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Advancing Chemical Characterization of Feedstuffs Commonly Included in Dairy Cow Rations

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ADVANCING CHEMICAL CHARACTERIZATION OF FEEDSTUFFS COMMONLY INCLUDED IN DAIRY COW RATIONS

by

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Chemical composition of feedstuffs commonly included in dairy cow rations is variable within a feed type, which raises a potential for nutrient inadequacies for an animal. There are multiple methods to characterize and determine chemical composition including in vitro and in situ methods, as well as refining processing procedures to produce a consistent feed product. Feed component digestibilities can also be evaluated using similar methods.

Research reported in Chapter 2 focuses on characterizing chemical composition and determining the digestibility of fiber and protein fractions of corn dried distillers grains with solubles (DDGS) originating from seven different production sites across Michigan, South Dakota, and Nebraska. Results indicate that chemical composition of DDGS, including amino acid composition and fatty acid composition, differed significantly among sources suggesting that processing procedures are variable and frequent analysis of chemical composition should be conducted to ensure quality control. Protein digestibility was determined using the Ross et al. (2013) method, with significant differences being reported among DDGS samples in the areas of rumen-undegradable protein (RUP), RUP digestibility, and total-tract digestible protein on both a crude protein and dry matter basis. Fiber digestibility was determined through a series of varying in vitro incubation periods, as well as using the Combs (2013) method of in vitro total-tract

neutral detergent fiber digestibility. Significant differences were observed across all DDGS samples for protein and fiber digestibility estimates across production sites.

Chapter 3 describes the second experiment, focusing on quantifying the extent of microbial contamination of eleven different feedstuffs with varying chemical compositions. Feeds used in this study included alfalfa hay, bloodmeal, wet brewers grain, canola meal, citrus pulp, corn silage, corn dried distillers grains with solubles, grass hay, soybean meal, soy hulls, and SoyPass®. Samples were weighed into nylon bags and ruminally incubated for 16 hours to determine DM digestibility (DMD) and the RUP fraction. After ruminal incubation, samples were lightly rinsed, DNA was extracted, and concentrations of DNA were obtained using spectrophotometry. DNA samples were then run through a droplet digitial polymerase chain reaction procedure in order to quantify the extent of microbial contamination on each feed sample. As expected, chemical composition and DMD differed across feedstuffs. Microbial contamination upwardly biased RUP estimates and the extent of this contamination differed across feedstuffs, suggesting there may be an opportunity to increase our understanding of chemical composition of dietary components and the effect they have on the extent of microbial contamination.

DEDICATION

This thesis is dedicated to my parents,

David and Irene Dufour,

whom I cannot thank enough

for the endless love, support,

and encouragement throughout

this project, and everything else I

have strived for in my life.

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"Moo."

-Lilac & Bugs-

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INTRODUCTION

The determination of nutrients contained in feedstuffs is determined through evaluation of chemical composition (NRC, 2001). Furthermore, the availability of these nutrients to the animal are commonly estimated using either in vitro or in vivo approaches (Stern et al., 1997). Together this information may be used to balance diets and predict production responses in animals consuming those diets (Van Soest and Robertson, 1985). Methods of evaluating the feeding value of individual feeds are important, because chemical composition and nutrient availability varies both within and across feedstuffs. Corn dried distillers grains with solubles (DDGS), a major byproduct feed from the growing ethanol production industry, is popular in both beef and dairy diets but is severely variable in both nutrient composition and nutrient availability from source to source, which has led to a need to more clearly establish accurate estimates of these parameters. Distillers grains contain large proportions of neutral detergent fiber (NDF) and crude protein (CP), approximately 34 % and 31 % of DM, respectively (NASEM, 2016), making accurate estimates of digestibility and composition invaluable to the industry.

Neutral detergent fiber is a measure of total fiber in a feedstuff and includes plant cell wall structural carbohydrates, cellulose and hemicellulose, and lignin (Van Soest et al., 1991). Digestibility of NDF is an important criteria in understanding the feeding value of byproduct feeds. Recent analytical advancements attempt to predict total tract fiber digestibility from in vitro measures of NDF digestion (Lopes et al., 2015a). Practically, these methods have been most frequently used to compare the feeding values of forages but may also be useful in understanding how production source may affect digestibility of fiber of byproducts.

In dairy cattle nutrition, protein is partitioned into rumen-degradable protein and rumen-undegradable protein (RUP). One technique to measure RUP is the in situ method (Ørskov, 1982). By incubating nylon bags containing a sample of feed in the rumen for 16 h, protein disappearance can be measured. Once the bags are removed from the animal, protein content can be measured to determine RUP. A limitation to this method is rumen microbes may contaminate the sample residue and upon analysis may lead to an upward and biased estimate of RUP. Erasmus et al. (1994) reported microbial nitrogen (N) contamination, expressed as a percentage of total N for a variety of rumen-exposed feedstuffs, varied between 8 to 26 % CP. The overestimation of CP and RUP in feedstuffs varies considerably, especially in those feeds with low CP and high NDF, as microbes strongly adhere to the fiber fraction of feeds (Arroyo and Gonzalez, 2011). Thus, to account for this contamination, samples may be analyzed using microbial markers to correct for their appearance. One common microbial marker is purines but this marker lacks specificity as purines may be of both feed and microbial origin. To overcome this limitation, DNA may be used as a microbial marker and the specific sequence may be detected using droplet digital polymerase chain reactions (ddPCR) to amplify and quantify specific microbial DNA present on the feedstuffs.

Consistent and accurate feed nutrient profiles are imperative for optimizing animal performance and for ensuring the nutritional requirements of the animal are properly met. Refining current techniques and assays to predict feed quality and digestibility of feed components is critical to meet these requirements. The objectives of this work were to 1) evaluate the chemical composition and estimate availability of CP and NDF of DDGS to determine if differences between sources exist, and 2) determine if in situ estimates of RUP can be improved by accounting for microbial contamination using ddPCR assays.

CHAPTER 1

Review of Literature

Nutrient Profile of Feedstuffs

Chemical composition is variable across and within feedstuffs and is undoubtedly one, if not the most important factor, attributing to proper nutrition and maximized performance of production ruminants. Unfortunately, there are inconsistencies in composition within feeds across the entire industry; thus, there is potential to over- or under-feed energy and other nutrients which could lead to cattle morbidity, mortality, or economic loss for the producer. Luckily, these irregularities have given researchers an opportunity to refine assays and processing methods to ensure the stated nutrient profiles are valid and accurate.

Protein Digestion in Ruminants

Protein digestion in ruminant species is a complex system involving interacting mechanisms that can be difficult to describe, predict, and measure. Proteins are large molecules present in cell walls and cell contents of both plant and animal tissues where they provide a variety of functions including structure and storage. Developmental events and stresses such as water and nutrient availability, temperature, competition, and sunlight exposure can alter both the chemical and physical composition of plant cell walls, which then can alter digestibility and degradation of the cell components in the ruminant animal. These proteins differ in shape, size, solubility, and amino acid (AA) composition. Proteins contain nitrogenous compounds needed for rumen microbes to synthesize proteins, allowing for growth and proliferation (Ørskov, 1982). Ruminal protein degradatioaminon by proteolytic microbes involves two steps. First, the protein chain is broken by hydrolysis of the peptide bonds, called proteolysis. This results in the formation of peptides, which are then transported into the microbe and further hydrolyzed into

AA. Next, the AA are used by the microbes or further degraded by deamination and decarboxylation, resulting in α-keto acids to produce volatile fatty acids (VFA), ammonia (NH_3), methane (CH_4), carbon dioxide (CO_2), or can be lost in the form of heat (Ahmed Mohammed, 1982). These end products are then released back into the lumen of the rumen and can be either absorbed through the rumen wall, passed on to the lower gastrointestinal tract, or expelled via belching (Tamminga, 1979). It would be beneficial if rumen microbes only degraded protein to the extent required for their optimized cell yields, but microbes derive energy from protein degradation therefore they carry degradation as far as possible (Ørskov, 1982). The Dairy NRC (2001) refers to dietary protein as crude protein (CP), which is further defined as the percent nitrogen (N) content of the feed × 6.25, and can be described in three different categories to account for differences in protein availability and utilization (Lanzas et al., 2007). The fractions include nonprotein N (NPN) (A fraction) which is arguably the most important source of N for the rumen microbes, true protein (B fraction), and rumen unavailable N (C fraction) (Van Soest et al., 1981).

Protein Fraction 'A'. Fraction A is assumed to be fully degraded in the rumen, more specifically being called rumen-degradable protein (RDP). This fraction includes all NPN compounds including free AA, amines, amides, nucleic acids, nitrates, and small peptides (Schwab et al., 2003), which are rapidly converted to NH_4 in the rumen. Pichard (1977), and Pichard and Van Soest (1977) reported that essentially all soluble protein (SOLP) in both silages and cut forages is in the form of NPN. Grasses and legume forages contain the highest and most variable amounts of NPN, and concentrations are typically higher in silages than in hays (Schwab et al., 2003). Estimates of Fraction A as a percent of SOLP are 65 % for canola meal,

69.8 % for soybean hulls, 4.9 % for bloodmeal, 77.3 % for dry corn distillers, and 55 % for soybean meal (Sniffen et al., 1992).

Protein Fraction 'B'. Fraction B is defined as insoluble CP that is potentially degradable in the rumen depending on the competition between the rate of digestion (kd) and the rate of passage (kp). More specifically, it is described as true protein. Lanzas et al. (2007) further divides the B fraction into B_1, B_2, and B_3 fractions, which are based on their inherent rates of ruminal degradation (Sniffen et al., 1992). Firstly, the B_1 fraction contains true proteins such as albumins and globulins, and is rapidly degraded in the rumen (Sniffen et al., 1992). The B_1 fraction consists of ~ 5 % of the total SOLP, and concentrate feeds contain double the amount of B_1 protein when compared to forages (Sniffen et al., 1992). The B_3 fraction contains prolamin proteins, is insoluble in neutral detergent but soluble in acid detergent, and is closely associated with the cell wall, causing it to be slowly degraded in the rumen. Forages, fermented grains, and byproduct feeds contain significant amounts of fraction B_3 (Sniffen et al., 1992). The B_2 fraction is estimated as buffer insoluble protein minus the protein insoluble in neutral detergent. B_2 protein is either fermented in the rumen or escapes to the lower gut, and this is dependent on both the kd and the kp in the rumen (Sniffen et al., 1992).

Protein Fraction 'C'. Fraction C is assumed to be completely unavailable in the rumen (Lanzas et al., 2007, Schwab et al., 2003), and does not provide AA post-ruminally. Fraction C contains proteins associated with lignin, tannin-protein complexes, and Maillard products highly resistant to both microbial and mammalian enzymes (Sniffen et al., 1992). Estimates of Fraction

A as a percent of CP are 6.4 % for canola meal, 14 % for soybean hulls, 1.2 % for bloodmeal, 20 % for dry corn distillers, and 2 % for soybean meal (Sniffen et al., 1992).

Protein Fraction Calculations. Sniffen et al. (1992) reported the following equations to calculate the various protein fractions. They include:

PA_j (% CP) = NPN_j (% SOLP) × 0.01 × $SOLP_j$ (% CP)

PB_{1j} (% CP) = $SOLP_j$ (% CP) – A_j (% CP)

PC_j (% CP) = $ADICP_j$ (% CP)

PB_{3j} = $NDICP_j$ (% CP) – $ADICP_j$ (% CP)

PB_{2j} = 100 – A_j (% CP) – B_{1j} (% CP) – B_{3j} (% CP) – C_j (% CP);

where,

CP_j (% DM) = percentage of crude protein of the j^{th} feedstuff;

NPN_j (% CP) = percentage of crude protein of the j^{th} feedstuff that is NPN × 6.25;

$SOLP_j$ (% CP) = percentage of the crude protein of the j^{th} feedstuff that is soluble protein;

$NDICP_j$ (% DM) = percentage of the j^{th} feedstuff that is neutral detergent insoluble protein;

$ADICP_j$ (% DM) = percentage of the j^{th} feedstuff that is acid detergent insoluble protein;

PA_j (% CP) = percentage of crude protein in the j^{th} feedstuff that is nonprotein nitrogen;

PB_{1j} (% CP) = percentage of crude protein in the j^{th} feedstuff that is rapidly degraded protein;

PB_{2j} (% CP) = percentage of crude protein in the j^{th} feedstuff that is intermediately degraded protein;

PB_{3j} (% CP) = percentage of crude protein in the j^{th} feedstuff that is slowly degraded protein; and

PC_j (% CP) = percentage of crude protein in the j^{th} feedstuff that is bound protein.

This system has been updated to account for the Protein A_1 fraction, which has been previously classified as NPN in the Sniffen et al. (1992) report (Higgs et al., 2015). This protein fraction has been assumed to be completely degraded in the rumen (Lanzas et al., 2007), although more recent studies report small peptides and free AA making up 10 to 20 % (Choi et al., 2002; Velle et al., 1997) of dietary NPN flow to the small intestine, thus the Protein A_1 fraction has been redefined to NH_3 (Higgs et al., 2015).

Protein Requirements of Dairy Cattle. According to the Dairy NRC (2001), the protein required for lactation is based on the amount of protein secreted in milk. Milk protein (YProtn) is calculated as:

YProtn (kg/d) = Milk production (kg/d) × (Milk true protein / 100).

The MP requirement for lactation (MPLact) is:

MPLact (g/d) = (YProtn / 0.67) × 1000

Where 0.67 represents the efficiency of use of MP which is based off of 206 diet treatments fed to cows in early- to mid-lactation and averaging 30.9 kg/d of milk (NRC, 2001).

Microbes in the Rumen.

The anaerobic rumen microbial ecosystem is a complex network of different microbial groups living in a symbiotic relationship with the host (Kamra, 2005). The efficiency of ruminants to utilize a wide variety of feeds is due to a highly diversified rumen microbial population,

consisting predominantly of bacteria, protozoa, and fungi (Kamra, 2005; Martin, 1994), as well as viruses, although their contribution to rumen degradation and their effect on the microbial community is less understood. Both the host animal and the microbial community benefit from the other, as outlined in Table 1.1 by Millen et al. (2016). The rumen provides anaerobic conditions for the microbes, while continuously supplying the microbial populations with substrates and the removal of waste products. The microbial community has the ability to ferment otherwise indigestible complex carbohydrates such as cellulose, which is the single largest source of energy in the world if made accessible. Due to the anaerobic state of the rumen, full oxidation of microbial fermentative products cannot be achieved, thus creating partially oxidized VFA, most notably acetate, propionate, and butyrate. Once dissociated, VFA are absorbed through the rumen wall, they are further utilized by the host for energy, in which they supply approximately 70 % of the animal's energy needs (Millen et al., 2016).

Rumen Bacteria. Bacteria in the rumen are the most abundant microbe in the rumen, with a population varying between 10^8 to 10^{11} /g of rumen digesta based on the available energy in a diet (Millen et al., 2016). Kamra (2005) lists common features of rumen bacteria which include: 1) 80 to 90 % of bacteria are Gram-negative, while the remaining 10 to 20 % are Gram-positive, although this ratio is diet dependent, 2) most rumen bacteria are obligate anaerobes, although there are some facultative bacteria present which are typically associated with the rumen epithelium, 3) the optimum pH for growth of rumen bacteria lies between 6.0 and 6.9, 4) the optimum temperature is 39 °C, and 5) rumen bacteria can tolerate a high concentration of organic acids without adversely affecting their metabolism. The majority of rumen bacteria (70 %) are associated with feed particles and actively participate in digestion of feedstuffs, while a

small portion is associated with the rumen epithelium (Millen et al., 2016). Free-floating bacteria constitute a minor component (30 %) of the rumen bacterial population, and actively participate in the digestion of feedstuffs (Millen et al., 2016). In addition to supplying energy to the host in the form of VFA, rumen bacteria contribute a large proportion of the microbial CP (MCP) available for digestion and absorption through the small intestine of the host. Depending on the composition of the diet, MCP may make up 80 % of the metabolizable protein (MP) flowing out of the rumen, while RUP makes up the remaining 20 % (Castillo-Lopez, 2012).

Rumen Protozoa. Like bacteria, rumen protozoa are anaerobic although they are much larger in size relative to bacteria. Protozoa are broadly classified into flagellated and ciliated protozoa, in which flagellated protozoa are smaller in size and lesser in number compared to ciliated. Flagellated protozoa are motile due to the presence of flagella, and utilize only soluble nutrients, making their contribution to ruminal fermentation insignificant (Millen et al., 2006). Flagellated protozoa can be categorized under five genera, including *Chilomastix*, *Monocercomonas*, *Monocercomonoides*, *Pentratrichomonas*, and *Tetratrichomonas*. Ciliated protozoa have small, thin cilia that function in motility in the rumen and aid in moving feed particles to the mouth (Millen et al., 2016), where eight genera have been identified, including *Dasytricha*, *Diplodinium*, *Entodinium*, *Epidinium*, *Eudiplodinium*, *Isotricha*, *Metadinium*, and *Ophryoscolex* (Kamra, 2005). Ciliated protozoa can be sub-divided into two morphological groups, holotrichs and entodiniomorphs, depending on their morphological characteristics including ciliary arrangement, location of the nucleus, and presence or absence of a skeletal plate (Hungate, 1966). Ciliates actively participate in ruminal digestion. Holotrichs are primary users of soluble sugars including starch and pectin, while entodiniomorphs use a wide variety of

substances, including the use of cell wall carbohydrates (Millen et al., 2005). The presence of protozoa is both advantageous and disadvantageous to bacteria. Protozoa work in stabilizing ruminal pH due to consumption and slow degradation of starch granules which mitigates starch availability in the rumen. Entodiniomorphs specifically function to stabilize pH by metabolizing lactate to produce butyrate (Brossard et al., 2004). Fibrolytic bacteria are sensitive to low pH, thus the stabilization of ruminal pH by protozoa promotes fiber degradation by fibrolytic bacteria. Unfavorable interactions between protozoa and the host firstly include predation on bacteria, resulting in a potential loss of MCP synthesis, and secondly, protozoa produce relatively high amounts of hydrogen, which may increase CH_4 emission from the rumen and create energetic losses for the host. The absence of ruminal protozoa, called defaunation, can also have positive and negative impacts on the microbial community including, 1) decreased pH stabilization, 2) increased concentrations of lactic acid and propionic acid, 3) reduced methanogenesis, 4) significant increase in bacterial and fungal numbers due to decreased predation and competition, and 5) increased feed conversion efficiency on some diets, especially high roughage diets (Kambra, 2005).

Rumen Fungi. Fungi present in the rumen are the only known anaerobic fungi in nature, and function in solubilizing lignin contained in plant cell walls. Fungi produce hemicellulases and cellulases, and have the ability to solubilize the cross-linkages between hemicellulose and lignin by producing lignin modifying enzymes (Millen et al., 2016). This provides increased enzyme accessible space for fiber digestion by both fungi and bacteria. Fungi can be classified into three groups, including Group 1) Facultatively anaerobic or aerobic fungi, which consists of yeasts and molds and are transient in the rumen, Group 2) Parasite ophryoscoleid ciliates, which

are non-flagellated spore forming fungi, and Group 3) Obligately anaerobic zoosporic fungi, which play an active role in fiber digestion and have a two-stage life cycle consisting of a non-motile vegetative stage, and a motile, flagellated stage. Fungi prefer large particle diets with lignified stem tissue. Fermentation products of fungi include acetic acid, formic acid, and hydrogen. Fungi do not produce propionic acid.

Passage Rate and Digestion Rate.

When feed enters the rumen, the extent of degradation and digestion is controlled by the relationship between kp and the kd, where:

% digested = kd / (kd + kp), and

% passing = kp / (kd + kp)

This relationship affects digestibility, intake, and fermentation end-products. Rate of passage involves both the indigestible and the potentially digestible fraction of the feed, is related to feed intake and the type of feed consumed, and is influenced by particle size and shape of the feed (Russell, 2008). The limiting process for passage rate is particle size reduction, the majority of which is done via mastication during the initial intake of feedstuffs and then again during rumination. The average size of particles passing through the rumino-omasal orifice are less than 1 mm in size (Welch, 1882). Cuboidal particles such as alfalfa tend to pass through the rumen faster than long, flat particles like grass type feeds (Engels and Jung, 1998). The specific gravity of feed particles also has an influence on passage rate. Cell wall particles are denser than water but float atop the rumen mat layer which prevents particles from passing from the rumen into the omasum. Forage feed particles contain air-filled pockets which are expulsed during the rumination processes of eructation and re-mastication, increasing the specific gravity which then

drops them from the mat layer into the rumino-omasal orifice for passage (Engels and Jung, 1998). Passage rate is extremely diet dependent and can highly influence the site of digestion. According to the AFRC (1993), maintenance diets pass at 2 %/h, growing diets at 5 %/h, concentrate diets at 2 to 7 %/h, roughage diets at 1 to 6 %/h, high-yielding dairy cows at 8 %/h, and the liquid fraction between 4 to 10 %/h. Other factors involved in passage rate include pregnancy, stage of lactation, environmental factors such as temperature, the presence of ionophores, and the neutral detergent fiber (NDF) content of the diet, where increasing the indigestible NDF fraction decreases kp (Haugan et al., 2006). Rate of digestion can also be influenced by type of feed, rumination behavior, and processing methods of the feed.

