vitro procedure is used and data are coupled with calculations to predict the total tract NDF digestibility (TTNDFD) of different feeds. The first step is to conduct in vitro assays to determine 24, 30, 48, and 240 h NDFD. The 240 h in vitro estimates iNDF concentration of the feed while the short-term fermentations allow for determination of the k_d of NDF. Then using a standardized k_p (2.67%/hr) and the predicted k_d, the TTNDFD is calculated using a first order model, and is shown below:

Equation 1.2. $TTNDFD,\ \%NDF = [pdNDF\ \times (kd/(kd + kp\ ))] \div 0.9$

Where pdNDF is a % of NDF and is calculated as 1 – iNDF from a 240 hour in vitro fermentation, k_d is the rate of NDF digestion as %/hr, and k_p is rate of passage as a %/hr. Dividing the whole quantity by 0.90 is done because they assume about 90% of NDF digestion occurs in the rumen (Lopes et al., 2015a). However, as much as 27% of the cellulose digestion and 40% of the hemicellulose digestion may occur in the hindgut (Hoover, 1978). The k_p is assumed to be 2.67%/hr, which is based on a 630 kg cow

consuming 23.4 kg/d of DM of a 30% NDF diet. An example using the TTNDFD system is listed in Table 1.6, with alfalfa hay and corn silage nutrient composition from the Dairy One interactive feed library (https://dairyone.com/services/forage-laboratory-services/feed-composition-library/interactive-feed-composition-libraries/).

Determining NDF digested after certain amounts of time is useful in rumen modeling and certain NDFD measures may influence performance of dairy cattle. In a study evaluating the NDF concentration, NDFD after 48 hours in vitro (NDFD48), and their interaction, Kendall et al. (2009) fed straw that was treated or untreated with ammonia to lactating dairy cows. Dry matter intake decreased as NDF concentration increased while milk, fat, and protein yield were all increased with an increase in NDFD48 (Kendall et al., 2009). Digestibility increases also increased rumen retention time (Kendall et al., 2009). In a similar study Fustini et al. (2017) evaluated the effects of undigested NDF after 24 and 240 hours (uNDF24; uNDF240). The authors altered uNDF through inclusions of a high and low digestible alfalfa with high and low inclusions of soybean hulls. Dry matter intake and milk production increased by 5% and 3%, respectively when feeding alfalfa with greater digestibility (Fustini et al., 2017). These results support the sugestions of Kendall et al. (2009) that NDFD measured at shorter time points (24 and 48 hours) are more related to animal performance than measuring NDFD or uNDF at later times. Balancing diets based on uNDF240 has had mixed results; in some cases there are no effects of changing uNDF on milk production (Fustini et al., 2017) while increasing uNDF240 has decreased milk and milk solids production (Hosseini et al., 2019).

Feeding fiber. The nutritional impact of NDF is dependent on many factors such as form, digestibility, and chemical composition of the TMR (Allen, 1996, Oba and Allen, 1999, Allen, 2000). Digestibility of forages, co-products, and other fibrous feeds vary greatly which affects energy supply, but NDFD is also affected by the rumen environment; as starch concentration increases, NDFD decreases (Sanchez-Duarte, 2017).

Extensive research efforts have been undertaken to enhance the value of NDF for dairy cattle. Improvements in NDFD have been realized through plant breeding or gene editing technology that delays the onset of lignin formation or reduces the lignin concentration of the plant. In a meta-analysis conducted by Ferraretto and Shaver (2015) it was demonstrated that increasing digestibility of corn silage and BMR corn silage hybrids leads to an increase in DMI, milk yield, and TTNDFD. The recent the introduction of low lignin alfalfa has created interest as a method to improve NDFD, but controlled feeding studies are still needed. Other methods that improve NDFD, include chemical and physical treatments such as shredding or alkaline treatments, but these have resulted in variable success (Adesogan et al., 2019).

Nutritional models

As our understanding of animal nutrition and needs for increasing efficiency have grown, nutritional models have become more prominent. These complex models allow for many ingredients and numerous feed characteristics to be included and integrated into solutions that predict animal performance. Within the dairy industry, the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992, Van Amburgh et al., 2015) and the NRC (NRC, 2001) are most common. The CNCPS model is used as the framework for commercial platforms such as AMTS (https://agmodelsystems.com/;

AMTS LLC., Groton, NY), CPM (Ithaca, NY), and NDS (https://www.rumen.it/registered/index.html; RU.M.&N Nutritional Dynamic Systems, Emilia-Romagna, Italy). Spartan (https://www.canr.msu.edu/spartandairy/; Michigan State University, East Lansing, MI) and Formulate2 (http://www.formulate2.com/; Diet Formulation Systems LLC, Visalia, CA) are based on NRC (2001) model.

Cornell Net Carbohydrate and Protein System Thorough understanding of the NDFD model within CNCPS requires that we understand how it partitions carbohydrates (CHO) for modelling purposes. The original model was published in a series of papers describing the rumen submodel (Russell et al., 1992), the carbohydrate and protein availability models (Sniffen et al., 1992), and animal requirements (Fox et al., 1992). Within this model, the CHO fraction was estimated by subtraction of crude protein, fat, and ash from 100. Once the total CHO pool was determined, the pool was partitioned and assigned degradation rates. The original model had 4 pools; Fraction A was the most rapidly digested and composed of sugars, fraction B1 was starch and pectin, fraction B2 was digestible NDF, and the C fraction was iNDF (Sniffen et al., 1992, Krämer et al.). The CHO fractions were all calculated by difference according to determination of NDF with α-amylase and without sodium sulfite (Sniffen et al., 1992).

The current CNCPS model has expanded to an 8 pool model which is as follows: A1 = Volatile fatty acids, A2 = lactic acid, A3 = organic acids, A4 = sugars, B1 = starch, B2 = soluble fiber, B3 = digestible NDF, C = iNDF (Lanzas et al., 2007). The most recent update to this scheme came from Van Amburgh et al. (2015) when they modified the model by how the C fraction was determined. Under the original model (Sniffen et

al., 1992) and the updated scheme (Lanzas et al., 2007) the C fraction was determined as follows;

Equation 1.3. $C,\ indigestible\ NDF, g\ /\ kg\ of\ DM = [NDF\ (g\ /\ kg\ DM) \times Lignin\ (g\ /\ kg\ \ NDF) \times 2.4] \div 1000$

where NDF is assayed with α-amylase and without sodium sulfite (% DM) and lignin is the lignin concentration of a feed (% NDF). The relationship between iNDF and lignin was originally extracted by Chandler et al. (1980). The relationship of NDFD and lignin is inconsistent and is influence by plant maturity and forage type. Lignin and NDFD was most strongly related for mature grasses (Pearson correlation coefficient of -0.90) but was much poorer for conventional and BMR corn silage hybrids (-0.27 and -0.14; Raffrenato et al., 2017). Further evidence demonstrated inconsistency of this relationship; the ratio of uNDF and lignin ranged from 1.00 to 6.71 for various forages (Raffrenato et al., 2018).

The updated model (Van Amburgh et al., 2015) uses uNDF as an input as determined from a 240 h in vitro (uNDF240; Palmonari et al., 2017, Raffrenato et al., 2018). This modification is expected to account for differences that occur in feedstuffs due to growing conditions and hybrid. Using the uNDF240 determined in vitro, B3 is determined by subtracting uNDF240 from total NDF. This yields digestible and uNDF pools, but modeling of NDFD may be improved by separating digestible NDF into quickly and slowly digesting pools (Raffrenato et al., 2019) which has been incorporated into the updated CNCPS model.

Each of these pools of carbohydrate are assigned a unique degradation rate, which when paired with an estimate of k_p (Seo et al., 2006) estimates the amount nutrient digested. The rate constants for each nutrient fraction are listed in Table 1.7**.** Digestion rates of 0 represent a constituent that is undigested and passes out of the rumen. Total

rumen degradation is determined by integrating the k_p and k_d. Within CNCPS, liquid and solid fractions in the rumen are assigned different k_p; liquid fractions are associated with soluble nutrients while the solid fractions are composed of the remaining potentially digestible nutreints (Seo et al., 2006, Van Amburgh et al., 2015). The soluble nutrients of feeds are associated with liquid k_p which are 5 to 10 times faster than solids (Seo et al., 2006). The solid k_p for forages or concentrates contains the remaining fractions associated with each ingredient (Van Amburgh et al., 2015).

The k_d of digestible NDF is variable (1-18%), and this is important because NDF is not broken down by mammalian enzymes like starch and sugar so increasing k_d of digestible NDF increases energy from fibrous feeds. For example, conducting a simulation using the CNCPS model to determine the effect of a highly digestible and a poorly digestible corn silage demonstrate how estimates change. According to DairyOne feed composition library (https://dairyone.com/services/forage-laboratory-services/feed-composition-library/interactive-feed-composition-libraries/) corn silage averages 53 %, 67 %, and 72% digestible NDF as %NDF at 30, 120, and 240 hrs respectively. Within CNCPS this generates a k_d of 4.5 %/hr. A BMR corn silage hybrid may be 67 %, 85% and 86% digestible at 30, 120, and 240 hrs which creates a k_d of 5.4 %/hr. The model assumes a postruminal NDF digestibility of 20% (Sniffen et al., 1992). Adjusting the k_d does not affect the k_p, so any increases in energy, protein, or milk are associated with improved NDF degradation from corn silage. The increase in k_d of digestible NDF resulted in a 3 and 4% increase in ME and MP supplied to the animal and 6% increases in ME and MP allowable milk, which is listed in Table 1.8. This demonstrates the model's

sensitivity to the k_d of digestible NDF and the importance of accurately estimating NDFD of feed ingredients.

Digestible NDF affects predicted ME and MP supplies. The ME intake of a diet is calculated according to total digestible nutrients (TDN). Both TDN and ME calculations are described below

Equation 1.4. $$TDN, g/d = (CP\ intake - Fecal\ protein\ output) + (CHO\ intake - Fecal\ CHO\ output) + 2.25(Fat\ intake - Fecal\ fat\ output)$$

Equation 1.5. $$ME,\ mcal\ /\ d = 0.001\ \times TDN, g\ /\ d \times 4.409\ \times 0.82\ \times DMI$$

where intake and outputs are in g/d. Increasing digested NDF increased CHO digested which increased the ME supply (Tylutki et al., 2008). One flaw in this approach is using 4.409 kcal/g because fat, protein, and CHO do not contain the same energy concentration. The ME intake is then multiplied by 0.644 to compute the net energy for lactation. The MP supply is based on the degradation rate of the CHO in the diet, so increasing the k_d digestible NDF will increase bacterial CP (BCP) yield. The increase BCP production increased MP supply and MP allowable milk. The whole system of equations used to determine microbial growth in the rumen are listed by Fox et al. (2004).

In summary, CNCPS is a mechanistic model that functions using k_d and k_p estimates within the rumen to determine rumen digestion, microbial growth, and nutrients that escape the rumen. Then fractions that escaped the rumen are used to determine intestinal absorption of nutrients which leads to predictions of nutrients that are excreted. Using nutrient intake and nutrients excreted the ME and MP of diets can be predicted and used to predict animal performance.

NRC. The NRC model handles carbohydrates and fiber differently than CNCPS. Where CNCPS uses 8 pools, the NRC separates CHO into NDF and non – fiber

carbohydrates (NFC; NRC, 2001). The NFC fraction is equal to 100 less the sum of CP, fat, NDF, and ash.

The determination of digestible NDF also differs greatly from the CNCPS model, it is an empirical rather than a mechanistic model. The NRC (2001) equation to determine digestible NDF is as follows:

Equation 1.6. $digestible\ NDF, \%\ DM = 0.75\ \times [(NDF - NDICP) - L] \times [1 - (L \div [NDF - NDICP])^{0.667}$

where NDICP is neutral detergent insoluble CP and L is acid detergent lignin. Then, the model assumes 4.2 kcal/kg for digested NDF which contributes to the predicted digestible energy (DE) of a feed ingredient at maintenance intake. Before ME is determined, a discount factor that decreases digestibility as intake increases above maintenance yields DE at actual intake (DE_p). The DE_p value is then used to determine ME_p and eventually NE_{Lp} using two summative equations (NRC, 2001).

Changes in digestible NDF in NRC are determined solely from chemical composition. Lignin has been well established as having a negative impact on fiber digestibility (Jung and Allen, 1995, Buxton and Redfearn, 1997), but the relationship is inconsistent (Raffrenato et al., 2017). Using the chemical composition alone to determine digestible NDF may be an area to be improved upon within the NRC (2001).

The NRC model accounts for carbohydrates in a simpler manner and determines digestibility of CHO fractions based on chemical composition alone. The model does not assume rate of passage in determining rumen degradation of CHO like CNCPS, instead the model employs a discount system based on level of intake which lowers diet digestibility as intake increases. Also, the determination of microbial protein is simpler and relies on TDN and RDP supply. Much like CNCPS, NDFD also contributes to MP

under the NRC model. Increased NDFD increases TDN of a diet. Then, assuming N is not limited in the rumen, TDN is used to calculate MCP by multiplying the TDN by 0.13 (NRC, 2001).

SUMMARY

Ethanol production has affected the energy and agricultural landscapes in the United States. Specifically, increased demand for ethanol and clean energy has increased ethanol production which in turn has led to large supplies of DDGS which has been used to feed livestock. The ethanol manufacturing process has continued to evolve by improving methods of grain processing, enzymes and yeast strains. These changes to ethanol manufacturing have also resulted in changes in the feed produced. These include differences in the concentration of protein, fat and fiber, but all types of DDGS made from dry grind processing have proven to be suitable supplements for dairy cattle.

Feeding DDGS is often done because of its digestible NDF concentration. It is also an effective source of protein, the protein within DDGS is mostly RUP. A possible challenge when feeding DDGS to dairy cattle may be the AA concentration because DDGS are a poor source of Lys. Although DDGS has less Lys than many dietary recommendations for lactating dairy cattle, supplementation with rumen-protected Lys has demonstrated mixed results. Plasma concentrations of Lys do decrease when fed DDGS, but milk production is generally unaffected by the decreased Lys supply. Mixing protein supplements has been more successful than supplementing RPAA to improve performance when feeding DDGS which may be due to providing a mix of RDP and RUP or a more complete AA profile.

The fat concentrations of DDGS have decreased as more ethanol producers removed corn oil for use in other biofuel production or as a livestock feed. Decreasing the

fat concentration proved valuable for the dairy industry because it has reduced the risk of MFD when feeding dairy cattle without decreasing the energy supplied by DDGS. Fat within DDGS is mostly unsaturated which may be a risk factor for MFD, but MFD is avoidable. Contrary to frequent industry perceptions, research has demonstrated that DDGS supplementation maintains or even increases milk fat yield, especially in cows that had low milk fat yield on control diets. When feeding DDGS it is prudent to monitor total diet unsaturated fatty acids, starch, and peNDF to avoid MFD.

The NDF in DDGS is highly fermentable and provides energy to the dairy cow. Feeding DDGS often increases TTNDFD, especially when DDGS replaces forages that are poorly digestible. Even though it is possible to use DDGS to replace some of the forages present in a diet, adequate peNDF must be also maintained. Also, when feeding diets greater in non-forage fiber it is beneficial to reduce starch concentrations which reduces diet fermentability. Concentrates can also be replaced by DDGS, but the increase in NDF concentration from DDGS may limit intake because NDF adds bulk to the diet.

The NDF that is digested from DDGS and forages is an important energy source for cattle, and it allows them to capitalize on their niche of converting human in-edible feedstuffs to high quality protein. Understanding how NDF is digested is a complex interaction of plant, animal, and dietary factors. Lignin is the main plant factor limiting digestion of NDF, but the relationship between lignin and digestibility is variable. Even though NDF, lignin, and digestibility to do not have a static relationship it has been used to predict digestibility. In vitro estimates of NDFD are encouraged because it captures differences in digestibility that chemical composition may not capture. Once the NDFD at fixed time points is determined, a k_d can be estimated. The k_d and k_p allow for

predictions of digested NDF. Using this mechanistic approach, feeds with greater k_d and reduced k_p increase digested NDF. Feeding studies have also indicated that modifying the particle size, using chemical treatments, or decreasing dietary starch may increase NDFD. Improving NDFD when feeding ruminants will increase their net contribution to the food supply and it will enhance the viability of the dairy industry for years to come.

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TABLES AND FIGURES

Table 1.1. Timeline of significant legislative actions that spurred the growth of ethanol demand and consumption in the United States

Year	Policy	Description
1978	Energy Tax Act of 1978	Exemption from the gasoline excise tax for fuels that are greater than 10% ethanol blends
1990	Clean Air Act	Demanded use of oxygenated fuels to combat ozone degradation and carbon monoxide emissions
1990	Small Ethanol Producer Tax Credit	An income tax credit for plants on their first 57,000 m^3 of ethanol
1997	Ethanol Mandate	Minnesota required all fuel sold in the state to contain at least 10% ethanol (Other states have implemented similar policies since)
1999	MTBE[1] Phase Out	California passed a law phasing out and banning the use of MTBE as a fuel oxygenate
2004	Volumetric Ethanol Excise Tax Credit	Changed tax credit from incentivizing blends to requiring a certain total volume be used in the fuel supply
2005	Renewable fuel standard	Set amounts and sources (corn, cellulosic, etc) of renewable fuels to be used in the fuel supply
2010	Renewable fuel standard 2	Separated renewable fuels based on GHG[2] emission reductions relative to petroleum based fuels

[1]MTBE = methyl tertiary butyl ether. MTBE is a fuel oxygenate.
[2]GHG = Greenhouse gas emissions.

Table 1.2. Nutrient composition of dried distillers grains with solubles fed to lactating dairy cattle in research experiments

Study	DM%	Fat, %DM	NDF, %DM	Starch, %DM	CP, %DM
Abdelqader et al., 2009	91.0	9.9	32.7	- [1]	30.9
Castillo-Lopez et al., 2014	90.3	5.53	33.9	7.50	31.9
Christen et al., 2010	89.9	9.57	26.1	-	29.8
	90.4	3.42	28.7	-	44.5
Foth et al., 2015	89.1	6.16	31.4	7.45	32.3
Hubbard et al., 2009	91.4	4.63	26.4	-	46.1
Janicek et al., 2008	88.5	9.55	37.3	8.25	29.3
	88.5	10.6	34.3	6.67	33.3
Kelzer et al., 2009	91.4	4.63	26.4	-	46.1
Kleinschmit et al., 2006	91.3	10.8	44.0	-	30.3
	88.4	10.6	39.8	-	29.9
	89.2	10.5	39.1	-	31.4
Mjoun et al., 2010a	87.7	10.8	31.2	8.9	31.3
	87.5	3.5	42.8	5.6	34.0
Mjoun et al., 2010b	87.5	3.5	42.8	5.6	34.0
Morris et al., 2018	88.4	7.4	27.5	-	34.4
Mulrooney et al., 2009	90.0	10.1	36.9	-	32.1
Paz et al., 2013	90.3	11.7	31.6	5.58	28.2
Paz and Kononoff, 2014	90.7	6.35	31.4	7.70	32.2
Ramirez-Ramirez et al., 2015	90.5	11.9	31.3	5.5	28.3
Ramirez-Ramirez et al., 2016a	89.8	6.6	37.7	6.7	31.5
	91.6	12.0	35.3	7.0	29.1
Ranathunga et al., 2010	89.9	13.8	32.6	9.7	30.8
Ranathunga et al., 2018	89.0	13.2	35.3	5.53	32.5
Reynolds et al., 2019	89.8	6.05	32.2	6.68	30.8
	89.7	10.0	31.8	2.60	32.2
Mean ± SD	***88.7 ±1.23***	***8.6 ± 3.17***	***33.4 ± 5.08***	***6.7 ±1.67***	***33.0 ± 5.02***

[1] – = Not reported.

Table 1.3 Protein characterization of various DDGS using the modified 3-step procedure or the mobile bag technique

Study	Method[1,2]	CP, % DM	CP Disappearance, % CP			RUP, % CP	dRUP[6], % RUP	TTCPD[7], % CP
			A[3]	B[4]	C[5]			
Cao et al., 2009	MS	34.3	4.3	88.6	7.19	66.1	66.5	77.9
		32.9	11.1	88.9	0	62.8	62.3	76.2
		32.0	12.8	87.2	0	60.9	65.1	78.7
		30.1	15.8	83.5	0.62	58.1	62.3	78.2
Kelzer et al., 2010	MB	26.9	17.0	66.6	4.16	33.2	92.1	97.4
		45.4	7.42	78.84	0.85	55.2	97.7	98.7
		25.9	17.9	54.27	27.9	56.3	91.9	95.4
Kleinschmit et al., 2007b	MS	31.3	5.21	71.66	23.14	71.7	59.2	70.7
		32.1	7.89	82.75	9.36	63.7	76.8	85.3
		32.8	5.63	84.09	10.27	59.1	74.2	84.9
		33.5	7.01	81.1	11.88	67.5	63.0	74.9
		30.6	9.84	82.71	7.45	60.3	68.1	80.8
Mjoun et al., 2010c	MS	30.8	18.4	75.2	6.4	52.3	92.4	96.0
		34.0	17.2	73.7	9.0	60.4	91.4	94.8
		41.5	11.1	84.7	4.2	54.5	93.5	96.5
Paz et al., 2014	MB	31.4	-[8]	-	-	23.1	89.7	97.6
Mean ± SD		***32.8 ± 4.78***	***11.2 ± 4.99***	***78.9 ± 9.37***	***8.2 ± 8.08***	***56.6 ± 12.3***	***77.9 ± 14.21***	***85.8 ± 9.74***

[1] MS = Modified three step procedure described by Gargallo et al., 2006.
[2]MB = Mobile bag technique described by Paz et al., 2014.
[3]A = Soluble CP.
[4]B = Potentially degradable CP.
[5]C = Undegradable CP.
[6] dRUP = Digestible rumen undegradable protein.
[7]TTCPD = Total tract crude protein digestibility.
[8]-= not reported

Table 1.4. Effect of supplementing rumen protected amino acids to lactating dairy cows consuming DDGS

	-RPAA[1]			+RPAA		
Study	Milk yield, kg	Protein, kg	Protein, %	Milk yield, kg	Protein, kg	Protein, %
Nichols. et al., 1998	35.3	1.07	3.02	36.7	1.13*	3.08*
Paz et al., 2013	31.0	0.99	3.22	30.7	0.99	3.20
Paz and Kononoff, 2014	25.4	0.92	3.41	25.1	0.92	3.48
Liu et al., 2000	32.6	1.05	3.23	31.7	1.02	3.26

[1]-RPAA = not supplemented with rumen protected AA, +RPAA = supplemented with rumen protected AA
*indicates treatment differences between treatments

Table 1.5. Summary of design, treatments, substitution strategies, and animal performance from research studies feeding corn co-products to lactating dairy cattle

Study	Design[1]	Treatment	Substitution[2,3]	DMI, kg	Milk, kg	Fat, %	Fat, kg	Protein, %	Protein, kg
Abdelqader et al., 2009	LS	CON	-GC, SBH, HPDDGS, RIF	23.2	34.0	3.88	1.31	3.24	1.10
		CG		24.3	35.2	3.80	1.33	3.19	1.12
		DDGS		23.7	35.8	3.59	1.30	3.21	1.14
		CO		22.3	34.7	3.50	1.20	3.15	1.08
Castillo-Lopez et al., 2014	LS	CON	-CS, AHL, AH, BH, CTS, GC, SBM, ESBM, BM +SBH, RI	25.0	34.4	3.59	1.24	3.08	1.06
		10%		23.8	33.2	3.74	1.23	3.18	1.04
		20%		25.9	34.5	3.64	1.25	3.15	1.07
		30%		27.9	34.2	3.67	1.26	3.19	1.09
Christen et al., 2010	LS	CON	-SBM, Fat,	24.1	31.7	4.21	1.33	3.33	1.04
		DDGS		23.6	32.7	3.78	1.24	3.23	1.05
		HPDDGS		24.6	31.2	4.21	1.31	3.36	1.05
Foth et al., 2015	RS	CON	-GC, SBM	21.3	29.8	4.32	1.24	3.56	1.04
		Co-P		21.4	30.9	4.34	1.28	3.41	1.02
Hubbard et al., 2009	CO	CON	-CS, AHL, GC, SBM, ESBM,	22.6	31.6	3.85	1.21	3.05	0.95
		HPDDGS		21.2	33.4	4.07	1.35	3.02	1.0
Janicek et al., 2008	LS	CON	-CS, AH, AHL, GC, CTS, ESBM	21.4	27.4	3.70	1.00	3.18	0.86
		10%		22.4	28.5	3.64	1.03	3.19	0.91
		20%		23.0	29.3	3.73	1.09	3.16	0.92
		30%		24.0	30.6	3.55	1.10	3.14	0.95
Janicek et al., 2008	CO	CON	-CS, AH, AHL, GC, CTS, ESBM	22.8	33.2	3.67	1.22	2.98	0.98
		DDGS		24.1	34.2	3.65	1.24	2.99	1.02

Kelzer et al., 2009	LS	CON	-CS, AHL, AH, GC, SBM, ESBM,	22.9	30.6	3.73	1.13	2.97	0.90
		DDGS		23.8	30.9	3.72	1.13	2.99	0.90
		HPDDGS		22.4	30.3	3.90	1.17	2.98	0.98
Kleinschmit et al., 2006	LS	CON	-GC, SBM	21.7	31.2	3.69	1.14	3.28	1.02
		DDGS1		21.2	35.0	3.60	1.26	3.13	1.09
		DDGS2		21.5	34.3	3.53	1.22	3.19	1.09
		DDGS3		21.1	34.6	3.67	1.29	3.17	1.09
Kleinschmit et al., 2007	LS	CS	N/A	21.9	26.5	3.67	0.96	3.36	0.88
		CSAH		24.9	28.4	3.55	1.02	3.33	0.94
		AH		20.9	29.0	3.49	1.01	3.33	0.96
Mjoun et al., 2010a	RCB	CON	-SBM, ESBM, SBH, RIF, DCP	24.8	39.2	3.63	1.33	2.82	1.07
		DDGS		24.7	38.9	3.24	1.34	2.88	1.15
		RFDDGS		24.6	39.8	3.57	1.40	2.89	1.14
Mjoun et al., 2010b	CR	CON	-GC, SBM, ESBM, SBH, DCP, +LS, RIF	22.7	34.5	3.18	1.08	2.99	1.03
		10%		23.0	34.8	3.40	1.19	3.06	1.07
		20%		23.7	35.5	3.46	1.23	3.13	1.10
		30%		22.2	35.2	3.72	1.32	2.99	1.06
Morris et al., 2018a	RCB	CON	-SBM, SBH, RIF, DCP	26.4	40.8	3.81	1.55	3.26	1.32
		DDGS		25.4	41.3	3.00	1.23	3.11	1.28
		DDGS+ MON		24.4	39.2	2.77	1.08	3.06	1.20
Mulrooney et al., 2009	LS	CM	-GC, CM, RIF	25.2	35.2	3.81	1.34	3.05	1.08
		2/3CM		25.4	35.8	4.05	1.45	3.06	1.10
		1/3CM		25.9	34.5	3.97	1.37	3.06	1.05
		DDGS		25.1	34.3	3.87	1.32	3.01	1.03

