12-2021

Feeding a New Corn Milling Coproduct to Lactating Dairy Cattle; Examination of Whole Animal Energy and Nitrogen Balance

Addison Carroll
University of Nebraska-Lincoln, acarroll11@huskers.unl.edu

FEEDING A NEW CORN MILLING COPRODUCT TO LACTATING DAIRY CATTLE; EXAMINATION OF WHOLE ANIMAL ENERGY AND NITROGEN BALANCE

by

Addison Lee Carroll

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfilment of Requirements

For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Paul J. Kononoff

Lincoln, Nebraska

December, 2021

FEEDING A NEW CORN MILLING COPRODUCT TO LACTATING DAIRY CATTLE; EXAMINATION OF WHOLE ANIMAL ENERGY AND NITROGEN BALANCE

Addison Lee Carroll, M.S.

University of Nebraska, 2021

Advisor: Paul J. Kononoff

New coproducts are continually developed in the ethanol production industry. With that, the new product originates from the dry milling process which is unlike the traditional process as nitrogenous based particles are concentrated from the residual fiber by sieving post fermentation. Although high protein coproducts have been available since the mid 2000's. These new products must be evaluated for chemical composition so that they can be accurately described in feed libraries that are used in commercial ration formulation software. While chemical composition provides us an initial and useful description of a feed product, in vivo nitrogen and energy balance studies are needed to examine the utilization and efficiency of converting the nutrients within a given feed product to milk. Therefore, it is integral to analyze both components for accurate and effective ration formulation in the field.

The first experiment analyzed 10 samples of a new high protein coproduct that were obtained from a singular production site over one months period for chemical composition and nutrient availability. Samples were analyzed for DM, CP, Soluble CP, ADICP, NDICP, ADF, aNDF, lignin, EE, sugar, starch, minerals, amino acids, and fatty acids. Also, aNDF was determined for the samples by 3 different commercial fiber systems including refluxing method, bagged sample method, and a confined refluxing and filtering method. For nutrient availability, RUP was determined with in situ and mobile procedures and NDF digestibility at 24, 30, 48, and 240 h. Total tract NDF

digestibility was also estimated. Results suggest that the new high protein coproduct contains increased concentration of protein and lysine and reduced fiber when compared to a traditional DDGS.

The second experiment utilized twelve multiparous lactating Jersey cattle in a triplicated 4 × 4 Latin square design. Animals were assigned to 4 different treatments diets with increasing inclusion from 0 % to 8 % of the new high protein coproduct replacing non-enzymatically browned soybean meal. The experiment aims to test the effects of formulation of the new product as well as quantify the whole animal energy and nitrogen balance. Results indicate that increasing inclusion of the new high protein coproduct increased dietary fatty acids. However, it had no effect nutrient digestibility. The utilization of energy for NE_L increased with increasing inclusion of the HPCoP with subsequent increases in milk fat production. Results indicate that the new high protein coproduct is able to effectively replace non-enzymatically browned soybean meal in lactating dairy rations.

“Three rules to live by: work hard, be honest, and always do what you believe is right.”

-AV Carroll

ACKNOWLEDGEMENTS

I would like to thank everyone who was a part of the last two years of my life. Many people have inspired me and pushed me to get me where and I am today. I am so very blessed and grateful to each one of you. I would not be where I am without the amazing support system that has surrounded me.

Firstly, thank you to the dairy staff Erin Marotz and Darren Strizek. The dairy wouldn't function without you both.

Next, I would like to thank my advisor, Paul Kononoff. You have taught me valuable lessons about life as well as research. I have always felt comfortable coming to you with any situation I faced. You have encouraged me to remain curious about the things around me while also challenging me to streamline my thoughts. Both things have been instrumental in my development as a young researcher.

Thank you to the undergraduate students as well as the graduate students who have helped, inspired, and made me laugh along the way. K^3 you three have a special place in my heart. Kirby your guidance through my first few months were invaluable, you are the best hype man anyone could ask for. I admire your confidence and persistence. Kyle you always made laugh and brought a light and fun attitude to the group. More importantly, you made me feel like I was at home from the moment I stepped foot in the office. Kassidy thank you for always being willing to share snacks or look at my writing. Your knowledge and skill in the craft will take you big places. Logan, thank you for becoming one of the most valuable mentors. Your way of thinking challenged me to grow and develop. Lastly, Jason, thank you for being the best officemate I could ask for.

You always brought a new way of thinking to the questions I posed. I feel like this has really contributed to my ability to think on physiological level outside of my applied skillset. Also thank you for always going on afternoon walks with me and allowing me to fire every research idea at you no matter how wild.

Lastly, I want to thank my family both those who are blood related or not. Thank you for always listening to me no matter the topic and encouraging to move forward with any task. You all have provided so much support and love through this journey. I am so thankful to have you all in my life and I know there will always be a good laugh around the corner when I am with you all.

TABLE OF CONTENTS

Page

CHAPTER 2

CHAPTER 3

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

The global population is expected to increase to 9.7 billion by 2050 with a projected growth of milk production to 1,020 metric megaton by 2030 (United Nations, 2019; Patterson, 2021). Due to a unique gastrointestinal tract, ruminants convert fibrous human-inedible inputs and produce high value human-edible outputs, namely milk and meat (Karlsson et al., 2018). Milk production alone provides sufficient energy, protein and calcium to meet the annual nutritional needs of 71, 169, and 245 million people, respectively (Liebe et al., 2020). Although in the current system, dairy cattle contribute markedly to global production of protein, their nitrogen use efficiency averages 25 % (Huhtanen and Hristov, 2009). Therefore, dairy cattle are considered poor nitrogen utilizers when compared to other livestock species including swine and poultry with nitrogen use efficiencies of approximately 35 % (Kohn et al., 2005) and 60 %, respectively (Belloir et al., 2017). However, due to advancements in our understanding of nitrogen utilization in the last 10 years, dairy diets may be balanced to increase average nitrogen use efficiency from 25% to 30% (Huhtanen and Hristov, 2009; LaPierre et al., 2019). In accordance with protein, milk energy accounts for 22 – 34 % of gross energy consumed (Morris, 2020). The energetic efficiency of milk production accounts for approximately ¼ of gross energy consumed while ¾ are associated with losses in the feces, urine, and heat production of the animal. Producers rely on the energetic conversion of feed to milk to produce profit, as such it is estimated dairy farms in the United States need to contain 686 lactating animals producing on average 10,730 kg to produce a profit (USDA, 2020). Accordingly, the average dairy cow across breeds in the United States is projected to produce 10,783 kg of milk in 2020 and 10,893 kg in 2021

which ultimately limits the profitability of dairy operations (Cessna and Teran, 2021). Due to narrow margins and increasing feed costs the use of ethanol coproducts provide an economic advantage due to the reduced cost compared to traditional protein and fiber sources (Bradford and Mullins, 2012). However, coproducts are created from corn-ethanol production, and they contain variable feed chemical composition which must first be defined prior to utilization in ration formulation.

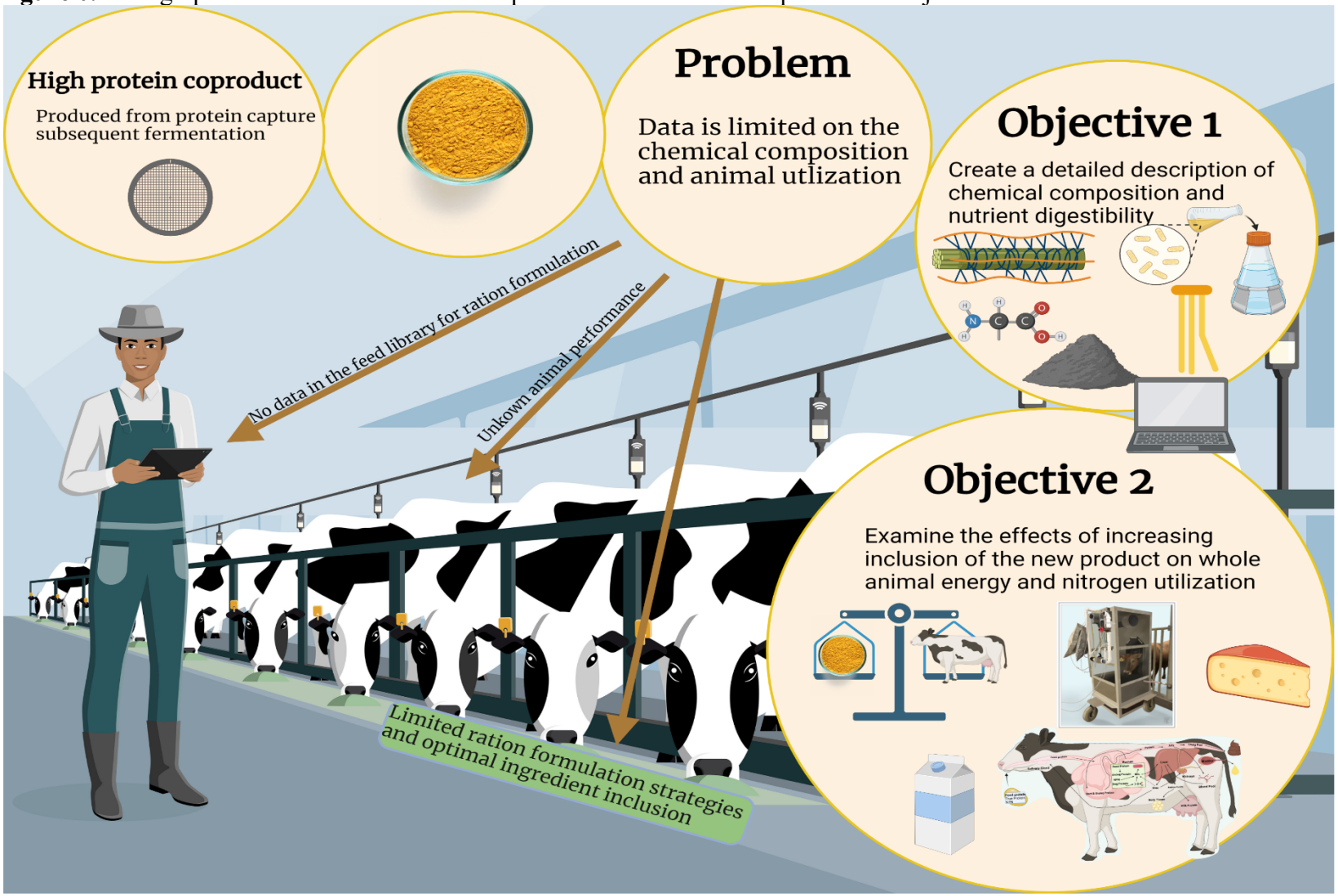
In the past 10 years high protein coproducts have predominantly been produced through methods of pre-fractionization of the corn grain (Hubbard et al., 2009; Christen et al., 2010). Recently, technology has focused on isolating protein subsequent fermentation. This innovation expands coproduct production and increases the marketability of a product as energy and metabolizable protein account for 90% of ration formulation costs in lactating dairy rations (Tebbe, 2020). Consequently, resulting feed products have yet to be extensively evaluated. This is important if they are to be accurately characterized in commercial feed libraries for further utilization by nutritionists in dairy rations.

While chemical composition provides us an initial understanding of a feed product, in vivo nitrogen and whole animal energy balance studies are needed to comprehensively understand the efficiency of converting the nutrients within a given feed product to milk. Overall, a feedstuffs effectiveness of converting gross energy to net energy of lactation (**NE_L**) is a reflection of the products chemical composition and subsequent interaction with other dietary ingredients (Weiss and Tebbe, 2019). To the producer, the cost associated with dietary NE_L is $ 0.16/Mcal (Tebbe, 2020). This translates into $ 474 per d for a 100 head of lactating Jersey cattle (weighing 450 kg and producing 33 kg of energy

corrected milk (**ECM**)(Morris, 2020). Since NE_L represents a large cost to dairy producers, controlled feeding experiments testing different formulation strategies are needed to determine the energetic utilization of new feed ingredients which when coupled with the improvements of genetics and animal management strategies, maximize animal production.

To date, studies have effectively characterized high protein coproducts (**HPCoP**) through the feed evaluation system. However, data is limited on the chemical composition and animal utilization of an emerging HPCoP produced from protein capture subsequent fermentation. Therefore, the objectives of these experiments were to 1) create a detailed description chemical composition for the new HPCoP for use in commercial ration formulation software and to 2) examine the effects of increasing inclusion the new HPCoP while replacing non-enzymatically browned soybean meal on whole animal energy and nitrogen utilization in lactating dairy cattle.

Figure 0.1 Infographic of the current thesis with problem statement and experimental objectives



CHAPTER 1

LITERATURE REVIEW

Ethanol Production

Earliest mention of the use of the dry milling process describes the revolving stone mill in the early 1600's (Hardeman, 1983). At the most simplistic level, the milling process transforms whole grains into forms which are utilizable for conversion into palatable food products (Rosentrater and Evers, 2018). Over time, use of dry milling in flour mills has expanded into industrialized ethanol production. This has in part occurred due to increasing demand of fuel grade ethanol and subsequent utilization of coproducts by the feed industry.

Dry Corn Milling

The usage of dry milling in ethanol production seeks to expose starch in the corn kernel so that through enzymatic reactions it may be converted to glucose and then fermented by yeast to ultimately yield ethanol. Dry grind ethanol production occurs in five steps these include grinding, cooking, liquefaction, saccharification and fermentation (Bothast and Schlicher, 2005). The process begins as whole corn kernels are ground with a hammer or roller mill so that the particle size is reduced, and starch trapped within the endosperm is exposed. The ground corn is then mixed with water and thermostable α-amylase to a *mash* (Murthy et al., 2006). Mash is then cooked in a two-part system, where the temperature is increased from approximately 80- 85° C to 104- 107° C in high pressure jet cookers (Singh et al., 2010). Jet cookers enable the application of heat and mechanical shear to break apart the insoluble, partially crystalline endosperm and allows

for preliminary starch degradation via the thermostable α-amylase. During liquefaction temperature is maintained at 80-85º C and the addition of α-amylase separates long chain sugars such as dextrose into sugar monomers. Finally, during saccharification, glucoamylase enzyme is used to aid in the conversion of starch to glucose throughout fermentation. Glucose is the primary energy source for yeast cells driving glycolysis and ATP production. However, from glycolysis, pyruvate degrades enzymatically by pyruvate decarboxylase and alcohol dehydrogenase creating ethanol and CO_2. Therefore, with the addition of *Saccharomyces cerevisiae* and supplemental fermentation aids such as antibiotics, protease enzymes, yeast nutrients, and nitrogen ethanol production proceeds (Singh et al., 2001; Bothast and Schlicher, 2005).

The process of ethanol extraction begins after fermentation has occurred over a 48-to-72-hour period. The concentration of ethanol in the fermentation vessel needs purified from 14-20 % ethanol to 95 % at the end of distillation for industrial utilization (Kumar and Singh, 2019). After removal of ethanol and CO_2, whole stillage flows from the fermentation vessel and the coarse ground solids or coproducts, known as wet distillers grains, are separated from the liquid via centrifugation. On average wet distillers grains contain 31 % DM and can be further dried to create modified distillers grains at 48 % DM or dried distillers grains at 90 % DM (NASEM, 2016). The liquid fraction, or thin stillage, is be dried and condensed into solubles. The soluble fraction can then be added back to create modified (**MDGS**) or dried distillers grains with solubles (**DDGS**). Although in dry milling, ethanol production is the primary goal, use of coproducts as animal feed contributes to the economic sustainability of the corn-ethanol industry.

Corn Milling Coproduct Production

Distillers grains are a byproduct of the production of ethanol by fuel industry and such coproducts are not the main source of revenue for a dry milling plant. However, ethanol byproduct utilization contributes to the sustainability of food production as only 1 to 3 % of energy efficiency is lost in the conversion of crops into biofuels and animal feed products (Shurson, 2017). Interestingly, the population is predicted to reach 9 billion by 2050, and the Unites States alone creates 32 to 93 million metric tons of food waste. Therefore, the utilization of coproducts in animal nutrition mitigates food loss of a valuable feed product (Chatzifragkou et al., 2015; Bellemare et al., 2017). In current ethanol production systems, ethanol represents 1/3 of the final mass while the remaining 2/3 is made up of spent grains and CO_2 (Hall and Kononoff, 2002; Roth et al., 2019).

Over time biorefineries have evolved to produce fiber, syrup, oil, and protein supplement from fractionation of corn grain. One of the first innovations to occur in the ethanol industry was the centrifugation and separation of corn oil from thin stillage. Overall, advances in corn oil separation and production have been widely implemented in the ethanol industry, as 85 % plants in the United States currently utilize a form of corn oil separation (Kumar and Singh, 2019). When corn oil is removed from the thin stillage the oil content of DDGS is reduced 8-12 % to approximately 4-8 % (Reis et al., 2017). Corn oil is feedstuff which could be highly valuable to increase energy density in ruminant rations. However, extracted corn oil contains a majority of the oil as unsaturated fatty acids and these may have the capability to alter rumen biohydrogenation (Jenkins, 1993; Bauman and Griinari, 2003). The alteration produces intermediates such as *trans*-10 *cis*-12 CLA which have the capacity to inhibit de novo synthesis of milk fat in the

mammary gland (Baumgard et al., 2002). However, this interaction of oil and rumen biohydrogenation is complex as early data saw no difference in milk fat production with the use of corn oil in ad libitum forage fed animals (Sutton et al., 1932). This concept was further by Griinari et al. (1998) who observed that milk fat depression occurred when 40 g/kg corn oil was fed with a dietary NDF content of 14.8 % but was mitigated at 32.1% NDF. Similar, Leonardi et al. (2005) found that milk fat yield and percentage were not statistically different when corn oil was fed at 1.5 % dietary DM and NDF was maintained at 27.8 %. Despite these findings, hesitancy has surrounded the utilization of corn oil to increase energy density in dairy rations. However, the byproduct of corn oil extraction has sparked interest in the dairy community.

Another facet related to the reduction of oil in distillers grains has been the production of reduced fat distillers grains (**RFDDGS**). In dairy rations when RFDDGS were fed at 30 % of dietary DM, RFDDGS inclusion was found to support or increase DMI and milk yield in mid lactation cattle (Mjoun et al., 2010b; Foth et al., 2015; Ramirez-Ramirez et al., 2016). However, milk component values have been variable depending upon how RFDDGS were utilized in ration formulation.

When replacing soybean meal completely and ground corn partially, a 29 % inclusion of RFDDGS decreased milk protein percent but had no effect on other components (Foth et al., 2015). Similarly, when RFDDGS replaced ground corn, soybean meal and non-enzymatically browned soybean meal at 10 % and 20 %, an increase in milk protein percent was observed with a subsequent decrease in milk protein at 30 % inclusion (Mjoun et al., 2010b). It was suggested in both these experiments that the decrease in milk protein was due to the low concentration of lysine in the RFDDGS (Paz

and Kononoff, 2014). However, in the experiment by Mjoun et al. (2010b) an effect of increased milk fat concentration and percentage was observed. Nonetheless, Mjoun et al. (2010b) utilized increasing inclusion of rumen inert fats (**RIF**) containing 85 % saturated fatty acids in conjecture with increasing inclusion of RFDDGS, likely contributing to the linear increase in milk fat content across treatments. However, the milk fat response could not be directly attributed to either the RFDDGS or the RIF. Additional exploration was described comparing DDGS to RFDGS as well as a RFDGS combined with a RIF (Ramirez-Ramirez et al., 2016). These investigators observed that treatments including RFDGS as well as RFDGS and RIF simulated milk fat synthesis independently potentially due to increased energy content (Foth et al., 2015). These data suggested that the fat contained in RFDGS may be at least partially protected by the germ or associated with the fiber fraction (Abdelqader et al., 2009). Indicating RFDDGS could effectively be utilized in dairy diet formulation similar to other high protein products.

The use of modern technology for corn oil fractionization and RFDDGS production has led to further innovations in the coproduct production sector of the ethanol industry. Since the mid 2000's high protein distillers grains (**HPCoP**) have been produced to create a feed product for the ruminant, pet food, and aquaculture industry. However, HPCoPs are produced through various methods of ethanol production including, prefractionation of the corn grain and post-fractionation of the spent grains. Of these methods, one of the earliest high protein distillers grains was created as a result of hexane extraction of corn oil which modestly increased the protein content of DDGS to approximately 35 % CP (Mjoun et al., 2010a; Morris et al., 2018). During this time, HPCoP were also created through the fractionation of the germ, bran, and endosperm

prior to fermentation. Pre-fermentation fractionization increased ethanol yield and resulted in a feed byproduct containing approximately 45 % CP (Singh et al., 2005; Hubbard et al., 2009; Christen et al., 2010). Fractionization subsequent fermentation through sieving and elutriation has created products which contain approximately 40 % (Srinivasan et al., 2005). With cellulosic ethanol production producing HPCoP containing 50 % CP (Kim et al., 2008). Therefore, recently ethanol producers have combined technologies to produce novel HPCoPs with increased protein content of approximately 56 % CP (Brown and Bradford, 2020). Consequently, the definition of high protein distillers grains includes a wide range of products of varying chemical composition which have yet to be defined by AAFCO or in a commercial feed library.

While the data are limited, four studies have explored the use of high protein coproducts versus other high protein feedstuffs when fed to lactating dairy cattle. In three experiments, HPCoPs were included from 12-20 % DM and dry matter intake and milk production were maintained relative to a soybean meal and non-enzymatically browned soybean meal control (Hubbard et al., 2009; Kelzer et al., 2009; Christen et al., 2010). Alternatively, in the experiment of Brown and Bradford (2020) testing a novel HPCoP at 9.4 % dietary DM investigators observed a decrease protein digestibility and DMI resulting in a decrease in milk yield and milk protein concentration. Although milk protein percentage decreased milk fat percentage was maintained and this was similar to other experiments (Kelzer et al., 2009; Christen et al., 2010; Brown and Bradford, 2020b). Similarly, Hubbard et al. (2009) observed that when feeding a diet containing 20 % HPCoP resulting from the removal of bran prior to fermentation an increase in milk fat yield was observed. The authors proposed that the increase in milk fat may be a result of

increased fat content in the product, however there were no further explorations of energy or nitrogen utilization. Between the Hubbard et. al (2009) experiment and that of Brown and Bradford (2020) there was a difference of 10 % CP between the products which were both consider HPCoPs (46 % CP vs 56 % CP). As a result, coproducts are important to the advancement of the biofuel industry; but accurate and defined nutrient characterization needs to be completed on new products for them to be effectively utilized in ration formulation.

Nutrient Characterization of Feedstuffs

The essential criterion of any feed evaluation system is the ability to predict animal responses based on the nutrient characterization and inclusion of the feedstuffs within a given ration. The goal of chemical composition analysis is to create accurate and reproducible values with expense and time in mind for the laboratory. Since 1809, producers have tried to characterize their feeds and predict the resulting effects on animal performance (Flatt et al., 1967). However, variability occurs in feed production processes, nutritive assays, and modeling tools which limit our ability to predict the productive responses of the animal. Since nutrient characterization continues to evolve, these limitations have bolstered interest in the effective creation of feed characterization outputs which represent the nutrient profile of a given feedstuff.

Book Values

The term “book values” arises as a slang term for utilizing the feed characterization values of a given feedstuff from a feed library or database. Book values are a comparative tool originating from “hay values” utilized in the 1800’s to compare

any forage to what was referred to as “good quality meadow hay” (Flatt et al., 1967). Today, book values are derived from the proximate analysis of a large number of submitted samples from commercial laboratories, literature data, other NRC publications, or unpublished data (NRC, 2001). As a result, book values are valuable estimates that allow nutritionist to formulate diets prior to feed analysis. However, when assessing whether to use book values for feedstuffs in diet formulation, one must recognize the contributing factors which affect the chemical composition of the feedstuff.

Variation in characterization of a feed can occur due to plant genetics, environment, soil, and manufacturing techniques (Weiss and St-Pierre, 2009). Overall differences from farm-to-farm accounts for 70-90 % of total nutrient variation across concentrates and forages (St-Pierre and Weiss, 2015). However, a portion can also be due to analytical variance as well as sampling techniques (Weiss and St-Pierre, 2007). When analyzing forages St. Pierre and Weiss (2015) determined daily differences in chemical composition for haycrop and corn silages only accounted for 20–60 % of within farm variance with 40–80 % being attributed to analytical variation and sampling practices. Interestingly, in the same experiment monthly changes in chemical comprised 50-90 % of within farm variation with 10-40 % occurring in part to analytical and sampling variation. Indicating that sampling and analytical variation may play a substantial role in the differences between chemical composition from day to day and month to month. This indicates the need for duplicate samples and averaging across both timeframes. Since forages are produced in environments specific to a singular farm setting, data should be taken on farm and summarized. Similarly, care should be taken in analytical and sampling practices for effective diet formulation (Table 1.1).

For other common feed ingredients commodity prevalence, manufacturing, and ability to effectively subsample determine the need for on farm values (St-Pierre and Weiss, 2015). Overall dry corn and soybean are national commodities and as a result are well represented in feed library values with lower standard deviations when compared to forages and byproduct feeds (Table 1.2). Wet byproducts contain greater variance as obtaining a representative sample of a wet feed is inherently more difficult than a dry. However, variability in the nutrient composition of DDGS have be outlined in the literature but the current data does not separate the variation which occurs through sampling and lab analysis from that of the feedstuff (Spiehs et al., 2002; Belyea, 2004). As a result, if the DDGS are purchased as a pure commodity from an unknown plant, the use of book values are favorable for ration formulation. If the plant is known using the summarized values from the specific plant may be warranted. Therefore, book values are a valuable input in diet formulation prior to analysis for forages and wet byproducts. However, book values may be directly utilized for national commodities and DDGS of unknown origin.

Crude Protein

In diet formulation, protein is one of the limiting factors to dairy cattle production. This limitation occurs as protein interacts with energy derived from carbohydrates to increase microbial crude protein production, carbohydrate digestion, and subsequent amino acid absorption. Feedstuffs contain a wide variety of structural, storage, catalytic, transport, and contractile proteins (NRC, 2001). All of which differ in physical characteristics including 3-d structure, inter and intra molecular bonding, amino acid composition, and inert barriers (Schwab et al., 2003). Although there are large

structural variations in proteins, they are all described as the proximate nutrient crude protein (**CP**) in the feed library. Crude protein refers directly to the nitrogen content of the feedstuff multiplied by a factor of 6.25. However, the factor comes under scrutiny as it assumes protein typically found in animal feedstuffs contain on average 16 % nitrogen per 100 g of true protein. As such, feed protein is a function of the amino acid and non-alpha amino nitrogen content of the feedstuff and may not be accurately characterized through the use of 16 % N (Mariotti et al., 2008). However, the ruminant animal utilizes protein heterogeneously and relies on both microbial and endogenous enzymatic degradation. Based on the differences in location and type of digestion crude protein is further divided into fractions in the NRC (2001) including non-protein nitrogen (**NPN**; **A**), true protein (**B**), and unavailable protein (**C**). Furthermore, the NRC (2001) model divides the A, B, and C fractions into two pools, being namely, rumen degradable protein (**RDP**) and rumen undegradable protein (**RUP**) (Schwab et al., 2003). Rumen undegradable protein includes the sum of non-protein nitrogen plus the digestible true protein fraction. Whereas rumen undegradable protein is calculated by difference and depends on accurate characterization of the RDP fraction. For post absorptive amino acids, metabolizable protein (**MP**) and scurf originates from the microbial crude protein created from RDP and the feed protein which has escaped ruminal digestion; both of which are degraded in the small intestine directly supplying amino acids to peripheral tissue and the blood pool (Burroughs et al., 1975).

Another ration formulation software, The Cornel Net Carbohydrate and Protein System (**CNCPS**) v6.5 aims to effectively characterize the protein fraction differently than that of the NRC (2001). The new version of CNCPS recharacterized the protein

fraction and shifted away from the utilization of NPN to ammonia nitrogen (**A1**) due to the amino acid content of the peptides within the NPN fraction (Higgs et al., 2015; Van Amburgh et al., 2015). This re-characterization caused a shift of a large proportion of protein from A1 to the soluble true protein (**PA2**) fraction by limiting the definition A1 solely to ammonia N. Protein which is not highly degradable in the rumen is denoted as with a "B" including moderately degradable protein (**B1**) and slowly degradable protein which is bound to NDF (**B2**) . Finally indigestible protein is a function of the acid detergent insoluble crude protein (**ADICP**) within the given feedstuff (**C**; Table 1.3; Van Amburgh et al., 2015).

Soluble Protein

Soluble protein contains proteins which can be degraded in the rumen which aid in supplying nitrogen to the rumen microbial population for MCP synthesis but contribute little to the amino acid requirements of the animal. Since soluble proteins are rapidly degradable in the rumen, early efforts in laboratory analysis led to buffers which mimicked the rumen pH (NRC, 2001). However, these solvent systems had unstable pH, rumen fluid as a reagent, or enzyme reagent limitations rendering the methods variable in the laboratory environment (Wohlt et al., 1973; Crooker et al., 1978; Waldo and Goering, 1979). To solve the pH variability, the procedure was modified to include a pH stable bicarbonate-phosphate buffer and was later updated to differentiate the NPN and true protein fraction with a subsequent precipitation with trichloroacetic acid (**TCA**) (Krishnamoorthy et al., 1982; NRC, 2001). As such, HPCoP from pre-fermentation fractionization have been found to have 7 % soluble protein on a DM basis contributing

to a smaller soluble protein fraction when compared to an average of 19 % soluble protein in DDGS (Kelzer et al., 2010).

Non-protein Nitrogen

Non-protein nitrogen composes the A fraction in modeling scenarios in the NRC due to immediate solubilization by rumen microbes (Ørskov, 1982). Non-protein nitrogen contains smaller compounds which include peptides, free AA, nitrate, ammonia, amides, and amines (Schwab et al., 2003). In feed analysis, methods for determining NPN utilize the principle of precipitation of true protein and the subsequent difference between total crude protein and true protein nitrogen (Krauss, 1927; Licitra et al., 1996). While the use of difference may not be the most favorable method, due to the heterogeneity of the NPN fraction one method of analysis may not accurately precipitate out specific fractions or different lengths of peptides (Greenberg and Shipe, 1979; Krishnamoorthy et al., 1982). Since non-protein nitrogen composes 95 % of the soluble nitrogen in silages and cut forages, soluble protein has been utilized to estimate the non-protein nitrogen (Pichard, 1977; Schwab et al., 2003). However, NPN only contribute 52 % of the soluble protein in HPCoPs and differentiation may be needed to characterize new HPCoP (Kelzer et al., 2010).

Neutral Detergent Insoluble Crude Protein

While soluble protein and NPN may occur in free form associated with the rumen fluid, some proteins are bound within the NDF fraction of plant cell walls (NRC, 2001). The neutral detergent insoluble crude protein (**NDICP**) fraction is the nitrogen content of the NDF residue and multiplied by 6.25 to create a crude protein value (Schwab et al.,

2003). Neutral detergent insoluble crude protein contains extensin proteins that are covalently bonded with the hemicellulose which links carbohydrates to the cell wall (Fry, 1988). Although NDICP is directly associated with the NDF fraction, when compared with the traditional NDF assay, the assay for NDICP utilizes the NDF solution but omits the use sodium sulfite and urea amylase as sodium sulfite cleaves disulfide bridges in cystine in a non-biological manner which would ultimately reduce the NDICP fraction (Van Soest et al., 1991). When modeling the protein digestibility in CNCPS, the B2 fraction is calculated by the difference between NDICP and acid detergent insoluble crude protein and assigned a Kd of 1 to 18 %/h due to slow degradation (Higgs et al., 2015). In heat treated products like DDGS, B2 proteins which are partially fermented in the rumen and the lower gut can be denatured increasing the C fraction (Sniffen et al., 1992; Licitra et al., 1996). As a result, coproducts can contain up to 40 % NDICP causing negative correlation with rumen degradable protein but a positive correlation with MP due to increased RUP and subsequent amino acid uptake in the hind gut (Weiss et al., 1989; Schwab et al., 2003).

Acid Detergent Insoluble Crude Protein

Acid detergent insoluble crude protein (**ADICP**) comprises the unavailable protein fraction (**C**) and helps quantify the amount of insoluble nitrogen in ADF residue (Firkins et al., 1984). Acid detergent insoluble crude protein has long been assumed to be indigestible in concentrates due to the negative association with crude protein digestibility in forages caused by ADICP's association with lignin (Kleinschmit et al., 2007). Aside from forages, in coproducts, a portion of the ADICP is a product of the heat applied during production resulting in the Maillard reaction cross linking carbohydrate

and proteins (Kajikawa et al., 2012). As a result of variation in production and concentration of ADICP in DDGS, the negative relationship between rumen availability of protein and ADICP has been nonexistent, moderate, or strong (Nakamura et al., 1994; Klopfenstein, 1996; Harty et al., 1998). However, there have been no observed milk yield responses directly attributed to increased ADICP concentration (Weiss et al., 1989; Machacek and Kononoff, 2009). Therefore, the assumption of 0 % digestibly in ADICP may not hold true as Maillard products associated with the ADICP fraction in DDGS may be relatively more digestible when compared ADICP associated with lignin. Therefore, modeling programs have moved away from the chemical fraction of ADICP and towards the utilization of undegraded nitrogen which aims to quantify the residual nitrogen after in vitro fermentation and simulated hind gut digestion with the Ross Assay.

Amino Acids

Since the discovery of essential amino acids in 1935, and subsequent confirmation of "essentiality" in 1952, nutritionists have grown substantially in their understanding of AA requirements in ruminants (Schwab and Broderick, 2017). Amino acids are a product of MCP, RUP, and endogenous crude protein which are digested in the abomasum. In the abomasum, digestion with pepsin and hydrochloric acid (**HCL**) breaks down the peptide bonds and creates free amino acids for the goal of supporting resynthesis of proteins and immune processes (Harmon, 1993). Microbial crude protein, RUP, and endogenous flows contribute 35-65 %, 20-45 %, and 10-20 % of MP requirements, respectively (Clark et al., 1992). As a result of the impact of MCP on amino acid requirements, early thoughts prevailed where MCP was able to provide all the amino acid needs of the animal. While possibly true at the time, as animals increased in production the demand for post ruminal

amino acid absorption also increased (Broderick et al., 1970; Clark, 1975). Overall, there are twenty amino acids which each contain a common nitrogen-carbon-carbon back bone with a chemically unique side chain. Essential amino acids include Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Tryp, and Val. Whereas non-essential include Ala, Asn, Asp, Gln, Gly, Pro, Ser, Tau, and Tyr. Essential and non-essential are divided as essential amino acids either cannot be synthesized by the animal, or if synthesis is possible, it is not at a large enough rate to meet the requirements of the animal (NRC, 2001). However, non-essential are synthesized via intermediary metabolism or from surplus AA amino groups.

Corn Zein

Zein is the structural prolamin which accounts for 60-70 % of the endosperm protein in corn grain (Larkins, 2019). Zein serves the plants as a storage protein by surrounding starch granules providing structural integrity and a hydrophobic barrier (Figure 1; Gibbon et al., 2003). In terms of nutrition, zein protein has been found to be rich in amino acids such as glutamic acid, leucine, and alanine consisting of relative amounts 22 %, 18 %, and 12 % of total amino acid content (Gianazza et al., 1977; Shukla and Cheryan, 2001). However, zein contains < 1 % of the essential amino acid lysine (Gianazza et al., 1977). As a result of containing a high proportion of nonpolar amino acids, zein is insoluble in water unless alcohol, high concentrations of urea, or high concentrations of alkali are present (Shukla and Cheryan, 2001). Accordingly, in ruminants, zein protein are not soluble in solutes which are found in the rumen environment likely contributing to a portion of increased RUP content of DDGS (Lawton, 2002). Therefore, corn zein decreases the rate of starch digestion from 0.06 %/h to 0.026 %/h when compared with globulin-albumin proteins (Hoffman and Shaver,

2009). This slower rate of digestion occurs due to the matrixes formed between the starch and zein protein as proteolysis must occur before amylolytic activity can begin (Seckinger, 1973). As a result, zein's inhibit starch digestion and are limited in their lysine content and considered a low-quality protein source for ruminant animals.

Measuring rumen and intestinal protein digestion

Measuring rumen and intestinal protein digestion aims to accurately quantify the extent and location of feed protein digestion in the ruminant animal. Three main assays are currently utilized to determine protein digestibility of feedstuffs including the Mobile Bag (**MOB**; Paz et al., 2014), Modified Three-Step (**MTS**; Gargallo et al., 2006), and Ross (Ross et al., 2013). Each assay aims to mimic the ruminant digestive tract through in situ or in vitro fermentation. The MOB assay was first used in situ for determining protein digestibility in ruminants by Hveplund (1985). Consequently, in situ methods allow for almost full contact with the ruminant digestive tract animals. As ruminally and duodenally cannulated animals are necessary to carry out the assay, increased labor and cost are expected limiting the assays commercial utilization. As a result, the MTS assay was developed to mitigate cost and labor. The MTS like the MOB procedure utilizes nylon bags to suspend feed samples in the rumen fluid for ruminal protein degradation (Gargallo et al. 2006). However, criticism has been made that bags utilized in MOB and MTS procedures may cause increased lag time for microbial attachment decreasing digestibility values (Ross, 2013). Whereas feed particle loss from the bag may occur not a result of ruminal or intestinal digestion increasing digestibility values (Ross, 2013). As a result, the Ross assay was developed as an in vitro assay performed in Erlenmeyer flasks where feed particles were fermented in direct contact with rumen fluid from a

donor cow (Ross et al., 2013). While the Ross assay provides standardized enzymes to decrease variation, replicating the ruminant digestive tract in a laboratory proves difficult and data should be carefully vetted prior to use.

Measuring Rumen NDF digestion

Neutral detergent fiber (**NDF**) is composed of cell wall structural carbohydrates, hemicellulose, cellulose, and lignin which all contain varied levels of digestibility (Van Soest et al., 1991). As a result, NDF digestibility (**NDFD**) can range from 20 to 80 % in fiber sources and contribute 0.23 kg of milk per unit of enhanced NDFD (Oba and Allen, 1999). Since measurements of the chemical composition of fiber cannot be used to describe the degradation of fiber in the ruminant animal, the use of NDFD measurements contribute heavily to modeling productive responses in dairy cattle (Raffrenato et al., 2019). As NDFD measurements are highly influenced by method of in vitro fermentation and subsequent NDF analysis, creating equipment and protocol which mimic rumen function is integral to gain accurate results for diet formulation (Goeser and Combs, 2009; Coblentz et al., 2019).

In Vitro System Production

In vitro systems attempt to mimic the dynamic rumen environment in a lab setting. The laboratory setting has advantages of economy, convenience, and can be a method of determining promising treatments prior to full animal experiments (Danielsson et al., 2017). However, replicating the dynamic microbial population, pH, and anaerobicity of the rumen has proved difficult. The first continuous culture artificial rumen was developed in 1949, but the size, difficulty of preparation, and constant need

for buffering lead to the development of the miniature artificial rumen in 1953 by Huhtanen et al. (Figure 2,3). At the point in time miniature artificial rumens produced crude fiber digestibility values. However, the method laid the framework for in vitro batch culture NDFD laboratory analysis utilized today.

During laboratory analysis 10 years after the development of the miniature artificial rumen the homogenous components of in vitro fermentation across 17 laboratories included the use of glass containers, McDougal's buffer, and rumen fluid strained through cheese cloth (Barnes, 1967). However, differences arose in sample size, cannulated ruminant animal, animal diet, CO_2 flushing, fermentation timing, and fluid to inoculum ratio. The variation in procedures observed ranged from 40 – 64 % for the mean 24-hour cellulose digestibility across 3 forage samples with significant differences observed between labs and within runs (Barnes, 1967). Therefore, the preliminary standard method was developed by Tilley and Terry (1963).

The protocol developed by Tilley and Terry (1963) addressed many of the variables of concern during the time, including a set sample size of 0.5 g, buffer to inoculum ratio of 4:1, 48 h fermentation timepoint, CO_2 flushing prior to sealing, crimped closed system, and Bunsen valve addition to flasks for microbial gas release (Figure 4). It is noted in the methods of Tilley and Terry (1963) the set sample size of 0.5 g limits the amount of herbage necessary to carry out the assay aimed to mimic larger digestibility trials. Also 0.5 g is integral for in vitro fermentation as too large of a sample size may not be degraded in the fermentation vessel whereas too small of a sample may not leave enough residue if further analysis is required. In this assay a buffer to inoculum ratio of 4:1 allows for the microbes to remain at an ideal pH as VFA concentrations increase in

the closed system. With that Tilley and Terry (1963) noted along with the 40 mL of buffer solution 10 mL of rumen liqor would provide the necessary microbes, protein, and cofactors for effective fermentation (Barnett and Reid, 1961). Since rumen microorganisms consist of anaerobes or facultative anaerobes CO_2 flushing prior to sealing and crimped closed system allow for maintenance of anerobic environment throughout fermentation. Flushing and crimping was chosen over continuous CO_2 as the continuous CO_2 baths of the time were small, ran only a few flask and the procedure was time consuming (Figure 5; Hoorn et al., 1957). Since no gas outlet was present Bunsen valve addition allowed for pressure to build in the system without incurring loss of anaerobicity through displacement of the top stopper. While all of these factors work together, the assay outlined by Tilley and Terry (1963) was later modified by Goering and Van Soest (1970) with removal of the centrifugation, pepsin solution, addition of continuous gassing, and the use of NDF solution in order to simplify the assay for larger utilization in the industry (Figure 6).

While these methods have been widely used for decades, modifications to the procedures often aim to reduce the inherent variability associated with vitro fermentation. In vitro fermentation can occur in glass Erlenmeyer flasks, polyethylene tubes with gas release valves, or stoppered serum vials (Goering and Van Soest, 1970; Moore and Mott, 1976; Pell and Schofield, 1993). However, Hall and Mertens (2008) found vessel type had little effect on NDFD and the main factor influencing digestibility was the increased CO_2 pressure. This finding strengthened the earlier observations of Grant and Mertens (1992b) as carbon dioxide pressure and reducing solutions also decreased microbial lag time and stabilized pH increasing NDF digestion (Figure 7, 8). Overall, fibrolytic bacteria

are sensitive to ruminal changes therefore various methods are utilized to create an ideal environment via additions of bicarbonate, biotin, urea, branch chain VFAs, and trace minerals (Russell et al., 1992; Millen et al., 2016; Roman-Garcia et al., 2021) Similarly, host animal diet also plays a role in the effectiveness of in vitro fermentation as the microbial population responds to dietary shifts which cause pH decline (Grant and Mertens, 1992a; Klop et al., 2017). As inoculum has been attributed to the largest amount of run to run variation, collection timing, rumen fluid pooling, fluid preservation, and rumen fluid priming techniques have been proposed (Hervás et al., 2005; Rymer et al., 2005; Goeser and Combs, 2009). While these improvements function in controlled environments, the confounding factor of laboratory has continued to lead to variability in the assay (Hall and Mertens, 2012). As a result, we still lack standard methodology after 70 years of experimentation.

Short Term Fermentation

Short term fermentations aim to accurately replicate two of the three components of rumen NDF digestibility being namely initial lag time, where microbial attachment occurs, and the rate of digestion of potentially digestible fraction (Fahey Jr. and Berger, 1988). Lag time occurs in in vitro fermentation as growth factors are limited until co-factor production and cell death releases trapped nutrients into general fermentation (Van Soest and Robertson, 1985). In the preliminary phase of fermentation cellulolytic microbial attachment occurs through stomata, lenticels or damaged areas of the fiber (Varga and Kolver, 1997). Since microbial and fungal attachment and proliferation completes after 12 h, short term fermentations display greater variation due to smaller amounts of residue disappearance (Pell and Schofield, 1993; McAllister et al., 2018).

However, 24 h and 48 h were used as timepoints likely as a result of preliminary rumen dry matter disappearance experiments and the early assumption that fiber was fully degraded by 48 h (Walker, 1959; Tilley and Terry, 1963; Tonroy and Perry, 1974). At the 48-hour time point, cell wall components are digested to a considerable amount; however this timepoint may not be physiologically accurate as 48 hours assumes low animal intake and slow passage rate out of the rumen environment (Singh et al., 1989). Therefore, 30 h NDFD was explored however it resulted in a lack of correlation with observed total-tract NDFD (Lopes et al., 2015). The 24 h timepoint produces the most accurate gas volume correlated with in vivo ruminal fermentation and provides convenience in field laboratories (Menke et al., 1979; Van Amburgh et al., 2003). As a result, short term fermentation may allow for initial view of microbial lag time and digestible fractions of a given feedstuff. Still multiple timepoints and long-term fermentations are necessary to describe the full rate and extent at which a feedstuff can be degraded in the ruminant animal.

Long Term Fermentation

The third and final pillar to NDF digestion quantifies the maximum extent of digestion (Fahey Jr. and Berger, 1988). The maximum extent of digestion quantifies the indigestible fiber fraction related to rumen functions (Palmonari et al., 2016). The timeframe for determining the indigestible fiber fraction has ranged from 3 days to weeks of fermentation (Mertens, 2005; Palmonari et al., 2017). The long timeframe occurs as the long-term fermentation approximates the amount of fiber that would remain undigested if it resided within the total tract indefinitely (Raffrenato et al., 2019). As a result, the current standard procedure includes a 240-hour fermentation as undigested

aNDFom did not differ between the 240-hour and 504-hour fermentation timepoint (Raffrenato and Van Amburgh, 2011). As batch culture analysis is generally limited to singular vessels, reinoculation has been explored to continue the longer fermentations in a manner indicative of the rumen environment. Preliminary protocols outlined fresh rumen fluid reinoculation occur every 72 hours after 96 hours of fermentation (Van Soest et al., 2005). However, it was later determined reinoculation increased the procedural error and was not necessary to carry out long term fermentations (Palmonari et al., 2017). Therefore, during long term fermentation cellular recycling may allow for the microbial population to remain intact as cofactor production and cell death may account for the microbial requirements, negating the necessity for reinoculation (Van Soest and Robertson, 1985).

Nitrogen utilization

The primary goal of understanding nitrogen utilization in ruminants aims to achieve the maximal productive output of protein via milk protein or body tissue accretion while minimizing nitrogenous waste via the urine and feces. Ruminant animals are relatively inefficient nitrogen utilizers as milk nitrogen efficiency varies from 14-45 % depending on diet chemical composition and animal inputs for lactating dairy cattle (Huhtanen and Hristov, 2009). The wide range in nitrogen utilization can be associated with both management and nutritional factors including, animal breed and size, available feed ingredients, forage management and diet formulation (Kebreab et al., 2000; Jonker et al., 2002; Spek et al., 2013; Sears et al., 2020). As a result, the typical dairy diet in the United States has a milk nitrogen efficiency averaging 25 % (Hristov et al., 2019). Consequentially, nitrogen excretion in the urine and feces contributes to environmental

concerns on a global scale including water pollution and gaseous nitrogen emissions (Külling et al., 2001; Hristov, 2011; Hristov et al., 2011). As a result, research has focused on understanding nitrogen metabolism and the contributing interactions that occur through dietary manipulation.