Laboratory Methods of Measuring Degradability of Feed Nitrogen and Fiber.

The rumen degradability of both feed protein and feed fiber fractions can be measured using multiple methods, including in situ studies and in vitro studies, each having advantages and disadvantages when compared to the other.

In Situ Method. The in situ bag technique is a low-cost method for estimating nutrient disappearance in the rumen, making it widely used in the industry today (Stern et al., 1997). This method was originally done with silk bags, but was changed to polyester and nylon bags after discovering that silk was not fully resistant to microbial degradation (Ørskov, 1982). This technique involves filling nylon bags of known size and known porosity with a measured amount of feedstuff, followed by suspending these bags in the rumen of ruminally cannulated animals in order to measure the disappearance of nutrients at various time intervals (Stern et al., 1997). It is important to note that the suspending cord should be long enough to allow incubated bags to

move freely within the digesta, both in the liquid and solid fractions, and additional weights may be added to ensure the bags do not float atop the rumen mat. Unfortunately, this technique possesses several factors that affect disappearance estimates, thus they need to be controlled in order for the method to be standardized (Stern et al., 1997). Michalet-Doreau and Ould-Bah (1992) recommend that the pore size of the bags must be large enough for microbes to enter and access the feed, yet sufficiently small enough to limit the loss of undegraded fine feed particles. Ørskov (1982) suggests that nylon bags with mesh size 20 to 40 μm with pore sizes between 400-1600 μm^2 should be used. The size of the nylon bag relative to the sample size of the feedstuff is vital. The incubated feed must be able to freely move within the bag in order to avoid the formation of microenvironments in the bag, resulting in poor replication (Ørskov, 1982). Unfortunately, the physical nature of a feedstuff may impede particle movement in the bag. For example, corn gluten meal is a glutinous material that tends to stick to itself in the presence of moisture, thus resulting in less exposure of surface area to the microbes (Stern et al., 1983). Particle size of feedstuffs within the bag must mimic the effect of mastication, increasing the total surface area of the feed for microbial attachment (Bailey et al., 1990). The diet of the animal receiving the incubated bags should be consistent and well documented, as rumen microbial communities are extremely different for animals receiving a high concentrate diet versus a high forage diet. Some assumptions must also be taken into account. For example, when studying protein disappearance, the technique assumes that all SOLP is completely and instantaneously degraded in the rumen, and that all protein that disappears at zero time is SOLP which is rarely correct (Stern et al., 1997). Ørskov (1982) states that the most appropriate times for incubated bags to be withdrawn from the rumen in order to best describe the rate of disappearance should depend on the shape of the degradation curve with time, therefore there is

no suggested incubation time that suites all substrates. The degradation curve for protein supplements is adequate for 2, 6, 12, 24, and 36 h incubation times, whereas longer incubation times are required for more fibrous materials, and shorter incubation times are sufficient for some succulent feeds (Ørskov, 1982). The simplest form of rumen degradation of feed incubated in the rumen can be used with the assumption that the feed will begin degrading as soon as it is incubated, and that the feed does not contain a water-soluble fraction, thus in time will degrade completely (Ørskov, 1982). The equation formulated by Ørskov (1982) is as follows:

$$p = 100\ (1 - e^{-ct})$$

where,

p = amount degraded at time (t); and

c = the degradation rate for N disappearance,

One of the greatest difficulties of the in situ technique is the error by contamination of rumen incubated residues with strongly attached microbes (Gonzalez et al., 2006). Microbial contamination in incubated residues can amount to as much as 95 % of the residual N, and microbial DM can account for up to 22 % of residual DM (Olubobokun et al., 1990). One way to attempt to correct for this microbial contamination is via the washing procedure once incubated bags are removed from the rumen. Unfortunately, relatively little progress has been made during the last 70 years, and there is no industry wide protocol for the washing of nylon bags. Vanzant et al. (1998) conducted a literature survey and found that 70 % of 63 studies reported rinsing nylon bags in tap water either until rinse water ran clear, rinsed for a defined time interval, or rinsed until a certain number of rinse cycles (water changes) were used. The other 30 % used machine rinsing to rinse their bags. Paine et al. (1982) evaluated the effects of hand- vs machine-rinsing incubated bags on N and DM disappearance and found greater amounts of N and DM

washed out of the bags during the machine procedure. Coblentz et al. (1997) reported that N concentration decreases following a first order kinetic model with increased amounts of machine rinses. They also found that increasing the rumen incubation time caused greater initial concentrations and greater rates of decline in concentration of N.

In Vitro Method. The two-step in vitro laboratory assay was developed by Tilley and Terry (1963), and involves two-stages in which forages are subjected to a 48 h fermentation in a buffer solution containing rumen fluid, followed by a 48 h digestion with pepsin in an acid solution (Getachew et al., 1998). Since then, countless modifications have been made to improve the accuracy and precision of the in vitro procedure. Currently, in vitro procedures can be used to determine a variety of feed component digestibilities and disappearances including DM digestibility, starch disappearance, NDF digestion, protein digestion, NH_3, N release, and gas production. Calsamiglia and Stern (1995) developed a three-step in vitro procedure for estimating the intestinal digestibility of RUP in a feed fraction. The procedure consists of 1) incubating ruminally undegraded feed residues for 1 h in 0.1N HCl solution containing 1 g/L of pepsin, 2) neutralizing the mixture with 1N NaOH and a pH of 7.8 phosphate buffer containing pancreatin followed by a 24 h incubation, and 3) precipitation of undigested proteins with a 100 % (wt/vol) trichloracetic acid solution (Calsamiglia and Stern, 1995; NRC, 2001). Calsamiglian and Stern (1995) reported a strong correlation ($r = 0.91$) with in vivo estimates of intestinal CP digestion of undegraded feed residues. Although widely used, in vitro techniques appear to have disadvantages, such as each measurement gives only one observation, the technique does not account for absorption, the technique does not provide information on kd, and the residue determination destroys the sample therefore a large number of replicates are needed (Getachew

et al., 1998). Dewhurst et al. (1995) conducted a study comparing in situ and in vitro techniques for estimating the extent of rumen fermentation on a range of plant-derived dietary ingredients. The in vitro technique used followed that of Tilley and Terry (1963), with incubations being stopped at 2, 4, 8, 24, and 48 h by the addition of saturated mercuric chloride solution. The in situ method involved weighing 10 g (DM basis) of feed into porous synthetic fiber bags and ruminally incubating for periods of 2, 4, 8, 12, 18, 24, 48, and 72 h. Results showed significant differences in organic matter (OM) digestibility, with in situ treatments showing higher digestibilities across all feeds aside from soy hulls which showed similar OM digestibilities between both in situ and in vitro methods. Dewhurst et al. (1995) concluded the in situ method overestimates fermentability, and the overestimation is strongly related to the carbohydrate composition of the feeds, especially at short incubation times. They also identified a problem with potential loss of indigestible materials from the incubated bag.

Microbial Contamination.

A popular research topic in ruminant nutrition is the estimation of MCP synthesis in the rumen, as MCP supplies the majority of MP available to the host. Methods to estimate MCP synthesis include using real time polymerase chain reaction (PCR) nucleic acids, diaminopimelic acid (DAPA), aminoethylphosphonic acid, ATP, total purines (Obispo and Dehority, 1999), as well as isotopes such as labeled sulfur (S), and N (Bates et al., 1985). Dehority (1995) suggests that the ideal microbial marker should, 1) be absent in feed, 2) be unabsorbed, 3) be biologically stable, 4) have a simple assay procedure, 5) occur in a similar percentage between types of microbes, 6) be a constant percentage of the microbial cell in all growth stages, and 7) all forms should flow at a similar rate. A limitation of measuring protein degradability and RUP content using the in

situ technique is that of microbial contamination. Contamination of in situ samples may upwardly bias estimates of RUP of feedstuffs. Because of the potentially high instance of microbial contamination, researchers can use modified MCP markers to quantify the degree of microbial contamination. Alexandrov (1998) conducted a study to determine errors associated with bacterial contamination of feed residues using DAPA as a marker in five feedstuffs with different physical and chemical characteristics, including alfalfa hay, wheat straw, maize, sunflower meal, and fish meal. The research showed that bacterial DM as a percent of total residue DM and bacterial CP as a percentage of total CP increased linearly with increasing in situ ruminal incubation of the feeds (Alexandrov, 1998). Bacterial DM as a percentage of total DM of residues in bags was 1.0 to 6.2 %, 2.8 to 6.7 %, 5.6 to 13.6 %, 3.8 to 16.7 %, and 4.4 to 28.6 % for maize, fish meal, alfalfa hay, sunflower meal and wheat straw, respectively (Alexandrov, 1998). Beckers et al. (1993) conducted a similar study with the goal to quantify bacterial contamination using the N isotope as a marker in three feedstuffs, including soybean meal, meat and bone meal, and wheat bran. Rumen bacterial N in meat and bone meal showed no differences of N concentrations between samples corrected and uncorrected for microbial contamination, while rumen bacterial N increased from 15 to 57 % N as a percent of feed residue CP over a 48 h incubation for wheat bran (Beckers et al., 1993). Bacterial N in soybean meal residues increased from 8 to 33 % N as a percent of feed residue N (Beckers et al., 1993). Erasmus et al. (1994) reported microbial N contamination, expressed as a percentage of total N for a variety of rumen-exposed feedstuffs varied between 8 to 26 %. The overestimation of CP and RUP in feedstuffs varies considerably, especially in those feeds with low CP and high NDF, as microbes strongly adhere to the fiber fraction of feeds (Arroyo and Gonzalez, 2011). The following are methods that can be used to estimate and quantify microbial contamination.

Polymerase Chain Reaction (PCR). Real-time PCR is a highly sensitive method that can be used for the detection and quantification of microbial populations without the need to cultivate those populations using anaerobic processes and environmental samples (Yu et al., 2005). This technique is advantageous, as it allows for rapid detection of microbial DNA and allows a large number of samples to be processed simultaneously.

Compared to conventional quantitative PCR methods employing a forward and reverse primer and a dye, an additional fluorescent probe is required in probe-based real-time PCR (Castillo-Lopez, 2009). Forward and reverse primers are oligonucleotides, both containing 20 to 24 base pairs, where the 5' end of the forward primer anneals to the 3' end of the target amplicon, and the 3' end of the target amplicon is complimentary to the 5' end of the reverse primer (Yu et al., 2005). Real-time PCR also involves a fluorescent Taqman probe described by Wilhelm and Pingoud (2003) as an oligonucleotide designed with a high energy dye called a 'reporter' at the 5' end, and a low molecule dye deemed a 'quencher' at the 3' end. The fluorescent probe is intact and excited by a light source, which can be suppressed by the close proximity of the reporter and quencher. The final component to real-time PCR is the polymerase enzyme which is used widely for PCR as it 1) can generate new strands of DNA using a DNA template and primers, and 2) they are heat resistant (Valasek and Repa, 2005). Droplet digital PCR (ddPCR) is a method for performing digital PCR that is based on water-oil emulsion droplet technology. Amplification occurs in each DNA droplet, which allows for rapid microfluidic analysis of thousands of droplets per sample (Hindson et al., 2013).

Amplification of the target amplicon is performed by temperature cycling. Holland et al. (2001) describes real-time PCR temperature cycling, which begins with a high temperature (95 °C) being applied to separate the strands of double helical DNA. The temperature is then lowered

to 60 °C to let forward and reverse primers anneal to the template. Finally, the temperature is set to 72 °C, which is optimum for the polymerase that extends the primers by incorporating deoxy-nucleotide-triphosphates (dNTP). This temperature cycling is also performed when using the ddPCR method. The amount of product doubles with every cycle, creating an exponential increase with increasing cycles. As the product increases, it develops a signal, thereafter the signal levels off and saturates. The signal saturation is due to the reaction using up a needed component, which may be primers, reporters, quenchers, or dNTPs. The number of cycles required to reach this fluorescence threshold is called the CT value, which can be compared to standards to determine concentrations of bacterial N (Holland et al., 2001). Droplet digital PCR has the capability to obtain absolute quantification without external references and standards (Hindson et al., 2013), making it advantageous over real-time PCR. Figure 1.1 depicts the process of real-time PCR (Valasek and Repa, 2005).

Purines. Zinn and Owens (1986) described the purine procedure as different from analytical procedures involving DNA and RNA, as those methods involve the measurement of either intact polymers or component sugars, but not the purines. By combining the purine procedure, involving silver nitrate which precipitates free purines, with the hydrolysis procedure developed by Marshak and Vogel (1951), Zinn and Owens (1986) were able to develop a rapid procedure for separation and quantification of purines which can be applied to estimating microbial contamination of in situ residues. Table 1.2 describes the laboratory protocol for rapid assay of purine content of feed and digesta described by Zinn and Owens (1986). Purines have a high nucleic acid content of adenine and guanine bases, which are abundant in microbes and low in feeds.

Assumptions for the purine procedure include, 1) all dietary purines are degraded in the rumen, 2) all microbes have a constant purine:N ratio, and 3) all duodenal purines are of microbial origin (Zinn and Owens, 1986). Unfortunately, like all marker procedures, limitations to the purine method exist and must be accounted for including, 1) purine:N ratio varies with bacterial growth and digesta components, 2) purines are found in protozoa, 3) microbial nucleic acids can be ruminally degraded, 4) nucleic acids are present in some feedstuffs including bloodmeal and fishmeal, and 5) purines are present in animal tissue such as sloughed rumen epithelial cells (Zinn and Owens, 1986). Values reported for purines in mixed ruminal bacteria vary widely. Clark et al. (1992) found bacterial purine concentrations ranging from 2.4 % to 13.02 %. Obispo and Dehority (1999) reported considerable variation of purine concentrations as a percent of DM, ranging from 0.69 to 5.57 %. Thus, it is important to understand assumptions and limitations when conducting samples for purine analysis.

Carbohydrate Digestion in Ruminants.

The Dairy NRC (2001) describes carbohydrates as the major source of energy in diets fed to dairy cattle, and are usually included at 60-70 % of the total diet. Carbohydrates can be classified according to degradation rate (Sniffen et al., 1992), and are also sub-divided into 2 categories consisting of structural carbohydrates and nonstructural carbohydrates (NSC). Total carbohydrate concentration of a feedstuff can be calculated by difference of the total CP, fat, and ash contents using the equation, 100 – CP – Fat – Ash (Sniffen et al., 2002).

Carbohydrate Fraction 'A'. Fraction A consists of sugars, short oligosaccharides, silage acids and organic acids and are rapidly fermented by ruminal microorganisms as an energy

source, although the sugar content of most dairy diets is normally low unless sugar byproduct feeds or fresh, lush grasses are fed (Sniffen et al., 2002). Fraction A is solubilized in neutral detergent.

Carbohydrate Fraction 'B'. Fraction B can be divided into 2 sub-fractions, B_1 and B_2. Fraction B_1 contains starch and pectin, which fall under NSC (Sniffen et al., 1992). Pectin is a complex monosaccharide rich in polygalacturans. Legume forages, seed products, citrus pulp, and beet pulp all contain significant amount of pectin, which are rapidly fermented in the rumen (Sniffen et al., 1992). As a percent of nonfibrous carbohydarate (NFC), beet pulp contains 65 % pectin and 2 % starch, alfalfa silage contains 33 % pectin and 25 % starch, and grass hay contains 49 % pectin and 15 % starch (NRC, 2001). Starch is the main storage carbohydrate in plants and has an abundant concentration in cereal grains (NASEM, 2016). Processing cereal grains can have a large impact on starch digestibility. For example, dry-rolled corn (DRC) has a ruminal starch digestibility of 76 %, and a total tract starch digestibility of 96 %, compared to steam-flaked corn, which has a ruminal starch digestibility of 90 % and total-tract starch digestibility of 99 %, compared to high-moisture corn that has a ruminal starch digestibility of 92 % and a total tract starch digestibility of 99 % (Richards et al., 2002). Fraction B_2 is available cell wall, slowly fermented by bacteria requiring NH_3 as their N source, and can be determined by subtracting fraction C from ash-free NDF that has been corrected for associated protein (Sniffen et al., 1992). Fraction B_1 is fermented by fast growing bacteria that utilize either NH_3 or peptides as a N source (Russel et al., 1992).

Nonstructural carbohydrates contain the A and B Fractions of carbohydrates and are a major source of energy for lactating dairy cattle as they are readily fermented by rumen microbes

which causes an increase in MCP synthesis and in turn supplies more N to be absorbed by the host animal. The concentration of NSC is commonly determined as the sum of sucrose, glucose, fructose, and starch, or as the difference between total DM and NDF of the feedstuff (Fox et al., 2004; Pelletier et al., 2010). Increased concentrations of NSC in a forage decrease both the acid detergent fiber (ADF) and NDF fractions. Starchy NSC are generally increased in the diet to meet the energy demands of high producing cattle (NRC, 2001). The concentration of NSC within forage species is variable and can alter due to environmental growth conditions and harvest management. Pelletier et al. (2010) conducted a study in which they grew eight forage species under the same growth conditions, and harvested them either in the AM or the PM and compared NSC and nutritive value within species. They found that the concentration of NSC of all species increased with PM cutting, although the extent of the increase varied among forage species. They also found a significant increase in in vitro true digestibility, suggesting that both species selection and cutting time can be used to increase forage NSC concentrations (Pelletier et al., 2010). The optimal concentration of NSC in diets of high producing dairy cows are related to 5 components including 1) the effects of rapidly degradable starch on ruminal fiber digestion, 2) the amount of NSC that replaces NDF in the diet, 3) the site of starch digestion, 4) DMI and physiologic state of the animal, and 5) processing and conservation methods used to alter rate and extent of NSC digestion (NRC, 2001). Nocek and Russel (1988) suggested that 40 % dietary NFC was optimal for lactating dairy cows from an evaluation based on alfalfa silage, corn silage, and a 50:50 blend of alfalfa:corn silage. MacGregor et al. (1983) conducted a study in which they fed early lactation cows concentrate:silage diets at a 60:40 blend. The first diet contained 24.9 % NSC, while the second treatment diet contained 32.9 % NSC, both diets having similar

CP, soluble N, and ADIN content. Results showed that cows receiving the high NSC diet had a greater DMI, greater milk yield, and greater net energy for lactation (MacGregor et al., 1983).

Carbohydrate Fraction 'C'. Fraction C is unavailable to rumen microbes and includes fiber bound lignin. Lignin is insoluble in sulfuric acid (H_2S) and acid detergent, and contains ester linkages between the phenolic acids and polysaccharide chains that limits bacterial adherence and digestion. Lignin can be divided into core and non-core lignin. Core lignin is linked to the plant cell wall carbohydrates via esters of ferulic acid. Non-core lignin is considered an extractable phenolic, composed of p-coumaric and ferulic acid esters cross-linking between lignin and structural carbohydrates. Legumes tend to have a higher lignin concentration than grasses, although this can greatly vary depending on maturity of the plant type. According to the NASEM (2016), the lignin concentration of various feeds includes 6.8 % for alfalfa hay, 11.0 % for almond hulls, 8.8 % for canola meal, 3.2 % for corn silage, 5.0 % for DDGS, and 7.4 % for wheat straw.

Sniffen et al. (1992) reported the following equations to calculate the various carbohydrate fractions. They include:

$$CHO_j\ (\%\ DM) = 100 - CP_j\ (\%\ DM) - Fat_j\ (\%\ DM) - Ash_j\ (\%\ DM)$$

$$CC_j\ (\%\ CHO) = 100 \times (NDF_j\ (\%\ DM) \times 0.01 \times Lignin_j\ (\%\ NDF) \times 2.4) / CHO_j\ (\%\ DM)$$

$$CB_{2j}\ (\%\ CHO) = 100 \times ((NDF_j\ (\%\ DM) - NDIP_j\ (\%\ CP) \times 0.01 \times CP_j\ (\%\ DM) - NDF_j\ (\%\ DM) \times 0.01 \times Lignin_j\ (\%\ NDF) \times 2.4) / CHO_j\ (\%\ DM)$$

$$CNSC_j\ (\%\ CHO) = 100 - B_{2j}\ (\%\ CHO) - C_j\ (\%\ CHO)$$

$$CB_{1j}\ (\%\ CHO) = Starch_j\ (\%\ NSC) \times (100 - B_{2j}\ (\%\ CHO) - C_j\ (\%\ CHO)) / 100$$

$$CA_j\ (\%\ CHO) = (100 - Starch_j\ (\%\ NSC)) \times (100 - B_{2j}\ (\%\ CHO) - C_j\ (\%\ CHO)) / 100$$

where,

CP_j (% DM) = percentage of crude protein of the j^{th} feedstuff;

CHO_j (% CHO) = percentage of carbohydrate of the j^{th} feedstuff;

Fat_j (% DM) = percentage of fat in the j^{th} feedstuff;

Ash_j (% DM) = percentage of fat in the j^{th} feedstuff;

NDF_j (% DM) = percentage of the j^{th} feedstuff that is neutral detergent fiber

$NDIP_j$ (% DM) = percentage of neutral detergent insoluble protein of the j^{th} feedstuff;

$Lignin_j$ (% NDF) = percentage of lignin of the j^{th} feedstuff's NDF;

$Starch_j$ (% NSC) = percentage of starch in the nonstructural carbohydrate of the j^{th} feedstuff;

$Sugar_j$ (% NSC) = percentage of sugar in the nonstructural carbohydrate of the j^{th} feedstuff;

CA_j (% CHO) = percentage of carbohydrate of the j^{th} feedstuff that is sugar;

CB_{1j} (% CHO) = percentage of carbohydrate of the j^{th} feedstuff that is starch and nonstarch polysaccharide;

CB_{2j} (% CHO) = percentage of carbohydrate of the j^{th} feedstuff that is available fiber; and

CC_j (% CHO) = percentage of carbohydrate in the j^{th} feedstuff; that is unavailable fiber.