Paz et al., 2013	LS	CON 10% 20%	-GC, SBH, SBM, BM	25.2 25.9 25.7	30.1 30.2 31.8	3.81 3.65 3.73	1.14 1.11 1.19	3.15 3.23 3.21	0.94 0.98 1.01
Paz and Kononoff, 2014	LS	15% 30%	-GC, SBM	25.8 24.7	26.3 27.4	3.70 3.73	0.97 1.02	3.49 3.40	0.91 0.93
Ramirez-Ramirez et al., 2015	LS	CON OL STR COMBO	N/A	25.2 25.5 24.1 22.0	35.2 34.9 32.5 30.4	3.19 2.75 2.88 2.21	1.10 0.97 0.91 0.69	2.89 2.92 3.00 2.94	0.99 1.00 0.95 0.90
Ramirez-Ramirez et al., 2016a	LS	CON DDGS RFDDGS RFDDGS+ RIF	-CS, AHL, GC, AH, CTS, ESBM, SBM, BM, +SBH	21.6 25.8 26.1 26.1	32.2 33.8 33.8 34.0	3.69 3.27 3.65 3.70	1.18 1.11 1.22 1.25	3.07 3.22 3.21 3.12	1.00 1.10 1.07 1.06
Ramirez-Ramirez et al., 2016b	LS	CO0+ SHORTP CO0+ LONGP CO2+ SHORTP CO2+ LONGP	N/A	28.1 26.7 26.3 24.9	33.3[4] 35.8 25.0 28.1	3.62 3.62 2.27 3.02	1.18 1.27 0.70 0.92	NR[5] NR NR NR	NR NR NR NR
Ranathunga et al., 2010	CR	29%Starch 26% Starch 23% Starch 20% Starch	+SBH, -GC, SBM, ESBM, RIF	25.6 25.0 23.4 22.9	39.4 37.4 37.7 38.3	3.14 3.23 3.29 3.24	1.24 1.23 1.22 1.22	2.97 2.96 3.01 2.94	1.17 1.11 1.10 1.13

Ranathunga et al., 2018	LS	LF+ 0DG	-GC, ESBM, ESB	25.6	42.8	3.07	1.30	3.09	1.32
		LF+18DG		26.1	43.7	2.99	1.30	3.13	1.36
		HF+0DG		25.1	41.7	3.42	1.43	3.00	1.25
		HF+18DG		25.1	41.3	3.34	1.37	2.96	1.22
Reynolds et al., 2019	LS	CON	-GC, SBM	17.5	23.4	6.23	1.46	3.67	0.86
		RFDDGS		17.4	24.2	6.11	1.48	3.63	0.87

[1]LS = Latin square, RS = reverse switchback, CO = crossover, RCB = randomized complete block, CR = completely randomized.
[2] Ingredients that were added (+) or subtracted (-) when feeding DDGS in research studies.
[3]GC = ground corn, SBH = Soybean Hulls, HPDDGS = High-protein dried distillers grains, RIF = Rumen inert fat, CS = Corn silage, AHL = Alfalfa haylage, AH = Alfalfa hay, BH = Brome Hay, CTS = Cottonseed, SBM = Soybean meal, ESBM = Extruded soybean meal, ESB = extruded soybeans, DCP = dicalcium phosphate , LS = limestone, BM = Bloodmeal.
[4]Fat corrected milk yield.
[5]NR = Not reported

Table 1.6. Estimated total tract NDF digestibility of corn silage and alfalfa haylage

Item, %NDF unless noted otherwise	Corn silage[1]	Alfalfa haylage
NDF, % DM	42	45
dNDF[2]		
24	47	45
30	54	52
48	64	57
240	71	60
pdNDF[3]	71	60
iNDF[4]	29	40
k_d	5.1	6.4
TTNDFD[5]	51.7	47.1

[1]Nutrient composition obtained from Dairy One feed library (https://dairyone.com/services/forage-laboratory-services/feed-composition-library/interactive-feed-composition-libraries/).
[2]NDF digestion after certain amount of time (24, 30, 48, 240 h).
[3]pdNDF = potentially digestible NDF.
[4]iNDF = indigestible NDF.
[5]TTNDFD = total tract NDF digestibility according to Lopes et al., 2015.

Table 1.7. Carbohydrate fractionation and degradation rates range for all feeds defined by the Cornell Net Carbohydrate and Protein System[1]

Nutrient	Identification[2]	K_d, %/hr
Volatile fatty acids[3]	CA1	0
Lactic acid	CA2	7
Other organic acids	CA3	5
Sugars	CA4	40 – 60
Starch	CB1	20 -40
Soluble Fiber	CB2	20 – 40
Degradable NDF	CB3	1 – 18
Undegradable NDF	CC	0

[1]Adapted from Van Amburgh et al. (2015).
[2]Identification = code used to identify each feed fraction within CNCPS.
[3]Volatile fatty acid = acetic, propionic. butyric acids.

Table 1.8. The effects on predicted energy supply, protein supply, ME allowable milk, and MP allowable milk when changing the k_d of corn silage by changing 30, 120 and 240 h in vitro NDF digestibility

Item[1,2,3]	CNCPS Report	
Assumptions, kg		
DMI	26.0	
Milk yield	38.0	
Diet composition, % DM		
Corn silage	37.5	
Alfalfa silage	17.9	
Grass hay	5.4	
Concentrate mix	39.2	
Nutrient composition, % DM		
CP	17.4	
NDF	36.1	
Starch	20.6	
Type of silage used	Conventional Corn Silage	BMR Corn Silage
NDF digestibility[4]		
$NDFD_{30}$	53	67
$NDFD_{120}$	67	85
$NDFD_{240}$	72	86
Corn silage NDF k_d, %/hr[5]	4.5	5.4
Metabolizable energy, kcal/d	65.9	68.6
Metabolizable protein, g/d	2783	2864
Metabolizable energy allowable milk, kg	95.7	101.4
Metabolizable protein allowable milk, kg	88.9	95.4

[1]Assuming a cow with 26 kg DMI producing 38 kg milk.
[2]Ingredient composition, %DM = Corn silage, 37.5%; alfalfa silage, 17.9%; grass hay, 5.4%; concentrate mix, 39.2%.
[3]Nutrient composition, % DM = CP, 17.4%; NDF, 36.1%; starch 20.6% .
[4]NDF digestibility at 30, 120 and 240 h in vitro. Conventional silage dNDF from www.dairyone.com and BMR silage results obtained from Raffrenato et al., 2018.
[5]Calculated within CNCPS using 30, 120, 240 h in vitro NDFD.

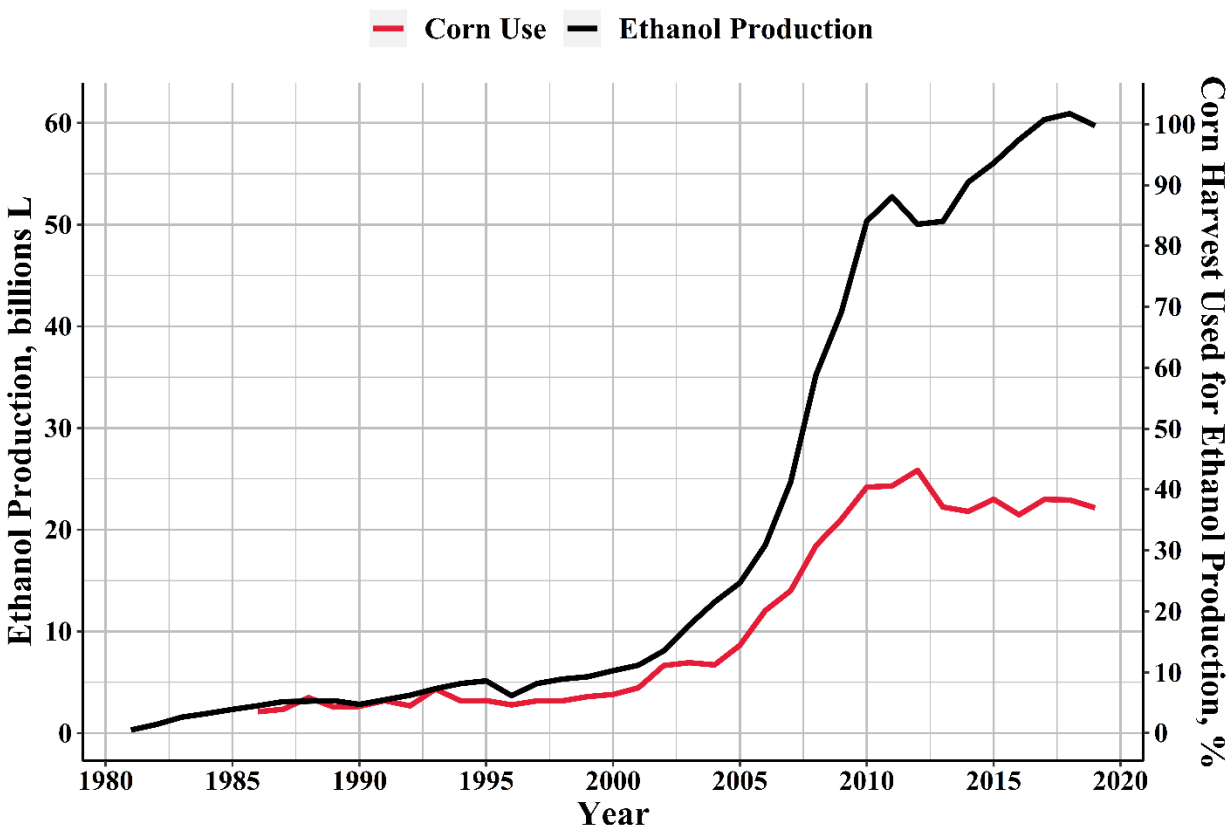

Figure 1.1. Growth in ethanol production and proportion of corn harvest used for ethanol production from 1981 to 2019. Data were obtained from the U.S. Department of Agriculture Economic Research Service and the U.S. Energy Infomration Administration

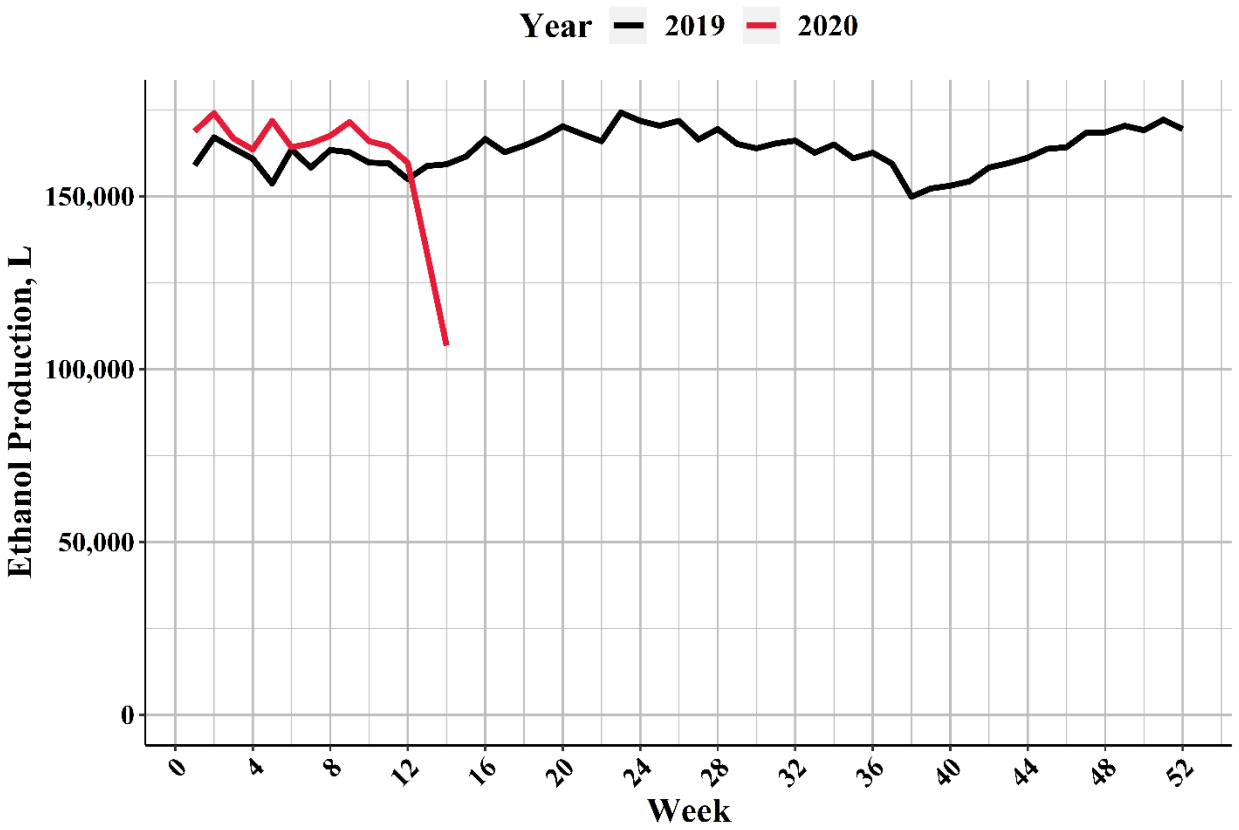

Figure 1.2. Weekly U.S. ethanol production during 2019 and 2020. COVID-19 was declared a pandemic March 11, 2020, since that day ethanol production has sharply declined as a response to decreased demand. Data were obtained from U.S. Energy Information Adminstration Weekly Oxygenate Report

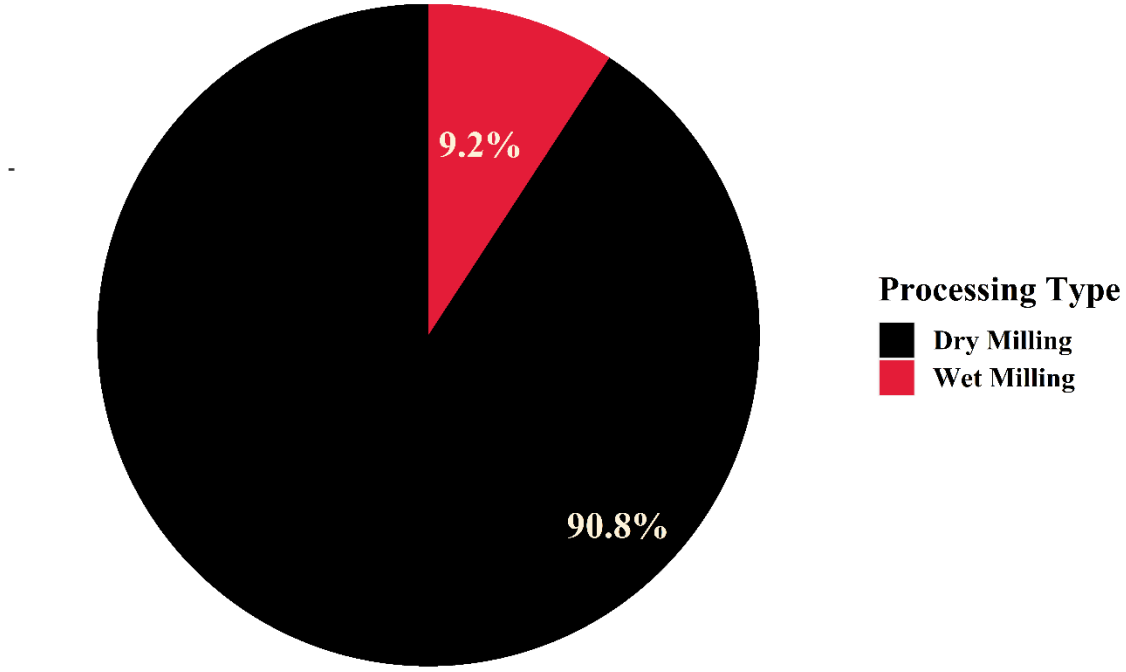

Figure 1.3. Proportion of ethanol produced by dry or wet milling processes. Data were obtained from the United States Department of Agriculture Monthly Grain Use for U.S. Ethanol Production report.

CHAPTER 2

INTERPRETIVE SUMMARY: "Use of 30 hour in vitro NDF digestibility of feedstuffs in dairy ration formulation software: evaluation of predictions for milk and methane production in lactating dairy cows." In vitro lab procedures to determine NDF digestibility are able to be used in ration formulation systems used in the dairy industry. Improving the accuracy of these ration models would allow for lower ration costs and reduced nutrient and greenhouse gas excretion, improving understanding of NDF digested in the dairy cow would help improve these models. In a study evaluating milk production predictions and observed milk production demonstrated that incorporating NDF digestibility estimates did not improve milk production estimates, but it did improve methane production estimates.

RUNNING HEAD: EVALUATION OF DAIRY RATION SOFTWARE PREDICTIONS

Use of 30 hour in vitro NDF digestibility of feedstuffs in dairy ration formulation software: evaluation of predictions for milk and methane production in lactating dairy cows

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ABSTRACT

Determining if the addition of 30-hour in vitro NDF digestibility (NDFD30) of fibrous ingredients included in rations fed to lactating dairy cattle improves the accuracy of milk and CH_4 production predictions from CNCPS (v. 6.5) will provide valuable data for field nutritionists. Animal performance from 8 energy balance studies were compiled into a database along with the treatments fed during those studies. Observed animal performance was compared to milk and CH_4 predictions from CNCPS (v. 6.5) when using CNCPS feed library k_d values and when using k_d calculated from NDFD30. The in vitro analysis was conducted according to Goering and Van Soest (1970) and aNDFom of the residue was determined according to methods outlined by Mertens (2002). Predictions of milk production were poorer with NDFD30, the CCC decreased from 0.87 to 0.82. Methane production predictions improved with NDFD30; the CCC increased from 0.33 to 0.38. These results indicate that including NDFD30 to calculate the k_d of NDF may not improve ME allowable milk production predictions but may improve CH_4 production predictions. Our results show that predictions from CNCPS (v. 6.5) are reliable but including NDFD30 may not provide additional value to ration formulation. Adding in vitro measurements of NDFD30 may improve CH_4 predictions which may prove important as environmental regulations strengthen. Next steps should evaluate including long-term fermentations (240 hours) to determine if altering the total pool of potentially digestible NDF would improve predictions from CNCPS.

Key Words: neutral detergent fiber, digestibility, model evaluation, CNCPS

INTRODUCTION

Ration formulation software is an important tool for dairy nutritionists. Ration balancing software allows for least cost diet formulations that also meet nutrient requirements of livestock. These tools are considered useful because they support precision feeding practices that may reduce nutrient excretion and lessen the environmental impact of the dairy industry (Alocilja, 1998; Cerosaletti et al., 2004). Given the widespread use of formulation software, evaluation and improvement of these models is important to advancing their predictive ability. One model used in dairy ration formulation software is the Cornell Net Carbohydrate and Protein System (CNCPS; v. 6.5; Higgs et al., 2015; Van Amburgh et al., 2015). The CNCPS platform incorporates environmental, animal, and feed inputs and these are used to predict animal performance (Lanzas et al., 2007). Research has demonstrated that CNCPS can be used to formulate diets that reduce nutrient loss and maintain projected income over feed cost (IOFC; Wang et al., 2000).

The digestibility of NDF (NDFD) is important to lactating dairy cows because it is an important source of energy; digested NDF (dNDF) is assumed to have an energy density of 4.11 Mcal/kg (NRC, 2001). It has been estimated that a one unit increase in NDFD results in a 0.17 kg increase in DMI and a 0.25 kg increase in energy-corrected milk (ECM; Oba and Allen, 1999). The CNCPS model is equipped with a calculator in which the user can input an in vitro NDFD estimate which is used to predict the rate of NDF digestion of that feed ingredient. Rate of NDF digestion is denoted as CB3 k_d within CNCPS. Since rate and extent of NDF digestion varies and is dependent on factors such

as plant hybrid and agronomic conditions, it is possible that conducting in vitro assays to estimate NDFD at 30 hours (NDFD30) to estimate CB3 k_d may improve the model's prediction accuracy (Higgs et al., 2015). The objectives of this work were to evaluate if inclusion of NDFD30 improved milk production and CH_4 predictions. The CO_2 production and DMI predictions were are also evaluated.

MATERIALS AND METHODS

Energy Balance Studies

The experimental observations used in our study were compiled from 8 past energy balance experiments conducted at the University of Nebraska–Lincoln (Foth et al., 2015; Drehmel et al., 2018; Judy et al., 2018ab; Judy et al., 2019ab; Knoell et al., 2019; Reynolds et al., 2019). This database contained 32 treatment means, 6 from Holstein cows and 26 from Jersey cows. During these energy balance experiments milk production, DMI, total fecal output, total urine output, CO_2 production, CH_4 production, and O_2 consumption were measured directly. All gas measurements were obtained through indirect calorimetry which is described in greater depth by Foth et al. (2015). Total fecal and urine output were collected over 4 consecutive days. Gross energy of the treatments and ingredients was obtained via bomb calorimetry (Parr 6400 Calorimeter, Moline, IL). The gas measurements, total fecal collection, and total urine collection were used to calculate the digestible energy (DE), metabolizable energy (ME), and net energy (NE) of each treatment. More detailed descriptions of the energy calculations are described by Judy et al., (2019b) and Reynolds et al., (2019).

Feed Sample and Analysis

In the current study, we evaluated 34 composited feed samples. The samples were originally collected by period or by period and block, and composites were made using equal parts from each of the original feed samples. In all, 8 corn silage, 8 alfalfa hay, 6 brome grass hay, 1 wheat straw, 3 dried distillers' grains with solubles (DDGS), 3 reduced-fat DDGS (RFDDGS), 3 soybean hulls, 1 canola meal, and 1 beet pulp sample were evaluated. All the samples were dried for 48 hours in a 60°C forced air oven and ground to pass through a 1 mm screen using a Wiley Mill (Arthur A. Thomas CO., Philadelphia, PA). The 34 feed samples were sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) and analyzed for CP (method 990.03; AOAC International, 2000), SP (Krishnamoorthy et al., 1982), ADICP, NDICP, aNDFom (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2000), lignin (Goering and Van Soest, 1970) starch (Hall, 2009), sugar (Dubois et al., 1956), fat (2003.05l AOAC International, 2000), ash (943.05; AOAC International, 2000) , and minerals (985.01; AOAC International, 2000).

In Vitro Fermentation

The feed samples were fermented in vitro for 30 hours using methods outlined by Goering and Van Soest (1970). Rumen fluid was harvested and filtered through 4 layers of cheesecloth from 2 ruminally cannulated, dry, Holstein cows weighing 646 ± 54.5 kg. The cows were fed a diet composed of 61 % corn silage, 23% grass hay, and 16 % concentrate on a DM basis. The rumen fluid was transported to the laboratory in a prewarmed thermos and then transferred to separatory funnels and held in a water bath at 39ºC until inoculation of the feed samples. McDougall's Buffer (McDougall, 1948),

containing 1 g/L of urea, was reduced and held in a 39ºC water bath. Samples were fermented in triplicate and in three independent in vitro runs. A 0.3 g sample of each feed was placed into a 30 ml polypropylene tube and 1 ml of ddH_2O was added to each tube to minimize feed loss when adding the inoculum. Tubes were then randomly placed into the 39ºC water bath. Once the rumen fluid had separated and the temperature of the buffer reached 39ºC, they were mixed in a 5-gallon bucket; the solution was 80% buffer and 20% rumen fluid. 30 ml of solution was added to each tube using an automatic pipetting system (Unispense, Wheaton Instruments, Millville, NJ). This mixture was reduced with CO_2 throughout the duration of dispensing inoculum into each tube. To maintain anaerobic conditions, each tube was purged with CO_2 and capped with a rubber stopper. The rubber stoppers were equipped with a small hole to allow gas to escape during fermentation. The system was under positive pressure, so the production of gas from fermentation forces gas out of the tube and prevents O_2 from entering the tube. During fermentation, tubes were manually agitated at 0, 4, 19, and 27 h of incubation. After completion of the fermentation, each test tube was immediately placed into a freezer and held at -20ºC for later analysis. Residues were then thawed at room temperature and analyzed for aNDFom according to Mertens (2002) with an adjustment by using Whatman 934-AH glass filter paper with 1.5 µm pore size (Whatman Limited, GE Healthcare, Maidstone, UK) because of its ability to work efficiently under vacuum and to retain small particles (Raffrenato et al., 2018).

Data Collection

To conduct comparisons of animal performance and ration model predictions, we used the Cornell Net Carbohydrate and Protein System (CNCPS; v 6.5; Van Amburgh et

al., 2015). Each experiment represented an individual farm within CNCPS and each treatment within each experiment was created as a separate group of cattle. The DIM, milk production, milk fat percent, milk protein percent, BCS, breed, and BW from each treatment group were entered into CNCPS. All other inputs not collected were left at the default settings of the software.

All ration information were obtained from the published literature of the respective experiments (Foth et al., 2015; Drehmel et al., 2018; Judy et al., 2018ab; Judy et al., 2019ab; Knoell et al., 2019; Reynolds et al., 2019). Chemical analysis of the forages and fibrous byproducts from Cumberland Valley Analytical Services (CVAS; Hagerstown, MD) were entered into the feed library for each ingredient that was analyzed, any remaining items not characterized by CVAS were left as the default feed library values (Higgs et al., 2015). Then each ingredient was copied to create a second version where the measured NDFD30 was used to estimate CB3 k_d for each ingredient. This created two sets of ingredients, a control (**CON**) group that used the feed library CB3 k_d values, and a second group (**DIG**) that used the NDFD30 values to calculate the CB3 k_d of each ingredient. The predictions that were generated from the CON or DIG ingredients in CNCPS were compared to observations from animal studies to determine if including NDFD30 to calculate CB3 k_d improved the predictions of animal performance.

Rations from each study were then inputted at the observed DMI from each treatment group within each study. Once the ration was inputted into CNCPS using CON ingredients, we recorded predicted ME and MP allowable milk, CH_4 production, CO_2 production, and predicted DMI. Then, using DIG ingredients we recorded the same outputs from CNCPS. This resulted in two sets of predictions for each experimental

treatment mean (n=32). These predictions that were collected were compared to the associated observed animal performance to measure model fit and bias.

Statistical Analysis

Comparison statistics were generated using R (v 3.5.2) and predictions were evaluated based upon the root mean square error (**RMSE**) which was then decomposed into mean and slope biases. A concordance correlation coefficient (**CCC**; Lawrence and Lin, 1989) was also used to evaluate agreement between predicted and observed animal performance.