Feed Factors

Animals ingest feeds which are comprised of both true protein and non-protein nitrogen with varying amino acid composition and digestibility (Figure 1.9). Based on these factors, fecal nitrogen excretion can be directly manipulated through the diet due to its composition of undigested feed nitrogen, undigested microbial nitrogen, and endogenous nitrogen (Tamminga, 1992). However, microbial nitrogen and endogenous nitrogen only contribute 19 % of fecal nitrogen excretion and on average 81 % originates from undigested feed material (Ouellet et al., 2002). Therefore, when determining the contribution of a feedstuff to fecal nitrogen excretion, the chemical composition fraction of soluble protein may be considered a good determinant due to immediate degradation and subsequent utilization by rumen microbes (NRC, 2001). However, microbial attachment, proteolysis, and protozoal engulfment occur with the insoluble fraction of protein (Mahadevan et al., 1980; NRC, 2001). Therefore, a potentially more effective indicator of ruminal and total tract digestibility of nitrogen hinges on the number of secondary and tertiary structures and the density of the disulfide cross linkages in the feed itself (Nolan and Dobos, 2005). For some coproducts, Maillard products are created via the Maillard reaction increasing cross linkages of the epsilon amino group with compounds such as carbohydrates, lipids, vitamins, and polyphenols (Rutherfurd and Moughan, 2018). These complexes lower the digestibility ultimately increasing fecal

nitrogen excretion (Brown and Bradford, 2020). As a result, fecal nitrogen hinges on the physical characteristics of the feed itself and more importantly the degree of complexing.

Urinary nitrogen excretion is dictated by the utilization of dietary nitrogen by rumen microbes as well as amino acids derived from MCP and MP in the animal. Rumen microbes require nitrogen and energy to utilize the carbon skeleton from fiber to produce MCP. Therefore, by feeding RDP in excess of energy, ruminal microbes will be unable to integrate the available nitrogen into the carbon skeleton. As a result, excess ammonia which is not converted to microbial protein causes increased ammonia diffusion across the rumen wall into the blood pool. Accordingly, on average, 43 % of excess ammonia not utilized in MCP synthesis detoxifies in the liver and converted to urea for subsequent excretion in the urine (Figure 1.9; Hristov et al., 2005; Lapierre et al., 2005). Therefore, it has been demonstrated by increasing dietary RDP from 7 % DM to 10 % DM urinary nitrogen excretion was increased from 140.5 to 245.8 g of N/d (Gressley and Armentano, 2007).

While RDP is commonly associated with urinary nitrogen excretion, dietary MP supply must also be considered in urinary nitrogen excretion. Excess dietary metabolizable protein will enter the blood pool as amino acids from the small intestines and if not utilized by the mammary gland, are recycled to the splanchnic tissues. If in excess after this point amino acids are further deaminated by the liver and excreted as urea in the urine similar to RDP. One example of this phenomena occurred in an experiment conducted by Wang et al. (2007) who fed increasing inclusions of MP ranging from 8 to 10 % dietary DM, while holding RDP, NEL, and NDF as a % DM steady across treatments. By holding energy and RDP constant Wang et al. (2007) was

able to isolate the effects of increasing MP on nitrogen utilization and observed a 25 % increase in urinary nitrogen from the lowest to the highest level of MP. Indicating dietary MP could directly influence urinary nitrogen excretion in the ruminant animal. In the literature, decreasing the dietary RDP and MP content has subsequent implications on the crude protein content provided in the diet. Therefore, decreasing dietary CP has long been recognized as the best method for reducing urinary nitrogen excretion as it affects both sources which directly contribute to urinary nitrogen excretion (Raggio et al., 2004; Agle et al., 2010). Overall, nitrogen excretion can be directly influenced by feed and diet composition. However, animal requirements have a direct impact on intake and metabolism of absorbed amino acids for milk protein synthesis which contributes directly to the profitability of dairy producers.

Animal Factors

Intake is subject to chemical interactions with the feed but also energetic requirements of the animal. As a result, nitrogen utilization has been found to be highly correlated with nitrogen intake by the animal as large influxes of nitrogen cannot be effectively captured in milk protein leading to nitrogen excretion in the urine and feces (Huhtanen et al., 2008; Reynolds and Kristensen, 2008). Overall, data has shown a positive linear relationship between nitrogen intake and output in feces, urine, and milk until 400 g/d, after which, urinary excretion increases exponentially (Castillo et al., 2000). This was further substantiated as nitrogen use efficiency was increased by 5 % when dietary nitrogen intake was decreased from 600 g/d to 300 g/d (Kebreab et al., 2010). However, the most economically important animal factor for nitrogen utilization relies on the utilize metabolized amino acids for milk protein synthesis.

For milk protein synthesis the goal of ration balancing would be to create the "perfect amino acid blend" to match milk protein from feed and MCP. However, amount and alteration of EAA profiles from feed and MCP occur due to splanchnic affinities limiting our ability to effectively accomplish this goal (Arriola Apelo et al., 2014). Once feeds are digested by the animal amino acids are deaminated by the liver and free amino acids are created. Free amino acids are circulated in the blood pool where they are subsequently extracted by splanchnic tissues and then the mammary gland for protein synthesis as well as cellular catabolism (DePeters and Cant, 1992). Historically, due to increased interest in milk protein, infusion trials were conducted to determine potentially limiting amino acids for milk synthesis. As a result, lysine and methionine were determined as first and second limiting in corn-based diets. (Schwab et al., 1992). However, a review by Lapierre et al. (2005) determined that the post liver uptake of lysine was 0.65 indicating metabolism in other tissues and lysine's utilization in NEAA synthesis for milk protein. This may contribute to variable observed responses in milk protein when coproduct diets were balanced and included rumen protected lysine (Paz et al., 2013b; Paz and Kononoff, 2014).

Energy Utilization

During the introductory session of the symposium on energy metabolism in 1958 three fundamental problems were identified. The second and third were creating vitamin requirements and determining the development of anatomical structures in children and animals. However, the first, and most applicable to the current discussion, being determining the nutritive value of feedstuffs via a single quantitative unit (MØllgaard, 1958). Twelve years later, scientists began to determine the energy value of a singular

feed in terms of net energy which has become the defining productive unit across ruminant animals (Holter et al., 1970). Accordingly, the energy utilization field has grown rapidly in the past 60 years; yet it is still governed by feed determination and subsequent animal utilization experiments as nutritive value is a biological measurement (Figure 1.10; Blaxter, 1956).

Feed Factors

The gross energy (**GE**) content of feed is comprised of the macronutrients protein, fatty acids, and carbohydrates. When totally combusted in a bomb calorimeter the three macro nutrients provide 5.6, 9.4, and 4.2 Mcal/kg DM, respectively (NRC, 2001). Depending on structure, amino acids have a wide range of energy from 3.34 Mcal/kg to 7.17 Mcal/kg based on branching and chain length (Milgen et al., 2018). Similarly, lignin contains a gross energy content of 6.0 Mcal/kg. However, as lignin remains completely indigestible in the animal it and theoretically contributes 0 Mcal to the average value of 4.2 Mcal/kg for carbohydrates. Although fats generally comprise 3-5 % of dietary DM in dairy rations, fatty acids contribute the largest amount of energy from a gross energy standpoint. Therefore, it has been demonstrated increased fatty acid content from distillers products are able to increase the gross energy value of the diet by 15 % when compared to older NRC values (Birkelo et al., 2004). As a result, gross energy measures the total chemical energy within a feedstuff. However, energy availability can be changed based on feed processing methods and feed interactions. Therefore, gross energy derived from bomb calorimetry should not be utilized directly for diet formulation.

Fecal energy composes the largest loss of energy accounting for thirty percent of total gross energy intake (Morris et al., 2020). Therefore, digestible energy (**DE**) retained

with in the animal directly reflects the ration's apparent digestibility (Figure 1.11). Apparent digestibility considers the gross energy in fecal matter as we are currently unable to separate gross energy derived from endogenous waste from that of the feeds themselves. When describing the factors effecting apparent digestibility a negative relationship occurs with digestibility, DMI, and concentrate fat intake (Huhtanen et al. 2009). Since dry matter intake and fat supplementation suppresses ruminal digestion of feed materials. However, Huhtanen et al. (2009) observed a positive correlation with in vitro organic matter digestibility and crude protein concentration. As such, if we were to increase dietary starch by 5 percentage units while subsequently decreasing NDF by 5 percentage units assuming 91 % and 48 % digestibility respectively, DE concentration would be expected to increase 3 % (Weiss and Tebbe, 2019).

Metabolizable energy (**ME**) is calculated by difference of digestible energy and chemical energy lost in urine and methane. Energetic losses associated with urine and methane account for 4 % and 5 % of gross energy loss, respectively (Morris, 2020). In terms of urinary energy loss, a strong relationship occurs between urinary nitrogen and urinary energy output (Morris et al., 2021b). However urinary energy loss occurs as a result of not only synthesized urea but also endogenous purine derivative, creatinine and creatine, hippuric acid and 3-methyl histidine (Lapierre et al., 2020; Morris et al., 2021b). However, most of these molecules contain nitrogen as well as energy. Therefore, feed factors which affect urinary nitrogen production like excess ruminal ammonia, inefficient incorporation of AA into milk protein, and excess MP ending in ureagenesis directly affect urinary energy excretion as discussed previously. For methane production, high dietary fat inclusion has been demonstrated to reduce methane by 10-25 % through

inhibition of cellulolytic bacteria (Beauchemin et al., 2008). Therefore, increasing dietary fat through DDGS has also demonstrated a decrease in methane production (Benchaar et al., 2013). Similarly, methane can be decreased by reducing the amount of cellulose as cellulose creates 3 times more methane per gram of substrate digested (Moe and Tyrrell, 1979). Therefore, by decreasing urinary and methane energy losses we can increase ME. As ME subsequent net energy are an interaction between diet and animal, it is necessary to explore the animal factors which affect energy utilization.

Animal Factors

Metabolizable energy represents the energy of the nutrients absorbed by the animal and available for metabolism. At this point in the energy cascade feed has been broken down and metabolizable energy provides energy for anabolic or catabolic processes. Within ME energetic losses in heat result from the breakdown of glycosidic linkages, peptide bonds, and ester linkages during hydrolysis of carbohydrates, protein, and lipids. Therefore, the loss of heat accounts for 25-32 % of total gross energy ingested by the animal and exists as the second largest loss after fecal energy (Drehmel et al., 2018; Judy et al., 2018; Reynolds et al., 2019a). As a result, two of the largest contributing factors to heat increment directly related to the animal are metabolic body weight and DMI (Morris et al., 2021a). Increased DMI will increase the amount of substrate to hydrolyze, and metabolic body weight carries out metabolic functions increasing the animal's capability to carry out metabolic reactions which generate heat.

Subsequent the removal of heat increment from the ME fraction, net energy supports maintenance, milk, conceptus, or body tissues. Heat increment encompasses the heat

produced through the consumption of food, production of milk, and maintenance of body tissues (Figure 1.11). While subtly different in name, heat production is the portion of heat increment that pertains to the heat released to conserve body function and directly reflects maintenance energy requirement of an animal at a fasting and dormant state and is considered set per unit of metabolic body weight (**MBW**; Baldwin, 1995). Similarly, fetal energy contributes to animal energy requirements but assumed at zero prior to 190 days of gestation (NRC, 2001). However, in the lactating animal's body, a push and pull occurs between energy for tissue (**TE**) and energy for milk production (**NEL)**. This can be influenced by stage of lactation, energy balance, or energetic substrate provided. In early lactation, animals will mobilize tissue stores due to reduced DMI and increased production resulting in negative energy balance. During negative energy balance animals are able to convert TE to milk energy at an efficiency of 0.89 which is actually greater than the conversion of ME to milk energy at 0.75 likely as a result of the efficiency of conversion of fats (Moraes et al., 2015). However, we are ultimately unable to separate if NE_L results from tissue energy or that of the diet alone. Therefore, the utilization of calorimetry, particularly indirect calorimetry gives insight to the location from which energy derived then utilized for milk production

Calorimetry Methods

The word calorimetry originates from the Latin word 'calor' (heat) and the Greek word 'Metrion' (measure). As such, calorimetry can measure all life processes including growth, work and animal production from the energy consumed as food and released into the environment as heat (Agnew and Yan, 2005). Energy released as heat can be accounted for via direct physical methods or estimated from measurement of byproducts

of metabolism. As such both direct and indirect calorimetry are utilized for animal research.

Direct Calorimetry

Direct calorimetry measures the direct dissipation of heat from the body due to bioenergetics (McLean and Tobin, 1988). Although there are disputes on the first use of direct calorimetry, priority is given to the works of Lavoisier and LaPlace who utilized direct calorimetry to estimate heat production from guinea pigs (Kaiyala, 2011; Kenny et al., 2017). Lavoisier utilized a chamber system known as a gradient layer calorimeter to house a guinea pig, surround it with ice, and collect the water melting due to the body heat of the animal (Shephard and Aoyagi, 2012). Accordingly, direct calorimetry is considered the "gold standard" for heat dissipation research due to lack of hinderance by gas production assumptions. However, as animals must be contained within the chamber use of direct calorimetry for farm animals has been limited. As a result, the use of direct calorimetry has led to the standardization of indirect calorimetry for large animals.

Indirect Calorimetry

Indirect calorimetry methods are dependent on measured of oxygen consumption, carbon dioxide, methane, and urea production. All of which are influenced by metabolism to meet energy requirements of the animal through cellular processes and metabolism of carbohydrates, lipids and protein. The preliminary form of indirect calorimetry was close circuit calorimetry by Regnault and Reiset who found that when comparing monogastric animals the ratio off carbon dioxide to oxygen or reaction quotient (**RQ**) was dependent on type of food eaten rather than the species of animal within the chamber (Figure 1.12; McLean and Tobin, 1988). Similarly, Carl von Voit was

a pioneer in indirect calorimetry as he took on previous respiratory exchange data from Lavoisier and created the open circuit method of indirect calorimetry (Battley, 1995). Voit's work established the relationship between protein metabolism and the excretion of urinary nitrogen. Thus, the Brouwer equation was created in order to quantify heat production based on the combustion of 1g of fat, carbohydrate and protein (Brouwer, 1965; Gerrits et al., 2015). However, later work by Brouwer added a negative correction factor for methane emission for applicability in ruminants.

$HP = 16.18\ O_2 + 5.02\ CO_2 - 2.17\ CH_4 - 5.99\ N$

HP= metabolic heat production rate

O2= Oxygen consumption rate, mL/s, STPD

CO2= Carbon Dioxide production rate, mL/s, STPD

CH4= Methane production rate, mL/s, STPD

N= nitrogen excretion rate, g/s

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

Indirect Calorimetry Method

Headboxes

Open circuit indirect calorimetry provides the opportunity to estimate heat production through the concentration of gases consumed and produced by the animal during respiration (Reynolds and Tyrrell, 2000). Since 1909, ways of measuring the concentration of respired gases have been conducted through diverse types of ventilated hood and chamber systems (McLean and Tobin, 1988). However, since that time, methods like headboxes containing air pumps have been developed to mitigate issues with increased CO_2 concentration stress and decrease error associated with respired air loss. During gas collection in the headbox system, a pump applies a slight negative

pressure on the air surrounding the head and neck of the animal and a composite of the respired gas collected (Birkelo et al., 2004). Measurements of carbon dioxide, methane produced, consumption of oxygen and urinary nitrogen are then taken over the collection period and utilized to calculate heat production with the Brouwer equation (1965). When compared with open chamber systems, headboxes are advantageous as data in full chamber systems must be discarded during animal care and maintenance and allow animals to remain in similar conditions to the dietary adaptation period. Also, headboxes are relatively inexpensive and simple to run when compared to full chamber systems. However, headboxes are not without faults as they cover only the front of the animal and approximately 7 % methane produced in the hind gut is lost through the rectum (Johnson et al., 1994).

SUMMARY OF LITERATURE

Distillers grains are a widely utilized feedstuff in the ruminant nutrition industry. Accordingly new products are continually being produced to expand the ethanol industry and reduce waste. As such, new products aim to concentrate the protein relative to the fiber fraction. However, these high protein coproducts (**HPCoP**) lack exact definition in the feed library based on processing technology and nutritive components. Nutrient characterization is integral to providing exact chemical composition measures for utilization in the field. Nonetheless, for nutritionists book values provide place holders prior to on farm feed analysis. However, we must be careful to match the coproduct in use with that of the product on farm to mitigate economic losses or environmental effects.

For feed characterization, crude protein is a proximate nutrient; however, it composes a wide range of proteins with varying utilization based on solubility as well as

association with the fiber fraction. Accordingly, protein can either provide nitrogen for microbial crude protein synthesis or post ruminal amino acids to the animal. For DDGS, corn zein is the main prolamin protein however zein is deficient in the essential amino acid lysine and moreover the available lysine may be complexed with sugar during the production process. As a result, feed evaluation determines the content of nutrients but not the interactions that occur within the ruminant animal.

To determine the effectiveness of a feed product we must first determine the nutritive availability based on current methodology. For protein nutrition the use of the mobile bag procedure aims to determine protein degradation through completely *in situ* methodology where feeds are subjected to ruminal and intestinal digestibility via dacron bags within dual cannulated animals. On the other hand, fiber digestibility can be determined through in vitro fermentation at short- and long-term time points to determine the full assumed digestibility of a feed ingredient. Although, there are two main assays for IVNDFD which are considered standard, there have been quite a few modifications to the procedures leading to variability across laboratories. Accordingly, we still lack homogenous methodology after 70 years of experimentation.

The final pillar of the feed evaluation system hinges on nitrogen and energy balance studies to determine the productive animal response based on feed characterization and nutrient availability of the new product. Feed factors affect nitrogen utilization through protein solubility as well as secondary and tertiary structures and disulfide cross linkages. However, ruminant animals have developed mechanisms to deal with nitrogen utilization through intake shifts and fecal, urinary, and milk nitrogen excretion based on dietary nitrogen intake and dietary nitrogen composition. Energy

derived from feedstuffs are composed of a gross energy value based on the type of macronutrients provided. However, losses of gross energy are encountered through the feces, urine and gas production which are preliminary manipulated by the feed chemical composition and later by animal metabolism. As such, calorimetry, particularly indirect calorimetry can be utilized to determine the heat increment associated with the digestion of a given ration ultimately coming full circle with the feed evaluation system.

PRACTICAL PRROBLEMS AND OBJECTIVES

Advancing technology of the corn dry-milling ethanol production process mechanically separates the fiber from the protein fraction of DDGS. This new HPCoP of post fermentation fractionization it has yet to be accurately described in the feed library or with animal responses. Therefore, it is necessary to examine both the chemical composition and the subsequent animal measures to characterize the feed product for utilization in the field.

The objectives of this research were to:

1) Fully characterize a new high protein processed corn product to be used as inputs for commercial ration balancing software
2) Examine the effects of replacing non-enzymatically browned soybean meal with the HPCoP on DMI, energy utilization, nitrogen utilization and production of lactating Jersey cows

REFERENCES

Abdelqader, M.M., A.R. Hippen, K.F. Kalscheur, D.J. Schingoethe, and A.D. Garcia. 2009. Isolipidic additions of fat from corn germ, corn distillers grains, or corn oil in dairy cow diets. Journal of Dairy Science 92:5523–5533. doi:10.3168/jds.2008-1867.

Agle, M., A.N. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V.K. Vaddella. 2010. The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. Journal of Dairy Science 93:1625–1637. doi:10.3168/jds.2009-2579.

Agnew, R.E., and T. Yan. 2005. Calorimetry. 2nd ed. CABI.

Arriola Apelo, S.I., J.R. Knapp, and M.D. Hanigan. 2014. Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. Journal of Dairy Science 97:4000–4017. doi:10.3168/jds.2013-7392.

Baldwin, R.L. 1995. Modeling ruminant digestion and metabolism. Chapman & Hall, London, UK.

Barnes, R.F. 1967. Collaborative in vitro Rumen Fermentation Studies on Forage Substrates. Journal of Animal Science 26:1120–1130. doi:10.2527/jas1967.2651120x.

Barnett, M.J.F., and R.L. Reid. 1961. Reactions in the Rumen.

Battley, E.H. 1995. The advantages and disadvantages of direct and indirect calorimetry. Thermochimica Acta 250:337–352. doi:10.1016/0040-6031(94)01963-H.

Bauman, D.E., and J.M. Griinari. 2003. Nutritional Regulation of Milk Fat Synthesis. Annu. Rev. Nutr. 23:203–227. doi:10.1146/annurev.nutr.23.011702.073408.

Baumgard, L.H., E. Matitashvili, B.A. Corl, D.A. Dwyer, and D.E. Bauman. 2002. trans-10, cis-12 Conjugated Linoleic Acid Decreases Lipogenic Rates and Expression of Genes Involved in Milk Lipid Synthesis in Dairy Cows. Journal of Dairy Science 85:2155–2163. doi:10.3168/jds.S0022-0302(02)74294-X.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T.A. McAllister. 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48:21. doi:10.1071/EA07199.

Bellemare, M.F., M. Çakir, H.H. Peterson, L. Novak, and J. Rudi. 2017. On the Measurement of Food Waste. American Journal of Agricultural Economics 99:1148–1158. doi:10.1093/ajae/aax034.

Belloir, P., B. Méda, W. Lambert, E. Corrent, H. Juin, M. Lessire, and S. Tesseraud. 2017. Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilization. Animal 11:1881–1889. doi:10.1017/S1751731117000660.

Belyea, R. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresource Technology 94:293–298. doi:10.1016/j.biortech.2004.01.001.

Benchaar, C., F. Hassanat, R. Gervais, P.Y. Chouinard, C. Julien, H.V. Petit, and D.I. Massé. 2013. Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation,

digestion, N balance, and milk production. Journal of Dairy Science 96:2413–2427. doi:10.3168/jds.2012-6037.

Birkelo, C.P., M.J. Brouk, and D.J. Schingoethe. 2004. The Energy Content of Wet Corn Distillers Grains for Lactating Dairy Cows. Journal of Dairy Science 87:1815–1819. doi:10.3168/jds.S0022-0302(04)73338-X.

Blaxter, K.L. 1956. The Nutritive Value of Feeds as Sources of Energy: A Review. Journal of Dairy Science 39:1396–1424. doi:10.3168/jds.S0022-0302(56)94865-2.

Bothast, R.J., and M.A. Schlicher. 2005. Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biotechnol 67:19–25. doi:10.1007/s00253-004-1819-8.

Bradford, B.J., and C.R. Mullins. 2012. Invited review: Strategies for promoting productivity and health of dairy cattle by feeding nonforage fiber sources. Journal of Dairy Science 95:4735–4746. doi:10.3168/jds.2012-5393.

Broderick, G.A., T. Kowalczyk, and L.D. Satter. 1970. Milk Production Response to Supplementation with Encapsulated Methionine per Os or Casein per Abomasum,. Journal of Dairy Science 53:1714–1721. doi:10.3168/jds.S0022-0302(70)86468-2.

Brouwer, E. 1965. Report of Sub-Committee on Constants and Factors. In: Blaxter, K.L., Ed. Proceedings of the 3rd Symposium on Energy Metabolism. Pages 441–443. Academic Press, London.

Brown, W.E., and B.J. Bradford. 2020a. Effects of a high-protein corn product compared with soy and canola protein sources on nutrient digestibility and production responses in mid-lactation dairy cows. Journal of Dairy Science 103:6233–6243. doi:10.3168/jds.2019-17939.

Brown, W.E., and B.J. Bradford. 2020b. Effects of a high-protein corn product compared with soy and canola protein sources on nutrient digestibility and production responses in mid-lactation dairy cows. Journal of Dairy Science 103:6233–6243. doi:10.3168/jds.2019-17939.

Burroughs, W., D.K. Nelson, and D.R. Mertens. 1975. Evaluation of Protein Nutrition by Metabolizable Protein and Urea Fermentation Potential. Journal of Dairy Science 58:611–619. doi:10.3168/jds.S0022-0302(75)84617-0.

Castillo, A., E. Kebreab, D. Beever, and J. France. 2000. A review of efficiency of nitrogen utilisation inlactating dairy cows and its relationship withenvironmental pollution. J. Anim. Feed Sci. 9:1–32. doi:10.22358/jafs/68025/2000.

Cessna, J., and A. Teran. 2021. Dairy.

Chatzifragkou, A., O. Kosik, P.C. Prabhakumari, A. Lovegrove, R.A. Frazier, P.R. Shewry, and D. Charalampopoulos. 2015. Biorefinery strategies for upgrading Distillers' Dried Grains with Solubles (DDGS). Process Biochemistry 50:2194–2207. doi:10.1016/j.procbio.2015.09.005.

Christen, K.A., D.J. Schingoethe, K.F. Kalscheur, A.R. Hippen, K.K. Karges, and M.L. Gibson. 2010. Response of lactating dairy cows to high protein distillers grains or 3 other protein supplements. Journal of Dairy Science 93:2095–2104. doi:10.3168/jds.2009-2687.

Clark, J.H. 1975. Lactational Responses to Postruminal Administration of Proteins and Amino Acids. Journal of Dairy Science 58:1178–1197. doi:10.3168/jds.S0022-0302(75)84696-0.

Clark, J.H., T.H. Klusmeyer, and M.R. Cameron. 1992. Microbial Protein Synthesis and Flows of Nitrogen Fractions to the Duodenum of Dairy Cows. Journal of Dairy Science 75:2304–2323. doi:10.3168/jds.S0022-0302(92)77992-2.

Coblentz, W.K., M.S. Akins, R.K. Ogden, L.M. Bauman, and A.J. Stammer. 2019. Effects of sample size on neutral detergent fiber digestibility of triticale forages using the Ankom DaisyII Incubator system. Journal of Dairy Science 102:6987–6999. doi:10.3168/jds.2019-16681.

Crooker, B.A., C.J. Sniffen, W.H. Hoover, and L.L. Johnson. 1978. Solvents for Soluble Nitrogen Measurements in Feedstuffs. Journal of Dairy Science 61:437–447. doi:10.3168/jds.S0022-0302(78)83618-2.

Danielsson, R., M. Ramin, J. Bertilsson, P. Lund, and P. Huhtanen. 2017. Evaluation of a gas in vitro system for predicting methane production in vivo. Journal of Dairy Science 100:8881–8894. doi:10.3168/jds.2017-12675.

DePeters, E.J., and J.P. Cant. 1992. Nutritional Factors Influencing the Nitrogen Composition of Bovine Milk: A Review. Journal of Dairy Science 75:2043–2070. doi:10.3168/jds.S0022-0302(92)77964-8.

Drehmel, O.R., T.M. Brown-Brandl, J.V. Judy, S.C. Fernando, P.S. Miller, K.E. Hales, and P.J. Kononoff. 2018. The influence of fat and hemicellulose on methane production and energy utilization in lactating Jersey cattle. Journal of Dairy Science 101:7892–7906. doi:10.3168/jds.2017-13822.

Fahey Jr., G.C., and L.L. Berger. 1988. Carbohydrate Nutrition of Ruminants. Prentice-Hall, Inc., Englewood Cliffs, New Jersey 07632.

Firkins, J.L., L.L. Berger, G.C.F. Jr, and N.R. Merchen. 1984. Ruminal Nitrogen Degradability and Escape of Wet and Dry Distillers Grains and Wet and Dry Corn Gluten Feeds 67:9.

Flatt, W.R., P.W. Moe, L.A. Moore, and P.J. Van Soest. 1967. Estimation and Prediction of the Energy Value of Feeds for Ruminants. European Association For Animal Production.

Foth, A.J., T. Brown-Brandl, K.J. Hanford, P.S. Miller, G. Garcia Gomez, and P.J. Kononoff. 2015. Energy content of reduced-fat dried distillers grains with solubles for lactating dairy cows. Journal of Dairy Science 98:7142–7152. doi:10.3168/jds.2014-9226.

Fry, S.C. 1988. The Growing Plant Cell Wall: Chemical andMetabolic Analysis. Wiley.

Gargallo, S., S. Calsamiglia, and A. Ferret. 2006. Technical note: A modified three-step in vitro procedure to determine intestinal digestion of proteins. Journal of Animal Science 84:2163–2167. doi:10.2527/jas.2004-704.

Gerrits, W.J.J., J.J.G.C. van de Borne, and E. Labussiere. 2015. Deriving heat production from gaseous exchange: validity of the approach. Wageningen Academic, The Netherlands.

Gianazza, E., V. Viglienghi, P.G. Righetti, F. Salamini, and C. Soave. 1977. Amino acid composition of zein molecular components. Phytochemistry 16:315–317. doi:10.1016/0031-9422(77)80054-X.

Gibbon, B.C., X. Wang, and B.A. Larkins. 2003. Altered starch structure is associated with endosperm modification in Quality Protein Maize. Proceedings of the National Academy of Sciences 100:15329–15334. doi:10.1073/pnas.2136854100.

Goering, H.K., and P.J. Van Soest. 1970. Forage Fiber Analysis. U.S Dept of Agriculture. Superintendent of Documents, US Government Printing Office, Washington D.C. 20402, USDSA Agriculture Research Service.

Goeser, J.P., and D.K. Combs. 2009. An alternative method to assess 24-h ruminal in vitro neutral detergent fiber digestibility. Journal of Dairy Science 92:3833–3841. doi:10.3168/jds.2008-1136.

Grant, R.H., and D.R. Mertens. 1992a. Influence of Buffer pH and Raw Corn Starch Addition on In Vitro Fiber Digestion Kinetics. Journal of Dairy Science 75:2762–2768. doi:10.3168/jds.S0022-0302(92)78039-4.

Grant, R.J., and D.R. Mertens. 1992b. Impact of In Vitro Fermentation Techniques upon Kinetics of Fiber Digestion. Journal of Dairy Science 75:1263–1272. doi:10.3168/jds.S0022-0302(92)77876-X.

Greenberg, N.A., and W.F. Shipe. 1979. Comparison of the Abilities of Tricholoracetic, Picric, Sulfosalicylic, and Tungstic Acids to Precipitate Protein Hydrolysates and Proteins. J Food Science 44:735–737. doi:10.1111/j.1365-2621.1979.tb08487.x.

Gressley, T.F., and L.E. Armentano. 2007. Effects of Low Rumen-Degradable Protein or Abomasal Fructan Infusion on Diet Digestibility and Urinary Nitrogen Excretion in Lactating Dairy Cows. Journal of Dairy Science 90:1340–1353. doi:10.3168/jds.S0022-0302(07)71621-1.

Griinari, J.M., D.A. Dwyer, M.A. McGuire, D.E. Bauman, D.L. Palmquist, and K.V.V. Nurmela. 1998. Trans-Octadecenoic Acids and Milk Fat Depression in Lactating Dairy Cows. Journal of Dairy Science 81:1251–1261. doi:10.3168/jds.S0022-0302(98)75686-3.

Hall, M.B., and P.J. Kononoff. 2002. Feed Concentrates: Co-Product Feeds 7.

Hall, M.B., and D.R. Mertens. 2008. In Vitro Fermentation Vessel Type and Method Alter Fiber Digestibility Estimates. Journal of Dairy Science 91:301–307. doi:10.3168/jds.2006-689.

Hall, M.B., and D.R. Mertens. 2012. A ring test of in vitro neutral detergent fiber digestibility: Analytical variability and sample ranking. Journal of Dairy Science 95:1992–2003. doi:10.3168/jds.2011-4802.

Hardeman, N.P. 1983. Shucks, Shocks, and Hominy Blocks: Corn As a Way of Life in Pioneer America. The Annals of Iowa 46:550–551. doi:10.17077/0003-4827.8921.

Harmon, D.L. 1993. Nutritional Regulation of Postruminal Digestive Enzymes in Ruminants. Journal of Dairy Science 76:2102–2111. doi:10.3168/jds.S0022-0302(93)77545-1.

Harty, S.R., J.-M. Akayezu, J.G. Linn, and J.M. Cassady. 1998. Nutrient Composition of Distillers Grains with Added Solubles 22.

Hervás, G., P. Frutos, F.J. Giráldez, M.J. Mora, B. Fernández, and Á.R. Mantecón. 2005. Effect of preservation on fermentative activity of rumen fluid inoculum for in vitro gas production techniques. Animal Feed Science and Technology 123–124:107–118. doi:10.1016/j.anifeedsci.2005.05.004.

Higgs, R.J., L.E. Chase, D.A. Ross, and M.E. Van Amburgh. 2015. Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs. Journal of Dairy Science 98:6340–6360. doi:10.3168/jds.2015-9379.

Hoffman, P.C., and R.D. Shaver. 2009. Corn Biochemistry: Factors Related to Starch Digestibility in Ruminants.

Holter, J.B., N.F. Colovos, and W.E. Urban Jr. 1970. Energy Utilization in the Ruminant Fed Maise Silages as the Sole Forage. Vitznau, Switzerland.

Hoorn, R., A.M. Frens, and K. Stolk. 1957. DE INVLOED VAN HET RUWVOEDERRANTSOEN OP HET CELLULOSE-AFBREKEND VERMOGEN VAN DE PENSFLORA IN EEN KUNSTPENS 12.

Hristov, A.N. 2011. Technical note: Contribution of ammonia emitted from livestock to atmospheric fine particulate matter (PM2.5) in the United States. Journal of Dairy Science 94:3130–3136. doi:10.3168/jds.2010-3681.

Hristov, A.N., A. Bannink, L.A. Crompton, P. Huhtanen, M. Kreuzer, M. McGee, P. Nozière, C.K. Reynolds, A.R. Bayat, D.R. Yáñez-Ruiz, J. Dijkstra, E. Kebreab, A. Schwarm, K.J. Shingfield, and Z. Yu. 2019. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. Journal of Dairy Science 102:5811–5852. doi:10.3168/jds.2018-15829.

Hristov, A.N., M. Hanigan, A. Cole, R. Todd, T.A. McAllister, P.M. Ndegwa, and A. Rotz. 2011. Review: Ammonia emissions from dairy farms and beef feedlots. Can. J. Anim. Sci. 91:1–35. doi:10.4141/CJAS10034.

Hristov, A.N., J.K. Ropp, K.L. Grandeen, S. Abedi, R.P. Etter, A. Melgar, and A.E. Foley. 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows1. Journal of Animal Science 83:408–421. doi:10.2527/2005.832408x.

Hubbard, K.J., P.J. Kononoff, A.M. Gehman, J.M. Kelzer, K. Karges, and M.L. Gibson. 2009. Short communication: The effect of feeding high protein distillers dried grains on milk production of Holstein cows. Journal of Dairy Science 92:2911–2914. doi:10.3168/jds.2008-1955.

Huhtanen, C.N., R.K. Saunders, and L.S. Gall. 1953. Fiber Digestion Using the Miniature Artificial Rumen 8.

Huhtanen, P., and A.N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science 92:3222–3232. doi:10.3168/jds.2008-1352.

Huhtanen, P., J.I. Nousiainen, M. Rinne, K. Kytölä, and H. Khalili. 2008. Utilization and Partition of Dietary Nitrogen in Dairy Cows Fed Grass Silage-Based Diets. Journal of Dairy Science 91:3589–3599. doi:10.3168/jds.2008-1181.

Huhtanen, P., M. Rinne, and J. Nousiainen. 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. Journal of Dairy Science 92:5031–5042. doi:10.3168/jds.2008-1834.

Hveplund, T. 1985. Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep and by in sacco procedure.. Acta Agriculture Scandanavia.

Jenkins, T.C. 1993. Lipid Metabolism in the Rumen 13.

Johnson, Kristen., Mark. Huyler, Hal. Westberg, Brian. Lamb, and Pat. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a sulfur

hexafluoride tracer technique. Environ. Sci. Technol. 28:359–362. doi:10.1021/es00051a025.

Jonker, J.S., R.A. Kohn, and J. High. 2002. Dairy Herd Management Practices that Impact Nitrogen Utilization Efficiency. Journal of Dairy Science 85:1218–1226. doi:10.3168/jds.S0022-0302(02)74185-4.

Judy, J.V., G.C. Bachman, T.M. Brown-Brandl, S.C. Fernando, K.E. Hales, P.S. Miller, R.R. Stowell, and P.J. Kononoff. 2018. Energy balance and diurnal variation in methane production as affected by feeding frequency in Jersey cows in late lactation. Journal of Dairy Science 101:10899–10910. doi:10.3168/jds.2018-14596.

Kaiyala, K.J. 2011. Direct animal calorimetry, the underused gold standard for quantifying the fire of life. Comparative Biochemistry and Physiology 13.

Kajikawa, H., K. Miyazawa, A. Yanase, Y. Tanabe, Y. Tsuchida, Y. Mitsumoto, Y. Kozato, and M. Mitsumori. 2012. Variation in chemical composition of corn dried distillers grains with solubles in relation to in situ protein degradation profiles in the rumen. Animal Science Journal 83:299–304. doi:10.1111/j.1740-0929.2011.00956.x.

Karlsson, J., R. Spörndly, M. Lindberg, and K. Holtenius. 2018. Replacing human-edible feed ingredients with by-products increases net food production efficiency in dairy cows. Journal of Dairy Science 101:7146–7155. doi:10.3168/jds.2017-14209.

Kebreab, E., A.R. Castillo, D.E. Beever, D.J. Humphries, and J. France. 2000. Effects of Management Practices Prior to and During Ensiling and Concentrate Type on Nitrogen Utilization in Dairy Cows. Journal of Dairy Science 83:1274–1285. doi:10.3168/jds.S0022-0302(00)74994-0.

Kebreab, E., A. Strathe, J. Dijkstra, J. Mills, C.K. Reynolds, L.A. Crompton, and J. Yan Tan France. 2010. Energy and protein interactions and their effect on nitrogen excretion in dairy cows. GM Crovetto. Wageningen Academic Publishers, Wageningen, The Netherlands.

Kelzer, J.M., P.J. Kononoff, A.M. Gehman, L.O. Tedeschi, K. Karges, and M.L. Gibson. 2009. Effects of feeding three types of corn-milling coproducts on milk production and ruminal fermentation of lactating Holstein cattle. Journal of Dairy Science 92:5120–5132. doi:10.3168/jds.2009-2208.

Kelzer, J.M., P.J. Kononoff, L.O. Tedeschi, T.C. Jenkins, K. Karges, and M.L. Gibson. 2010. Evaluation of protein fractionation and ruminal and intestinal digestibility of corn milling co-products. Journal of Dairy Science 93:2803–2815. doi:10.3168/jds.2009-2460.

Kenny, G.P., S.R. Notley, and D. Gagnon. 2017. Direct calorimetry: a brief historical review of its use in the study of human metabolism and thermoregulation. Eur J Appl Physiol 117:1765–1785. doi:10.1007/s00421-017-3670-5.

Kim, Y., R. Hendrickson, N.S. Mosier, M.R. Ladisch, B. Bals, V. Balan, and B.E. Dale. 2008. Enzyme hydrolysis and ethanol fermentation of liquid hot water and AFEX pretreated distillers' grains at high-solids loadings. Bioresource Technology 99:5206–5215. doi:10.1016/j.biortech.2007.09.031.

Kleinschmit, D.H., J.L. Anderson, D.J. Schingoethe, K.F. Kalscheur, and A.R. Hippen. 2007. Ruminal and Intestinal Degradability of Distillers Grains plus Solubles

Varies by Source. Journal of Dairy Science 90:2909–2918. doi:10.3168/jds.2006-613.

Klop, G., S. van Laar-van Schuppen, W.F. Pellikaan, W.H. Hendriks, A. Bannink, and J. Dijkstra. 2017. Changes in in vitro gas and methane production from rumen fluid from dairy cows during adaptation to feed additives in vivo. Animal 11:591–599. doi:10.1017/S1751731116002019.

Klopfenstein, T. 1996. Distillers grains as an energy source and effect of drying on protein availability. Animal Feed Science and Technology 60:201–207. doi:10.1016/0377-8401(96)00978-9.

Kohn, R.A., M.M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats1. Journal of Animal Science 83:879–889. doi:10.2527/2005.834879x.

Krauss, W.E. 1927. The Non-Protein Nitrogen in Certain Dairy Rations and the Partition of Nitrogen in the Urine Produced Thereon. Journal of Dairy Science 10:400–415. doi:10.3168/jds.S0022-0302(27)93858-2.

Krishnamoorthy, U., T.V. Muscato, C.J. Sniffen, and P.J. Van Soest. 1982. Nitrogen Fractions in Selected Feedstuffs. Journal of Dairy Science 65:217–225. doi:10.3168/jds.S0022-0302(82)82180-2.

Külling, D.R., H. Menzi, T.F. Kröber, A. Neftel, F. Sutter, P. Lischer, and M. Kreuzer. 2001. Emissions of ammonia, nitrous oxide and methane from different types of dairy manure during storage as affected by dietary protein content. J. Agric. Sci. 137:235–250. doi:10.1017/S0021859601001186.

Kumar, D., and V. Singh. 2019. Bioethanol Production From Corn. Elsevier.

Lapierre, H., R. Berthiaume, G. Raggio, M.C. Thivierge, L. Doepel, D. Pacheco, P. Dubreuil, and G.E. Lobley. 2005. The route of absorbed nitrogen into milk protein. Anim. Sci. 80:11–22. doi:10.1079/ASC41330011.

Lapierre, H., R. Martineau, M.D. Hanigan, H.J. van Lingen, E. Kebreab, J.W. Spek, and D.R. Ouellet. 2020. Review: Impact of protein and energy supply on the fate of amino acids from absorption to milk protein in dairy cows. Animal 14:s87–s102. doi:10.1017/S1751731119003173.

LaPierre, P.A., D. Luchini, D.A. Ross, and M.E. Van Amburgh. 2019. Effects of Precision Essential Amino Acid Formulation on a Metabolizable Energy Basis for Lactating Dairy Cows 11.

Larkins, B.A. 2019. Proteins of the Kernel. Elsevier.

Lawton, J.W. 2002. Zein: A History of Processing and Use. Cereal Chemistry Journal 79:1–18. doi:10.1094/CCHEM.2002.79.1.1.

Leonardi, C., S. Bertics, and L.E. Armentano. 2005. Effect of Increasing Oil from Distillers Grains or Corn Oil on Lactation Performance. Journal of Dairy Science 88:2820–2827. doi:10.3168/jds.S0022-0302(05)72962-3.

Licitra, G., T.M. Hernandez, and P.J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57:347–358. doi:10.1016/0377-8401(95)00837-3.

Liebe, D.L., M.B. Hall, and R.R. White. 2020. Contributions of dairy products to environmental impacts and nutritional supplies from United States agriculture. Journal of Dairy Science 103:10867–10881. doi:10.3168/jds.2020-18570.

Lopes, F., K. Ruh, and D.K. Combs. 2015. Validation of an approach to predict total-tract fiber digestibility using a standardized in vitro technique for different diets fed to high-producing dairy cows. Journal of Dairy Science 98:2596–2602. doi:10.3168/jds.2014-8665.

Machacek, K.J., and P.J. Kononoff. 2009. The Relationship Between Acid Detergent Insoluble Nitrogen and Nitrogen Digestibility in Lactating Dairy Cattle. The Professional Animal Scientist 25:701–708. doi:10.15232/S1080-7446(15)30778-6.

Mahadevan, S., J.D. Erfle, and F.D. Sauer. 1980. Degradation of Soluble and Insoluble Proteins by Bacteroides Amylophilus Protease and by Rumen Microorganisms2. Journal of Animal Science 50:723–728. doi:10.2527/jas1980.504723x.

Mariotti, F., D. Tomé, and P. Mirand. 2008. Converting Nitrogen into Protein – Beyond 6.25 and Jones' Factors. Crit. Rev. Food Sci. Nutr. 22.

McAllister, T.A., H.D. Bae, and G.A. Jones. 2018. Microbial Attachment and Feed Digestion in the Rumenl12 15.

McLean, J.A., and G. Tobin. 1988. Animal and Human Calorimetry- Indirect Calorimetry. 1st ed. Cambridge University Press.

Menke, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci. 93:217–222. doi:10.1017/S0021859600086305.

Mertens, D.R. 2005. Rate and Extent of Digestion. 2nd ed. CABI.

Milgen, J. van, E. Labussiere, J. Noblet, and C.F.M. de Lange. 2018. Bioavailability of the dietary energy component. Wageningen Academic Publishers.

Millen, D.D., M. De Beni Arrigoni, and R.D. Lauritano Pacheco eds. . 2016. Rumenology. Springer International Publishing, Cham.

Mjoun, K., K.F. Kalscheur, A.R. Hippen, and D.J. Schingoethe. 2010a. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains products. Journal of Dairy Science 93:4144–4154. doi:10.3168/jds.2009-2883.

Mjoun, K., K.F. Kalscheur, A.R. Hippen, D.J. Schingoethe, and D.E. Little. 2010b. Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grains with solubles. Journal of Dairy Science 93:288–303. doi:10.3168/jds.2009-2377.

Moe, P.W., and H.F. Tyrrell. 1979. Methane Production in Dairy Cows 62:4.

Mollgaard, H. 1958. The opening lecture. Copenhagen.

Moore, J.E., and G.O. Mott. 1976. Fermentation Tubes for In Vitro Digestion of Forages. Journal of Dairy Science 59:167–169. doi:10.3168/jds.S0022-0302(76)84173-2.

Moraes, L.E., E. Kebreab, A.B. Strathe, J. Dijkstra, J. France, D.P. Casper, and J.G. Fadel. 2015. Multivariate and univariate analysis of energy balance data from lactating dairy cows. Journal of Dairy Science 98:4012–4029. doi:10.3168/jds.2014-8995.

Morris, D. 2020. Energy metabolism in Jersey cows: Improving our understanding of energy requirements and utilization 309.

Morris, D.L., T.M. Brown-Brandl, K.E. Hales, K.J. Harvatine, and P.J. Kononoff. 2020. Effects of high-starch or high-fat diets formulated to be isoenergetic on energy

and nitrogen partitioning and utilization in lactating Jersey cows. Journal of Dairy Science 103:4378–4389. doi:10.3168/jds.2019-17638.

Morris, D.L., T.M. Brown-Brandl, P.S. Miller, W.P. Weiss, R.R. White, and P.J. Kononoff. 2021a. Factors that affect heat production in lactating Jersey cows. Journal of Dairy Science 104:346–356. doi:10.3168/jds.2020-18976.

Morris, D.L., J.L. Firkins, C. Lee, W.P. Weiss, and P.J. Kononoff. 2021b. Relationship between urinary energy and urinary nitrogen or carbon excretion in lactating Jersey cows. Journal of Dairy Science 104:6727–6738. doi:10.3168/jds.2020-19684.

Morris, D.L., S.H. Kim, P.J. Kononoff, and C. Lee. 2018. Continuous 11-week feeding of reduced-fat distillers grains with and without monensin reduces lactation performance of dairy cows. Journal of Dairy Science 101:5971–5983. doi:10.3168/jds.2017-14170.

Murthy, G.S., V. Singh, D.B. Johnston, K.D. Rausch, and M.E. Tumbleson. 2006. Evaluation and Strategies to Improve Fermentation Characteristics of Modified Dry-Grind Corn Processes. Cereal Chemistry Journal 83:455–459. doi:10.1094/CC-83-0455.

Nakamura, T., T.J. Klopfenstein, and R.A. Britton. 1994. Evaluation of acid detergent insoluble nitrogen as an indicator of protein quality in nonforage proteins. Journal of Animal Science 72:1043–1048. doi:10.2527/1994.7241043x.

National Academies of Sciences, E., and Medicine. 2016. Nutrient Requirements of Beef Cattle: Eighth Revised Edition. The National Academies Press, Washington, DC.

Nolan, J.V., and R.C. Dobos. 2005. Nitrogen Transactions in Ruminants. 2nd ed. CABI.