Carbohydrate and protein fractions of feed can be summed to determine intake of each fraction (Sniffen et al., 1992).

Total Tract NDF Digestibility. The digestibility of NDF is more variable than the digestibility of any other feed component, and can profoundly affect intake and milk production in the dairy industry (Combs, 2017). Fiber digestion is affected by both plant and animal

characteristics. Plant characteristics include plant maturity at harvest, soil quality, nutrient availability, water stress, plant population, and precipitation. Plant maturity has the biggest impact on forage quality and digestibility. Animal characteristics affecting forage digestibility include animal age, breed, diet, intake, microbial community present in rumen, heat stress, pregnancy, lactation, and energy requirements. Fiber digestion is also heavily influenced by kp and kd, which are confounding factors. Researchers at the University of Wisconsin-Madison have developed an in vitro assay and model, which uses measures of the proportion of potentially digestible (pdNDF) and k_d to predict total tract NDF digestibility (TTNDFD) (Lopes et al., 2015a). The TTNDFD model predicts fiber digestion of alfalfa, corn silage, and grass forages, in cattle and has been validated against directly measured NDF digestibility in lactating dairy cattle (Combs, 2013). Total tract NDF digestibility values can be used not only to predict in vivo fiber utilization, but also predict forage digestible energy (DE), net energy (NE), and total digestible nutrients (TDN) by accounting for pdNDF, kd, kp, and hindgut fermentation of NDF (Combs, 2013). Lopes et al. (2013) compared in vivo TTNDFD and in vitro TTNDFD of corn silage and alfalfa hay and found they were similar. In vivo TTNDFD of alfalfa hay was reported as 43.8 %, compared to in vitro TTNDFD of 45.0 %. In vivo TTNDFD of corn silage was reported as 38.3 %, compared to in vitro TTNDFD of 38.0 %. Combs (2013) outlined guidelines and limitations to the TTNDFD method which include:

1) TTNDFD is intended to evaluate NDF digestibility of feeds and rations in animals fed an otherwise balanced diet. Inadequacies or excesses of other dietary components or nutrients other than fiber are not accounted for.
2) TTNDFD can be used to compare fiber utilization across forage or fiber sources. For example, fiber digestibility of alfalfa can be compared to fiber digestibility of corn silage, grass, or byproduct feeds.

3) TTNDFD does not account for differences in physical form of forages, influencing effective fiber.
4) TTNDFD estimates total tract fiber digestibility of a dairy cow consuming approximately 54 lbs/d of DM.
5) In vitro NDF digestibility values at specific time points should not be used as a single indicator to compare fiber digestibility of forages. These values do not factor in indigestible fiber or NDF concentration. Single time NDF digestibility values are poorly correlated to TTNDFD.
6) Total NDF and TTNDFD must be considered when comparing forages for quality.

Corn Milling Industry.

Corn-based ethanol production has surged over the last three decades, as depicted in Figure 1.2 (Liu, 2011), and is poised to continue expanding. Byproducts from ethanol production have become major feedstuffs used in feeding cattle, providing them with a readily available and highly digestible starch source. Bothast and Schlicher (2005) stated that corn is the most important and economical source of starch in the US, of which starch comprises 70 to 72 % (DM basis) of the corn kernel weight. Starch is converted into glucose in the rumen, which then converts to available energy for the ruminant animal. There are currently two main types of milling processes which includes dry milling to primarily produce distillers grains with solubles, and the wet milling process to produce a multitude of feedstuffs including corn gluten feed.

Wet Corn Milling Process. The conventional process of corn wet milling is designed to recover and purify starch and other byproducts including germ, gluten meal, fiber, corn gluten feed, and steep liquor. The process is divided into six main sections, as seen in Figure 1.3 including (1) grain handling, (2) steeping, (3) germ separation and recovery, (4) fiber separation and recovery, (5) gluten separation and recovery, and (6) starch washing and recovery (Ramirez

et al., 2008). In short, clean corn grain is steeped in a dilute, aqueous sulfur dioxide solution for 24 to 48 h, which causes a breakdown of the cytoplasmic matrix triggering a release of starch granules and other grain components to form a slurry (Haros et al., 2003). Light steepwater (4 to 8 % solids) is classified as the material remaining after the steeping process, and can be further concentrated via evaporation into heavy steepwater (35 to 40 % solids). Steepwater solids contain water-soluble proteins originating from the corn germ and other corn components (Rausch and Belyea, 2006). The heavy steepwater contains 45 to 50 % protein, where much of the N portion is in the form of AA (Christianson, 1965). This starchy slurry contains low-density high oil corn germ, and therefore can be separated from the remaining components through a series of coarse grinds to crack the corn kernels, which then flow through centrifuges where the difference in density and particle size during germ separation allows for proper germ recovery (Ramirez et. al., 2008). Corn oil, the most valuable portion of the corn kernel, can then be separated from the germ at an extraction plant and sold for $0.40 to $0.50 /lbs. Germ meal is defined as the protein fraction of fiber and is also produced from this extraction process, which is a high protein feed (20 to 22 % CP) and has about the same energy content of DRC. This germ meal can be sold as a feed ingredient mainly to be used in poultry/egg-layer diets or dairy diets, or can be added to other gluten feed products. The degermed components are further separated through centrifuges and screens into gluten, starch, and fiber. The starch portion can be sent out as dry starch to the animal feed industry, or can be converted to dextrose which serves as an intermediate for many products including high-fructose corn syrup which is a popular sweetener in the human food industry. The starch can also be fermented and made into ethanol, condensed distillers solubles (CDS), or sent as over-the-fence products of the distilling industry. The gluten portion is dried and becomes corn gluten meal which is a high protein source and is consistent in

AA profile. Corn gluten meal is popular in both the pet food and egg-laying industry and can be sold into these industries, or enzymes can be used to remove residual starch to create Empyreal, a product that contains ~80 % protein. The fiber and fiber-bound starch and gluten are finely ground and washed through a series of tanks and fiber wash screens to free starch and gluten while minimizing fiber breakup. The cleaned fiber is then dewatered to a final moisture of 60 %, and then combined with the concentrated steep liquor portion, dried to 10 % moisture and sold as dry corn gluten feed (DCGF) to the livestock industry as a high-protein animal feed (Bothast and Schlichler, 2005; Ramirez et al., 2008). Dry corn gluten feed has a chemical composition of 88.92 % DM, 22.64 % CP, 72.8 % TDN, 35.05 % NDF, 11.18 % ADF, 1.86 % lignin, 3.32% fat, 37.10 % RUP (NASEM, 2016), 85 % RUP digestibility, and is also a valuable source of first-limiting AA methionine in dairy diets at 1.76 % CP (NRC, 2001). Wet corn gluten feed (WCGF) is a mixture of wet bran, heavy corn oil, and germ meal and is a readily available non-forage source of fiber (Boddugari et al., 2001) containing 43.76 % DM, 21.70 % CP, 86.0 % TDN, 38.53 % NDF, 11.78 % ADF, 1.60 % lignin, 4.29 % fat, and 34.11 % RUP (NASEM, 2016). SweetBran is a branded WCGF-like product produced by Cargill containing heavy steep water, germ meal, CDS and dried bran with a chemical composition of 60.07 % DM, 23.76 % CP, 89.0 % TDN, 26.75 % NDF, 9.79 % ADF, and 4.65 % fat (NASEM, 2016).

Wet Corn Gluten Feed Inclusion in Dairy Diets. With the increase in popularity of the wet and dry corn milling industries, more and more byproducts have been incorporated into both beef and dairy diets. Kononoff et al. (2006) conducted a study where they fed Holstein cattle a control diet or a WCGF treatment (38 % of diet on a DM basis) during either lactation or the dry period. Their results showed that cows consuming WCGF during lactation consumed more feed

compared with the control (25.4 vs 21.2 kg/d). They also found that milk production was 4 kg higher, and total milk protein yields were 0.15 kg/d more for cows consuming WCGF when compare to the control group, although the consumption of WCGF during the dry period did not influence lactational performance (Kononoff et al., 2006). Similar results were observed in a study by VanBaale et al. (2001), where they replaced a mix of alfalfa hay, corn silage, and corn grain with WCGF at different inclusions. They found that cows fed 20 % WCGF (DM basis) consumed more DM and produced more energy-corrected-milk (ECM), and had higher milk protein and lactose yields than control cattle (VanBaale et al., 2001). Interestingly, both studies found that including WCGF in the diet resulted in significant reductions in the concentration of milk fat, although total milk fat yield was not different from control diets. Results from Kononoff et al. (2006) indicate that diets for lactating dairy cows may be formulated to contain as much as 37.5 % WCGF on a DM basis.

Dry Corn Milling Process. One of the main goals of the biofuel production industry, specifically the ethanol industry, is to produce fuels from grain sugars with optimal fuel properties such as corn, sorghum, wheat, or a combination of grains dependent on both grain price and grain availability (Kang and Lee, 2015). The process begins, as seen in Figure 1.4, by grinding the whole grain into a coarse flour or meal through a hammer mill to pass through a 30 mm mesh screen, and then soaked with water containing added enzymes, N, minerals, and lactic acid producing microbes to convert the grain starch to sugar, specifically dextrose, which is chemically identical to glucose, in a mash. Each bushel of corn generates about 22 gallons of mash (Bothast and Schichler, 2005). Ammonia is added to the mash for pH control and to act as a nutrient source for the yeast. The mash is then cooked to hinder bacterial growth, and placed in

a fermentation tank once cooled. Ethanol is a 2-carbon primary metabolite whose production is coupled with the growth of yeast cells, specifically *S. cerevsiae* and *Z. mobilis,* thus a strain of yeast is added to the mash which initiates glycolysis to produce 3-carbon pyruvate molecules from 6-carbon glucose molecules (Bai et al., 2008). These pyruvate molecules are then further reduced to 2-carbon ethanol with the release of CO_2. This process occurs in a distillation column where the ethanol is captured, and whole stillage remains. Whole stillage is a wet product consisting of 90 % water and 10 % feed, which is centrifuged to remove the water resulting in wet grains and thin stillage. Wet grains, also known as wet distillers grains (WDG) is a very dense mash having ~31 % DM, and can be sold as-is or dried further into modified distillers grains (MDG, ~48 % DM), or dried distillers grains (DDG, ~90 % DM) (NASEM, 2016). The thin stillage contains about 95 % water and goes through a multi-stage evaporator to produce CDS, also commonly known as syrup having a DM of ~30 % which is variable depending on the efficacy of the evaporator. The chemical composition of condensed corn distillers solubles (CCDS) specifically, is 30.89 % DM, 18.94 % CP, 16.85% fat, 4.71 % NDF, 3.81 % ADF, 9.11 % ash, 0.11 % Ca, and 1.52 % P as reported by the NASEM (2016). The CDS can be sold as-is to the liquid feed industry, can be added to the distillers grains at various amounts to produce wet distillers grains with solubles (WDGS), dried together to produce DDGS, or added onto dried WDGS to produce modified dried distillers grains (MDGS**)** which has become increasingly popular within the last decade. The condensed chemical compositions of DDGS, MDGS, and WDGS listed in Table 1.3 are highly inconsistent due to differences in dry milling techniques across and within ethanol plants, and due to unregulated quality standards and unspecified amounts of CDS to be added back to the distillers grains. The handling of solubles is highly variable by production facility. Due to its high fiber content, DDGS are mainly used as a feed

ingredient in both beef and dairy diets and is a low valued byproduct when compared to byproducts produced from the wet milling industry (Murthy et al., 2009). Liu (2011) conducted a review of gross chemical composition of DDGS from different plants, different years (1993, 2002, 2004, and 2008), and different sources as reported from a multitude of studies and observed ranges of 87.2 to 92.7 % DM, 25.8 to 33.3 % CP, 3.7 to 8.1 % ash, 33.1 to 49.1 % NDF, 11.4 to 20.8 % ADF, 2.64 to 9.9 mg/g P, and 0.05 to 7.1 mg/g Ca DM basis.

Sulfur. Sulfur is a mineral found in several AA including methionine, cysteine, homocysteine and taurine, in the B-vitamins biotin and thiamin, as well as in a number of other organic compounds (NASEM 2016), and makes up about 0.15 % of a ruminants body weight (NRC, 2001). It is essential that thiamin, biotin, and methionine are provided in cattle diets, as the animal cannot synthesize these vitamins and AA on their own therefore they must either be fed or synthesized by microbes in the rumen (NRC, 2001). Ruminal microorganisms have the ability to synthesize necessary inorganic (sulfate) and organic S-containing AA compounds required by the animal but S is initially required for microbial growth and regular microbial cellular metabolism, thus dietary requirements of S in the animal is to provide necessary substrate for maximal MCP synthesis (NASEM, 2016; NRC, 2001). Sulfur incorporated into MCP is absorbed through the small intestine as methionine and cysteine. Nonprotein N such as urea is commonly used in ruminant diets that are high in RUP in order to meet RDP requirements. As a NPN source, urea does not contain AA, specifically S-containing AA, thus, there is a potential for S deficiencies to occur when cattle are fed low RDP, among other deficiency risks. Mature forages, corn silage, and forages grown in S-deficient soils can be low in S (NASEM, 2016). Sulfur deficiencies are uncommon when well-balanced diets are fed, but

deficiencies can decrease feed intake, digestibility, and MCP synthesis as well as cause excessive salivation, weakness, or death (Starks et al., 1953). Excess dietary S has been shown to reduce the absorption and bioavailability of other minerals, specifically selenium, which is an important trace mineral of the cellular antioxidant system, and copper, an important mineral involved in several biologic processes including electron transport during aerobic respiration, the transport of iron for hemoglobin synthesis, and phagocytic cell function (NRC, 2001). Ivancic Jr. and Weiss (2001) conducted a study in which they found increasing dietary S from 2.1 to 7.0 g S/kg of diet linearly decreased plasma Se and apparent absorption of Se in lactating dairy cows. According to the NASEM (2016), S toxicity has historically been a non-issue, as it was considered a non-toxic mineral until 1956 when Adams et al. (1956) discovered that excessive S can cause polioencepahlomalacia (PEM), a neurologic disorder in ruminants. As free form S is released in the rumen, it combines with hydrogen gas to produce gaseous H_2S. Sulfuric acid is then released through the mouth as the ruminant eructates for further feed mastication, and is inhaled through the nasal cavity, transported into the lungs, and is absorbed into the bloodstream causing PEM. The National Research Council (1996) reported that the maximum tolerable S concentration was 0.40 % for potential occurrence of PEM. As H_2S is used to clean holding tanks in the dry milling process, there is concern of increased S concentrations in distillers grains and other dry milling byproducts. Buckner et al. (2011) randomly sampled S concentrations in WDGS and MDGS from 6 ethanol plants across Nebraska, and found that the S content from plant to plant ranged from 0.71 to 0.84 % DM, with average S content across the 6 plants being 0.77 % DM basis, which is greater than the 0.40 % reported by the NRC (1996).

Dry Milling Innovation. Ethanol companies are refining dry milling processes to improve efficiency of ethanol production, as dry mills produce less valued byproducts than wet mills (Rajagopalan et al., 2005). In a conventional dry mill, energy is used in jet cooking and liquefaction of dextrins to over 90 °C for 1 to 2 hrs using liquefaction enzymes (Wang et al., 2007). The dextrins are then hydrolyzed into fermentable sugars using saccharification enzymes during simultaneous saccharification and fermentation (SSF) (Wang et al., 2007). Recently, a raw starch hydrolyzing (RSH) enzyme has been developed which can convert starch into dextrins at under 48 °C, which is significantly cooler than conventional dry milling processes. The RSH enzyme can also hydrolyze dextrin into fermentable sugars during SSF (Wang et al., 2007). Robertson et al. (2006) reported that the decrease in temperature using RSH enzymes in ethanol production causes a 10 to 20 % decrease in energy used. With the removal of the high temperature steps, corn grains are less exposed to conditions prone to cause heat damage. Therefore, the protein within the byproducts from this alternative milling process may be more readily available in the digestive tract of ruminant animals (Kelzer, 2008). Kleinschmidt et al. (2006) reported that generally these corn byproducts are of higher quality and contain more nutritional value than that of the conventional dry milling process. Additional innovations to dry milling includes ethanol production from cellulosic feedstuffs of, or in addition to, corn and wet grains (Tyner, 2008), as well as centrifuging the cracked corn germ prior to grinding in order to maximize the quantity of corn oil collected, and decreasing the fat content of the end product.

Distillers Grains Inclusion in Dairy Diets. Corn dried distillers grains with solubles have historically been included at 10 % of the diet DM of dairy cows due to its high fat content, which typically ranges from 10 – 12 % (Janicek et al., 2008). This dietary fat can negatively affect fiber

digestion (Van Soest, 1994) as unsaturated fatty acids are toxic to the microbial community, and may also contribute to milk fat depression (Pantoja et al., 1994). As grain markets have changed and byproducts of the ethanol industry have become cheaper than corn, there has been a significant amount of research done on feeding lactating dairy cows more than the usual 10 % inclusion of DDGS. Janicek et al. (2008) conducted a study to evaluate the effects of feeding increasing amounts of DDGS on both the feed intake and milk production of Holstein dairy cows. They fed treatment groups either 0 %, 10 %, 20 %, or 30 % DDGS, replacing a portion of both forages and concentrates and found that DM intake (DMI) and milk production increased linearly with increasing concentrations of DDGS (21.4, 22.4, 23.0, and 24.0 kg/d DMI, 27.4, 28.5, 29.3, and 30.6 kg/d milk). Another study conducted by Nebchaar et al. (2013) studied the effects of increasing inclusion of DDGS from 0 %, 10 %, 20 %, and 30% on and CH_4 production, ruminal fermentation, N balance, and milk production in lactating Holstein dairy cows. They found that DMI increased linearly, whereas apparent-total tract digestibility of DM and gross energy declined linearly as DDGS inclusion increased. Milk yield also increased linearly, whereas milk fat composition, milk protein composition, and CH_4 production decreased linearly with increasing inclusions of DDGS (Benchaar et al., 2013). With the benefits in milk production and decreased CH_4 emissions from dairy cattle, studies suggest that dairy rations may be formulated to contain as much as 30 % of dietary DM as DDGS (Benchaar et al., 2013; Janicek et al., 2008).

Fractionation Process. Since the wet milling industry produces more valued feed byproducts than the dry milling industry, several corn fractionation processes have been developed to recover the germ and fiber fractions as valuable byproducts prior to fermentation in

the dry grind process (Murthy et al., 2009), and to reduce the amount of distillers grains produced. Wet fractionation involves soaking corn for a short amount of time before milling to recover germ and pericarp fiber before fermentation of the degermed and defibered slurry (Murthy et al., 2006). The main unit operations used in wet fractionation methods are soaking/steeping, size reduction, density separation, and sieving as briefly described by Murthy et al. (2009). Dry fractionation involves a dry degerm defiber process similar to conventional dry milling, which is used to separate germ and pericarp fiber before fermentation of the endosperm fraction (Murthy et al., 2006). Murthy et al. (2009) briefly describes the main unit operations in the dry fractionation method as tempering, size reduction, sieving, aspiration, and density separation. These fractionation processes can reduce the amount of distillers grains produced by up to 66 %, based on type of fractionation and number of byproducts recovered (Wang et al., 2005).

Color Analysis. The amount of wet grains and CDS blended together during drying affects nutritive value and physical characteristics of DDGS, MDGS, and WDGS (Kingsly et al., 2010). Chemical properties of these products are used as market value indicators, and directly impact market price. The color of distillers products can give an indication of grain maturity, storage conditions, the presence of toxins, contamination, and possible use of insecticides and fungicides. The current color analysis scale (Hunter Laboratory Analysis, 2008) evolved during the 1950s and 1960s, and uses 3 variables as depicted in Figure 1.5 to describe the color space including:

L-axis (L) : runs vertically from top to bottom in Figure 1.5. The maximum for L is 100 which is described as white, and the minimum is 0 which is described as black.

A-axis (*a*) : has no specific numerical limit. Negative *a* is green, positive *a* is red.