RESULTS AND DISCUSSION

Animal Characteristics and Production

Our database consisted of cows in various stages of lactation, body condition, and production cycle (Table 2.1). The mean DIM of the cattle was 176 ± 58.1 and ranged between 118 and 372 DIM. Body weight (BW) of the cattle was 485 ± 73.9 kg and ranged between 426 kg and 679 kg. Holsteins (n=60) included in the data base weighed 629 ± 51.0 kg, Jerseys (n=261) weighed 467 ± 46.4 kg. In experiments where both Holsteins and Jersey cattle were fed (Foth et al., 2015, Judy et al., 2019b), they were inputted separately within CNCPS. This data set covers a wide range of production, with the minimum being 16.8 kg of milk and the max being 38.4 kg. Holsteins produced 33.3 ± 3.44 kg of milk with 3.75 ± 0.122 % fat and 2.94 ± 0.211 % protein and Jerseys produced 25.0 ± 5.25 kg of milk with 5.45 ± 0.672 % fat and 3.53 ± 0.327 % protein. Mean CH_4 production was observed to be 15.8 ± 2.65 L/kg of milk and ranged from11.7

L/kg of milk to 22.2 L/kg of milk. Carbon dioxide production averaged 193.3 ± 31.12 L/kg of milk and ranged from 153.0 L/kg of milk to 280.0 L/kg of milk.

Nutrient Composition of Treatments

A summary of the treatment diets fed during each of the energy balance studies are listed in Table 2.2. Reported energy values were determined experimentally. When the diets were entered into CNCPS, MP allowable milk was greater than ME allowable milk for each experimental treatment. This demonstrated that, according to the model, metabolizable protein was in greater excess which is why fit statistics were conducted using ME allowable milk.

Model Evaluation

Carbohydrates (CHO) are the most abundant nutrient fraction fed to cattle and the CNCPS model partitions these along with products of CHO metabolism found in feeds into 8 fractions (Lanzas et al., 2007). Briefly these fractions are as follows; 1) volatile fatty acids (VFA; CA1), 2) lactic acid (CA2), 3) other organic acids (CA3), 4) sugars (CA4), 5) starch (CB1), 6) soluble fiber (CB2), 7) dNDF (CB3), and 8) indigestible NDF (iNDF; CC; Lanzas et al., 2007). Although CA1, CA2, and CA3 are not carbohydrates, they are considered a carbohydrate fraction because they are more closely related to carbohydrates than either fat or protein (Lanzas et al., 2007). In version 6.5 of CNCPS, the rate at which NDF is digested in the rumen can be determined from 30, 120 and 240-h in vitro fermentations. Users may input one or all these time points into the software which in turn computes the CB3 k_d in the rumen and ultimately total dNDF (Raffrenato et al., 2019; Van Amburgh et al., 2015). This is important because model predictions of ME

allowable milk and rumen microbial N flow are sensitive to the k_d of CB3 (Fox et al., 2004; Lanzas et al., 2007). As stated previously, 34 feed ingredients were evaluated for NDFD30 for use in CNCPS, and the observations are listed by ingredient type in Table 2.3. In evaluating NDFD we did not alter the iNDF estimate of feeds, which is used to compute the amount of potentially digestible NDF (pdNDF) that is available for rumen fermentation (Palmonari et al., 2017, Raffrenato et al., 2018). Since we did not estimate iNDF of the ingredients, we did not alter the pool size of pdNDF. We did however measure NDFD30 and this can be used to estimate k_d of CB3 which is used to account for energy contributions from NDF by altering the extent and site of digestion. For example, assume a cow is eating 26 kg of DM of a diet containing 35% DM as corn silage. Increasing the k_d of corn silage, which was assumed to have 40 % NDF, from 3 to 6 %/hr increased total dNDF by 20%. Increasing dNDF by 20% would increase the TDN and ME predicted from CNCPS (Sniffen et al., 1992; Fox et al., 2004).

Determination of NDFD30 to calculate the k_d CB3 of the ingredients affected the predictions of animal performance from CNCPS. Results from the comparison of experimental observations and CNCPS predictions using CON and DIG ingredients are listed in Table 2.4. The CON ingredients used CNCPS feed library values for the k_d of CB3 while DIG used k_d calculated from the measured NDFD30, which is listed in Table 2.3. Predictions of milk and CH_4 production decreased by 6% and 3% with DIG ingredients. This response is a result of a lower CB3 k_d that was calculated from NDFD30 when compared to the default CB3 k_d of the CNCPS feed library.

When evaluating the predictions of milk production, we chose to use ME allowable milk because in comparison to MP, it was predicted to be in less excess in each

observation. According to CNCPS, cows consumed 101% of the energy required for milk production while they received 127% of their MP requirements. Across all treatments milk production averaged 25.8 ± 6.00 kg; The prediction of the CON was 26.1 ± 5.12 kg and the prediction of DIG was 24.5± 4.77 kg. Compared to CON, DIG had a poorer mean bias (-0.23 versus 1.30 kg for CON and DIG respectively); slope bias was also poorer (0.04 versus 0.09 for CON and DIG respectively). The CCC, a measure of accuracy and precision (Tedeschi, 2004), was 0.87 and 0.82 for CON and DIG, respectively, indicating that DIG did not improve predictions of ME allowable milk production. This observation is supported by the RMSE, which was lower for CON (10.7% vs 12.5%). These measures suggest that CNCPS is effective in predicting milk production but the addition of NDFD30 did not improve model predictions of milk production. Further evaluation when including a measure of iNDF may be warranted because that would alter the total pdNDF pool available for ruminal and hindgut fermentation.

The CNCPS model employs an equation extracted by Mills et al. (2003) to predict CH_4 production. The equation is as follows:

Equation 2.1. $CH_4,\ MJ\ /\ d = 45.98 - (45.98e^{(-1\times\left(-0.0011\times\frac{starch}{ADF}\right)+0.0045\times ME\ Intake])})$

where starch and ADF are the kg consumed per day and ME intake is measured in MJ consumed per day. Digestibility of NDF is indirectly accounted for in the CH_4 equation by including ME intake. Reducing the CB3 k_d reduces the ME intake which ultimately reduces CH_4 production predictions. The observed mean for CH_4 production was 15.8 ± 2.65 L/kg of milk, the prediction of CON was 20.2 ± 3.04 L/kg, and DIG was 19.6 ± 2.95 L/kg of milk. Figure 2.1 illustrates that production of CH_4 was over-predicted in all

experimental treatments of these 8 studies. The mean bias was observed to be -4.38 L/kg and -3.76 L/kg for CON and DIG, respectively. Using the DIG ingredients also reduced the slope bias. The CCC was 0.33 and 0.38 for CON and DIG, respectively. Taken together, these results suggest that that DIG improved the CH_4 predictions compared to using CON ingredients. This suggestion is also supported by decreased RMSE when using DIG (27.0% vs. 30.7% for DIG and CON respectively).

The Mills et al. (2003) equation of CH_4 production requires a small number of inputs, but a recent evaluation of CH_4 prediction equations demonstrated that this equation does not perform as well as others that have been developed (Appuhamy et al., 2016). These investigators compared predictions of CH_4 to a database of 55 treatment means from North America. Appuhamy et al. (2016) presented alternative equations that could, in theory, be used within CNCPS. Specifically in that evaluation, subsequent rankings of models to predict CH_4 using a database of North American cattle resulted in a the Mills et al. (2003) equation being ranked outside the top 10 but the specific fit statistics were not reported. Nielsen et al. (2013) derived an equation with a CCC of 0.72; with a slight adjustment the CCC increased to 0.78 (Appuhamy et al., 2016). The adjusted equation from Nielsen et al. (2013) was the top ranked model to predict CH_4 for cattle in North America (Appuhamy et al., 2016) and is a good candidate because it requires few inputs and CNCPS would be able to provide the needed inputs to predict CH_4. Their adjusted equation (Nielsen et al., 2013, Appuhamy et al., 2016) requires DMI, fatty acids (FA), and total tract NDF digestibility (TTNDFD) and is as follows:

Equation 2.2. $$CH_4\ ,\ g\ /cow/day = [1.23\ \times DMI - 1.45\ \times FA + 0.171 \times TTNDFD]/0.05565$$

Where DMI is kg/d, FA is dietary fatty acid concentration as a % of DM, and TTNDFD is as a % of DM. Practically, the above equation may be more suitable for estimating CH_4 production in field conditions because DMI is believed to have the largest influence on CH_4 production, and this equation includes DMI as a parameter while the Mills et al. (2003) equation does not (Knapp et al., 2014). Additionally, a measure of TTNDFD is a measure of the amount digestible carbohydrate leading to the production of CO_2 and H_2 which are used to synthesize CH_4 in the rumen and hindgut. Using TTNDFD may be considered a limitation because in the field TTNDFD may not be readily available. Appuhamy et al. (2016) overcame this challenge by using the TTNDFD equation from the NRC (2001). The inclusion of FA is likely due fat's effect on the rumen environment (Nagaraja et al., 1997) but research has demonstrated that fat, through biohydrogenation, does not significantly reduce CH_4 production in lactating dairy cattle (Jenkins et al., 2008; Judy et al., 2019). Surprisingly, when evaluated against our database the Nielsen et al. (2013) equation performed poorly (CCC of 0.12 and the mean and slope bias of -5.44 and -0.70, respectively). The discrepancy in model performance of the Nielsen et al. (2013) equation may be a result of the database used to construct the equation. The current database has a mean CH_4 intensity of 15.8 ± 2.65 L/kg of milk while Nielsen et al. (2013) had a mean observed value of 21.2 ± 3.08 L/kg of milk. Total CH_4 production was 33% lower for our database than the Nielsen et al. (2013) data. The current database also had 8% greater DMI and 5% greater MY then data reported by Nielsen et al. (2013). These results indicate that the Mills et al. (2003) equation led to better predictions of CH_4 production and may be the most suitable for use in CNCPS.

Predictions of CO_2 were also evaluated but they are not affected by altering NDFD30 because the equation to predict CO_2 does not include NDFD30 or measures impacted by the input of it. The equation to predict CO_2 in CNCPS is from Casper and Mertens (2010) and is as follows:

Equation 2.3. $CO_2,\ g\ /\ d = 821.3 + 126.0\ \times DMI\ (kg\ /\ d) - 1.18\ \times milk\ (kg\ /\ d)$

This equation was selected because it was simple to implement and milk yield was a factor which allowed for greater range in CO_2 predictions (Van Amburgh et al., 2015). The CCC of the CO_2 equation was 0.48. Similar to the CH_4 predictions, this equation over predicted CO_2 production for each treatment mean. The mean bias accounted for 76% of the RMSE (Table 2.4). Our database had a mean CO_2 production of 193.3 ± 32.12 L/kg of milk produced, while the equation predicted 227.9 ± 30.27 L/kg of milk.

CONCLUSIONS

In summary, incorporating NDFD30 into CNCPS did not improve the prediction of milk production and did improve the prediction of CH_4. Additionally, inclusion of NDFD30 did not appear to improve the predictions of animal performance and may not be required to characterize feed ingredients for accurate ration formulation. The next step that should be done in evaluating the effect of NDFD on CNCPS predictions is the use of long-term fermentation times such as 240 hours to estimate iNDF. Adjusting the iNDF of feeds affects the estimate of substrate available for fermentation in the rumen and hindgut and may enhance the accuracy of the predictions by more accurately predicting the amount of energy supplied by the diet.

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TABLES AND FIGURES

Table 2.1. Summary statistics of cattle and their performance during energy balance studies conducted at the University of Nebraska-Lincoln

Item	n[1]	Mean	SD	Minimum	Maximum
DIM	32	176	58.1	119	373
BW[2], kg	32	485	73.9	426	697
BCS[3]	32	3.30	0.19	2.98	3.78
DMI, kg	32	18.8	2.26	15.0	25.1
Milk, kg	32	25.8	6.00	16.8	38.4
Milk fat, %	32	5.28	0.825	3.57	6.23
Milk protein, %	32	3.48	0.349	2.78	4.09
CH_4[4], L	32	413	64.2	335	566
CH_4, L/kg of milk	32	15.8	2.65	11.7	22.2
CO_2[4], L	32	5050	867.2	3938	7173
CO_2, L/kg of milk	32	193	31.1	153	280

[1]Number of treatment means.
[2]BW = Body weight.
[3]BCS = 1 to 5 scale according to Wildman et al. (1982).
[4]CH_4 and CO_2 production measured using headbox style indirect calorimeters.

Table 2.2. Summary statistics of nutrient composition of treatment diets fed during energy balance studies at the University of Nebraska-Lincoln

Item[1]	n[2]	Mean	SD	Minimum	Maximum
DM	25	62.4	6.77	53.9	76.3
CP	25	17.7	0.63	16.8	18.8
ADF	25	20.8	1.89	16.6	23.5
NDF	25	32.2	2.65	25.6	37.1
Starch	25	24.4	2.82	18.9	28.7
Crude fat	25	4.20	0.703	2.60	5.63
Ash	25	7.76	0.381	6.88	8.41
ME[3], Mcal/kg of DM	23	2.55	0.153	2.27	2.78

[1]Expressed as a % DM unless denoted otherwise.
[2]Number of experimental diets.
[3]ME= Metabolizable energy determined experimentally (Foth et al., 2015; Judy et al., 2019a,b).

Table 2.3. 30 hour in vitro NDFD and calculated rate of digestion of forages and fibrous co-products included in treatments during energy balance studies

Feed	n[1]	NDFD[2], %NDF	SD	CB3 k_d, %/hr[3]	CB3 k_d, %/hr[4]
Alfalfa hay	8	34.3	4.43	3.52	6.50
Corn Silage	8	34.4	4.88	2.16	3.80
Brome Hay	5	41.3	7.15	2.88	4.50
DDGS[3]	3	27.7	9.07	1.57	5.00
RFDDGS[4]	3	41.1	8.02	2.54	5.00
Canola Meal	1	44.3		3.00	4.00
Soybean Hulls	3	53.7	2.58	2.74	8.00
Beet Pulp	1	62.9		4.90	8.00
Wheat Straw	1	26.9		1.67	3.00

[1]Number of samples evaluated.
[2]NDFD = % digested NDF after 30 hours in vitro.
[3]DDGS = dried distillers' grains with solubles.
[4]RFDDGS = Reduced-fat dried distillers' grains with solubles.

Table 2.4. Fit statistics of CNCPS predictions vs. observed performance from energy balance studies conducted at the University of Nebraska-Lincoln[1,2]

	ME allowable milk, kg		CH_4, L/kg of milk		DMI, kg			CO_2, L/kg of milk
Item	CON	DIG	CON	DIG	Lower Bound[3]	Upper Bound[4]	Mean[5]	
Observed mean	25.8	25.8	15.8	15.8	18.8	18.8	18.8	193.3
Predicted mean	26.1	24.5	20.2	19.6	18.5	21.4	20.0	227.9
RMSE	2.77	3.23	4.84	4.26	1.08	2.96	1.65	39.7
RMSE[6], % mean	10.7	12.5	30.7	27.0	5.78	15.9	8.79	20.6
Mean bias, % RMSE	0.71	16.2	81.7	77.9	5.57	80.5	53.0	76.0
Slope bias, % RMSE	0.44	1.77	4.93	5.20	0.48	1.64	1.12	1.92
Mean bias	-0.23	1.30	-4.38	-3.73	0.26	-2.66	-1.20	-34.6
Slope bias	0.04	0.09	-0.36	-0.33	-0.04	-0.17	-0.08	-0.18
CCC[7]	0.87	0.82	0.33	0.38	0.87	0.48	0.75	0.48

[1]CON = predictions from ingredients using feed library CB3 K_d, %/hr, DIG = predictions from ingredients using NDFD30 to estimate CB3 K_d, %/hr

[2]Fit statistics done using R (v. 3.5.2).

[3]Lower bound of DMI range given by CNCPS (v. 6.5).

[4]Upper bound of DMI range given by CNCPS (v. 6.5).

[5]Mean= mean of lower and upper bound of DMI given by CNCPS (v. 6.5).

[6]RMSE = residual mean square error.

[7]CCC = concordance correlation coefficient.

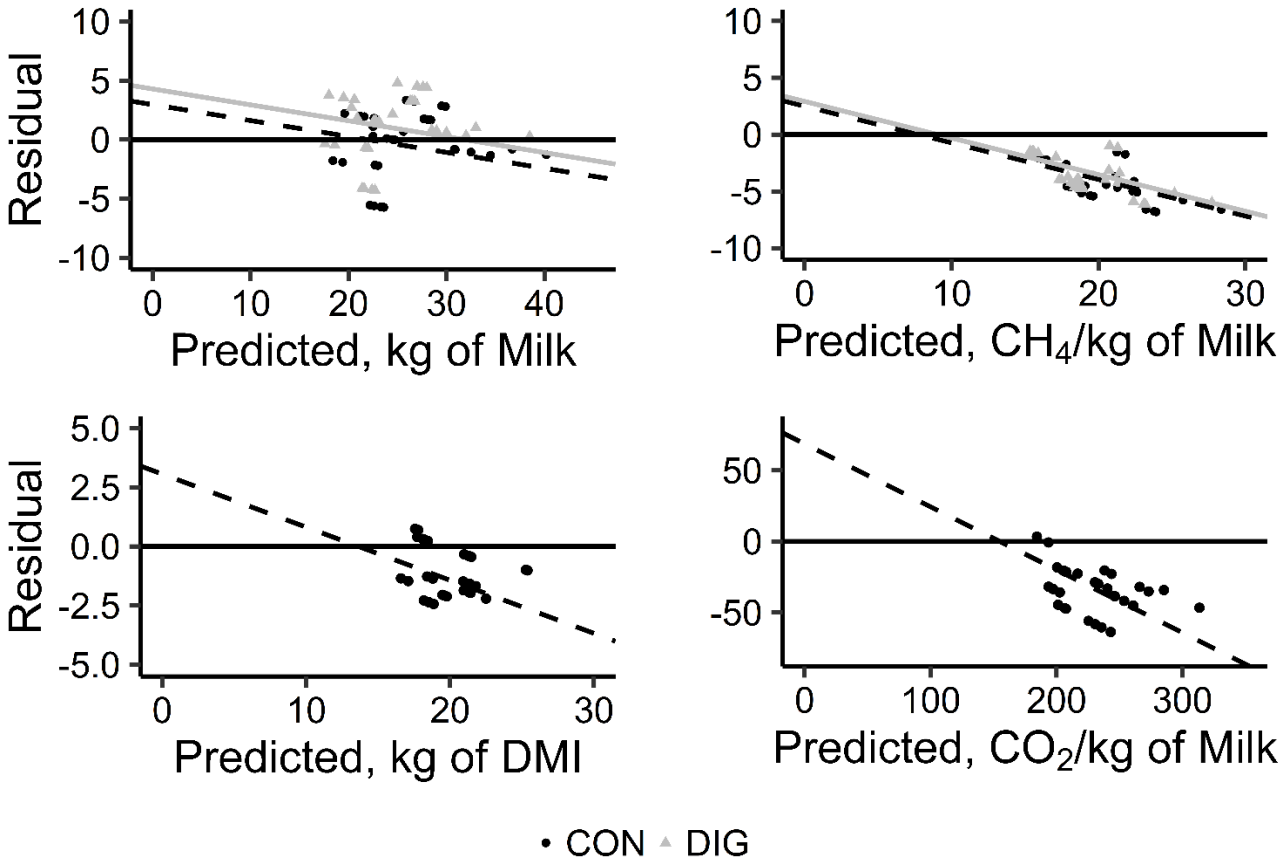

Figure 2.1. Plot of residual vs. predicted values of milk yield, CH_4, CO_2, and DMI obtained from CNCPS (v. 6.5) using CON ingredients, which predicted CB3 K_d, %/hr from default feed library inputs, or DIG ingredients, which predicted CB3 K_d, %/hr using NDFD30 from an in vitro lab assay. All slope and intercepts were different from 0, but treatment only affected the y-intercept of milk yield ($P < 0.01$).

CHAPTER 3

INTERPRETATIVE SUMMARY: Krogstad and Kononoff (20XX). "The effects of pelleted dried distillers grains and solubles (DDGS) fed in a ration containing different concentrations of forage on milk production, nutrient digestibility, rumen passage rate, rumen fermentation, and chewing behavior of lactating dairy cows," Pelleting feed may affect its feeding value. Pelleted or meal DDGS were fed with low or high forage concentrations to seven lactating Jersey cows that were fitted with rumen cannulae. Pelleting DDGS had no effect on milk yield, composition, or rumen characteristics. Pelleting DDGS increased energy and NDF digestibility, it also increased the cows' preference for particles > 8 mm and reduced preference for particles < 8 mm. Pelleting may improve the feeding value of DDGS by improving digestibility of the diet.

RUNNING HEAD: EFFECT OF PELLETING AND FORAGE CONCENTRATION

The effects of pelleted dried distillers grains and solubles fed with different forage concentrations on milk production, nutrient digestibility, passage rate, rumen characteristics, and chewing behavior of lactating dairy cows

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ABSTRACT

Physical form of feeds can influence animal chewing behavior, rumen characteristics, and rumen passage rate. Changing particle size is usually done through grinding or chopping forages, but pelleting feed ingredients also changes particle size. Our objective was to determine if pelleting DDGS affected its feeding value for lactating dairy cattle. Seven lactating Jersey cows that were each fitted with a rumen cannula averaging 56 ± 10.3 DIM and 462 ± 75.3 kg of BW were used in a cross over design. The treatments contained 15% DDGS in either meal or pelleted form with 45% or 55% forage on a DM basis. The factorial treatment arrangement was meal DDGS and low forage (**mDDGS-LF**), pelleted DDGS and low forage (**pDDGS-LF**), meal DDGS and high forage (**mDDGS-HF**), and pelleted DDGS and high forage (**pDDGS-HF**). Dry matter intake and energy corrected milk were both unaffected by treatment averaging 19.8 ± 2.10 kg and 33.9 ± 1.02 kg. Fat yield was unaffected averaging 1.7 ± 0.13 kg, but protein yield was affected by the interaction of forage and DDGS. Protein yield was similar for LF treatments (1.08 kg and 1.05 kg) but was 0.99 and 1.05 kg for mDDGS-HF and pDDGS-HF respectively (P = 0.081). Starch digestibility increased by 1.9 units, CP digestibility increased 1.1 units and residual organic matter digestibility decreased 3.4 units when forage concentration was increased. Pelleting the DDGS increased aNDFom and energy digestibility by 1.4 and 1.6 units, respectively. Passage rate slowed from 2.84 ± 0.205 to 2.65 ± 0.205 when feeding HF compared to LF. Rumination time was increased from 417 min to 454 min due to increasing forage concentration but was

unaffected by the form of DDGS. Eating time increased with pDDGS (P = 0.11) and total chewing time increased for HF (P = 0.15). Rumen pH and ammonia increased due to increasing forage concentration; pH increased from 5.86 to 5.92 (P = 0.04) and rumen ammonia increased from 16.8 to 19.1 mg/dL. Pelleting DDGS affected sorting by increasing preference for particles retained on the 8 mm sieve and decreased preference for particles on the 1.18 mm sieve and in the pan. Outcomes confirm that increasing forage concentration increases rumen pH, rumination time, and slows passage rate but contrary to our hypothesis it did not increase NDF digestibility. Results also suggest that pelleting DDGS do not appear to affect milk production, rumen characteristics, or passage rate, but pelleting DDGS may increase sorting behavior of lactating Jersey cows. Pelleting DDGS may add value to DDGS when feeding dairy cattle because it improved NDF and energy digestibility.

Key words: distillers grains and solubles, pellet, digestibility, passage rate

INTRODUCTION

Ethanol is an important source of fuel within the United States, even more so with increased ethanol blending allowances (Mills, 2019). Ethanol production continues to provide dried distillers grains with solubles (DDGS) which is used as livestock feed. Although DDGS has been studied in depth (Foth et al., 2015, Ranathunga et al., 2019), the ethanol industry continues to employ novel processing methods that may affect the feeding value (Singh et al., 2005). Recently, researchers developed a pelleting process for DDGS that does not require the addition of either binders or other feeds (Yoder et al., 2019). Pelleting feed improves feed handling, reduces feed waste, and increases feed

density. Because the physical form of a feed may affect sorting behavior (Leonardi and Armentano, 2003), chewing activity (Kononoff and Heinrichs, 2003a), and DMI (Bonfante et al., 2016) when fed to dairy cattle this new form of DDGS should be investigated.

The concentration of forage contained in a TMR is an important factor affecting digestion and overall metabolism in dairy cattle. Increasing forage concentration has been shown to increase rumen pH (Yang and Beauchemin, 2007a). Also, increasing forage concentration is often thought to reduce passage rate and if DMI is unchanged or reduced this in turn, may enhance digestibility, especially of NDF. Consequently, we wanted to investigate if there was an interaction between forage concentration and form of DDGS when feeding dairy cattle. Increasing NDF digestibility is of particular importance because it increases the conversion of human inedible nutrients to human edible nutrients (Karlsson et al., 2018).

Forage particle size and its effects on rumination behavior have been investigated with a variety of forages (Kononoff and Heinrichs, 2003b, Beauchemin and Yang, 2005) but few studies have evaluated the impacts of changing the particle size of concentrates such as DDGS. Additionally, increasing forage particle size has increased sorting behavior in dairy cows (Kononoff et al., 2003b) but we are unaware of any studies that evaluate how sorting is affected when pelleting DDGS. The current experiment will provide insights into the effects of pelleting on sorting and rumination behavior of lactating cows.

The objectives of this study were to first determine if pelleting DDGS affects digestibility, rumen fermentation, and lactation performance, second if pelleting DDGS

interacts with forage concentration, and third to determine if feeding pellets or increasing forage concentration reduces rumen passage rate and positively affects digestibility of NDF. We hypothesized that pelleting DDGS would have no effect on digestibility, rumen characteristics or milk production while increasing forage concentration would reduce passage rate, increase NDF digestibility, and increase milk production.

MATERIALS AND METHODS

Cows, Experimental Design and Diets

Experimental cows were cared for according to protocols approved by the University of Nebraska Institutional Animal Care and Use Committee. Cows were housed in individual tie stalls, milked at 0700 and 1800 h, and had access to an exercise area for 2 h after each milking. Cows were fed once per day for ad libitum consumption and 10% refusals which were collected, weighed, and recorded daily. Seven rumen cannulated Jersey cows averaging 56 ± 10.3 DIM and 462 ± 75.3 kg of BW were used in a cross-over design composed of a 2 × 2 factorial treatment arrangement of treatments. Treatments for period 1 were randomly assigned using the random number generator in Excel (Microsoft, Redmond, WA) and treatments for the remaining periods were assigned such that each cow received a unique treatment sequence. The treatments were developed to test the effects of forage concentration and form of DDGS on digestibility, rumen passage rate, milk production, and rumen fermentation. Over four 28 d periods cows were offered 1 of 4 TMR, which are listed in Table 3.1. The first 21 d of each period were assumed to be a phase of adaptation while the final 7 d were for used for sample and data collection.