NRC. 2001. Nutrient Requirements of Dairy Cattle. National Academies Press, Washington, D.C.

Oba, M., and M.S. Allen. 1999. Evaluation of the Importance of the Digestibility of Neutral Detergent Fiber from Forage: Effects on Dry Matter Intake and Milk Yield of Dairy Cows. Journal of Dairy Science 82:589–596. doi:10.3168/jds.S0022-0302(99)75271-9.

Ørskov, E.R. 1982. Protein Nutition in Ruminants. Acad. Press Inc, New Your, NY.

Ouellet, D.R., M. Demers, G. Zuur, G.E. Lobley, J.R. Seoane, J.V. Nolan, and H. Lapierre. 2002. Effect of Dietary Fiber on Endogenous Nitrogen Flows in Lactating Dairy Cows. Journal of Dairy Science 85:3013–3025. doi:10.3168/jds.S0022-0302(02)74387-7.

Palmonari, A., G. Canestrari, E. Bonfante, M. Fustini, L. Mammi, and A. Formigoni. 2017. Technical note: In vitro digestibility of amylase-treated, ash-corrected neutral detergent fiber, with addition of sodium sulfite, at 240 hours with or without rumen fluid reinoculation. Journal of Dairy Science 100:1200–1202. doi:10.3168/jds.2016-11368.

Palmonari, A., A. Gallo, M. Fustini, G. Canestrari, F. Masoero, C.J. Sniffen, and A. Formigoni. 2016. Estimation of the indigestible fiber in different forage types. Journal of Animal Science 94:248–254. doi:10.2527/jas.2015-9649.

Patterson, M. 2021. OECD-FAO Agricultural Outlook 2021-2030 12.

Paz, H.A., T.J. Klopfenstein, D. Hostetler, S.C. Fernando, E. Castillo-Lopez, and P.J. Kononoff. 2014. Ruminal degradation and intestinal digestibility of protein and

amino acids in high-protein feedstuffs commonly used in dairy diets. Journal of Dairy Science 97:6485–6498. doi:10.3168/jds.2014-8108.

Paz, H.A., and P.J. Kononoff. 2014. Lactation responses and amino acid utilization of dairy cows fed low-fat distillers dried grains with solubles with or without rumen-protected lysine supplementation. Journal of Dairy Science 97:6519–6530. doi:10.3168/jds.2014-8315.

Paz, H.A., M.J. de Veth, R.S. Ordway, and P.J. Kononoff. 2013. Evaluation of rumen-protected lysine supplementation to lactating dairy cows consuming increasing amounts of distillers dried grains with solubles. Journal of Dairy Science 96:7210–7222. doi:10.3168/jds.2013-6906.

Pell, A.N., and P. Schofield. 1993. Computerized Monitoring of Gas Production to Measure Forage Digestion In Vitro. Journal of Dairy Science 76:1063–1073. doi:10.3168/jds.S0022-0302(93)77435-4.

Pichard, D.G.R. 1977. Forage nutritive value. Continuous and batch in vitro rumen fermentatios and nitrogen solubility. Cornell University,.

Raffrenato, E., C.F. Nicholson, and M.E. Van Amburgh. 2019. Development of a mathematical model to predict pool sizes and rates of digestion of 2 pools of digestible neutral detergent fiber and an undigested neutral detergent fiber fraction within various forages. Journal of Dairy Science 102:351–364. doi:10.3168/jds.2018-15102.

Raffrenato, E., and M.E. Van Amburgh. 2011. Technical note: Improved methodology for analyses of acid detergent fiber and acid detergent lignin. Journal of Dairy Science 94:3613–3617. doi:10.3168/jds.2010-3701.

Raggio, G., D. Pacheco, R. Berthiaume, G.E. Lobley, D. Pellerin, G. Allard, P. Dubreuil, and H. Lapierre. 2004. Effect of Level of Metabolizable Protein on Splanchnic Flux of Amino Acids in Lactating Dairy Cows. Journal of Dairy Science 87:3461–3472. doi:10.3168/jds.S0022-0302(04)73481-5.

Ramirez-Ramirez, H.A., E. Castillo Lopez, C.J.R. Jenkins, N.D. Aluthge, C. Anderson, S.C. Fernando, K.J. Harvatine, and P.J. Kononoff. 2016. Reduced-fat dried distillers grains with solubles reduces the risk for milk fat depression and supports milk production and ruminal fermentation in dairy cows. Journal of Dairy Science 99:1912–1928. doi:10.3168/jds.2015-9712.

Reis, C.E.R., A. Rajendran, and B. Hu. 2017. New technologies in value addition to the thin stillage from corn-to-ethanol process. Rev Environ Sci Biotechnol 16:175–206. doi:10.1007/s11157-017-9421-6.

Reynolds, C.K., and N.B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis1. Journal of Animal Science 86:E293–E305. doi:10.2527/jas.2007-0475.

Reynolds, C.K., and H.F. Tyrrell. 2000. Energy metabolism in lactating beef heifers.. Journal of Animal Science 78:2696. doi:10.2527/2000.78102696x.

Reynolds, M.A., T.M. Brown-Brandl, J.V. Judy, K.J. Herrick, K.E. Hales, A.K. Watson, and P.J. Kononoff. 2019. Use of indirect calorimetry to evaluate utilization of energy in lactating Jersey dairy cattle consuming common coproducts. Journal of Dairy Science 102:320–333. doi:10.3168/jds.2018-15471.

Roman-Garcia, Y., K.E. Mitchell, B.L. Denton, C. Lee, M.T. Socha, B.A. Wenner, and J.L. Firkins. 2021. Conditions stimulating neutral detergent fiber degradation by

dosing branched-chain volatile fatty acids. II: Relation with solid passage rate and pH on neutral detergent fiber degradation and microbial function in continuous culture. Journal of Dairy Science S0022030221006871. doi:10.3168/jds.2021-20335.

Rosentrater, K.A., and A.D. Evers. 2018. Wet milling. Elsevier.

Ross, D. 2013. Methods to Analyze Feeds for Nitrogen Fractions And Digestibility For Ruminants With Application For The Cncps. Cornell University,.

Ross, D., M. Gutierrez, and M.V. Amburgh. 2013. Development of an in vitro intestinal digestibility assay for ruminant feeds 24.

Roth, M., M. Jekle, and T. Becker. 2019. Opportunities for upcycling cereal byproducts with special focus on Distiller's grains. Trends in Food Science & Technology 91:282–293. doi:10.1016/j.tifs.2019.07.041.

Russell, J.B., J.D. O'Connor, D.G. Fox, P.J. Van Soest, and C.J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Animal Science 70:3551–3561. doi:10.2527/1992.70113551x.

Rutherfurd, S.M., and P.J. Moughan. 2018. The chemical analysis of proteins and amino acids. Wageningen Academic.

Rymer, C., J.A. Huntington, B.A. Williams, and D.I. Givens. 2005. In vitro cumulative gas production techniques: History, methodological considerations and challenges. Animal Feed Science and Technology 123–124:9–30. doi:10.1016/j.anifeedsci.2005.04.055.

Schwab, C.G., C.K. Bozak, N.L. Whitehouse, and M.M.A. Mesbah. 1992. Amino Acid Limitation and Flow to Duodenum at Four Stages of Lactation. 1. Sequence of Lysine and Methionine Limitation. Journal of Dairy Science 75:3486–3502. doi:10.3168/jds.S0022-0302(92)78125-9.

Schwab, C.G., and G.A. Broderick. 2017. A 100-Year Review: Protein and amino acid nutrition in dairy cows. Journal of Dairy Science 100:10094–10112. doi:10.3168/jds.2017-13320.

Schwab, C.G., T.P. Tylutki, R.S. Ordway, C. Sheaffer, and M.D. Stern. 2003. Characterization of Proteins in Feeds. Journal of Dairy Science 86:E88–E103. doi:10.3168/jds.S0022-0302(03)74042-9.

Sears, A., O. Gonzalez, A. Alberto, A. Young, J. de Souza, A. Relling, and F. Batistel. 2020. Effect of feeding a palmitic acid–enriched supplement on production responses and nitrogen metabolism of mid-lactating Holstein and Jersey cows. Journal of Dairy Science 103:8898–8909. doi:10.3168/jds.2020-18232.

Seckinger, H.L. 1973. Sorghum protein ultrastructure as it relates to composition. Cereal chemistry v. 50:455–0.

Shephard, R.J., and Y. Aoyagi. 2012. Measurement of human energy expenditure, with particular reference to field studies: an historical perspective. Eur J Appl Physiol 31.

Shukla, R., and M. Cheryan. 2001. Zein: the industrial protein from corn. Industrial Crops and Products 13:171–192. doi:10.1016/S0926-6690(00)00064-9.

Shurson, G.C. 2017. The Role of Biofuels Coproducts in Feeding the World Sustainably. Annu. Rev. Anim. Biosci. 5:229–254. doi:10.1146/annurev-animal-022516-022907.

Singh, B., H.P.S. Makkar, and S.S. Negi. 1989. Rate and Extent of Digestion and Potentially Digestible Dry Matter and Cell Wall of Various Tree Leaves. Journal of Dairy Science 72:3233–3239. doi:10.3168/jds.S0022-0302(89)79482-0.

Singh, V., D.B. Johnston, K. Naidu, K.D. Rausch, R.L. Belyea, and M.E. Tumbleson. 2005. Comparison of Modified Dry-Grind Corn Processes for Fermentation Characteristics and DDGS Composition. Cereal Chemistry Journal 82:187–190. doi:10.1094/CC-82-0187.

Singh, V., D.B. Johnston, K.D. Rausch, and M.E. Tumbleson. 2010. Improvements in Corn to Ethanol Production Technology UsingSaccharomyces cerevisiae. A.A. Verts, N. Qureshi, H.P. Blaschek, and H. Yukawa, ed. Blackwell Publishing Ltd., Oxford, UK.

Singh, V., K.D. Rausch, P. Yang, H. Shapouri, R.L. Belyea, and M.E. Tumbleson. 2001. Modified Dry Grind Ethanol Process 43.

Sniffen, C.J., J.D. O'Connor, P.J. Van Soest, D.G. Fox, and J.B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science 70:3562–3577. doi:10.2527/1992.70113562x.

Spek, J.W., J. Dijkstra, G. van Duinkerken, W.H. Hendriks, and A. Bannink. 2013. Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: A meta-analysis. Journal of Dairy Science 96:4310–4322. doi:10.3168/jds.2012-6265.

Spiehs, M.J., M.H. Whitney, and G.C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota 7.

Srinivasan, R., R.A. Moreau, K.D. Rausch, R.L. Belyea, M.E. Tumbleson, and V. Singh. 2005. Separation of Fiber from Distillers Dried Grains with Solubles (DDGS) Using Sieving and Elutriation. Cereal Chemistry Journal 82:528–533. doi:10.1094/CC-82-0528.

St-Pierre, N.R., and W.P. Weiss. 2015. Partitioning variation in nutrient composition data of common feeds and mixed diets on commercial dairy farms. Journal of Dairy Science 98:5004–5015. doi:10.3168/jds.2015-9431.

Sutton, T.S., J.B. Brown, and E.W. Johnston. 1932. The Effect of Corn Oil on Milk and Butter Fat Production and on the Composition of Butter Fat in the Dairy Cow. Journal of Dairy Science 15:209–211. doi:10.3168/jds.S0022-0302(32)93405-5.

Tamminga, S. 1992. Nutrition Management of Dairy Cows as a Contribution to Pollution Control 13.

Tebbe, A.W. 2020. Effects of Dietary Protein and Amino Acids and Their Labile Stores in Dairy Cows. PhD Dissertation Thesis. The Ohio State University,.

Tilley, J.M.A., and R.A. Terry. 1963. A Two-Stage Technique for the In Vitro Digestion of Forage Crops. Grass and Forage Sci 18:104–111. doi:10.1111/j.1365-2494.1963.tb00335.x.

Tonroy, B.R., and T.W. Perry. 1974. In Vitro Digestion of Raw, Roasted, and Pressure-Flaked Corn. Journal of Dairy Science 57:1508–1511. doi:10.3168/jds.S0022-0302(74)85097-6.

United Nations. 2019. World Population Prospects Highlights, 2019 Revision Highlights, 2019 Revision.

USDA. 2020. Milk Cost of Production estimates.

Van Amburgh, M.E., E.A. Collao-Saenz, R.J. Higgs, D.A. Ross, E.B. Recktenwald, E. Raffrenato, L.E. Chase, T.R. Overton, J.K. Mills, and A. Foskolos. 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. Journal of Dairy Science 98:6361–6380. doi:10.3168/jds.2015-9378.

Van Amburgh, M.E., P.J. Van Soest, J.B. Robertson, and W.F. Knaus. 2003. Corn Silage Neutral Detergent Fiber: Refining a Mathematical Approach for In Vitro Rates of Digestion. Pages 99–108 Cornell Nutr. Conf., Syracuse, NY.

Van Soest, P.J., and J.B. Robertson. 1985. Analysis of Forages and Fiberous Foods. Cornel University.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Van Soest, P.J., M.E. Van Amburgh, J.B. Robertson, and W.F. Knaus. 2005. Validation of the 2.4 Times Lignin Factor For Ultimate Extent of NDF Digestion, and Curve Peeling Rate of Fermentation Cureces Into Pools 139–149.

Varga, G.A., and E.S. Kolver. 1997. Microbial and Animal Limitations to Fiber Digestion and Utilization. The Journal of Nutrition 127:819S-823S. doi:10.1093/jn/127.5.819S.

Waldo, D.R., and H.K. Goering. 1979. Insolubility of Proteins in Ruminant Feeds by Four Methods. Journal of Animal Science 49:1560–1568. doi:10.2527/jas1979.4961560x.

Walker, D.M. 1959. The in vitro digestion of roughage dry matter. J. Agric. Sci. 53:192–197. doi:10.1017/S0021859600020256.

Wang, C., J.X. Liu, Z.P. Yuan, Y.M. Wu, S.W. Zhai, and H.W. Ye. 2007. Effect of Level of Metabolizable Protein on Milk Production and Nitrogen Utilization in Lactating Dairy Cows. Journal of Dairy Science 90:2960–2965. doi:10.3168/jds.2006-129.

Weiss, B., and N. St-Pierre. 2007. Understanding and Managing Variation in Nutrient Composition 11.

Weiss, W.P., D.O. Erickson, G.M. Erickson, and G.R. Fisher. 1989. Barley Distillers Grains as a Protein Supplement for Dairy Cows. Journal of Dairy Science 72:980–987. doi:10.3168/jds.S0022-0302(89)79192-X.

Weiss, W.P., and N.R. St-Pierre. 2009. Impact and Management of Variability in Feed and Diet Composition 15.

Weiss, W.P., and A.W. Tebbe. 2019. Estimating digestible energy values of feeds and diets and integrating those values into net energy systems. Translational Animal Science 3:953–961. doi:10.1093/tas/txy119.

Wohlt, J.E., C.J. Sniffen, and W.H. Hoover. 1973. Measurement of Protein Solubility in Common Feedstuffs. Journal of Dairy Science 56:1052–1057. doi:10.3168/jds.S0022-0302(73)85304-4.

TABLES AND FIGURES

Table 1.1 Recommendations regarding level of sampling and data summarization for the utilization of book values in ration formulation (St-Pierre and Weiss, 2015)

Farm Variation	Feeds	Reccomendation
1. Farm was a significant source of variation	Corn silage Haycrop silage Wet corn gluten feed Wet brewers grains Wet distillers grains	Multiple samples to be taken at the farm level and summarized by farm
2. Farm was not a significant source of variation	Dry corn grain Soybean meal Dry corn gluten feed Whole cotton seed	Feed composition tables can be used or laboratory summaries across farms
3. Farm was often not a significant source of variation	Dried distillers grains	If feed is from a nonspecified production plant, the feed composition tables should be use. Otherwise, data should be summarized by production plant origin.

Table 1.2 Average chemical composition values and standard deviations for common feeds utilized in ration formulation on commercial dairy farms. Values from accumulated years Dairy One Feed Composition Library[1]

Feed	DM (± SD)	CP (± SD)	NDF (± SD)	ADF (± SD)	EE (± SD)	Ash (± SD)
Corn silage	33.3 (6.08)	8.27 (1.068)	43.0 (5.68)	25.4 (3.90)	3.29 (0.484)	4.34 (1.254)
Grass silage	38.1 (14.05)	15.5 (4.07)	57.4 (7.30)	37.3 (4.93)	3.97 (0.988)	9.52 (2.680)
Legume silage	40.2 (10.66)	22.0 (2.95)	43.7 (5.59)	33.8 (4.07)	3.80 (0.755)	11.2 (2.14)
Wet corn gluten feed	46.6 (15.58)	25.7 (6.13)	38.7 (7.56)	12.7 (3.37)	4.19 (2.546)	7.70 (2.364)
Wet brewers grains	23.7 (7.18)	28.7 (4.71)	49.3 (6.50)	24.4 (3.70)	9.77 (1.535)	4.49 (0.7)
Wet distillers grains	35.8 (15.92)	30.4 (8.64)	32.0 (8.49)	15.9 (4.93)	12.0 (3.71)	5.58 (1.836)
Dry corn grain	88.7 (3.85)	8.86 (1.400)	9.95 (2.792)	3.73 (1.383)	4.14 (1.062)	1.63 (1.188)
Soybean meal	90.8 (2.78)	51.0 (4.67)	13.5 (5.36)	8.57 (2.911)	4.23 (4.543)	7.29 (1.007)
Dry corn gluten feed	89.5 (2.76)	23.6 (6.00)	36.2 (5.61)	11.8 (2.65)	4.29 (1.41)	7.57 (1.726)
Whole cotton seed	91.5 (1.95)	23.5 (3.74)	55.7 (6.73)	41.3 (6.23)	18.2 (3.93)	4.29 (1.015)
Dried distillers grains	89.4 (6.00)	31.4 (4.46)	34.1 (4.60)	16.5 (3.30)	11.5 (3.29)	6.26 (1.361)

[1] https://dairyone.com/services/forage-laboratoryservices/feed-composition-library/interactive-feed-composition-libraries/ Accessed 09/13/2021.

Table 1.3 Definition, rate and equation for protein fractions in the Cornel Net Protein and Carbohydrate System (Higgs et al., 2015)

Fraction name	**Definintion**	**Kd, %/h**	**Equation**
PA1	Ammonia N	200	$Ammonia_j \times (SP_j / 100) \times (CP_j / 100)$
PA2	Soluble true protein	10-40	$SP_j \times (CP_j / 100) – PA1_j$
PB1	Moderately degraded protein	3-20	$CP_j – (PA1_j – PA2_j – PB2_j - PC_j)$
PB2	Slowly degradable protein, bound in NDF	1-18	$(NDICP_j - ADICP_j) \times CP_j / 100$
PC	Indigestible protein	0	$ADICP_j \times CP_j / 100$

[1]Subscript *j* means the *j*th feed in the library.

Figure 1.1 Corn starch heavily embedded in corn zein (A), Starch granules with less extensive zein encapsulation (B)

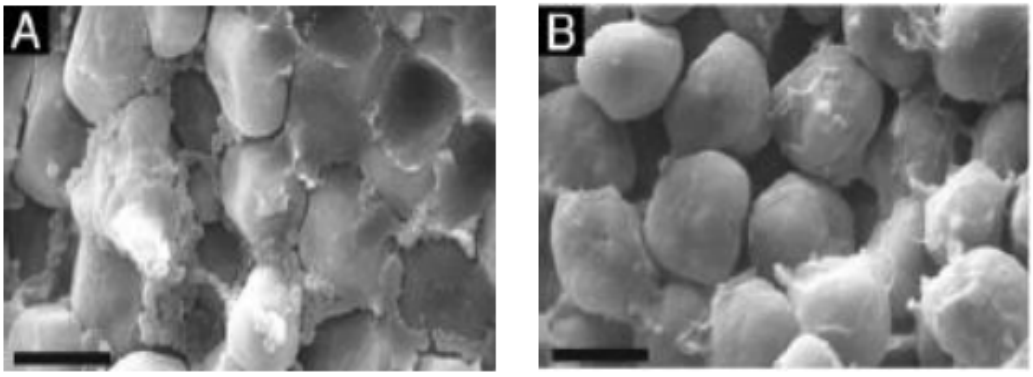


Figure 1.2 Miniature artificial rumen including dialysis bag with rumen fluid

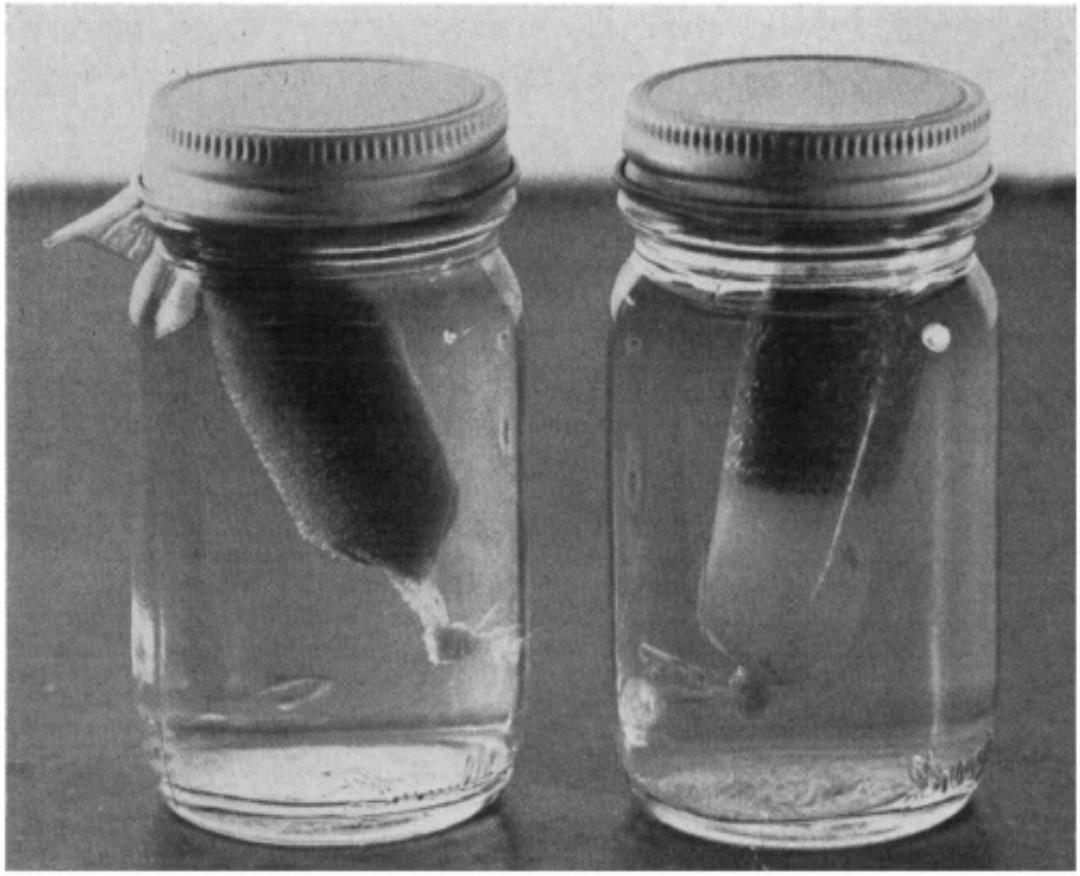


Figure 1.3 Schematic diagram for Rusitec system for long term artificial rumen from Czerkawski and Breckenridge (1977)

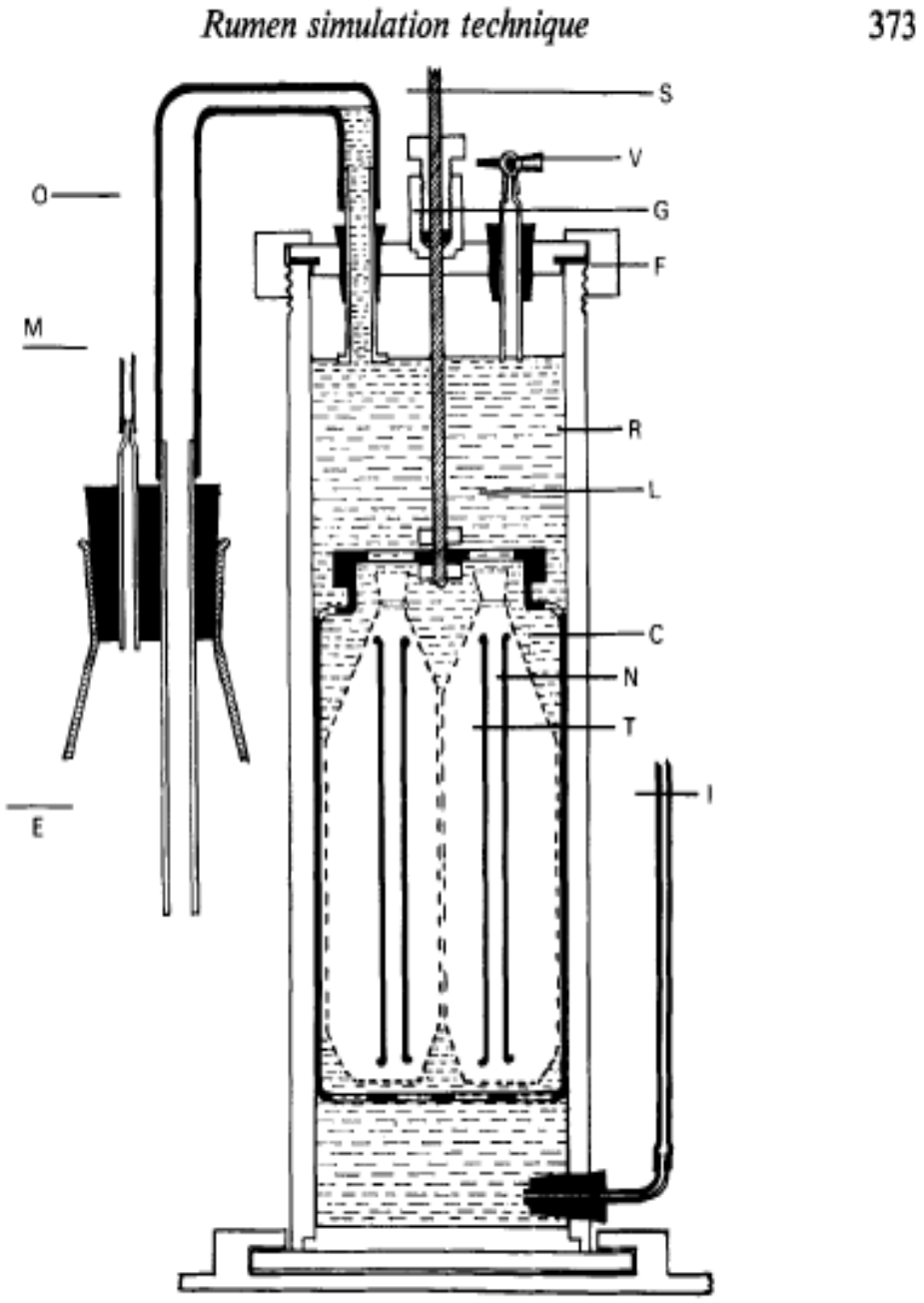


Fig. 1. Schematic diagram of one unit of the four-vessel long-term artificial rumen. (□), Made of perspex, (■), made of rubber or polyethylene. The driving shaft (S) was made of stainless steel. V, Sampling valve; G, gland (gas-tight); F, flange; R, main reaction vessel; L, rumen fluid; C, perforated food container; N, nylon gauze bag; T, rigid tube; I, inlet of artificial saliva; O, outlet through overflow; M, line to gas-collection bag; E, vessel for collection of effluent.

Figure 1.4 Diagram of Bunsen release valve in rubber stopper for in vitro fermentation

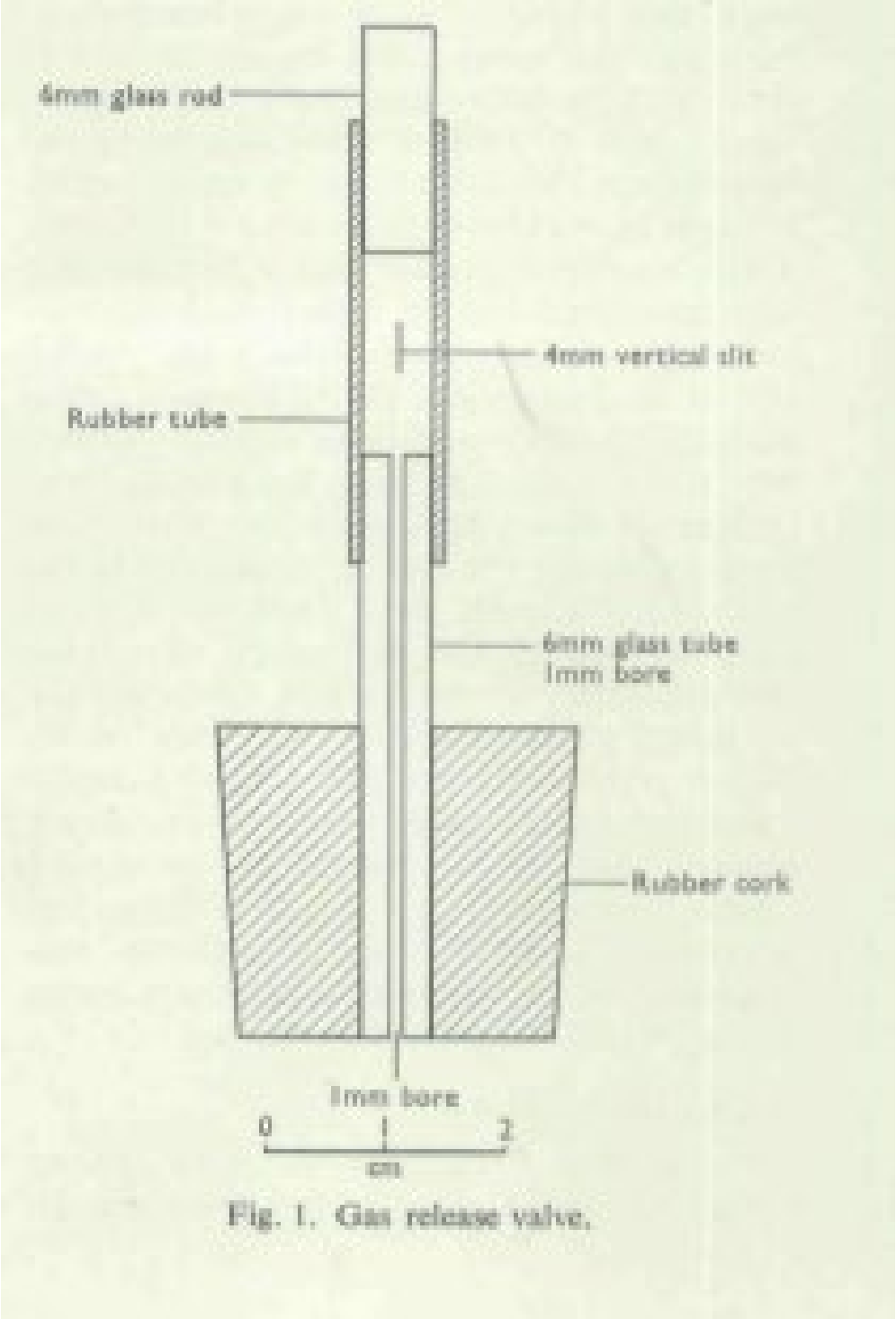


Fig. 1. Gas release valve.

Figure 1.5 Early continuous CO_2 flushing in vitro system with CO_2 input and CO_2 scrubbing jars in temperature-controlled water bath from Hoorn et al. (1957)

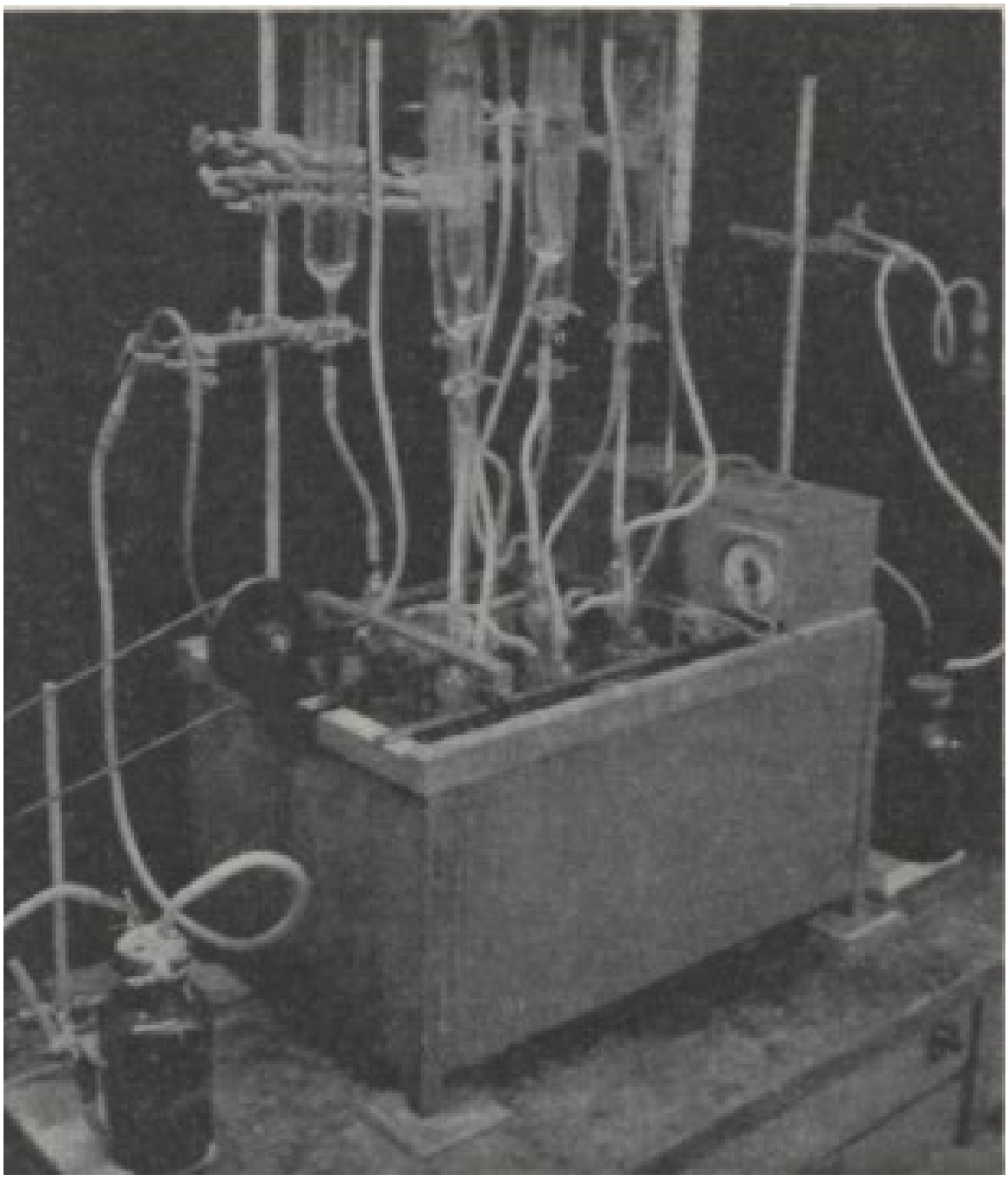


Figure 1.6 In vitro batch culture system with CO_2 input, Bunsen valve gas release, and sample inlet in Goering and Van Soest (1970)

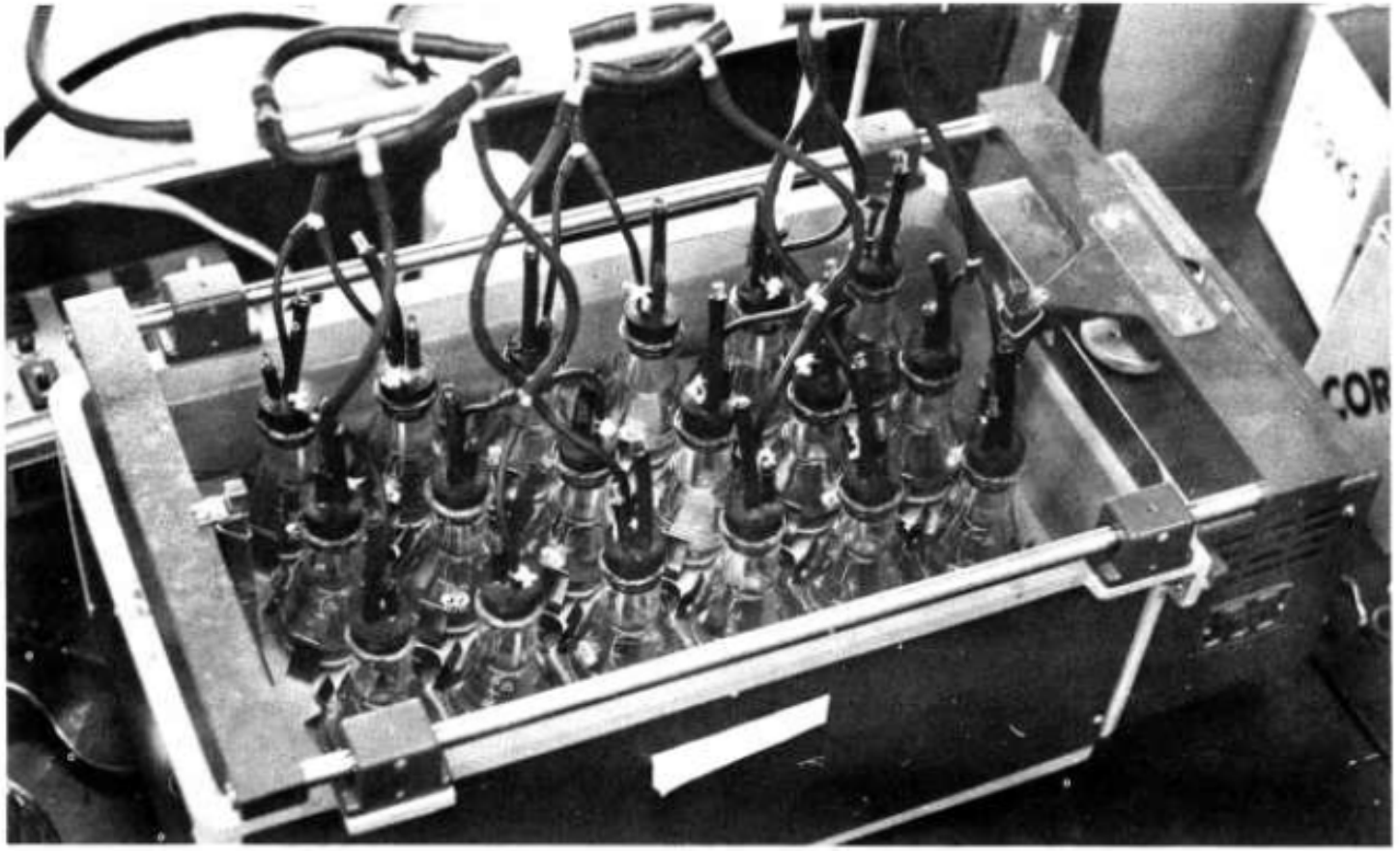


Figure 1.7 Resazurin indicator color change from initial dosing to anerobic conditions and aerobic conditions

Figure 1.8 UNL batch culture system with continual CO_2 manifold and CO_2 delivery lines, and check valve for gas release

Figure 1.9 Graphic for feed protein metabolism, utilization, and excretion in the lactating dairy cow. Modified from (Owens and Zinn, 1988)

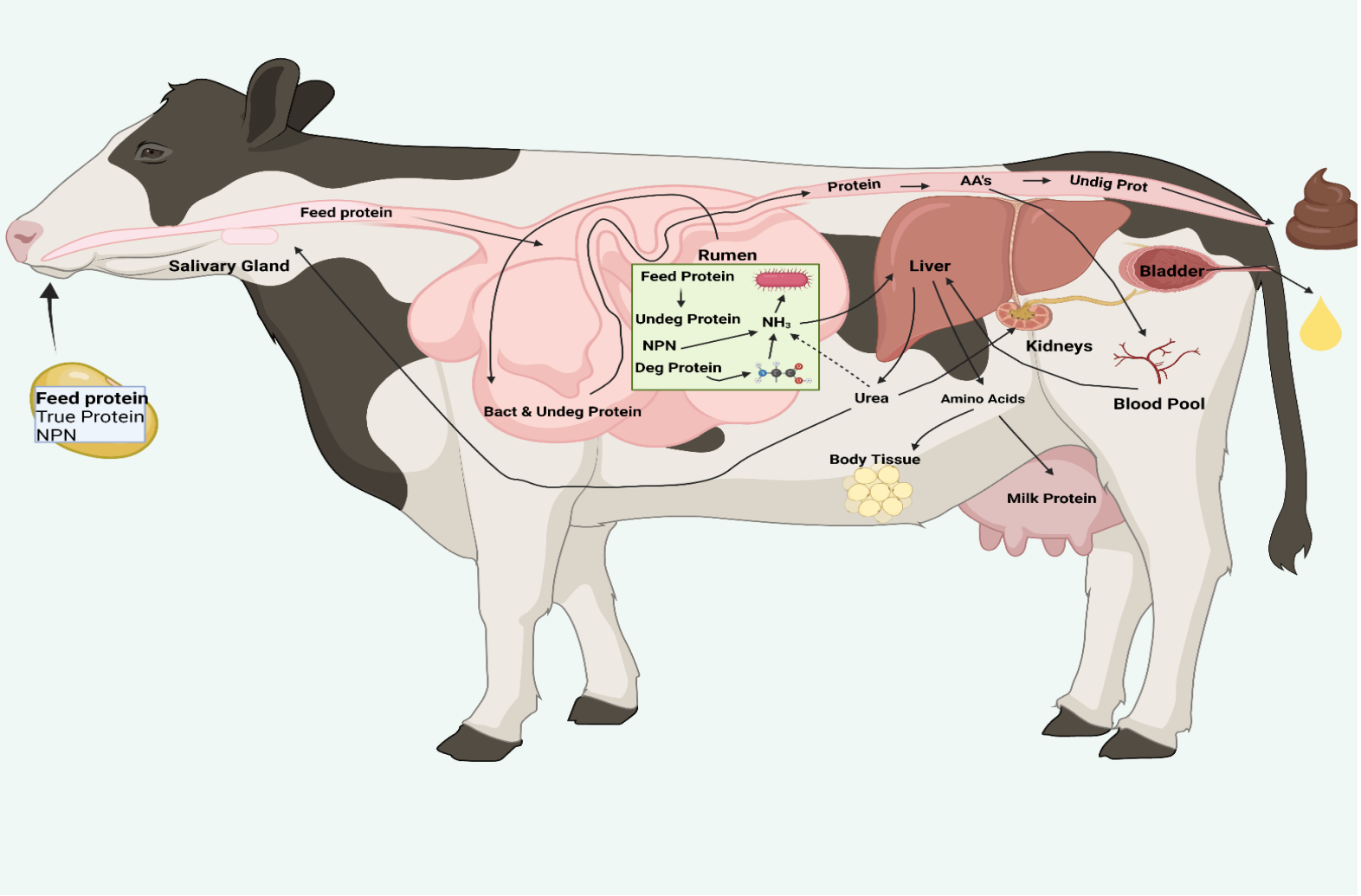


Figure 1.10 Diagram of the inputs for the feed evaluation system including animal description, feed characterization, nutrient digestibility, nutrition response and exchange of feed value

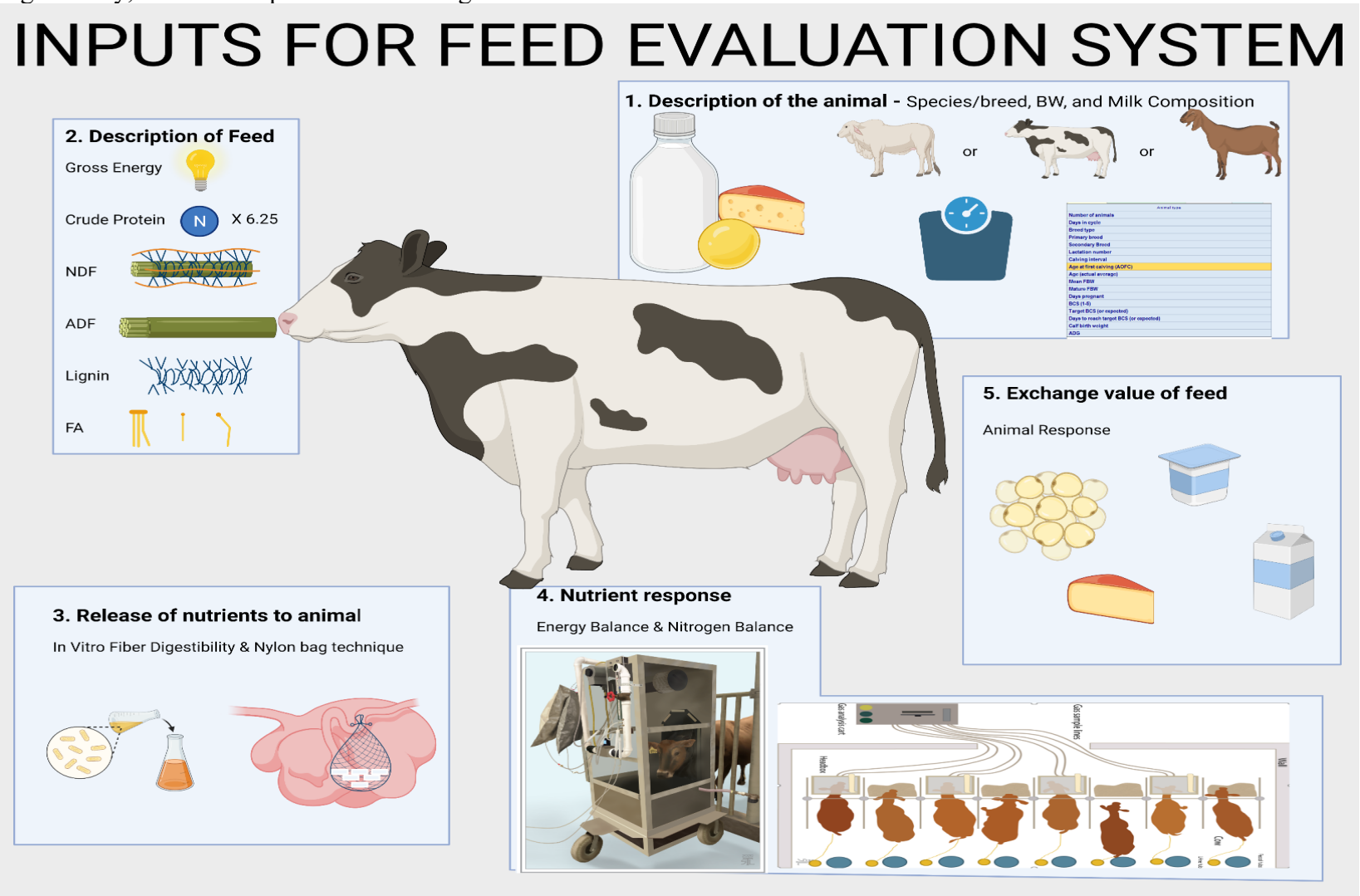


Figure 1.11 Outline of the Net Energy System. Modified from Morris (2020)