B-axis (*b*) : has no specific numerical limit. Negative *b* is blue, positive *b* is yellow.

Delta values (ΔL, Δa, Δb) are associated with the color scale and indicate how much a standard and a sample differ from one another in Hunter *L*, Hunter *a*, and Hunter *b* fractions. These delta values are used for quality control, and values that are out of tolerance indicate too much difference between the standard and the sample and adjustments are needed in the sample. The total color difference is denoted as ΔE during color analysis procedures (Hunter Laboratory Analysis, 2008). Kingsly et al. (2010) conducted a study where color parameters were analyzed for varying amounts of CDS being blended with WDG and dried to become DDGS. The research found that increasing amounts of CDS dried on to WDG produced darker colored DDGS when dried at the same temperature, and that the Maillard reaction between sugars and protein in WDG and CDS during drying was the primary cause of the browning (Kingsly et al., 2010; Labuza and Baisier, 1992). There has been recent research done describing a linkage between WDG color and microbial growth over time, which has the potential to be used to determine WDG spoilage. Rosentrater and Lehman (2010) conducted a study where they analyzed the color and microbial counts of WDG at 0, 1, 2, 4, and 7 days post-production and found that Hunter *L* values did not exhibit high correlations with the microbial parameters. On the other hand, Hunter *a* and Hunter *b* values were highly related to microbial measures, which had a strong negative correlation with aerobic heterotroph numbers ($R^2 = 0.751$), yeast and mold counts ($R^2 = 0.668$), and CO_2 production ($R^2 = 0.816$) (Rosentrater and Lehman, 2010). A study conducted on pigs and chicks reported that rate and efficiency of gain were highly correlated with the color score of DDGS (Cromwell et al., 1993). To date, there is limited research and high debate on if there is a correlation between the color and the nutritional quality of distillers grains.

Summary.

Rumen-undegradable protein and MCP combine into MP, which is protein available to be absorbed through the small intestine of the host animal, of which MCP contributes more than 50 % of the MP. Rumen-undegradable protein can be estimated in feeds via in vitro and in situ methods, but microbial contamination of feedstuffs flowing into the small intestine can upwardly bias our estimates of RUP. For accurate assessment of MP available to the host, we must first understand the extent of microbial contamination of feeds, which differs depending on feed composition and the microbial community present in the rumen.

Fiber digestibility is another feed characteristic that is difficult to measure, although new methods including TTNDFD have been shown to accurately measure the rate of fiber digestibility of several feeds. More research in this area is needed to create a more intensive library of TTNDFD values, as the data are limited to alfalfa hay, grass hay, and corn silage.

Another factor needed to accurately understand protein and fiber digestibility is to understand the chemical composition of feedstuffs in the diet. With the increase in ethanol production over the last decade and the subsequent use of ethanol production byproducts in production animal diets, it is imperative to refine ethanol production methods in order to produce consistent byproducts with consistent nutrient profiles and chemical compositions.

Research Objectives.

The objectives of this research were to:

1) Characterize chemical composition; and evaluate differences in protein and fiber digestibility of DDGS originating from seven different dry milling facilities across Michigan, South Dakota, and Nebraska.

2) Correct for the microbial contamination of RUP estimated from in situ residues of 11 feeds with varying physical and chemical composition using quantitative PCR.

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TABLES AND FIGURES

Table 1.1. Symbiotic relationship between the ruminal microorganisms and the host animal (Millen et al., 2016).

Host contributions and their outcomes to the microbes	Microbial contributions and their outcome to the host
Mastication and rumination of the feed breakdown large feed particles into smaller particles (comminution), which increase surface area for microbial attachment and digestion	Host is absolutely dependent on microbes to digest fiber. Only microbes have fibrolytic enzymes to degrade cellulose and hemicellulose
Salivary input to the rumen provides aqueous environment necessary for microbial growth, nutrients (nitrogen), but more importantly supplies the major buffering compounds (bicarbonates and phosphates) essential for regulation of ruminal pH	Microbes can use nonprotein nitrogen (urea, nitrate, nucleic acids) as a source of ammonia and synthesize amino acids and protein
Ruminal contractions (peristalsis and antiperistalsis) help mix digesta, which brings microbes into contact with fresh substrate, and facilitates passage of digesta to make room for additional feed	Production of fermentations products particularly VFA, which serve as the major source of energy to the host
Removal of fermentation products by eructation (gases) and absorption (acids) are critical for maintaining optimal conditions (pH) for microbial growth	Production of microbial cells which in the lower tract (abomasum and small intestine) serve as the major source of proteins and vitamins

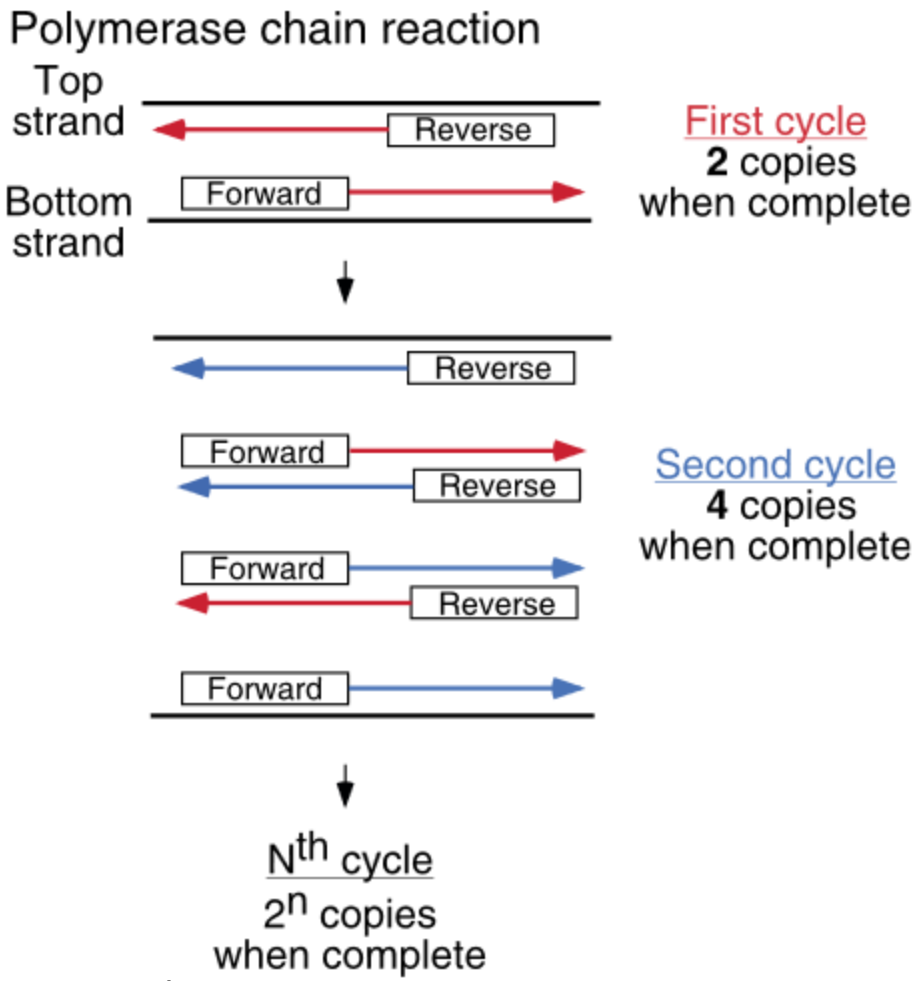
Table 1.2. Laboratory protocol for rapid assay of purine content of feed and digesta (Zinn and Owens, 1986).

1. Weigh 0.2 g dried, group sample into 25 or 50 mL screw-cap tubes. Hydrolysis may be incomplete if sample is wet. Sample weight may be adjusted.
2. Add 2.5 mL 2M $HClO_4$, tightly cap tube and incubate in 90-95°C water bath for 1 h. Break pellet for more complete extraction after 30 min.
3. Add 17.5 mL of 28.5 mM H_6NPO_4. Add half the needed volume, vortex, and add the remaining half. Mix. Re-insert tubes into 90-95°C water bath for 10-15 min.
4. Filter through Whatman No. 1 filter paper (Whatman Ltd., Engl.) into 60 × 125 mm disposable glass tubes. Filtrate should have a pH near 2.
5. Transfer 2 mL of filtrate into a 16 × 125 mm tube and add 2 mL 0.4 M $AgNO_3$* and 6 mL 0.2 M H_6NPO_4. Allow samples to stand overnight at 5 °C for increased precision.
   *Amount of 0.4 M $AgNO_3$ and 0.2 M H_6NPO_4 added dependent on initial sample size.
6. Centrifuge for 10 min at 1,000 × *g* and draw off supernatant liquid being careful to not disturb pellet.
7. Wash pellet with 4.5 mL washing solution and 250 µL $AgNO_3$. Repeat step 6.
8. Add 5 mL of 0.5 N HCl and vortex until thoroughly mixed.
9. Cover tubes with marbles and incubate in 90-95 °C water bath for 30 min. Centrifuge samples again. Do not disturb or re-suspend the pellet.
10. Read absorbance of supernatant fluid and standards at 260 nm.

Table 1.3. Condensed chemical composition of DDGS, MDGS, and WDGS (NASEM, 2016).

Component, % DM	DDGS	MDGS	WDGS
DM (% as fed)	88.93 ± 2.07	47.83 ± 4.09	31.44 ± 8.02
CP	30.79 ± 2.67	29.08 ± 2.45	30.63 ± 3.22
Ash	5.32 ± 0.88	6.65 ± 0.72	5.13 ± 1.13
Fat	10.73 ± 2.05	10.22 ± 2.21	10.84 ± 1.75
Sugar	1.16 ± 1.26	-----	0.90 ± 1.04
Starch	5.88 ± 2.43	3.36 ± 1.07	6.06 ± 2.61
NDF	33.66 ± 3.51	28.73 ± 3.67	31.52 ± 5.57
ADF	16.17 ± 3.15	14.81 ± 3.06	15.27 ± 4.46
Lignin	4.96 ± 1.52	-----	4.70 ± 1.39
Ca	0.05 ± 0.04	0.08 ± 0.05	0.05 ± 0.05
P	0.86 ± 0.11	0.94 ±0.14	0.81 ± 0.18
S	0.66 ± 0.16	0.67 ± 0.16	0.64 ± 0.54

Figure 1.1. Enzyme reactions that make real-time PCR possible (Valasek and Repa, 2005).



[1]mRNA = messenger RNA.
[2]ssDNA = single-stranded DNA.

Figure 1.2. U.S annual ethanol and distillers dried grains with solubles (DDGS) production between 1980 and 2010 (Liu, 2011).

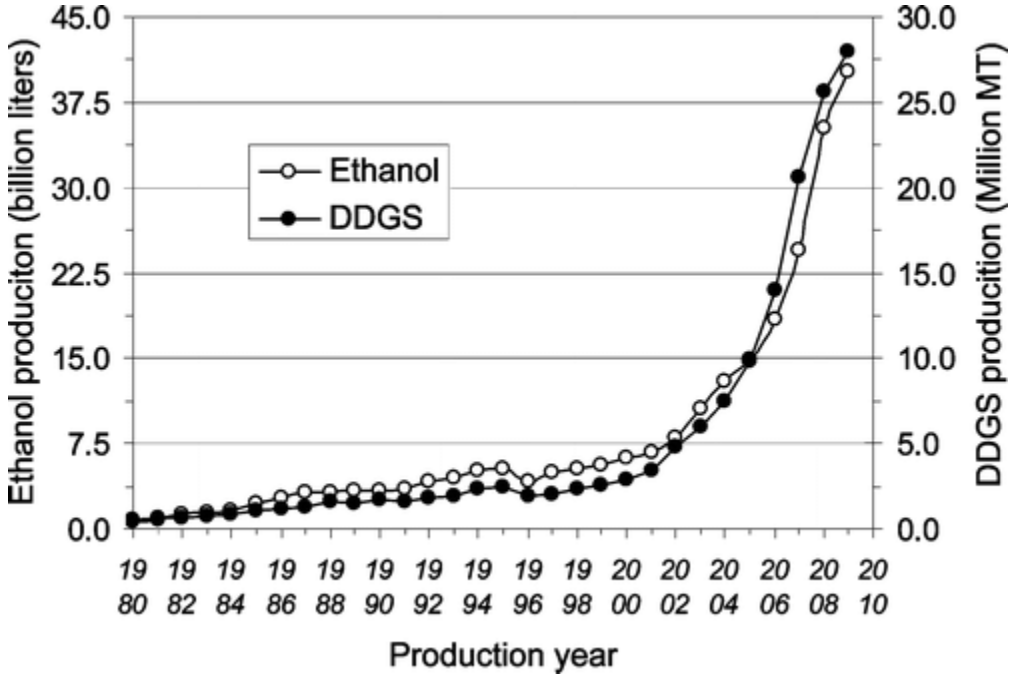


Figure 1.3. Process of wet milling illustrating byproducts produced, including de-oiled condensed distillers solubles (CDS).

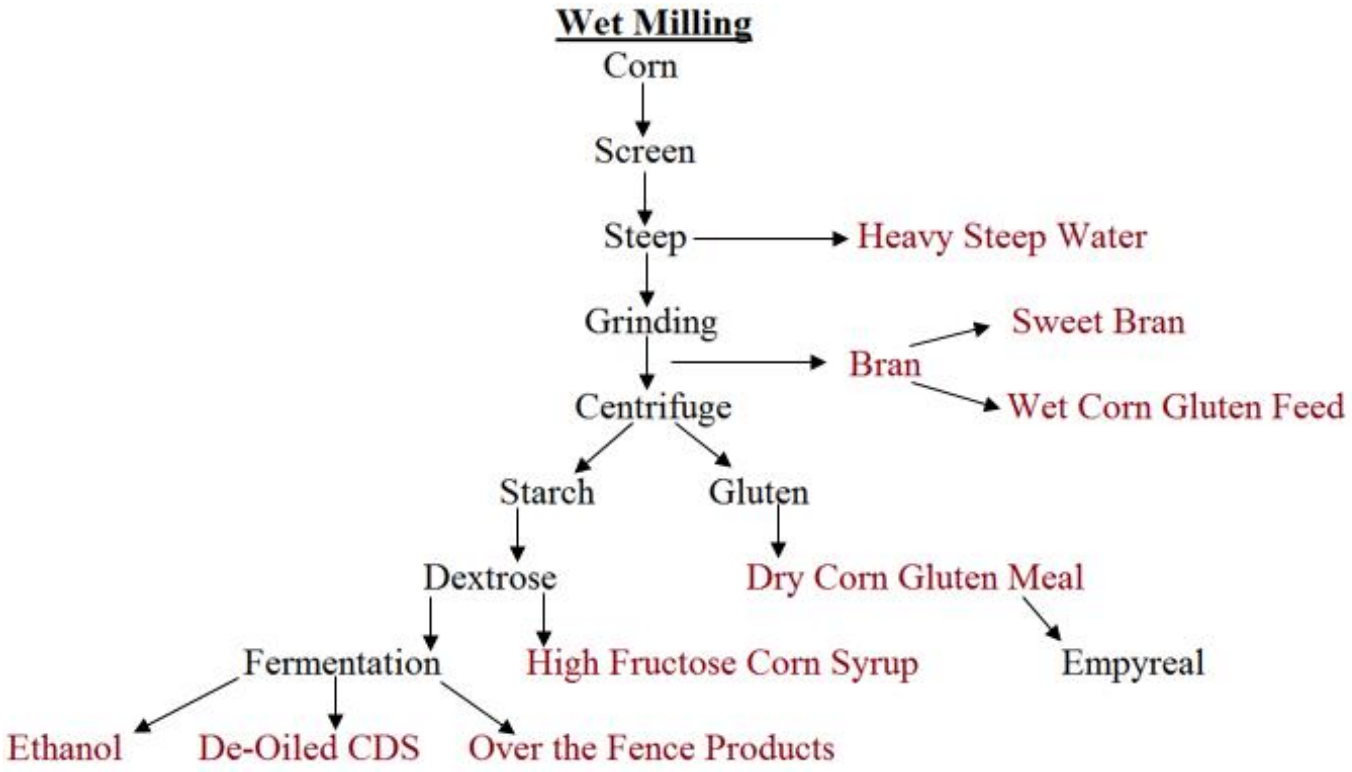


Figure 1.4. Process of dry milling illustrating byproducts produced, including corn dried distillers grains with solubles (DDGS), modified corn dried distillers grains with solubles, wet corn distillers grains with solubles (WDGS), and condensed distillers solubles (CDS).

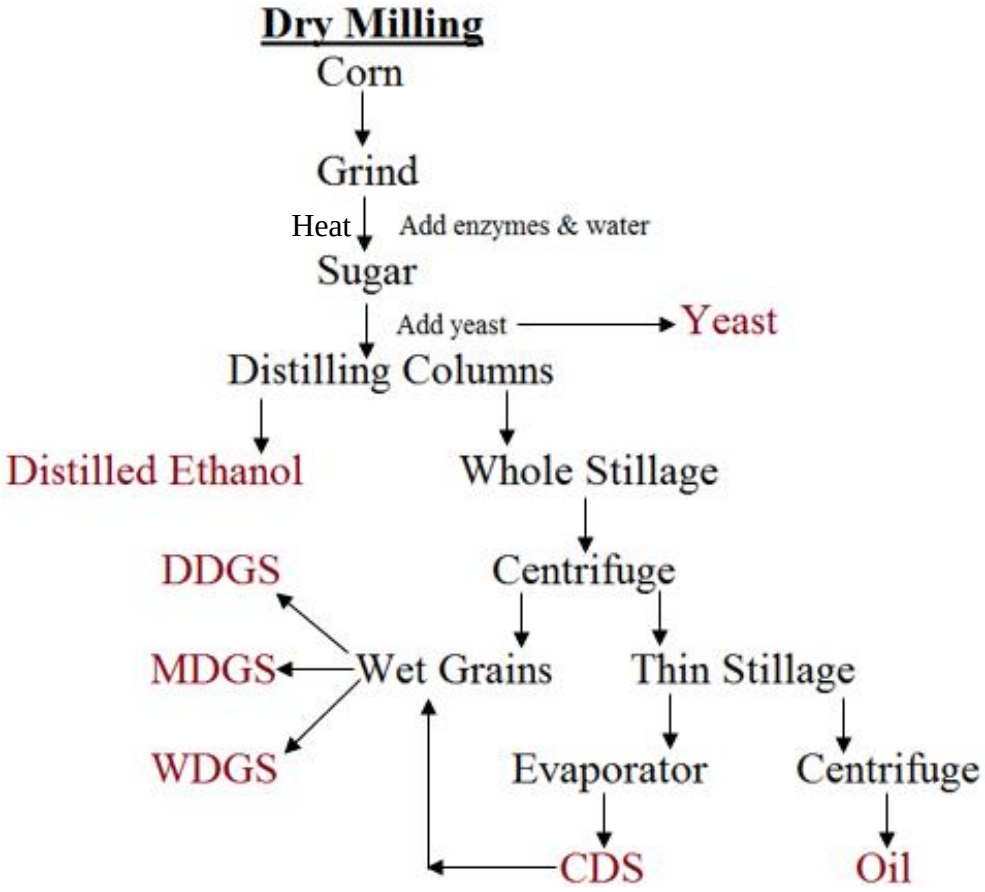
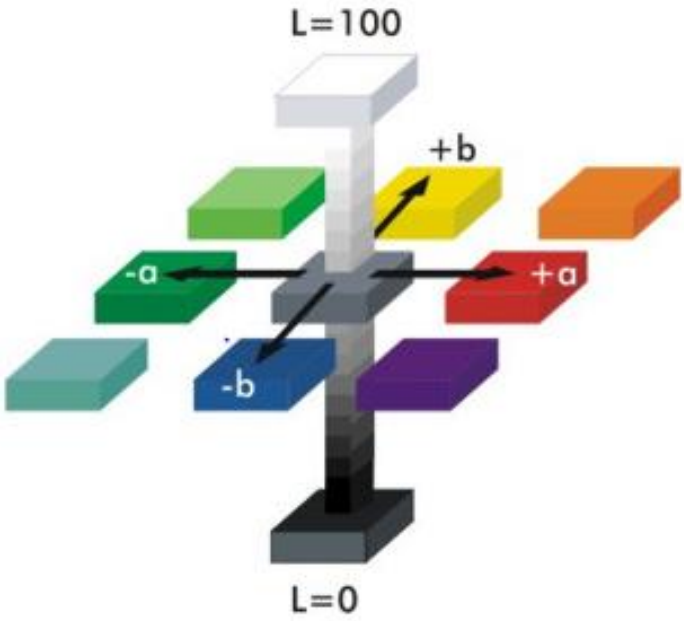


Figure 1.5. Variables used to determine color analysis of feeds (Hunter Color Analysis, 2008).