Each diet included 15% DDGS (DM basis) in either a meal or pelleted form (**mDDGS**, **pDDGS**) and 45% or 55% forage (DM basis); the resulting treatments were mDDGS (Dakota Gold, POET, Mitchell, SD) with low forage concentration (mDDGS-LF), pDDGS (Dakota Gold Pro-Pellet, POET, Mitchell, SD) with low forage concentration (pDDGS-LF), mDDGS with high forage concentration (mDDGS-HF), and pDDGS with high forage concentrations (pDDGS-HF). The mDDGS and pDDGS used throughout the study were from the same production batch at the biorefinery. Forage concentration was increased by increasing corn silage and alfalfa hay concentrations while also including wheat straw at 2% of the diet DM. While increasing forage, soybean hulls and beet pulp were reduced. The diets were balanced to provide similar amounts of CP, NDF, starch, and fat using commercially available but courtesy licensed software (AMTS, version 4.10.4.1, Agricultural Modeling and Training Systems, LLC, Groton, NY).

Data Collection and Sample Analysis

Samples of forages, mDDGS, pDDGS, and grain mixes were collected on d 25, 26, 27, and 28 and composited by period for nutrient analysis. They were sent to Cumberland Valley Analytical Systems (CVAS; Waynesboro, PA) for analysis of DM (AOAC International, 2000) CP (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MO), NDF (Van Soest et al., 1991), aNDFom (Mertens, 2002), in vitro NDF digestibility (Goering and Van Soest, 1970), ADF (method 973.18; AOAC International, 2000), starch (Hall, 2009), sugar (Dubois et al., 1956), crude fat (2003.05; AOAC International, 2006), fatty acids (Sukhija and Palmquist, 1988) , and minerals (2003.05; AOAC International, 2006). The original TMR was analyzed for particle size using the

Penn State Particle Separator (PSPS) according to Kononoff et al. (2003a). Refusals were collected on the last 4 days of the period and composited by weight and period. They were sent to CVAS for analysis of DM, CP, NDF, aNDFom, starch, crude fat and total fatty acids. Refusals were also evaluated using the PSPS to evaluate sorting behavior (Leonardi and Armentano, 2003).

Total fecal collections were conducted during the last 4 d of each period. A 137 × 76 cm rubber mat (Snake River Supply, Idaho Falls, ID) was placed behind the cow to collect feces. The feces were deposited multiple times a day from the rubber mats into a large plastic container (Rubbermaid, Wooster, OH) with a black garbage bag covering the top to reduce any nitrogen losses. The feces were then subsampled each day and composited by period. Feces were sent to CVAS for analysis of DM, CP, NDF, aNDFom, starch, crude fat and total fatty acids. Rumen evacuations were conducted 2 hours pre-feeding on d 27 and 2 hours post-feeding on d 28. A subsample of 5% of the total rumen weight was collected each day and composited for analysis. A subsample was sent to CVAS for analysis of DM, NDF, and aNDFom. Rumen fluid samples were collected into conical vials every two hours on d 21 (0, 2, 4, 6, 8 , 10, 12, 14, 16, 18, 20, 22 h post-feeding). The 10 mL of rumen fluid were mixed with 2 mL meta-phosphoric acid (25%, w/v) and frozen for later analysis of VFA (Erwin et al., 1961) and rumen ammonia concentrations (Smith and Murphy, 1993). The gas chromatography settings for VFA analysis were as follows; the oven was set to 145˚C, the injector was 185˚C, and the detector was 200˚C with nitrogen and hydrogen flows of 20 mL/min.

Milk was sampled were during the AM and PM milking on the final 4 days of the treatment period and were composited by milk yield for the period. Milk was preserved

using a pellet of 2-bromo-2-nitropropane-1,3 diol and sent to Heart of America DHIA (Kansas City, MO) for analysis of fat, protein, lactose, SNF, MUN, and SCC using a B2000 Infrared Analyzer (Bentley Instruments, Chaska, MN). Yields of milk components were estimated from the product of milk composition and milk yield for each milking.

Chewing, eating and ruminating behaviors were observed on d 25 according to Kononoff et al. (2002) every 5 min after feeding. Behaviors were classified as eating, ruminating, or other. The behavior observed was assumed to last over 5 min intervals. The number of observations of each behavior observed was then multiplied by 5 and the product was used as the observed time spent for each behavior. Eating and ruminating were then summed over 24 h to obtain total chewing time.

Calculations and Statistical Analysis

Predictions of rumen passage rate were conducted according to Robinson et al. (1987) Rumen passage rate was calculated as follows:

Equation 3.1. $$Passage\ rate\ (kp),\ \%\ /\ hr = [1 \div 24] \times [fecal\ output\ (kg\ /\ d) \div rumen\ pool\ size\ (kg)] \times 100$$

where fecal output and rumen pool size are both in kg of DM. Rumen pool size was estimated by averaging the weights obtained from rumen evacuations. Predictions of rumen pH were conducted using a set of equations developed by White et al. (2017a).

Production data were analyzed as a crossover design using the GLIMMIX procedures of SAS (9.4; SAS Institute, Cary NC). Treatment was considered a fixed effect while cow and period were treated as random effects. Rumen fluid data were analyzed as repeated measures using an autoregressive heterogenous covariance structure which was chosen based on Bayesian information criterion. Rumen fluid data were

analyzed using MIXED procedures in SAS (9.4; SAS Institute, Cary NC). Treatment and time post-feeding were treated as fixed effects while cow and period were random effects. Statistical significance for all treatments effects was declared at $P \leq 0.05$; trends are discussed at $P \leq 0.15$ All results are presented as least squares means ± the largest standard error of the mean.

RESULTS AND DISCUSSION

Feed and Diet Nutrient Composition

The mDDGS and pDDGS used in this study originated from an identical production batch, and their composition is listed in Table 3.2. These feeds contained similar concentrations of CP, NDF, and total fatty acids (TFA). Differences in 30 h vitro NDF digestibility (NDFD30) were observed, specifically NDFD30 was greater in pDDGS compared to mDDGS (73.9 ± 14.11% vs. 70.4 ± 12.85%; Mean ± SD). In the field, determination of NDFD of individual ingredients is often used to balance diets for lactating dairy cattle (Fustini et al., 2017, Kahyani et al., 2019). In vitro observations are valuable because they have been used to predict in vivo NDFD (Lopes et al., 2015b). The LF and HF concentrate mixes contained similar concentrations of CP but differed in starch and aNDFom by 9 and 7 units, respectively. This difference was by design to ensure similar concentrations of CP, aNDFom, and starch across treatments. This aim was achieved with the exception of starch which was 2 units greater in the HF treatments. The TMR did differ in NDFD30 with the LF diets being approximately 10% greater than HF diets (Table 3.1). Particle size distribution only differed appreciably on the 8 mm sieve and pan proportions (Table 3.1). Specifically, the addition of pDDGS increased the proportion of material retained on the 8 mm sieve by 34% and reduced the proportion

retained on the pan by 43%. This increased peNDF by 20% when including pDDGS (20.9 ± 0.93% vs. 24.8 ± 0.89%; Mean ± SD). Increasing forage concentration did slightly increase feed retained on the 8 mm sieve, but similar amounts were retained on the 19 mm and 1.18 mm sieve in both LF and HF. The addition of straw in the HF treatments may be why feed retained on the 8 mm sieve increased.

Nutrient Intake and Digestibility

Increasing forage concentration may decrease nutrient intakes of lactating dairy cattle (Kalscheur et al., 1997, Li et al., 2020) but in the current study neither increasing forage concentration or form of DDGS affected the intake of DM, OM, NDF, TFA or gross energy (Table 3.3). Decreased nutrient intake that is a result of increased forage inclusion may partially be attributed to increased particle size when forage concentration increases; increasing forage particle size has reduced DMI in dairy cattle (Kononoff et al., 2003b, Haselmann et al., 2019). Additionally, increasing forage concentration often increases dietary NDF and increased NDF concentration can limit DMI (West et al., 1999). Similar nutrient intakes observed in our experiment were likely observed because NDF and TMR particle size were similar. Starch concentration of the HF treatments increased which resulted in an increase in starch intake ($P = 0.01$). Although starch intake increased for HF, both gross and digestible energy intake were unaffected ($P = 0.36$) which may be a result of increased CP ($P = 0.13$) and ROM ($P < 0.01$) intake when LF was fed.

Apparent total tract digestibility was affected by forage concentration and form of DDGS (Table 3.3). Specifically, starch digestibility increased from 93.3 ± 0.83% to 95.3 ± 0.83% in cows fed HF ($P < 0.01$). We speculate that the improvement in starch

digestibility may have been a result of the effect ensiling has on the starch-protein matrix within the corn kernel (Hoffman et al., 2011). Similar proportions (51% of dietary starch) and amounts (2.4 kg) of starch originated from corn silage in both LF and HF treatments, which suggests that source of starch in the treatments was not the cause of increased starch digestibility when HF was fed. An alternative explanation for increased starch digestibility when HF was fed is that HF contained 9% less NDFD30. Providing less digestible NDF when feeding HF may have allowed for more extensive ruminal degradation of starch leading to increased starch digestibility. This suggestion is supported by data from Voelker and Allen (2003) who observed that increasing NDFD decreased the rate of starch digestion in vivo. The authors speculated that this result may be due to a reduction in starch digesting and an increase in fiber digesting bacteria. An increase in fiber digesting bacteria and reduction in starch digesting bacteria when LF was fed may have reduced starch digestibility when LF was fed. Crude protein digestibility tended to increase as the proportion of forage increased ($P = 0.12$), this may be a result of additional alfalfa hay which is known to contain readily digestible protein (Balde et al., 1993).

Increasing forage concentration has resulted in mixed observations on total tract aNDFom digestibility (TTNDFD; Kalscheur et al., 1997, Ranathunga et al., 2019, Li et al., 2020). The inconsistent response of TTNDFD may due to increased NDF concentration or reduced DM and NDF intake when increasing forage in TMR's. In the current study, TTNDFD was unaffected by forage concentration ($P = 0.38$; Table 3.3) even though retention time increased when HF was fed ($P = 0.11$; Table 3.4). Interestingly, TTNDFD observed in our study is similar to TTNDFD predictions

generated by AMTS (AMTS, version 4.10.4.1, Agricultural Modeling and Training Systems, LLC, Groton, NY); TTNDFD estimates were 48.1% and 48.7% for LF and HF respectively. Compared to mDDGS, the inclusion of pDDGS tended to result in a 4% increase in TTNDFD ($P = 0.15$). This observation may be a result of improved NDFD of the pDDGS when compared to mDDGS which was observed in vitro; specifically NDFD30 was 70.4 ± 12.85 for mDDGS and 73.9 ± 14.11 for pDDGS (Mean ± SD). Pelleting oats has resulted in similar increases in TTNDFD when dairy cows were fed oats that were either rolled, flaked, or pelleted (Tosta et al., 2019). However a positive response from pelleting is not always observed as pelleting a whole TMR has been observed to reduce TTNDFD (Bonfante et al., 2016) but this observation may have been confounded by increased passage rate due to decreased particle size of the entire TMR. Particle size reduction increases DMI and rumen passage rate while reducing TTNDFD (Teimouri Yansari et al., 2004). Although the positive impact of pelleting on digestibility is not readily apparent we know that the process involved the addition of steam and pressure which are applied to propel feed through a screen. Interestingly, steam and pressure treatments have been observed to improve in vitro DM digestibility of forages (Rangnekar et al., 1982). Although at higher temperatures than used during pelleting, steam pretreatment has also been used as a method to improve degradation of fiber from residues for biofuel production (Lizasoain et al., 2017). Steam treatments are also known to hydrolyze portions of hemicellulose and associated ferulic acids, and this may improve access to and degradability of cell wall constituents (Hendriks and Zeeman, 2009, Auxenfans et al., 2017). Temperatures used during pelleting are much lower than steam explosion as a pretreatment before biofuel production (82°C vs. 150°C), which may be

why the concentration of NDF was similar, but we suggest that the temperature may be high enough to solubilize some cross linkages such as those in ferulic acids. Possible solubilization of lignin in pDDGS is supported by decreased lignin concentration of the pDDGS compared to mDDGS (1.83% vs. 2.14%). Further research should be conducted to determine if pelleting feed improves NDFD through hydrolyzing lignin or by some other means.

Total fatty acid digestibility was similar across treatments, a trend for increased TTNDFD ($P = 0.15$) led to a trend for increased energy digestibility when feeding pDDGS compared to mDDGS ($P = 0.07$). Although energy digestibility tended to increase, this did not lead to an increase in milk production. Forage concentration had no effect on energy digestibility, but compared to HF, LF treatments resulted in increased residual organic matter (ROM) digestibility. The ROM fraction may contain sugars, pectin, β-glucans, and organic acids; the true digestibility of ROM was estimated to be 87% to 96% (Tebbe et al., 2017). Tebbe et al. (2017) partitioned non fiber carbohydrates into starch and ROM, doing so increased precision of estimating true digestibility and metabolic fecal excretion. Increased apparent ROM digestibility of LF may be due to increased ROM intake which dilutes any endogenous contributions of ROM in the feces leading to increased digestibility.

Rumen Characteristics and Passage Rate

Rumen mass and NDF concentration were unaffected by treatment (Table 3.4). Rumen pH and rumen ammonia increased when feeding HF. Increasing forage concentration often results in increased rumen pH (Kalscheur et al., 1997, Yang and Beauchemin, 2009) which was attributed to increased rumination and saliva production

but the authors also suggest decreased DMI and diet fermentability (reduced starch concentration) as factors resulting in reduced acid production and increased rumen pH. Decreasing diet fermentability may be of greater consequence than increasing rumination activity (Krause and Combs, 2003), but in the current study, pH increased when forage was increased despite a concurrent increase in starch concentration and intake. Starch intake is negatively related to rumen pH (White et al., 2017) and the increased pH when forage concentration, and starch intake, increased may be a result of increased rumination activity. Rumen ammonia production may have increased due to greater alfalfa hay inclusion with HF however total diet soluble, crude, and degradable protein were similar between LF and HF. Interestingly compared to mDDGS, pDDGS contained half the soluble protein concentration (5.7 ± 1.28% vs. 11.3 ± 12.00%), indicating that pelleting may affect soluble protein of the feed but it did not affect rumen concentrations of ammonia ($P = 0.285$) which has been observed to increased when soluble protein concentration increases (Gabler and Heinrichs, 2003). Although CP fractions of pDDGS may have been altered, total tract CP digestibility was unaffected due to form of DDGS ($P = 0.33$). There is evidence that heat, especially with the addition of moisture (Peng et al., 2014) may decrease protein solubility and rumen degradability but heat has not affected apparent total tract digestibility consistently (Arieli et al., 1989, Doiron et al., 2009)

White et al. (2017) developed 8 equations to predict ruminal pH and observed ruminal pH was less than each model prediction (Table 3.5). The models were broken down based on how particle size measurements were integrated into each. Models 1 through 4 combined particle size measurements with NDF concentration, much like

peNDF, while models 5 through 8 used particle size measurements independent of NDF concentration, a system referred to as physically adjusted NDF (paNDF; White et al., 2017). The models also differed in whether they used DM or as fed particle size measurements and if the inclusion of diet or diet and rumen measurements were included as parameters. Our results indicate that model 5 was the best predictor of rumen pH being 103% of the observed pH; this model included mean particle size, proportion of particles > 8 mm, forage NDF, NDF, starch, ADF:NDF ratio, and starch × mean particle size. Additionally, our results demonstrate that using the paNDF system improved ruminal pH predictions, paNDF models were 105% of the observed pH while peNDF models were 110% of observed pH. Contrary to the findings of Li et al. (2020), we observed that rumen measurements did not improve predictions of rumen pH even though increased starch (Lopes et al., 2009) and NDF digestibility (Ramirez et al., 2012) are known to have a reducing influence on rumen pH.

Rumen passage rate has important effects on nutrient digestion because the amount of time feed is available for digestion in the rumen will determine the extent it is digested. In the current study rumen passage tended to slow from 2.84 ± 0.205%/hr to 2.65 ± 0.205 %/hr and retention tended to increase from 36.2 ± 2.76 hr to 39.0 ± 2.76 hr when forage concentration was increased ($P < 0.13$). Total rumen passage rate often ranges from 2.6 %/h to 3.7%/h and has been observed to be affected by forage concentration, particle size, digestibility, and NDF concentration (Teimouri Yansari et al., 2004, Kendall et al., 2009, Ramirez Ramirez et al., 2016). As DMI increases, passage out of the rumen becomes more rapid (Seo et al., 2006). There is also evidence suggesting that as NDFD increases, rumen passage rate decreases (Kendall et al., 2009).

In the current experiment, increasing forage concentration did not affect DMI, NDF concentration, or particle size of the TMR but passage rate was reduced. Past studies have indicated that particle size may play a larger role in slowing passage than forage concentration alone (Zebeli et al., 2007), but since particle size between LF and HF was similar we can conclude that increasing forage concentration may slow passage rate independently of particle size. Increasing forage concentration reduced passage even though it had a lower concentration of NDFD30, which is contrary to observations from Kendall et al. (2009). Furthermore, even though passage rate slowed and retention time increased when HF was fed, TTNDFD did not increase as hypothesized. Similar TTNDFD for HF and LF may be a result of providing less digestible feeds like wheat straw in place of readily digestible NDF sources like soybean hulls and beet pulp in HF which would negate the benefits of slowing passage rate.

Total volatile fatty acid (VFA) concentration tended to decrease by 3.5% when increasing forage concentration ($P = 0.15$). Increasing forage concentration often decreases total rumen VFA concentration (Kalscheur et al., 1997, Li et al., 2020), which is attributed to a decrease in rumen degradable carbohydrates, often decreased starch concentration and intake. In our study, when HF was fed starch intake and digestibility increased, but total VFA still decreased. We speculate that this effect may be due to differences in ROM intake and digestibility. Residual organic matter may contain β-glucans, organic acids, and pectin (Tebbe et al., 2017) which are rapidly degraded in the rumen (Van Soest, 1994). Rumen concentrations of acetate were unaffected which differs from published observations demonstrating that increasing forage concentration increases molar proportions of acetate (Kalscheur et al., 1997, Ranathunga et al., 2019). When

increasing forage, these experiments also increased NDF concentration and reduced starch of the diet when increasing forage, but in our study the starch and NDF concentrations across treatments was similar which may explain why we observed similar acetate proportions. Butyrate proportions increased when cows consumed LF ($P < 0.01$), which was similar to the response observed by Kalscheur et al. (1997) but different from Li et al. (2020) who observed a decrease in butyrate when forage concentration increased. In the current study, increased butyrate concentration may be related to increased ROM concentration and intake which is related to increased intake of β-glucans, organic acids, and pectin (Table 3.1, 3.3). Morvay et al. (2011) observed that molar proportion of butyrate was correlated with soluble carbohydrates and a "rest" category defined as 100 - NDF - starch - ash - VFA - lactate which is similar to how ROM is calculated and would encompass β-glucans, organic acids, and pectin. Feeding pDDGS also increased butyrate concentrations ($P = 0.025$), which may be a result of solubilization of NDF components due to the pelleting process. Propionate, isobutyrate, valerate, and isovalerate proportions were affected by the interaction between forage and form of DDGS. When LF diets were fed, pDDGS reduced each VFA concentration but when feeding HF diets, pDDGS increased propionate, isobutyrate valerate, and isovalerate concentrations. The reason for this interaction is unclear.

Eating, Ruminating, and Sorting Behavior

Pelleting DDGS did not affect eating, rumination, or total chewing time but increased forage concentration tended to increase rumination time by 9% (Table 3.6; $P = 0.08$). Increasing forage concentration has consistently increased chewing and rumination time in cattle (Zebeli et al., 2007, Yang and Beauchemin, 2009). The response of time

spent eating is less predictable, increasing forage concentration has either increased (Zebeli et al., 2007) or not affected eating time (Yang and Beauchemin, 2009). Increased particle size also increases total chewing time, but effects on time spent eating are less obvious (Teimouri Yansari et al., 2004, Yang and Beauchemin, 2009). We did observe that feeding pDDGS tended to eating time.

Forage concentration did not affect sorting behavior but pDDGS appeared to increase sorting behavior. Forage particle size may influence sorting (Kononoff et al., 2003b) but in the current study there was little difference in particle size when the proportion of forage was increased and consequently reducing the likelihood of particles being sorted (Miller-Cushon and DeVries, 2017). Including pDDGS in place of mDDGS resulted in a preference for particles retained on the 8 mm sieve and sorting against particles on the 1.18 mm sieve (Table 3.6). Since the pellets were retained on the 8 mm sieve of the PSPS, these results indicate that cows sorted for the pDDGS. Dried distillers grains are highly palatable (Rodriguez-Hernandez, 2018), and we suggest that pelleting allows them to be more readily selected for. Additionally, it is possible that the pellet absorbed moisture and this softened the pellet throughout the day which made it crumble back into a meal form. This response would lead to an increase in the proportion of feed refusals retained on the 1.18 mm. Given that DDGS is palatable, we suggest that pelleting the feed enhanced the cow's ability to sort for the DDGS leading to preferential consumption of the pDDGS.

Milk yield, Composition, and Feed Efficiency

Increasing forage concentration tended to reduce milk yield ($P = 0.14$) and increase milk fat percent ($P = 0.11$; Table 3.7) but had no effect on ECM, feed efficiency,

or milk fat yield. Increasing the proportion of forage in a TMR often increases milk fat percent and reduces milk yield due to increased rumen pH, increased proportions of acetate and reduced DMI (Yang and Beauchemin, 2007b, Li et al., 2020). These studies both reduced starch as forage proportion increased and observed reduced ruminal propionate concentrations when HF diets were fed. Both a reduction in DMI and molar proportion of propionate, which is a precursor for glucose and eventually lactose, may reduce milk yield. We observed neither of those scenarios in the current study, which makes the cause for decreased milk yield less apparent. In the current study, there was a trend for an interaction between forage concentration and form of DDGS on milk protein yield (P = 0.08). Protein yield was 1.08, 1.05, 0.99, and 1.05 kg for mDDGS-LF, pDDGS-LF, mDDGS-HF, and pDDGS-HF respectively. The increase in milk protein yield of pDDGS-HF compared with mDDGS-HF may have been a result of the improved TTNDFD of pDDGS. Similarly, Kendall et al. (2009) observed milk protein yield increased when in vitro NDFD concentration and TTNDFD numerically increased. When pDDGS was fed TTNDFD increased and we speculate that this may have resulted in increased microbial protein flow out of the rumen by increasing fermentation of carbohydrates in the rumen. This explanation is supported by increased rumen ammonia concentration when feeding HF because the increased N concentration may indicate that rumen fermentable carbohydrates were limiting microbial crude protein production. Hristov and Ropp (2003) observed that feeding rumen degradable NDF increased propensity for rumen ammonia to be transferred to milk protein, which would support our hypothesis. Consequently, increased TTNDFD when feeding pDDGS may have resulted in greater milk protein yield in HF diets.

CONCLUSIONS

In our study, we fed low and high forage concentrations with either mDDGS or pDDGS and each diet had similar concentrations of NDF, CP, and fatty acids while starch was slightly increased for HF diets. Our results indicate that the form of DDGS and forage concentration did not affect DMI or ECM. Similar to previous published results, increasing forage concentration increased rumination time which would increase salivary buffering leading to increased rumen pH. We also observed that pelleting DDGS did not affect either rumination, total chewing time, or rumen pH. Additionally, pDDGS increased feed sorting behavior which is likely a result of DDGS palatability and the ease of sorting the pellet compared to the meal form. Even though starch intake and digestibility were greater when HF was fed, total VFA decreased which may be a result of decreased intake of soluble fiber components like pectin and β-glucans when compared to cows fed LF diets. As we hypothesized, increasing forage concentration slowed passage rate but it did not increased TTNDFD which was likely a result of less fermentable NDF sources in the HF diets thereby negating the positive effects of slower passage rate on digestibility. Total tract NDF and energy digestibility both increased because of the pDDGS and we hypothesized that may be due to solubilization of certain components of NDF during the pelleting process leading to improved digestibility. In summary, pelleting DDGS resulted in an increase in sorting behavior, TTNDFD, and energy digestibility which warrants further investigation into the effects the pelleting process has on different feeds.

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TABLES AND FIGURES

Table 3.1. Ingredient composition, nutrient composition and particle size distribution of diets fed during the experiment

	Treatment[1]			
	LF		HF	
Item	LD	PD	LD	PD
Ingredient, % DM				
Corn silage	31.6	31.6	34.9	34.9
Alfalfa hay	15.3	15.3	18.0	18.0
Wheat straw	0	0	2.0	2.0
DDGS[2]	15.0	0	15.0	0
Pelleted DDGS[3]	0	15.0	0	15.0
Ground corn grain	13.9	13.9	13.4	13.4
Soybean meal	6.44	6.44	6.62	6.62
Soybean hulls	4.62	4.62	1.52	1.52
Cane molasses	1.77	1.77	1.73	1.73
Beet pulp	6.37	6.37	1.90	1.90
Rumen-protected Met[4]	0.17	0.17	0.17	0.17
Rumen-protected Lys[5]	0.55	0.55	0.54	0.54
Rumen-inertfat[6]	1.55	1.55	1.52	1.52
Mineral-vitamin mix[7]	2.68	2.68	2.72	2.72
Chemical composition[8], % DM unless noted otherwise				
DM	61.3 (0.48)	61.3 (0.46)	59.7 (0.50)	59.9 (0.49)
CP	16.8 (0.43)	16.8 (0.37)	16.6 (0.30)	16.6 (0.35)
ADF	19.6 (1.29)	19.6 (1.23)	19.5 (1.14)	19.5 (1.09)
NDF	30.5 (1.42)	30.5 (1.38)	30.7 (0.84)	30.7 (0.85)
aNDFom	29.9 (1.12)	29.9 (1.08)	29.9 (0.75)	29.9 (0.76)
dNDF30, % NDF	59.0 (2.58)	58.3 (3.63)	53.3 (1.61)	53.8 (2.02)
dNDF240, % NDF	73.4 (1.66)	73.4 (1.79)	69.0 (1.68)	69.1 (1.59)
Starch	23.1 (1.27)	23.0 (1.31)	25.0 (0.43)	25.0 (0.46)
Crude fat	4.56 (0.23)	4.72 (0.24)	4.27 (0.24)	4.42 (0.25)
Total fatty acids	4.88 (0.20)	4.86 (0.14)	4.93 (0.28)	4.92 (0.24)
Ash	8.46 (0.74)	8.46 (0.78)	8.86 (0.68)	8.87 (0.72)
ROM	15.2 (2.11)	15.3 (1.95)	13.7 (0.77)	13.9 (0.60)
Particle size				
>19.0 mm, % as-is	3.09 (0.51)	4.08 (1.51)	2.86 (1.13)	3.86 (1.59)
8.0–19.0 mm, % as-is	33.7 (2.20)	44.5 (2.71)	35.4 (1.94)	48.6 (3.27)
1.18–8.0 mm, % as-is	32.9 (1.73)	33.7 (2.64)	31.6 (2.78)	30.9 (3.31)
<1.18 mm, % as-is	30.3 (2.41)	17.7 (1.38)	30.1 (0.55)	16.6 (1.23)
>19.0 mm, % of DM	3.33 (0.75)	3.31 (1.08)	2.74 (1.07)	3.50 (1.64)
8.0–19.0 mm, % of DM	27.1 (1.99)	41.3 (3.42)	29.0 (1.58)	45.4 (2.34)
1.18–8.0 mm, % of DM	37.2 (3.09)	36.1 (2.79)	35.1 (2.67)	32.8 (2.77)
<1.18 mm, % of DM	32.4 (3.51)	19.3 (1.55)	33.2 (1.07)	18.2 (0.96)

[1]LF = low forage (45%), HF = high forage (55%), LD = loose DDGS, PD = pelleted DDGS.
[2]Dakota Gold (POET Nutrition, Sioux Falls, SD).
[3]Dakota Gold Pro Pellet (POET Nutrition, Sioux Falls, SD).
[4]Ajipro (Ajinomoto, Chicago, IL).
[5]Smartamine M (Adisseo, Alpharetta, GA).
[6]Energy Booster Merge (Milk Specialties, Eden Prairie, MN).
[7]Contained per kg of premix: 245g of $NaHCO_3$, 224 g of $CaCO_3$, 148g of CaHPO4, 127 g of salt, 127 g of MgO, 97g of $NaSO_4$, 21g of vitamin premix (14,850 IU/g Vitamin A, 3,850 IU/g Vitamin D, and 90 IU/g of Vitamin E), and 13g of trace mineral premix (180,000 mg/kg Zn, 150,000 mg/kg Mn, 25,000 mg/kg Cu, 2,600 mg/kg I, 2,300 mg/kg Co, 1,000 mg/kg Fe, and 820 mg/kg Se).
[8]aNDFom(alpha amylase treated) = NDF – NDF ash, dNDF30 = aNDFom digested after 30 h in vitro, dNDF240 = aNDFom digested after 240 h in vitro, ROM (residual organic matter) = 100 − %CP − % TFA − %Ash − %Starch − %NDF.