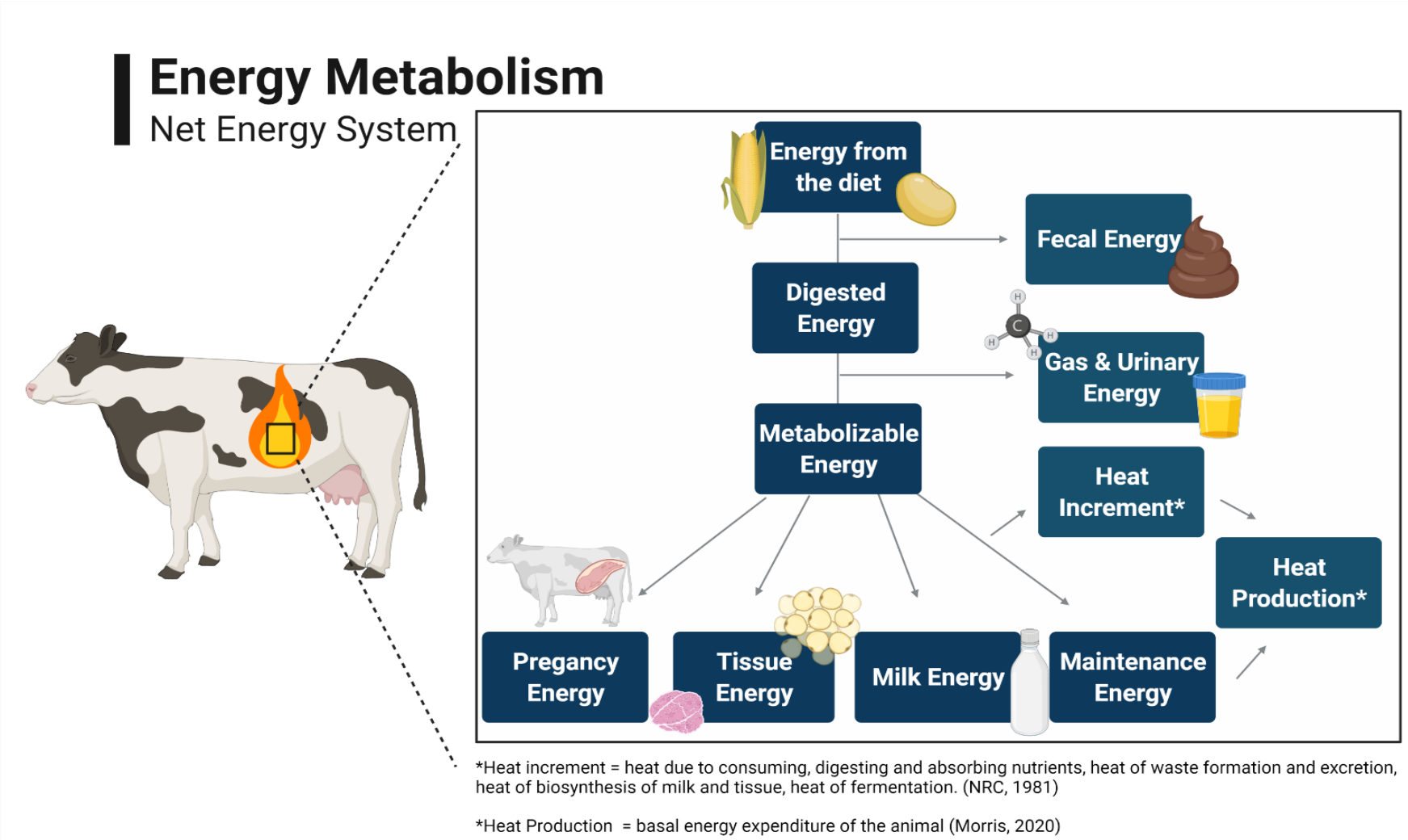
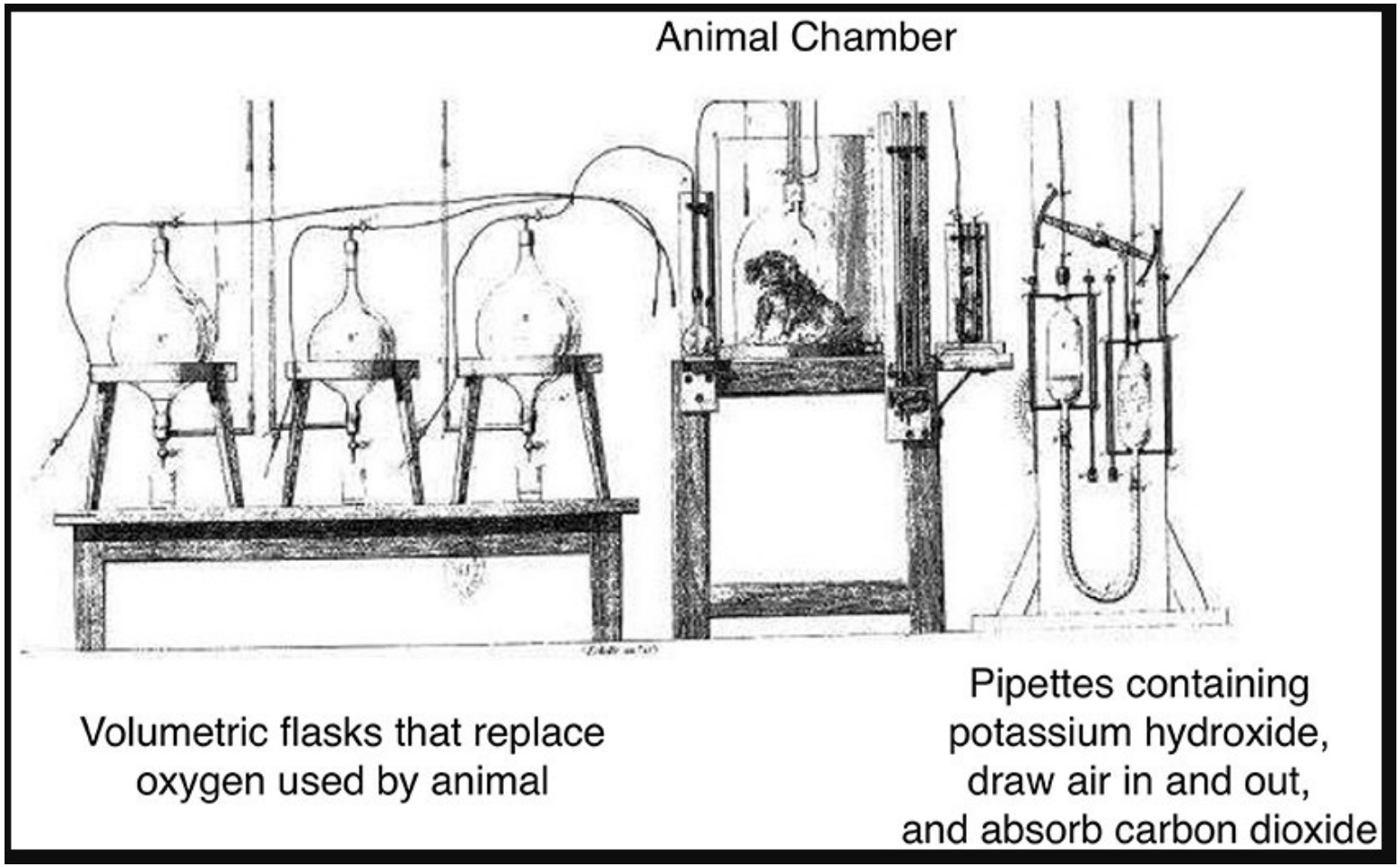


Figure 1.12 Regnault and Reiset's closed circuit indirect calorimeter from Mtaweh et al. (2017)



CHAPTER 2

RUNNING HEAD: HIGH PROTEIN COPRODUCT NUTRIENT COMPOSITION AND DIGESTIBILITY

Defining the chemical composition and nutrient digestibility of new high protein coproduct

Department of Animal Science, University of Nebraska–Lincoln, Lincoln 68583

*Corresponding author: P.J. Kononoff, Department of Animal Science C220, Fair St, Lincoln, NE, 68583, Phone number: 402-472-6442, Fax number: 402-472-6362, E-mail: pkononoff2@unl.edu

ABSTRACT

Objective: Our objective was to chemically characterize a novel high protein coproduct and evaluate the nutritionally significant components compared to traditional DDGS.

Materials and Methods: A total of 10 samples were collected over a month's time from The Flint Hill Resources plant in Fairmont, NE. Samples were analyzed by Cumberland Valley Analytical laboratory for DM, CP, soluble CP, acid detergent insoluble CP, neutral detergent insoluble CP, ADF, amylase treated NDF, lignin, EE, sugar, starch, minerals, amino acids, and fatty acids. Rumen undegradable protein content was determined with in situ and mobile bag procedures. NDF digestibility on an organic matter basis was determined in vitro at 24, 30, 48 and 240 hours. Amylase treated NDF was determined by three commercially available fiber systems including the refluxing method (**aNDF$_{R}$**), bagged sample method (**aNDF$_{B}$**), and a confined refluxing and filtering method (**aNDF$_{CR}$**).

Results and Discussion: Traditionally DDGS contain 38.8 % NDF$_{R}$, 6.51% TFA, 29.7% CP of which is 2.98% Lysine. (NRC, 2001; Dufour, 2017). In this study the new coproduct contained 31.2% NDF$_{R}$, 7.17% TFA, 53.6% CP and 3.6% Lysine on a CP basis. Rumen undegradable protein was determined on a CP basis 46.1 ± 13.92 % respectively.

Implications and Applications: Result indicates the new product contains increased protein and lysine and decreased NDF relative to traditional DDGS and may be able to successfully replace other high protein products in dairy rations.

Key Words: chemical composition, coproducts, DDGS

INTRODUCTION

Obtaining chemical compositions of new feed products are key to the success of the livestock industry, whether it be maintaining feed libraries, describing the content of nutrients in a new product, or creating inputs to be utilized in ration formulation. Over the past 110 years, DDGS have gone from a waste product of the brewing industry to a staple in diets of ruminants (Loosli et al., 1952). Due to recent technological advancements, the corn milling industry has begun to hybridize wet and dry milling practices and this results in higher value coproducts fractionated from the dry milling process (NRC, 2001). In the new high protein coproduct (**HPCoP**) production, nitrogenous based particles are separated from the residual fiber by multiple stages of sieving post fermentation (Srinivasan et al., 2005). While earlier HPCoP were a product of fractionization prior to fermentation, the newer method results in a feed coproduct which contains a substantial but unknown proportion of yeast particles as well as corn protein (Hubbard et al., 2009; Christen et al., 2010; Shurson, 2018). As a result of these technological advancements, we currently lack knowledge on the nutrient composition and nutrient digestibility for the new HPCoP. Since both factors play distinct roles in ration formulation, lack of data ultimately limits the ability of nutritionists to utilize the feed ingredient in ration formulation.

The objective of the experiment was to characterize the chemical composition and nutrient availability of a new high protein corn milling coproduct through wet chemistry analysis, in situ incubation, mobile bag assay and in vitro fermentation. Our hypothesis was that chemical composition of this new product would differ from that of traditional DDGS.

MATERIALS AND METHODS

The new high protein coproduct evaluated in this experiment was acquired from Flint Hills Resources and was produced at the biorefinery located in Fairmont, NE now owned by POET. All chemical composition assays are outlined in Figure 1.

A total of 10 samples (n=10) were collected over a month period and analyzed for DM (method 930.15, AOAC, 2000), CP (method 990.03, AOAC, 2000), soluble CP (Krishnamoorthy et al., 1982) ADICP and NDICP (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085), and amino acids (method 982.30, AOAC, 2006) by Cumberland Valley Analytical Services Inc. (Waynesboro, PA).

Rumen undegradable protein was analyzed using the in situ and mobile bag procedure (Kononoff et al., 2007). Prior to conducting the experiment all procedures using animals were approved by the University of Nebraska-Lincoln IACUC. Two multiparous dry Jersey cows fitted with flexible ruminal and duodenal cannula were a fed a diet listed in Table 2.7 once daily at 0930h and had an average intake of 10.2 ± 2.02 kg/d DMI. For each sample obtained from the production site 1.5 g was weighed and placed in 10 R510 Ankom concentrate bags (Ankom Technologies) with a pore size of 50 μm and dimensions of 5 cm × 10cm the heat sealed. Dacron bags were placed in a mesh bag (48 Dacron bags/mesh bag) then in a secondary bag that contained a 100 g weight and placed within the ventral sac of the rumen for a total incubation time of 16 hours. Subsequent this time, all bags were removed from the rumen and gently rinsed in a commercial washing machine using 5 cycles that were 1 minute of agitation and 2 minutes spin. After washing, four bags per sample denoted with an “R” for rumen of each

HPCoP sample were rinsed and dried in a 45° C oven for 24 hours. The remaining 6 bags per sample were then transferred into a pepsin – HCl solution (1g pepsin/L of 0.01M HCL) in a 39° C water bath for 3 hours while stirring every 15 minutes according to Kononoff et al. (2007). At 1000 h bags were rolled from the top to the bottom and then inserted in the duodenal canula at a rate of 1 bag per 5 minutes. Mats were placed behind the animal at 1730 h and fecal matter was checked and bags were recovered at 200 h. After bags were recovered, they were gently rinsed to remove excess fecal matter, refrigerated, washed in the procedure described, and dried in a 45° C oven for 24 h. After drying, bags were weighed to determine the weight of the residue, then composited utilizing a mortar and pestle by sample, mobile or rumen, and cow. Composites were then sent to Cumberland Valley Analytical Services (Waynesboro, PA) to be analyzed for DM (method 930.15, AOAC, 2000) and nitrogen (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000).

The same 10 samples (n=10) were analyzed for ADF (method 973.18, AOAC, 2000), aNDF (Van Soest et al., 1991), lignin (method 973.18, AOAC, 1977), sugar (Hall, 2009) , starch (Hall, 2009) by Cumberland Valley Analytical Services Inc. (Waynesboro, PA). Samples were also analyzed for α-amylase treated NDF by two analytical laboratories and the UNL ruminant nutrition lab to determine the difference between three commercially available fiber systems. Refluxing method (Van Soest et al., 1991) was performed at Cumberland Valley Analytical Services modified to utilize a 1.5 µm filter (Whatman 934-AH glass micro-fiber filter; Cytiva, Marlborough, MA) and the confined refluxing and filtering method (AOAC, 2002.04) was determined by Minnesota Valley Testing Laboratories (New Ulm, MN). The method utilizing bagged samples

(Ankom, 2017) was determined at the University of Nebraska-Lincoln Ruminant Nutrition Lab utilizing the Ankom Fiber Analyzer (A2000; Ankom Technologies, Macedon, NY). For the bagged sample procedure approximately 0.50 g of sample was placed into dried and tared filter bags with 25 μm pore size in quadruplicate (n = 40) (Ankom F57). Bags were then placed in the suspender trays (n = 20) with 20 g of sodium sulfite, and 4 mL of undiluted α-amylase, and 2 L of neutral detergent solution. Samples were heated to 100º C for 1.5 h followed by four 5-minute rinses with boiling water and 8 mL of diluted α-amylase split between the first and second rinse. Bags were then soaked in acetone for five minutes, allowed to air dry for 20 minutes, then dried at 105 ° C overnight. Each aNDF procedure was denoted based on methodology in Table 2.1. Similarly, in vitro NDF digestibility was also used to analyze the amylase treated NDF digestibility on an organic matter basis (**aNDFDom**) of the samples. Samples were sent for analysis by Cumberland Valley Analytical Services (Waynesboro, PA) where samples underwent fermentations of 24, 30, 48 and 240 hours according to Van Soest et al. (1970) then were analyzed for aNDFDom according to the refluxing method (Van Soest et al., 1991) with modifications utilizing a 1.5 μm filter (Whatman 934-AH glass micro-fiber filter; Cytiva, Marlborough, MA). Ash content of the samples were obtained through method 942.05 (AOAC, 2000) to determine organic matter. Three lactating dairy cattle were used for rumen fluid collection by Cumberland Valley Analytical Services (Waynesboro, PA) averaging 136 ± 46.3 DIM, with diets formulated for 26.3 kg DMI and herd averaging 40.8 kg of milk per day. The total tract NDF digestibility on an organic matter basis (**TTNDFDom$_R$**), Indigestible NDF (**iNDF**), Potentially digestible NDF (**pdNDF**), and rate of digestibility (**kd**) was calculated according to (Lopes et al.,

2015). Indigestible NDF was determined as the aNDFom content of the sample after a 240-h in vitro fermentation, the aNDFom content was determined as described in the method previously stated. Potentially digestible NDF was calculated as the difference between total aNDFom and the iNDF. The rate of digestibility of the pdNDF was calculated from the aNDFom measurements at 24, 30, and 48 hours of in vitro fermentation (Goering and Van Soest, 1970) using a first order kinetic model (Mertens, 1993) assuming the iNDF residue does not disappear and the pdNDF disappears at a rate proportional to its mass. Rate of passage (**kp**) in this model was assumed at 2.67 %/h based on a 630 kg dairy cow consuming 23.4 kg/d of a diet that includes 30 % NDF in order to calculate $TTNDFDom_R$ (Lopes et al., 2015).

Samples were analyzed for EE (method 2003.05 AOAC, 2000) and fatty acids (Sukhija and Palmquist, 1988) by Cumberland Valley Analytical Services Inc. (Waynesboro, PA).

Samples were analyzed for ash (method 942.05, AOAC, 2000) and minerals (method 985.01, AOAC, 2000) by Cumberland Valley Analytical Services Inc. (Waynesboro, PA).

Data for the comparison of fiber methods were analyzed using the GLIMMIX procedure in SAS 9.4 with $P \leq 0.05$ being designated as significant. The model for the dependent variable of aNDF is as follows:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}.$$

Where y_{ij} represented the observation, μ the overall mean, a_i the effect of method *i*, and e_{ij} the residual term.

RESULTS AND DISCUSSION

During d1 of the mobile bag experiment one animal (5505) failed to pass 50% of the inserted mobile bags. Therefore, data collected for that animal during that time was discarded. Bag placements were halted until the cow returned to normal health. Samples were then rerun in the healthy animal and cows were synced for the remaining 4 periods of the experiment. Data are reported as mean ± SD where all samples were averaged

Protein Composition

This experiment was designed to determine the chemical composition of a new high protein coproduct to be utilized in the feed library and ration formulation software. A summary of the chemical composition is listed in Table 2.2. As we expected, crude protein values were increased for the new HPCoP at 53.6 ± 1.13 % CP relative to tradition DDGS at 30 % CP (Schingoethe et al., 2009). Similarly, the new HPCoP contains increased protein relative to other HPCoPs produced from the removal of bran and germ prior to fermentation with protein fractionization occurring post fermentation at approximately 45 % CP (Tedeschi et al., 2009). However, the new product contained 4.52 ± 0.818 % soluble protein which is decreased from the 6.24 % utilized for DDGS in NDS ration formulation software. Decreased soluble protein content relative to DDGS decreases the fraction nitrogen in the HPCoP immediately available for microbial crude protein synthesis (Russell and Hespell, 1981; Kajikawa et al., 2012). However, this increases the potentially degradable fraction which contribute to the metabolizable protein requirements of the animal (Higgs et al., 2015). During the production process of

the new HPCoP, fiber is removed post fermentation via sieving, this step concentrates the CP and energy relative to traditional DDGS (Birkelo et al., 2004; Loy and Lundy, 2019). The protein stream is then purified leaving corn protein and spent yeast cells remaining (Shurson, 2018). Similar to reduced fat DDGS, HPCoPs produced from protein capture subsequent fermentation increases CP as a result of removal of other chemical fractions (Morris et al., 2018). Since non-starch polysaccharides average 10 % of the dry corn mass and the percent fiber increase threefold in the final mash, we speculate the removal of large fiber particles, namely bran, contributed to the increase in CP (Li et al., 2012; Hamaker et al., 2019; Kumar and Singh, 2019). Similarly, an unknown but substantial proportion of spent brewers yeasts captured in the protein stream contain 45-60% CP and likely contributed to the increased CP content of the HPCoP (Jaeger et al., 2020).

Lysine is often considered to be the first limiting amino acid for diets containing high proportions of corn and corn coproducts such as DDGS (Schingoethe et al., 2009). However, other essential amino acids must be considered for ration formulation. Surprisingly, lysine was higher at 3.70 ± 0.188 % of CP in the new product compared to an average of 2.56 % lysine on a CP basis for traditional DDGS (Table 2.3; Cromwell et al., 1993; NRC, 2001; Spiehs et al., 2002). Similarly, amino acids including leucine, threonine, and valine contributed 12.2 ± 0.54 % CP, 4.21 ± 0.150 % CP, and 6.56 ± 0.415 % CP, respectively. These values are increased when compared with DDGS which contain 9.59 % CP leucine, 3.44 % CP threonine and 4.70 % CP valine (NRC, 2001). Generally, the deficiency in lysine and increased leucine content occurs as corn protein contains 60% zein protein (Larkins, 2019). Also, during the production of DDGS, lysine may be complexed with sugars during the heating stage, and this is thought to decrease

bioavailability of the AA (Larkins, 2019). Therefore, over time, we speculate as processing technologies change and heat damage decreases, the content of lysine in DDGS may increase. Additionally, unlike traditional DDGS, we further speculate the increase in lysine, threonine, and valine content in this product may be attributed to an increased proportion of yeast cells. In the new production process, yeast cells could contribute approximately 29% of the material after fiber is removed (Shurson, 2018). According to Liu (2011) yeasts used in dry grind ethanol production contain 6.96% lysine, 4.99 % threonine, and 4.55 % valine on a CP basis. Since fiber was removed in the production process yeast cells account for a larger proportion of the protein increasing the relative lysine, threonine, and valine values of the product. Methionine was also increased to 2.51 ± 0.161 % CP compared to the 1.82 % value in the Dairy NRC (2001). The increase may be partially attributed to analytical error as historical methods of acid hydrolysis converts some methionine to methionine sulfoxide which cannot be recovered (Higgs et al., 2015). Although this may contribute to the decreased value for methionine in DDGS in the NRC (2001) the magnitude of the difference may not be fully explained by analytical error alone. An additional factor which may increase the methionine content may be a result of other nitrogen containing components of the corn protein in the HPCoP.

The RUP content of the new product was numerically decreased at 46.1 ± 13.92 % CP compared to traditional DDGS at 55.1 % (Table 2.6; Janicek et al., 2008). In the literature, RUP has ranged from 53.2 – 87.2 % CP for DDGS products (Brouk, 1994; Kleinschmit et al., 2007; Mjoun et al., 2010b). Although differences in technique may explain some of this difference, we also speculate that a portion of the variation may be

due to processing differences of the new HPCoP. However, based on previous research, during the in situ procedure, several sources of variation occur due to bag pore size and sample particle size (Vanzant et al., 1998). The HPCoP's particle size was much finer than the 2 mm grind size which is traditionally suggested in the procedure, therefore there was potential washout of the feed during washing or material was released into the rumen and intestinal tract during the incubation time (Vanzant et al., 1998). We speculate decreased RUP content in this experiment was likely an effect of feed particle size and subsequent washout (Van Hellen and Ellis, 1977; Nocek and Kohn, 1988; Gierus et al., 2005).

Neutral Detergent Fiber

Neutral detergent fiber is a heterogeneous mixture of fiber components with varying levels of digestibility. Therefore, one cannot assume the digestion of the feedstuff via the NDF content alone (Mertens, 1977). In this experiment the $aNDF_R$ (Table 2.1) most closely aligns with the assay outlined by Van Soest et al. (1991) as such it was utilized for comparison to DDGS. The $aNDF_R$ content of the HPCoP was 31.2 ± 3.53 % which is similar to that reported in DDGS by Krogstad et al. (2021) and Tran et al. (2020) ranging from 31.0 % to 33.8 % $aNDF_R$. Interestingly, when subtracting the $aNDF_R$ fraction from ADF, the new product contained 12% hemicellulose whereas tradition DDGS contain an average of 22 % (Mulrooney et al., 2009; Christen et al., 2010; Krogstad et al., 2020). The reduction of hemicellulose may be a result of the removal of fibrous bran of the corn kernel which contains approximately 70 % hemicellulose (Sandstead et al., 1978). Therefore, we speculate during multistage sieving, the large fibrous bran is at least partially removed, and this has a reducing effect on hemicellulose

content of the feed. In the new HPCoP decreased hemicellulose content may limit NDF digestibility due to a lower hemicellulose to cellulose ratio when compared to DDGS (Andrighetto et al., 1993). However, the NDF value for the new product was statistically different ($P < 0.01$; Table 2.5) across the three different commercial fiber systems including the $aNDF_R$, $aNDF_B$, and $aNDF_{CR}$ (Table 2.1) which produced values of 31.2 ± 3.53, 47.1 ± 4.32 and 22.5 ± 5.28 % aNDF, respectively. Overall, bagged sample methods have been shown to be effective when determining the $aNDF_B$ content of forage samples (Schlau et al., 2021). The experiment by Schlau et al. (2021) obtained similar $aNDF_B$ and aNDF values for grass hay, corn silage, and alfalfa averaging 43.4 and 44.7 %, respectively when utilizing the bagged sample method and a modified refluxing method (Mertens, 2002). We speculate values are similar due to the filtering pore size at 25 μm and 50 μm. However, in the experiment by Schlau et al. (2021) differences were observed when the pore size of the bagged sample method was decreased to 6 μm from 25 μm increasing the percent $aNDF_B$ from 43.4 to 46.6 %. Therefore, in the current experiment decreased $aNDF_{CR}$ content in confined refluxing and Gooch crucible filtering method is likely a result of the larger filtering pore size of 40 – 100 μm utilizing ashed sea sand when compared to that of the filters used in the refluxing method and bags at 1.5 and 25 μm, respectively. Interesting, if pore size was the only factor the refluxing method should have the largest $aNDF_R$ fraction due to the 1.5 μm filter pore size, but this was not the case. In the literature $aNDF_B$ has been shown to have large deviations from $aNDF_R$ for DDGS (Mertens, 1998). Therefore, an additional factor that may contribute to the observed effect are differences in the nature of immersion of sample in solution. In refluxing the sample has increased surface area contact with the NDF solution caused by

rolling agitation. Rolling agitation is not present in the bagged sample methods as bags are placed within suspender trays which move vertically within the extraction chamber. We speculate the difference in aNDF between the three methods may be attributed to two factors, being namely the filtering agent and the ability to reflux without constraints within the NDF solution. The 24.6 % difference in aNDF content between methods limits the ability to accurately define the contribution of digestible aNDF to predicted milk yield in ration formulation. Based on calculations from the Dairy NRC (2001) a 24.6 % difference in aNDF content would lead to a range in predicted milk yield from 0.60 kg/d to 1.25 kg/d from $aNDF_{CR}$ to $aNDF_{B}$ when 1 kg of the HPCoP was fed.

Animal performance does not directly hinge on the NDF content of a given feedstuff but more so the quality of the forage which can be estimated by in vitro NDF digestibility and calculated total tract NDF digestibility. In this experiment, the aim was to determine the extent of $NDFom_{R}$ digestion (**$NDFDom_{R}$**) at time points including 24, 30, 48 h, and the maximal extent of digestion at 240 h of in vitro fermentation (Goering and Van Soest, 1970). Also, estimated total tract NDF digestibility on an organic matter basis (**$TTNDFDom_{R}$**) was calculated (Lopes et al., 2015). The values for $NDFDom_{R}$ were 77.8 ± 2.63, 81.9 ± 2.20, 83.8 ± 1.79, and 85.8 ± 1.17 % $NDFom_{R}$ for 24, 30, 48, and 240 h respectively (Table 2.5). Total tract NDF digestibility on an organic matter basis (**$TTNDFDom_{R}$**) was estimated at 74.4 ± 4.23 %. Neutral detergent fiber digestibility values were calculated on an organic matter basis as ash can compose 1 to 3% of NDF and does not directly contribute to digestible energy (Mertens, 2002; Tebbe et al., 2017). The digestible $NDFom_{R}$ values in the current experiment are increased relative to the findings of Krogstad et al. (2021) where four samples of DDGS with

solubles were 70.4 ± 12.9 % digestible at 30 hours and 86.4 ± 4.18% digestible at 240 h. While there was increased digestibility at the 30-hour mark, there was no difference in digestibility at the 240-hour mark. We speculate the difference observed in the shorter incubation period is an effect of the smaller particle size of the new product, as smaller particle size has been suggested to improve fermentation with shorter incubation times (Huntingon and Givens, 1995). Another explanatory factor for increased digestibility in the HPCoP relative to DDGS is the lignin content (1.96 % DM vs 4.3 % DM)(NRC, 2001). Lignin is negatively correlated with in vitro NDF digestibility at 24 hours based on the findings of Raffrenato et al. (2017). Therefore, the reduced lignin content in the HPCoP likely improved in vitro NDF digestibility in the early fermentation timepoints.

Fatty Acids

When evaluating fatty acid content in the product the most abundant fatty acid was C18:2w6 followed by C18:1w9 and C16:0. These fatty acids accounted for 53.9 ± 1.37 % TFA, 22.8 ± 1.54 % TFA, and 17.3 ± 0.26 %, respectively in the new product (Table 2.4). These values have a slight difference relative to those reported in NDS ration formulation software with DDGS containing 56.1 % C18:2, 24.6 % C18:1, and 14.0 % C16:0. The NRC has no reported value for TFA in DDGS, therefore a comparison was made to Moreau et al. (2011) who examined DDGS from 7 different plants. The values for C18:2, C18:1, and C16:0 averaged 54.7 % TFA, 26.2 % TFA, and 15.7 % TFA, respectively for DDGS. As a result, C16:0 was numerically increased in the new HPCoP, and C18:1w9 was numerically decreased. Fatty acids can be found in two general forms in dietary feed ingredients including, saturated fatty acids and unsaturated fatty acids. Unsaturated fatty acids undergo biohydrogenation in the rumen, have been linked with

decreases in NDF digestibility and milk fat depression when certain isomers of conjugated linoleic acid are present (Baumgard et al., 2002; Weld and Armentano, 2017). The current product contained 79% unsaturated fatty acids, which is similar to the findings of Dufour (2017) who reported 80% unsaturated fatty acid in DDGS from 7 different production processing sites in the midwestern United States. As HPCoP and traditional DDGS contain corn oil as the base fat, composition of fatty acids were similar to other reports with linoleic being the major fatty acids, followed by oleic and palmitic (Moreau et al., 2011). However, a slight increase in C16:0 was observed averaging 17.3 ± 0.26 % of TFA in the current experiment compared to 13.7 % TFA and 14.7 % TFA in the experiments by Cao et al. (2009) and Ranathunga et al. (2010). We speculate that this change in composition can also be linked to the increased percentage of spent brewers yeast. As yeast cells average 44.2 % C16:0 on a TFA basis (Ahvenainen, 1982; Blagovi et al., 2001).

Minerals

In our analysis we observed that the new HPCoP contained 0.71 ± 0.097 % DM sulfur, 0.03 ± 0.012 % DM calcium, and 0.52 ± 0.026 % DM potassium (Table 2.2). Buckner et al. (2011) observed that sulfur varied from 0.71 to 0.84 % DM across 6 Nebraska ethanol plants. Similarly, in a review by Liu (2011) mean values across 5 studies totaling over 142 samples averaged a sulfur content of 0.64 %. Generally, sulfur is utilized as a cleaning agent and pH control in the dry milling process. We speculate that the rise of the use of sulfuric acid as a cleaning agent likely contributed to the increased value from the average 0.44 % and 0.48 % reported by the Dairy NRC (2001) and Holt and Pritchard (2004). However, calcium and potassium were both decreased

relative to the average 0.05 % and 1.02 % DM values given to DDGS in a review of distillers products by Schingoethe et al. (2009). Calcium is a stable element and is generally retained during cooking and storage (Dunn et al., 2014). However, calcium can form complexes with phytic phosphorus (Dei, 2017). As phytate is decreased when cereals are fermented this may have attributed to the loss of the calcium in the final product (Acosta-Estrada et al., 2019). Similarly, brewers yeast contains averages 27.1 mg of calcium/100 g of dry weight lowering the average value of calcium in the product (Jaeger et al., 2020). There are limited data on corn processing on potassium content, but we speculated the centrifugation and removal of solubles during production likely contributed to the decreased K content in the HPCoP compared to DDGS as solubles contain 2.87 % K on a DM basis (Cao et al., 2009). Historically grains and byproducts have been used to examine the effects of decreased dietary potassium content on milk production (Dennis et al., 1976; Dennis and Hemken, 1978). Dietary cationic difference (DCAD) in ration balancing is calculated based on the dietary inclusion of sodium and potassium minus dietary chloride and sulfur and is -32.4 mEq for the HPCoP. As such, research has displayed a 0.1 % increase in milk fat per 100 mEq/kg increase in DCAD (Iwaniuk and Erdman, 2015). Due to the strong cationic nature of potassium in acid-base balance and osmotic regulation it directly contributes to the DCAD which could have subsequent effects on milk production.

APPLICATIONS

Continued evaluation of novel coproducts is integral to our ability to estimate subsequent animal performance. Overall results indicate that the new high protein coproduct contains increased protein and lysine relative to traditional DDGS. As protein

is a costly component of dairy diets and care should be taken in understanding the digestibility and amino acid content of the product for accurate ration balancing. Also, as lysine is typically limiting in corn coproducts increased lysine content in the new product may be valuable for amino acid balancing. However, the new product contained decreased hemicellulose content relative to traditional DDGS because of reduction in fibrous bran material during sieving. Overall, the new product may be able to replace other high protein products in dairy rations.

ACKNOWLEDGMENTS

The authors thank the University of Nebraska Dairy Metabolism (Lincoln, NE) staff and students for care of the experimental animals and assistance with collections, Flint Hills Resources (Wichita, KS) for financial support.

REFERENCES

Acosta-Estrada, B.A., J.A. Gutiérrez-Uribe, and S.O. Serna-Saldivar. 2019. Minor Constituents and Phytochemicals of the Kernel. Elsevier.

Ahvenainen, J. 1982. Lipid Composition of Aerobically and Anaerobically Propagated Brewer's Bottom Yeast. Journal of the Institute of Brewing 88:367–370. doi:10.1002/j.2050-0416.1982.tb04123.x.

Andrighetto, I., L. Bailoni, G. Cozzi, H.F. Tolosa, B. Hartman, M. Hinds, and D. Sapienza. 1993. Observations on In Situ Degradation of Forage Cell Components in Alfalfa and Italian Ryegrass. Journal of Dairy Science 76:2624–2631. doi:10.3168/jds.S0022-0302(93)77598-0.

AOAC. 1977. Official Methods of Analysis. AOAC International, Gaithersburg, MD.

AOAC. 2000. Official Methods of Analysis. AOAC International, Gaithersburg, MD.

AOAC. 2002. Official Methods of Analysis. AOAC International, Gaithersburg, MD.

AOAC. 2006. Official Methods of Analysis. AOAC International, Gaithersburg, MD.

Baumgard, L.H., E. Matitashvili, B.A. Corl, D.A. Dwyer, and D.E. Bauman. 2002. trans-10, cis-12 Conjugated Linoleic Acid Decreases Lipogenic Rates and Expression of Genes Involved in Milk Lipid Synthesis in Dairy Cows. Journal of Dairy Science 85:2155–2163. doi:10.3168/jds.S0022-0302(02)74294-X.

Belloir, P., B. Méda, W. Lambert, E. Corrent, H. Juin, M. Lessire, and S. Tesseraud. 2017. Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilization. Animal 11:1881–1889. doi:10.1017/S1751731117000660.

Birkelo, C.P., M.J. Brouk, and D.J. Schingoethe. 2004. The Energy Content of Wet Corn Distillers Grains for Lactating Dairy Cows. Journal of Dairy Science 87:1815–1819. doi:10.3168/jds.S0022-0302(04)73338-X.

Blagovi, B., J. Rupi, M. Mesari, K. Georgiú, and V. Mari. 2001. Lipid Composition of Brewer's Yeast 8.

Bradford, B.J., and C.R. Mullins. 2012. Invited review: Strategies for promoting productivity and health of dairy cattle by feeding nonforage fiber sources. Journal of Dairy Science 95:4735–4746. doi:10.3168/jds.2012-5393.

Brouk, M.J. 1994. Net energy for lactation and ruminal digradability of wet corn distillers grains. PhD Diss. Thesis. South Dakota State Univ., Brookings, SD.

Brown, W.E., and B.J. Bradford. 2020. Effects of a high-protein corn product compared with soy and canola protein sources on nutrient digestibility and production responses in mid-lactation dairy cows. Journal of Dairy Science 103:6233–6243. doi:10.3168/jds.2019-17939.

Buckner, C.D., M.F. Wilken, J.R. Benton, S.J. Vanness, V.R. Bremer, T.J. Klopfenstein, P.J. Kononoff, and G.E. Erickson. 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products1. The Professional Animal Scientist 27:57–64. doi:10.15232/S1080-7446(15)30445-9.

Cao, Z.J., J.L. Anderson, and K.F. Kalscheur. 2009. Ruminal degradation and intestinal digestibility of dried or wet distillers grains with increasing concentrations of condensed distillers solubles. Journal of Animal Science 87:3013–3019. doi:10.2527/jas.2009-1894.

Cessna, J., and A. Teran. 2021. Dairy.

Christen, K.A., D.J. Schingoethe, K.F. Kalscheur, A.R. Hippen, K.K. Karges, and M.L. Gibson. 2010. Response of lactating dairy cows to high protein distillers grains or 3 other protein supplements. Journal of Dairy Science 93:2095–2104. doi:10.3168/jds.2009-2687.

Cromwell, G.L., K.L. Herkelman, and T.S. Stahly. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. Journal of Animal Science 71:679–686. doi:10.2527/1993.713679x.

Dei, H.K. 2017. Assessment of Maize (Zea mays) as Feed Resource for Poultry. M. Manafi, ed. InTech.

Dennis, R.J., and R.W. Hemken. 1978. Potassium Requirements of Dairy Cows in Early and Midlaction. Journal of Dairy Science 61:757–761. doi:10.3168/jds.S0022-0302(78)83644-3.

Dennis, R.J., R.W. Hemken, and D.R. Jacobson. 1976. Effect of Dietary Potassium Percent for Lactating Dairy Cows. Journal of Dairy Science 59:324–328. doi:10.3168/jds.S0022-0302(76)84204-X.

Dufour, E.I. Advancing Chemical Characterization of Feedstuffs Commonly Included in Dairy Cow Rations 129.

Dunn, M.L., V. Jain, and B.P. Klein. 2014. Stability of key micronutrients added to fortified maize flours and corn meal. Ann. N.Y. Acad. Sci. 1312:15–25. doi:10.1111/nyas.12310.

Gierus, M., L. de Jonge, and G.A.L. Meijer. 2005. Physico-chemical characteristics and degradation rate of soluble protein obtained from the washout fraction of feeds. Livestock Production Science 97:219–229. doi:10.1016/j.livprodsci.2005.05.002.

Goering, H.K., and P.J. Van Soest. 1970. Forage Fiber Analysis. U.S Dept of Agriculture. Superintendent of Documents, US Government Printing Office, Washington D.C. 20402, USDSA Agriculture Research Service.

Hall, M.B. 2009. Determination of Starch, Including Maltooligosaccharides, in Animal Feeds: Comparison of Methods and a Method Recommended for AOAC Collaborative Study. Journal of AOAC INTERNATIONAL 92:42–49. doi:10.1093/jaoac/92.1.42.

Hamaker, B.R., Y.E. Tuncil, and X. Shen. 2019. Carbohydrates of the Kernel. Elsevier.

Higgs, R.J., L.E. Chase, D.A. Ross, and M.E. Van Amburgh. 2015. Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs. Journal of Dairy Science 98:6340–6360. doi:10.3168/jds.2015-9379.

Holt, S.M., and R.H. Pritchard. 2004. Composition and Nutritive Value of Corn Co-Products from Dry Milling Ethanol Plants 8.

Hubbard, K.J., P.J. Kononoff, A.M. Gehman, J.M. Kelzer, K. Karges, and M.L. Gibson. 2009. Short communication: The effect of feeding high protein distillers dried grains on milk production of Holstein cows. Journal of Dairy Science 92:2911–2914. doi:10.3168/jds.2008-1955.

Huhtanen, P., and A.N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science 92:3222–3232. doi:10.3168/jds.2008-1352.

Huntingon, J.A., and D.I. Givens. 1995. The in situ technique for studying the rumen degreadation of feeds: A review of the procedure. Pages 64–93. Nutr. Abs. Rev. Ser. B.

Iwaniuk, M.E., and R.A. Erdman. 2015. Intake, milk production, ruminal, and feed efficiency responses to dietary cation-anion difference by lactating dairy cows. Journal of Dairy Science 98:8973–8985. doi:10.3168/jds.2015-9949.

Jaeger, A., E.K. Arendt, E. Zannini, and A.W. Sahin. 2020. Brewer's Spent Yeast (BSY), an Underutilized Brewing By-Product. Fermentation 6:123. doi:10.3390/fermentation6040123.

Kajikawa, H., K. Miyazawa, A. Yanase, Y. Tanabe, Y. Tsuchida, Y. Mitsumoto, Y. Kozato, and M. Mitsumori. 2012. Variation in chemical composition of corn dried distillers grains with solubles in relation to in situ protein degradation profiles in the rumen. Animal Science Journal 83:299–304. doi:10.1111/j.1740-0929.2011.00956.x.

Karlsson, J., R. Spörndly, M. Lindberg, and K. Holtenius. 2018. Replacing human-edible feed ingredients with by-products increases net food production efficiency in dairy cows. Journal of Dairy Science 101:7146–7155. doi:10.3168/jds.2017-14209.

Kleinschmit, D.H., J.L. Anderson, D.J. Schingoethe, K.F. Kalscheur, and A.R. Hippen. 2007. Ruminal and Intestinal Degradability of Distillers Grains plus Solubles Varies by Source. Journal of Dairy Science 90:2909–2918. doi:10.3168/jds.2006-613.

Kohn, R.A., M.M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats1. Journal of Animal Science 83:879–889. doi:10.2527/2005.834879x.

Kononoff, P.J., S.K. Ivan, and T.J. Klopfenstein. 2007. Estimation of the Proportion of Feed Protein Digested in the Small Intestine of Cattle Consuming Wet Corn Gluten Feed. Journal of Dairy Science 90:2377–2385. doi:10.3168/jds.2006-552.

Krishnamoorthy, U., T.V. Muscato, C.J. Sniffen, and P.J. Van Soest. 1982. Nitrogen Fractions in Selected Feedstuffs. Journal of Dairy Science 65:217–225. doi:10.3168/jds.S0022-0302(82)82180-2.

Krogstad, K.C., J.L. Anderson, and K.J. Herrick. 2020. In situ rumen dry matter, neutral detergent fiber, and crude protein degradability in dairy cows and in vitro intestinal digestibility of dried distillers grains with solubles with varying fat concentrations. Applied Animal Science 36:503–508. doi:10.15232/aas.2020-01994.

Krogstad, K.C., K.J. Herrick, D.L. Morris, K.J. Hanford, and P.J. Kononoff. 2021. The effects of pelleted dried distillers grains and solubles fed with different forage concentrations on rumen fermentation, feeding behavior, and milk production of lactating dairy cows. Journal of Dairy Science S0022030221004318. doi:10.3168/jds.2020-19592.

Kumar, D., and V. Singh. 2019. Bioethanol Production From Corn. Elsevier.

LaPierre, P.A., D. Luchini, D.A. Ross, and M.E. Van Amburgh. 2019. Effects of Precision Essential Amino Acid Formulation on a Metabolizable Energy Basis for Lactating Dairy Cows 11.

Larkins, B.A. 2019. Proteins of the Kernel. Elsevier.
Li, C., J.Q. Li, W.Z. Yang, and K.A. Beauchemin. 2012. Ruminal and intestinal amino acid digestion of distiller's grain vary with grain source and milling process. Animal Feed Science and Technology 175:121–130. doi:10.1016/j.anifeedsci.2012.05.011.
Liebe, D.L., M.B. Hall, and R.R. White. 2020. Contributions of dairy products to environmental impacts and nutritional supplies from United States agriculture. Journal of Dairy Science 103:10867–10881. doi:10.3168/jds.2020-18570.
Liu, K. 2011. Chemical Composition of Distillers Grains, a Review. J. Agric. Food Chem. 59:1508–1526. doi:10.1021/jf103512z.
Loosli, J.K., K.L. Turk, and F.B. Morrison. 1952. The Value of Distillers Feeds for Milk Production. Journal of Dairy Science 35:868–873. doi:10.3168/jds.S0022-0302(52)93768-5.
Lopes, F., K. Ruh, and D.K. Combs. 2015. Validation of an approach to predict total-tract fiber digestibility using a standardized in vitro technique for different diets fed to high-producing dairy cows. Journal of Dairy Science 98:2596–2602. doi:10.3168/jds.2014-8665.
Loy, D.D., and E.L. Lundy. 2019. Nutritional Properties and Feeding Value of Corn and Its Coproducts. Elsevier.
Mertens, D. 1993. Kinetics of Cell Wall Digestion and Passage in Ruminants. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, USA.
Mertens, D.R. 1977. Dietary fiber components: reelationship to the rate and exent of ruminal digestion. 36th ed. Fed Proc.
Mertens, D.R. 1998. Effect of Method Variation on the Determination of aNDF Using the ANKOM Filter Bag System 3.
Mertens, D.R. 2002. Gravimetric Determination of Amylase-Treated Neutral Detergent Fiber in Feeds with Refluxing in Beakers or Crucibles: Collaborative Study 25.
Mjoun, K., K.F. Kalscheur, A.R. Hippen, D.J. Schingoethe, and D.E. Little. 2010. Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grains with solubles. Journal of Dairy Science 93:288–303. doi:10.3168/jds.2009-2377.
Moreau, R.A., K. Liu, J.K. Winkler-Moser, and V. Singh. 2011. Changes in Lipid Composition During Dry Grind Ethanol Processing of Corn. J Am Oil Chem Soc 88:435–442. doi:10.1007/s11746-010-1674-y.
Morris, D. 2020. Energy metabolism in Jersey cows: Improving our understanding of energy requirements and utilization 309.
Morris, D.L., S.H. Kim, P.J. Kononoff, and C. Lee. 2018. Continuous 11-week feeding of reduced-fat distillers grains with and without monensin reduces lactation performance of dairy cows. Journal of Dairy Science 101:5971–5983. doi:10.3168/jds.2017-14170.
Mulrooney, C.N., D.J. Schingoethe, K.F. Kalscheur, and A.R. Hippen. 2009. Canola meal replacing distillers grains with solubles for lactating dairy cows. Journal of Dairy Science 92:5669–5676. doi:10.3168/jds.2009-2276.

Nocek, J.E., and R.A. Kohn. 1988. In Situ Particle Size Reduction of Alfalfa and Timothy Hay as Influenced by Form and Particle Size. Journal of Dairy Science 71:932–945. doi:10.3168/jds.S0022-0302(88)79639-3.

NRC. 2001. Nutrient Requirements of Dairy Cattle. National Academies Press, Washington, D.C.

Patterson, M. 2021. OECD-FAO Agricultural Outlook 2021-2030 12.