CHAPTER II

Evaluation of chemical composition and in vitro protein and fiber digestibility of corn dried distillers grains with solubles originating from seven sources

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ABSTRACT

The chemical composition and nutrient digestibility of corn dried distillers grains with solubles (DDGS) may vary by source. The objectives of this study were to characterize chemical composition and evaluate differences in digestibility of DDGS originating from seven different dry milling facilities. Data were analyzed as a completely randomized design assuming treatment was production site, which was replicated by collecting 2 to 4 independent samples over an approximate 1-month period. Significant ($P < 0.05$) differences between sources were observed in crude protein (CP) (ranging from 29.6 to 31.2 ± 0.32 % dry matter (DM)), neutral detergent fiber (NDF) (ranging from 29.4 to 32.7 ± 0.52 % DM), ash-free NDF on an organic matter basis (aNDFom) (ranging from 28.98 to 32.25 ± 0.53 % DM), and crude fat (ranging from 5.53 to 7.52 ± 0.27 %). Rumen-undegradable protein (RUP) and its intestinally digestible fraction (dRUP), were determined according to the methods described by Ross et al. (2013). Additionally, total tract NDF digestibility (TTNDFD) was tested using the methods described by Combs (2013) which included fermentations at 24, 30, 48, and 240 h. Significant differences ($P < 0.01$) in RUP and dRUP were observed across sources ranging from 65.3 to 89.1 ± 2.77 % of CP and 56.4 to 77.5 ± 0.24 % of CP for RUP and dRUP, respectively. As determined by 240 h fermentations, the proportion of potentially digestible NDF (pdNDF) also differed ($P < 0.01$) by source and ranged from 83.0 to 86.8 ± 0.40 % of NDF. Greater differences ($P < 0.005$) were observed between sources in TTNDFD which ranged from 60.0 to 70.5 ± 1.59 % of NDF across sources with rate of pdNDF ranging from 4.79 to 6.82 ± 0.48 %/h. Results indicate that both nutrient composition and digestibility vary across production sites of DDGS. Fiber and protein digestibilities should be analyzed to ensure appropriate feeding and inclusion of DDGS in high-producing dairy diets for optimal performance.

Key Words: DDGS, in vitro intestinal digestion, rumen NDF digestion, rumen undegraded protein.

INTRODUCTION

Corn dried distillers grains with solubles (DDGS) as a byproduct feed ingredient is used as a readily available energy source and over the last 15 years has become an increasingly popular feed ingredient for both beef and dairy feeding programs. Although there has been large technological advancements in ethanol production and corn milling byproduct industries, a number of sources of variation exist which may affect the chemical composition of the feed. These include starting grain variability, extent of starch extraction, grain harvesting method, extent of drying and temperature, and amount of solubles. In addition to chemical composition, these factors likely also affect digestibility and in turn nutrient availability to the animal (Kleinschmit et al., 2007). When considering the protein and fiber fraction of DDGS, drying differences during the milling process may affect the concentration and composition of proteins namely, acid detergent insoluble CP (ADICP). This is believed to increase with the occurrence of Maillard reactions (Kajikawa et al., 2012). These heat-damaged proteins are generally believed to be poorly digested, although the ruminal degradation profiles of the protein fractions of DDGS is not widely investigated (Kajikawa et al., 2012).

Corn dried distillers grains with solubles contain approximately 30 % CP (DM basis), of which 68 % of the protein is RUP (NASEM, 2016). This high RUP content along with ruminal microbial CP and endogenous protein contribute to the AA supply available for absorption through the small intestine of the dairy cow. Accurate estimates of RUP and dRUP in the small intestine are important when formulating diets to avoid under- or over- supplying metabolizable protein (MP). Under-supplying MP can result in an AA deficiency, especially of methionine and lysine, which are often believed to be a limiting AA for milk protein synthesis resulting in a decrease in milk protein yield. In comparison, over-supplying MP may increase ration cost for a

producer and can increase urinary nitrogen (N) losses, causing environmental concerns (Klopfenstein and Erickson, 2002). There are several methods commonly used to estimate RUP content and the extent of dRUP in ruminants. In situ methods using nylon bags can be used to determine the RUP fraction of feeds. Following ruminal incubation, in situ nylon bags are washed and soaked in a pepsin-hydrochloric acid (HCl) solution to mimic abomasal secretions, then inserted into the small intestine of the animal via a duodenal cannula. Once bags exit the animal in the feces, they can be washed and residues can be analyzed to determine the dRUP of the feed (Hvelplund and Weisbjerg, 2000). In vitro methods to measure the intestinal digestibility of the RUP fraction also exist. Calsamiglia and Stern (1995) developed a three step in vitro procedure (TSP) to estimate intestinal dRUP, which was adopted by the NRC (2001) as a reference method (Gargallo et al., 2006). The TSP possesses limitations on determining the digestibility of individual AAs and involves the use of trichloroacetic acid to terminate digestion, which is a highly corrosive and toxic substance, thus several modifications have been made to this assay to both improve the safety and the accuracy of the assay. Updated assays include a modified three step procedure (MTSP) (Gargallo et al., 2006), a precision fed-cecectomized rooster bioassay (Boucher et al., 2009), and the Ross et al. (2013) method.

Both in situ and in vitro methods have limitations, for example, when relying on in situ bags a microbial barrier may be created thus prolonging the lag phase and limiting the extent of ruminal digestion. Additionally the loss of feed particles from bags during incubation may occur, sometimes being as high as 30 % loss of the initial sample (Ørskov, 1982). Thus, there has been increased interest in developing an improved assay in order to better predict protein digestibility in the ruminant animal. Ross et al. (2013) recently developed an in vitro assay designed to

minimize or eliminate sample loss out of the nylon bag to more accurately predict protein digestibility.

The digestibility of NDF may affect intake based on fill capacity of the rumen and milk production in high producing dairy cows, and is more variable than the digestibility of other feed components (Combs, 2013). As with protein digestibility estimates, fiber digestibility is difficult to quantify although it has been recently suggested that the model developed to measure in vitro total-tract NDF digestibility (TTNDFD) predicts similar coefficients to in vivo values for corn silage and alfalfa silage (Lopes et al., 2015a,b). This in vitro model estimates potentially digestible NDF (pdNDF), rate of NDF digestion (kd), and rate of passage (kp) of NDF to predict total tract digestibility for lactating dairy cattle. Corn dried distillers grains with solubles contains approximately 30 % NDF (DM basis), making this feed a relatively good source of fiber for dairy cattle. Understanding the source variation and digestibility coefficients of DDGS components and incorporating this information into ration formulations should improve our ability to optimize milk production. The objectives of this study were: 1) characterize chemical composition; and 2) evaluate differences in protein and fiber digestibility of DDGS originating from seven different dry milling facilities across Michigan, South Dakota, and Nebraska.

MATERIALS AND METHODS

Feedstuffs

Feedstuffs evaluated in this experiment included DDGS from 7 different locations throughout Michigan, South Dakota, and Nebraska including POET Biorefining, Caro, MI (DDGS-1); POET Biorefining, Groton, SD (DDGS-2); POET Biorefining, Chancellor, SD (DDGS-3); POET, Sioux Falls, SD (DDGS-4); POET Biorefining, Mitchell, SD (DDGS-5);

POET Biorefining, Big Stone, SD (DDGS-6); and E Energy Adams, Adams, NE (DDGS-7). These ethanol production plants used energy for jet cooking and liquefaction of dextrins to ≥ 90 °C for 1 to 2 hrs using liquefaction enzymes (Wang et al., 2007). The dextrins were then hydrolyzed into fermentable sugars using saccharification enzymes during simultaneous saccharification and fermentation (Wang et al., 2007), which is a dry milling innovation technique unique to these production sites. Two to 4 batches from each location were obtained over a 1 month period for a total of 25 samples. Products from the Sioux Falls location, SD (DDGS-4) utilized a high oil corn hybrid, thus it was expected this would result in a product with higher fat content from this site.

Feedstuffs were analyzed for DM (method 930.15; AOAC, 2000), CP, ADICP, and neutral detergent insoluble CP (NDICP) (Leco FP-528 N Combustion Analyzer; Leco Corp., St. Joseph, MI 49085), soluble CP (Krishnamoorthy et al., 1982), nonprotein N (NPN) (method 941.04 AOAC, 2000), acid detergent fiber (ADF) and lignin (method 973.18; AOAC, 2000), NDF using sodium sulfite and ash-free NDF on an OM basis (aNDFom) (Van Soest et al., 1991), NDF digestibility (Goering and Van Soest, 1970), sugar (DuBois et al., 1956), starch (Hall, 2009), crude fat (method 2003.05; AOAC, 2006), ash (method 942.05, AOAC, 2000), and minerals (method 985.01; AOAC, 2000) by Cumberland Valley Analytical Services Inc. (Hagerstown, MD), and color was analyzed using the Hunter ColorFlex spectrophotometer (Hunter Associates Laboratory, Reston, VA). Additionally, feedstuffs were analyzed for AA using a Hitachi L-8800 AA analyzer (Hitachi Co., Tokyo, Japan) by the Experimental Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO), and fatty acid analysis was performed at an external laboratory (Department of Animal Science, The Pennsylvania State University, University Park) as described by Rico and Harvatine (2013).

Furthermore, RUP, dRUP, and total tract protein digestibility on both a CP and DM basis were analyzed using an in vitro intestinal digestibility assay developed by Ross et al. (2013). Briefly, 0.5 g of sample was placed in 125 mL Erlenmeyer flasks along with 40 mL of rumen buffer and 10 mL of rumen fluid. Flasks were incubated in a water bath at 39 °C for 16 h under continuous CO_2 to maintain anaerobic conditions. Samples were then acidified with 3M HCl to reduce the pH to 2, and incubated in a shaking bath for 1 h after the addition of 2 mL of pepsin and pH 2 HCl. The pepsin reaction was then ceased with the addition of 2 mL 2M NaOH, and an enzyme mix containing trypsin, chymotrypsin, lipase, and amylase was added to the flask and incubated for 24 h in a shaking bath set at 39 °C. After this incubation, samples were filtered through a 1.5 µM glass filter with boiling water. Nitrogen content of the residue was determined by Kjeldahl and expressed as a percent of total N in the sample (Gutierrez et al., 2014). In short, this in vitro assay to estimate intestinal protein digestion was developed using an enzyme mix of trypsin, chymotrypsin, lipase, and amylase at activities found in cattle digesta to replace pancreatin (Ross et al., 2013). This assay was developed to reduce sample loss and variation among samples by eliminating the use of bags, using Erlenmeyer flasks and small pore size filter papers to improve recoveries of undegraded feed N, which can further allow for recovery of residue for analysis of AA, as well as allow for comparison with assay conditions of other published assays and ADICP (Ross et al., 2013). Total tract fiber digestibility was determined by the TTNDFD model, which is based on the concept that fiber digestion is a 2-step process, beginning in the rumen followed by fiber digestion in the hindgut (Lopes et al., 2015b). Total tract NDF digestibility uses in vitro measures of the proportion of total pdNDF, kd of pdNDF, and kp of pdNDF (Combs, 2013), and is predicted by in vitro incubation of feed samples at several time points using a predetermined endpoint of digestion, and measuring the rate of fiber digestion. The predetermined endpoint of

digestion is termed the indigestible NDF (iNDF) fraction, which can be approximated using a measurement of undigested NDF at varying incubation times including 24, 30, 48, and 240 h incubations. As described by Lopes et al. (2015a), the kd is calculated from NDF residue measurements taken at 24, 30, and 48 h in vitro incubation in rumen fluid (Goeser and Combs, 2009) using a first-order kinetics model with an indigestible fraction as described by Mertens (1993), which assumes that the indigestible residue does not disappear, and pdNDF residue disappears at a rate proportional to its mass at any time. The kp is predicted from a regression model developed by Krizsan et al. (2010) for iNDF, which is adjusted to account for the selective retention of pdNDF (Lund et al., 2007) determined using the flux-compartment pool method described by Ellis et al. (1994) (Lopes et al., 2015a). Total-tract NDF digestibility is expressed as a percentage of total NDF: TTNDFD = pdNDF × [kd / (kd + kp)] / 0.9.

Statistical Analysis

Data were analyzed by using MIXED procedure of SAS (version 9.1, SAS Inst. Inc., Cary, NC). The model included a fixed effect for location and a random effect of sample within location. Statistical significance for treatment effects was declared at $P \leq 0.05$, and trends were discussed at $P \leq 0.10$. The PDIFF option was used to separate and compare differences of least squares means when the *P*-value for the treatment effect was at $P \leq 0.10$. Treatment means are presented as least squares means, and the smallest standard error of the mean (SEM) is reported.

RESULTS

Chemical Composition

As listed in Table 2.1, significant differences in chemical composition were observed. Sample DDGS-7 from a Nebraska production site shows significant differences from the Michigan and South Dakota production site samples (DDGS-1 through DDGS-6), having a lower DM (*P*-value < 0.01) of 87.2 ± 0.199 % DM and a higher soluble CP concentration (*P* < 0.01) at 5.75 ± 0.252 % DM. DDGS-1 shows a significantly lower CP content of 29.6 ± 0.323 % DM when compared to the other production site samples, which all contained approximately 31% CP on a DM basis. Both DM and CP concentrations are similar to those reported in the Dairy NRC (2001) for DDGS, which are 90.2 % DM and 29.7 % DM, respectively.

Significant differences between production site samples also exist in the components of ADICP (*P* < 0.01), ADF (*P* < 0.01), NDF (*P* < 0.01), aNDFom (*P* = 0.01), starch (*P* = 0.02), and ash (*P* = 0.01) content all on a % DM basis. DDGS-4 is produced from a high oil hybrid corn grain and has a crude fat concentration of 7.43 ± 0.267 %, which is significantly higher than the other DDGS samples (*P* < 0.01) aside from DDGS-7, of which has a crude fat content of 7.52 % ± 0.267. Color analysis on the L-value showed significant differences (*P* < 0.01) across samples. The manufacturing process of DDGS may damage a portion of the protein due to excessive heat during the drying process, causing a darkened color of the product and potentially making protein unavailable to the animal (Kleinschmit et al., 2006). DDGS-3 is the darkest with a color score of 49.2, and DDGS-4 is the highest with a color score of 58.5. Tendencies for differences in the components of NDICP (*P* = 0.09), lignin (*P*-value = 0.08), and sugar (*P* = 0.06) also exist between sources. The sole chemical composition component showing no differences across samples is NPN (*P* = 0.95). Although statistical differences exist in the majority of components

across DDGS, these differences are numerically small and may be insignificant in terms of practical application of this information to the dairy industry.

Mineral Composition

Table 2.2 lists mineral composition of DDGS across sources, with DDGS-5 having a significantly higher content of magnesium (% DM) ($P < 0.01$), sodium (% DM) ($P < 0.01$) and manganese (mg/kg) ($P < 0.01$). DDGS-3 shows significantly different ($P < 0.01$) manganese content (mg/kg) when compared to other production site samples. The Nebraska samples (DDGS-7) showed significantly lower mineral concentrations in both sulfur (% DM) ($P = 0.01$) and chlorine (% DM) ($P < 0.01$) than the Michigan and South Dakota production sites. Significant differences also exist across sources for calcium (% DM) ($P < 0.01$), phosphorous (% DM) ($P < 0.01$), and zinc (mg/kg) ($P < 0.01$) content.

Amino Acid Composition

Amino acid composition listed in Table 2.3 reports consistent AA profiles across sources, although methionine content (% DM) of DDGS-5 (0.61 ± 0.010 % DM) was significantly higher ($P < 0.01$) than the other production sites. Significant differences ($P < 0.01$) across sources are also observed in ornithine content (% DM), however DDGS contain trace amount of ornithine, thus this difference can be considered negligible. Total essential AA content ranges from 12.0 to 12.5 % DM, and total AA content (essential and non-essential) ranges from 27.5 to 28.5 % DM.

Fatty Acid Composition

Fatty acid composition is listed in Table 2.4. Total fatty acid (TFA) content was not significantly different among samples ($P = 0.13$), although significant differences were seen in specific fatty acid concentrations. DDGS-5 was significantly different in concentration of several fatty acids including a lower concentration of palmitoleic acid (C16:1c9) at 0.09 % of TFA, compared to 0.13 % of TFA for the remaining 6 samples ($P = 0.03$), as well as significantly lower concentrations of behenic acid (C22:0) ($P = 0.03$). Stearic acid (C18:0) was significantly higher ($P < 0.01$) when compared to the other 6 samples, as well as unknown fatty acid percent ($P = 0.02$). Palmitic acid (16:0) and a conjugated linoleic acid (C18:1c9) were of the highest abundance of specific fatty acids, averaging 14.9 % and 53.8 % respectively across samples, although both components list significant differences across samples ($P < 0.01$).

Protein Content and Digestibility

Table 2.5 lists RUP content, dRUP, and total tract protein digestibility of DDGS across production sites. Interestingly, significant differences in RUP content exist between sources on both a DM and CP basis, ranging from 65.3 to 89.1 ± 2.772 % CP ($P < 0.01$). Surprisingly, the digestibility of RUP in this study ranges from 56.4 to 77.5 ± 2.404 % CP ($P < 0.01$). Total tract digestible protein ranged from 87.2 to 92.4 ± 0.604 % CP ($P < 0.01$) across sources.

NDF Digestibility

NDF content and digestibilities listed in Table 2.6 show significant differences across all time points. Specifically, samples originating from Nebraska (DDGS-7) had a significantly lower 24 h NDF digestibility on both a DM basis and NDF basis when compared to DDGS-1 through

DDGS-6 ($P = 0.01$, $P < 0.01$, respectively). DDGS-7 also showed a decreased 30 h NDF digestibility of 60.6 % NDF basis when compared with the other 6 sources ($P < 0.01$). 24 h NDF digestibility on a DM basis ranged from 16.0 to 20.8 $\pm$ 0.600 % ($P = 0.01$). 30 h NDF digestibility on a DM basis ranged from 19.6 to 22.6 $\pm$ 0.377 % ($P < 0.01$), while 48 h NDF digestibility ranged from 22.9 to 24.8 $\pm$ 0.559 % DM ($P = 0.43$), and 240 h NDF digestibility ranged from 24.2 to 27.5 $\pm$ 0.477 % DM ($P = 0.01$). Total tract NDF digestibility ranged from 60.0 to 70.5 $\pm$ 1.593 % DM ($P < 0.05$), with DDGS-7 having the lowest TTNDFD across samples.

DISCUSSION

As expected, DDGS were observed to contain approximately 30 % fiber and 30 % protein across samples, with small numerical differences in these concentrations being observed by production site. Chemical composition and mineral composition showed significant differences for the majority of the listed components, suggesting this could either be due to production processes or starting grain material of ethanol production from corn. Despite significant differences, these differences are small and likely to be of little practical significance.

Amino acid composition was relatively constant across samples, aside from methionine and ornithine. Methionine is an essential AA, meaning the animal cannot synthesize this AA itself. Methionine is one of the most important AAs to supply to the animal, as it is commonly the first-limiting AA for dairy cattle. Lysine is typically the second-limiting AA for lactating cows consuming a high forage diet (NRC, 2001). Unlike methionine, lysine concentrations showed no significant differences across production sites. Although some statistical differences exist in both methionine and ornithine content, the averages across all sources are similar to the

values the Dairy NRC (2001) reports, although the method to determine the methionine concentration has been altered since the publication of the NRC (2001), which consistently results in higher methionine estimated than previous methods. When comparing concentrations (Dairy NRC (2001) vs. average of DDGS-1 through DDGS-7), results for arginine are 1.21 vs. 1.21, 0.74 vs 0.84 for histidine, 1.01 vs 1.07 for isoleucine, 2.48 vs 3.38 for leucine, 0.67 vs 1.01 for lysine, 0.54 vs 0.54 for methionine, 0.54 vs 0.56 for cysteine, 1.45 vs 1.41 for phenylalanine, 1.02 vs 1.14 for threonine, 0.26 vs 0.21 for tyrosine, and 1.40 vs 1.39 for valine, all on a % DM basis. When compared to DDGS samples analyzed by Kelzer (2008), results from this study showed higher concentrations of EAA. Kelzer (2008) reported 0.41 ± 0.005 %, 0.56 ± 0.005, 0.76 ± 0.008 %, 0.77 ± 0.008 %, 2.32 ± 0.024 %, 0.62 ± 0.005 %, 0.83 ± 0.005 %, 0.23 ± 0.005, 0.91 ± 0.011 %, and 0.10 ± 0.000 % DM for methionine, lysine, arginine, threonine, leucine, isoleucine, valine, histidine, phenylalanine, and tryptophan, respectively. These data suggest that analyzing DDGS for methionine concentrations may be beneficial to ensure an adequate supply to cattle, although these differences between samples of this study are not practically important to consider when feeding DDGS.