Table 3.2. Nutrient composition of corn silage, alfalfa hay, wheat straw, DDGS, pDDGS, and grain mixes used in the experiment[1]

	Corn silage		Alfalfa hay		Wheat Straw		DDGS		pDDGS		LF conenctrate		HF concentrate	
Item[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, % as-is	36.4	1.10	87.4	2.07	90.2	2.7	91.5	0.23	90.7	0.81	89.5	0.33	89.4	0.38
CP	8.4	0.42	20.3	1.12	4.30	0.14	32.3	0.24	32.1	0.46	16.4	0.89	16.9	1.07
ADF	24.1	3.07	36.1	0.77	58.2	1.13	11.3	1.15	11.0	1.82	12.7	1.11	6.2	0.30
NDF	38.3	2.35	42.9	1.18	77.0	0.07	31.0	1.23	31.0	1.78	19.0	2.76	11.3	0.38
aNDFom	37.7	2.20	41.4	1.52	74.5	0.78	30.6	1.36	30.6	1.84	18.6	2.41	11.1	0.51
dNDF30, %NDF	54.2	2.45	41.0	1.70	43.3	0.14	70.4	12.85	73.9	14.11	76.3	3.38	57.5	4.96
dNDF240, %NDF	73.3	3.05	47.9	3.01	64.5	0.14	86.4	4.18	87.4	4.08	87.7	3.24	76.8	3.08
ADICP	0.80	0.10	1.76	0.40	1.13	0.36	1.26	0.18	1.09	0.23	0.73	0.34	0.36	0.10
NDICP	1.08	0.25	24.9	0.56	1.41	0.13	2.74	0.22	2.79	0.21	1.55	0.21	1.09	0.50
Lignin	3.01	0.61	7.96	0.41	10.1	0.51	2.14	0.57	1.83	0.95	1.23	0.08	1.32	0.19
Sugar	0.88	0.15	4.18	0.73	0.30	0.28	2.63	0.44	2.80	0.67	7.33	0.82	5.55	1.06
Starch	37.1	0.63	1.95	0.53	4.3	0.28	4.80	0.22	4.73	0.17	27.1	2.98	36.0	1.11
Crude fat	3.13	0.18	1.58	0.21	1.04	0.13	5.73	0.14	6.75	0.15	6.50	0.58	6.70	0.58
Total fatty acids	2.60	0.21	1.16	0.05	0.83	0.34	7.50	0.37	7.39	0.29	7.24	0.22	8.90	0.57
Ash	4.96	0.82	11.38	0.25	8.79	0.51	5.76	0.67	5.81	0.90	11.25	1.16	13.48	1.45
Ca	0.22	0.01	1.15	0.08	0.22	0.01	0.07	0.01	0.05	0.01	1.94	0.19	2.39	0.19
P	0.24	0.02	0.31	0.01	0.09	0.00	0.98	0.09	1.00	0.13	0.40	0.05	0.46	0.07
Mg	0.16	0.04	0.24	0.01	0.07	0.00	0.42	0.01	0.42	0.01	0.27	0.02	0.26	0.01
K	1.04	0.10	3.53	0.07	1.15	0.01	1.38	0.02	1.41	0.02	1.17	0.01	1.12	0.01
S	0.14	0.02	0.25	0.01	0.07	0.00	1.09	0.09	1.13	0.13	0.35	0.05	0.35	0.07

[1]n = 4.

[2] aNDFom = alpha amylase treated NDF – NDF ash, dNDF30 = aNDFom digested after 30 h in vitro, dNDF240 = aNDFom digested after 240 h in vitro, ADICP = acid detergent insoluble CP, NDICP = neutral detergent insoluble CP.

[3]Low forage TMR grain mix.

[4]High forage TMR grain mix.

Table 3.3. Apparent total tract nutrient digestibility of diets containing 15% DDGS in a loose or pelleted form with a 45% or 55% forage concentration fed to lactating jersey cows

Item	Treatment[1]				SEM[2]	*P* – Value[3]		
	LF		HF					
	mDDGS	pDDGS	mDDGS	pDDGS		FGE	DGF	FGE× DGF
Intake, kg/d								
DM	20.4	19.8	19.4	19.8	0.85	0.33	0.85	0.34
OM	18.6	18.1	17.7	18.0	0.66	0.27	0.87	0.37
CP	3.42	3.31	3.26	3.21	0.152	0.13	0.75	0.36
Starch	4.21	4.05	4.41	4.45	0.154	<0.01	0.59	0.39
NDF	6.22	6.12	5.93	6.06	0.197	0.23	0.91	0.45
aNDFom[4]	6.08	6.00	5.81	5.95	0.196	0.25	0.84	0.42
TFA[5]	1.00	0.96	0.96	0.97	0.040	0.64	0.89	0.30
Energy, Mcal/d								
GE[6]	88.3	86.1	84.1	85.9	3.24	0.36	0.92	0.40
DE[7]	59.2	58.7	56.3	58.4	2.61	0.36	0.66	0.46
ROM[8]	4.00	3.93	3.57	3.29	0.201	<0.01	0.48	0.24
Digestibility, %								
DM	67.3	67.5	67.2	68.3	1.10	0.54	0.19	0.35
OM	69.0	69.4	68.9	69.6	1.08	0.94	0.24	0.81
CP	67.6	68.9	69.3	69.4	1.06	0.12	0.33	0.40
Starch	93.7	93.0	95.4	95.2	0.83	<0.01	0.41	0.67
NDF	45.7	46.5	44.4	46.4	2.05	0.52	0.21	0.60
aNDFom[4]	47.8	49.9	47.2	48.5	1.85	0.38	0.15	0.70
TFA[5]	73.0	74.0	73.6	72.2	1.65	0.72	0.89	0.42
16C fatty acids	76.6	78.7	77.7	77.6	1.80	0.40	1.00	0.35
18C fatty acids	74.1	74.4	75.1	72.8	1.78	0.54	0.83	0.43
GE[6]	67.2	68.2	67.0	68.1	1.18	0.81	0.07	0.89
ROM[8]	80.8	81.6	77.0	77.8	1.78	<0.01	0.34	0.82

[1]LF = low forage (45%), HF = high forage (55%), mDDGS= meal DDGS, pDDGS = pelleted DDGS.

[2]Least square means; largest standard error of the treatment mean is listed.

[3]FGE = effect of forage concentration, DGF = effect of form of DDGS (meal or pelleted), FGE × DGF = interaction of forage concentration and form of DDGS.

[4]aNDFom = alpha amylase treated NDF – NDF ash.

[5]TFA = total fatty acids.

[6]GE = gross energy.

[7]DE = digestible energy.

[6]ROM (residual organic matter) = 100 − %CP − % TFA − %Ash − %Starch − %NDF.

Table 3.4. Fecal output, rumen mass, passage rate, and rumen retention of diets containing 15% DDGS in a loose or pelleted form with a 45% or 55% forage concentration fed to lactating jersey cows

Item	Treatment[1]				SEM[2]	*P* – Value[3]		
	LF		HF					
	mDDGS	pDDGS	mDDGS	pDDGS		FGE	DGF	FGE× DGF
Feces, kg	43.2	40.5	40.9	41.9	2.42	0.76	0.53	0.18
Feces, % DM	15.5	15.9	15.6	15.0	0.32	0.16	0.80	0.07
Feces, kg DM	6.7	6.5	6.4	6.3	0.39	0.24	0.46	0.76
Rumen, % DM	14.7	15.1	15.5	14.7	0.87	0.63	0.68	0.29
Rumen, kg DM	10.0	9.6	10.5	9.6	0.73	0.61	0.21	0.62
aNDFom[4], % DM	49.9	52.2	51.6	52.0	1.19	0.37	0.13	0.30
Rumen aNDFom, kg	4.99	4.99	5.43	5.00	0.326	0.33	0.37	0.39
Passage rate, %/hr[5]	2.83	2.85	2.56	2.74	0.205	0.13	0.39	0.52
Retention, hr	36.4	35.9	40.5	37.4	2.76	0.11	0.29	0.46
pH	5.83	5.90	5.92	5.91	0.057	0.04	0.25	0.17
Time pH<5.8, hr/d	13.1	11.0	8.2	8.2	1.89	0.15	0.38	0.49
Time pH<5.6, hr/d	6.1	4.4	3.1	3.0	1.45	0.25	0.40	0.51
NH_3, mg/dL	17.0	16.6	19.8	18.5	3.22	<0.01	0.29	0.56
Total VFA, m*M*	94.3	91.5	88.1	91.2	5.09	0.15	0.95	0.20
VFA, mol/100 mol								
Acetate	55.9	55.1	53.2	54.3	2.91	0.20	0.93	0.48
Propionate	23.8	21.9	21.8	23.2	1.72	0.68	0.78	0.07
Butyrate	11.1	12.0	10.2	10.7	0.78	<0.01	0.03	0.57
Isobutyrate	0.69	0.64	0.67	0.70	0.096	0.38	0.92	0.05
Valerate	1.44	1.35	1.19	1.32	0.095	<0.01	0.64	0.03
Isovalerate	1.00	0.88	0.95	1.08	0.103	0.07	0.97	0.03

[1]LF = low forage (45%), HF = high forage (55%), mDDGS= meal DDGS, pDDGS = pelleted DDGS.

[2]Least square means; largest standard error of the treatment mean is listed.

[3] FGE = effect of forage concentration, DGF = effect of form of DDGS (meal or pelleted), FGE × DGF = interaction of forage concentration and form of DDGS.

[4]aNDFom = alpha amylase treated NDF – NDF ash.

[5]rumen passage rate measured according to Robinson et al., 1987.

Table 3.5. Comparing observed rumen pH with predicted rumen pH from models that include particle size, nutrient composition, nutrient fermentability, and rumination behavior

Item	Model number[1]	Treatment			
		LF		HF	
		mDDGS	pDDGS	mDDGS	pDDGS
Observed pH		5.83	5.90	5.92	5.91
peNDF					
Diet					
DM	1	6.25	6.20	6.34	6.28
AF	2	6.26	6.29	6.41	6.40
Diet + rumen					
DM	3	6.64	6.95	6.74	7.11
AF	4	6.37	6.40	6.52	6.50
paNDF					
Diet					
DM	5	5.99	6.08	6.09	6.19
AF	6	6.26	6.29	6.38	6.36
Diet + rumen					
DM	7	6.11	6.17	6.18	6.24
AF	8	6.26	6.29	6.38	6.36
AMTS[2]		6.15	6.15	6.24	6.24

[1]Models from White et al., 2017; Model 1, pH = 13.8 - 0.124(mean particle size, mm) + 0.279(MPS × NDF) + 0.00727(Wet forage, %DM) + 0.0107(legume forage, %DM) – 0.0352(starch, %DM) + 0.000345(starch, %DM × starch, %DM) – 0.723(CP, %DM) + 0.0183(CP, %DM × CP, %DM) – 0.069(Fat, %DM) + 0.0017(Starch, %DM × mean particle size, mm); Model 2, pH = 12.0 + 0.0112(forage NDF, %DM) – 0.019(starch, %DM) + 0.0003448(starch, %DM × starch, %DM) – 0.679(CP, %DM) + 0.0186(CP, %DM × CP, %DM) 0.0152(rumination/DMI, min/kg); Model 3, pH = 4.21 – 0.0739(mean particle size, mm) + 0.0275(%>8mm × NDF, %DM) + 0.0589(forage NDF, %DM) – 0.000852(forage NDF, %DM × forage NDF, %DM) – 0.00794(starch, %DM) + 1.055(ADF, %DM÷NDF, %DM) + 0.00903(rumen digested NDF, % digestible NDF) + 0.0016(starch, %DM × mean particle size, mm); Model 4, pH = 6.72 + 0.0137(forage NDF, %DM) + 0.00798(starch, %DM) – 0.0456(CP, %DM) – 0.00835(rumen digested starch, % starch) + 0.0204(rumination/DMI, min/kg); Model 5, pH = 4.15 – 0.0712(mean particle size, mm) + 0.0108(>8mm, % retained) + 0.0594(forage NDF, %DM) – 0.000875(forage NDF, %DM × forage NDF, %DM) – 0.00849(starch, %DM) + 0.0198(NDF, %DM) + 0.786(ADF, %DM÷NDF, %DM) + 0.0533(starch, %DM × mean particle size, mm); Model 6, pH = 12.0 + 0.0122(forage NDF, %DM) – 0.019(starch, %DM) + 0.000348(starch, %DM × starch, %DM) – 0.679(CP, %DM) + 0.0186(CP, %DM × CP, %DM) + 0.0152(rumination/DMI, min/kg); Model 7, pH = 4.53 – 0.0708(mean particle size, mm) + 0.00955(>8mm, %retained) + 0.0204(forage NDF, %DM) – 0.00708(starch, %DM) + 0.967(ADF, %DM÷NDF, %DM) + 0.0114(rumen digested NDF, % digestible NDF) + 0.0015(starch, %DM × mean particle size, mm); Model 8, pH = 12.0 + 0.0122(forage NDF, %DM) – 0.019(starch, %DM) +

0.000348(starch, %DM × starch, %DM) – 0.679(CP, %DM) + 0.0186(CP, %DM × CP, %DM) + 0.0152(rumination/DMI, min/kg).
[2]Agricultural Modeling and Training Systems LLC., version 4.10.4.1, Groton, NY.

Table 3.6. Effect of feeding pelleted DDGS and different forage concentrations on eating, ruminating, total chewing, and sorting behavior in lactating Jersey cows

Item	Treatment[1]				SEM[2]	*P* – Value[3]		
	LF		HF					
	mDDGS	pDDGS	mDDGS	pDDGS		FGE	DGF	FGE×DGF
Eating, min	206	235	211	236	19.8	0.88	0.11	0.91
Ruminating, min	421	413	468	440	49.4	0.08	0.39	0.62
Total chewing, min	629	648	679	673	57.0	0.15	0.81	0.62
Sorting index[4], %								
As is								
>19.0 mm	100.0	100.5	98.30	99.40	2.72	0.42	0.64	0.86
8.0–19.0 mm	101.1	103.1	100.8	102.7	1.32	0.62	0.02	0.89
1.18–8.0 mm	100.0	97.60	99.30	99.80	0.91	0.72	0.03	0.14
<1.18 mm	101.5	95.90	100.6	96.30	2.06	0.87	0.01	0.69
DM								
>19.0 mm	101.7	100.3	98.5	98.6	2.93	0.17	0.74	0.67
8.0–19.0 mm	100.1	103.3	99.6	102.8	1.56	0.59	<0.01	0.98
1.18–8.0 mm	99.9	89.4	100.2	92.4	3.13	0.48	<0.01	0.58
<1.18 mm	100.8	102.6	100.9	102.4	0.88	0.91	0.06	0.90

[1]LF = low forage (45%), HF = high forage (55%), mDDGS= meal DDGS, pDDGS = pelleted DDGS.

[2]Least square means; largest standard error of the treatment mean is listed.

[3] FGE = effect of forage concentration, DGF = effect of form of DDGS (meal or pelleted), FGE × DGF = interaction of forage concentration and form of DDGS.

[4]Calculated as measured particle size intake/predicted particle size intake × 100 (Leonardi and Armentano, 2003).

Table 3.7. Effect of feeding pelleted DDGS and different forage concentrations on milk production in lactating Jersey cows

Item	Treatment[1]				SEM[2]	*P*-Value[3]		
	LF		HF					
	mDDGS	pDDGS	mDDGS	pDDGS		FGE	DGF	FGE×DGF
Milk yield, kg/d	28.3	28.4	26.8	27.8	1.22	0.14	0.41	0.56
ECM[4], kg/d	34.7	34.0	32.9	34.2	1.02	0.31	0.72	0.23
ECM/DMI	1.71	1.74	1.71	1.73	0.058	0.93	0.41	0.95
Fat, %	4.85	4.70	4.96	4.93	0.283	0.11	0.37	0.58
Fat, kg/d	1.36	1.32	1.32	1.35	0.056	0.79	0.91	0.32
Protein, %	3.83	3.72	3.72	3.81	0.133	0.89	0.83	0.22
Protein, kg/d	1.08	1.05	0.99	1.05	0.035	0.09	0.53	0.08
Lactose, %	4.78	4.83	4.78	4.78	0.068	0.29	0.25	0.33
Lactose, kg/d	1.35	1.38	1.29	1.33	0.072	0.12	0.36	0.74
MUN, mg/dL	13.0	13.6	13.8	14.7	0.77	0.02	0.53	0.68
BW, kg[5]	468	459	464	457	36.68	0.40	0.04	0.93
BCS[6]	3.13	3.11	3.15	3.11	0.134	0.70	0.43	0.76

[1]LF = low forage (45%), HF = high forage (55%), mDDGS= meal DDGS, pDDGS = pelleted DDGS.

[2]Least square means; largest standard error of the treatment mean is listed.

[3] FGE = effect of forage concentration, DGF = effect of form of DDGS (meal or pelleted), FGE × DGF = interaction of forage concentration and form of DDGS.

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

[5]Average from 2 measurements during last 4 d of each period.

[6]Scored 1–5 by 3 independent observation.

GENERAL SUMMARY AND CONCLUSIONS

In livestock industries, we measure fiber using the neutral detergent fiber (NDF) system. The NDF of feed is compose of plant cell wall components that are indigestible by mammalian enzymes, but ruminants' microbial populations digest NDF and provide energy to the host. Thus, increasing the ability of microbes to digest NDF increases energy supplied to the host. Improving our understanding of NDF and integrating this knowledge into nutrition models is important to the dairy industry because NDF can comprise as much as one-third of a ration fed to dairy cows. In vitro lab assays to estimate NDF digestibility are now commonly incorporated into ration modeling software. We evaluated if these in vitro assays improved ration model predictions of animal performance. Improving modeling of NDF digestion or how models incorporate NDF may improve our ability to precision feed cattle. Fibrous byproducts, such as dried distillers grains with solubles (DDGS), are excellent fermentable fiber sources. Novel pelleting has enabled ethanol producers to pellet DDGS without binders, and the pelleting process may enhance digestibility due to the use of steam and pressure. Evaluating this new feed, and how well it is digested, may provide an avenue for dairy producers to provide more energy to cows from a human inedible ingredient. Additionally, ration formulation strategies, like increasing forage concentration or adding wheat straw, may improve rumen health, reduce passage rate, and enhance NDF digestion.

To evaluate the predictions of milk yield and CH_4 production with and without the use of in vitro NDF digestibility (NDFD) of feeds, we compiled data from past energy balance studies conducted at the University of Nebraska-Lincoln. We also determined NDFD at 30 h (NDFD30) of forages and non-forage fibrous feeds fed during each energy balance study. Then, we used this information and the Cornell Net Carbohydrate and

Protein System (CNCPS) to predict milk production. The objectives of this study were to determine if using NDFD30 improved milk and methane production estimates of the CNCPS. We hypothesized that NDFD30 measurements would improve the model predictions of milk and methane production. Results from this experiment suggested that CNCPS estimates of milk and methane production are sensitive to NDFD30 estimates. When using NDFD30 in CNCPS, milk production predictions were poorer which was demonstrated by a reduced CCC (0.87 vs. 0.82), but methane production predictions improved (0.33 vs. 0.38). It should be noted that our observations on NDFD30 results were lower than some reported values of the same feeds and it is possible that using our system may have made it difficult to maintain anaerobicity or release gas produced during fermentation, both of which may depress NDFD30.

Continued evaluation of models like CNCPS is important because these models are periodically updated to integrate recent research. By using 30 h fermentations we evaluated model options that varied the rate of NDF digestion in CNCPS, but we did not estimate 240 h fermentations that would allow is to evaluate manipulations in indigestible NDF (iNDF). Future model evaluations should investigate whether conducting 240 h in vitro fermentations to estimate iNDF improves model predictions. Adjusting the iNDF of individual feeds within CNCPS would alter the total pool of digestible NDF that is available which changes the amount of NDF available for ruminal and hindgut fermentation. Also, any further investigations using in vitro systems should employ an apparatus that has a continuous influx of CO_2 and continuous outflow of fermentation gasses. This should aid in maintaining anaerobicity and microbial activity during the in vitro fermentation. Additionally, further investigation should be carried out on equations

that could be used to predict enteric methane production. The current equation used by CNCPS was observed to overpredicted methane production for each observation.

Research studies have demonstrated that dried distillers grains with solubles are an effective feed for dairy cattle. A novel pelleting process has led to the ability to pellet DDGS without the use of either binders or additional feeds. Pelleting feed increases its bulk density which is useful when hauling feed. Pelleting is also done to improve handling of feed by reducing bridging that may occur. Our goal was to determine if the form DDGS (pelleted vs. meal) affected digestibility and ultimately milk production. Furthermore, we evaluated if forage concentration and the addition of wheat straw to the TMR reduced rumen passage rate and increased total tract NDF digestibility (TTNDFD). Our hypothesis was that feeding pelleted DDGS (pDDGS) would not affect digestibility, behavior, rumen kinetics, or performance while increasing forage concentration would slow passage rate, increase TTNDFD, and increase milk production. In general, when lactating dairy cows consumed pDDGS they performed similar to cows fed DDGS in a meal form (mDDGS), with a few exceptions. First, pDDGS appeared to affect feeding behavior and our results indicated that cows selected for pDDGS. This observation may have been a result of cattle being more able to sort large particle feeds, like pellets. Second, results of this experiment suggest that the pelleting process may improve the NDF digestibility of DDGS. Specifically, NDFD30 increased from 70% to 73% at 30 h for pDDGS vs. mDDGS, respectively, and in vivo TTNDFD increased by 4% when pDDGS was fed. Results from this study also support the suggestion that increasing forage concentration increases rumination activity, rumen pH, and slows rumen passage

rate. Although rumen passage rate was reduced as we hypothesized, increasing forage inclusion from 45% to 55% did not increase TTNDFD.

The next steps in evaluating the effect of the pelleting process on DDGS digestibility should be to conduct an in vitro digestibility experiment. Nutrient analysis and in vitro NDFD of mDDGS and pDDGS samples from multiple batches would allow for a controlled evaluation of the effect of pelleting on DDGS. In addition, if pelleting is observed to have positive effects on DDGS digestibility, studies designed to elucidate the mode of action should be conducted. Furthermore, other fibrous feedstuffs should also be tested to determine if the pelleting process increases NDF digestibility. If pelleting an ingredient increases the rate or extent of NDF digestion of a feed, it will increase the energy concentration of that feed. This is of importance for fibrous byproducts where a significant portion of the energy when fed to ruminants originates from NDF. It may also be useful to investigate how adjustments in the pelleting process, such as conditioning temperature or die size, affect digestibility. Additionally, conducting an animal study with more experimental units that is designed to evaluate milk production when feeding a pelleted compared to a meal product would also be valuable. Increasing the NDFD is important because it will allow for increased inclusion human inedible products when feeding cattle which will increase the net contribution of cattle to the human food supply.

APPENDIX A: EVALUATION OF DMI PREDICTIONS FROM CNCPS

Predictions of DMI were also evaluated, but like CO_2, it is not affected by determination of NDFD30. This could be an area for improvement within the model because NDFD of forages has been shown to impact DMI (Allen, 2000). The equation used in CNCPS was originally developed by Milligan et al. (1981) and was modified by Fox et al. (2004) and is listed below:

$$DMI = (0.0185 \times FBW + 0.305 \times FCM) \times DMIAF \times Mud \times Lag$$

Where FBW is full body weight (kg), FCM is fat corrected milk (kg), DMIAF is dry matter intake adjustment factor based on temperature (kg), and Mud is mud depth (cm). Lag is an exponential function to adjust the intake of cows that are in early lactation, the equation and adjustments are shown by Roseler et al. (1997). The mean DMI from our database was 18.8 ± 2.26 kg, the mean predicted DMI was 20.0 ± 2.13 kg. The DMI prediction yielded a CCC of 0.75 but the lower bound predicted DMI from CNCPS had a CCC of 0.87. This over prediction may have been due to the feeding procedure employed when collecting data in these energy balance studies; cows were fed at 95% ad libitum intake during collection periods of the energy balance experiments (Judy et al., 2019b; Reynolds et al., 2019).