Raffrenato, E., R. Fievisohn, K.W. Cotanch, R.J. Grant, L.E. Chase, and M.E. Van Amburgh. 2017. Effect of lignin linkages with other plant cell wall components on in vitro and in vivo neutral detergent fiber digestibility and rate of digestion of grass forages. Journal of Dairy Science 100:8119–8131. doi:10.3168/jds.2016-12364.

Ranathunga, S.D., K.F. Kalscheur, A.R. Hippen, and D.J. Schingoethe. 2010. Replacement of starch from corn with nonforage fiber from distillers grains and soyhulls in diets of lactating dairy cows. Journal of Dairy Science 93:1086–1097. doi:10.3168/jds.2009-2332.

Russell, J.B., and R.B. Hespell. 1981. Microbial Rumen Fermentation. Journal of Dairy Science 64:1153–1169. doi:10.3168/jds.S0022-0302(81)82694-X.

Sandstead, H.H., J.M. Muñoz, R.A. Jacob, L.M. Klevay, S.J. Reck, G.M. Logan, F.R. Dintzis, G.E. Inglett, and W.C. Shuey. 1978. Influence of dietary fiber on trace element balance. The American Journal of Clinical Nutrition 31:S180–S184. doi:10.1093/ajcn/31.10.S180.

Schingoethe, D.J., K.F. Kalscheur, A.R. Hippen, and A.D. Garcia. 2009. Invited review: The use of distillers products in dairy cattle diets. Journal of Dairy Science 92:5802–5813. doi:10.3168/jds.2009-2549.

Schlau, N., D.R. Mertens, K. Taysom, and D. Taysom. 2021. Technical note: Effects of filter bags on neutral detergent fiber recovery and fiber digestion in vitro. Journal of Dairy Science 104:1846–1854. doi:10.3168/jds.2020-18731.

Shurson, G.C. 2018. Yeast and yeast derivatives in feed additives and ingredients: Sources, characteristics, animal responses, and quantification methods. Animal Feed Science and Technology 235:60–76. doi:10.1016/j.anifeedsci.2017.11.010.

Spiehs, M.J., M.H. Whitney, and G.C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota 7.

Srinivasan, R., R.A. Moreau, K.D. Rausch, R.L. Belyea, M.E. Tumbleson, and V. Singh. 2005. Separation of Fiber from Distillers Dried Grains with Solubles (DDGS) Using Sieving and Elutriation. Cereal Chemistry Journal 82:528–533. doi:10.1094/CC-82-0528.

Sukhija, P.S., and D.L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206. doi:10.1021/jf00084a019.

Tebbe, A.W. 2020. Effects of Dietary Protein and Amino Acids and Their Labile Stores in Dairy Cows. PhD Dissertation Thesis. The Ohio State University,.

Tebbe, A.W., M.J. Faulkner, and W.P. Weiss. 2017. Effect of partitioning the nonfiber carbohydrate fraction and neutral detergent fiber method on digestibility of carbohydrates by dairy cows. Journal of Dairy Science 100:6218–6228. doi:10.3168/jds.2017-12719.

Tedeschi, L.O., P.J. Kononoff, K. Karges, and M.L. Gibson. 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. Journal of Dairy Science 92:401–413. doi:10.3168/jds.2008-1141.

United Nations. 2019. World Population Prospects Highlights, 2019 Revision Highlights, 2019 Revision.

USDA. 2020. Milk Cost of Production estimates.

Van Hellen, R.W., and W.C. Ellis. 1977. Sample Container Porosities for Rumen In Situ Studies. Journal of Animal Science 44:141–146. doi:10.2527/jas1977.441141x.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Vanzant, E.S., R.C. Cochran, and E.C. Titgemeyer. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation.. Journal of Animal Science 76:2717. doi:10.2527/1998.76102717x.

Weiss, W.P., and A.W. Tebbe. 2019. Estimating digestible energy values of feeds and diets and integrating those values into net energy systems. Translational Animal Science 3:953–961. doi:10.1093/tas/txy119.

Weld, K.A., and L.E. Armentano. 2017. The effects of adding fat to diets of lactating dairy cows on total-tract neutral detergent fiber digestibility: A meta-analysis. Journal of Dairy Science 100:1766–1779. doi:10.3168/jds.2016-11500.

TABLES AND FIRGURES

Figure 2.1 Methods for evaluating protein composition, protein digestibility, ether extract, fatty acid content and composition, fiber content and digestibility, non-fiber polysaccharide content, and the inorganic fraction of new HPCoP (n=10)

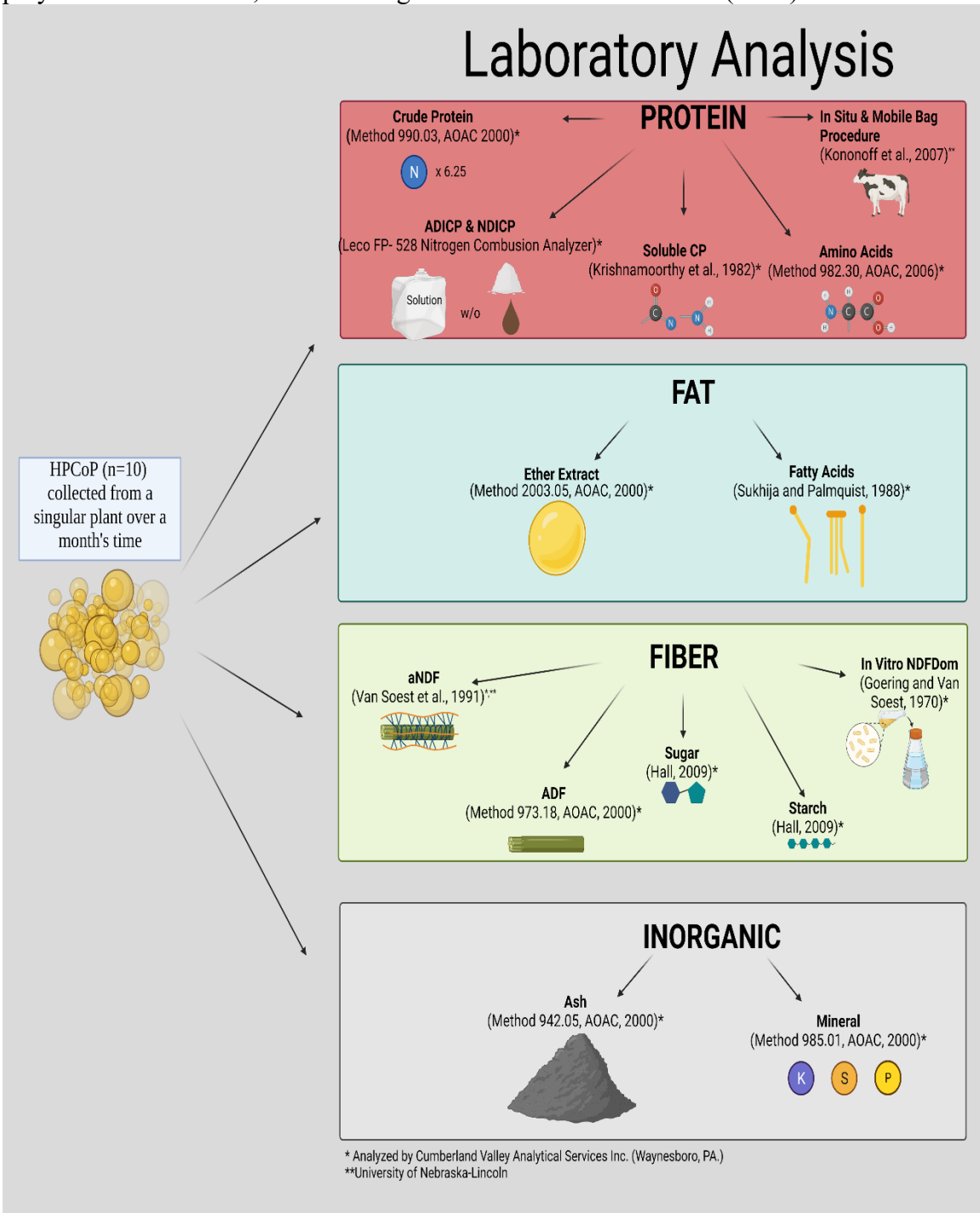


Table 2.1 Defining NDF based upon three different methods of analysis and organic matter basis for a new high protein coproduct

NDF Method	NDF[1]	NDFom[2]	TTNDFD[3]	TTNDFDom[4]
Reflux [5]	NDF_R	$NDFom_R$	$TTNDFD_R$	$TTNDFDom_R$
Bagged Sample[6]	NDF_B	-	-	-
Confined refluxing and filtering[6]	NDF_{CR}	-	-	-

[1] Neutral detergent fiber.
[2]Neutral detergent fiber, organic matter basis.
[3]Total tract neutral detergent fiber digestibility.
[4]Total tract neutral detergent fiber digestibility, organic matter basis.
[5]Van Soest et al. (1991) modified using a 1.5 μm filter.
[6] AOAC Official Method 2002.04.

Table 2.2 Protein, fiber, non-fiber polysaccharide, ether extract and mineral content of new high protein coproduct (n=10) Flint Hills Resources, Wichita, KS.[1]

	HPCoP	
Item %DM	Mean	SD
% DM	92.1	2.57
CP	53.6	1.13
Sol Protein	4.52	0.818
NDCIP[2]	5.00	2.220
ADCIP[3]	3.73	1.463
aNDF[4]	31.2	3.53
ADF	19.2	2.43
Lignin	1.96	0.756
Sugar	1.25	0.391
Starch	1.47	0.276
Crude Fat	5.81	0.461
Minerals		
Ash	3.47	0.373
Ca	0.03	0.012
P	0.72	0.155
Mg	0.22	0.081
K	0.52	0.026
S	0.71	0.097
Na	0.12	0.032
Cl	0.08	0.005
Fe, mg/kg	120	12.9
Mn, mg/kg	16.7	7.51
Zn, mg/kg	116	67.8
Cu, mg/kg	3.80	0.980

[1] Values determined by Cumberland Valley Analytical Service (Waynesborough, PA.).
[2] Neutral detergent-insoluble crude protein.
[3] Acid detergent-insoluble crude protein.
[4] Van Soest et al. (1991) modified using a 1.5 µm filter.

Table 2.3 Amino Acid composition on a dry matter and crude protein basis for new high protein coproduct (n=10) Flint Hills Resources, Wichita, KS.[1]

	HPCoP			
Amino Acids	Mean, % DM	SD	Mean, % CP	SD
EAA[2]	23.6	1.27	45.8	2.08
Arg	2.29	0.132	4.27	0.228
His	1.39	0.078	2.60	0.124
Ile	1.83	0.170	3.41	0.301
Leu	6.53	0.341	12.2	0.54
Lys	1.99	0.126	3.70	0.188
Met	1.34	0.087	2.51	0.161
Phe	2.81	0.125	5.25	0.203
Thr	2.26	0.096	4.21	0.150
Trp	0.62	0.031	1.15	0.076
Val	3.51	0.236	6.56	0.415
NEAA[3]	30.8	1.26	57.4	2.03
Ala	3.86	0.161	7.21	0.271
Asp	3.96	0.147	7.39	0.279
Cys	1.23	0.065	2.29	0.104
Glu	9.37	0.433	17.5	0.71
Gly	2.11	0.085	3.93	0.157
Pro	4.89	0.286	9.12	0.462
Ser	3.00	0.126	5.60	0.195
Try	2.33	0.099	4.35	0.167
TEAA[4]	55.3	2.49	103.2	4.01

[1]AOAC Official Method 994.12.

[2]EAA= Sum of essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Try, Val).

[3]NEAA= Sum of non-essential AA (Ala, Asp, Cys, Glu, Gly, Pro, Ser, Try).

[4]TEAA = EAA+ NEAA.

Table 2.4 Fatty Acid composition of novel high protein co-product (n=10) Flint Hills Resources, Wichita, KS.[1]

	HPCoP			
Fatty Acid	Mean, %DM	SD	Mean, %TFA	SD
Total FA	7.17	0.498	100.01	0.051
C14:0	0.01	0.005	0.08	0.007
C16:0	1.24	0.087	17.3	0.26
C16:1	0.01	0.003	0.19	0.004
C17:0	0.01	0.003	0.07	0.005
C18:0	0.17	0.010	2.35	0.134
C18:1w9	1.63	0.156	22.8	1.54
C18:2w6	3.87	0.298	53.9	1.37
C18:3w3	0.15	0.012	2.10	0.104
C20:0	0.02	0.005	0.33	0.005
C20:1w9	0.02	0.005	0.21	0.008
C22:0	0.01	<0.001	0.07	0.005
C24:0	0.02	0.005	0.16	0.004
C24:1	0.01	0.007	0.26	0.005

[1]Assay determined by Sukjija et al. (1988).

Table 2.5 In vitro NDF digestibility, potentially digestible NDF fraction, indigestible NDF fraction, rate of digestion, total tract digestibility and comparison of aNDF methodology of novel high protein coproduct (n=10) Flint Hills Resources, Wichita, KS.

Item	HPCoP	
	Mean	SD
aNDF[1] % DM		
NDF_R[2]	31.2	3.53
NDF_A[8]	47.1	4.32
NDF_{FT}[8]	22.5	5.28
$NDFom_R$[2], % DM	31.8	4.31
Dig $NDFom_R$[2,3,4]		
24 h	77.8	2.63
30 h	81.9	2.20
48 h	83.8	1.79
240 h	85.8	1.17
pdNDFom[5]	85.8	1.23
iNDFom[6]	14.2	1.23
K_d	10.10	3.54
TTNDFDom%[7]	74.4	4.23

[1]Methods differ $P < 0.01$.
[2]Van Soest et al. (1991) with modified using a 1.5 µm filter.
[3] Neutral detergent fiber digestibility on an organic matter basis ($NDFDom_R$).
[4] NDF digestion at set timepoints (24, 30, 48, 240 hours).
[5]pdNDFom = potentially digestible NDF (om basis).
[6]iNDFom= indigestible NDF (om basis).
[7]TTNDFDom= total tract NDF digestibility based on Lopes et al. (2015).
[8]AOAC Official Method 2002.04.

Table 2.6 Rumen dry matter digestibility, rumen degradable protein, rumen undegradable protein, and total tract dry matter digestibility of novel high protein coproduct (n=10) Flint Hills Resources, Wichita, KS.

	HPCoP	
Item	Mean	SD
RDMD[1] % DM	62.3	12.57
RDP[2] % CP	53.9	13.88
RUP[3] % CP	46.1	13.92
TTDMD[4] % DM	99.5	0.78

[1]Rumen dry matter digestibility.
[2]Rumen degradable protein.
[3]Rumen undegradable protein.
[4]Total Tract Dry Matter Digestibility.
[5]Non-enzymatically browned soybean meal: 49.3 ± 4.06 RDMD % DM, 16.41 ± 9.13 RDP % CP, 83.59 ± 9.13 RUP % CP, and 49.5 ± 5.33 TTDMD % DM.

Table 2.7 Ingredient inclusion and chemical composition of experimental diets for in situ and mobile bag experiment (% of diet DM)[1]

Item	% diet DM
Ingredient	
Corn silage	38.5
Alfalfa hay	14.1
Corn grain, ground fine	16.0
Corn Dried Distillers Grains	10.3
Soybean meal	9.40
Non-enzymatically browned soybean meal[2]	2.82
Soybean hulls	1.79
Rumen Protected LYS[3]	0.41
Rumen Protected MET[4]	0.11
Molasses, beet	1.23
Fat[5]	1.87
Vitamin-mineral mix[6]	3.42

[1]Multiparous dry Jersey cattle (n=2) averaging 10.2 ± 2.02 kg DMI.

[2]SoyPass, LignoTech, Overland Park, KS.

[3]AjiPro (Ajinomoto Co., Inc., Tokyo Japan).

[4]Smartamine (Adisseo, Alpharetta, GA).

[5]Porcine tallow.

[6]Contained per kilogram of premix: 393 g of CaCO3, 234 g of NaCO3, 179 g of salt, 97 g of MgO, 69 g of CaPO4, 14 g of vitamin premix (14,850 IU/g vitamin A, 3,850 IU/g vitamin D, and 90 IU/g vitamin E), and 14 g of trace mineral premix (180,000 mg/kg Zn, 1500,000 mg/kg Mn, 25,00 mg/kg Cu, 2,600 mg/kg I, 2,300 mg/kg Co, 1,000 mg/kg Fe, and 820 mg/kg Se).

APPENDIX A: AMTS RATION INPUTS FOR NEW HPCOP

AMTS

Feed Analysis Report (NexPro_LabAnalysis-ACARROLL-02008-AMTS)

UNL Dairy **11/19/2021**

Feed Type	Plant Protein
Purchased	True
On Farm	False
Available	False
Density (lbs/cu. ft):	0.00

DM (%)	92.10
Conc (%DM)	100.00
Forage (%DM)	0.00
CP (%DM)	53.60
SP (%CP)	8.45
Ether Extract (%DM)	5.81
CHO-B3 kd (%/hr)	18.00
Ammonia (%SP)	0.00
ADIP (%CP)	6.96
NDIP (%CP)	9.33
NFC (%DM)	5.32
Acetic (%DM)	0.00
Propionic (%DM)	0.00
Butyric (%DM)	0.00
Lactic (%DM)	0.00
Other OAs (%DM)	0.00
Sugar (%DM)	1.25
Starch (%DM)	1.47
Soluble Fiber (%DM)	2.60
ADF (%DM)	19.20
aNDFom (%DM)	31.80
peNDF(%)	20.00
Lignin (%NDF)	6.16
Ash (%DM)	3.47
NDIP (%DM)	5.00
ADIP (%DM)	3.73
Lignin (%DM)	1.96

Ca (%DM)	0.03
P (%DM)	0.72
Mg (%DM)	0.22
K (%DM)	0.52
S (%DM)	0.71
Na (%DM)	0.12
Cl (%DM)	0.08
Fe (ppm)	119.60
Zn (ppm)	116.20
Cu (ppm)	3.80
Mn (ppm)	16.70
Se (ppm)	0.40
Co (ppm)	0.00
I (ppm)	0.00
Vit-A (KIU/lb)	0.00
Vit-D (KIU/lb)	0.00
Vit-E (IU/lb)	0.00
Niacin (%DM)	0.00
Biotin (ppm)	0.00
Choline (%DM)	0.00
Chromium (%DM)	0.00
Organic Chromium (%Chromium)	0.00
Organic Zinc (%Zn)	0.00
Organic Copper (%Cu)	0.00
Organic Manganese (%Mn)	0.00
Organic Selenium (%Se)	0.00
Organic Cobalt (%Co)	0.00

Monensin (ppm)	0.00
Lasalocid (ppm)	0.00
Decoquinate (ppm)	0.00
Yeast (10^6 cfu/kg)	0.00
Beta Agonist (ppm)	0.00
Virginiamycin (ppm)	0.00
Aureomycin (ppm)	0.00
Chlortetracycline (ppm)	0.00
Oxytetracycline (ppm)	0.00
Salinomycin (ppm)	0.00
Zinc Bacitracin (ppm)	0.00
Enzymes (act/kg)	0.00
Toxin Binders (act/kg)	0.00
Flavor (%DM)	0.00

*AMTS only allows TFA to equal to 100% EE Value.

*TFA = 7.17 % DM

AMTS

Feed Analysis Report (NexPro_LabAnalysis-ACARROLL-02008-AMTS)

UNL Dairy 11/19/2021

MET (%CP)	2.51
LYS (%CP)	3.70
ARG (%CP)	4.27
THR (%CP)	4.21
LEU (%CP)	12.18
ILE (%CP)	3.41
VAL (%CP)	6.56
HIS (%CP)	2.60
PHE (%CP)	5.25
TRP (%CP)	1.15

TFA (%EE)	100.00
TFA (%DM)	5.81
Glycerol (%DM)	1.27
Pigment (%DM)	0.00
C12:0 (%TFA)	0.00
C14:0 (%TFA)	0.08
C16:0 (%TFA)	17.33
C16:1 (%TFA)	0.19
C18:0 (%TFA)	2.35
C18:1 Trans (%TFA)	0.01
C18:1 Cis (%TFA)	22.78
C18:2 (%TFA)	53.91
C18:3 (%TFA)	2.10
Other Lipid (%TFA)	1.25
Lipolysis Rate (%/hr)	500.00
Adjustment Factor	0.00

CHO-A1 kd (%/hr)	0.00
CHO-A2 kd (%/hr)	7.00
CHO-A3 kd (%/hr)	5.00
CHO-A4 kd (%/hr)	40.00
CHO-B1 kd (%/hr)	2.00
CHO-B2 kd (%/hr)	30.00
CHO-B3 kd (%/hr)	18.00
CHO-C kd (%/hr)	0.00
Prt-A1 kd (%/hr)	200.00
Prt-A21 kd (%/hr)	10.40
Prt-B1 kd (%/hr)	6.80
Prt-B2 kd (%/hr)	5.00
Prt-C kd (%/hr)	0.00

Ca Bioavailability (g/g)	0.60
P Bioavailability (g/g)	0.70
Mg Bioavailability (g/g)	0.16
K Bioavailability (g/g)	0.90
S Bioavailability (g/g)	1.00
Na Bioavailability (g/g)	0.90
Cl Bioavailability (g/g)	0.90
Fe Bioavailability (mg/mg)	0.10
Zn Bioavailability (mg/mg)	0.15
Cu Bioavailability (mg/mg)	0.04
Mn Bioavailability (mg/mg)	0.01
Se Bioavailability (mg/mg)	1.00
Co Bioavailability (mg/mg)	1.00
I Bioavailability (mg/mg)	0.85
Vit-A Bioavailability (IU/IU)	1.00
Vit-D Bioavailability (IU/IU)	1.00
Vit-E Bioavailability (IU/IU)	1.00

CHO-A1 ID (%DM)	100.00
CHO-A2 ID (%DM)	100.00
CHO-A3 ID (%DM)	100.00
CHO-A4 ID (%DM)	100.00
CHO-B1 ID (%DM)	75.00
CHO-B2 ID (%DM)	75.00
CHO-B3 ID (%DM)	5.00
CHO-C ID (%DM)	0.00
Prt-A1 ID (%DM)	100.00
Prt-A2 ID (%DM)	100.00
Prt-B1 ID (%DM)	100.00
Prt-B2 ID (%DM)	80.00
Prt-C ID (%DM)	0.00
Fat ID (%DM)	84.78
C12:0 ID (%DM)	95.39
C14:0 ID (%DM)	75.06
C16:0 ID (%DM)	72.48
C16:1 ID (%DM)	64.00
C18:0 ID (%DM)	72.80
C18:1 Trans ID (%DM)	78.56
C18:1 Cis ID (%DM)	89.25
C18:2 ID (%DM)	83.00
C18:3 ID (%DM)	77.55
Other Lipid ID (%DM)	58.17

APPENDIX B: NDS RATION INPUTS FOR NEW HPCOP

acarroll11

Full analysis **11/19/2021**

CZO510653083 - DDGS 1/1/0001

Nutrient	Unit	AF	DM
Moisture	%	7.9000	
D.M.	%	92.100	100.0000
CF	%	5.3510	5.8100
aNDFom	%	29.287	31.8000
ADF	%	17.683	19.2000
ADL	%	1.8052	1.9600
Forage aNDFom	%		
peNDF	%	5.8576	6.3600
NSC	%	1.3539	1.4700
NFC	%	3.6472	3.9600
Total CHO	%	32.935	35.7600
Acetic	%		
Propionic	%		
Butyric	%		
Lactic	%		
Organic Acids	%		
Sugar (WSC)	%		
Starch	%	1.3539	1.4700
Soluble Fiber	%	2.2938	2.4900
CHO B3 pdNDF Lig*2.4	%	24.955	27.0960
CHO C uNDF Lig*2.4	%	4.3324	4.7040
CHO C uNDF	%	7.8653	8.5400
CHO B3 pdNDF	%	21.422	23.2600
CHO B3 fast pool	%		
CHO B3 slow pool	%		
RD CHO 3x Level 1	%	14.748	16.0140
RD Starch 3xLevel 1	%	0.9757	1.0593
Forage	%		
Concentrate	%		100.0000
CP	%	49.365	53.6000
Soluble Protein	%	10.366	11.2560
Ammonia (Prot.A1)	%		
NDIP	%	9.5634	10.3837
ADIP	%	1.6142	1.7527
Prot. A2	%	10.366	11.2560
Prot. B1	%	29.435	31.9603
Prot. B2	%	7.9492	8.6310
RDP 3x Level 1	%	23.402	25.4095
RUP 1x Level 1	%	20.250	21.9878
RUP 3x Level 1	%	25.963	28.1905
PDI	%	23.751	25.7890
PDIA	%	19.062	20.6971
GE	Mcal/kg	4.7368	5.1431
DE	Mcal/kg	3.9316	4.2688
ME	Mcal/kg	3.2769	3.5580

Nutrient	Unit	AF	DM
NEl 3x NRC	Mcal/kg	2.1499	2.3343
NEm NRC	Mcal/kg	2.2569	2.4505
NEg NRC	Mcal/kg	1.5884	1.7246
UFL	unit/kg	1.1483	1.2468
UFV	unit/kg	1.1318	1.2289
TDN 1x	%	78.104	84.8034
EE	%	6.6036	7.1700
EE 1	%	6.6036	7.1700
EE 2	%		
EE 3	%		
TFA	%	6.6036	7.1700
Glycerol	%		
Pigment	%		
C12:0	%		
C14:0	%	0.0092	0.0100
C16:0	%	1.1420	1.2400
C16:1	%	0.0092	0.0100
C18:0	%	0.1566	0.1700
C18:1T	%	0.0004	0.0004
C18:1C	%	1.5012	1.6300
C18:2	%	3.5643	3.8700
C18:3 - ALA	%	0.1382	0.1500
C20:5 - EPA	%	0.0184	0.0200
C22:5 - DPA	%		
C22:6 - DHA	%		
Other LCFA	%	0.0641	0.0696
ID rfC12:0			95.3900
ID rfC14:0			75.0600
ID rfC16:0			72.4800
ID rfC16:1			72.0000
ID rfC18:0			72.8000
ID rfC18:1T			80.0000
ID rfC18:1C			80.0000
ID rfC18:2			83.0000
ID rfC18:3			77.5500
ID rfC20:5 n-3			77.5500
ID rfC22:5 n-3			77.5500
ID rfC22:6 n-3			77.5500
ID rf Other LCFA			58.7100
Ash	%	3.1959	3.4700
Ca	%	0.0276	0.0300
P	%	0.6631	0.7200
Mg	%	0.2026	0.2200
K	%	0.4789	0.5200
Na	%	0.1105	0.1200

Nutrient	Unit	AF	DM
Cl	%	0.0737	0.0800
S	%	0.6539	0.7100
NaCl	%		
S - Sulfates	%	0.6539	0.7100
Ca Bio.	g/g		0.6000
P Bio.	g/g		0.7000
Mg Bio.	g/g		0.1600
K Bio.	g/g		0.9000
Na Bio.	g/g		0.9000
Cl Bio.	g/g		0.9000
S Bio.	g/g		1.0000
Mn - total	ppm	15.380	16.7000
Cu - total	ppm	3.4998	3.8000
Fe - total	ppm	110.52	120.0000
Zn - total	ppm	106.83	116.0000
I - total	ppm		
Co - total	ppm	0.1566	0.1700
Se - total	ppm	0.5710	0.6200
Mo - total	ppm	0.9210	1.0000
Cr - total	ppm		
F - total	ppm		
Mn - added	ppm		
Cu - added	ppm		
Fe - added	ppm		
Zn - added	ppm		
I - added	ppm		
Co - added	ppm		
Se - added	ppm		
Mo - added	ppm		
Cr - added	ppm		
Mn Bio.	mg/mg		0.0100
Cu Bio.	mg/mg		0.0400
Fe Bio.	mg/mg		0.1000
Zn Bio.	mg/mg		0.1500
I Bio.	mg/mg		0.8500
Co Bio.	mg/mg		1.0000
Se Bio.	mg/mg		1.0000
Mo Bio.	mg/mg		1.0000
Cr Bio.	mg/mg		1.0000
Org. Mn	ppm		
Org. Cu	ppm		
Org. Zn	ppm		
Org. Co	ppm		
Org. Se	ppm		
Org. Cr	ppm		

Nutrient	Unit	AF	DM
MET	%	1.2391	1.3454
LYS	%	1.8265	1.9832
ARG	%	2.1079	2.2887
THR	%	2.0783	2.2566
LEU	%	6.0225	6.5392
ILE	%	1.6834	1.8278
VAL	%	3.2384	3.5162
HIS	%	1.2835	1.3936
PHE	%	2.5917	2.8140
TRP	%	0.5677	0.6164
MET	%CP		2.5100
LYS	%CP		3.7000
ARG	%CP		4.2700
THR	%CP		4.2100
LEU	%CP		12.2000
ILE	%CP		3.4100
VAL	%CP		6.5600
HIS	%CP		2.6000
PHE	%CP		5.2500
TRP	%CP		1.1500
Vit. A	IU/kg		
Vit. D3	IU/kg		
Vit. E	IU/kg		
Niacin	mg/kg		
Vit. B1	mg/kg		
Vit. B2	mg/kg		
Vit. B6	mg/kg		
Pantothenic Acid	mg/kg		
Vit. B12	mg/kg		
Choline	mg/kg		
Biotin	mg/kg		
Betain	mg/kg		
Vit. C	mg/kg		
Vit. H1	mg/kg		
Fumaric Acid	mg/kg		
Folic Acid	mg/kg		
Vit.A Bio	UI/UI		1.0000
Vit.D3 Bio.	UI/UI		1.0000
Vit.E Bio	UI/UI		1.0000
Kd CHO A1	%/hr		
Kd CHO A2	%/hr		7.0000
Kd CHO A3	%/hr		5.0000
Kd CHO A4	%/hr		60.0000
Kd CHO B1	%/hr		17.0000
Kd CHO B2	%/hr		30.0000
Kd CHO B3	%/hr		6.0000
Kd CHO B3 fast pool	%/hr		
TRUE PROTEIN	%		

Nutrient	Unit	AF	DM
Kd CHO C	%/hr		
Kd PRO A1	%/hr		200.0000
Kd PRO A2	%/hr		10.4000
Kd PRO B1	%/hr		6.8000
Kd PRO B2	%/hr		6.0000
Kd PRO C	%/hr		
Lipolysis	%/hr		500.0000
Adj Factor			
Int.Dig.CHO A1	%esca		100.0000
Int.Dig.CHO A2	%esca		100.0000
Int.Dig.CHO A3	%esca		100.0000
Int.Dig.CHO A4	%esca		100.0000
Int.Dig.CHO B1	%esca		75.0000
Int.Dig.CHO B2	%esca		75.0000
Int.Dig.CHO B3	%esca		20.0000
Int.Dig.CHO C	%esca		
Int.Dig.PRO A1	%esca		100.0000
Int.Dig.PRO A2	%esca		100.0000
Int.Dig.PRO B1	%esca		100.0000
Int.Dig.PRO B2	%esca		80.0000
Int.Dig.PRO C	%esca		
Int.Dig.FAT	%esca		79.8653
Monensin	mg/kg		
Lasalocid	mg/kg		
Decoquinate	mg/kg		
Chlortetracycline	mg/kg		
Oxytetracycline	mg/kg		
Virginiamycin	mg/kg		
Aureomycin	mg/kg		
Salinomycin	mg/kg		
Tylosin	mg/kg		
Diflubenzuron	mg/kg		
Zinc Bacitracin	mg/kg		
Beta-Agonists	mg/kg		
Melengestrol Acetate	mg/kg		
<Int.Dig. CHO B3 - v6.55>	%esca		5.0000

CHAPTER 3

INTERPRETIVE SUMMARY. Carroll et al (20XX). "Energy and nitrogen utilization of lactating dairy cattle fed increasing inclusion of a new high protein processed corn product." Increasing inclusion of a high protein processed corn product (**HPCoP**) replaced non-enzymatically browned soybean meal at 0, 2.6, 5.4 and 8% dietary DM. Increasing inclusion of HPCoP had no effect on nutrient digestibility. Increasing HPCoP increased the energy concentration in the diet and also milk fat and energy output in milk. Results of this study suggest that HPCoP can replace common feeds high in protein and support milk production.

RUNNING HEAD: HIGH PROTEIN PROCCESSED CORN PRODUCT AFFECT ENERGY

Energy and nitrogen utilization of lactating dairy cattle fed increasing inclusion of new high protein processed corn product.

Department of Animal Science, University of Nebraska–Lincoln, Lincoln 68583

*Corresponding author: P.J. Kononoff, Department of Animal Science C220, Fair St, Lincoln, NE, 68583, Phone number: 402-472-6442, Fax number: 402-472-6362, E-mail: pkononoff2@unl.edu

ABSTRACT

Advancing technologies of the corn dry-milling ethanol production process includes the mechanical separation of fiber containing particles from a portion of plant and yeast based nitrogenous particles. The resulting high protein processed corn product (HPCoP) contains approximately 52% CP, 36% NDF, 6.4% total fatty acids. The objective of this experiment was to examine the effects of replacing non-enzymatically browned soybean meal with the HPCoP on DMI, energy utilization, and production of lactating Jersey cows. Twelve multiparous Jersey cows were utilized in a triplicated 4x4 Latin square design consisting of 4, 28 d periods. Cows were blocked by milk yield and assigned randomly to 1 of 4 treatment diets that contained HPCoP (DM basis) at (1) 0% HPCoP (**CTRL**); (2) 2.6% HPCoP (**2.6L**); (3) 5.4% HPCoP (**5.4M**); and (4) 8.0% HPCoP (**8.0H**). Increasing the concentration of HPCoP tended to result in a quadratic effect on DMI (19.2, 19.9, 20.7, and 19.9 ± 0.62 kg for CTRL, 2.6L, 5.4M, and 8.0H). An increasing trend was observed for milk yield (27.8, 28.6, 29.8, and 29.0 ± 1.00 kg). While no difference was observed in the concentration of milk protein across treatments (3.40 ± 0.098 %) the concentration of fat increased with the inclusion of HPCoP (5.05, 5.18, 5.15, 5.47 ± 0.29). No differences were observed in the digestibility of DM, NDF, CP, TFA, and energy averaging 66.6 ± 0.63 %, 49.0 ± 2.13%, 66.1 ± 0.77 %, 73.6 ± 2.68 %, 66.3 ± 0.72 % across treatments. The concentration of GE linearly increased with increasing concentrations of HPCoP (4.25, 4.26, 4.28, and 4.31 ± 0.02 Mcal/kg), but no difference was observed in DE and ME across treatments averaging 2.83 ± 0.035 and 2.52 ± 0.039 Mcal/kg, respectively. An increasing trend was observed in concentration of NE_L (1.61, 1.72, 1.74, 1.71 ± 0.056 Mcal/kg) with the ratio of NE_L:ME increasing

linearly across treatments (0.648, 0.674, 0.685, 0.675 ± 0.0174). Results of this study suggests that the inclusion of the HPCoP can replace common feeds high in protein and support normal milk production.

Key Words: energy, corn product

INTRODUCTION

In 2020, the United States supplied 53% of the total global production of grain-based fuel ethanol and 33.1 million metric tons of distillers grains, gluten feed and gluten meal; together these contributed approximately $34.7 billion dollars to the nation's Gross Domestic Product (RFA, 2020). Development of new coproducts aim to expand the revenue stream of grain-ethanol production by creating specialized coproducts with concentrated protein content. The concentration of protein occurs through modifications of grain-ethanol production including sieving and elutriation of coproduct streams (Srinivasan et al., 2005). In this process, subsequent fermentation fiber from the spent grain is mechanically separated through sieving from kernel protein and yeast based nitrogenous particles (Srinivasan et al., 2005). The resulting high protein coproduct (**HPCoP**) contains 54% crude protein (**CP**), and 7.2 % total fatty acids (**TFA**) on a DM basis (Carroll et al., 2021). The CP of the new HPCoP increased relative to traditional dried distillers grains (**DDGS**) which contain approximately 30 % CP (NRC, 2001). Although a number of high protein corn milling coproducts have been available since the late 2000's they are produced through the removal of bran and germ prior to fermentation with protein fractionization occurring post fermentation and contain approximately 45 % CP (Tedeschi et al., 2009).

Whole animal energy and nitrogen balance experiments have examined both wet DGS (Birkelo et al., 2004) and reduced fat DDGS (Foth et al., 2015). However, innovations in the production process of DDGS concentrates nitrogenous based particles from the residual fiber by sieving post fermentation. Since the chemical composition of the HPCoP differs from that of DDGS controlled feeding experiments are necessary to examine ration formulation strategies, determine energy and nitrogen utilization, and animal production. As a result, the objective of this experiment was to examine the effects of replacing non-enzymatically browned soybean meal with the HPCoP on DMI, energy utilization, and production of lactating Jersey cows. We hypothesized that feeding HPCoP in an isonitrogenous and isoenergetic ration would maintain milk production across treatments.

MATERIALS AND METHODS

Animals and Treatments

The University of Nebraska- Lincoln Animal Care and Use Committee approved animal care and experimental procedures. Twelve multiparous Jersey cows 95 ± 7.3 DIM were sourced from a commercial diary. Cows were housed in individual tie stalls in a climate-controlled environment (20º C) at the University of Nebraska-Lincoln Dairy Metabolism Facility in the Animal Science Complex. Stalls were equipped with rubber mats and cows were milked at 0700 and 1800 h. All cows were less than 134 d pregnant at the end of the last experimental period thus fetal energy was assumed to be zero. (NRC, 2001)

The experimental design was a triplicated 4×4 Latin square design balanced for carryover effects consisting of 4 periods of 28-d in length. Prior to the start of the

experiment cows grouped by milk yield and DMI and were randomly assigned one of four TMRs. Treatment sequence was based on Kononoff and Hanford (2006). The high protein corn milling coproduct (HPCoP) originated from Flint Hill Resources, Fairmont, NE. Treatments were as follows: **CRTL** [0 % HPCoP]; **2.6L** [2.6 % HPCoP on DM basis]; **5.4M** [5.4 % HPCoP on DM basis]; or **8.0H** [8 % HPCoP on DM basis]. Two concentrate mixes were utilized in the study where concentrate mix one provided 0% HPCoP and the second provided 8% HPCoP. These two concentrate mixes were added in a ratio of 33.3 % and 66.7 % for the low **(2.6L)** and 66.7 % to 33.3 % for the medium **(5.4M).** Dietary ingredients (corn silage, alfalfa hay and concentrate) were placed in the Calan Data Ranger (American Calan, Inc. Northwood, NH), mixed and fed at 0930 h. The target refusal rate aimed to be 5% for the 24 d adaptation period of each period. During the 4 d collection period cattle were fed at 100% of the prior week's average intake to limit refusals.

Sample Collection and Analysis

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

amylase corrected for ash contamination (**aNDFom**) (Van Soest et al., 1991) , lignin (Goering and Van Soest 1970), crude fat (method 2003.05 AOAC, 2000), sugar (Hall, 2009), starch (Hall 2009), ash (method 942.05, AOAC, 2000), minerals (method 985.01, AOAC, 2000), total fatty acids (Sukhija and Palmquist, 1988). Feed samples were also analyzed for gross energy (**GE**) content using a bomb calorimeter (Parr 6400 Calorimeter, Moline, IL). The chemical composition of the diets and feed ingredients is listed in Table 3.1. Total mixed rations were sampled on d 1 of each collection period and used to determine particle size using the Penn State particle separator (Kononoff and Heinrichs, 2002) and reported on an as is and DM basis (60ºC for 48 h). During each collection period refusals were sampled and composited on a weight basis. Refusals were analyzed for DM, CP, NDF, aNDFom, starch, ash, fatty acids, and GE according the same methods as feeds described above.

Total fecal and urine output was collected from each individual cow during the collection period for 4 consecutive d as described by McLain et al. (2021). After collections, approximately ~ 600 g feces were dried at 60ºC for 48 h and ground to pass through a 1 mm screen (Wiley Mill; Aurthur A. Thomas Co., Philadelphia, PA). The ground feces were analyzed for DM, CP, NDF, aNDFom, ash, fatty acids and GE using the same methods as described for feeds. Milk production was measured daily, and milk samples were collected during the morning and evening milking of collection periods as described by McLain et al. (2021). Composited milk samples were analyzed for nitrogen and fatty acids as previously described for feeds. To determine body weight, cows were weighed before feeding at 800h the first day and 1000h the last day of each collection period.

Heat Production and Energy Utilization

Heat production was determined indirectly using the headbox-type indirect calorimeters as described previously (McLain et al., 2021). However, total volume of gas flow through the headbox was measured using mass flow meters (MCW Whisper, Alicat Scientific) and corrected to standard temperature and pressure (0ºC, 101.3 kPa) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). System efficiency (head box and gas analyzer) was determined by burning 100 % ethyl alcohol and measuring gas recoveries. Recoveries of O_2 and CO_2 were (average ± SD) 101 ± 1.1 and 99 ± 1.3 %, respectively.

Energy Calculations

The respiratory quotient (**RQ**) was calculated using the ratio of carbon dioxide produced to oxygen consumed (L/L). Methane energy was estimated by multiplying CH_4 production by its enthalpy (9.45 kcal/L). Calculations to estimate digested energy (**DE**), ME and NE_L were as follows:

DE (Mcal/d) = GE (Mcal/d) – fecal energy (Mcal/d) [1]

ME (Mcal/d) = DE (Mcal/d) – urine energy (Mcal/d) – methane energy (Mcal/d) [2]

Unaccounted for energy was assumed to represent tissue energy retention or mobilization which was corrected to an NE_L basis as follows:

Tissue energy (Mcal/d) = ME (Mcal/d) – heat production (Mcal/d) – milk energy (Mcal/d) [3]

Adjusted tissue energy (**TE**; Mcal of NE_L/d) = positive residual energy × k_L/k_G or negative residual energy × k_T [4]

Where k_T is the efficiency of utilizing body reserve energy for milk production, k_G is the efficiency of utilizing ME intake for tissue gain (Moe et al., 1970). Values of 0.66 and 0.74, and 0.89 were used for k_L, k_G, and k_T respectively (Moraes et al., 2015).

Net energy of lactation (NE_L; Mcal/d) = $0.08 \times BW^{0.75}$ + Milk E (Mcal/d) + Adjusted TE (NE_L Mcal/d) [5]

Statistical Analysis

The UNIVARIATE procedure of SAS (9.4) was used to determine outliers from the data set. An outlier was determined if an observation was greater than 2.5 standard deviations from the mean of milk production and DMI. Data were analyzed in SAS (9.4). The model includes fixed effect of treatment and the random effect of period, square and cow nested in square. A type III analysis of variance with Kenward-Rodger's denominator degrees of freedom was complete using the PROC GLIMMIX function of SAS. All data are presented as least-squares means ± largest standard error. Significance and trends was declared with a P-value ≤ 0.05 and P-value ≤ 0.10.

RESULTS

Out of the total of 48 planned observations 42 energy utilization, 47 DMI, 47 digestibility, and 47 nitrogen utilization observations were collected in the experiment. Forty-two energy observations were collected as one animal refused to drink water while in the headbox during the first period and was unresponsive to further training attempts. Also, another animal's observations were removed from energy, DMI, digestibility, and nitrogen utilization during the first period. This animal (4842) consuming the 2.6L treatment contracted mastitis and daily DMI dropped to 13.6 kg. This intake was 2.72

standard deviations from the mean DMI (19.8 ± 2.27 kg). Records also indicated that this animal displayed signs of mastitis on the day of observation this animal was removed prior to statistical analysis. The animal recovered and was utilized for the remaining 3 periods.

Chemical Composition and Feed Intake

Diet composition of the four diet treatments are listed in Table 3.1. Crude protein of the diets remaining similar with increasing inclusion averaging 16.1 % DM. Increasing inclusion of HPCoP increased the concentration of total fatty acids from 5.03 ± 0.32 % DM in CTRL to 5.27 ± 0.46 % DM in 8.0H. Similarly, 18C fatty acids increased from 2.86 ± 0.12 % DM in the CTRL to 3.07 ± 0.14 % DM in 8.0H. Chemical composition of corn silage, alfalfa hay, concentrate mixes and HPCoP are listed in Table 3.2. In the current experiment the HPCoP contained 36.2 ± 1.63 % NDF, 52.4 ± 0.35 % CP, 6.44 ± 0.099 % total fatty acids and 3.39 ± 0.342 % lysine on a CP basis.

Energy Utilization and Digestibility

Energy utilization is outlined in Table 3.3. Increasing inclusion of HPCoP linearly increased ($P \leq 0.01$) GE from 4.25 to 4.31 ± 0.020 Mcal/kg, however no difference ($P > 0.25$) was observed in the concentration of either DE or ME averaging 2.83 ± 0.035 and 2.52 ± 0.039 Mcal/kg, respectively. An increasing linear trend ($P = 0.09$) was observed for NE_L from 1.61 to 1.71 ± 0.056 Mcal/kg . These same effects were also reflected in measures of GE, DE, ME, and NE_L expressed as Mcal/d. The ratio of ME to DE tended ($P = 0.09$) to quadratically increase from CTRL to 5.4M then decrease at 8.0H (0.884 to 0.898 to 0.893 ± 0.0076) but the ratio of NE_L to ME increased linearly ($P = 0.03$) across

treatments from 0.648 to 0.675 ± 0.0174 (Table 3.3). Milk energy increased ($P \leq 0.01$) linearly across treatment from 23.5 to 25.8 ± 0.759 Mcal/d.

No difference ($P > 0.18$) was observed in O_2 consumption and CO_2 and CH_4 production averaging 4779 ± 245 L/d, 4927 ± 292 L/d and 414 ± 36 L/d, respectively across treatments (Table 3.3). However, a quadratic response ($P = 0.05$) in RQ was observed with an increase from CTRL (1.019 ± 0.014) to 5.4M (1.040 ± 0.014) and decrease to 8.0H (1.022 ± 0.014). Similarly, a quadratic tend ($P = 0.10$) was observed as tissue energy increased from CTRL to 5.4M then decreased to 8.0H (-0.20 to 2.73 to 0.66 ± 1.64 Mcal/d).

No difference (P > 0.12) was observed in DM, NDF, CP, Starch, Total fatty acid, and energy apparent total- tract digestibility, averaging 66.6 ± 0.63 %, 49.0 ± 2.13 %, 66.1 ± 0.77 %, 73.6 ± 2.68 %, 66.3 ± 0.72 % respectively across treatments (Table 3.4).

Nitrogen Utilization

No difference ($P = 0.11$) was observed in nitrogen intake averaging 512 ± 17.36 g/d across treatments (Table 3.5). Fecal nitrogen excretion tended to increase ($P = 0.07$) linearly from 167.5 to 177.2 ± 6.83 g/d. No difference ($P > 0.11$) was observed for urinary nitrogen or milk nitrogen excretion averaging 127.4 ± 13.6 g/d and 164.8 ± 6.83 g/d, respectively. Urinary nitrogen as a proportion of total nitrogen intake decreased ($P = 0.05$) quadratically from CRTL (25.8 ± 2.84 %) to 2.6L (22.9 ± 2.84 %) and then increased to 8.0H (27.3 ± 2.84 %).