High producing dairy cattle have large energy requirements that may exceed their ability to consume dietary energy, resulting in less than maximum milk production (Harvatine and Allen, 2006). Fats are considered a good source of energy in the dairy industry, thus the addition of fat to dairy diets may increase the energy density of the diet. Distillers grains with solubles contains both saturated and unsaturated fatty acids, which can be digested and metabolized by the cow if they pass through the rumen. Unsaturated fatty acids allow for biohydrogenation by the microbial community in the rumen. Milk fat synthesis is inhibited by fatty acid intermediates formed during ruminal biohydrogenation of unsaturated fatty acids and milk fat depression is

associated with diets containing higher concentrations of polyunsaturated fatty acids (Bauman and Griinari, 2003). For the 7 DDGS samples, unsaturated fatty acids make up over 80% of the total fatty acid content. The TFA content averages 6.29 % ± 0.0177 across DDGS samples from the various production sites, therefore unsaturated fatty acids comprise only 5 % of the DDGS samples. Ranathunga et al. (2010) reported a DDGS TFA content of 11.6 %, which is almost double the amount observed in this study. Total fatty acid concentration in this study is also lower than values listed by Moreau et al. (2011) and Noureddini et al. (2009), who reported 9.1 ± 0.4 % and 7.4 ± 0.6% TFA, respectively. This is due to a change in the DDGS production process, as current dry milling facilities centrifuge the corn oil out of the grain before grinding in order to sell the oil as a valuable byproduct. In general, the profile of fatty acids were very similar across sources, although DDGS-4 had increased crude fat content when compared to all other sources aside from the Nebraska source (DDGS-7) due to the high fat corn hybrid used in the production process.

The concentration of RUP in feedstuffs has been determined through in vivo (Castillo-Lopez et al., 2013, Vanzant et al., 1996), in situ (Ørskov and McDonald, 1979) and in vitro (Krishnamoorthy et al., 1983; Poos-Floyd et al., 1985) methods. The extent of protein from DDGS degraded in the rumen may be influenced by a number of factors (Aines et al., 1987) including production plant (Spiehs et al., 2002), degree of heat used to dry the feed (Kleinschmit et al., 2007a), amount of solubles added back to distillers grains (Corrigan et al., 2009), and particle size. Paz et al. (2013) summarized a number of studies in which the ruminal disappearance of CP from DDGS was estimated using either in situ or in vitro methods. Using meta-analytical techniques, Paz et al. (2013) calculated RUP using the parameters reported in each study and assumed a kp of 5 %/h. When studies employed the model of Ørskov and

McDonald (1979), mean RUP for corn dried distillers grains was reported to be 47.4 ± 12.6 % CP, while when studies employed the lag model of McDonald (1981) mean RUP for DDGS was reported to be 53.4 ± 8.2 %. Across models, RUP for corn dried distillers grains was observed to be 50.4 ± 10.4 % CP. On average, the RUP content of corn dried distillers grains reported in the current study ranged from 65.4 to 77.5 % CP and averaged 63.7 ± 2.404 % CP (Table 2.5). Interestingly, these values determined through in vitro methods are very similar to those reported by Castillo-Lopez et al. (2013), which used laborious in vivo measures. As expected, differences between sources were observed in RUP. More specifically, DDGS-1, DDGS-2, DDGS-3, and DDGS-4 were observed to have a similar RUP content (averaging 66.5 ± 2.772 % CP) while RUP was increased in remaining sources with DDGS-6 having the highest RUP content (89.1 % CP). It is difficult to identify the factor or factors responsible for these observed effects but results support the assentation that variability exists between corn-ethanol production facilities. A portion of this variation may be due to fermentation and processing technology used and also the amount of solubles added to the feedstuff. Practically, nutritionists should be aware of this and when possible test the chemical composition and nutrient availability (Spiehs et al., 2002).

The Dairy NRC (2001) assumes that intestinal dRUP in DDGS is 80%. Since this was published, the assumption has been tested in a number of recent studies also summarized by Paz et al. (2013). Analytically speaking, these studies can be grouped into three groups based on the adopted technique (several others exist but for simplicity are not included here).

1) The mobile bag technique (MB), this technique requires a small sample of the feed is first incubated in the rumen and then directly inserted through a duodenal cannula into the small intestine and ultimately recovered in the manure (Hvelplund, 1985).

2) The three step in vitro procedure (TSP), this technique requires rumen incubation followed by pepsin and pancreatic digestion (Calsamiglia and Stern, 1995).

3) The modified three step in vitro procedure (MTSP) (Gargallo et al., 2006), this technique is similar to the TSP but does not include the use of trichloroacetic acid and includes the use of a batch rumen incubator.

The current study employed an in vitro assay ("Ross Method") described by Ross et al. (2013); however, this method has not been used to estimate the dRUP content of DDGS previously. Overall in the dataset generated by Paz et al. (2013), the average dRUP in DDGS was observed to be 83.9 ± 10.5 %. This digestibility coefficient is similar to the NRC (2001) assumption of 80% but the reported estimates are also highly variable ranging from 59.2 to 95.0 %. Kelzer (2008) used an in situ mobile bag technique to determine the RUP content and dRUP of DDGS described by Kononoff et al. (2007), and reported an RUP content of 33.2 to 56.3 ± 2.54 % CP ($P < 0.01$) and the digestibility of this RUP averaged 92.0 ± 2.44 % CP. In the current study dRUP averaged 63.7 ± 2.404 % (Table 2.5). Similar to RUP, significant, expected, and unexplainable differences were observed between sources. Total tract digestible protein in this study ranged from 87.2 to 92.4 ± 0.604 % CP ($P < 0.01$) across sources, which is lower than values reported by Kelzer (2008) who reported a range of 95.4 to 97.4 ± 0.20 % CP for total tract digestible protein using the mobile bag technique described by Kononoff et al. (2007). Kleinschmit et al. (2007) evaluated the ruminal and intestinal degradability of DDGS from 5 different sources using the in situ method followed by the TSP method, and consistent to this study, reported a large range in RUP from 59.1 to 71.7 ± 1.6 % CP, a range in dRUP ranging from 59.2 to 76.8 ± 2.4 % CP, and a range in total tract digestible protein from 70.1 to 85.3 ± 1.4

% CP, further suggesting variation in DDGS composition from different production sites. Li et al. (2012) did similar work using in situ methods and MTSP to estimate RUP, dRUP, and total tract digestibile protein for DDGS. This study reported an RUP content of 48.2 ± 41.4 % CP, which is much lower than RUP estimates reported elsewhere, a dRUP estimate of 86.4 ± 0.0086 % CP, and a total tract digestible protein estimate of 93.5 ± 0.0047 % CP. Several other studies report variation in RUP, dRUP, and total tract digestible protein estimates, ranging from 33.3 to 48.7 % CP, 66.5 to 80.3 % CP, and 77.9 to 95 % CP, respectively (Boucher et al., 2009; Cao et al., 2009; Westreicher-Kristen et al., 2013).

Analytically speaking the TSP, MTSP, and the newly developed Ross method all appear to be promising techniques to commercially estimate dRUP because they do not require the use of cattle fitted with duodenal cannulas and may be used to analyze large numbers of samples rapidly and with precision. However, it must be noted that MB technique is the only technique that ensures samples are exposed to all physiological digestive processes. Future research methods should seek to identify the best method to estimate dRUP and this likely would require comparisons of observations to the MB method.

In vivo methods have been employed to estimate the kinetics of fiber digestion. As with RUP and dRUP, these methods are expensive and not applicable for routine analysis when characterizing feed samples. To address this challenge, an in vitro NDF fermentation assay has been developed to estimate TTNDFD (Lopes et al., 2015b;c). To do so, in vitro fermentations are conducted at 24, 30, 48, and 240 h to determine the proportion of pdNDF. Rate of NDF digestion is calculated and a fixed extent of hind gut digestion is inferred and together these are used to predict TTNDFD. In vitro conditions of this assay have been standardized to improve the repeatability of this assay (Goeser et al., 2009) and on average the TTNDFD of corn silage,

alfalfa, and grass forages have been observed to be 42, 43 and 47 % of total NDF, respectively (Lopes et al., 2015b). Although relatively new, the precision of this assay has been compared through in vivo measures of TTNDFD and results suggest good (R^2 = 0.68) agreement between these methods (Lopes et al., 2015b;c). In the current study, the TTNDFD assay was used to test for differences in fiber digestion between DDGS originating from different corn-ethanol facilities. To our knowledge this study represents the first to report TTNDFD on a large number of DDGS samples and on average TTNDFD was observed to be 65.5 ± 1.59 % of total NDF (Table 2.6). As was the case with RUP and dRUP, differences between production sites were observed with differences greater than 10 % being observed. Of the treatments tested, DDGS-7 was observed to have the lowest TTNDFD (60.0 % of total NDF), interestingly this sample also contained the lowest total tract digestible protein (Table 2.5) (87.2 % CP). As with previous in vitro measures, it is difficult to identify driving factors responsible for observed differences in TTNDFD. Nonetheless, results support the notion that in addition to differences in chemical composition (Spiehs et al., 2002), differences in nutrient availability also exist between production facilities. The TTNDFD method represents an important and powerful tool to estimate in vivo fiber digestibility, but it should also be noted that the method does not account for selective retention of feed particles in the rumen (Huhtanen et al., 2007; Lopes et al., 2015b) which is affected both by particle fragility and particle size (Grant, 2010) and as a result it may difficult to compare estimates of TTNDFD across feedstuffs.

CONCLUSIONS

Results indicate that both nutrient composition and digestibility vary across production sites of DDGS. In vitro assays assessing the digestibility of protein and fiber are useful in

detecting differences in sources of DDGS. Application of these estimates in formulation decisions should be further explored.

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Table 2.1. Chemical composition of DDGS from 7 sources across Michigan, South Dakota, and Nebraska

	Feedstuffs								
	DDGS-1[1]	DDGS-2[2]	DDGS-3[3]	DDGS-4[4]	DDGS-5[5]	DDGS-6[6]	DDGS-7[7]		
Item	Mean	Mean	Mean	Mean	Mean	Mean	Mean	SEM	*P*-value
% DM	89.5^{a}	89.2^{ba}	88.7^{b}	88.9^{b}	89.0^{ba}	88.7^{b}	87.2^{c}	0.199	<0.01
CP, % DM	29.6^{b}	30.7^{a}	30.6^{a}	30.9^{a}	31.2^{a}	31.2^{a}	31.2^{a}	0.323	0.03
Sol Protein, % DM	4.0^{c}	4.40^{cb}	3.90^{c}	3.83^{c}	4.20^{cb}	4.80^{b}	5.75^{a}	0.252	<0.01
NPN, % N	0.05^{a}	0.05^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.006	0.95
ADICP, % DM	1.86^{b}	1.69^{b}	2.57^{a}	2.00^{b}	1.64^{b}	1.81^{b}	2.10^{ba}	0.152	<0.01
NDICP, % DM	2.26^{bac}	2.03^{c}	2.81^{a}	2.28^{bac}	2.04^{c}	2.11^{bc}	2.81^{ba}	0.206	0.09
ADF, % DM	12.2^{ba}	10.8^{c}	11.2^{bc}	11.3^{bc}	10.1^{c}	12.1^{ba}	13.2^{a}	0.415	<0.01
NDF[8], % DM	31.9^{ba}	29.4^{d}	30.2^{dc}	31.8^{bac}	29.6^{d}	30.8^{bdc}	32.7^{a}	0.522	<0.01
aNDFom[9], % DM	31.3^{ba}	29.0^{c}	29.9^{bc}	31.2^{ba}	29.2^{c}	30.3^{bc}	32.3^{a}	0.531	0.01
Lignin, % DM	1.8^{b}	2.10^{ba}	2.32^{a}	2.34^{a}	1.85^{b}	2.09^{ba}	1.71^{b}	0.151	0.08
Sugar, % DM	4.4^{bc}	4.45^{bc}	4.53^{bac}	5.60^{ba}	3.80^{c}	5.90^{a}	5.60^{ba}	0.467	0.06
Starch, % DM	5.23^{bc}	7.28^{a}	6.00^{ba}	4.80^{bc}	6.80^{ba}	5.03^{bc}	3.05^{c}	0.652	0.02
Crude fat, % DM	6.26^{b}	5.74^{b}	5.95^{b}	7.43^{a}	5.53^{b}	6.04^{b}	7.52^{a}	0.267	<0.01
Ash, % DM	5.55^{a}	5.53^{a}	5.65^{a}	5.21^{b}	5.69^{a}	5.62^{a}	5.14^{b}	0.094	0.01
Color Analysis[10]	57.1^{ba}	54.9^{b}	49.2^{c}	58.5^{a}	55.9^{ba}	55.6^{ba}	57.1^{ba}	0.923	<0.01

[1]DDGS-1 = corn dried distillers dried grains with solubles from Caro, MI (n = 4).
[2]DDGS-2 = corn dried distillers dried grains with solubles from Groton, SD (n = 4).
[3]DDGS-3 = corn dried distillers dried grains with solubles from Chancellor, SD (n = 4).
[4]DDGS-4 = corn dried distillers dried grains with solubles from Sioux Falls, SD (n = 3).
[5]DDGS-5 = corn dried distillers dried grains with solubles from Mitchell, SD (n = 4).
[6]DDGS-6 = corn dried distillers dried grains with solubles from Big Stone, SD (n = 4).
[7]DDGS-7 = corn dried distillers dried grains with solubles from Adams, NE (n = 2).
[8]Assay described by Van Soest et al. (1991) using α-amylase and sodium sulfite.
[9]aNDFom = NDF determined on an organic matter basis.
[10]Color Analysis = reported as *L*-value by Hunter Lab Color spectrophotometer (Hunter Associates Laboratory, Reston, VA).

Table 2.2. Mineral composition of DDGS from 7 sources across Michigan, South Dakota, and Nebraska

	Feedstuffs								
	DDGS-1[1]	DDGS-2[2]	DDGS-3[3]	DDGS-4[4]	DDGS-5[5]	DDGS-6[6]	DDGS-7[7]		
Item	Mean	Mean	Mean	Mean	Mean	Mean	Mean	SEM	*P*-value
Ca, % DM	0.05^{cb}	0.05^{cb}	0.10^{a}	0.06^{b}	0.08^{a}	0.03^{c}	0.05^{cb}	0.006	<0.01
P, % DM	0.85^{ba}	0.80^{c}	0.81^{bc}	0.85^{bac}	0.87^{a}	0.85^{a}	0.90^{a}	0.015	<0.01
Mg, % DM	0.35^{b}	0.36^{b}	0.37^{b}	0.37^{b}	0.40^{a}	0.37^{b}	0.35^{b}	0.069	<0.01
K, % DM	1.33^{a}	1.26^{b}	1.27^{ba}	1.32^{ba}	1.28^{ba}	1.30^{ba}	1.28^{ba}	0.021	0.34
S, % DM	1.16^{ba}	1.15^{ba}	1.24^{a}	1.04^{ba}	1.22^{ba}	1.01^{b}	0.73^{c}	0.072	0.01
Na, % DM	0.24^{cb}	0.26^{b}	0.26^{b}	0.18^{cd}	0.36^{a}	0.23^{cb}	0.12^{d}	0.019	<0.01
Cl, % DM	0.20^{d}	0.20^{dc}	0.20^{bdc}	0.22^{ba}	0.23^{a}	0.22^{bac}	0.16^{e}	0.007	<0.01
Fe, mg/kg	76.8^{ba}	88.0^{ba}	87.0^{ba}	90.3^{a}	87.8^{ba}	74.3^{b}	90.0^{ba}	4.689	0.16
Mn, mg/kg	15.3^{d}	19.0^{c}	25.8^{a}	18.7^{c}	22.3^{b}	15.5^{d}	19.5^{c}	0.533	<0.01
Zn, mg/kg	83.3^{a}	55.3^{d}	58.0^{dc}	66.7^{bc}	63.0^{dc}	57.8^{dc}	75.5^{ba}	3.226	<0.01
Cu, mg/kg	3.00^{b}	3.00^{b}	3.25^{b}	3.33^{b}	3.50^{ba}	3.50^{ba}	4.50^{a}	0.287	0.13

[1]DDGS-1 = corn dried distillers dried grains with solubles from Caro, MI (n = 4).
[2]DDGS-2 = corn dried distillers dried grains with solubles from Groton, SD (n = 4).
[3]DDGS-3 = corn dried distillers dried grains with solubles from Chancellor, SD (n = 4).
[4]DDGS-4 = corn dried distillers dried grains with solubles from Sioux Falls, SD (n = 3).
[5]DDGS-5 = corn dried distillers dried grains with solubles from Mitchell, SD (n = 4).
[6]DDGS-6 = corn dried distillers dried grains with solubles from Big Stone, SD (n = 4).
[7]DDGS-7 = corn dried distillers dried grains with solubles from Adams, NE (n = 2).

Table 2.3. Amino acid composition of DDGS from 7 sources across Michigan, South Dakota, and Nebraska

	Feedstuffs								
	DDGS-1[1]	DDGS-2[2]	DDGS-3[3]	DDGS-4[4]	DDGS-5[5]	DDGS-6[6]	DDGS-7[7]		
Item, % DM	Mean	Mean	Mean	Mean	Mean	Mean	Mean	SEM	*P*-value
EAA[8]									
Arg	1.18^{b}	1.15^{ba}	1.20^{ba}	1.26^{a}	1.23^{ba}	1.21^{ba}	1.21^{ba}	0.038	0.27
His	0.84^{a}	0.83^{a}	0.85^{a}	0.84^{a}	0.85^{a}	0.84^{a}	0.84^{a}	0.013	0.95
Ile	1.08^{ba}	1.06^{ba}	1.08^{ba}	1.06^{ba}	1.10^{a}	1.07^{ba}	1.03^{b}	0.015	0.20
Leu	3.38^{a}	3.35^{a}	3.40^{a}	3.35^{a}	3.52^{a}	3.36^{a}	3.33^{a}	0.052	0.28
Lys	1.03^{a}	1.00^{a}	0.97^{a}	1.03^{a}	1.00^{a}	0.99^{a}	1.04^{a}	0.023	0.41
Met	0.50^{c}	0.55^{b}	0.55^{b}	0.54^{b}	0.61^{a}	0.56^{b}	0.50^{c}	0.010	<0.01
Phe	1.40^{a}	1.39^{a}	1.42^{a}	1.41^{a}	1.43^{a}	1.41^{a}	1.43^{a}	0.026	0.86
Thr	1.15^{a}	1.13^{ba}	1.16^{a}	1.15^{a}	1.15^{a}	1.16^{a}	1.09^{b}	0.014	0.11
Trp	0.21^{ba}	0.20^{b}	0.20^{ba}	0.21^{ba}	0.21^{a}	0.21^{ba}	0.21^{ba}	0.004	0.35
Val	1.39^{a}	1.38^{a}	1.41^{a}	1.41^{a}	1.43^{a}	1.39^{a}	1.36^{a}	0.022	0.57
TEAA[9]	12.2	12.0	12.2	12.3	12.5	12.2	12.0		
NEAA[10]									
Ala	2.14^{ba}	2.10^{bc}	2.13^{ba}	2.10^{ba}	2.19^{a}	2.12^{ba}	2.00^{c}	0.029	0.05
Asp	1.94^{a}	1.90^{a}	1.94^{a}	1.94^{a}	1.95^{a}	1.94^{a}	1.93^{a}	0.025	0.82
Cys	0.55^{ba}	0.55^{ba}	0.58^{ba}	0.52^{b}	0.60^{a}	0.56^{ba}	0.55^{ba}	0.019	0.20
Glu	4.93^{b}	4.96^{b}	5.01^{ba}	4.97^{ba}	5.16^{a}	4.96^{b}	5.02^{ba}	0.066	0.28
Gly	1.17^{bc}	1.16^{c}	1.21^{ba}	1.23^{a}	1.20^{bac}	1.19^{bac}	1.17^{bc}	0.015	0.07
Orn	0.06^{a}	0.03^{b}	0.03^{b}	0.03^{b}	0.03^{b}	0.05^{a}	0.03^{b}	0.004	<0.01
Pro	2.05^{c}	2.14^{bac}	2.14^{bac}	2.08^{bc}	2.24^{a}	2.21^{ba}	2.15^{bac}	0.041	0.07
Ser	1.47^{ba}	1.45^{b}	1.48^{ba}	1.48^{ba}	1.50^{a}	1.48^{ba}	1.47^{ba}	0.016	0.46
Tau	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.003	0.92
Tyr	1.10^{a}	1.08^{a}	1.12^{a}	1.10^{a}	1.10^{a}	1.08^{a}	1.10^{a}	0.022	0.93
Total AA[11]	27.6	27.5	27.9	27.8	28.5	27.8	27.5		

[1]DDGS-1 = corn dried distillers dried grains with solubles from Caro, MI (n = 4).
[2]DDGS-2 = corn dried distillers dried grains with solubles from Groton, SD (n = 4).
[3]DDGS-3 = corn dried distillers dried grains with solubles from Chancellor, SD (n = 4).
[4]DDGS-4 = corn dried distillers dried grains with solubles from Sioux Falls, SD (n = 3).
[5]DDGS-5 = corn dried distillers dried grains with solubles from Mitchell, SD (n = 4).

[6]DDGS-6 = corn dried distillers dried grains with solubles from Big Stone, SD (n = 4).
[7]DDGS-7 = corn dried distillers dried grains with solubles from Adams, NE (n = 2).
[8]EAA = essential AA.
[9]TEAA = total EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[10]NEAA = nonessential AA.
[11]Total AA = EAA + NEAA.