Research has suggested that that NDFD measured in vitro can influence DMI (Allen, 2000). More recent research that has focused on the effects of undigested NDF after 24 and 240 hours in vitro (uNDF24, uNDF240) has shown that the uNDF24 had an effect on DMI while uNDF240 did not appear to affect DMI (Fustini et al., 2017). Better understanding of how NDFD and uNDF affect DMI could improve our precision when estimating DMI. In two recent publications, DMI prediction equations were re-

visited (Allen et al., 2019; de Souza et al., 2019). Allen et al. (2019) demonstrated that dietary factors, along with animal factors, can be included to improve predictions of DMI. The authors acknowledged that ration formulation begins with predicting DMI from animal factors only, but they recommend using equations with dietary factors when ration changes are being made to evaluate possible changes to DMI, especially in cases of high milk production (Allen et al., 2019)

In an evaluation of DMI prediction equations, Krizsan et al. (2014) evaluated 5 DMI models, including the NRC (2001) and CNCPS (Fox et al., 2004). In this review they evaluated fit and bias for each equation. Their data showed that the equation of Fox et al. (2004) tended to under predict DMI, while the NRC (2001) tended to over predict DMI which is similar to the results seen from de Souza et al. (2019) when they evaluated the NRC (2001) equation. This is not in line with our results, DMI predictions from Fox et al. (2004) tended to over predict DMI. With the improvement in DMI predictions when using dietary factors being demonstrated, it may be beneficial to incorporate these factors in equations to predict DMI in ration balancing software (Huhtanen et al., 2011; Krizsan et al., 2014).

APPENDIX B: METHANE PREDICTION EQUATIONS EVALUATED BY APPUHAMNY ET AL., 2016[1]

Study	Data origin[2]	Model[3], CH_4 emissions (g/cow per d)
Moe and Tyrell (1979)	NA	= [3.41 + 0.511 × NSC + 1.74 × HC + 2.65 × CEL]/0.05565
Kirchgeßner et al. (1995)	EU	= 10.0 + 4.9 × Milk + 1.5 × BW0.75
IPCC (1997) Tier II		= [0.060 × GEI]/0.05565
IPCC (2006) Tier II		= [0.065 × GEI]/0.05565
Yan et al. (2000)	EU	= [3.23 + 0.055 × GEI]/0.05565
		= [3.32 + 0.071 × DEI]/0.05565
Corré (2002)	EU	= [50.0 + 0.01 × Milk × 365]/365 × 1000
Giger-Reverdin et al. (2003)	NA, EU	= [44.9 − 0.022 × DMIBW2] × DMI × (16/22.4)
		= [47.3 − 0.021 × DMI2 − 0.68 × EE] × DMIBW × (16/22.4)
Mills et al. (2003)	EU	= [5.93 + 0.92 × DMI]/0.05565
		= [1.06 + 0.87 × DMI + 10.27 × dietary forage proportion]/0.05565
		= [56.27 − (56.27 + 0) × e[−0.028 × DMI]]/0.05565
		= [8.25 + 0.07 × MEI]/0.05565
		= [45.89 − (45.89 + 0) × e[−0.003 × MEI]]/0.05565
Ellis et al. (2007)	NA	= [3.23 + 0.809 × DMI]/0.05565
		= [3.14 + 2.11 × NDFI]/0.05565
		= [2.16 + 0.493 × DMI − 1.36 × ADFI + 1.97 × NDFI]/0.05565
		= [4.08 + 0.068 × MEI]/0.05565
		= [1.21 + 0.059 × MEI + 0.093 × Forage]/0.05565
		= [1.64 + 0.040 × MEI + 1.45 × NDFI]/0.05565
		= [8.56 + 0.139 × Forage]/0.05565
		= [5.87 + 2.43 × ADFI]/0.05565
Moate et al. (2011)	NA, EU, AU, NZ	= [24.51 + 0.788 × EE] × DMI
		= [$e^{(3.15-0.035 \times EE)}$] × DMI
Hristov et al. (2013)	NA, EU, AU, NZ	= 2.54 + 19.14 × DMI
Nielsen et al. (2013)	EU	= [1.36 × DMI − 1.25 × FA − 0.20 × CP + 0.170 × NDF]/0.05565
		= [1.23 × DMI − 1.45 × FA + 0.120 × NDF]/0.05565
		= [1.23 × DMI − 1.45 × FA + 0.171 × dNDF]/0.05565
		= [1.39 × DMI − 0.91 × FA]/0.05565
		= [1.26 × DMI]/0.05565
		= [0.738 × DMIBW − 1.45 × FA + 0.130 × NDF]/0.05565
Ramin and Huhtanen (2013)	NA, EU	= [62 + 25 × DMI] × 16.0/22.4
		= [20 + 35.8 × DMI − 0.5 × DMI2] × 16.0/22.4
Storlein et al. (2014)	NA, EU, AU, NZ	= [−1.47 + 1.28 × DMI]/0.05565

		= [−2.76 + 3.74 × NDFI]/0.05565
Moraes et al. (2014)	NA	= [3.25 + 0.043 × GEI]/0.05565
		= [0.225 + 0.042 × GEI + 0.125 × NDF − 0.329 × EE]/0.05565
		= [−9.311 + 0.042 × GEI + 0.094 × NDF − 0.381 × EE + 0.008 × BW + 1.621 × mFat]/0.05565
Charmley et al. (2016)	AU	= 38.0 + 19.22 × DMI
		= [2.14 + 0.058 × GEI]/0.05565

[1]Table adapted from Appuhamy et al., 2016.

[2]Region of the world where data used for model development originated from. NA = North America, EU = Europe, and AU = Australia, NZ = New Zealand.

[3]NSC = nonstructural carbohydrate intake (kg), HC = hemicellulose intake (kg), CEL = cellulose intake (kg), Milk = milk yield (kg), BW = body weight (kg), GEI = gross energy intake (MJ), DEI = digestible energy intake (MJ), DMIBW = dry matter intake relative to BW (g/kg), DMI = dry matter intake (kg), MEI = metabolizable energy intake (MJ), NDFI = dietary neutral detergent fiber (NDF) intake (kg), Forage = dietary forage (% of DM), ADFI = dietary acid detergent fiber intake (kg), Ym = methane conversion rate (% of GEI), CP = dietary crude protein content (% of DM), NDF = dietary NDF content (% of DM), dNDF = dietary apparent total tract digestible NDF content (% of DM), EE = dietary ether extract content (% of DM), FA = dietary fatty acid content (% of DM), and mFat = milk fat percentage.

APPENDIX C: 2019 ANNUAL MEETING POSTER

Abstract M13: Using 30 h in vitro NDF digestibility of feedstuffs in ration formulation: evaluation of predictions for milk and methane production in lactating dairy cows

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INTRODUCTION

- Neutral detergent fiber (NDF) is a heterogenous component of feeds which makes accurate characterization of high NDF feeds important for use in ration software.
- Chemically, NDF is composed of hemicellulose, cellulose, and lignin; this nutrient is also characterized as being composed of rapidly digestible, slowly digestible, and indigestible fractions that impact rumen and total tract digestion (Raffrenato and Van Amburgh 2010).
- Rations fed to lactating dairy cattle diets contain approximately 1/3 NDF; accurate characterization and understanding of NDF and its role in animal performance is important.
- The Cornell Net Carbohydrate and Protein System (CNCPS) allows users to input lab derived values to estimate rate of digestion (K_d) of NDF in place of default values.
- Correct determination of user inputs may improve predictions of metabolizable energy and protein supply while also more accurately predicting nutrient loss and gaseous emissions (Cerosaletti et al., 2004)

OBJECTIVE

- To determine if including lab generated digestible NDF (dNDF) to calculate K_d of NDF and using it in place of default feed library values improves the prediction of ME allowable milk and methane production of lactating dairy cattle.

HYPOTHESIS

- Inclusion of lab determined dNDF to calculate K_d of NDF will improve the CNCPS prediction of ME allowable milk and methane production when compared to observed performance.

MATERIALS AND METHODS

- Data were compiled from 8 energy balance experiments conducted at the University of Nebraska-Lincoln Ruminant Nutrition Metabolism Unit.
- Thirty- four forages and co-products used in the experiments were composited in equal parts by period and were sent to Cumberland Valley Analytical Services (Hagerstown, MD) for analysis of DM, CP, SP, ADICP, NDICP, ADF, aNDF, lignin, ethanol soluble CHO, crude fat, macro minerals, and trace minerals.
- The 30 h in vitro NDF digestibility of the 34 ingredients was conducted according to Goering and Van Soest (1970) at the University of Nebraska-Lincoln.
- Upon completion of the in vitro, tubes were placed in a freezer until aNDFom analysis, according to Mertens (2002), could be done.

Figure 1. In-vitro system at University of Nebraska-Lincoln Ruminant Nutrition lab

- All of the ingredients, and their respective nutrient analysis, were entered into the feed library in CNCPS.
- Each ingredient was made in two versions, one with lab determined dNDF and one with the default feed library values.
- The ME allowable milk and methane production predictions were collected from each diet when default values and lab determined values were used.
- Each of these predictions were compared to observed data to compare goodness of fit of each set of predictions.
- Model fit was determined by RMSE and CCC which was conducted using the lmer function of R (v. 3.5.2).

RESULTS

Figure 2. Computer display of dNDF input screen within CNCPS

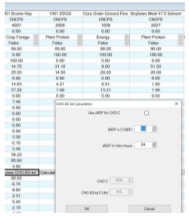


Table 1.30-hour in vitro NDF digestion of ingredients included in energy balance studies

Feed	n[1]	dNDF[2], %NDF	SD
Alfalfa Hay	8	34.3	4.43
Corn Silage	8	34.4	4.88
Brome Hay	6	42.1	6.72
DDGS	3	27.7	9.07
RFDDGS	3	41.1	8.02
Canola Meal	1	44.3	
Soybean Hulls	3	53.7	2.58
Beet Pulp	1	62.9	
Wheat Straw	1	26.9	

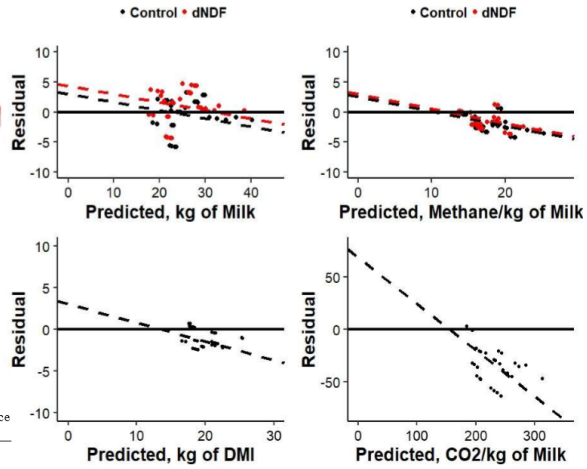
[1]n= number of treatments ingredient was included in.
[2]dNDF= digested NDF after 30 hours in vitro.

Table 2. Fit statistics of CNCPS predictions vs. the observed performance from energy balance studies[1,2]

	ME allowable milk, kg		CH_4, L/kg of milk		DMI, kg			CO_2, L/kg of milk
Item	Control	dNDF	Control	dNDF	Lower Bound[3]	Upper Bound[4]	Mean[5]	
Observed mean	25.8	25.8	15.8	15.8	18.8	18.8	18.8	193.3
Predicted mean	26.1	24.5	18.0	17.4	18.5	21.4	20.0	227.9
RMSE	2.77	3.2	2.91	2.48	1.08	3.00	1.65	39.7
RMSE[6], % mean	10.7	12.5	18.4	15.7	5.78	15.8	8.79	20.6
Mean bias, % RMSE	0.71	16.2	56.3	43.2	5.57	80.5	53.0	76.0
Slope bias, % RMSE	0.44	1.77	6.65	6.98	0.48	1.64	1.12	1.92
Residual error, % RMSE	98.9	82.0	37.1	49.9	94.00	17.8	45.9	-34.6
Mean bias	-0.23	1.30	-2.18	-1.63	0.26	-2.66	-1.20	-0.18
Slope bias	0.04	0.09	-0.28	-0.25	-0.04	-0.17	-0.08	-0.18
CCC[7]	0.87	0.82	0.55	0.62	0.87	0.48	0.75	0.48

[1]Predictions from CNCPS with and without lab determined dNDF for use in calculation of CHO - B3 Kd, %/hr, [2]Fit statistics done using R v 3.5.2, [3]Lower bound of DMI range given by CNCPS v. 6.5, [4]Upper bound of DMI range given by CNCPS v. 6.5, [5]Mean= mean of lower and upper bound of DMI given by CNCPS v. . 6.5, [6]RMSE = residual mean square error, [7]CCC = concordance correlation coefficient.

Figure 3. Plot of residual vs. predicted values. Predictions obtained from CNCPS (v. 6.5) and compared to observed data. In top row, 'Control' indicates predictions obtained with feed library default values and 'dNDF' indicates predictions were obtained from 30 h in vitro dNDF



RESULTS AND CONCLUSIONS

- Inclusion of lab determined dNDF to calculate K_d of NDF improved the CCC of methane (0.55 vs 0.62), but did not improve ME allowable milk predictions (0.87 vs. 0.82).
- Prediction of carbon dioxide production had a poor CCC with observed production (0.48).
- Prediction of DMI was good, it had a CCC of 0.75.
- Results suggest CNCPS users may not see additional benefit from including 30 h dNDF when formulating lactating dairy cow rations because model fit of ME allowable milk was not improved above control (0.82 vs 0.87).
- Sensitivity analysis when using these values may be important to determine if altering dNDF or uNDF values have a material impact on the predictions and rations fed to dairy cattle.
- Improving predictions of carbon emissions, especially methane, could provide value to farmers by formulating diets that decrease energy loss through methane emissions.
- Further research should evaluate including uNDF obtained from 240 hour in vitro fermentation to determine how altering the total pdNDF effects milk and methane predictions.

ACKNOWLEDGEMENTS

- The authors would like to acknowledge Erin Marotz and Darren Strizek for caring for the animals used in all 8 energy balance studies, as well as maintaining the cannulated cows used for rumen fluid to perform the in vitro assays.

APPENDIX D: DIET SUMMARIES FROM AMTS

Low Forage (mDDGS-LF, pDDGS-LF)

Nutrient Balances		
Nutrient	Balance	%Rqd
ME Mcal	-1.6	97
MP (g)	268	110
NH3-N (g)	39	122
Peptide-N (g)	138	181
peNDF lbs	-3.0	74
LYS (g)	18.0	109.8
MET (g)	19.8	130.5
Ca (g)	51.70	166
P (g)	-1.89	97
Mg (g)	3.94	149
K (g)	62.95	128

Total ME Avail. (Mcal/day)	61.46
ME Milk Prod (lbs/day)	72.5
MP Milk Prod (lbs/day)	86.0
MUN (mg/dl)	11.6
Urea Cost (Mcal)	0.31
Rumen pH	6.15
Milk:Feed	1.40
IOFC ($/hd)	15.23
IOpurFC ($/hd)	14.82

Excretion	
Fecal (lbs)	91
Urine (lbs)	48
Total Manure (lbs)	139
Fecal N (g)	229
Urine N (g)	202
Total Manure N (g)	432
Productive N:Total N	0.33:1
Productive N:Urinary N	1.00:1
Manure N:Total N	0.67:1
Fecal P (g)	48.4
Urine P (g)	1.0
Total Manure P (g)	49.4
Productive P:Total P	0.44:1
Manure P:Total P	0.56:1
CH4 (Mcal) / CH4 (L)	5.88 / 641.82

Diet Concentrations	
NFC (%DM)	41.1
CHO Ferm. (%DM)	42.1
CHO Ferm. (%CHO)	61.0
NDF Ferm. (%DM)	13.4
NDF Ferm. (%NDF)	46.5
Starch Ferm. (%DM)	17.9
Starch Ferm. (% Starch)	71.5
Sol. Fiber Ferm. (%DM)	7.7
Sol. Fiber Ferm. (% Sol. Fiber)	83.7
Sugar Ferm. (%DM)	3.2
Sugar Ferm. (% Sugar)	69.1
Sugar (A4) (%DM)	4.6
Starch (B1) (%DM)	25.0
Sol Fiber (B2) (%DM)	9.2
Ferm. Fiber (B3) (%DM)	22.1
Lig * 2.4 (C) (%DM)	6.6
aNDFom (%DM)	28.72
Forage NDF (%NDF)	56.29
Forage NDF (%FBW)	0.76
EE (%DM)	5.0
LCFA (%DM)	4.5
CP (%DM)	16.56
RDP (%DM)	8.36
LYS (%MP)	6.98
MET (%MP)	2.94
LYS:MET	2.38
TDN (%DM)	68.5
ME (Mcal/lb)	1.18
NEl (Mcal/lb)	0.76
Forage (%DM)	44.8
DM (%)	65.4
DCAD1 (meq/kg)	251
DCAD2 (meq/kg)	247

Ration Fed				
Ingredient	$/hd	%DM	DM lbs/day	AF lbs/day
UNL Corn Silage 2018 2019	0.00	41.5	16.4	39.5
Alfalfa Hay UNL 2018 2019	0.00	87.5	6.9	7.9
Wheat Straw 5 CP 79 NDF	0.00	92.0	0.0	0.0
Dakota Gold	0.00	88.7	7.792	8.781
Soybean Meal 47.5 Solvent	0.00	90.0	3.534	3.927
Corn Grain Ground Fine	0.00	88.0	7.892	8.968
Soybean Hulls Ground	0.00	91.0	2.569	2.823
Magnesium Ox	0.00	99.5	0.0210	0.0211
Calcium Phosphate Di (Dical)	0.00	99.5	0.0800	0.0804
Sodium Bicarbonate	0.00	99.5	0.6500	0.6533
Trace Mineral Premix	0.00	99.5	0.0201	0.0202
Vitamin Premix 1	0.00	99.5	0.0221	0.0222
Energy Booster 100	0.00	99.4	0.835	0.840
Molasses Cane	0.00	73.0	0.917	1.256
Smartamine M	0.00	98.0	0.088	0.090
AjiPro L v3	0.00	97.0	0.285	0.293
Calcium Carbonate	0.00	99.5	0.6500	0.6533
Beet Pulp Shreds	0.00	91.0	3.027	3.326
Salt White	0.00	99.5	0.2500	0.2513
Totals	0.00	65.4	51.8986	79.3627

Cost/ton As-Fed: $0.00

High Forage (mDDGS-HF, pDDGS-HF)

Nutrient Balances		
Nutrient	Balance	%Rqd
ME Mcal	-1.7	97
MP (g)	268	110
NH3-N (g)	36	119
Peptide-N (g)	139	178
peNDF lbs	-2.0	84
LYS (g)	17.9	109.8
MET (g)	20.0	130.9
Ca (g)	46.70	159
P (g)	-1.36	98
Mg (g)	3.61	145
K (g)	84.96	137

Total ME Avail. (Mcal/day)	61.41
ME Milk Prod (lbs/day)	72.5
MP Milk Prod (lbs/day)	86.0
MUN (mg/dl)	11.6
Urea Cost (Mcal)	0.31
Rumen pH	6.24
Milk:Feed	1.40
IOFC ($/hd)	15.22
IOpurFC ($/hd)	14.81

Excretion	
Fecal (lbs)	90
Urine (lbs)	48
Total Manure (lbs)	139
Fecal N (g)	229
Urine N (g)	203
Total Manure N (g)	432
Productive N:Total N	0.33:1
Productive N:Urinary N	1.00:1
Manure N:Total N	0.67:1
Fecal P (g)	49.4
Urine P (g)	1.0
Total Manure P (g)	50.4
Productive P:Total P	0.44:1
Manure P:Total P	0.56:1
CH4 (Mcal) / CH4 (L)	5.86 / 640.48

Diet Concentrations	
NFC (%DM)	40.8
CHO Ferm. (%DM)	42.7
CHO Ferm. (%CHO)	62.1
NDF Ferm. (%DM)	13.6
NDF Ferm. (%NDF)	47.4
Starch Ferm. (%DM)	18.6
Starch Ferm. (% Starch)	72.3
Sol. Fiber Ferm. (%DM)	7.6
Sol. Fiber Ferm. (% Sol. Fiber)	86.9
Sugar Ferm. (%DM)	2.9
Sugar Ferm. (% Sugar)	69.7
Sugar (A4) (%DM)	4.2
Starch (B1) (%DM)	25.7
Sol Fiber (B2) (%DM)	8.8
Ferm. Fiber (B3) (%DM)	21.1
Lig * 2.4 (C) (%DM)	7.6
aNDFom (%DM)	28.68
Forage NDF (%NDF)	71.76
Forage NDF (%FBW)	0.97
EE (%DM)	5.0
LCFA (%DM)	4.5
CP (%DM)	16.59
RDP (%DM)	8.67
LYS (%MP)	6.98
MET (%MP)	2.95
LYS:MET	2.37
TDN (%DM)	68.5
ME (Mcal/lb)	1.18
NEl (Mcal/lb)	0.76
Forage (%DM)	54.1
DM (%)	63.8
DCAD1 (meq/kg)	273
DCAD2 (meq/kg)	263

Ration Fed				
Ingredient	$/hd	%DM	DM lbs/day	AF lbs/day
UNL Corn Silage 2018 2019	0.00	41.5	17.9	43.0
Alfalfa Hay UNL 2018 2019	0.00	87.5	9.1	10.4
Wheat Straw 5 CP 79 NDF	0.00	92.0	1.1	1.2
Dakota Gold	0.00	88.7	7.609	8.575
Corn Grain Ground Fine	0.00	88.0	7.684	8.732
Soybean Meal 47.5 Solvent	0.00	90.0	3.372	3.746
Soybean Hulls Ground	0.00	91.0	0.662	0.727
Magnesium Ox	0.00	99.5	0.0206	0.0207
Calcium Phosphate Di (Dical)	0.00	99.5	0.0800	0.0804
Sodium Bicarbonate	0.00	99.5	0.6500	0.6533
Trace Mineral Premix	0.00	99.5	0.0200	0.0201
Vitamin Premix 1	0.00	99.5	0.0220	0.0221
Energy Booster 100	0.00	99.4	0.871	0.876
Molasses Cane	0.00	73.0	0.688	0.942
Smartamine M	0.00	98.0	0.086	0.088
AjiPro L v3	0.00	97.0	0.275	0.283
Calcium Carbonate	0.00	99.5	0.6500	0.6533
Salt White	0.00	99.5	0.2500	0.2513
Beet Pulp Shreds	0.00	91.0	0.856	0.941
Totals	0.00	63.8	51.8453	81.2262

Cost/ton As-Fed: $0.00

APPENDIX E: 2020 POET RESEARCH PRESENTATION

We can pellet DDGS, but does it change DDGS?

Kirby Krogstad, PAS

1

About me!

- Raised on dairy farms in Baltic, SD and Eyota, MN
- B.S from South Dakota State University
- Dairy cattle judge, golfer, and politics junky

2

My Journey with PNUT ☺

3

Feeding the ProPellet

•"… One of the speakers at the Southwest Nutrition Conference made the statement that we cannot make a 100% DDGS pellet"

•Wrong again!

DakotaGold PRO PELLET

4

Questions to Answer…

- Does pelleting Dakota Gold change
  - feeding value?
  - eating behavior of the cow?
  - the cow's rumen environment?
- Does adding straw to the diet slow passage rate?

5

Hypothesis

- ***Pelleting DDGS would not affect passage rate, but it would decrease nutrient digestibility.***
- ***Increasing forage and adding straw would slow passage and increase digestibility.***

6

How we did it!

The diets

Item	Treatment			
	Loose, Low Forage	Pellet, Low Forage	Loose, High Forage	Pellet, High Forage
Ingredient, % DM				
Corn silage	31.6	31.6	34.9	34.9
Alfalfa hay	15.3	15.3	18.0	18.0
Wheat straw	0	0	2.0	2.0
Dakota Gold	15.0	0	15.0	0
Dakota Gold ProPellet	0	15.0	0	15.0
Grain Mix	38.1	38.1	30.1	30.1

7

How we did it!

Nutrients of the diets

Item	Treatment			
	Loose, Low Forage	Pellet, Low Forage	Loose, High Forage	Pellet, High Forage
Chemical composition, % DM				
DM	61.3	61.3	59.7	59.9
CP	16.8	16.8	16.6	16.6
NDF	30.5	30.5	30.7	30.7
Digestible NDF, % of NDF	59.0	58.3	53.3	53.8
Starch	23.1	23.0	25.0	25.0
Total Fatty Acids	4.9	4.9	4.9	4.9

8

How we did it!

9

Dakota Gold vs. ProPellet

Item	Dakota Gold		ProPellet	
	Mean	SD	Mean	SD
DM, % as-is	91.5	0.23	90.7	0.81
CP	32.3	0.24	32.1	0.46
ADF	11.3	1.15	11.0	1.82
NDF	31.0	1.23	31.0	1.78
aNDFom	30.6	1.36	30.6	1.84
dNDF30, %NDF	70.4	12.85	73.9	14.11
dNDF240, %NDF	86.4	4.18	87.4	4.08
ADICP	1.26	0.18	1.09	0.23
NDICP	2.74	0.22	2.79	0.21
Lignin	2.14	0.57	1.83	0.95

Both indicate fiber digestibility

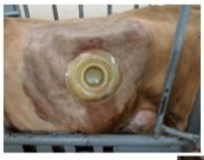
"Lignin is the most significant factor limiting the availability of plant cell wall material....."
-Van Soest, 1994

10

How we did it!

The cows....

- 7 rumen cannulated cows were fed for four months
- Fed a different diet for each month



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How we did it!

Crossover design

Cow	Month 1	Month 2	Month 3	Month 4
		Loose, Low Forage		
			Pellet, Low Forage	
	Loose, High Forage			
				Pellet, High Forage

12

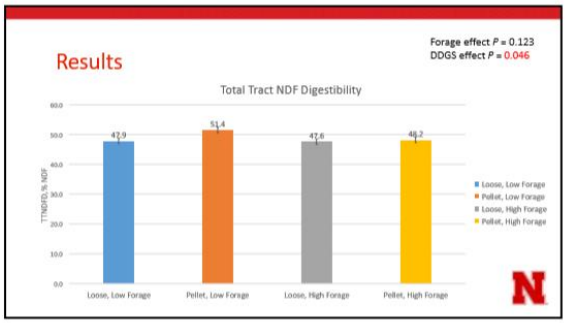
13

Results!