Milk Yield and Composition

Dry matter intake tended to increase ($P = 0.07$) quadratically from CRTL to 5.4M (19.2 to 20.7 to 19.9 ± 0.62 kg/d) and then decreased when cows consumed 8.0H (Table

3.6). Milk yield tended ($P = 0.08$) to increase linearly across treatments from 27.8 to 29.0 ± 1.00 kg/d from CTRL to 8.0H. No difference ($P > 0.14$) was observed in the concentration of protein which averaged 3.40 ± 0.098 % however milk protein yield tended ($P = 0.06$) to increase from 0.93 to 0.99 ± 0.033 kg/d across treatments. Milk fat percentage increased ($P < 0.01$) linearly from 5.05 to 5.47 ± 0.288 %, while milk fat yield increased ($P < 0.01$) linearly from 1.40 to 1.58 ± 0.065 kg/d. Concentration of C16:0 in the milk tended ($P = 0.08$) to decrease linearly across treatments from 38.5 to 37.7 ± 0.867 g/100 g of fat (Table 3.7). The concentration of LA increased linearly from CTRL to 8.0H (1.98 to 2.35 ± 0.099 g/100 g of fat). Similarly, ALA increased linearly from 0.23 to 0.25 ± 0.009 g/100 g of fat. No difference ($P = 0.20$) was observed for the concentration of < 16 carbon milk fatty acids averaging 25.3 ± 0.43 g/100g of fat. Greater than 16 carbon milk fatty acids tended ($P = 0.08$) to increase linearly from 32.0 to 32.9 ± 0.08 g/100 g of fat. *Trans*- 10 *cis*- 12 was not detected in any of the milk samples.

DISCUSSION

Chemical Composition

The objective of this experiment was to examine the effects of replacing traditionally used high-protein feeds with a new HPCoP and to examine the effects on DMI, energy and nitrogen utilization, and production of lactating Jersey cattle. During production of the new HPCoP a large portion of fiber is removed by sieving to purify the protein stream concentrating the protein and energy content relative to DDGS (Birkelo et al., 2004; Srinivasan et al., 2005). Later, the remaining nutrients are decanted to the fermenting vessel leaving corn protein and approximately 29% spent yeast which are dried to form the HPCoP (Shurson, 2018). Based upon data published in NRC (2001) DDGS typically

contain 39 % NDF, 6.5 % TFA (Dufour, 2017), and 30 % CP of which approximately 3.2 % is lysine (Mjoun et al., 2010b). In comparison, in the current study the test coproduct contained 36.2 ± 1.63 % NDF, 6.44 ± 0.099 % TFA, 52.4 ± 0.35 % CP and 3.39 ± 0.342 % lysine on a CP basis. As a result, the decrease in NDF content and subsequent increase in protein is likely an effect of the removal of fiber through sieving and increased concentration of yeast cells in the product.

Feed Intake

We hypothesized that there would be no difference in DMI across diets due to observations where DMI was maintained with dried DG inclusion (Paz et al. 2013). Overall, the Dairy NRC (2001) indicates that milk yield is the primary driver of intake based upon the influence of milk production within the DMI equation. This is further supported as milk yield has been found to be strongly correlated with DMI (Morris et al., 2021). In the current experiment, a quadratic trend was observed for DMI intake as HPCoP increased from 0.0 to 5.4 % of dietary DM but was reduced when it was included up to 8 % of dietary DM. Since milk yield is a primary driver of intake the observed increase in milk yield from 0.0 to 5.4 % and subsequent decrease at 8.0% may have led to the observed response in DMI.

Nitrogen Utilization

In the current experiment apparent total tract CP digestibility was not affected by diet but increasing the inclusion of HPCoP resulted in an increase in the total mass of nitrogen excreted through the feces. Fecal nitrogen is composed of undigested feed nitrogen, endogenous nitrogen, and undigested microbial nitrogen (Tamminga, 1991). As such DMI likely contributed to the observed effects of fecal nitrogen excretion as only 4.3 g/d

fecal nitrogen can be directly attributed to CP digestibility in 8.0H. Surprisingly, urinary nitrogen as a percent of nitrogen intake followed a quadratic pattern, with increased urinary N as a % of N intake in the CRTL and 8.0H diets relative to 2.6L and 5.4M. The increase in urinary nitrogen as a percent of nitrogen intake in the CTRL relative to the other treatments may have been an effect of tissue loss in the animal as no difference was observed in nitrogen intake.

Energy Supply and Utilization

The gross energy value of a feed is equivalent to the energy released during complete combustion (Forbes, 1996). Therefore, with increasing inclusion there was linear increase in dietary gross energy (**GE**) in response to increasing organic compounds in the diet such as fat, starch, and aNDFom. However, fecal energy increased with increasing inclusion as a function of DMI but had no subsequent effects on digestible energy (**DE**)(Mcal/d) across treatments. Metabolizable energy (**ME**)(Mcal/d) was similar with increasing inclusion as no differences were observed in energy losses associated with urinary energy (Mcal/d) and gaseous energy (Mcal/d). Energetic conversion efficiencies for DE to ME and ME to NEL are dependent on diet chemical composition, digestibility, nutrient flux, and metabolic status of the animal. However, fixed conversion efficiencies are utilized in nutrition models which may not accurately encompass the mechanisms and interactions associated with energetic losses. Prediction of animal responses may be improved through continued evaluation of the dietary and metabolic factors associated with energetic conversion efficiencies. In the current experiment we speculate that decreased efficiency of the conversion of DE to ME in the CTRL and the 8.0H were likely a result of increased amino acid catabolism for energy production

indicated by the increasing proportion of urinary energy loss and decreased tissue energy relative to 2.6L and 5.4M. The efficiency of conversion of DE to ME has been shown to decrease with negative energy balance and increased dietary CP (Reynolds, 2006; Weiss and Tebbe, 2019); this is in response to increased AA catabolism and subsequent urinary nitrogen excretion increasing urinary energy loss through metabolites such as urea. Increased dietary fat has been demonstrated to increase the conversion of DE to ME due to decreased methane production (Ellis et al., 2007). Although a decreasing linear tendency for CH_4:DMI L/kg was observed across treatments, the decreased CH_4:DMI likely resulted from the increase in fatty acids with increasing inclusion of HPCoP (Benchaar et al., 2013; Drehmel et al., 2018) but had little impact on DE to ME conversion. Decreased tissue energy in the 8.0H could have resulted in energy diverted to support lactation as manipulating dietary nutrients has been shown to influence energy partitioning between milk and tissue (van Knegsel et al., 2007; Nichols et al., 2018). The conversion of ME to NE_L can be increased by increasing TFA content in the diets, as some dietary fats can be directly converted to milk fat (Rico et al., 2014; Boerman et al., 2015). This occurs as de novo lipogenesis is less energetically efficient when compared with preformed fatty acid utilization in milk fat synthesis (70 - 75 % vs. 94 - 97 %; Baldwin et al., 1985). Therefore, our data suggests HPCoP increased dietary fatty acid supply and the subsequent utilization for NE_L when cows consumed the 8.0H diet. However, the increased milk energy response was not observed in the CTRL diet compared to the diets including HPCoP due to the decreased gross energy and fatty acid content in the diet containing solely non-enzymatically browned soybean meal.

Milk Yield and Composition

Increasing inclusion of HPCoP tended to increase milk yield, increased milk fat concentration, but had no effect milk protein concentration. In isoenergetic diets, increased conversion of ME to NE_L causes partitioning of feed energy for milk synthesis in mid lactation cows likely contributing to the increased milk yield with increasing inclusion of HPCoP (Boerman et al., 2015). In this experiment, increased milk fat concentration with increasing inclusion of HPCoP may be a multifaceted response of dietary fatty acids, adipose metabolism, and the yeast cells provided in the new HPCoP. Generally, dietary preformed > 16 carbon fatty acids are efficiently incorporated into milk fat (Bauman and Griinari, 2003). In this experiment we observed an increase in dietary 18 carbon fatty acids (**C18**) with a subsequent increase in C18:2 LA and C18:3 ALA milk fatty acids similar to other distillers products (Ramirez-Ramirez et al., 2016). Therefore, increasing dietary C18 and subsequent utilization in milk fat likely explains a portion of the increase in milk fat concentration with increasing inclusion of the HPCoP. In the 8.0H diet, DMI and C18 digestibility do not account for the total C18 output in the milk fat. Another contributing factor to increased C18 milk fatty acids may be a result of the decrease in TE in the 8.0H relative to 2.6L and 5.4M as > 16 carbon fatty acids can be derived from the adipose tissue of animals (Harvatine, 2018). However, with increasing duodenal flow of C18 Prado et al. (2019) observed C18 fatty acids had a negative impact on de novo milk fat synthesis. Nonetheless, we observed no difference in de novo fatty acids concentration with increasing dietary C18 from the HPCoP. Sustained de novo fatty acid concentration with increasing inclusion may be a function of the unknown but substantial proportion of yeast in the HPCoP. De novo fatty acids are produced by acetate and butyrate from bacterial fermentation (Barbano et al., 2017). Yeast cells contained

within DDGS have been observed to contribute little to the total omasal flow of MCP but are digested ruminally and may provide cofactors which can be utilized to increase bacterial protein (Castillo-Lopez et al., 2010).

Fluctuations in milk and milk protein yield, in diets containing DDGS, has been linked with DMI (Janicek et al., 2008; Paz and Kononoff, 2014) and this is often attributed to a decrease in the supply of lysine (Nichols et al., 1998). However, the HPCoP in this experiment contained increased lysine content relative to traditional DDGS due to increased yeast cell content. Therefore, we speculate the effects of milk protein yield was not singular effects of DMI or lysine imbalance but more likely an effect of the increasing intake of NE_L (kg/d). As all components are highly dependent on glucose availability, our results suggest increasing NE_L (kg/d) with increasing inclusion of HPCoP contributed to the increase in milk protein yield (Huang et al., 2021).

CONCLUSIONS

Lactating dairy cows were fed diets that supplied increasing inclusion of a new HPCoP while replacing non-enzymatically browned soybean meal Increasing inclusion of HPCoP increased DM intake when HPCoP was included at 5.4 % of the diet DM but no effects in nutrient digestibility were observed. However, increasing inclusion of HPCoP increased gross energy content, conversion of energy from ME to NEL, and energy utilization for milk and milk fat production. This response was likely an effect of increasing dietary fat provided by the HPCoP and the subsequent energetic efficiency of utilizing preformed long chain fatty acids for milk fat synthesis. Results indicate that the new high protein corn milling co-product can be effectively utilized in diet formulation similar to other high protein feedstuffs.

ACKNOWLEDGMENTS

The authors thank the University of Nebraska Dairy Metabolism (Lincoln, NE) staff and students for care of the experimental animals and assistance with collections, Flint Hills Resources (Wichita, KS) for financial support, and representatives from Fluid Quip Technologies, LLC (Cedar Rapids, IA) for experimental design and planning.

REFERENCES

Baldwin, B.R., N.E. Forsberg, and C.Y. Hu. 1985. Potential for Altering Energy Partition in the Lactating Cow. Journal of Dairy Science 68:3394–3402. doi:10.3168/jds.S0022-0302(85)81252-2.

Barbano, D.M., C. Melilli, H. Dann, and R. Grant. 2017. INFARED MAILK FATTY ACID ANALYSIS: EXPIERENCE IN THE FIELD FOR FARM MANAGEMENT.

Bauman, D.E., and J.M. Griinari. 2003. Nutritional Regulation of Milk Fat Synthesis. Annu. Rev. Nutr. 23:203–227. doi:10.1146/annurev.nutr.23.011702.073408.

Benchaar, C., F. Hassanat, R. Gervais, P.Y. Chouinard, C. Julien, H.V. Petit, and D.I. Massé. 2013. Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production. Journal of Dairy Science 96:2413–2427. doi:10.3168/jds.2012-6037.

Birkelo, C.P., M.J. Brouk, and D.J. Schingoethe. 2004. The Energy Content of Wet Corn Distillers Grains for Lactating Dairy Cows. Journal of Dairy Science 87:1815–1819. doi:10.3168/jds.S0022-0302(04)73338-X.

Boerman, J.P., S.B. Potts, M.J. VandeHaar, and A.L. Lock. 2015. Effects of partly replacing dietary starch with fiber and fat on milk production and energy partitioning. Journal of Dairy Science 98:7264–7276. doi:10.3168/jds.2015-9467.

Carroll, A.L., D.L. Morris, P.J. Kononoff, and M.L. Jolly-Breithaupt. 2021. Energy of lactating dairy cattle fed increasing inclusion of new high-protein processed corn product. Page ADSA 2021.

Castillo-Lopez, E., P.J. Kononoff, and J.L. Miner. 2010. Short communication: Detection of yeast DNA in omasal digesta of dairy cows consuming dried distillers grains and solubles. Journal of Dairy Science 93:5926–5929. doi:10.3168/jds.2010-3302.

Drehmel, O.R., T.M. Brown-Brandl, J.V. Judy, S.C. Fernando, P.S. Miller, K.E. Hales, and P.J. Kononoff. 2018. The influence of fat and hemicellulose on methane production and energy utilization in lactating Jersey cattle. Journal of Dairy Science 101:7892–7906. doi:10.3168/jds.2017-13822.

Dufour, E.I. Advancing Chemical Characterization of Feedstuffs Commonly Included in Dairy Cow Rations 129.

Ellis, J.L., E. Kebreab, N.E. Odongo, B.W. McBride, E.K. Okine, and J. France. 2007. Prediction of Methane Production from Dairy and Beef Cattle. Journal of Dairy Science 90:3456–3466. doi:10.3168/jds.2006-675.

Forbes, J.M. 1996. Integration of regulatory signals controlling forage intake in ruminants.. Journal of Animal Science 74:3029. doi:10.2527/1996.74123029x.

Foth, A.J., T. Brown-Brandl, K.J. Hanford, P.S. Miller, G. Garcia Gomez, and P.J. Kononoff. 2015. Energy content of reduced-fat dried distillers grains with solubles for lactating dairy cows. Journal of Dairy Science 98:7142–7152. doi:10.3168/jds.2014-9226.

Harvatine, K.J. 2018. Milk FA Analysis: Interpreting the data.

Huang, X., P.S. Yoder, I.A.M.A. Teixeira, and M.D. Hanigan. 2021. Assessing amino acid uptake and metabolism in mammary glands of lactating dairy cows intravenously infused with methionine, lysine, and histidine or with leucine and

isoleucine. Journal of Dairy Science S0022030221000047. doi:10.3168/jds.2020-18169.

Janicek, B.N., P.J. Kononoff, A.M. Gehman, and P.H. Doane. 2008. The Effect of Feeding Dried Distillers Grains Plus Solubles on Milk Production and Excretion of Urinary Purine Derivatives. Journal of Dairy Science 91:3544–3553. doi:10.3168/jds.2007-0777.

van Knegsel, A.T.M., H. van den Brand, J. Dijkstra, W.M. van Staalen, M.J.W. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary Energy Source in Dairy Cows in Early Lactation: Energy Partitioning and Milk Composition 90:10.

Kononoff, P.J., and K.J. Hanford. 2006. Technical Note: Estimating Statistical Power of Mixed Models Used in Dairy Nutrition Experiments. Journal of Dairy Science 89:3968–3971. doi:10.3168/jds.S0022-0302(06)72439-0.

Kononoff, P.J., and A.J. Heinrichs. 2003. The Effect of Corn Silage Particle Size and Cottonseed Hulls on Cows in Early Lactation. Journal of Dairy Science 86:2438–2451. doi:10.3168/jds.S0022-0302(03)73838-7.

McCaherty, J., C. Wilson, G. Cooper, and R. Schwarck. 2020. Neil Koehler RFA Chairman Pacific Ethanol Inc. www.pacificethanol.com 40.

McLain, K.A., D.L. Morris, and P.J. Kononoff. 2021. Effect of feeding hydrolyzed feather meal and rumen-protected lysine on milk protein and energy utilization in late-lactation Jersey cows. Journal of Dairy Science 104:8708–8720. doi:10.3168/jds.2020-19657.

Mjoun, K., K.F. Kalscheur, A.R. Hippen, D.J. Schingoethe, and D.E. Little. 2010. Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grains with solubles. Journal of Dairy Science 93:288–303. doi:10.3168/jds.2009-2377.

Moe, P.W., H.F. Tyrrell, and W.R. Flatt. 1970. Partial eficiency of energy use for maintenance, lactation, body gain and gestation in the dairy cow. Vitznau, Switzerland.

Morris, D.L., T.M. Brown-Brandl, P.S. Miller, W.P. Weiss, R.R. White, and P.J. Kononoff. 2021. Factors that affect heat production in lactating Jersey cows. Journal of Dairy Science 104:346–356. doi:10.3168/jds.2020-18976.

Nichols, J.R., D.J. Schingoethe, H.A. Maiga, M.J. Brouk, and M.S. Piepenbrink. 1998. Evaluation of Corn Distillers Grains and Ruminally Protected Lysine and Methionine for Lactating Dairy Cows. Journal of Dairy Science 81:482–491. doi:10.3168/jds.S0022-0302(98)75600-0.

Nichols, K., A. Bannink, S. Pacheco, H.J. van Valenberg, J. Dijkstra, and H. van Laar. 2018. Feed and nitrogen efficiency are affected differently but milk lactose production is stimulated equally when isoenergetic protein and fat is supplemented in lactating dairy cow diets. Journal of Dairy Science 101:7857–7870. doi:10.3168/jds.2017-14276.

NRC. 2001. Nutrient Requirements of Dairy Cattle. National Academies Press, Washington, D.C.

Paz, H.A., E. Castillo-Lopez, H.A. Ramirez-Ramirez, D.A. Christensen, T.J. Klopfenstein, and P.J. Kononoff. 2013. Invited Review: Ethanol co-products for dairy cows: there goes our starch … now what?. Can. J. Anim. Sci. 93:407–425. doi:10.4141/cjas2013-048.

Paz, H.A., and P.J. Kononoff. 2014. Lactation responses and amino acid utilization of dairy cows fed low-fat distillers dried grains with solubles with or without rumen-protected lysine supplementation. Journal of Dairy Science 97:6519–6530. doi:10.3168/jds.2014-8315.

Prado, L.A., Ph. Schmidely, P. Nozière, and A. Ferlay. 2019. Milk saturated fatty acids, odd- and branched-chain fatty acids, and isomers of C18:1, C18:2, and C18:3n-3 according to their duodenal flows in dairy cows: A meta-analysis approach. Journal of Dairy Science 102:3053–3070. doi:10.3168/jds.2018-15194.

Ramirez-Ramirez, H.A., E. Castillo Lopez, C.J.R. Jenkins, N.D. Aluthge, C. Anderson, S.C. Fernando, K.J. Harvatine, and P.J. Kononoff. 2016. Reduced-fat dried distillers grains with solubles reduces the risk for milk fat depression and supports milk production and ruminal fermentation in dairy cows. Journal of Dairy Science 99:1912–1928. doi:10.3168/jds.2015-9712.

Reynolds, C. 2006. Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. K. Sejrsen, T. Hvelplund, and M. O. Nielsen, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.

Rico, D.E., Y. Ying, and K.J. Harvatine. 2014. Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. Journal of Dairy Science 97:3739–3751. doi:10.3168/jds.2013-7341.

Shurson, G.C. 2018. Yeast and yeast derivatives in feed additives and ingredients: Sources, characteristics, animal responses, and quantification methods. Animal Feed Science and Technology 235:60–76. doi:10.1016/j.anifeedsci.2017.11.010.

Srinivasan, R., R.A. Moreau, K.D. Rausch, R.L. Belyea, M.E. Tumbleson, and V. Singh. 2005. Separation of Fiber from Distillers Dried Grains with Solubles (DDGS) Using Sieving and Elutriation. Cereal Chemistry Journal 82:528–533. doi:10.1094/CC-82-0528.

Sukhija, P.S., and D.L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206. doi:10.1021/jf00084a019.

Tamminga, S. 1992. Nutrition Management of Dairy Cows as a Contribution to Pollution Control 13.

Tedeschi, L.O., P.J. Kononoff, K. Karges, and M.L. Gibson. 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. Journal of Dairy Science 92:401–413. doi:10.3168/jds.2008-1141.

Weiss, W.P., and A.W. Tebbe. 2019. Estimating digestible energy values of feeds and diets and integrating those values into net energy systems. Translational Animal Science 3:953–961. doi:10.1093/tas/txy119.

TABLES

Table 3.1 Ingredient inclusion and chemical composition of experimental diets of lactating Jersey cattle fed increasing inclusion of a new high protein coproduct (HPCoP) (% of diet DM)

	Treatment[1]			
Item	CTRL	2.6L	5.4M	8.0H
Ingredient				
Corn silage	40.0	40.0	40.0	40.0
Alfalfa hay	18.1	18.1	18.1	18.1
Ground corn	14.3	14.3	14.3	14.3
High protein coproduct[2]	-	2.64	5.36	8.00
Soybean meal	2.66	2.66	2.66	2.66
Non-enzymatically browned soybean meal[3]	8.00	5.36	2.64	-
Soybean hulls	8.61	8.61	8.61	8.61
Urea	0.64	0.64	0.64	0.64
Salt	0.38	0.38	0.38	0.38
Sodium bicarbonate	0.60	0.60	0.60	0.60
Vitamin premix[4]	0.04	0.04	0.04	0.04
Molasses, beet	1.73	1.73	1.73	1.73
Fat supplement[5]	3.00	3.00	3.00	3.00
Trace mineral premix[6]	0.05	0.05	0.05	0.05
Calcium carbonate	1.11	1.11	1.11	1.11
Calcium phosphate	0.51	0.51	0.51	0.51
Magnesium oxide	0.40	0.40	0.40	0.40
Formulated chemical composition, % DM				
DM	59.6 (2.43)	59.4 (1.92)	60.3 (1.94)	59.4 (1.73)
CP	16.1 (0.43)	16.1 (0.35)	16.1 (0.36)	16.1 (0.45)
ADICP	0.80 (0.08)	0.92 (0.09)	1.04 (0.11)	1.15 (0.13)
Total fatty Acids	5.03 (0.32)	5.11 (0.36)	5.19 (0.41)	5.27 (0.46)
16C fatty acids	1.93 (0.24)	1.93 (0.27)	1.94 (0.30)	1.95 (0.33)
18C fatty acids	2.86 (0.12)	2.93 (0.12)	3.00 (0.13)	3.07 (0.14)
aNDFom[7]	30.7 (1.46)	31.0 (1.31)	31.3 (1.18)	31.6 (1.07)
ADF	20.9 (0.53)	21.2 (0.41)	21.5 (0.29)	21.8 (0.18)
Lignin	3.14 (0.24)	3.21 (0.22)	3.29 (0.21)	3.36 (0.21)
Ash	7.54 (0.33)	7.44 (0.36)	7.34 (0.41)	7.25 (0.46)
Na	0.33 (0.01)	0.33 (0.02)	0.33 (0.04)	0.33 (0.06)
K	1.46 (0.05)	1.41 (0.04)	1.35 (0.04)	1.29 (0.04)
Starch, % DM	27.2 (1.98)	27.4 (2.02)	27.7 (2.09)	28.0 (2.19)
Particle Size (%DM retained)				
>19.0 mm	2.9 (0.49)	2.6 (0.26)	3.1 (0.72)	2.5 (0.64)
19.0 to 8.00 mm	28.9 (4.08)	29.7 (2.70)	27.2 (5.23)	29.6 (2.62)
8.0 to 1.18 mm	44.4 (2.94)	44.2 (2.90)	42.7 (2.88)	42.0 (1.97)
<1.18 mm	23.8 (2.07)	23.5 (1.35)	27.0 (2.33)	25.9 (1.08)

[1]Treatments: CTRL = 0% high protein coproduct; 2.6L = 2.64% high protein coproduct; 5.4M = 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2]NexPro, Flint Hills Resources, Fairmont, NE.

[3]Soypass, LignoTech, Overland Park, KS.
[4]Formulated to supply approximately 1,133.79 KIU/d vitamin A, 181.41 KIU/d vitamin D and 53.51 IU/d vitamin E in total rations.
[5]Energy Booster Merge, Milk Specialties, Eden Prairie, MN.
[6]Formulated to supply approximately 2,000 mg/kg Co, 20,000 mg/kg Cu, 2,000 mg/kg I, 5 mg/kg Fe, 100,000 mg/kg Mn, 625 mg/kg Se and 15 mg/kg Zn in total rations.
[7]Amylase-treated NDF on organic matter basis.

Table 3.2 Chemical composition of corn silage, alfalfa hay, concentrate mixes of lactating Jersey cattle fed increasing inclusion of new high protein coproduct[1]

	Corn Silage		Alfalfa Hay		CTRL Concentrate[2]		8.0H Concentrate[2]		CoP[3,7,8]	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, as is	38.8	2.69	91.2	0.59	89.8	1.20	91.4	0.30	94.3	0.49
CP	8.23	0.59	17.3	1.27	23.2	0.56	23.0	0.90	52.4	0.35
ADF	20.9	0.56	38.2	2.64	13.4	1.31	15.7	1.00	21.6	7.57
NDF	34.0	1.56	46.6	2.23	23.4	3.00	25.6	1.53	36.2	1.63
$aNDF_{OM}$[4,5]	33.2	1.27	44.2	2.78	22.5	2.88	24.7	1.62	-[6]	-[6]
ADICP[5]	0.66	0.10	1.53	0.12	0.63	0.09	1.45	0.25	3.42	2.40
NDICP[5]	0.83	0.18	2.43	0.38	3.83	0.73	3.06	0.26	10.6	0.59
Lignin	2.63	0.31	8.61	0.54	1.29	0.33	1.80	0.27	3.63	0.48
Sugar	1.25	0.39	5.88	0.40	6.23	1.03	4.03	0.62	2.15	0.07
Starch	40.7	3.97	2.63	0.77	24.8	1.82	26.8	2.67	1.90	0.28
Total fatty acids	2.65	0.26	0.99	0.05	9.03	0.70	9.59	1.05	6.44	0.10
Ash	4.33	0.71	10.1	0.74	9.52	0.69	8.81	0.80	4.78	2.39
Ca	0.19	0.03	1.03	0.10	1.77	0.17	1.74	0.10	0.02	0.00
P	0.19	0.03	0.30	0.01	0.63	0.02	0.58	0.04	0.60	0.01
Mg	0.17	0.02	0.22	0.01	0.73	0.04	0.71	0.04	0.15	0.00
K	0.87	0.09	3.09	0.05	1.33	0.06	0.93	0.03	0.55	0.03
S	0.14	0.02	0.20	0.01	0.22	0.05	0.28	0.02	0.74	0.02
Na	0.01	0.01	0.06	0.01	0.74	0.02	0.75	0.14	0.02	0.00

[1] Mean and SD (n=4) for corn silage, alfalfa hay, and concentrate based on samples of feedstuff collected during each period and analyzed by commercial feed laboratory (Cumberland Valley Analytical Services, Waynesboro, PA.).

[2] CTRL concentrate= concentrate with 0% high protein coproduct; 8.0H= concentrate with 8% high protein coproduct.

[3]Novel high protein corn milling coproduct (n=2).

[4] Van Soest et al. (1991) modified using a 1.5 µm filter.

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

[6]Value not determined.
[7]Amino acid content of CoP all values reported as % CP ± SD; 3.39 ± 0.342 % Lys, 2.17 ± 0.055 % Met, 5.24 ± 0.762 %Arg, 3.59 ± 0.024 % Thr, 11.7 ± 1.05 % Leu, 4.12 ± 0.323 % Ile, 5.18 ± 0.019 % Val, 2.32 ± 0.002 % His, 4.92 ± 0.074 % Phe, 0.68 ± 0.387 % Trp.
[8]Fatty acid content CoP all values reported as % Total fatty acids ± SD; 17.39 ± 0.048 % C16:0, 0.16 ± 0.002 % C16:1, 2.41 ± 0.073 % C18:0, 22.44 ± 0.204 % C18:1, 53.27 ± 0.599 % C18:2, 2.17 ± 0.033% C18:3, 2.40 ± 0.292% Other.

Table 3.3 Oxygen consumption, carbon dioxide and methane production, respiratory quotient, and energy utilization of lactating Jersey cattle fed increasing inclusion of new high protein coproduct

	Treatments[1,2]				SEM	P-value[3]		
Item	CTRL	2.6L	5.4M	8.0H		L	Q	C
Gases, L/d								
O_2 consumption	4,892	4,674	4,779	4,770	245	0.50	0.25	0.28
CO_2 production	4,995	4,861	4,984	4,869	292	0.60	0.93	0.33
CH_4 production	436	403	413	402	36	0.18	0.46	0.38
RQ	1.019	1.040	1.040	1.022	0.014	0.82	0.05	0.96
Components, Mcal/d								
Feces	27.5	27.9	29.7	29.4	0.883	0.02	0.56	0.27
Urine	2.07	1.95	2.13	2.19	0.117	0.11	0.28	0.23
Methane	4.13	3.81	3.90	3.80	0.336	0.18	0.47	0.38
Heat	24.5	23.5	24.1	23.9	1.26	0.51	0.40	0.30
Milk	23.5	24.6	25.7	25.8	0.759	< 0.01	0.42	0.68
Tissue	-0.20	1.84	2.73	0.66	1.64	0.50	0.10	0.74
Fraction, Mcal/d								
GE	81.8	83.5	88.8	85.7	3.04	0.05	0.20	0.18
DE	54.1	55.9	58.8	56.4	2.36	0.13	0.18	0.34
ME	47.9	50.1	52.7	50.5	2.23	0.13	0.15	0.47
NE_L[4]	31.3	33.8	36.0	34.2	1.81	0.07	0.11	0.54
Fraction, Mcal/kg of DM								
GE	4.25	4.26	4.28	4.31	0.020	< 0.01	0.65	0.95
DE	2.81	2.84	2.83	2.83	0.035	0.56	0.53	0.69
ME	2.48	2.54	2.54	2.53	0.039	0.39	0.25	0.77
NE_L	1.61	1.72	1.74	1.71	0.056	0.09	0.12	0.90
Efficiencies								
ME/DE	0.884	0.896	0.898	0.893	0.0076	0.21	0.09	0.91
NE_L/ME	0.648	0.674	0.685	0.675	0.0174	0.03	0.07	0.90
CH_4/DMI, L/kg	22.9	20.9	19.8	20.4	1.73	0.06	0.20	0.85

[1]Treatments: CTRL = 0% high protein coproduct; 2.6L = 2.64% high protein coproduct; 5.4M = 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2] Least squares means; largest SEM is listed.
[3] L = Linear, Q = Quadradic, C = Cubic.
[4]$NE_L = 0.08 \times BW^{0.75}$ + Milk E (Mcal/d) + TE (Mcal NE_L)

Table 3.4 Apparent total-tract digestibility of nutrients of lactating Jersey cattle fed increasing inclusion of a new high protein coproduct

	Treatments[1,2]				SEM	P-value[3]		
Item	CTRL	2.6L	5.4M	8.0H		L	Q	C
DM	66.4	67.1	67.0	65.8	0.63	0.45	0.12	0.89
OM	68.4	69.0	68.8	67.6	0.65	0.31	0.15	0.95
NDF	47.8	50.0	49.0	49.2	2.13	0.45	0.27	0.30
CP	66.2	66.3	66.8	65.2	0.77	0.47	0.22	0.46
Starch	95.8	95.4	95.9	94.3	0.95	0.21	0.41	0.36
Total fatty acids	72.6	73.1	74.7	74.1	2.68	0.36	0.71	0.60
16C Fatty acids	72.3	72.5	73.6	72.3	4.07	0.89	0.65	0.63
18C Fatty acids	73.8	74.2	76.5	76.1	2.10	0.15	0.76	0.49
Energy	66.2	66.9	66.4	65.6	0.72	0.41	0.20	0.80

[1]Treatments: CTRL = 0% high protein coproduct; 2.6L = 2.64% high protein coproduct; 5.4M = 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2] Least squares means; largest SEM is listed.
[3] L = Linear, Q = Quadradic, C = Cubic.

Table 3.5 Fecal output, urine output and nitrogen (N) utilization of lactating Jersey cows fed increasing inclusion of a new high protein coproduct

	Treatments[1,2]				SEM	P-value[3]		
Item	CTRL	2.6L	5.4M	8.0H		L	Q	C
Output, kg/d (as is)								
Feces	40.9	41.6	43.8	42.9	1.46	0.09	0.45	0.35
Urine	22.4	23.0	22.0	21.8	1.96	0.44	0.60	0.51
Mass, g/d								
N intake	496.5	510.8	532.8	511.4	17.36	0.17	0.11	0.31
Fecal N	167.5	170.9	176.6	177.2	6.28	0.07	0.73	0.71
Urinary N	125.8	114.8	130.5	138.5	13.60	0.11	0.21	0.31
Milk N	160.4	165.6	172.9	160.4	6.83	0.78	0.14	0.41
N balance	42.8	59.7	52.8	35.5	17.62	0.55	0.13	0.79
As a proportion of total N intake, %								
Fecal N	33.8	33.7	33.2	34.8	0.767	0.47	0.22	0.46
Urinary N	25.8	22.9	24.7	27.1	2.84	0.32	0.05	0.49
Milk N	32.5	32.5	32.5	31.6	1.24	0.62	0.69	0.86
N balance	7.9	11.0	9.6	6.5	3.29	0.54	0.14	0.78
Efficiencies								
Total N[3]	40.4	43.5	42.1	38.1	3.01	0.22	0.02	0.78
Milk N[4]	32.5	32.5	32.5	31.6	1.24	0.62	0.69	0.86

[1]Treatments: CTRL = 0% high protein coproduct; 2.6 = 2.64% high protein coproduct; 5.4M = 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2]Least squares means; largest SEM is listed.
[3]Total N efficiency = (Milk N + N bal)/(Milk N + N bal + Urine N + fecal N).
[4]Milk N efficiency = (Milk N)/(Milk N + N bal + Urine N + fecal N).

Table 3.6 DMI, milk production and milk composition, water intake, BW and BCS of lactating Jersey cattle fed increasing inclusion of new high protein coproduct

	Treatments[1,2]				SEM	P-value[3]		
Item	CTRL	2.6L	5.4M	8.0H		L	Q	C
DMI, kg/d	19.2	19.9	20.7	19.9	0.62	0.11	0.07	0.38
Milk yield, kg/d	27.8	28.6	29.8	29.0	1.00	0.08	0.20	0.36
ECM, kg/d[4]	34.3	35.7	37.3	37.4	1.08	<0.01	0.40	0.64
ECM/DMI	1.80	1.81	1.81	1.89	0.042	0.05	0.28	0.44
Protein, %	3.35	3.43	3.40	3.40	0.098	0.22	0.14	0.16
Protein, kg/d	0.93	0.98	1.01	0.99	0.033	0.06	0.12	0.63
Fat, %	5.05	5.18	5.15	5.47	0.288	<0.01	0.35	0.26
Fat, kg/d	1.40	1.46	1.53	1.58	0.065	<0.01	0.87	0.81
Lactose, %	4.86	4.89	4.90	4.93	0.037	0.02	0.95	0.61
Lactose, kg/d	1.35	1.40	1.46	1.43	0.051	0.05	0.22	0.49
MUN, mg/dL	12.9	13.0	12.8	13.5	0.60	0.26	0.44	0.53
Free water intake, L/d	79.0	90.6	84.7	80.9	4.52	0.98	0.04	0.24
BW, kg	436	440	440	439	13	0.39	0.42	0.90
BCS[5]	3.05	3.04	3.16	3.04	0.074	0.51	0.14	0.05

[1]Treatments: CTRL = 0% high protein coproduct; 2.6L = 2.64% high protein coproduct; 5.4M= 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2] Least squares means; largest SEM is listed.
[3] L = Linear, Q = Quadradic, C = Cubic.
[4]ECM= 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × true protein (kg) (Tyrrell and Reid, 1965).
[5]Scored 1-5 by 2 independent observations.

Table 3.7 Milk fatty acid composition of lactating dairy cows fed increasing inclusion of novel high protein coproduct

Item, g/100 g of fat	Treatments[1,2]				SEM	P-value[3]		
	CTRL	2.6L	5.4M	8.0H		L	Q	C
C4:0	4.34	4.01	4.13	4.20	0.103	0.37	0.02	0.18
C6:0	1.99	1.91	1.94	1.98	0.048	0.90	0.09	0.44
C8:0	1.12	1.08	1.10	1.13	0.030	0.61	0.11	0.57
C10:0	2.49	2.46	2.50	2.58	0.080	0.16	0.26	0.87
cis-9 C10:1	0.25	0.25	0.25	0.25	0.010	0.51	0.51	0.84
C11:0	0.05	0.06	0.06	0.06	0.006	0.47	0.09	0.13
C12:0	2.94	2.94	2.96	3.04	0.103	0.22	0.56	0.91
iso C13:0	0.02	0.02	0.02	0.02	0.001	0.74	0.38	0.41
anteiso C13:0	0.06	0.07	0.07	0.07	0.003	0.42	0.05	0.68
C13:0	0.08	0.10	0.10	0.09	0.007	0.35	0.04	0.17
iC14:0	0.06	0.06	0.06	0.06	0.005	0.19	0.33	0.25
C14:0	9.89	9.78	9.85	9.83	0.208	0.76	0.60	0.49
C14:1c9	0.78	0.82	0.80	0.77	0.035	0.51	0.04	0.35
iso C15:0	0.17	0.16	0.17	0.17	0.007	0.81	0.09	0.13
anteiso C15:0	0.34	0.34	0.34	0.34	0.014	0.84	0.89	0.74
C15:0	0.87	1.00	0.93	0.91	0.050	0.68	0.03	0.13
iso C16:0	0.20	0.19	0.20	0.18	0.011	0.33	0.62	0.60
C16:0	38.5	38.9	38.3	37.7	0.867	0.05	0.20	0.53
cis-9 C16:1	1.78	1.85	1.78	1.73	0.080	0.24	0.15	0.42
iso C17:0	0.30	0.29	0.30	0.30	0.016	0.22	0.42	0.93
C17:0	0.56	0.57	0.55	0.56	0.025	0.80	0.83	0.48
cis-9 C17:1	0.18	0.19	0.18	0.18	0.014	0.54	0.45	0.47
C18:0	9.51	8.62	9.10	9.53	0.350	0.58	<0.01	0.18
trans-4 C18:1	0.02	0.02	0.02	0.02	0.001	0.27	0.21	0.53
trans-5 C18:1	0.01	0.01	0.01	0.02	0.002	0.48	0.84	0.34
trans-6 C18:1	0.25	0.26	0.26	0.26	0.011	0.11	0.76	0.66
trans-9 C18:1	0.19	0.20	0.19	0.19	0.007	0.85	0.19	0.60
trans 10 C18:1	0.35	0.39	0.38	0.37	0.037	0.52	0.13	0.47
trans-11 *C18:1*	0.53	0.54	0.53	0.53	0.037	0.94	0.61	0.68
trans-12 *C18:1*	0.34	0.37	0.37	0.36	0.013	0.11	<0.01	0.59
cis-9 C18:1	15.9	16.2	16.3	16.2	0.696	0.42	0.46	0.95
cis-11 C18:1	0.45	0.51	0.47	0.47	0.049	0.71	0.06	0.12
cis-12 C18:1	0.22	0.25	0.26	0.24	0.030	0.20	0.06	0.94
LA	1.98	2.10	2.21	2.35	0.099	<0.01	0.88	0.88
ALA	0.23	0.24	0.24	0.25	0.009	0.05	0.69	0.61
C20:0	0.12	0.11	0.12	0.12	0.005	0.53	0.06	0.55

cis-11 C20:1	0.03	0.04	0.04	0.03	0.004	0.46	0.14	0.84
C20:2n6	0.02	0.02	0.02	0.02	0.002	0.05	0.98	0.99
cis-9*, trans*-11 CLA	0.25	0.28	0.27	0.26	0.022	0.59	0.08	0.58
Total saturated fatty acids	73.7	72.7	72.8	72.9	1.03	0.18	0.19	0.59
Total unsaturated fatty acids	24.3	25.1	25.2	25.1	0.97	0.17	0.24	0.68
Milk fatty acids g/100 g fat								
<16 Carbon	25.5	25.0	25.3	25.5	0.43	0.79	0.20	0.54
16 Carbon	40.5	40.9	40.2	39.6	0.79	0.04	0.15	0.48
>16 Carbon	32.0	31.9	32.4	32.9	1.04	0.08	0.48	0.69
Milk fatty acids g/d								
<16 Carbon	356.9	365.4	387.4	403.3	21.48	<0.01	0.75	0.72
16 Carbon	564.1	597.5	614.9	626.2	32.88	<0.01	0.43	0.86
>16 Carbon	445.9	460.3	495.1	514.7	17.47	<0.01	0.84	0.55
Unknown	28.5	31.2	31.7	32.6		<0.01	0.20	0.45

[1]Treatments: CTRL = 0% high protein coproduct; 2.6L = 2.64% high protein coproduct; 5.4M = 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2] Least squares means; largest SEM is listed.
[3] L = Linear, Q = Quadradic, C = Cubic.

GENERAL SUMMARY AND CONCLUSIONS

The corn-ethanol industry an evolving field and consequently new products are created, and these diversify the industry and create new revenue streams. These new products must be evaluated for chemical composition so that they can be accurately described in feed libraries that are used in commercial ration formulation software. As a result, the objective of the first experiment was to examine the chemical composition and nutrient availability in 10 samples of a single new high protein coproduct (**HPCoP**)(Flint Hills Resources, NexPro). This product originated from the dry milling process which is unlike the traditional process as nitrogenous based particles are concentrated from the residual fiber by sieving post fermentation.

Chemical composition and in vitro nutrient availability. Results from this experiment indicate that the new HPCoP contained a greater concentration of protein and lysine on a dry matter basis when compared with a traditional DDGS. These results can be attributed to the partial removal of fiber in the production process and subsequent concentration of corn protein and an unknown but likely substantial proportion of yeast cells. Neutral detergent fiber digestibility of the HPCoP at 30 hours was also increased relative to DDGS likely due to reduced lignin content and smaller particle size of the HPCoP. Interestingly, amylase treated neutral detergent fiber (**aNDF**) content of the new HPCoP was different when analyzed by three commercial fiber systems. These systems included the traditional refluxing method (**aNDF$_{R}$**; Van Soest et al. 1991), a bagged sample method (**aNDF$_{B}$**; Ankom, 2017) and a method which refluxes then flushes material through ashed sea sand (**aNDF$_{CR}$**; Mertens, 2002). High protein coproducts pose an interesting challenge when it comes to NDF analysis, and all methods may not

accurately reflect the true NDF content of distillers grains. Based on 3 different processing methods we observed a range from 22.5 ± 5.28 % to 47.1 ± 4.32 % aNDF for $aNDF_R$, $aNDF_B$, and $aNDF_{CR,}$ respectively. While bag systems are convenient for analysis, they pose a risk of overestimating NDF content of coproducts as samples are not fully immersed in the NDF solution. Methods for determining aNDF content of HPCoP should allow the sample to reflux within solution and filter through material of 25 μm or less.

Whole animal nitrogen and energy utilization. While chemical composition provided us an initial and useful description of a feed product, in vivo nitrogen and energy balance studies are needed to examine the utilization and efficiency of converting the nutrients within a given feed product to milk. Therefore, in order to accomplish the goal of understanding the effects of HPCoP on DMI, energy and nitrogen utilization, and milk production we tested the replacement of non-enzymatically browned soybean meal (52 % CP) with HPCoP (52% CP) up to 8 % dietary DM. All rations were formulated to be isonitrogenous averaging 16.1 % CP. We observed that inclusion of HPCoP did not affect apparent nutrient digestibility. However, inclusion of the new HPCoP did increase gross energy content and total intake with no subsequent effects on digestible energy or metabolizable energy. Similarly, the utilization of energy for NE_L increased with increasing inclusion of the HPCoP with subsequent increases in milk fat production. These observations are likely an effect of the increases in TFA content.

Overall technical observations and recommendations. In the current energy balance experiment 1 animal was unable to adapt to the headbox system, and visual observations note some animals do not show full comfort in the headbox until the second

collection period. The importance of training animals to be comfortable around instrumentation used for total gas production measures during sample collection has been previously noted. Protocols should be developed so that future investigators are able to estimate normal gas production and consumption accurately. Previous recommendations suggest that animals should be exposed to 3 days of training in the headboxes. In the future, animals should demonstrate they can be comfortable within the headboxes for a period of 24 hours prior to enrollment in an experiment. Animal comfort is defined as the ability for the animal to drink from the waterer, lay down and stand comfortably, and consume at least 80 % of allotted TMR during a 24-hour period. After energy balance periods are completed, urine samples become difficult to homogenize after freezing. Currently, when analyzing urine samples for energy one will shake the conical tube however the question is whether this practice gives a representative sample due to floating particulate matter. This directly impacts the ability to obtain a representative sample for gross energy analysis. Currently freeze drying is not possible without extensive waiting time in our laboratory. A potentially more effective way to homogenize sample would be to vortex after thawing just prior to sampling for gross energy analysis.

APPENDIX C: EQUATIONS

DE (Mcal/d) = GE (Mcal/d) – fecal energy (Mcal/d) [1]

ME (Mcal/d) = DE (Mcal/d) – urine energy (Mcal/d) – methane energy (Mcal/d) [2]

Unaccounted for energy was assumed to represent tissue energy retention or mobilization which was corrected to an NE_L basis as follows:

Tissue energy (Mcal/d) = ME (Mcal/d) – heat production (Mcal/d) – milk energy (Mcal/d) [3]

Adjusted tissue energy (**TE**; Mcal of NE_L/d) = positive residual energy × k_L/k_G or negative residual energy × k_T [4]

Where k_T is the efficiency of utilizing body reserve energy for milk production, k_G is the efficiency of utilizing ME intake for tissue gain (Moe et al., 1970). Values of 0.66 and 0.74, and 0.89 were used for k_L, k_G, and k_T respectively (Moraes et al., 2015).

Net energy of lactation (NE_L; Mcal/d) = $0.08 \times BW^{0.75}$ + Milk E (Mcal/d) + Adjusted TE (NE_L Mcal/d) [5]

APPENDIX D: COMPARISON OF METHODS TO DETERMINE POSITIVE TE FOR INTEGRATION INTO ESTIMATES OF NEL Mcal/d

Description of this note:

Net Energy Lactation (NE_L) is calculated as follows

NE_L (Mcal/d) = 0.08 × $BW^{0.75}$ + Milk E (Mcal/d) + adjusted TE (Mcal NE_L) [1]

Where,

Maintenance energy = 0.08 × $BW^{0.75}$ [2]

Milk energy is (Mcal/d) = [(9.29 × Fat %/100) + (5.63 × Protein %/100) + (3.95 × Lactose %/100)] × Milk production (kg/d) [3]

Where adjusted TE (Mcal NE_L) is as follows,

When TE (Mcal/d) > 0, adjusted TE = TEp × (K_L/K_G) [4]

When TE (Mcal/d) < 0, adjusted TE = TEn × (K_T) [5]

K_L is equal to 0.66 based upon the average values from the 1974-1983 and 1984-1995 data sets (Moraes et al., 2015). This value is in accordance with those published in the NASEM, (2021).

K_G is equal to 0.74 based upon the average values from the 1974-1983 and 1984-1995 data sets (Moraes et al., 2015)This value is in accordance with those published in the NASEM, (2021).

K_T is equal to 0.89 and represents the conversion of tissue energy to milk energy. This value is equivalent to 0.89 as outlined by (Moraes et al., 2015) and in accordance with the values published in the NASEM (2021).

TEp is the positive tissue energy (Mcal/d) remaining after subtracting heat production (Mcal/d)(Brouwer, 1965) and milk energy (Mcal/d) from metabolizable energy (Mcal/d).