Table 2.4. Fatty acid composition of DDGS from 7 sources across Michigan, South Dakota, and Nebraska

	Feedstuffs								
	DDGS-1[1]	DDGS-2[2]	DDGS-3[3]	DDGS-4[4]	DDGS-5[5]	DDGS-6[6]	DDGS-7[7]		
Fatty Acid	Mean	Mean	Mean	Mean	Mean	Mean	Mean	SEM	*P*-value
Total FA, %	6.70^{a}	6.06^{b}	6.16^{b}	6.08^{b}	6.58^{ba}	6.36^{ba}	6.08^{ba}	0.177	0.13
C14:0	0.06^{c}	0.06^{b}	0.06^{b}	0.06^{cb}	0.07^{a}	0.07^{a}	0.06^{b}	0.001	<0.01
C15:0	0.03^{b}	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{ba}	0.001	0.04
C16:0	14.8^{de}	15.0^{bc}	15.2^{a}	15.2^{ba}	14.9^{dc}	14.7^{e}	14.7^{de}	0.052	<0.01
C16:1c9	0.13^{a}	0.13^{a}	0.13^{a}	0.13^{a}	0.09^{b}	0.13^{a}	0.13^{a}	0.007	0.03
C17:0	0.08^{a}	0.09^{a}	0.09^{a}	0.09^{a}	0.07^{b}	0.09^{a}	0.09^{a}	0.004	0.08
C18:0	2.06^{e}	2.12^{d}	2.20^{c}	2.21^{c}	2.30^{a}	2.25^{b}	2.22^{cb}	0.011	<0.01
C18:1t9	0.04^{a}	0.04^{ba}	0.04^{ba}	0.04^{ba}	0.03^{b}	0.00^{c}	0.00^{c}	0.002	<0.01
C18:1t11	0.00^{dc}	0.00^{d}	0.00^{d}	0.00^{dc}	0.01^{c}	0.04^{a}	0.02^{b}	0.002	<0.01
C18:1c9	24.3^{c}	24.3^{c}	24.3^{c}	24.3^{c}	25.2^{b}	25.5^{a}	25.3^{ba}	0.064	<0.01
C18:1c11	0.85^{a}	0.84^{a}	0.83^{b}	0.82^{b}	0.82^{b}	0.80^{c}	0.80^{c}	0.002	<0.01
C18:2c9c12	54.6^{a}	54.3^{b}	53.8^{c}	53.9^{c}	53.2^{e}	53.3^{ed}	53.5^{d}	0.057	<0.01
C20:0	0.41^{d}	0.42^{b}	0.43^{a}	0.43^{a}	0.42^{cb}	0.41^{cd}	0.40^{d}	0.002	<0.01
C20:1c11	0.29^{a}	0.29^{a}	0.28^{b}	0.28^{b}	0.26^{c}	0.27^{c}	0.25^{d}	0.002	<0.01
C18:3c9c12c15	1.45^{c}	1.48^{b}	1.54^{a}	1.55^{a}	1.48^{b}	1.48^{b}	1.50^{b}	0.007	<0.01
C20:2n6	0.06^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.04^{b}	0.06^{a}	0.05^{ba}	0.003	0.02
C22:0	0.18^{a}	0.19^{a}	0.20^{a}	0.20^{a}	0.14^{b}	0.18^{a}	0.19^{a}	0.011	0.03
C24:0	0.27^{d}	0.28^{bac}	0.29^{a}	0.28^{ba}	0.27^{bdc}	0.28^{bac}	0.27^{dc}	0.003	<0.01
Unknown	0.43^{b}	0.39^{b}	0.46^{b}	0.42^{b}	0.58^{a}	0.41^{b}	0.41^{b}	0.035	0.02

[1]DDGS-1 = corn dried distillers dried grains with solubles from Caro, MI (n = 4).
[2]DDGS-2 = corn dried distillers dried grains with solubles from Groton, SD (n = 4).
[3]DDGS-3 = corn dried distillers dried grains with solubles from Chancellor, SD (n = 4).
[4]DDGS-4 = corn dried distillers dried grains with solubles from Sioux Falls, SD (n = 3).
[5]DDGS-5 = corn dried distillers dried grains with solubles from Mitchell, SD (n = 4).
[6]DDGS-6 = corn dried distillers dried grains with solubles from Big Stone, SD (n = 4).
[7]DDGS-7 = corn dried distillers dried grains with solubles from Adams, NE (n = 2).

Table 2.5. Rumen-undegradable protein, intestinal digestibility, and total tract digestibility of dry matter and crude protein of DDGS from 7 sources across Michigan, South Dakota, and Nebraska

	Feedstuffs								
Item	DDGS-1[1]	DDGS-2[2]	DDGS-3[3]	DDGS-4[4]	DDGS-5[5]	DDGS-6[6]	DDGS-7[7]	SEM	*P*-value
RUP									
% CP	68.0^{c}	65.9^{c}	66.7^{c}	65.3^{c}	77.3^{b}	89.1^{a}	84.7^{ba}	2.772	<0.01
% DM	20.1^{c}	20.2^{c}	20.4^{c}	20.2^{c}	24.2^{b}	27.8^{a}	26.4^{ba}	0.872	<0.01
dRUP[8]									
% CP	57.5^{c}	56.4^{c}	56.7^{c}	57.7^{c}	68.0^{b}	77.5^{a}	71.9^{ba}	2.404	<0.01
% DM	17.0^{c}	17.3^{c}	17.3^{c}	17.8^{c}	21.2^{b}	24.2^{a}	22.5^{ba}	0.743	<0.01
Total Tract DP[9]									
% CP	89.6^{bc}	90.5^{ba}	90.0^{bc}	92.4^{a}	90.7^{ba}	88.5^{dc}	87.2^{d}	0.604	<0.01
% DM	26.5^{c}	27.8^{ba}	27.5^{ba}	28.5^{a}	28.3^{ba}	27.6^{ba}	27.2^{bc}	0.324	<0.01

[1]DDGS-1 = corn dried distillers dried grains with solubles from Caro, MI (n = 4).
[2]DDGS-2 = corn dried distillers dried grains with solubles from Groton, SD (n = 4).
[3]DDGS-3 = corn dried distillers dried grains with solubles from Chancellor, SD (n = 4).
[4]DDGS-4 = corn dried distillers dried grains with solubles from Sioux Falls, SD (n = 3).
[5]DDGS-5 = corn dried distillers dried grains with solubles from Mitchell, SD (n = 4).
[6]DDGS-6 = corn dried distillers dried grains with solubles from Big Stone, SD (n = 4).
[7]DDGS-7 = corn dried distillers dried grains with solubles from Adams, NE (n = 2).
[8]dRUP = RUP digestibility.
[9]Total Tract DP = total tract digestible protein.

Table 2.6. In vitro NDF digestibility of DDGS from seven different sources across Michigan, South Dakota, and Nebraska at various in vitro incubation times

	Feedstuffs								
	DDGS-1[1]	DDGS-2[2]	DDGS-3[3]	DDGS-4[4]	DDGS-5[5]	DDGS-6[6]	DDGS-7[7]		
Item	Mean	Mean	Mean	Mean	Mean	Mean	Mean	SEM	*P*-value
24 h NDF digestibility[8]									
% DM	20.8^{a}	18.8^{b}	18.9^{b}	20.0^{ba}	18.9^{b}	19.0^{ba}	16.0^{c}	0.600	0.01
% NDF	66.2^{a}	64.9^{a}	63.1^{a}	64.1^{a}	64.6^{a}	62.7^{a}	49.1^{b}	1.494	<0.01
30 h NDF digestibility[9]									
% DM	21.8^{ba}	20.3^{ecd}	21.4^{bc}	22.6^{a}	21.1^{bcd}	20.0^{ed}	19.6^{e}	0.377	<0.01
% NDF	69.7^{a}	70.3^{a}	71.4^{a}	72.5^{a}	72.4^{a}	66.1^{b}	60.6^{c}	0.889	<0.01
48 h NDF digestibility[10]									
% DM	24.8^{a}	22.9^{b}	23.6^{ba}	24.2^{ba}	23.9^{ba}	24.1^{ba}	24.0^{ba}	0.559	0.43
% NDF	79.1^{ba}	79.2^{ba}	78.8^{b}	77.7^{bc}	81.7^{a}	79.6^{ba}	74.1^{c}	0.938	0.01
240 h NDF digestibility[11]									
% DM	26.1^{bac}	24.2^{d}	25.3^{dc}	26.9^{ba}	25.4^{dc}	25.7^{bc}	27.5^{a}	0.477	0.01
% NDF	83.3^{d}	83.5^{d}	84.3^{dc}	86.2^{ba}	86.8^{a}	84.9^{c}	85.3^{bc}	0.403	<0.01
TTNDFD[12], % of total NDF	64.1^{bc}	66.6^{ba}	65.9^{ba}	67.0^{ba}	70.5^{a}	64.3^{bc}	60.0^{c}	1.593	<0.05

[1]DDGS-1 = corn dried distillers dried grains with solubles from Caro, MI (n = 4).
[2]DDGS-2 = corn dried distillers dried grains with solubles from Groton, SD (n = 4).
[3]DDGS-3 = corn dried distillers dried grains with solubles from Chancellor, SD (n = 4).
[4]DDGS-4 = corn dried distillers dried grains with solubles from Sioux Falls, SD (n = 3).
[5]DDGS-5 = corn dried distillers dried grains with solubles from Mitchell, SD (n = 4).
[6]DDGS-6 = corn dried distillers dried grains with solubles from Big Stone, SD (n = 4).
[7]DDGS-7 = corn dried distillers dried grains with solubles from Adams, NE (n = 2).
[8]24 h NDF dig = digested NDF after 24 hour incubation.
[9]30 h NDF dig = digested NDF after 30 hour incubation.
[10]48 h NDF dig = digested NDF after 48 hour incubation.
[11]240 h NDF dig = digested NDF after 240 hour incubation.
[12]TTNDFD = total tract NDF digestibility.

CHAPTER III

Estimating RUP content of various feeds using DNA as a marker to correct for microbial contamination

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ABSTRACT

A study was conducted on multiple sources of 11 different feeds of varying chemical composition, particularly neutral detergent fiber (NDF) percent. Feedstuffs included alfalfa hay (ALF), bloodmeal (BM), canola meal (CAN), corn silage (CS), corn dried distillers grains with solubles (DDGS), grass hay (GH), soybean meal (SBM), soyhulls (SH), wet brewers grain (WBG), citrus pulp (CIT), and lignosulfonate treated SBM (SoyPass® (SP)). A 16 h ruminal incubation was used to determine dry matter (DM) disappearance and rumen-undegradable protein (RUP) of these feeds. To correct for bacterial crude protein (BCP) contamination of the RUP across feedstuffs, DNA as a bacterial marker was used. As expected, chemical composition varied greatly across feedstuffs, with DM content ranging from 37.3 to 87.7 ± 29.0 % for forage feeds, and 25.8 to 90.3 ± 22.6 % for byproduct and protein feeds. Crude protein (CP) ranged from 7.25 to 95.0 ± 26.9 % DM, NDF content ranged from 10.5 to 66.7 ± 18.8 % DM, while non-fiber carbohydrates ranged from 0.00 to 60.2 ± 16.5 % DM. Dry matter digestibility (DMD) varied greatly between sources. Soybean meal had the highest DMD of 82.5 ± 2.45 % DM and lowest uncorrected RUP (uRUP) content of 19.7 ± 11.4 % CP, while BM had the lowest DMD of 3.86 ± 8.88 % DM and the highest uRUP content of 89.1 ± 8.82 % CP. The DMD and uRUP of remaining feedstuffs ranged from 32.2 to 77.8 ± 14.7 % DM, and 21.2 to 81.3 ± 21.4 % CP. The corrected RUP of wet in situ residues (wcRUP) was variable across feedstuffs, with BCP contamination contributing 0.0 to 1.4 ± 7.49 % CP to uRUP estimates. Similarly, the corrected RUP of lyophilized in situ residues (lcRUP) ranged from 18.5 to 83.1 % CP, with BCP contamination contributing 2.0 to 17.0 ± 8.03 % CP to uRUP estimates. The relationship between BCP contamination (% CP) and NDF concentration (% DM) had a strong positive correlation for wcRUP (R^2 = 0.7157), a weak positive correlation for lcRUP (R^2 = 0.1947), and

no correlation when results from wcRUP and lcRUP were averaged against NDF concentration (R^2 = 0.00007). These observations indicate that there is a relationship between the NDF content of a feed and the extent of BCP contamination when using DNA as a microbial maker on wet in situ residues.

Key words: in situ, microbial contamination, rumen degradation

INTRODUCTION

Duodenal flow of CP includes 3 major fractions: 1) RUP, 2) endogenous protein, and 3) microbial CP. Endogenous proteins originate from various sources, including mucoproteins, saliva, sloughed epithelial cells, and enzyme secretions (Tamminga et al., 1995). There are multiple methods to quantify the RUP fraction of feeds, including in situ and in vitro methods. The in situ method involves weighing a known amount of feed sample into a nylon bag with pore sizes ranging from 20 to 60 µm, followed by sealing and incubating these bags in the rumen of ruminally cannulated animals for an allotted amount of time (Ørskov, 1982). Once bags are removed from the rumen, they can be analyzed for DM digestibility and protein fractions. The protein disappearance from the rumen bag is considered rumen-degradable protein (RDP), while the remaining residue protein is assumed to be RUP (Ørskov, 1982). To avoid errors associated with bacterial contamination, in situ bags are washed in an attempt to free ruminal bacteria from feedstuff residue, however bacterial contamination may still remain (Beckers et al., 1995). It is known that fibrolytic bacteria adhere strongly to fiber particles in feedstuffs in order to digest and ferment structural carbohydrates in the cell wall, specifically cellulose and hemicellulose present in plant based feeds. Because of the strong adherence of a ligand bond or ionic charge attachment between the microbes and the feed particles, attached microbes may upwardly bias our estimates of RUP in feeds. Methods to quantify the extent of microbial contamination on in situ residues include the use of both purines and DNA as bacterial markers. Using DNA as a bacterial marker has not been well explored to correct for bacterial contamination across a variety of feeds in in situ studies, although Paz et al. (2014) conducted a similar study using bloodmeal, canola meal, low-fat corn dried distillers grains with solubles, soy bean meal, and expeller soy bean meal and observed a range of 0.1 to 1.6 % bacterial CP (BCP) contamination

of in situ residue RUP. Paz et al. (2014) also observed a strong positive correlation ($R^2 = 0.81$) between BCP contamination and NDF concentration of the feedstuffs. The objectives of this study were to 1) determine the rumen degradation of CP using the in situ technique, and 2) to evaluate the use of DNA as a marker to correct for BCP of 11 different feeds with varying NDF concentrations.

MATERIALS AND METHODS

Feedstuffs

Feedstuffs evaluated in this experiment included alfalfa hay (ALF), bloodmeal (BM), canola meal (CAN), corn silage (CS), distillers dried grains with solubles (DDGS), grass hay (GH), soy bean meal (SBM), soyhulls (SH), wet brewers grain (WBG), citrus (CIT), and lignosulfonate treated SBM (SoyPass® (SP)) all acquired from Cumberland Valley Analytical Services Inc. (Hagerstown, MD). One to 3 samples per feed were used in this study, for a total of 29 samples, and were assumed to contain no appreciable amount of bacterial DNA. All samples were ground to pass through a 2 mm screen using a Wiley Mill (Arthur A. Thomas Co., Philadelphia, PA). Feedstuffs were analyzed for DM (method 930.15; AOAC, 2000), nitrogen (N) (Leco FP-528 N Combustion Analyzer; Leco Corp., St. Joseph, MI 49085), acid detergent fiber (ADF) (method 973.18; AOAC, 2000), NDF using sodium sulfite (Van Soest et al., 1991), lignin (method 973.18; AOAC, 2000), sugar (DuBois et al., 1956), starch (Hall, 2009), crude fat (method 2003.05; AOAC 2006), non-fiber carbohydrates (NFC), ash (method 942.05, AOAC, 2000), and minerals (method 985.01; AOAC, 2000) by Cumberland Valley Analytical Services Inc. (Hagerstown, MD).

Animals and In Situ Bags

Two Angus steers (BW of 621 ± 11 kg) fitted with a flexible ruminal cannula with a daily DMI of 6.9 kg were used in this study. Steers were housed in box stalls with continuous access to water and fed once daily at 1000 h a diet of 70.5 % grass hay (DM basis), 5.8 % dry rolled corn (DM basis), 23.3 % DDGS (DM basis), and 0.4 % added minerals and vitamins (DM basis), with a total diet DM of 89.61 %. For each feed sample, approximately 1.50 g from each batch was weighed into 20 N-free polyester bags (R510, Ankom Technologies, Macedon, NY) with a mean pore size of 50 µm and a dimension of 5 ×10 cm. These 20 bags per batch were then divided in half, thus having 10 bags/batch/sample/steer, 5 of which will be referred to as rumen bags, and the other 5 being referred to as droplet digital polymerase chain reaction (ddPCR bags). All bags were heat-sealed using an Ankom Heat Sealer (Ankom Technologies) and then divided into mesh bags (46 × 38 cm) that contained 2 secured 100-g weights to prevent bags from floating up in the rumen mat. Each mesh bag contained 95-100 polyester bags so that all batches from every sample were present. At 1600 h, 3 mesh bags per steer were inserted through the rumen cannula, positioned in the ventral sac, and incubated for 16 h. Following rumen incubation, all mesh bags were gently rinsed with cold water to remove particulate matter and to cease microbial activity. All bags were then frozen at -80 °C. Rumen bags were then lyophilized (Freezemobile 25SL, Virtis, Gardiner, NY). Following freeze drying, rumen bags were weighed to determine the weight of the remaining feed residue, and residues were individually placed in 4 oz whirl-pak bags for later analysis of residue N and DM, while ddPCR bags were removed from the deep freeze, partially thawed, and residues were then placed in individual 4 oz whirl-pak bags. The ddPCR residues were then re-frozen at -80 °C for later analysis of bacterial N using DNA as a bacterial marker.

Calculations

The RDP for all samples was determined as the portion of CP that disappeared from the rumen bags following the 16 h in situ incubation. The RUP was calculated as 100 – RDP. The corrected RUP for all samples was calculated as uncorrected RUP (uRUP) – BCP.

Correction for Bacterial CP Contamination

Whole ruminal contents were collected for 2 consecutive days from 4 different locations within the rumen at 1600 h on the first day and at 0830 h on the second day, followed by techniques to isolate the rumen bacteria from the rumen contents.

Isolation of Ruminal Bacteria

Ruminal bacteria were isolated following the procedure described by Hristov et al. (2005). Whole ruminal contents were composited by steer and squeezed through 4 layers of cheesecloth and the filtrate was retained. Solids remaining on the cheesecloth were added to a volume of cold buffer (McDougall, 1948) equal to the volume of filtrate and shaken manually in a snap-lid plastic container to dislodge the ruminal microorganisms loosely associated with feed particles. This suspension was then squeezed through four layers of cheesecloth, and the 2 filtrates were combined in similar proportions and preserved with 5 % (vol/vol) formalin. From this sample, bacteria were harvested via differential centrifugation (Hristov and Broderick, 1996) with an initial low-speed centrifugation at 400 × *g* for 5 min at 4 °C and a subsequent high-speed centrifugation at 20,000 × *g* for 15 min at 4 °C. Samples were maintained on ice while being processed. The supernatant was then discarded and the isolated pellets were separated by steer and collection day, and frozen at -80 °C for further analysis. Bacterial pellets were thawed, lyophilized (Freezemobile 25SL, Virtis, Gardiner, NY), ground with a mortar and pestle and a

subsample was analyzed for N and DM (Leco FP-528 N Combustion Analyzer; Leco Corp., St. Joseph, NI).

Bacterial N using DNA as bacterial marker

For bacterial pellets, rumen bag residues, and ddPCR bag residues, bacterial DNA was extracted using the repeated bead beating column method detailed by Yu and Morrison (2004) for PCR-quality community DNA using a Mag-Bind® Soil DNA 96 extraction kit (Omega Bio-Tek Inc., Norcross, GA) Briefly, pellets and residues were mixed with a lysis buffer and garnet beads (Scientific Asset Management, LLC, Basking Ridge, NJ) to chemically lyse cell walls, and mechanically shear the cells walls to expose DNA contained in the cell contents. Nucleic acids were then precipitated and DNA was purified by a series of centrifugation steps which removed RNA and proteins. The DNA concentration in the samples was measured by spectrophotometry (DS-C Cuvette Spectrophotometer, DeNovix, Inc. Wilimington, DE) and samples were stored at -20 °C in 25 µL aliquots for later analysis of bacterial crude protein (BCP) using ddPCR (QX200 Droplet Digital PCR System, Bio-Rad Laboratories, Inc, Hercules, CA). The microbial DNA marker used in this study is reported elsewhere (Yu et al., 2005; Castillo-Lopez et al., 2013) and is part of the gene encoding the 16S rRNA, which has been shown to be highly preserved in bacteria (Ogier et al., 2002; Zimmerman et al., 2010).