Eating, ruminating, and sorting

Item	Treatment				SEM	*P* – Value		
	Loose, Low forage	Pellet, Low forage	Loose, High forage	Pellet, High forage		F	D	F×D
Eating, min	206	235	211	236	19.8	0.880	0.114	0.911
Ruminating, min	421	413	468	440	49.4	*0.080*	0.390	0.624
Total chewing, min	629	648	679	673	57.0	0.145	0.810	0.624
Sorting index, %								
DM								
>19.0 mm	101.7	100.3	98.5	98.6	2.93	0.174	0.739	0.671
8.0–19.0 mm	100.1	103.3	99.6	102.8	1.56	0.589	*0.003*	0.983
1.18–8.0 mm	99.9	89.4	100.2	92.4	3.13	0.481	*0.001*	0.582
<1.18 mm	100.8	102.6	100.9	102.4	0.88	0.907	*0.060*	0.897

14



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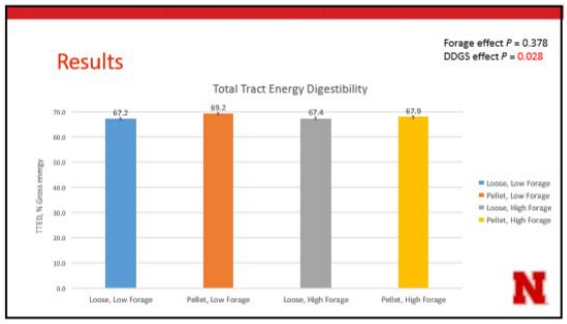

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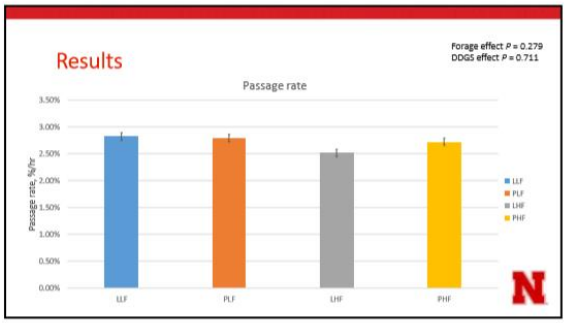


17

Results

Milk Production

Item	Treatment				SEM	P-Value		
	Loose, Low Forage	Pellet, Low Forage	Loose, High Forage	Pellet High Forage		F	D	F×D
Milk yield, kg/d	28.3	28.4	26.8	27.8	1.22	0.144	0.414	0.557
ECM[2], kg/d	34.7	34.0	32.9	34.2	1.02	0.310	0.719	0.227
ECM/DMI	1.71	1.74	1.71	1.73	0.058	0.925	0.410	0.946
Fat, %	4.85	4.70	4.96	4.93	0.283	0.110	0.368	0.579
Fat, kg/d	1.36	1.32	1.32	1.35	0.056	0.778	0.911	0.323
Protein, %	3.83	3.72	3.72	3.81	0.133	0.885	0.829	0.221
Protein, kg/d	1.08	1.05	0.99	1.05	0.035	0.089	0.531	*0.081*
BCS[3]	3.13	3.11	3.15	3.11	0.134	0.701	0.426	0.763

18

Conclusions

Feeding ProPellet was successful!

- Pelleting increased sorting behavior
- Pelleting increased NDF and energy digestibility
- Forage concentration did not affect production when feeding pellets
- Milk production was not affected by pelleting

19

Benefits of Pelleting?

16% increase!

- Reducing shrink = cost savings for producer
- Increase density = more product on a truck
- Increase digestibility = more energy for the cow

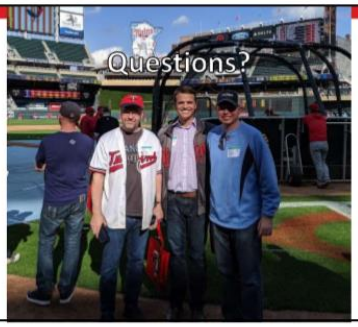
20

Next steps in DDGS research…

Where can we continue to improve and learn?

- Does pelleting improve NDF digestibility?
  - Lignin? Ferulic Acids? Something else?
- Robots? Will the ProPellet be a viable option for robot feeding?
- Can we isolate and separate certain fatty acids from DDGS?

21



22

APPENDIX F: 2020 ADSA ANNUAL MEETING PRESENTATION

The effects of feeding pelleted dried distillers' grains with different concentrations of forage on milk production, nutrient digestibility, passage rate, rumen characteristics, and chewing behavior of lactating Jersey dairy cows

K. C. Krogstad , K. J. Herrick, P. J. Kononoff

What we learned:

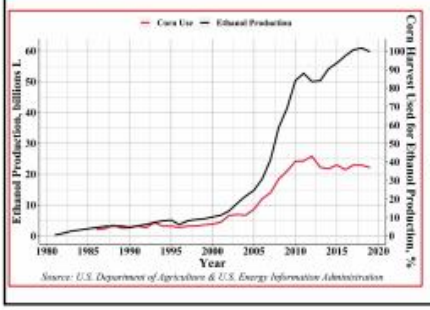
Forage

Increased forage → increased rumen pH, decreased total VFA, slowed passage, increased starch digestibility

DDGS

Pelleted DDGS → increased TTNDFD, CP, and energy digestibility, increased milk protein when fed with HF

1



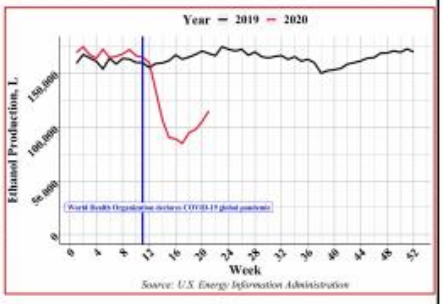
Introduction

- Ethanol is an important part of agriculture
- Ethanol production provides a steady supply of DDGS

2

The Pandemic

- COVID – 19 pandemic has drastically ethanol production and DDGS supply
- Production declined 33% and caused plant closures across the Midwest (Voegele, 2020ab)



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Pelleting DDGS

- Recently, a pelleting process was developed for DDGS (Yoder et al., 2019)
- Allows for 100% DDGS pellet – no additional feeds or binders

4

Objectives

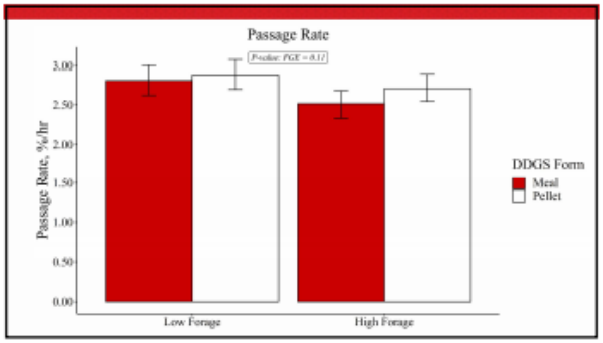
- Evaluate whether pelleted DDGS (**pDDGS**) affects rumen kinetics, rumen characteristics, digestibility, rumination, sorting, or lactation performance compared to meal DDGS **(mDDGS)**
- Evaluate whether mDDGS or pDDGS interacts with forage concentration to affect rumen kinetics, rumen characteristics, digestibility, rumination, sorting or lactation performance

5

Hypothesis

- pDDGS will have no affect on rumen kinetics, rumen characteristics, digestibility, rumination, sorting, or lactation performance but increasing forage concentration will slow passage rate, increase NDF digestibility, and increase milk production of lactating Jersey cows.

6



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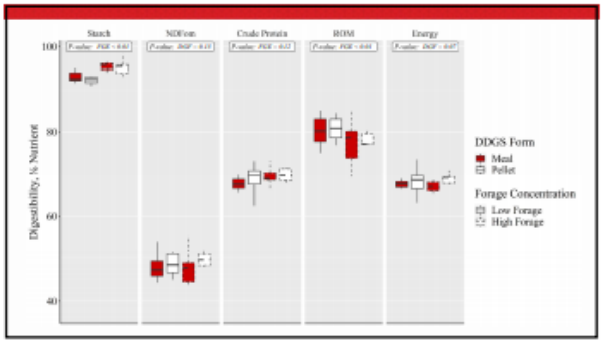


14

Results – Lactation Performance

Item	Treatment				SEM	P-Value		
	Low Forage		High Forage			F	D	F×D
	mDDGS	pDDGS	mDDGS	pDDGS				
Milk yield, kg/d	28.3	28.4	26.8	27.8	1.22	*0.14*	0.41	0.56
ECM[4], kg/d	34.7	34.0	32.9	34.2	1.02	0.31	0.72	0.23
ECM/DMI	1.71	1.74	1.71	1.73	0.058	0.93	0.41	0.95
Fat, %	4.85	4.70	4.96	4.93	0.283	*0.11*	0.37	0.58
Fat, kg/d	1.36	1.32	1.32	1.35	0.056	0.78	0.91	0.33
Protein, %	3.83	3.72	3.72	3.81	0.133	0.89	0.83	0.22
Protein, kg/d	1.08	1.05	0.99	1.05	0.035	0.09	0.53	*0.08*
BCS[5]	3.13	3.11	3.15	3.11	0.134	0.70	0.43	0.76

15

Conclusion

- Forage
  - Increased forage → increased rumen NH_3, increased rumen pH, decreased total VFA, slowed passage, increased starch, increased CP, and reduced ROM digestibility
- DDGS
  - Pelleted DDGS → increased sorting, increased NDF and energy digestibility, increased milk protein when fed with HF

- ***Final thought***: further research should be conducted to evaluate effect of pelleting on NDFD of fibrous feeds

16

Calculations and Statistics

- Passage rate calculation (Robinson et al., 1987)
  - $Passage\ rate\ (kp), \%/hr = \left[\frac{1}{24}\right] \times [fecal\ output\ (kg/d) \div rumen\ pool\ size\ (kg)] \times 100$
- Intake, digestibility, production, passage rate, sorting, rumination
  - GLIMMIX with fixed effects of forage concentration, DDGS form, and their interaction as well as random effects of cow & period
- Rumen pH, NH_3, VFA
  - MIXED as repeated measures with fixed effects of forage concentration, DDGS form, and their interaction as well as random effects of cow & period

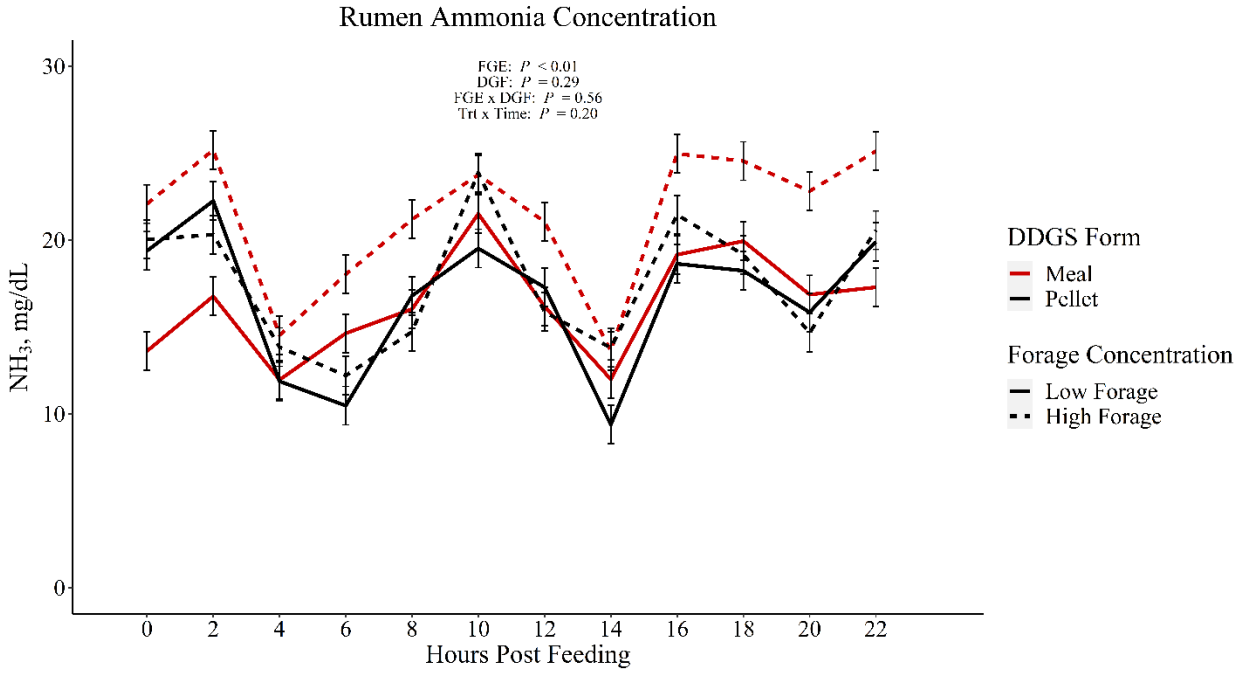
11

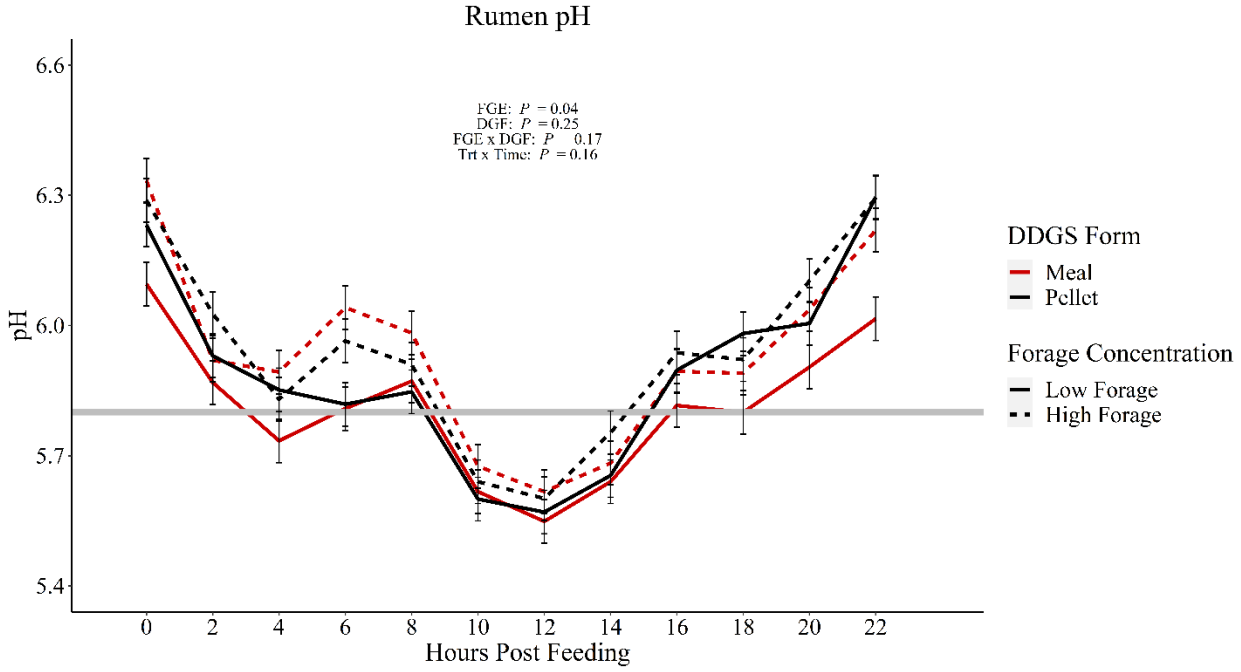
Results – rumination and sorting

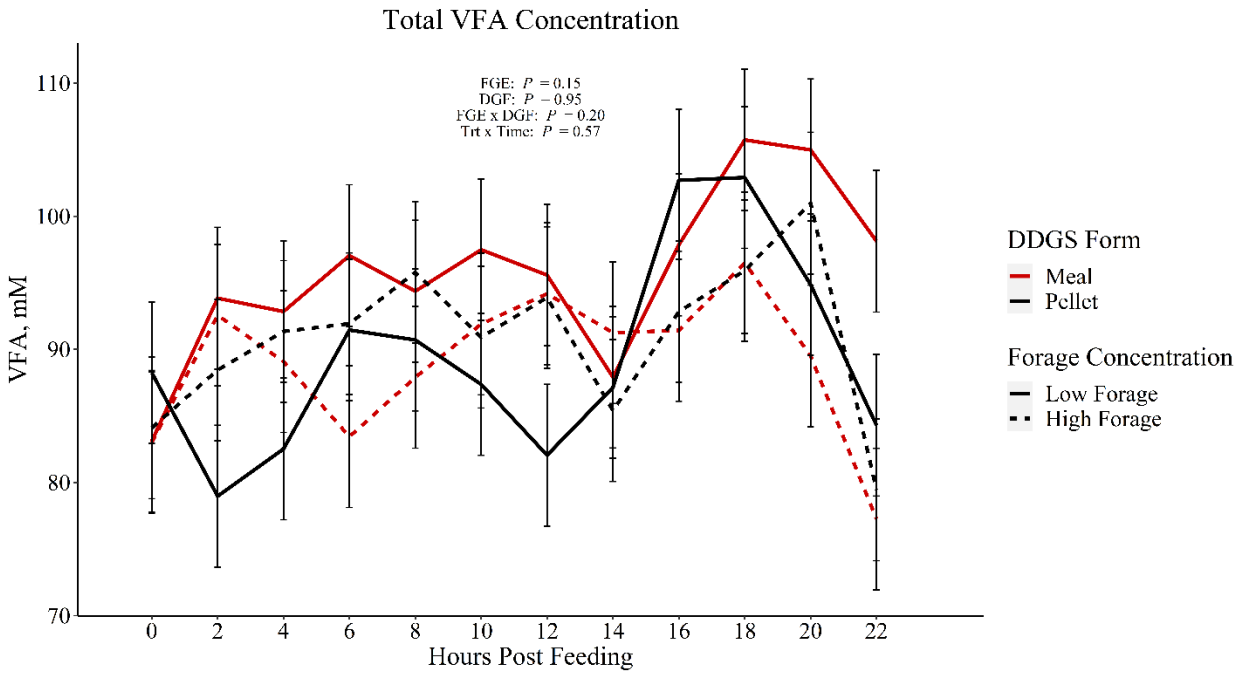
Item	Treatment				SEM	P - Value		
	Low Forage		High Forage			FGE	DGF	FGE×DGF
	mDDGS	pDDGS	mDDGS	pDDGS				
Eating, min	206	235	211	236	19.8	0.88	*0.11*	0.91
Ruminating, min	421	413	468	440	49.4	*0.08*	0.39	0.62
Total chewing, min	629	648	679	673	57.0	*0.15*	0.81	0.62
Sorting index, %								
>19.0 mm	101.7	100.3	98.5	98.6	2.93	0.17	0.74	0.67
8.0–19.0 mm	100.1	103.3	99.6	102.8	1.56	0.59	*0.03*	0.98
1.18–8.0 mm	99.9	89.4	100.2	92.4	3.13	0.48	*<0.01*	0.58
<1.18 mm	100.8	102.6	100.9	102.4	0.88	0.91	*0.06*	0.90

12

APPENDIX G: RUMEN pH, VFA CONCENTRATION, AND AMMONIA CONCENTRATION OVER TIME FOR COWS FED 15% DDGS IN MEAL OR PELLET FORM WITH 45% OR 55% FORAGE






Total VFA Concentration
FGE: P = 0.15
DGF: P = 0.95
FGE x DGF: P = 0.20
Trt x Time: P = 0.57
VFA, mM
110
100
90
80
70
0
2
4
6
8
10
12
14
16
18
20
22
Hours Post Feeding
DDGS Form
Meal
Pellet
Forage Concentration
Low Forage
High Forage

APPENDIX H: JOURNAL OF DAIRY SCIENCE REFLECT STATEMENT

Checklist for REFLECT statement: Reporting guidelines For randomized control trials in livestock and food safety. ***Bold text are modifications from the CONSORT statement description (Altman DG et al . Ann Intern Med 2001; 134(8):663-694).***

Paper section and topic	**Item**	**Descriptor of REFLECT statement item**	**Reported on Page #**
Title & Abstract	1	How **study units** were allocated to interventions (eg, "random allocation," "randomized," or "randomly assigned"). **Clearly state whether the outcome was the result of natural exposure or was the result of a deliberate agent challenge.**	2,3
Introduction Background	2	Scientific background and explanation of rationale.	4-5
Methods Participants	3	Eligibility criteria **for owner/managers and study units at each level of the organizational structure,** and the settings and locations where the data were collected.	6,7
Interventions	4	Precise details of the interventions intended for each group, **the level at which the intervention was allocated,** and how and when interventions were actually administered.	6
	4b	**Precise details of the agent and the challenge model, if a challenge study design was used.**	NA
Objectives	5	Specific objectives and hypotheses. **Clearly state primary and secondary objectives (if applicable).**	5
Outcomes	6	Clearly defined primary and secondary outcome measures and the levels at which they were measured, and, when applicable, any methods used to enhance the quality of measurements (eg, multiple observations, training of assessors).	In Hypothesis
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules. **Sample-size considerations should include sample-size determinations at each level of the organizational structure and the assumptions used to account for any non-independence among groups or individuals within a group.**	6
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence **at the relevant level of the organizational structure**, including details of any restrictions (eg, blocking, stratification)	6

Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence **at the relevant level of the organizational structure**, (eg, numbered containers ~~**or central telephone**~~**),** clarifying whether the sequence was concealed until interventions were assigned.	6
Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled **study units,** and who assigned **study units** to their groups **at the relevant level of the organizational structure**.	6
Blinding (masking)	11	Whether or not ~~**participants**~~ those administering the interventions, **caregivers** and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated**. Provide justification for not using blinding if it was not used.**	NA
Statistical methods	12	Statistical methods used to compare groups for all outcome(s); Clearly state the level of statistical analysis **and methods used to account for the organizational structure, where applicable;** methods for additional analyses, such as subgroup analyses and adjusted analyses.	8,9
Results **Study** flow	13	Flow **of study units** through each stage **for each level of the organization structure of the study** (a diagram is strongly recommended). Specifically, for each group, report the numbers of **study units** randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	6
Recruitment	14	Dates defining the periods of recruitment and follow-up.	NA
Baseline data	15	Baseline demographic and clinical characteristics of each group, **explicitly providing information for each relevant level of the organizational structure. Data should be reported in such a way that secondary analysis, such as risk assessment, is possible.**	6
Numbers analyzed	16	Number **of study units** (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat." State the results in absolute numbers when feasible (eg, 10/20, not 50%).	25-31
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, **accounting for each relevant level of the organizational structure**, and the estimated effect size and its precision (e.g., 95% confidence interval)	9-17

Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	NA
Adverse events	19	All important adverse events or side effects in each intervention group.	NA
Discussion Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision, and the dangers associated with multiplicity of analyses and outcomes. **Where relevant, a discussion of herd immunity should be included. If applicable, a discussion of the relevance of the disease challenge should be included.**	12-21
Generalizability	21	Generalizability (external validity) of the trial findings.	9-17
Overall evidence	22	General interpretation of the results in the context of current evidence.	17,18

APPENDIX I: DISTILLERS GRAINS TECHNOLOGY COUNCIL POSTER

Dry matter, protein and fiber digestion of DDGS with varying fat content from different ethanol plants.

K. C. Krogstad*[1], J. L. Anderson[1], J. S. Osorio[1], K. J. Herrick[2]

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[2]POET Nutrition, Sioux Falls, SD

INTRODUCTION

- Distillers dried grains with solubles (DDGS) is a popular dairy cattle feed and a co-product of the ethanol industry.
- Popularity stems from its high content of rumen undegradable protein which provides metabolizable protein in dairy cattle diets. It is also a good source of digestible fiber.
- Due to the nature of the changing ethanol industry and evolving processing methods, DDGS have changed in quality and digestibility.

HYPOTHESIS

- We hypothesized that nutrient digestibility would differ by source and fat content of the DDGS

OBJECTIVES

- Our objectives were to 1) determine if the fat content or source of DDGS impacted the digestibility of DM, NDF and CP, 2)see if DM and NDF digestibility differed between types of bags used for ruminal incubation, 3) compare in situ and in vitro methods for evaluating digestibility.

MATERIALS AND METHODS

In Situ:

- Three primiparous, ruminally cannulated, cows were used to incubate 7 feedstuffs to determine their respective digestibilities. The cows weighed 601 ± 17.0 kg and averaged 33.2 ± 3.41 kg/d of milk while on trial.
- The cows were housed in individual box stalls which allowed for feed intake measurements and constant access to fresh water. They were bedded with wheat straw and fed a TMR on an ad libitum basis.
- Seven feedstuffs were evaluated including 6 DDGS samples (DG1 to DG6) and a soybean meal (SBM) sample. The SBM served as the control.
- Five grams of feed were placed in 10 x 20 cm Dacron bags and 0.5 g in F57 fiber bags. The bags ruminally incubated for 0, 2, 4, 8, 16, 24, 48, 72, and 120 h.
- Crude protein digestibility was determined using the method from Gargallo et al., 2006.
- Degradation rates were analyzed using NLIN procedures in SAS 9.4. Means were compared using Tukey's test. Significance was declared at $P < 0.05$.

In Vitro:

- An Ankom RF Gas Production system (Ankom Technology, Macedon, NY) was used to compare 6 samples of DDGS (DG1 to DG6) and 1 soybean meal (SBM) which served as a control.
- The bottles held 250 mL of McDougall's buffer and 50 mL of rumen fluid from 3 lactating Holstein cows, which was strained through four layers of cheese cloth.
- F57 filter bags were used and they held 1 gram of feed. Each bottle held 2 bags of feed along with 1 blank bag.
- Once the bottles were filled, they were purged with CO_2 to create an anaerobic environment in the bottle.
- Fermentation bottles were placed in a water bath at 39°C and shaking at 45 movements per min for 24 h. Gas production was measured every 10 min.
- Fluid samples were collected from each bottle at 24 h for VFA and ammonia analysis. Bags were rinsed and dried to determine DM loss and NDF digestibility.

RESULTS

Table 1. Nutrient composition of DDGS from different ethanol plants and soybean meal

	Feedstuff						
Item[1]	DG1	DG2	DG3	DG4	DG5	DG6	SBM
DM, %	92.1	93.2	92.9	91.9	91.5	92.0	90.4
CP	29.5	28.0	27.4	29.7	28.5	30.2	49.5
Ross RUP	58.1	71.5	71.6	63.8	65.9	65.2	36.6
NDF	29.3	34.6	34.5	31.7	28.7	29.2	9.39
ADF	16.2	13.6	10.8	11.8	16.0	13.9	9.24
EE, Pet.	**7.62**	**9.52**	**10.5**	**5.63**	**5.79**	**4.97**	**0.85**
EE, Diethyl	**12.6**	**14.9**	**14.7**	**8.90**	**9.28**	**9.70**	**2.92**
NFC[2]	27.0	25.9	26.3	31.5	35.6	33.8	67.6
NFC[3]	22.4	20.5	22.1	28.2	32.1	29.1	64.7
Ash	4.90	5.34	4.98	6.13	5.58	6.32	6.63
S	0.87	0.52	0.56	0.89	0.66	0.87	0.41

[1] % of DM unless otherwise noted; [2]NFC= 100-(%NDF + % CP + EE + % Ash) (NRC, 2001), using Petroleum Ether Extract values; [3]NFC= 100-(%NDF + % CP + EE + % Ash) (NRC, 2001), using Diethyl Ether Extract values.