TEn is the negative tissue energy (Mcal/d) remaining after subtracting heat production (Mcal/d)(Brouwer, 1965) and milk energy (Mcal/d) from metabolizable energy (Mcal/d).

Focus will be on the **positive tissue energy conversion**. Comparing a personal correspondence from a contributor to early NE_L calculations and a later examination of the correspondence and resulting data.

Notation of pervious errors made in the calculation of TE when TE was > 0

Personal correspondence from colleague to our early work in NE_L calculations suggested positive TE conversion was a function of the efficiency of ME to gain (K_G) divided by the conversion of tissue energy to milk energy (K_T).

1. Where K_G was 0.74 for all lactating animals (Reynolds, personal correspondence) and K_T was 0.84 in negative energy balance animals (Moe et al., 1971).
2. The above yields a conversion efficiency of 0.74 (K_G) / 0.84 (K_T) = 1/1.135 or rounded to 1/1.14 shown in the work of Reynolds (2000)

   As adjusted TE= TE / 1.14 if TE > 0 [6]
3. Using the above, erroneous calculations were published by (Morris and Kononoff, 2021; Morris et al., 2021a; b) and (McLain et al., 2021).

   TE (NE_L Mcal/d) = TEp × (K_G/K_T) [7]

   Where K_G is updated to 0.75 and K_T is updated to 0.89, respectively (Moraes et al., 2015)
4. When using data generated from in this thesis (Carroll, 2021) and the equation [7] to estimate TE above, NE_L (Mcal/d and Mcal/kg), and NE_L/ME are reported in the table below.

	Treatments[1,2]				SEM	P-value[3]		
Item	CTRL	2.6L	5.4M	8.0H		L	Q	C
NE_L[4], Mcal/d	31.20	33.63	35.89	34.05	1.773	0.07	0.11	0.51
NE_L, Mcal/kg DM	1.608	1.708	1.732	1.704	0.0543	0.09	0.12	0.92
NE_L/ME	0.6476	0.6712	0.6824	0.6735	0.01715	0.03	0.08	0.86

[1]Treatments: CTRL = 0% high protein coproduct; 2.6L = 2.64% high protein coproduct; 5.4M = 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2] Least squares means; largest SEM is listed.
[3] L = Linear, Q = Quadradic, C = Cubic.
[4]NE_L= 0.08 × $BW^{0.75}$+ Milk E (Mcal/d) + adjusted TE (Mcal NE_L).

This calculation is likely incorrect as it considers the conversion of tissue energy to milk in negative energy balance. During positive energy balance animals will not need to convert tissue energy to milk as milk energy requirements will be accounted for by dietary ME. Any residual ME left after milk energy is subtracted will be utilized for tissue gain. Secondly, the value 0.74 was for all lactating animals not solely animals in positive tissue.

Corrected approach:

Based on Moe et al. (1971)(see table below)

5. K_G of all lactating animals has been observed to be 0.747 and K_T has been observed to be 0.84 in animals in negative energy balance animals (Moe et al., 1971). Using these (correct) estimates of conversion efficiency (0.747 (K_G) / 0.84 (K_T) = 1/1.12 and this is different that the value as listed in the works of Reynolds (2000) which was TE/1.14
6. Data for the conversion of ME to milk energy (K_L) and the efficiency of ME to gain (K_G) was available for animals in **positive energy balance**.

TABLE 3. Estimates of partial efficiencies and maintenance requirement.

	$Kg^{3/4}$	Milk from ME	Gain from ME	Milk from tissue
	(kcal)	(%)		
Lactating, negative tissue	128	66.1		84.0
Lactating, positive tissue	118	63.5	72.6	
All lactating	122	64.4	74.7	82.4

7. These two values can be used to derive a conversion efficiency of 0.635 (K_L) / 0.726 (K_G) = 0.875 or 1/1.14

   This supports the values outlined by and uses partial efficiencies of milk from ME (0.635) and gain from ME (0.726) derived from animals in positive tissue and does not use the conversion of TE to milk (K_T or 0.84) during negative energy balance .

8. Therefore, using data generated from this thesis (Carroll, 2021) the correct estimate or TE when TE > 0 is as follows:

   $$TE\ (NE_L\ Mcal/d) = TEp \times (K_L/K_G) \quad [4]$$

   Where K_L is updated from 0.635 to 0.66 and K_G is updated from 0.726 to 0.74 as these updated estimates are observed in (Moraes et al., 2015) and this is based upon the average K_L and K_G values from the 1974-1983 and 1984-1995 data sets. These values are in accordance with those published in the NASEM, (2021).

9. The resulting NE_L (Mcal/d and Mcal/kg) and NE_L/ME for equation [4] are listed in the below table.

	Treatments[1,2]				SEM	P-value[3]		
Item	CTRL	2.6L	5.4M	8.0H		L	Q	C
NE_L[4], Mcal/d	31.27	33.79	36.04	34.16	1.810	0.07	0.11	0.54
NE_L, Mcal/kg DM	1.611	1.716	1.741	1.709	0.0561	0.09	0.12	0.90
NE_L/ME	0.6480	0.6738	0.6850	0.6754	0.01744	0.03	0.07	0.90

[1]Treatments: CTRL = 0% high protein coproduct; 2.6L = 2.64% high protein coproduct; 5.4M = 5.36% high protein coproduct; 8.0H = 8% high protein coproduct.
[2] Least squares means; largest SEM is listed.
[3] L = Linear, Q = Quadradic, C = Cubic.
[4]$NE_L = 0.08 \times BW^{0.75}$ + Milk E (Mcal/d) + adjusted TE (Mcal NE_L).

References

Brouwer, E. 1965. Report of Sub-Committee on Constants and Factors. In: Blaxter, K.L., Ed. Proceedings of the 3rd Symposium on Energy Metabolism. Pages 441–443. Academic Press, London.

McLain, K.A., D.L. Morris, and P.J. Kononoff. 2021. Effect of feeding hydrolyzed feather meal and rumen-protected lysine on milk protein and energy utilization in late-lactation Jersey cows. Journal of Dairy Science 104:8708–8720. doi:10.3168/jds.2020-19657.

Moe, P.W., H.F. Tyrrell, and W.P. Flatt. 1971. Energetics of Body Tissue Mobilization. Journal of Dairy Science 54:548–553. doi:10.3168/jds.S0022-0302(71)85886-1.

Moraes, L.E., E. Kebreab, A.B. Strathe, J. Dijkstra, J. France, D.P. Casper, and J.G. Fadel. 2015. Multivariate and univariate analysis of energy balance data from lactating dairy cows. Journal of Dairy Science 98:4012–4029. doi:10.3168/jds.2014-8995.

Morris, D.L., T.M. Brown-Brandl, K.E. Hales, K.J. Harvatine, and P.J. Kononoff. 2021a. Corrigendum to "Effects of high-starch or high-fat diets formulated to be isoenergetic on energy and nitrogen partitioning and utilization in lactating Jersey cows" (J. Dairy Sci. 103:4378–4389). Journal of Dairy Science 104:11334. doi:10.3168/jds.2021-104-10-11334.

Morris, D.L., J.V. Judy, and P.J. Kononoff. 2021b. Corrigendum to "Use of indirect calorimetry to evaluate utilization of energy in lactating Jersey dairy cattle consuming diets with increasing inclusion of hydrolyzed feather meal" (J. Dairy Sci. 103:4206–4217). Journal of Dairy Science 104:11333. doi:10.3168/jds.2021-104-10-11333.

Morris, D.L., and P.J. Kononoff. 2021. Corrigendum to "Effects of rumen-protected lysine and histidine on milk production and energy and nitrogen utilization in diets containing hydrolyzed feather meal fed to lactating Jersey cows" (J. Dairy Sci. 103:7110–7123). Journal of Dairy Science 104:11335. doi:10.3168/jds.2021-104-10-11335.

NASEM. 2021. Nutrient Requirements of Dairy Cattle. National Academies Press, Washington, D.C.

Reynolds, C.K. 2000. Measurement of Energy Metabolism. M.K Throdorou and J. France.

APPENDIX F: NDS HIGH PROTEIN COPRODUCT INPUT FROM ENERGY AND NITROGEN BALANCE EXPERIMENT

acarroll11

Full analysis 10/14/2021

CIRT25543290 - [FLINT HILLS RESOURCES]. NexPro - Corn distillers grains dehydrated 29-30% CP (DDG USA) as fed 1/13/2021

Nutrient	Unit	AF	DM
Moisture	%	7.9000	
D.M.	%	92.100	100.0000
CF	%	5.2681	5.7200
aNDFom	%	28.735	31.2000
ADF	%	17.683	19.2000
ADL	%	1.8052	1.9600
Forage aNDFom	%		
peNDF	%	5.7470	6.2400
NSC	%	2.5051	2.7200
NFC	%	5.4523	5.9200
Total CHO	%	34.187	37.1200
Acetic	%		
Propionic	%		
Butyric	%		
Lactic	%		
Organic Acids	%		
Sugar (WSC)	%	1.1513	1.2500
Starch	%	1.3539	1.4700
Soluble Fiber	%	2.9472	3.2000
CHO B3 pdNDF Lig*2.4	%	24.402	26.4960
CHO C uNDF Lig*2.4	%	4.3324	4.7040
CHO C uNDF	%	7.8653	8.5400
CHO B3 pdNDF	%	20.869	22.6600
CHO B3 fast pool	%		
CHO B3 slow pool	%		
RD CHO 3x Level 1	%	15.971	17.3417
RD Starch 3xLevel 1	%	0.9757	1.0593
Forage	%		
Concentrate	%		100.0000
CP	%	49.365	53.6000
Soluble Protein	%	4.1629	4.5200
Ammonia (Prot A1)	%		
NDIP	%	4.6050	5.0000
ADIP	%	3.4353	3.7300
Prot. A2	%	4.1629	4.5200
Prot. B1	%	40.597	44.0800
Prot. B2	%	1.1697	1.2700
RDP 3 xLevel 1	%	8.9617	9.7304
RUP 1 xLevel 1	%	36.354	39.4731
RUP 3 xLevel 1	%	40.403	43.8696
PDI	%	24.666	26.7821
PDIA	%	19.783	21.4807
GE	Mcal/kg	4.5910	4.9848
DE	Mcal/kg	4.0952	4.4465
ME	Mcal/kg	3.1125	3.3795

Nutrient	Unit	AF	DM
NEl 3x NRC	Mcal/kg	2.0269	2.2008
NEm NRC	Mcal/kg	2.1459	2.3299
NEg NRC	Mcal/kg	1.4966	1.6249
UFL	unit/kg	1.2346	1.3405
UFV	unit/kg	1.2482	1.3552
TDN 1x	%	74.257	80.6272
EE	%	5.3510	5.8100
EE 1	%		
EE 2	%	5.3510	5.8100
EE 3	%		
TFA	%	4.6050	5.0000
Glycerol	%	0.5066	0.5500
Pigment	%	0.2395	0.2600
C12:0	%	0.0054	0.0059
C14:0	%	0.0092	0.0100
C16:0	%	1.1420	1.2400
C16:1	%	0.0092	0.0100
C18:0	%	0.1566	0.1700
C18:1T	%	0.0006	0.0006
C18:1C	%	1.5012	1.6300
C18:2	%	3.5643	3.8700
C18:3 - ALA	%	0.1382	0.1500
C20:5 - EPA	%		
C22:5 - DPA	%		
C22:6 - DHA	%		
Other LCFA	%		
ID rfC12:0			95.3900
ID rfC14:0			75.0600
ID rfC16:0			72.4800
ID rfC16:1			72.0000
ID rfC18:0			72.8000
ID rfC18:1T			80.0000
ID rfC18:1C			80.0000
ID rfC18:2			83.0000
ID rfC18:3			77.5500
ID rfC20:5 n-3			77.5500
ID rfC22:5 n-3			77.5500
ID rfC22:6 n-3			77.5500
ID rf Other LCFA			58.7100
Ash	%	3.1959	3.4700
Ca	%	0.0276	0.0300
P	%	0.6631	0.7200
Mg	%	0.2026	0.2200
K	%	0.4789	0.5200
Na	%	0.1105	0.1200

Nutrient	Unit	AF	DM
Cl	%	0.0737	0.0800
S	%	0.6539	0.7100
NaCl	%		
S - Sulfates	%	0.6539	0.7100
Ca Bio.	g/g		0.6000
P Bio.	g/g		0.7000
Mg Bio.	g/g		0.1600
K Bio.	g/g		0.9000
Na Bio.	g/g		0.9000
Cl Bio.	g/g		0.9000
S Bio.	g/g		1.0000
Mn - total	ppm	15.380	16.7000
Cu - total	ppm	3.4998	3.8000
Fe - total	ppm	110.52	120.0000
Zn - total	ppm	106.83	116.0000
I - total	ppm		
Co - total	ppm	0.1566	0.1700
Se - total	ppm	0.5710	0.6200
Mo - total	ppm	0.9210	1.0000
Cr - total	ppm		
F - total	ppm		
Mn - added	ppm		
Cu - added	ppm		
Fe - added	ppm		
Zn - added	ppm		
I - added	ppm		
Co - added	ppm		
Se - added	ppm		
Mo - added	ppm		
Cr - added	ppm		
Mn Bio.	mg/mg		0.0100
Cu Bio.	mg/mg		0.0400
Fe Bio.	mg/mg		0.1000
Zn Bio.	mg/mg		0.1500
I Bio.	mg/mg		0.8500
Co Bio.	mg/mg		1.0000
Se Bio.	mg/mg		1.0000
Mo Bio.	mg/mg		1.0000
Cr Bio.	mg/mg		1.0000
Org. Mn	ppm		
Org. Cu	ppm		
Org. Zn	ppm		
Org. Co	ppm		
Org. Se	ppm		
Org. Cr	ppm		

Nutrient	Unit	AF	DM
MET	%	1.2341	1.3400
LYS	%	1.8328	1.9900
ARG	%	2.1091	2.2900
THR	%	2.0815	2.2600
LEU	%	6.0141	6.5300
ILE	%	1.6854	1.8300
VAL	%	3.2327	3.5100
HIS	%	1.2802	1.3900
PHE	%	2.5880	2.8100
TRP	%	0.5710	0.6200
MET	%CP		2.5000
LYS	%CP		3.7127
ARG	%CP		4.2724
THR	%CP		4.2164
LEU	%CP		12.1828
ILE	%CP		3.4142
VAL	%CP		6.5485
HIS	%CP		2.5933
PHE	%CP		5.2425
TRP	%CP		1.1567
Vit. A	IU/kg		
Vit. D3	IU/kg		
Vit. E	IU/kg		
Niacin	mg/kg		
Vit. B1	mg/kg		
Vit. B2	mg/kg		
Vit. B6	mg/kg		
Pantothenic Acid	mg/kg		
Vit. B12	mg/kg		
Choline	mg/kg		
Biotin	mg/kg		
Betain	mg/kg		
Vit. C	mg/kg		
Vit. H1	mg/kg		
Fumaric Acid	mg/kg		
Folic Acid	mg/kg		
Vit.A Bio.	UI/UI		1.0000
Vit.D3 Bio.	UI/UI		1.0000
Vit.E Bio	UI/UI		1.0000
Kd CHO A1	%/hr		
Kd CHO A2	%/hr		7.0000
Kd CHO A3	%/hr		5.0000
Kd CHO A4	%/hr		60.0000
Kd CHO B1	%/hr		17.0000
Kd CHO B2	%/hr		30.0000
Kd CHO B3	%/hr		6.0000
Kd CHO B3 fast pool	%/hr		
TRUE PROTEIN	%		

Nutrient	Unit	AF	DM
Kd CHO C	%/hr		
Kd PRO A1	%/hr		200.0000
Kd PRO A2	%/hr		8.0000
Kd PRO B1	%/hr		1.3250
Kd PRO B2	%/hr		6.0000
Kd PRO C	%/hr		
Lipolysis	%/hr		500.0000
Adj Factor			
Int.Dig.CHO A1	%esca		100.0000
Int.Dig.CHO A2	%esca		100.0000
Int.Dig.CHO A3	%esca		100.0000
Int.Dig.CHO A4	%esca		100.0000
Int.Dig.CHO B1	%esca		75.0000
Int.Dig.CHO B2	%esca		75.0000
Int.Dig.CHO B3	%esca		20.0000
Int.Dig.CHO C	%esca		
Int.Dig.PRO A1	%esca		100.0000
Int.Dig.PRO A2	%esca		100.0000
Int.Dig.PRO B1	%esca		100.0000
Int.Dig.PRO B2	%esca		100.0000
Int.Dig.PRO C	%esca		
Int.Dig.FAT	%esca		106.9660
Monensin	mg/kg		
Lasalocid	mg/kg		
Decoquinate	mg/kg		
Chlortetracycline	mg/kg		
Oxytetracycline	mg/kg		
Virginiamycin	mg/kg		
Aureomycin	mg/kg		
Salinomycin	mg/kg		
Tylosin	mg/kg		
Diflubenzuron	mg/kg		
Zinc Bacitracin	mg/kg		
Beta-Agonists	mg/kg		
Melengestrol Acetate	mg/kg		
<Int.Dig. CHO B3 - v6.55>	%esca		5.0000

APPENDIX G: PHOTO OF HIGH PROTEIN COPRODUCT

APPENDIX H: AMTS DIET SUMMARY FOR CTRL AND 8.0H DIET FORMULATION

Diet summary CTRL:

Nutrient Balances		
Nutrient	Balance	%Rqd
ME Mcal	0.8	102
MP (g)	117	105
NH3-N (g)	86	151
Peptide-N (g)	126	180
peNDF lbs	-1.2	89
LYS (g)	1.3	100.8
MET (g)	-5.9	89.6
Ca (g)	59.99	191
P (g)	3.32	106
Mg (g)	33.00	595
K (g)	97.13	148

Total ME Avail. (Mcal/	54.34
ME Milk Prod (lbs/day)	67.4
MP Milk Prod (lbs/day)	70.9
MUN (mg/dl)	11.3
Urea Cost (Mcal)	0.29
Rumen pH	6.29
Milk:Feed	1.43
IOFC ($/hd)	0.00
IOpurFC ($/hd)	0.00

Excretion	
Fecal (lbs)	83
Urine (lbs)	46
Total Manure (lbs)	129
Fecal N (g)	208
Urine N (g)	199
Total Manure N (g)	407
Productive N:Total N	0.31:1
Productive	0.88:1
Manure N:Total N	0.69:1
Fecal P (g)	48.5
Urine P (g)	1.0
Total Manure P (g)	49.5
Productive P:Total P	0.39:1
Manure P:Total P	0.61:1
CH4 (Mcal) / CH4	5.40 / 589.42

Diet Concentrations	
NFC (%DM)	39.0
CHO Ferm. (%DM)	42.0
CHO Ferm. (%CHO)	61.0
NDF Ferm. (%DM)	14.5
NDF Ferm. (%NDF)	47.2
Starch Ferm. (%DM)	19.8
Starch Ferm. (%	74.8
Sol. Fiber Ferm. (%DM)	4.4
Sol. Fiber Ferm. (% Sol.	85.0
Sugar Ferm. (%DM)	3.3
Sugar Ferm. (% Sugar)	70.6
Sugar (A4) (%DM)	4.7
Starch (B1) (%DM)	26.5
Sol Fiber (B2) (%DM)	5.2
Ferm. Fiber (B3) (%DM)	22.6
Lig * 2.4 (C) (%DM)	8.1
aNDFom (%DM)	30.73
Forage NDF (%NDF)	70.25
Forage NDF (%FBW)	0.92
EE (%DM)	5.7
LCFA (%DM)	4.9
CP (%DM)	16.74
RDP (%DM)	10.16
LYS (%MP)	6.70
MET (%MP)	2.10
LYS:MET	3.19
TDN (%DM)	67.0
ME (Mcal/lb)	1.15
NEl (Mcal/lb)	0.74
Forage (%DM)	58.0
DM (%)	57.3
DCAD1 (meq/kg)	306
DCAD2 (meq/kg)	301

Ration Fed				
Ingredient	$/hd	%DM	DM lbs/day	AF lbs/day
UNL Alf Hay 2020	0.00	90.0	8.5	9.4
UNL Corn Silage 2020	0.00	37.0	18.8	50.8
Corn Grain Ground Fine	0.00	88.0	6.705	7.619
Soy Pass	0.00	90.1	3.765	4.176
Soybean Meal 47.5 Sol...	0.00	90.0	1.250	1.389
Soybean Hulls Ground	0.00	91.0	4.050	4.451
Salt White	0.00	99.5	0.1790	0.1799
Sodium Bicarbonate	0.00	99.5	0.2800	0.2814
Vitamin Premix 1	0.00	99.5	0.0190	0.0191
Molasses Beet	0.00	75.0	0.815	1.087
Energy Booster 100	0.00	99.4	1.410	1.419
Trace Mineral Premix	0.00	99.5	0.0240	0.0241
Calcium Carbonate	0.00	99.5	0.5200	0.5226
Calcium Phosphate Di ...	0.00	99.5	0.2440	0.2452
Magnesium Ox	0.00	99.5	0.1900	0.1910
Urea 281 CP	0.00	99.0	0.300	0.303
Totals	0.00	57.3	47.0530	82.1672

Cost/ton As-Fed: $0.00

Diet Summary 8H:

Nutrient Balances		
Nutrient	Balance	%Rqd
ME Mcal	1.6	103
MP (g)	-32	99
NH3-N (g)	107	160
Peptide-N (g)	155	202
peNDF lbs	-1.2	89
LYS (g)	-16.7	89.5
MET (g)	-3.5	93.7
Ca (g)	54.77	183
P (g)	2.24	104
Mg (g)	32.61	590
K (g)	63.94	132

Total ME Avail. (Mcal/	55.06
ME Milk Prod (lbs/day)	68.7
MP Milk Prod (lbs/day)	64.7
MUN (mg/dl)	11.5
Urea Cost (Mcal)	0.31
Rumen pH	6.29
Milk:Feed	1.37
IOFC ($/hd)	0.00
IOpurFC ($/hd)	0.00

Excretion	
Fecal (lbs)	83
Urine (lbs)	46
Total Manure (lbs)	129
Fecal N (g)	210
Urine N (g)	202
Total Manure N (g)	412
Productive N:Total N	0.30:1
Productive	0.87:1
Manure N:Total N	0.70:1
Fecal P (g)	46.9
Urine P (g)	1.0
Total Manure P (g)	47.9
Productive P:Total P	0.39:1
Manure P:Total P	0.61:1
CH4 (Mcal) / CH4	5.55 / 606.18

Diet Concentrations	
NFC (%DM)	38.1
CHO Ferm. (%DM)	42.4
CHO Ferm. (%CHO)	61.7
NDF Ferm. (%DM)	15.7
NDF Ferm. (%NDF)	49.7
Starch Ferm. (%DM)	19.8
Starch Ferm. (%	74.5
Sol. Fiber Ferm. (%DM)	4.2
Sol. Fiber Ferm. (% Sol.	85.4
Sugar Ferm. (%DM)	2.8
Sugar Ferm. (% Sugar)	69.7
Sugar (A4) (%DM)	4.0
Starch (B1) (%DM)	26.5
Sol Fiber (B2) (%DM)	5.0
Ferm. Fiber (B3) (%DM)	23.1
Lig * 2.4 (C) (%DM)	8.4
aNDFom (%DM)	31.50
Forage NDF (%NDF)	68.53
Forage NDF (%FBW)	0.92
EE (%DM)	6.0
LCFA (%DM)	5.1
CP (%DM)	16.87
RDP (%DM)	10.89
LYS (%MP)	6.35
MET (%MP)	2.34
LYS:MET	2.71
TDN (%DM)	67.7
ME (Mcal/lb)	1.17
NEl (Mcal/lb)	0.75
Forage (%DM)	58.0
DM (%)	57.3
DCAD1 (meq/kg)	261
DCAD2 (meq/kg)	255

Ration Fed				
Ingredient	$/hd	%DM	DM lbs/day	AF lbs/day
UNL Alf Hay 2020	0.00	90.0	8.5	9.4
UNL Corn Silage 2020	0.00	37.0	18.8	50.8
Corn Grain Ground Fine	0.00	88.0	6.705	7.619
NexPro_LabAnalysis	0.00	92.1	3.765	4.087
Soybean Meal 47.5 Sol...	0.00	90.0	1.250	1.389
Soybean Hulls Ground	0.00	91.0	4.050	4.451
Salt White	0.00	99.5	0.1790	0.1799
Sodium Bicarbonate	0.00	99.5	0.2800	0.2814
Vitamin Premix 1	0.00	99.5	0.0190	0.0191
Molasses Beet	0.00	75.0	0.815	1.087
Energy Booster 100	0.00	99.4	1.410	1.419
Trace Mineral Premix	0.00	99.5	0.0240	0.0241
Calcium Carbonate	0.00	99.5	0.5200	0.5227
Calcium Phosphate Di ...	0.00	99.5	0.2440	0.2452
Magnesium Ox	0.00	99.5	0.1900	0.1910
Urea 281 CP	0.00	99.0	0.300	0.303
Totals	0.00	57.3	47.0530	82.0782

Cost/ton As-Fed: $0.00

APPENDIX I: NDS DIET SUMMARY FOR EXPERIMENTAL DIETS AND ANIMAL OUTPUTS

Diet Summary CTRL:

acarroll11

First Working group - NexPro - Lactation Barn - CTRL NexPro - Lactating Dairy Cow

Recipe: Flint Hills NexPro UNL CTRL 2021 01 14				
Ingredients	D.M. %	A.F. kg	DM kg	% DM
Corn silage 34.4033 medium	38.800	19.794	7.680	40.00
Alfalfa hay 45.18 Trial	91.200	3.811	3.475	18.10
Corn grain	87.620	3.134	2.746	14.30
Soybean meal solv. 48%	89.750	0.569	0.511	2.66
SoyPass MP	87.100	1.763	1.536	8.00
Soybean Hulls	90.200	1.833	1.653	8.61
Urea	99.000	0.124	0.123	0.64
Sodium Choride	99.850	0.073	0.073	0.38
Sodium Bicarbonate	95.600	0.121	0.115	0.60
Vitamin Premix ADE	99.500	0.008	0.008	0.04
Beet molasses	79.500	0.418	0.332	1.73
Trace Mineral Premix	99.500	0.010	0.010	0.05
Calcium Carbonate	99.200	0.190	0.188	0.98
Calcium Phos. Mono-Dical	95.500	0.103	0.098	0.51
MAGOX 54%	99.500	0.077	0.077	0.40
Energy Booster 100	99.360	0.580	0.576	3.00
Totals		**32.605**	**19.200**	**58.9 %DM**

Summary analysis: Lactating Dairy Cow				
Nutrient	**Unit**	**DM**	**Supply**	**Unit**
CP	%	15.9679	3,065.8430	g
Soluble Protein	%	6.3117	1,211.8440	g
aNDFom	%	30.9364	5,939.7820	g
Forage aNDFom	%	21.2802	4,085.7990	g
CHO C uNDF	%	8.7342	1,676.9720	g
Sugar (WSC)	%	4.5633	876.1617	g
Starch	%	26.7530	5,136.5710	g
Soluble Fiber	%	5.3419	1,025.6400	g
NFC	%	40.5916	7,793.5850	g
EE	%	5.4813	1,052.4150	g
TFA	%	4.7962	920.8674	g
Ash	%	7.8310	1,503.5510	g
Ca	%	0.8853	169.9835	g
P	%	0.3789	72.7418	g
Mg	%	0.4073	78.2107	g
K	%	1.4061	269.9721	g

Diet Summary 2.6L:

acarroll11

First Working group - NexPro - Lactation Barn - LoCoP NexPro - Lactating Dairy Cow

Recipe: Flint Hills NexPro UNL LCoP 2021 01 14				
Ingredients	D.M. %	A.F. kg	DM kg	% DM
Corn silage 34.4033 medium	38.800	20.515	7.960	40.00
Alfalfa hay 45.18 Trial	91.200	3.949	3.602	18.10
Corn grain	87.620	3.248	2.846	14.30
NexPro [FHR] Trial	92.100	0.570	0.525	2.64
SoyPass MP	87.100	1.225	1.067	5.36
Soybean Hulls	90.200	1.900	1.713	8.61
Urea	99.000	0.129	0.127	0.64
Sodium Choride	99.850	0.076	0.076	0.38
Sodium Bicarbonate	95.600	0.125	0.119	0.60
Vitamin Premix ADE	99.500	0.008	0.008	0.04
Beet molasses	79.500	0.433	0.344	1.73
Trace Mineral Premix	99.500	0.010	0.010	0.05
Calcium Carbonate	99.200	0.197	0.195	0.98
Calcium Phos. Mono-Dical	95.500	0.106	0.101	0.51
MAGOX 54%	99.500	0.080	0.080	0.40
Energy Booster 100	99.360	0.601	0.597	3.00
Soybean meal solv. 48%	89.750	0.589	0.529	2.66
Totals		**33.761**	**19.900**	**58.9 %DM**

Summary analysis: Lactating Dairy Cow				
Nutrient	**Unit**	**DM**	**Supply**	**Unit**
CP	%	16.1416	3,212.1140	g
Soluble Protein	%	6.3218	1,258.0130	g
aNDFom	%	30.9156	6,152.1010	g
Forage aNDFom	%	21.2806	4,234.7600	g
CHO C uNDF	%	8.8901	1,769.0930	g
Sugar (WSC)	%	4.3137	858.4199	g
Starch	%	26.7790	5,328.9330	g
Soluble Fiber	%	5.4100	1,076.5800	g
NFC	%	40.4363	8,046.6800	g
EE	%	5.5952	1,113.4240	g
TFA	%	4.8894	972.9819	g
Ash	%	7.7196	1,536.1740	g
Ca	%	0.8756	174.2357	g
P	%	0.3767	74.9711	g
Mg	%	0.4052	80.6408	g
K	%	1.3644	271.5058	g

Diet Summary 5.4M:

acarroll11

First Working group - NexPro - Lactation Barn - MdCoP NexPro - Lactating Dairy Cow

Recipe: Flint Hills NexPro UNL MCoP 2021 01 14				
Ingredients	D.M. %	A.F. kg	DM kg	% DM
Corn silage 34.4033 medium	38.800	21.340	8.280	40.00
Alfalfa hay 45.18 Trial	91.200	4.108	3.747	18.10
Corn grain	87.620	3.378	2.960	14.30
NexPro [FHR] Trial	92.100	1.205	1.110	5.36
SoyPass MP	87.100	0.627	0.546	2.64
Soybean Hulls	90.200	1.976	1.782	8.61
Urea	99.000	0.134	0.132	0.64
Sodium Choride	99.850	0.079	0.079	0.38
Sodium Bicarbonate	95.600	0.130	0.124	0.60
Vitamin Premix ADE	99.500	0.008	0.008	0.04
Beet molasses	79.500	0.450	0.358	1.73
Trace Mineral Premix	99.500	0.010	0.010	0.05
Calcium Carbonate	99.200	0.204	0.203	0.98
Calcium Phos. Mono-Dical	95.500	0.111	0.106	0.51
MAGOX 54%	99.500	0.083	0.083	0.40
Energy Booster 100	99.360	0.625	0.621	3.00
Soybean meal solv. 48%	89.750	0.614	0.551	2.66
Totals		**35.084**	**20.700**	**59.0 %DM**

Summary analysis: Lactating Dairy Cow				
Nutrient	**Unit**	**DM**	**Supply**	**Unit**
CP	%	16.3224	3,378.7910	g
Soluble Protein	%	6.3323	1,310.8140	g
aNDFom	%	30.8931	6,394.9860	g
Forage aNDFom	%	21.2798	4,405.0010	g
CHO C uNDF	%	9.0502	1,873.4320	g
Sugar (WSC)	%	4.0570	839.8056	g
Starch	%	26.8045	5,548.6310	g
Soluble Fiber	%	5.4809	1,134.5710	g
NFC	%	40.2757	8,337.2210	g
EE	%	5.7123	1,182.4600	g
TFA	%	4.9853	1,031.9810	g
Ash	%	7.6048	1,574.2210	g
Ca	%	0.8655	179.1597	g
P	%	0.3746	77.5398	g
Mg	%	0.4031	83.4345	g
K	%	1.3214	273.5425	g

Diet Summary 8.0H:

acarroll11

First Working group - NexPro - Lactation Barn - HiCoP NexPro - Lactating Dairy Cow

Recipe: Flint Hills NexPro UNL HCoP 2021 01 14				
Ingredients	D.M. %	A.F. kg	DM kg	% DM
Corn silage 34.4033 medium	38.800	20.515	7.960	40.00
Alfalfa hay 45.18 Trial	91.200	3.949	3.602	18.10
Corn grain	87.620	3.248	2.846	14.30
NexPro [FHR] Trial	92.100	1.729	1.592	8.00
Soybean Hulls	90.200	1.900	1.713	8.61
Urea	99.000	0.129	0.127	0.64
Sodium Choride	99.850	0.076	0.076	0.38
Sodium Bicarbonate	95.600	0.125	0.119	0.60
Vitamin Premix ADE	99.500	0.008	0.008	0.04
Beet molasses	79.500	0.433	0.344	1.73
Trace Mineral Premix	99.500	0.010	0.010	0.05
Calcium Carbonate	99.200	0.197	0.195	0.98
Calcium Phos. Mono-Dical	95.500	0.106	0.101	0.51
MAGOX 54%	99.500	0.080	0.080	0.40
Energy Booster 100	99.360	0.601	0.597	3.00
Soybean meal solv. 48%	89.750	0.589	0.529	2.66
Totals		**33.694**	**19.900**	**59.1 %DM**

Summary analysis: Lactating Dairy Cow				
Nutrient	**Unit**	**DM**	**Supply**	**Unit**
CP	%	16.4953	3,282.5120	g
Soluble Protein	%	6.3424	1,262.1090	g
aNDFom	%	30.8727	6,143.5680	g
Forage aNDFom	%	21.2806	4,234.7600	g
CHO C uNDF	%	9.2062	1,832.0040	g
Sugar (WSC)	%	3.8072	757.6224	g
Starch	%	26.8310	5,339.2800	g
Soluble Fiber	%	5.5489	1,104.2060	g
NFC	%	40.1206	7,983.8550	g
EE	%	5.8262	1,159.3960	g
TFA	%	5.0787	1,010.6340	g
Ash	%	7.4934	1,491.1620	g
Ca	%	0.8557	170.2892	g
P	%	0.3725	74.1178	g
Mg	%	0.4009	79.7875	g
K	%	1.2797	254.6528	g

APPENDIX J: 2021 DISTILLERS GRAINS TECHNOLOGY COUNCIL POSTER

Energy of lactating dairy cattle fed increasing inclusion of new high protein processed corn product

A. L. Carroll[1], D. L. Morris[1], M. L. Jolly-Breithaupt[2], P. J. Kononoff[1]
[1]Department of Animal Science University of Nebraska-Lincoln
[2]POET Sioux Falls, SD

INTRODUCTION

- As of 2020, the United States supplied 33.1 million metric tons of distillers grains, contributing $34.7 billion dollars to nation's Gross Domestic Product.[1]
- Since the value of distillers grains is typically based on their protein and energy content, new coproducts aim to concentrate the protein relative to the fiber fraction.
- New technology results in new products which need both analyzed for chemical composition and animal productive responses. [2]

OBJECTIVES

- To examine the effects of replacing non-enzymatically browned soybean meal (NEBSBM) with a high processed corn product (HPCoP) on DMI, energy utilization, and production of lactating Jersey cows.

MATERIALS and METHODS

- 12 multiparous Jersey cows (95 ± 7.5 DIM)
- Triplicated 4 × 4 Latin square with 4 periods of 28 d
- Cow were blocked by production and randomly assigned to diets with 4 different levels of HPCoP inclusions:
  - 0% HPCoP (**CTRL**)
  - 2.6% HPCoP (**2.6L**)
  - 5.4% HPCoP (**5.4M**)
  - 8.0% HPCoP (**8.0H**)
- Measured:
  - DMI, milk production and components (4 d)
  - Urine and fecal output (4 d total collection)
  - O_2 consumption, CO_2 and CH_4 production (1 d headboxes-type indirect calorimeter)

- Data analyzed with GLIMMIX procedure of SAS (9.4)
  - Fixed effects: HPCoP inclusion; Random Effects: period, square and cow nested in square
  - Tested linear, quadratic, and cubic effects of level of HPCoP inclusion

RESULTS

Table 1. Ingredient and chemical composition of the diets fed to all experimental cows

Item (% DM)	CTRL	2.6L	5.4M	8.0H
Ingredient				
Corn silage	40.0	40.0	40.0	40.0
Alfalfa hay	18.1	18.1	18.1	18.1
Corn grain, ground	14.3	14.3	14.3	14.3
HPCoP[1]	-	2.64	5.36	8.0
Soybean Meal	2.66	2.66	2.66	2.66
NEBSBM[2]	8.0	5.36	2.64	-
Soybean hulls	8.61	8.61	8.61	8.61
Remaining mix[3]	8.46	8.46	8.46	8.46
Composition (% DM, mean ± SD)				
DM	59.6 (2.43)	59.4 (1.92)	60.3 (1.94)	59.4 (1.73)
CP	16.14 (0.43)	16.11 (0.35)	16.09 (0.35)	16.06 (0.45)
aNDFom	30.67 (1.46)	30.97 (1.31)	31.28 (1.18)	31.59 (1.07)
Starch	27.15 (1.98)	27.42 (2.02)	27.70 (2.09)	27.98 (2.19)
Fatty Acids				
Total fatty acids	5.03 (0.32)	5.11 (0.36)	5.19 (0.41)	5.27 (0.46)
C16 fatty acids	1.93 (0.24)	1.93 (0.27)	1.94 (0.30)	1.95 (0.33)
C18 fatty acids	2.86 (0.12)	2.93 (0.12)	3.00 (0.13)	3.07 (0.14)
MP supply (g)[4]	2410.0	2360.5	2309.5	2260.0

[1]HPCoP = High protein processed corn product
[2]NEBSBM = Non-enzymatically browned soybean meal
[3]Supplied (% of the total diet): 0.64% urea, 1.73% beet molasses, 3.00% Energy Booster Merge (Milk Specialties, Eden Prairie, MN), 3.09% mineral-vitamin mix.
[4]Value determined assuming 21.34 kg DMI

Table 3. Effect of increasing inclusion of HPCoP on apparent total-tract digestibility (%)

	Treatment[1]					P-Value[2]		
Item	CTRL	2.6L	5.4M	8.0H	SEM	L	Q	C
DM	66.4	67.1	67.0	65.8	0.63	0.45	0.12	0.89
OM	68.4	69.0	68.8	67.6	0.65	0.31	0.15	0.95
CP	66.2	66.3	66.8	65.2	0.77	0.47	0.22	0.46
NDF	47.8	50.0	49.0	49.2	2.13	0.45	0.27	0.30
Starch	95.8	95.4	95.9	94.3	0.95	0.21	0.41	0.36
Fatty acids								
Total fatty acids	72.6	73.1	74.7	74.1	2.68	0.36	0.71	0.60
C16 fatty acids	72.3	72.5	74.7	74.1	4.07	0.89	0.65	0.63
C18 fatty acids	73.8	74.2	76.5	76.1	2.10	0.15	0.76	0.49
Energy	66.2	66.9	66.4	65.6	0.72	0.41	0.20	0.80

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear, Q = quadratic, C = cubic.

Table 2. Chemical composition of HPCoP

Item (% DM ± SD)	HPCoP[1,2]
DM	94.3 ± 0.49
CP	52.4 ± 0.35
NDF	36.2 ± 1.63
Starch	1.90 ± 0.28
Total fatty acids	6.44 ± 0.099
Ash	4.78 ± 2.39

[1]NexPro (Flint Hills Resources, Wichita, KS).
[2]Mean and SD (n = 2)

Table 4. The effects of increasing inclusion of HPCoP on energy partitioning

	Treatment[1]					P-Value[2]		
Item	CTRL	2.6L	5.4M	8.0H	SEM	L	Q	C
Fractions, Mcal/d								
GE	81.8	83.5	88.8	85.7	3.04	0.05	0.20	0.18
DE	54.1	55.9	58.8	56.4	2.36	0.13	0.18	0.34
ME	47.9	50.1	52.7	50.5	2.23	0.13	0.15	0.47
NE_L	31.2	33.6	35.9	34.1	1.77	0.07	0.11	0.51
Fractions, Mcal/kg of DM								
GE	4.25	4.26	4.28	4.31	0.020	< 0.01	0.65	0.95
DE	2.81	2.84	2.83	2.83	0.035	0.56	0.53	0.69
ME	2.48	2.54	2.54	2.53	0.039	0.39	0.25	0.77
NE_L	1.61	1.71	1.73	1.70	0.054	0.09	0.12	0.92

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear, Q = quadratic, C = cubic.

Table 5. Effects of HPCoP on intake, milk production and components

	Treatment[1]					P-Value[2]		
Item	CTRL	2.6L	5.4M	8.0H	SEM	L	Q	C
DMI, kg	19.2	19.9	20.7	19.9	0.62	0.11	0.07	0.38
Milk yield, kg	27.8	28.6	29.8	29.0	1.00	0.08	0.20	0.36
ECM[3], kg	34.3	35.7	37.3	37.4	1.08	<0.01	0.40	0.64
ECM/DMI	1.80	1.81	1.81	1.89	0.042	0.05	0.28	0.44
Fat, %	5.05	5.18	5.15	5.47	0.288	<0.01	0.35	0.26
Fat, kg	1.40	1.46	1.53	1.58	0.065	<0.01	0.87	0.80
Protein, %	3.35	3.43	3.40	3.40	0.098	0.22	0.14	0.16
Protein, kg	0.93	0.98	1.01	0.99	0.033	0.06	0.12	0.63

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear, Q = quadratic, C = cubic.
[3]ECM = Energy corrected milk

CONCLUSIONS

- DM, OM, CP, NDF, Starch, Fatty Acid and Energy digestibility was unaffected by increasing inclusion of HPCoP
- GE in Mcal/d and Mcal/kg increased with increasing inclusion but did not affect DE or ME in Mcal/d or Mcal/kg
- Increasing inclusion of HCoP increased ECM and milk fat production

ACKNOWLEDGEMENTS

Flint Hills Resources
Wichita, KS for funding this project.

Citations

[1]Renewable Fuels Association, 2020
[2]Birkelo et al. 2004

APPENDIX K: FULL MATERIALS AND METHODS FROM ENERGY BALANCE AND NITROGEN UTILIZATION EXPERIMENT

Animals and Treatments

The University of Nebraska- Lincoln Animal Care and Use Committee approved animal care and experimental procedures. Twelve multiparous Jersey cows 95 ± 7.3 DIM were sourced from a commercial diary. Sample size was based on previous work at the University of Nebraska-Lincoln (Reynolds et al., 2019b). Cows were housed in individual tie stalls in a climate-controlled environment (20º C) at the University of Nebraska-Lincoln Dairy Metabolism Facility in the Animal Science Complex. Stalls were equipped with rubber mats and cows were milked at 0700 and 1800 h. All cows were less than 134 d pregnant at the end of the last experimental period thus fetal energy was assumed to be zero. (NRC, 2001)

The experimental design was a triplicated 4x4 Latin square design balanced for carryover effects consisting of 4 periods of 28-d. In the experiment cows grouped by milk yield and were randomly assigned one of four TMRs. Treatment sequence was based on Kononoff and Hanford, 2006. The high protein corn milling coproduct (HPCoP) was sourced from Flint Hill Resources, Wichita, KS. Treatments were as follows: **CRTL** [0% HPCoP]; **LCoP** [2.6% HPCoP on DM basis]; **MCoP** [5.4% HPCoP on DM basis]; or **HCoP** [8% HPCoP on DM basis]. Two concentrate mixes were utilized in the study where concentrate mix one provided 0% HPCoP and the second provided 8% HPCoP. These two concentrate mixes were added in a ratio of 33.3% and 66.7% for the **LCoP** and 66.7% to 33.3% for **MCoP.** Both concentrate mixes were mixed at the University of Nebraska-Lincoln feed mill. Dietary ingredients (corn silage, alfalfa hay and concentrate)

were placed in the Calan Data Ranger (American Calan, Inc. Northwood, NH), mixed and fed at 0930 h. The target refusal rate was set at 5% for the 24 d adaptation period of each period. During the 4 d collection period cattle were fed at 100% of the prior week's average intake to limit refusals.

Sample collection and analysis

Individual feed ingredients were sampled daily during collection periods and frozen at -20º C. All feed ingredients were dried at 60º C and were ground through a 1mm screen. (Wiley Mill; Aurthur A. Thomas Co., Philadelphia, PA). A subsample of ground feed was sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis of DM (method 930.15, AOAC, 2000), CP (method 990.03, AOAC, 2000), nitrogen (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085), soluble CP (Krishnamoorthy et al. 1982) ADICP and NDICP (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085), ADF (method 973.18, AOAC, 2000), NDF with sodium sulfite and α amylase corrected for ash contamination (**aNDFom**) (Van Soest et al., 1991) , Lignin (Goering and Van Soest 1970), EE (method 2003.05 AOAC, 2000) sugar (Hall, 2009), starch (Hall 2009), ash (method 942.05, AOAC, 2000), minerals (method 985.01, AOAC, 2000), fatty acids (Sukhija and Palmquist, 1988). Feed samples were also analyzed for gross energy (**GE**) content (Parr 6400 Calorimeter, Moline, IL). The chemical composition of the diets and feed ingredients is listed in table 3.1. Total mixed rations were sampled on d 1 of each collection period and used to determine particle size using the Penn State particle separator on an (Kononoff and Heinrichs, 2002) as is and DM basis (60ºC for 48 h). During each collection period refusals were sampled and

composited on a weight basis. Refusals were analyzed for DM, CP, NDF, aNDFom, starch, ash, fatty acids, and GE vias the same methods as feeds.

Total fecal and urine output was collected from each individual cow during the collection period for 4 consecutive d. A 137× 76 cm mat was placed behind the cow to aid in fecal collection. Feces were manually collected by personnel during defecation or occasionally were picked up from the rubber mat and deposited into a trach can (Rubbermaind). Then a trash bag was placed on the top of the trash can to minimize nitrogen volatilization of the feces. Daily feces were subsampled (~500 g as-is), composited on a weight basis and frozen between collection events. After collections, feces were dried at 60ºC for 48 h and ground to pass through a 1-mm screen (Wiley Mill; Aurthur A. Thomas Co.). The ground feces were analyzed as described for refusals. Total urine was collected by inserting a 30 French Foley catheter into each cow's bladder with a stylus. The balloon was inflated to 55mL with physiological saline. The catheter was drained into a 55-L plastic container via Tygon tubing (Saint Gobain, La Defense). Acid (50% HCl) was added to the urine collection container at the beginning of the collection d. Urine pH was measured at the end of each d and the quantity of acid was adjusted to maintain a urinary pH of < 5. Urine was subsampled daily and composited on a wet-weight basis. Urine samples were frozen (-20º C) until analysis for GE. Urine GE was determined by drying (60º C) 4 mL of sample in a bomb capsule and allowed to dry until tacky (4 h) then combusted (Parr 6400 Calorimeter). Urine subsamples were sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis of nitrogen (Leco FP-528 Nitrogen Combustion Analyzer. Leco).