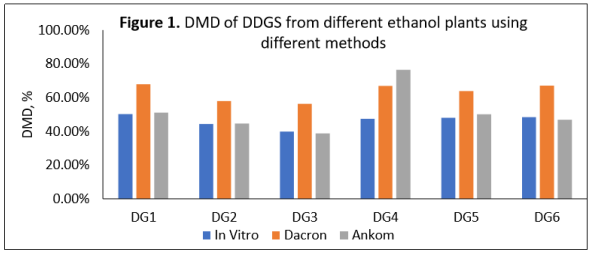


Table 2. Ruminal DM and NDF degradation of test feeds ruminally incubated in Dacron bags.

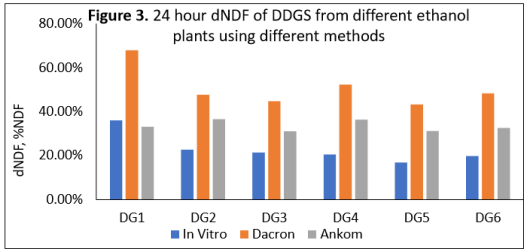
	Feedstuff								
Item[1]	DG1	DG2	DG3	DG4	DG5	DG6	SBM	SEM	*P*-value
DM dis., %									
A[2]	38.74^{b}	31.63^{d}	26.94^{d}	41.54^{a}	43.05^{a}	35.62^{c}	29.42^{d}	1.12	**<0.01**
B[3]	56.67^{c}	63.39^{b}	70.88^{a}	57.39^{c}	53.91^{d}	58.00^{c}	70.35^{a}	1.20	**<0.01**
C[4]	4.59^{a}	4.97^{a}	2.18^{bc}	1.07^{bc}	3.04^{b}	6.38^{a}	0.23^{c}	1.20	**0.03**
K_d[5], %/h	3.42^{b}	2.64^{b}	2.48^{b}	2.86^{b}	2.92^{b}	3.25^{b}	7.21^{a}	0.57	**<0.01**
RDDM[6]	58.36^{b}	49.74^{d}	46.36^{e}	59.13^{b}	59.88^{b}	55.07^{c}	66.00^{a}	1.90	**<0.01**
NDF Dis., %									
K_d[7], %/h	8.89^{ab}	6.83^{ab}	4.49^{b}	3.83^{b}	13.62^{a}	5.44^{b}	-	1.70	**0.01**
RDNDF[8]	33.10^{ab}	34.66^{ab}	31.00^{b}	40.81^{a}	34.69^{ab}	34.98^{ab}	-	2.46	**0.04**
RUNDF[9]	66.99^{ab}	65.34^{ab}	69.00^{a}	59.19^{b}	65.19^{ab}	65.02^{ab}	-	2.46	0.04

[1] Units expressed in % of DM or NDF unless otherwise noted; [2] Soluble DM.; [3] Potentially degradable DM.; [4] Undegradable DM.; [5] Rate of DM degradation.; [6] Ruminally degradable DM.; [7]Rate of NDF degradation; [8]Ruminally degradable NDF; [9]Ruminally undegradable NDF; [abcd] Values with unlike superscripts differ by $P < 0.05$ using Tukeys.

Table 3. Ruminal and intestinal CP degradability of DDGS from different ethanol plants and soybean meal

	Feedstuff								
Item	DG1	DG2	DG3	DG4	DG5	DG6	SBM	SEM	*P*-value
CP dis., %									
A[2]	41.1^{a}	25.0^{c}	20.0^{d}	39.5^{a}	41.1^{a}	30.7^{b}	20.6^{d}	1.56	**<0.01**
B[3]	55.5^{c}	68.7^{b}	77.2^{a}	60.5^{bc}	58.0^{c}	61.7^{bc}	79.4^{a}	2.39	**<0.01**
C[4]	3.4^{abc}	6.3^{ab}	2.8^{abc}	0.01^{c}	0.9^{bc}	7.6^{a}	0.1^{c}	1.77	**0.04**
K_d[5], % h	3.6^{b}	2.7^{b}	2.7^{b}	2.7^{b}	2.6^{b}	3.3^{b}	7.0^{a}	0.55	**<0.01**
RDP[6], % of CP	61.1^{a}	44.9^{cd}	41.8^{c}	57.7^{ab}	58.0^{ab}	51.8^{bc}	61.4^{a}	2.58	**<0.01**
RUP[7], % of CP	38.9^{d}	55.1^{ab}	58.2^{a}	42.3^{cd}	42.0^{cd}	48.2^{bc}	38.6^{d}	2.58	**<0.01**
IDP[8], % of RUP	59.1^{b}	48.7^{b}	56.0^{b}	68.0^{ab}	72.8^{ab}	62.8^{b}	94.0^{a}	4.80	**<0.01**
IADP[9], % of CP	22.7	26.7	32.0	28.3	30.3	30.2	37.3	3.92	**0.26**
TDP[10], % of CP	84.1^{bc}	72.3^{d}	75.0^{cd}	86.7^{b}	88.6^{ab}	82.2^{bc}	97.6^{a}	2.08	**<0.01**

[1] Ruminal CP disappearance of test feeds ; [2] Soluble CP; [3] Potentially degradable CP; [4] Undegradable CP; [5] Rate of ruminal CP degradation; [6] Ruminally degradable protein (RDP); [7] Ruminally undegradable protein (RUP); [8] Estimated intestinal digestible protein (IDP) after 12 h rumen incubation and 1 h pepsin and 24 h pancreatin digestion; [9] Intestinally absorbable digestible protein (IADP) = Rumen undegradable protein (RUP, % of CP) × intestinal CP digestion (% of RUP); [10] Total Digestible Protein (TDP) = RDP + IADP; [abcde] Values with unlike superscripts differ by $P < 0.05$ using Tukey's test.



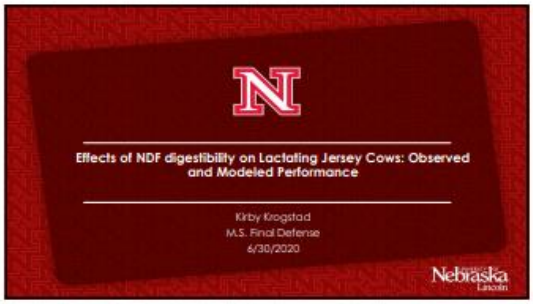
SUMMARY AND CONCLUSIONS

- The source and processing effected the nutrient profile in regards to fat, fiber, and crude protein.
- DDGS have changed over time, particularly in portion of soluble dry matter. Results show an increase of 8-18% in soluble DM (Cao et. al. 2009).
- DG1, DG4, and DG5 had the greatest RDDM when incubated in both bag types indicating that source and ethanol process effect DDGS digestibility.
- Fiber digestion was greatest in DG4 and least in DG3, with other DG intermediary when using residue from the Dacron bags, but was similar among all DG when F57 were used for incubation.
- There was variability of RUP and TDP by source of the DDGS.
- Further investigation needs to be done as to the proper method for testing ruminal fiber digestibility of DG as bag type did impact digestibility values.
- Overall, DDGS do vary in digestibility, but it may be more related with other processing effects rather than total fat content.

ACKNOWLEDGEMENTS

This work was supported by the SDSU College of ABS through an Undergraduate Research Engagement Award and POET Nutrition in Sioux Falls, SD.

APPENDIX J: FINAL DEFENSE PRESENTATION



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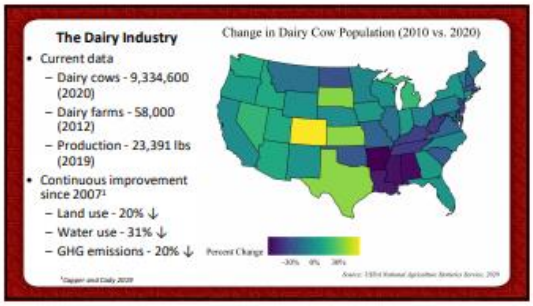

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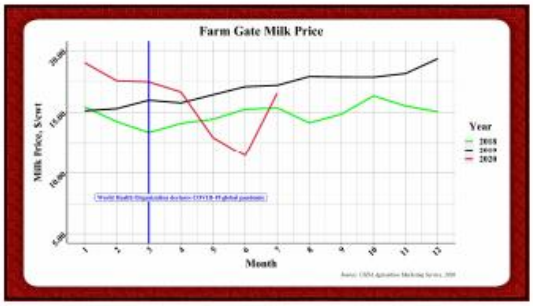

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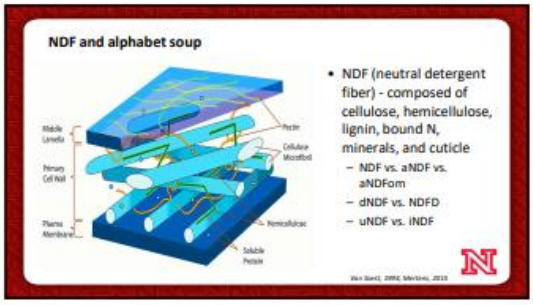

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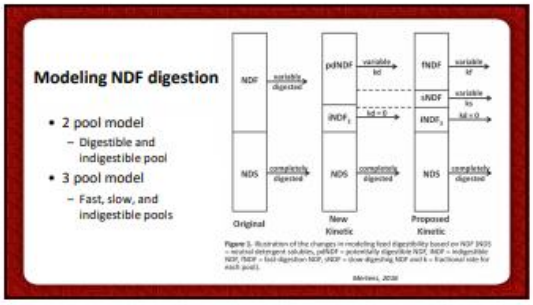


6

USE OF 30 HOUR IN VITRO NDF DIGESTIBILITY OF FEEDSTUFFS IN DAIRY RATION FORMULATION SOFTWARE: EVALUATION OF PREDICTIONS FOR MILK AND METHANE PRODUCTION IN LACTATING DAIRY COWS

K. C. Krogstad, D. L. Morris, P. J. Kononoff

7

Introduction

- Ration formulation software enable formulation of least cost rations with many ingredients
- Many possible nutrient inputs
- Correct determination of nutrients may improve predictions and precision feeding

8

NDF digestibility

- Cornell Net Carbohydrate and Protein System (CNCPS) has unique NDF system
- Allows input of in vitro NDFD at different times
- Rate (kd) of NDFD predicted from in vitro estimates of NDFD

9

Rate (kd) of NDFD in CNCPS

- Increase kd of NDFD increased model predicted milk output by ~3 kgs

10

Objectives

- To determine if including lab generated NDFD (**DIG**) to calculate kd of NDF and using it in place of default feed library values (**CON**) affects the prediction of ME allowable milk and methane production of lactating dairy cattle.

Hypothesis

- Inclusion of lab determined NDFD to calculate kd of NDF will improve the CNCPS prediction of ME allowable milk and methane production when compared to observed performance.

11

Materials and Methods

- Compiled data from 8 past energy balance experiments
  - Each experiment created as an individual farm
  - Each treatment created as separate pen of cattle
- Nutrient analysis of 34 forages and fibrous feeds
- Conducted 30-hour in vitro on each ingredient to estimate NDFD

12

Materials and Methods

- 2 sets of ingredients created
  - CON – feed library kd of NDFD
  - DIG – kd estimated from 30-hour in vitro NDFD
- Recorded DMI, milk, methane, and carbon dioxide production for each treatment group
- Predictions were compared to observed values
- Model fit determined using RMSE and CCC with lmer function of R (v 3.5.2)

13

Animal and diet summaries (8 experiments)

Item	n	Mean	SD	Min	Max
DIM	32	176	58.1	119	373
BW, kg	32	685	73.9	426	697
BCS	32	3.30	0.19	2.98	3.78
DMI, kg	32	18.8	2.26	15.0	25.1
Milk, kg	32	25.8	6.00	16.8	38.4
Milk fat, %	32	5.28	0.825	3.57	6.23
Milk protein, %	32	3.48	0.349	2.78	4.09
CH_4, L	32	413	64.2	335	566
CH_4, L/kg of milk	32	15.8	2.65	11.7	22.2
CO_2, L	32	5050	867.2	3938	7173
CO_2, L/kg of milk	32	193	31.1	153	280

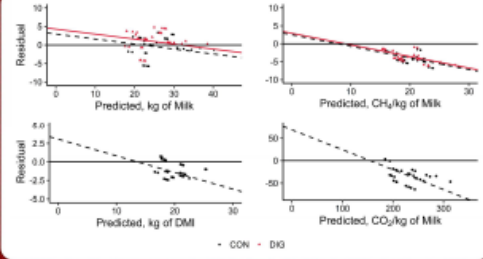
Item	n	Mean	SD	Min	Max
DM	25	62.4	6.77	53.9	76.3
CP	25	17.7	0.63	16.8	18.8
ADF	25	20.8	1.89	16.6	23.5
NDF	25	32.2	2.65	25.6	37.1
Starch	25	24.4	2.82	18.9	28.7
Crude fat	25	4.20	0.703	2.60	5.63
Ash	25	7.76	0.381	6.88	8.41
ME, Mcal/kg of DM	23	2.55	0.153	2.27	2.78

14

Model fit statistics

	ME allowable milk, kg		CH_4, L/kg of milk		DMI, kg			CO_2, L/kg of milk
Item	CON	DIG	CON	DIG	Lower Bound	Upper Bound	Mean	
Observed mean	25.8	25.8	15.8	15.8	18.8	18.8	18.8	193.3
Predicted mean	26.1	24.5	20.2	19.6	18.5	21.4	20.0	227.9
RMSE	3.77	3.23	4.84	4.26	1.08	2.96	1.65	29.7
RMSE, % mean	10.7	12.5	30.7	27.0	5.78	15.9	8.79	20.6
Mean bias, % RMSE	0.71	16.2	81.7	77.9	5.57	80.5	53.0	76.0
Slope bias, % RMSE	0.44	1.77	4.93	5.20	0.48	1.64	1.12	1.92
Mean bias	-0.23	1.30	-4.38	-3.73	0.26	-2.66	-1.20	-34.6
Slope bias	0.04	0.09	-0.36	-0.33	-0.04	-0.17	-0.08	-0.18
CCC	0.87	0.82	0.33	0.28	0.87	0.48	0.75	0.48

15



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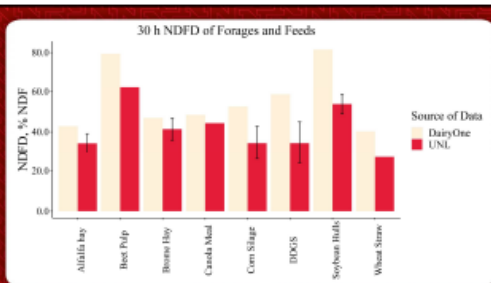


17

Conclusions

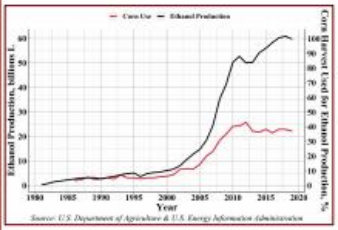
- In vitro values were low compared to commercial lab (DairyOne)
  - Recommend updating in vitro system to include continuous CO_2
- Milk production predictions not improved with NDFD
- Methane production predictions were slightly improved with NDFD
  - Overpredicted CH_4 in every instance
- Further research should be conducted to determine if uNDF240 improves model output
  - New body of research regarding uNDF240

18

THE EFFECTS OF FEEDING PELLETED DRIED DISTILLERS' GRAINS WITH DIFFERENT CONCENTRATIONS OF FORAGE ON MILK PRODUCTION, NUTRIENT DIGESTIBILITY, PASSAGE RATE, RUMEN CHARACTERISTICS, AND CHEWING BEHAVIOR OF LACTATING JERSEY DAIRY COWS

K. C. Krogstad , K. J. Herrick, P. J. Kononoff

19



Introduction

- Ethanol is an important part of agriculture
- Ethanol production provides a steady supply of DDGS

20

The Pandemic

- COVID – 19 pandemic has drastically ethanol production and DDGS supply
- Production declined 33% and caused plant closures across the Midwest (Voegele, 2020ab)

21

Pelleting DDGS

- Recently, a pelleting process was developed for DDGS (Yoder et al., 2019)
- Allows for 100% DDGS pellet – no additional feeds or binders

22

Objectives

- Evaluate whether pelleted DDGS (**pDDGS**) affects rumen kinetics, rumen characteristics, digestibility, rumination, sorting, or lactation performance compared to meal DDGS (**mDDGS**)
- Evaluate whether mDDGS or pDDGS interacts with forage concentration to affect rumen kinetics, rumen characteristics, digestibility, rumination, sorting, or lactation performance
- Evaluate whether forage concentration and straw affect passage rate

23

Hypothesis

- pDDGS will have no affect on rumen kinetics, rumen characteristics, digestibility, rumination, sorting, or lactation performance, but increasing forage concentration will slow passage rate, increase NDF digestibility, and increase milk production of lactating Jersey cows.

24

Materials & Methods

- 7 rumen cannulated Jersey cows (56 ± 10.3 DIM, and 462 ± 75.3 kg of BW)
- Housed in individual tie stalls, fed ad libitum once, and milked twice daily
- 2 × 2 factorial treatment arrangement

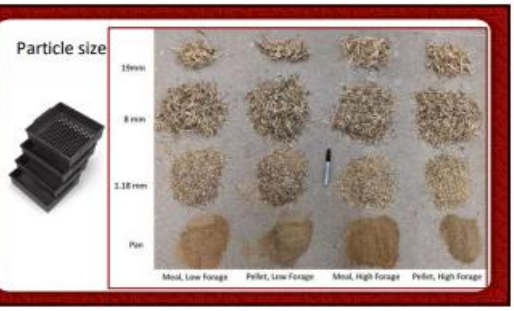
	Low Forage (LF)	High Forage (HF)
mDDGS	LF - mDDGS	HF - mDDGS
pDDGS	LF - pDDGS	HF - pDDGS

25

Item	Treatment			
	Low Forage		High Forage	
	mDDGS	pDDGS	mDDGS	pDDGS
Ingredient, % DM				
Corn silage	31.6	31.6	34.9	34.9
Alfalfa hay	15.3	15.3	18.0	18.0
Wheat straw	0	0	2.0	2.0
mDDGS	15.0	0	15.0	0
pDDGS	0	15.0	0	15.0
Grain Mix	38.1	38.1	30.1	30.1
Chemical composition, % DM				
DM	61.3	61.3	59.7	59.9
CP	16.8	16.8	16.6	16.6
NDF	30.5	30.5	30.7	30.7
NDFD, % of NDF	59.0	58.3	53.3	53.8
Starch	23.1	23.0	25.0	25.0
Total Fatty Acids	4.9	4.9	4.9	4.9
Residual organic matter	15.2	15.3	13.7	13.9

26

Particle size



27

mDDGS vs. pDDGS

- Indications of improved NDFD
- "Lignin is the most significant factor limiting the availability of plant cell wall material….."
  -Van Soest, 1994

Item	mDDGS		pDDGS	
	Mean	SD	Mean	SD
DM, % as-is	91.5	0.23	90.7	0.81
CP	32.3	0.24	32.1	0.46
ADF	11.3	1.15	11.0	1.82
NDF	31.0	1.23	31.0	1.78
aNDFom	30.6	1.36	30.6	1.84
NDFD30, %NDF	70.4	12.85	73.9	14.11
NDFD240, %NDF	86.4	4.18	87.4	4.08
ADICP	1.26	0.18	1.09	0.23
NDICP	2.74	0.22	2.79	0.21
Lignin	2.14	0.57	1.83	0.95

28

- BW – d 25, 28
- DMI, milk production, total fecal collection – d 25-28,
- Sorting, rumination = d 25
- Rumen evacuations = d 26, 27

Rumen sampling – pH, rumen NH_3, VFA

ADAPTATION

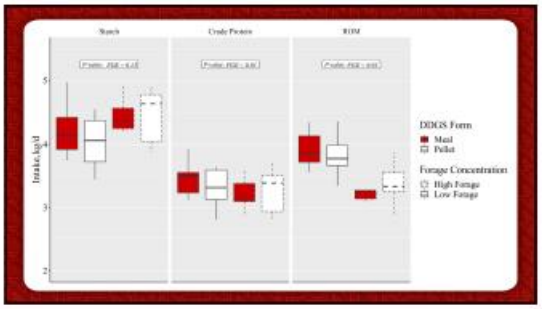
Day 0 | Day 21 | Day 25 | Day 28

29

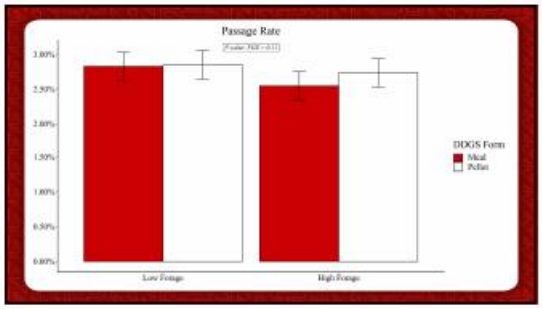
Calculations and Statistics

- Passage rate calculation (Robinson et al., 1987)
  - $Passage\ rate\ (kp), \%/hr = \left[\frac{1}{24}\right] \times [fecal\ output\ (kg/d) \div rumen\ pool\ size\ (kg)] \times 100$
- Intake, digestibility, production, passage rate, sorting, rumination
  - GLIMMIX with fixed effect of treatment, random effect of cow & period
- Rumen pH, NH_3, VFA
  - MIXED as repeated measures with fixed effect of treatment and time post feeding and random effects of cow and period

30



31



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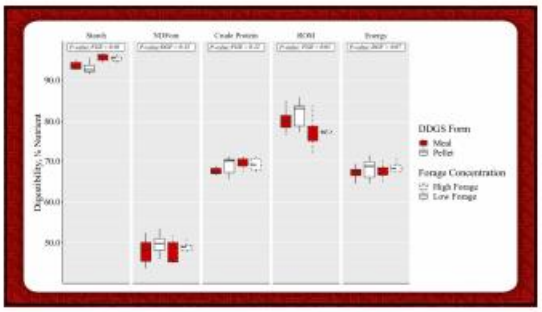

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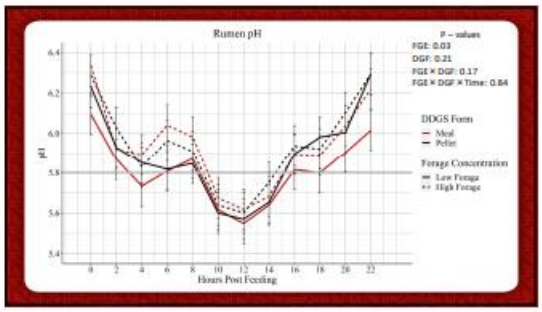
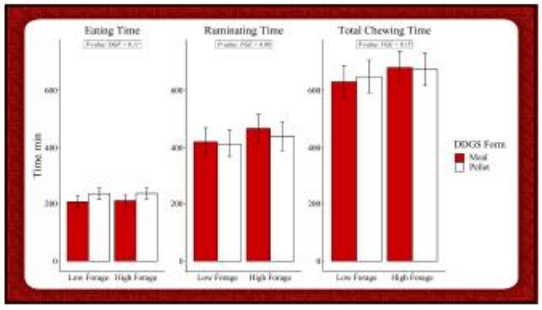


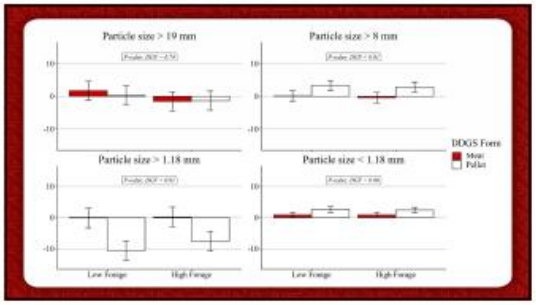
34

Item	Treatment				SEM	P - Value		
	Low Forage		High Forage			FGE	DGF	FGE×DGF
	mDDGS	pDDGS	mDDGS	pDDGS				
NH_3, mg/dL	17.0	16.6	19.8	18.5	3.22	*0.002*	0.285	0.563
Total VFA, mM	94.3	91.5	88.1	91.2	5.09	0.152	0.951	0.197
VFA, mol/100 mol								
Acetate	55.9	55.1	53.2	54.3	2.91	0.200	0.931	0.475
Propionate	23.8	21.9	21.8	23.2	1.72	0.675	0.779	*0.073*
Butyrate	11.1	12.0	10.2	10.7	0.78	*<0.001*	*0.025*	0.565
Isobutyrate	0.69	0.64	0.67	0.70	0.096	0.378	0.916	*0.047*
Valerate	1.44	1.35	1.19	1.32	0.095	*<0.001*	0.637	*0.003*
Isovalerate	1.00	0.88	0.95	1.08	0.103	*0.072*	0.967	*0.003*

35



36



37

Results – Lactation Performance

Item	Treatment				SEM	P-Value		
	Low Forage		High Forage			FGE	DGF	FGE ✖ DGF
	mDDGS	pDDGS	mDDGS	pDDGS				
Milk yield, kg/d	28.3	28.4	26.8	27.8	1.22	*0.14*	0.41	0.56
ECM[a], kg/d	34.7	34.0	32.9	34.2	1.02	0.31	0.72	0.23
ECM/DMI	1.71	1.74	1.71	1.73	0.058	0.93	0.41	0.95
Fat, %	4.85	4.70	4.96	4.93	0.283	*0.11*	0.37	0.58
Fat, kg/d	1.36	1.32	1.32	1.35	0.056	0.78	0.91	0.33
Protein, %	3.83	3.72	3.72	3.81	0.133	0.89	0.83	0.22
Protein, kg/d	1.08	1.05	0.99	1.05	0.035	0.09	0.53	*0.04*
BCS[b]	3.13	3.11	3.15	3.11	0.134	0.70	0.43	0.76

38

Conclusion

- Forage
  - Increased forage → increased rumen NH_3 , increased rumen pH, decreased total VFA, slowed passage, increased starch and CP digestibility, reduced ROM digestibility
- DDGS
  - Pelleting → increased sorting, slightly increased in vitro AND in vivo NDFD, increased energy digestibility, increased milk protein when fed with HF
- ***Final thought***: further research should be conducted to evaluate effect of pelleting on NDFD of fibrous feeds

39

Overall conclusions

- Incorporating in vitro measurements changed model predictions with mixed results
  - Need consistent methodology outlined for use of in vitro results in ration models
  - Explore CH_4 equations to improve predictions from CNCPS
  - CNCPS has improved over time
- Pelleting feed improved NDFD and increased sorting behavior
- Increasing forage concentration reduced passage rate, increased pH, and decreased VFA

40

Next steps

- Evaluate long-term fermentations (240 h) as estimate of iNDF for use in ration models
- Reliability of NIR to predict NDFD?
- Investigate effects of pelleting on NDFD
  - Look into other fibrous feeds like soyhulls, beet pulp, wheat middlings, corn residue, wheat straw
  - Effects of pelleting settings like conditioning time, temperature, and die size
- Continue investigating low forage diets

41

Thank you…

- ***Advisor***: Dr. Paul Kononoff
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42

43