Milk production was measured daily, and milk samples were collected during the morning and evening milking of collection periods. Milk from individual milking events were preserved with 2-bromo-2-nitropropane-1,3 diol and sent to Heart of America DHIA (Kasnsas City, MO) Milk samples were analyzed for fat, protein, lactose, SNF, MUN and SCC using Bentley FTS/FCM Infrared Analyzer (Bentley Instruments). Additionally, milk from each milking event was composited on a weight basis. Composited milk samples were analyzed for nitrogen and fatty acids as previously described. Cows were weighed, before feeding on the first and last day of each collection period.

Heat production was determined through the headbox-type indirect calorimeters as described previously (Freetly et al., 2006; Foth et al., 2015). For each cow, a collection period of 23-h was used to measure O_2 consumption and CO_2 and CH_4 production. Gas data were adjusted to a 24-h period. Four headboxes were used and data were collected across 3-d during the 4-d collection period. Cows were adapted to headboxes for a minimum for 3 d prior to the start of the experiment. Feed was placed in the bottom of the headbox and cows were allowed ad libitum access to water from a water bowl placed inside the headbox. Free water intake was measured using a water meter (Model DLJSJ75, Daniel L. Jerman Co.) while each cow was inside the headbox. Within the headbox, temperature and dew point were measured every minute during the 23-h collection interval using a probe (Model TRH-100, Pace Scientific Inc.) and recorded using a data logger (Model XR440, Pace Scientific Inc.). Line pressure was measured using a u-tube manometer (Item # 1221–8, Park Supply of America, Inc.) and barometric pressure of the room was measured using a barometer (Chaney Instruments Co.). Total

volume of gas flow through the headbox was measured using a gas meter (MCW Whisper, Alicat Scientific) and corrected to standard temperature and pressure (0ºC, 101.3 kPa) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). From the headbox, continuous samples of incoming and outgoing air were collected into separate bags (44 L, LAM-JAPCON-NSE; Pollution Measurement Corp.) using glass tube rotameters (Model 1350E Sho-Rate "50," Brooks Instruments). Gas bags were analyzed for O_2, CO_2 and CH_4 using an Emerson X-stream 3-channel analyzer (Solon, OH) according to the method of Nienaber and Maddy, 1985. System efficiency (head box and gas analyzer) was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O_2 and CO_2 were (average ± SD) 101 ± 1.1 and 99 ± 1.3%, respectively. Gas measurements were adjusted to 100% using recoveries for individual headboxes. Heat production was estimated as follows. (Brouwer, 1965):

Heat production (HP, kcal/d) = 3.866 × O_2 (L/d) + 1.200 × CO_2 (L/d) – 0.518 × CH_4 (L/d) –1.431 × Urinary nitrogen excretion (g/d)

Energy Calculations

The respiratory quotient (**RQ**) was calculated using the ratio of carbon dioxide produced to oxygen consumed (L/L). Methane energy was estimated by multiplying CH_4 production by its enthalpy (9.45 kcal/L). Calculations to estimate digested energy (**DE**) and ME were as follows:

DE (Mcal/d) = GE (Mcal/d) – fecal energy (Mcal/d) [1]

ME (Mcal/d) = DE (Mcal/d) – urine energy (Mcal/d) – methane energy (Mcal/d) [2]

Unaccounted for energy was assumed to represent tissue energy retention or mobilization which was corrected to an NE_L basis as follows:

Tissue energy (Mcal/d) = ME (Mcal/d) – heat production (Mcal/d) – milk energy (Mcal/d) [3]

Adjusted tissue energy (**TE**; Mcal of NE_L/d) = positive residual energy × k_L/k_G or negative residual energy × k_T [4]

Where k_T is the efficiency of utilizing body reserve energy for milk production, k_G is the efficiency of utilizing ME intake for tissue gain (Moe et al., 1970). Values of 0.66 and 0.74, and 0.89 were used for k_L, k_G, and k_T respectively (Moraes et al., 2015).

Net energy of lactation (NE_L; Mcal/d) = 0.08 × $BW^{0.75}$+ Milk E (Mcal/d) + adjusted TE (Mcal of NE_L/d) [5]

Statistical Analysis

The UNIVARIATE procedure of SAS (9.4) was used to determine outliers from the data set. An outlier was determined if an observation was greater than 2.5 standard deviations from the mean of milk production and DMI. The result of the outlier test indicated one DMI outlier cow number 4842 during the first period. This animal had a dry matter intake of 13.60 kg/d which was 2.72 standard deviations away from the mean DMI of 19.77 kg/d. The animal displayed signs of mastitis however no treatment was applied. As a result of the statistical analysis the observation from this animal was removed prior to statistical analysis.

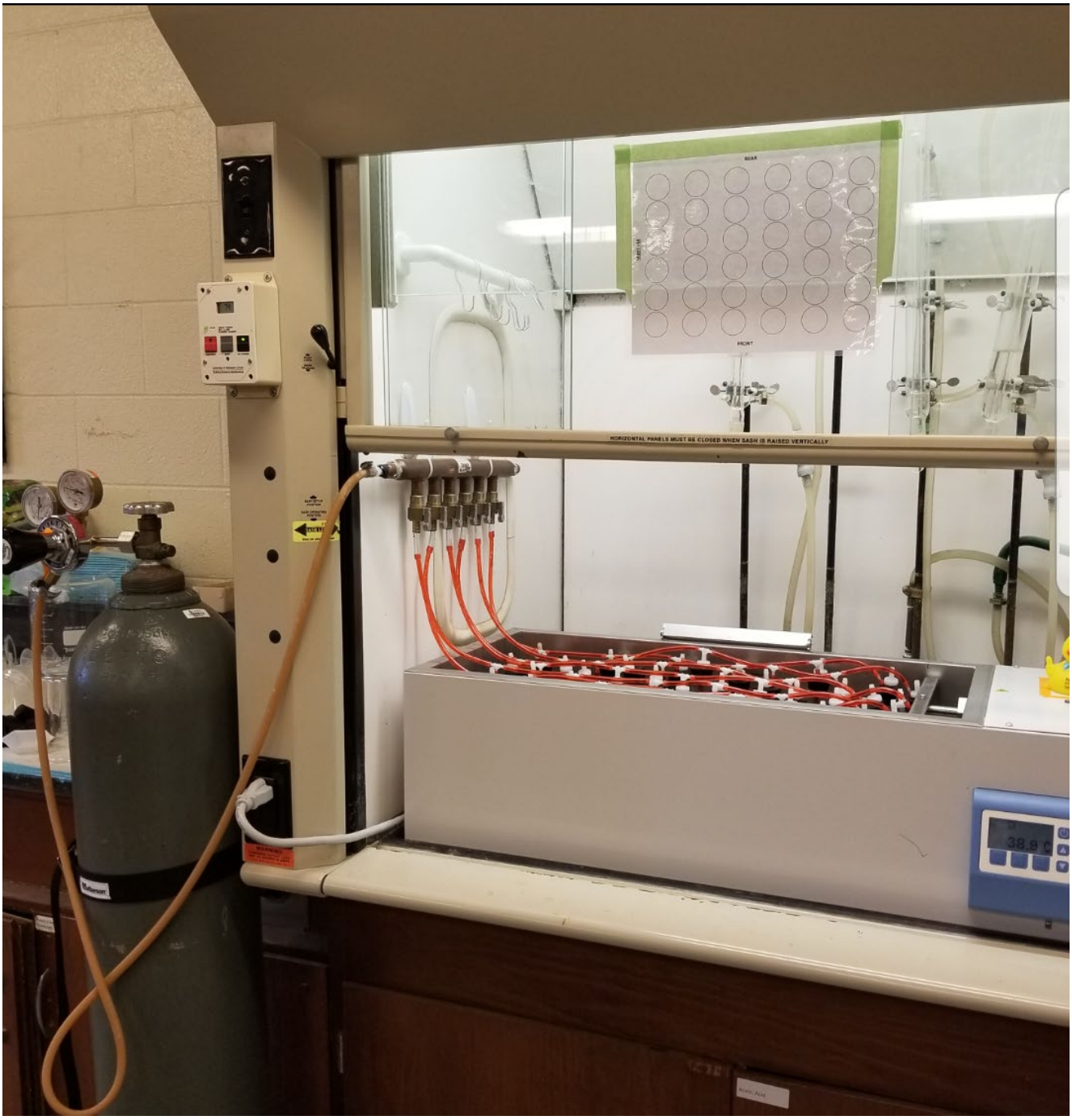
Data were analyzed in SAS (9.4). The model include fixed effect of treatment and the random effect of period, square and cow nested in square. A type III analysis of variance with Kenward-Rodger's denominator degrees of freedom was complete using the PROC GLIMMIX function of SAS. All data are presented as least-squares means ± largest

standard error. Significance and trends was declared with a P-value ≤ 0.05 and P-value ≤ 0.10.

APPENDIX L: IN VITRO SYTEM PARTS

Part Name	Part #	Quantity	Cost	Location of Purchase
Thermo Scientific™ TSSWB27	TSSWB27	1	$4,480	Nebraska EShop
Matheson CO2 Tank	CD 50	1	$6.37	Nebraska EShop
Matheson CO2 Table Mount	SEQ 708	1	$52.85	Nebraska EShop
Matheson CO2 Regulator	SEQ 18320	1	$211.03	Nebraska EShop
Kimble KIMAX 125ml Erlenmeyer Flasks	26650	36	$276 (12 for $92)	Nebraska EShop
Two Hole #6 Rubber Stoppers	501531471	36	$97.38 (12 for $32.46)	Nebraska EShop
1/4 Inch Check Valve	Eurob-0514-1133-1	36	$131.89 (4 per pack @ 11.99)	Amazon
1/4 OD 1/8 ID Micro fuel Line	4101549	18 ft.	$8.46 (10ft for $4.80)	Home Depot
1/8" Nylon Hose Barb Splicer	CBSHM1800BG1	6	$14.34 (1 for $2.39)	ACE Hardware
1/8" Nylon Hose Barb TEE	CBT18BG1	30	$71.70 (1 for $2.39)	ACE Hardware
Resazurin	418900050	1	$74.30	Nebraska EShop
3/4" Pex Manifold with Valve	6807197	1	$69.99	Menards

APPENDIX M: IN VITRO SYTEM PICTURES



APPENDIX N: IN VITRO SYTEM PROTOCOL

In Vitro Protocol- 10/13/2021

24 prior to fermentation:

- Remove shaking platform from bath and set to the side.
- Fill water bath to blue sharpie line with deionized water.
- Turn water bath on to warm to 39 degrees C 12-24 hours in advance of desired fermentation start time.
    - Press the ⏻ to start.
    - Press the ⇄ and then the down arrow to slow the water bath to zero rpm and press enter.

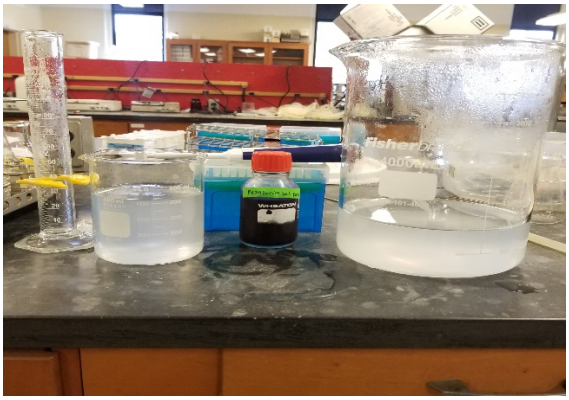
Calculations:

- Calculate the number of runs
    - (Number of samples × 3 (triplicate) × Number of time points)/33 = runs
    - 33 = number of flasks available for sample fermentation
    - Round runs up to the next whole number.
        - Ex: (10 samples × 3 × 4 timepoints)/33 = 3.63 runs
            - 4 runs will be needed.
- Calculate the amount of buffer needed.
    - Each sample will need 40 mL of buffer + 310mL of extra
        - EX: 36 Flasks = 40mL × 36= 1.44L + 310mL = 1.75 L
- Calculate urea inclusion.
    - Add 1 gram of urea per L of buffer used and stir into buffer
        - EX: 1.75 L buffer = 1.75 g Urea
- Calculate the rumen fluid needed.
    - You will need 10mL per flask and it is best to add 100mL extra. The total mL will need to be divided by 2 as rumen fluid is composited from two cows.

- Ex: 36 Flasks = 10ml × 36= 360mL + 100mL= 460mL
- 460mL/2=230mL
    - 230 mL Cow 1
    - 230 mL Cow 2

Materials:

- Buffer:
    - McDougal's Buffer (calculated)
    - Urea (calculated)
    - 500mL Beaker
    - 4 L Beaker
    - 100 mL Graduated Cylinder
    - 10-100 microliter pipette
    - 10-100 microliter pipette tip
    - Resazurin solution (0.1% wt/vol, 50 µL per flask)



- Rumen Fluid collection:
    - Collection thermos filled with warm H2O in the lab
    - 1 Square of cheese cloth folded to 4 layers
    - 1 Large Plastic graduated cylinder from the metabolism area
    - 1 Metal funnel from metabolism area
- Flask Inoculation:
    - 1000 mL Separatory funnel
    - #6 rubber stopper
    - Rubber band

- 500mL beaker
- 30 mL syringe

Sample Preparation:

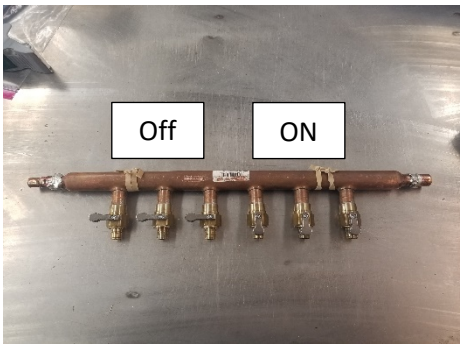
- Weigh out .5000-.5010g of sample that has been ground through a 1mm Wiley Mill screen.
- Order the flasks on the shaking platform based on desired run set up.

- Place the sample in a designated flask.
- Once all flasks are filled with sample set the platform to the side.

Buffer Prep:

- Obtain McDougal's buffer in beaker from outside lab refrigerators.
- Stir urea into buffer and situate the 4 L Beaker in 39 degree C water bath for **3 hours**.
  - Cover top of beaker with tin foil.
  - Stir occasionally to fully incorporate the urea.
- After 3 hours have passed use a 100 mL graduated cylinder and pour 40 ml of buffer in each flask.
- Add 50 µL of resazurin indicator solution to each flask and swirl until color is distributed

- Place shaking platform in water bath and adjust water levels to the upper marked line.
- Push stoppers into each flask and turn on the CO2 tank.
    - Do not touch the settings on the regulator, just open the tank.
    - Check the tank to make sure you have 500 Psi available in tank, that will get you through an NDF 240 run.
- Make sure that the manifold is turned to the on position for each line.



- Allow beakers with feed to reduce for **2 hours prior to inoculation**.

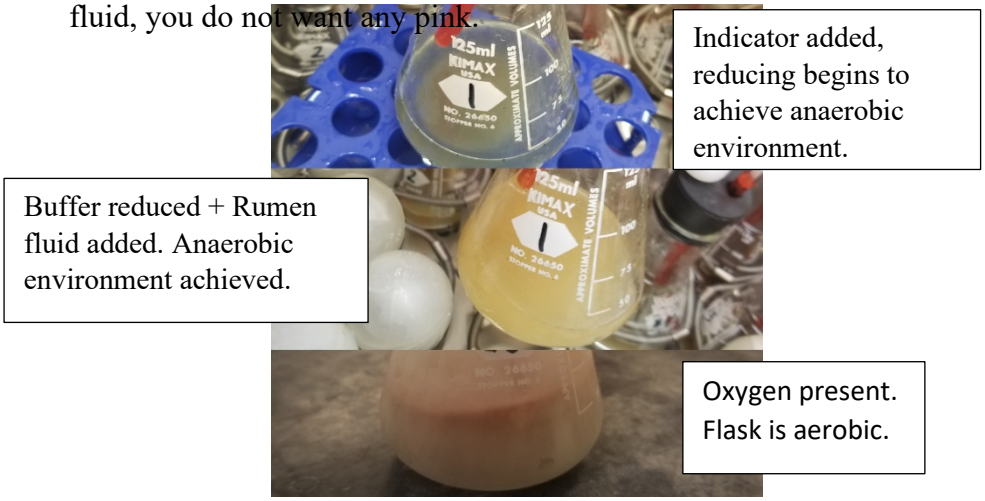
Rumen Fluid Collection:

- Take thermos containing warm H_2O to the dairy, on your way grab the metal funnel and large plastic graduated cylinder from the metabolism area.
- Once in the dairy cut and fold a piece of cheese cloth so you strain the rumen contents through four layers.
- Use two cows fitted with a rumen cannula from the dairy.
    - Open cannula and grab handful samples from front, middle and back of the rumen at random getting both mat layer and fluid layer.
    - Place contents in cheesecloth and squeeze until all fluid is released, continue to grab samples until you have reached 50% of the needed rumen fluid.
    - Return rumen fiber back into the rumen and seal the cannula.
- Go to the second cow and repeat the process until all fluid is collected.

- Dump the warm water out of the thermos and fill the thermos with the rumen fluid.
- Return to the lab quickly, inoculation should be finished within 30 minutes of collection to keep microorganisms alive.

Back in the lab:

- Funnel the rumen fluid into a separatory funnel and submerge all but the stopper in a 39 degree C water bath.
- Allow the rumen fluid to separate for 5 minutes or until there is a distinct layer between the aqueous and fiber layer. (within 30-minute window).
- After separation has occurred release fluid into a 500 mL beaker and utilize a 50mL graduated cylinder and remove 10mL of fluid and distribute it quickly into flasks in a random order.
    - Check to see that flasks have gone from blue to the normal color of rumen fluid, you do not want any pink.



- Once all flasks are inoculated record start time.
    - Press the ⇄ and then the up arrow to 45 rpm and press enter.
- Record the start time.
- Take out flasks at designated time points and freeze them.

For NDFD- Follow UNL Protocol for NDF procedure

APPENDIX O: ANIMAL HEADBOX TRAINING PROTOCOL

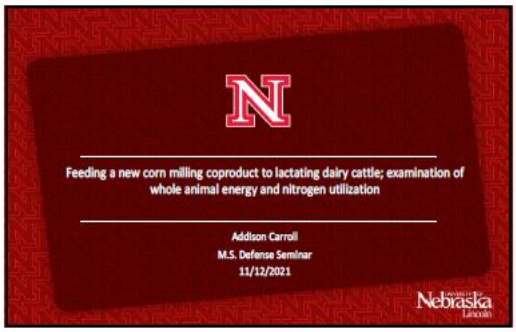
New cow training protocol:

1. Allow cows to adapt to surroundings for one week prior to beginning of headbox training.
2. Set up training schedule. Animals should begin training at least 3 weeks prior to the first period. To be enrolled in the experiment animals must show comfort in the headbox for a 24-hour period.
    a. Training at the minimum must include:
        i. First day training: 8 hours within headbox.
        ii. Second: 24 hours within headbox.
3. Animals who demonstrate "comfort" during the 24-hour measurement may be enrolled in the experiment.
4. Indicators of comfort include:
    a. Ability to lie down and stand comfortably
    b. Ability to consume water
    c. Consumption of 80% of allotted daily TMR while within headbox for 24 hours
5. Animals who do not meet the criteria will need to be subjected to more training until the criteria is met.

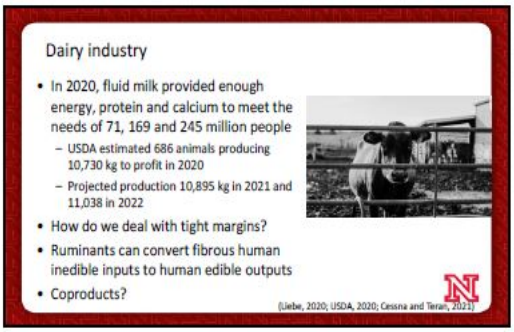
After the first period of experiment:

1. The lead of the experiment will examine the data from the collection period.
2. If animals regress and no longer meet one or more of the comfort criteria more training will be necessary prior to the second period collection week.

APPENDIX P: FINAL DEFENSE PRESENTATION



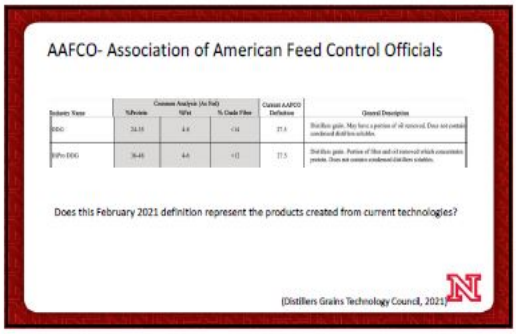
1



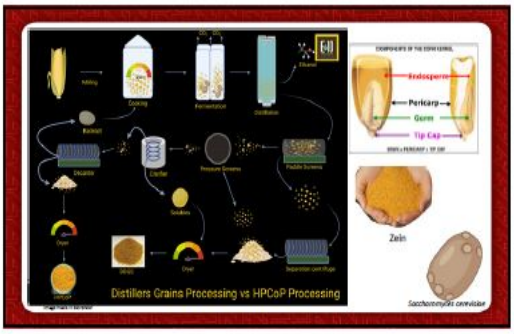
2

3

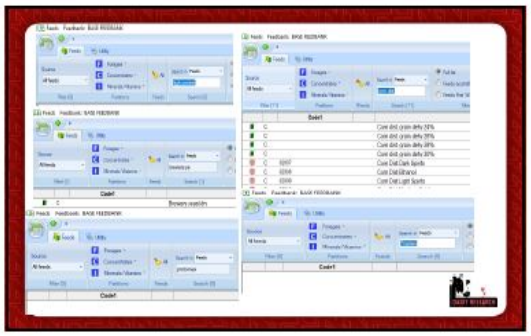
4



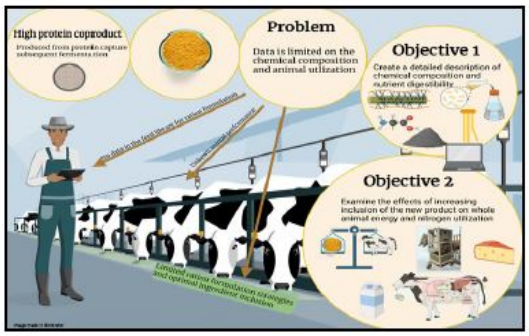
5



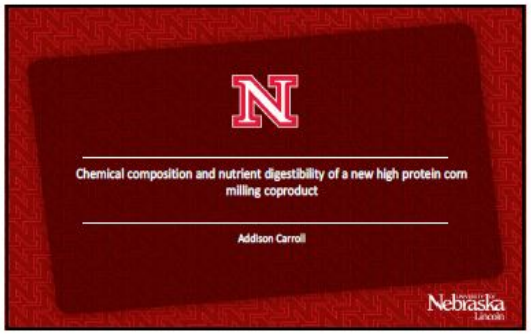
6



7


High protein coproduct
Problem
Data is limited on the chemical composition and animal utilization
Objective 1
Create a detailed description of chemical composition and nutrient digestibility
Objective 2
Examine the effects of increasing inclusion of the new product on whole animal energy and nitrogen utilization

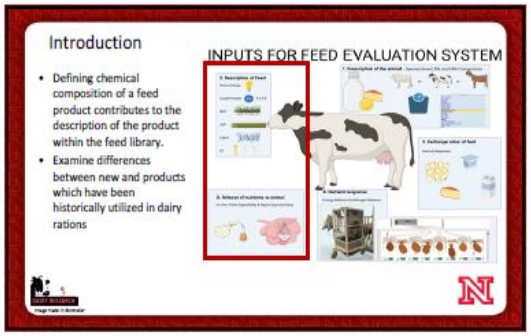
8


Chemical composition and nutrient digestibility of a new high protein corn milling coproduct
Addison Carroll
University of Nebraska Lincoln

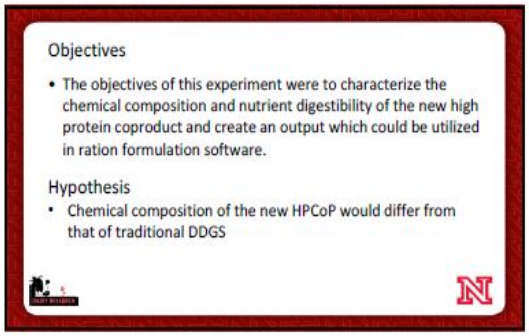
9


1933 – Earl Coryell Co. Lincoln, Nebraska
(Abbe, 2008)

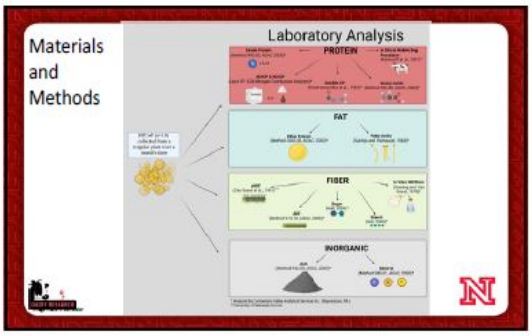
10


Introduction
Defining chemical composition of a feed product contributes to the description of the product within the feed library.
Examine differences between new and products which have been historically utilized in dairy rations
INPUTS FOR FEED EVALUATION SYSTEM

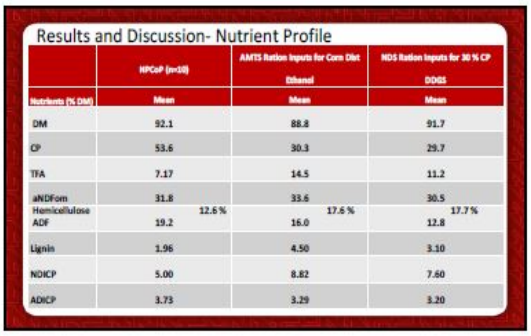
11


Objectives
The objectives of this experiment were to characterize the chemical composition and nutrient digestibility of the new high protein coproduct and create an output which could be utilized in ration formulation software.
Hypothesis
Chemical composition of the new HPCoP would differ from that of traditional DDGS

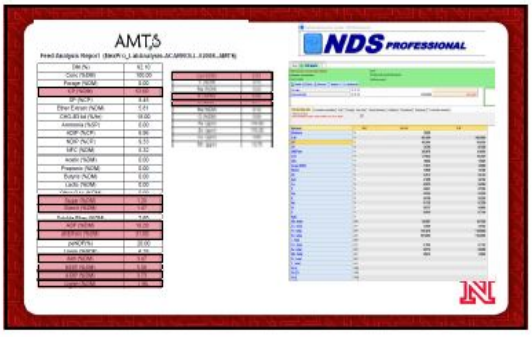
12



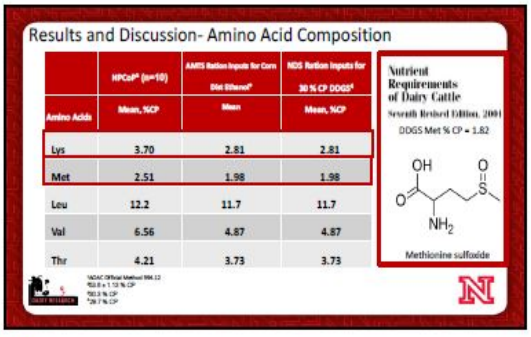
13



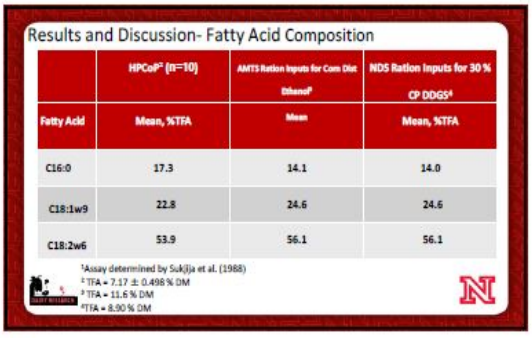
14



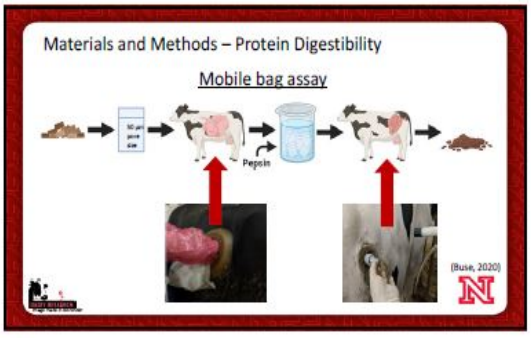
15



16



17



18

Results and Discussion – Protein Degradability

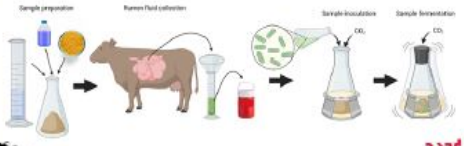
Item	HPCoP (n=10) Mean	NEBSBM[5] Mean
RDMD[1] % DM	62.3	49.3
RDP[2] % CP	53.9	16.4
RUP[3] % CP	46.1	83.6
TTDMD[4] % DM	99.5	49.5

[1]Rumen dry matter digestibility
[2]Rumen degradable protein
[3]Rumen undegradable protein
[4]Total Tract Dry Matter Digestibility
[5]Non-enzymatically browned soybean meal

19

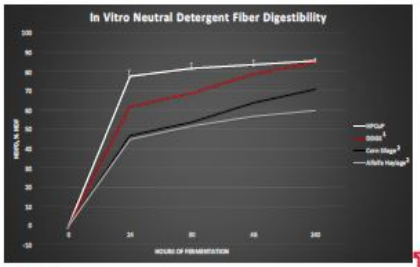
Materials and Methods – Neutral Detergent Fiber Digestibility

In vitro fermentation



20

In Vitro Neutral Detergent Fiber Digestibility



[1]Dufour, 2017
[2]Krogstad,2020

21

Materials and Methods- Fiber Analysis Methods

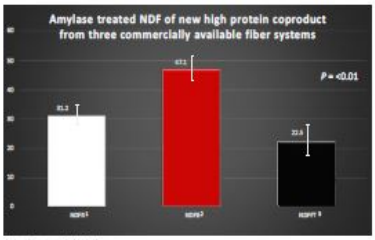

Refluxing method


Fiber Bag


Confined refluxing and filtering method

22

Amylase treated NDF of new high protein coproduct from three commercially available fiber systems



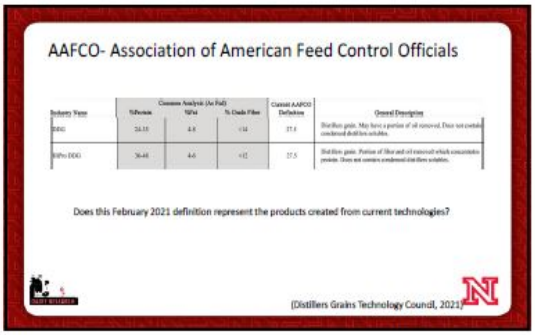
[1] Van Soest et al. (1991)
[2]Ankom method (2017)
[3]AOAC Official Method 2002.04

23

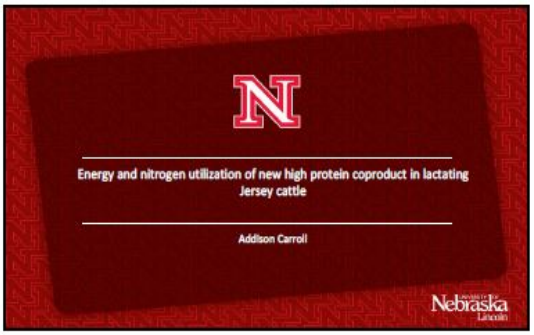
Conclusions

- Continued evaluation of novel coproducts is integral to the continued growth of the feed library
- Overall results indicate the new high protein coproduct contains increased protein and lysine content relative to traditional DDGS
- Palmitic acid may be slightly increased relative to traditional DDGS
- The product contained decreased hemicellulose and lignin content relative to traditional DDGS because of the reduction in fibrous bran material during sieving

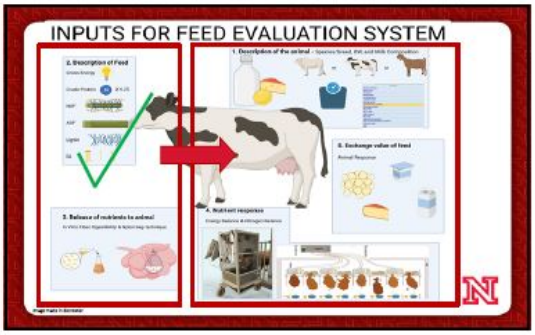
24



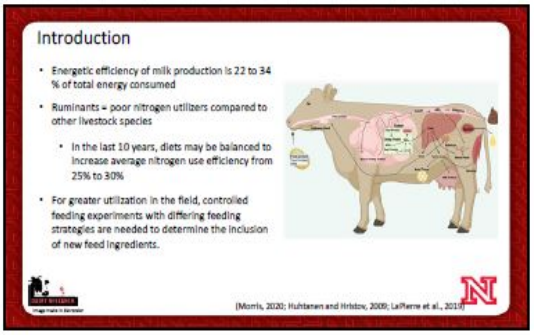
25



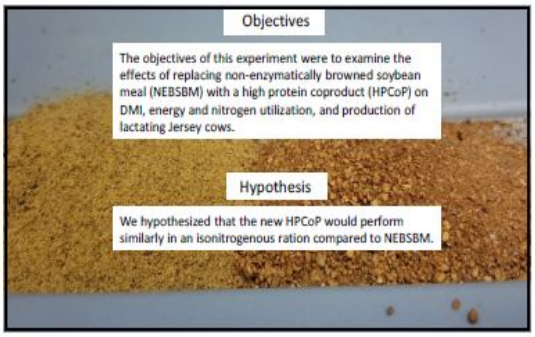
26



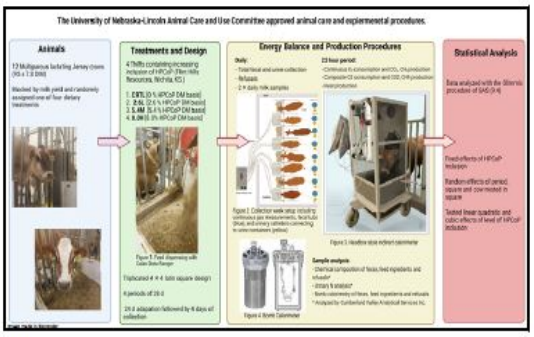
27



28



29



30

Treatments and TMR composition

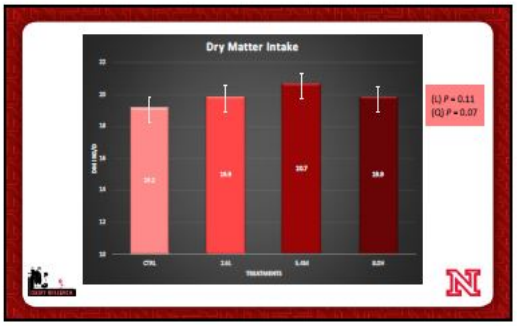
Table 1. Ingredient inclusion and chemical composition of the diets fed to all experimental cows

Item (% DM)	CTRL	2.6L	5.4M	8.0H
Ingredient				
Corn silage	40.0	40.0	40.0	40.0
Alfalfa hay	18.1	18.1	18.1	18.1
Corn grain, ground	14.3	14.3	14.3	14.3
MPLUS[1]		2.66	5.38	8.0
Soybean Meal	2.66	2.48	2.66	2.66
Non-enzymatically browned SBM[2]	8.0	5.38	2.66	
Soybean hulls	8.61	8.61	8.61	8.61
Remaining mix[3]	8.66	8.66	8.66	8.66
Composition (% DM, mean ± SD)				
CP	14.1 (0.49)	14.1 (0.36)	14.1 (0.36)	14.1 (0.49)
aNDFom	30.7 (1.48)	31.0 (1.91)	31.3 (1.58)	31.4 (1.07)
Starch	27.3 (1.68)	27.4 (2.02)	27.7 (2.06)	28.0 (2.19)
Fatty Acids				
Total fatty acids	5.69 (0.43)	6.11 (0.96)	6.16 (0.41)	6.27 (0.48)
C16 fatty acids	1.45 (0.13)	1.63 (0.27)	1.81 (0.30)	1.86 (0.33)
C18 fatty acids	2.84 (0.12)	2.99 (0.22)	3.00 (0.19)	3.07 (0.14)

Table 2. Chemical composition of HPCoP

Item (% DM ± SD)	HPCoP[1,2]
DM	94.3 ± 0.49
CP	52.4 ± 0.35
NDF	36.2 ± 1.63
Starch	1.90 ± 0.28
Total fatty acids	6.44 ± 0.099
Ash	4.78 ± 2.39

31



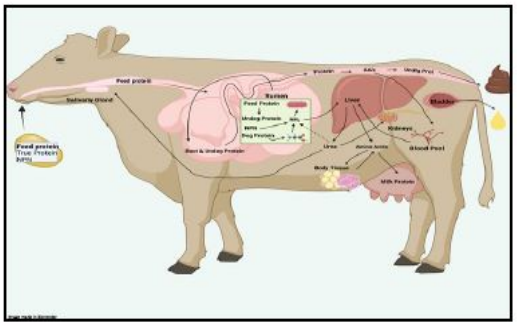
32

Results and Discussion- Digestibility

Item	Treatment[1] CTRL	2.6L	5.4M	8.0H	SEM	P-Value[2] L	Q	C
DM	66.4	67.1	67.0	65.8	0.63	0.45	0.12	0.89
OM	68.4	69.0	68.8	67.6	0.65	0.31	0.15	0.95
CP	66.2	66.3	66.8	65.2	0.77	0.47	0.22	0.46
NDF	47.8	50.0	49.0	49.2	2.13	0.45	0.27	0.30
Starch	95.8	95.4	95.9	94.3	0.95	0.21	0.41	0.36
Fatty acids								
Total fatty acids	72.6	73.1	74.7	74.1	2.68	0.36	0.71	0.60
C16 fatty acids	72.3	72.5	74.7	74.1	4.07	0.89	0.65	0.63
C18 fatty acids	73.8	74.2	76.5	76.1	2.10	0.15	0.76	0.49
Energy	66.2	66.9	66.4	65.6	0.72	0.41	0.20	0.80

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear, Q = quadratic, C = cubic.

33



34

Results and Discussion- Nitrogen Utilization

Item (% DM)	Treatment[1] CTRL	2.6L	5.4M	8.0H	SEM	P-Value[2] L	Q	C
Mass, g/d								
N intake	496.5	510.8	532.8	511.4	17.36	0.17	0.11	0.31
Fecal N	167.5	170.9	176.6	177.2	6.28	0.07	0.73	0.71
Urinary N	125.8	114.8	130.5	118.5	13.60	0.11	0.21	0.31
Milk N	160.4	165.6	172.9	160.4	6.83	0.78	0.14	0.41
N balance	42.8	59.7	52.8	55.5	17.62	0.55	0.13	0.79
As a Proportion of N Intake, %								
Fecal N	33.8	33.7	33.2	34.8	0.767	0.47	0.22	0.46
Urinary N	25.8	22.9	24.7	27.1	2.84	0.32	0.05	0.49
Milk N	32.5	32.5	32.5	31.6	1.24	0.62	0.69	0.86
N balance	7.9	11.0	9.6	6.5	3.29	0.54	0.14	0.78

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear, Q = quadratic, C = cubic.

35

Results and Discussion – Energy Partitioning

Item	Treatment[1] CTRL	2.6L	5.4M	8.0H	SEM	P-Value[2] L	Q	C
Components, Mcal/d								
Feces	27.5	27.9	29.7	29.4	0.883	0.02	0.56	0.27
Urine	2.07	1.95	2.13	2.19	0.117	0.11	0.28	0.23
Methane	4.13	3.81	3.90	3.80	0.336	0.18	0.47	0.38
Heat	24.5	23.5	24.1	23.9	1.26	0.51	0.40	0.30
Milk	23.5	24.6	25.7	25.8	0.759	< 0.01	0.42	0.68
Tissue	-0.20	1.84	2.73	0.66	1.64	0.50	0.10	0.74

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear, Q = quadratic, C = cubic.

36

Results and Discussion – Energy Partitioning

Item	Treatment[1]				SEM	P-Value[2]		
	CTRL	2.6L	5.4M	8.0H		L	Q	C
Fractions, Mcal/d								
GE	81.8	83.5	86.8	85.7	3.04	0.05	0.20	0.18
DE	54.1	55.9	58.6	56.4	2.36	0.13	0.18	0.34
ME	47.9	50.1	52.7	50.5	2.23	0.13	0.15	0.47
NE_L	31.2	33.6	35.9	34.1	1.77	0.07	0.11	0.51
Fractions, Mcal/kg of DM								
GE	4.25	4.26	4.28	4.31	0.020	< 0.01	0.65	0.95
DE	2.81	2.84	2.83	2.83	0.035	0.56	0.53	0.69
ME	2.48	2.54	2.54	2.53	0.039	0.39	0.25	0.77
NE_L	1.61	1.71	1.73	1.70	0.054	0.09	0.12	0.92

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear; Q = quadratic; C = cubic.

37

Results and Discussion – Energy Partitioning

Item	Treatment[1]				SEM	P-Value[2]		
	CTRL	2.6L	5.4M	8.0H		L	Q	C
Efficiencies								
ME:DE	0.664	0.696	0.698	0.693	0.0076	0.21	0.09	0.91
NE_L:ME	0.648	0.671	0.682	0.674	0.0172	0.03	0.08	0.86

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear; Q = quadratic, C = cubic.

38

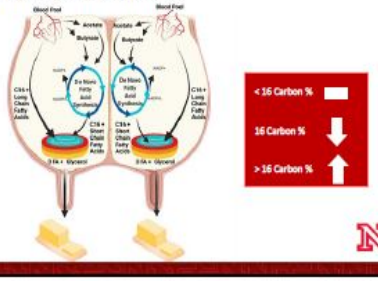
Results and Discussion - DMI, Milk production and components

Item	Treatment[1]				SEM	P-Value[2]		
	CTRL	2.6L	5.4M	8.0H		L	Q	C
DMI, kg	19.2	19.9	20.7	19.9	0.62	0.11	0.07	0.38
Milk yield, kg	27.8	28.6	29.8	29.0	1.00	0.08	0.20	0.36
ECM[3], kg	34.3	35.7	37.3	37.4	1.08	<0.01	0.40	0.64
ECM/DMI	1.80	1.81	1.81	1.89	0.042	0.05	0.28	0.44
Protein, %	3.35	3.43	3.40	3.40	0.038	0.22	0.14	0.16
Protein, kg/d	0.93	0.98	1.01	0.99	0.033	0.06	0.12	0.63
Fat, %	5.05	5.18	5.15	5.47	0.288	<0.01	0.35	0.26
Fat, kg/d	1.40	1.46	1.53	1.58	0.065	<0.01	0.87	0.81

[1]CTRL = Control Diet (0% HPCoP); 2.6L = Low Diet (2.6% HPCoP); 5.4M = Medium Diet (5.4% HPCoP); 8.0H = High diet (8.0% HPCoP).
[2]L = linear; Q = quadratic, C = cubic.
[3]ECM = Energy corrected milk.

39

Results and Discussion- Milk Fatty Acids



40

Conclusions

- DM, OM, CP, NDF, starch, fatty acid, and energy digestibility was unaffected by increasing inclusion of HPCoP
- GE in Mcal/d and Mcal/kg increased with increasing inclusion but did not affect DE or ME in Mcal/d or Mcal/kg
- Increasing inclusion of HCoP increased ECM and milk fat production likely as a function of an increasing NEL:ME ratio

41

Overall Conclusions

- The new product was different than that of traditional DDGS
  - Increased protein and lysine content
  - Increased NDFD at 24, 30, and 48 hours
  - aNDF content of HPCoP was variable based on analytical method
- The new high protein coproduct can effectively be utilized in ration formulation similar to other high protein products
  - Increasing inclusion of HPCoP:
    - No difference in digestibility
    - Increased gross energy content and intake
    - Increasing utilization of energy for NEL with subsequent increase in milk fat production

42

Next Steps

- Create AAFCO definitions for the high protein products
- Examine the best method to determine the NDF content of byproduct feeds
- Look at ruminal effect of the high protein processed corn product
- Push the limits of high protein processed corn product inclusion in lactating dairy rations

43

Thank you!

- **Advisor:** Dr. Paul Kononoff
- **Committee:** Dr. Andrea Watson and Dr. Mary Drewnowski
- **Barn Staff:** Erin Marotz and Darren Strizek
- **Graduate Students:** Logan, Kirby, Kyle, Kassidy, Chad, Jason
- **Undergraduate Students:** Jade, Garrett, Brynn, Jake

44

45

Questions?

46

References

- Abebe, M. 2008. History of Ethanol 6.
- Buse, K. 2020. Characterization of protein and fat in dairy feeds and implications on digestibility and milk composition 163.
- Cesana, L., and A. Tarpo. 2021. Livestock Dairy and Poultry Outlook
- Distillers Technology Council. 2021. Distiller & Biorefinery Products- February 2021.
- Dufour, E.J. 2017. Advancing Chemical Characterization of Feedstuffs Commonly Included in Dairy Cow Rations 129.
- Goeser, J.P., and D.K. Combs. 2006. An alternative method to assess 24-h ruminal in vitro neutral detergent fiber digestibility. Journal of Dairy Science 92:3833–3841.
- Goering, H.K., and P.J. Van Soest. 1970. Forage Fiber Analysis. U.S Dept of Agriculture. Superintendent of Documents, US Government Printing Office, Washington D.C. 20402, USDA Agriculture Research Service.
- Hall, M.B. 2006. Determination of Starch, Including Maltooligosaccharides, in Animal Feeds: Comparison of Methods and a Method Recommended for AOAC Collaborative Study. Journal of AOAC INTERNATIONAL 92:42–49.
- Huhtanen, P., and A.N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science 92:3222–3232.
- Kononoff, P.J., S.K. Ivan, and T.J. Klopfenstein. 2007. Estimation of the Proportion of Feed Protein Digested in the Small Intestine of Cattle Consuming Wet Corn Gluten Feed. Journal of Dairy Science 90:2377–2385.
- Krishnamoorthy, U., T.V. Muscato, C.J. Sniffen, and P.J. Van Soest. 1982. Nitrogen Fractions in Selected Feedstuffs. Journal of Dairy Science 65:217–225.

47

References

- Krogstad, K. 2020. Effects of NDF digestibility on Lactating Jersey Cows: Observed and Modeled Performance 170.
- LaPierre, P.A., D. Luchini, D.A. Ross, and M.E. Van Amburgh. 2019. Effects of Precision Essential Amino Acid Formulation on a Metabolizable Energy Basis for Lactating Dairy Cows 11.
- Liebe, D.L., M.B. Hall, and R.B. White. 2020. Contributions of dairy products to environmental impacts and nutritional supplies from United States agriculture. Journal of Dairy Science 103:10867–10881. doi:10.3168/jds.2020-18570.
- McCafferty, J., F. Huschitt, R. Schwarck, C. Wilson, G. Cooper, and N. Bennnet. 2020. RFA Board of Directors 40.
- Mertens, D.R. 2002. Gravimetric Determination of Amylase-Treated Neutral Detergent Fiber in Feeds with Refluxing in Beakers or Crucibles: Collaborative Study 25.
- Morris, D. 2020. Energy metabolism in Jersey cows: Improving our understanding of energy requirements and utilization 309.
- Sukhija, P.S., and D.L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206.
- USDA. 2020. Milk Cost of Production estimates.
- Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74:3583–3597.

48