Droplet digital PCR technology uses a combination of microfluidics and surfactant chemistries to divide PCR samples into water-in-oil droplets, which can then be analyzed for absolute quantitation of DNA copy numbers (Hindson et al., 2011). Briefly, 11 µL of 2X QX200 ddPCR Evagreen Supermix containing a dsDNA-binding dye allowing for double-stranded DNA detection following amplification was combined with 0.22 µL of a 10 mM forward primer, 0.22

µL of a 10 mM reverse primer, 5.56 µL RNase-free water, and 5 µL 0.015 ng/µL diluted DNA sample for a total of 22 µL. This solution was then vortexed, centrifuged, and 20 µL was pipetted into droplet generator cartridges containing ddPCR droplet reader oil (Bio-Rad Laboratories, Inc, Hercules, CA) to form droplets. Droplets of each sample were then carefully pipetted into a 96 well PCR plate, covered, and thermocycled using the C100 Touch Thermal Cycler under the following conditions at a 2 ºC/sec ramp rate: 1) 1 cycle at 95 ºC for 5 min to activate enzymes, 2) 40 cycles at 95 ºC for 30 sec/cycle to allow for denaturation to occur, 3) 40 cycles at 60 ºC for 1 min/cycle for annealing and extension, 4) 1 cycle of a 5 min signal stabilization at 4 ºC, followed by further signal stabilization at 90 ºC for 5 min, and 5) an infinite hold at 4 ºC to keep products stable. Afterwards, the PCR plate was placed into ddPCR (QX200 Droplet Digital PCR System, Bio-Rad Laboratories, Inc, Hercules, CA), gated, and analyzed for copies/µL of final ddPCR reaction, allowing for concentration analysis of DNA abundance per gram of residue sample using calculations described in Appendix II.

Statistical Analysis

Data were analyzed by using MIXED procedures of SAS (version 9.1, SAS Inst. Inc., Cary, NC). The model included a fixed effect for feed and a random effect of replication within feed. Means and standard deviations are reported. Line of best fit and correlation were analyzed by using Microsoft Excel (Microsoft Office, Redmond, WA).

RESULTS

Chemical Composition and Ruminal Disappearance of DM

The chemical composition and DM digestibility (DMD) of forage and non-forage feedstuffs are listed in Table 3.1 and 3.2, respectively. As expected, chemical composition across feedstuffs varied. Across feeds, DM and CP ranged from 25.8 to 91.3 ± 23.5 % DM, and 7.25 to 95.0 ± 26.9 % DM, respectively. In forage feeds, NDF content of ALF, CS, and GH was 39.7 ± 7.88 %, 37.8 ± 2.11 %, and 65.6 ± 9.06 % DM, respectively. In byproduct and protein feeds, NDF content ranged from 8.20 to 66.7 ± 19.0 % DM. Starch content had a large range across feeds, ranging from 0.17 to 34.2 ± 9.96 % DM. Overall, chemical compositions of the feedstuffs were comparable to reported values (NRC, 2001).

Dry matter digestibility also showed a large range throughout feedstuffs. Dry matter digestibility of forage feeds was 59.7 ± 10.0 %, 59.6 ± 2.85 %, and 32.2 ± 14.4 % for ALF, CS, and GH, respectively. Of the byproduct and protein feeds, BM had the lowest DMD of 3.86 ± 8.88 %, and SBM had the highest DMD of 82.5 ± 9.53 %, with all other feeds ranging from 36.9 to 77.8 ± 15.2 %.

Uncorrected and Corrected RUP

Uncorrected rumen-undegradable protein after 16 h in situ incubation, corrected RUP of wet in situ residues (wcRUP), and corrected RUP of lyophilized in situ residues (lcRUP) using DNA as a microbial marker are listed in Table 3.3 and 3.4 for forage feeds and byproduct and protein feeds, respectively. The uRUP of incubated forage feeds was 24.4 ± 7.00, 27.3 ± 2.85, and 70.1 ± 11.8 % CP for ALF, CS, and GH, respectively. The corrected RUP of wet in situ samples was observed to be more similar to uRUP estimates when compared to corrected RUP

of lyophilized samples. When comparing concentrations (wcRUP vs. lcRUP), results for ALF were 24.2 vs. 23.2, 27.1 vs. 23.8 for CS, 68.7 vs. 53.0 for GH, 89.1 vs. 83.1 for BM, 30.0 vs. 26.5 for WBG, 21.2 vs. 20.7 for CAN, 27.8 vs. 27.8 for CIT, 44.5 vs. 42.0 for DDGS, 34.0 vs. 33.4 for SH, and 81.1 vs. 75.8 for SP, all on a % CP basis. The lyophilized residue of SBM did not provide enough sample for a DNA extraction. Averaging the wet residue and lyophilized residue corrected RUP estimates (acRUP) resulted in a range of 23.8 to 67.8 ± 24.6 % CP for forage feeds, and a range of 19.2 to 83.1 ± 25.8 % CP for byproduct and protein feeds.

Figure 3.1 lists the relationship between BCP contamination (% CP) and NDF concentration (% DM) of the feedstuffs using wet in situ residues for DNA extraction and ddPCR analysis. Bloodmeal and SBM were observed to have the lowest NDF concentrations, and the lowest extent BCP contamination, while GH and SH were observed to have the highest NDF concentrations and the highest % BCP contamination. The other 7 feed residues were intermediate, resulting in a strong positive correlation ($R^2 = 0.7157$) between BCP contamination and the NDF concentration of the feed. Figure 3.2 lists the relationship between BCP contamination (% CP) and NDF concentration (% DM) of the feedstuffs using lyophilized in situ residues for DNA extraction and ddPCR analysis. Results were observed to have a weak positive correlation between ($R^2 = 0.1947$), although similarly to Figure 3.1, GH had the largest impact from BCP contamination when compared to all other feeds. Figure 3.3 lists the relationship between BCP contamination (% CP) and NDF concentration (% DM) of the averaged results from wet residues and lyophilized residues, and no correlation ($R^2 = 0.00007$) was observed across feed samples. Interestingly, BM was observed to have the highest BCP contamination (% CP) when both methods were averaged together.

DISCUSSION

As expected, chemical composition, DMD, uncorrected RUP, corrected RUP, and extent of BCP contamination varied across feeds, as all feeds used in this study had different chemical compositions including fiber content, starch content, and protein content, including the RDP and RUP fractions of that protein. The DMD of the forage feeds are similar to a study conducted by Holden (1999), who used traditional in vitro methods to determine the DMD of ten different feeds, where he reported DMD of 58.8, 63.9, and 49.7 % for ALF, CS, and GH, respectively.

Accurate estimates of RUP is an important parameter for protein nutrition in ruminants (NRC, 2001) and proper estimates of this RUP could improve the use of protein in dairy cow diets. The rumen-undegradable protein content of the feeds in this study showed a large range, as expected. Bloodmeal had the highest RUP content, whereas SBM had the lowest RUP content. This result was anticipated, as BM and SBM had the lowest and highest DMD, respectively. Aside from GH, all other feedstuffs reported similar uRUP values to the NRC (2001). Surprisingly, GH, with an NDF of 65.6 ± 9.06 % DM had an uRUP content of 70.1 ± 11.8 % CP, whereas the NRC (2001) reports the uRUP content for mature hay with over 60 % NDF as 43.7 % CP. The increase in uRUP content of GH seen in this study could be due to various reasons, including access of the microbes to the feedstuffs, extent of microbial contamination, and location of the in situ bags when incubated in the rumen. Hoffman et al. (1993) describes factors that may influence the ruminal disappearance of forage CP including stage of plant maturity, forage species, and preservation method. Plant maturity may affect the uRUP estimate of GH, as immature hay tends to have less uRUP when compared to mature hay (NRC, 2001).

Microbial contamination of rumen-incubated residues may lead to large underestimations of RDP and upwardly bias RUP estimates (González et al., 2014), especially in feeds with a low

CP and high fiber contents (Michalet-Doreau and Ould-Bah, 1989), although studies have shown that the degree of microbial contamination is negligible for feedstuffs with a high CP content and low degradation rates such as cereal grains (Varvikko and Lindberg, 1985; Katzy et al., 1993).

Determination of RUP can be done using an array of methods, including in vivo, in situ, and in vitro procedures. In vivo methods are expensive, time-consuming, and labor-intensive for routine analysis (Stern et al., 1997), therefore in situ and in vitro methods are more commonly used. The current methodologies used for correction of microbial contamination are based on removal of bacterial cells from the in situ feed residue, or tagging microbial cells (González et al., 1998; Krawielitzki et al., 2006) with the use of markers including diaminopimelic acid, sulfur isotopes, N isotopes (^{15}N), purines, and DNA. The use of the N isotope ^{15}N has been a widely used as a marker because of its low environmental hazard, low cost, is not found naturally in protein of feeds, and does not label the proteins of animals until the labeled microbial amino acids are absorbed and incorporated into their tissues (Broderick and Merchen, 1992). A recent study by Machado et al. (2013) attempted to use ^{15}N as a marker to label microbial cells for estimating microbial contamination after ruminal in situ incubation of forages. The study found that differences in the A and B protein fractions differed significantly between feeds corrected and uncorrected for microbial contamination, and an equation to correct the non-degraded residue of ruminally incubated forages was developed to attempt to replace the use of microbial markers as follows: CNDR = NDR × [(100 - % C)/100], where CNDR = corrected residue of incubation (g), NDR = apparent residue of incubation (g), and % C = the percentage of microbial contamination in relation to the initial sample incubated (Machado et al., 2013).

Constraints exist with all markers, including purines which is commonly used to determine microbial CP contamination. Challenges that exist with purines include 1) dietary

purines which escape ruminal degradation may cause microbial contamination to be overestimated, and 2) input from sloughed epithelial cells may contribute to purines in the digesta (Zinn and Owens., 1986). In addition, the challenge of using total purines as a bacterial marker seem to be whether they are present in 1) a similar percentage in the different species, and 2) in all stages of growth. (Castillo-Lopez, 2009). The use of purines and DNA as microbial markers have been recently compared in studies that attempt to quantify the presence of microbial protein (Castillo-Lopez et al., 2010; Paz et al., 2014). Paz et al. (2014) compared in situ residue microbial contamination of various feeds including 3 BM sources, CAN, SBM, low-fat DDGS, and expeller SBM, and found that estimations of microbial contamination using purines and DNA were similar across all three BM samples and SBM, averaging 0.71, 0.41, 0.59, and 1.98 %, respectively, although compared with purines, estimates of microbial contamination using DNA as a bacterial marker were lower for low-fat DDGS and expeller SBM, and higher for CAN. Paz et al. (2014) concluded that estimations of microbial contamination were positively correlated ($y = 0.50 + 0.10x$; $R^2 = 0.81$) with NDF concentration of the feedstuff using DNA, suggesting an opportunity to more thoroughly research a potential relationship between microbial CP contamination of residues and NDF concentration across a variety of feeds.

In the current study, the ratio of microbial abundance to CP of isolated rumen bacteria averaged 23.2 ± 3.01 %. This is lower than that of Paz et al. (2014) who observed this relationship to be 101.1 ± 43.4 %. Differences in this estimate are not surprising, given analytical differences and the fact that the samples were collected from different animals consuming different diets, factors are likely to affect the nature of the microbial population in the rumen. When correcting for BCP contamination, different results were observed for wet in situ

residues when compared to lyophilized in situ residues (Tables 3.3 and 3.4). Although the extent of BCP contamination showed a strong positive correlation ($R^2 = 0.7157$) with the NDF concentration of the feed, the lyophilized residues resulted in increased BCP contamination observations across all feeds (0.0 to 1.4 ± 0.53 % BCP for wet residues (Figure 3.1) vs. 2.0 to 17.4 ± 4.43 % BCP for lyophilized residues (Figure 3.2)). Interestingly, the averaged results from both methods listed in Figure 3.3 were observed to have no correlation ($R^2 = 0.00007$) between the NDF concentration of feeds and the extent of BCP contamination. To our knowledge, this study represents the first to compare corrected RUP estimates using DNA as a microbial marker between wet in situ residues and lyophilized in situ residues. It should be noted that the lyophilized samples were ground with a mortar and pestle, sent to an out-of-state laboratory for CP analysis where they were further ground through a 1 mm screen, thus the probability of both microbial cell lysing and sample contamination is high when compared to the wet residues. Currently, there is no recommendation whether DNA should be extracted from wet residues or lyophilized residues.

Although the use of microbial DNA appears to be a powerful and specific way to estimate microbial contamination of in situ residues we suggest that the assay is also very sensitive to variation in sampling and isolation of DNA resulting in varying estimations of microbial protein. Results illustrated in Figure 3.1 lead us to accept our hypothesis that concentration of NDF in a feedstuff is highly and positively related to the extent of BCP contamination in a residue after rumen incubation. Moving forward, more research should be conducted to determine the expected ratio of microbial abundance to CP of isolated rumen bacteria on animals consuming both forage and concentrate-based diets, as calculations to determine the extent of microbial contamination is based off of this ratio. Further research should

be conducted on the form of the residue, wet or lyophilized, prior to DNA extraction to determine the effect this may have on abundance calculations.

CONCLUSIONS

Dry matter digestibility and RUP content differs among feedstuffs, and the extent of microbial contamination on in situ residues can have substantial impacts on the estimate of RUP. Different methods to correct for microbial contamination provide different estimations on the extent of this contamination. In the present study, observations indicate that using wet in situ residues results in a strong positive correlation and lyophilized in situ residues results in a weak positive correlation between BCP contamination and NDF concentration, leading us to accept our hypothesis that increased NDF concentrations in a feed contain increased protein from a microbial source after rumen incubation. Previous research has reported that using DNA as a microbial marker estimates lower contaminations when compared to using purines as a marker on the same feeds. These lower estimates may be attributed to an underestimation of the microbial mass, since the DNA marker used may not be present across all microbial species.

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Table 3.1. Chemical composition and dry matter digestibility of forage feedstuffs

	Feeds[1]					
	ALF		CS		GH	
Item[2]	Mean	SD	Mean	SD	Mean	SD
DM	87.3	2.35	37.3	0.51	87.7	1.86
CP	19.9	3.16	8.80	0.10	8.90	0.56
ADF	34.3	7.17	23.9	1.57	41.6	4.65
NDF	39.7	7.88	37.8	2.11	65.6	9.06
Lignin	8.10	2.51	3.11	0.11	6.15	1.54
Sugar	7.17	2.59	0.63	0.12	9.00	2.26
Starch	2.03	0.35	34.2	3.56	1.03	1.02
Crude Fat	1.42	0.37	3.12	0.56	1.61	1.04
NFC[3]	31.6	4.83	45.3	3.68	17.7	8.96
Ash	9.59	2.10	5.98	2.51	8.07	1.23
Ca	1.43	0.14	0.24	0.07	0.38	0.07
P	0.31	0.05	0.28	0.02	0.28	0.07
Mg	0.24	0.04	0.18	0.03	0.16	0.08
K	2.75	0.95	1.25	0.23	2.48	0.50
S	0.26	0.06	0.13	0.01	0.17	0.03
Na	0.11	0.12	0.03	0.03	0.01	0.01
Cl	0.56	0.69	0.34	0.28	0.33	0.02
Fe (mg/kg)	266	188	258	184	192	51.1
Mn (mg/kg)	41.0	8.72	31.3	4.16	87.7	86.9
Zn (mg/kg)	24.7	6.03	28.7	10.4	20.0	1.73
Cu (mg/kg)	9.00	2.00	5.33	1.15	7.33	1.15
DM digestibility	59.7	10.0	59.6	2.85	32.2	14.4

[1]ALF = alfalfa from 3 sources; CS = corn silage from 3 sources; and GH = grass hay from 3 sources.
[2]Values expressed as % DM unless otherwise noted.
[3]NFC = non fiber carbohydrate.

Table 3.2. Chemical composition and dry matter digestibility of byproduct and protein feedstuffs

	Feeds[1]															
	BM		WBG		CAN		CIT		DDGS		SBM		SH		SP	
Item[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	89.4	6.62	25.8	2.47	91.3	4.71	88.6	0.35	88.9	1.71	88.4	1.82	90.3	0.97	91.1	---
CP	95.0	9.61	23.6	0.85	43.3	3.39	7.25	0.64	29.6	0.98	53.0	1.19	13.0	1.64	52.2	---
ADF	0.77	0.57	21.6	5.87	19.7	4.14	19.1	0.14	13.7	2.17	4.73	1.19	48.7	2.46	9.90	---
NDF	10.5	15.3	46.1	13.9	28.5	5.51	25.9	0.07	31.1	2.14	8.20	0.61	66.7	2.52	38.1	---
Lignin	0.86	0.82	6.1	3.10	8.96	0.24	2.75	0.33	2.36	0.02	0.89	0.10	2.54	0.45	3.23	---
Sugar	0.87	0.81	9.55	10.8	9.33	1.18	25.9	6.01	6.77	1.27	9.83	0.10	2.23	0.84	8.60	---
Starch	0.17	0.12	6.60	4.53	0.97	0.06	0.45	0.35	4.13	0.76	0.83	0.06	0.53	0.42	0.90	---
Crude Fat	3.16	4.38	8.66	1.69	5.73	4.39	1.20	0.07	9.62	0.76	1.20	1.10	1.65	0.25	0.968	---
NFC[3]	0	9.82	20.2	14.8	21.2	1.30	60.2	0.14	27.9	6.93	34.6	0.57	17.3	0.21	12.5	---
Ash	4.44	1.49	5.80	1.63	7.38	1.48	8.04	0.20	6.06	1.99	6.43	3.18	5.45	0.32	6.29	---
Ca	0.57	0.90	0.23	0.06	0.83	0.22	2.35	0.10	0.03	0.00	0.30	0.30	0.66	0.08	0.44	---
P	0.74	0.92	0.53	0.01	1.16	0.09	0.11	0.00	0.92	0.05	0.82	0.02	0.15	0.04	0.80	---
Mg	0.08	0.10	0.18	0.05	0.66	0.06	0.14	0.00	0.36	0.03	0.35	0.02	0.28	0.04	0.35	---
K	0.23	0.16	0.11	0.01	1.28	0.11	1.03	0.04	1.27	0.06	2.55	0.03	1.65	0.10	2.54	---
S	0.66	0.16	0.29	0.01	0.86	0.08	0.08	0.00	0.88	0.15	0.45	0.03	0.13	0.01	0.57	---
Na	0.44	0.45	0.03	0.01	0.05	0.02	0.02	0.00	0.20	0.13	0.23	0.00	0.01	0.00	0.11	---
Cl	0.23	0.10	0.03	0.00	0.06	0.03	0.07	0.00	0.19	0.01	0.02	0.02	0.01	0.01	0.01	---
Fe (mg/kg)	2281	939	216	30.4	285	88.8	72.0	4.24	84.3	2.52	149	0.01	431	53.5	142	---
Mn (mg/kg)	7.33	8.50	47.0	26.9	81.3	1.15	10.0	0.00	13.7	1.53	35.0	39.8	21.3	9.71	45.0	---
Zn (mg/kg)	20.3	6.03	68.5	10.6	76.3	14.0	10.5	0.71	62.7	5.77	50.3	5.29	46.0	5.57	55.0	---
Cu (mg/kg)	6.00	2.64	5.00	1.41	6.67	1.15	7.00	0.00	4.67	0.58	17.3	3.79	8.33	2.31	20.0	---
DM digestibility	3.86	8.88	53.3	6.91	66.7	6.85	77.8	7.83	60.8	3.23	82.5	9.53	42.9	2.45	36.9	4.16

[1]BM = bloodmeal from 3 sources; WBG = wet brewers grains from 2 sources; CAN = canola meal from 3 sources; CIT = citrus pulp from 2 sources; DDGS = corn dried distillers grains with solubles from 3 sources; SBM = soybean meal from 3 sources; SH = soy hulls from 3 sources; and SP = SoyPass® from 1 source.

[2]Values expressed as % DM unless otherwise noted.

[3]NFC = non fiber carbohydrate.

Table 3.3. Rumen-undegradable protein and corrected rumen-undegradable protein of forage feedstuffs

	Feeds[1]					
	ALF		CS		GH	
Item, % CP	Mean	SD	Mean	SD	Mean	SD
uRUP[2]	24.4	7.00	27.3	2.85	70.1	11.8
wcRUP[3]	24.2	11.0	27.1	3.51	68.7	11.0
lcRUP[4]	23.2	11.7	23.8	4.7	53.0	16.0
acRUP[5]	23.8	10.5	26.9	3.19	67.8	10.2

[1]ALF = alfalfa from 3 sources; CS = corn silage from 3 sources; and GH = grass hay from 3 sources.
[2]uRUP = uncorrected RUP.
[3]wcRUP = wet residue corrected RUP using DNA as a microbial marker.
[4]lcRUP = lyophilized residue corrected RUP using DNA as a microbial marker.
[5]acRUP = average corrected RUP using DNA as a microbial marker.

Table 3.4. Rumen-undegradable protein and corrected rumen-undegradable protein of byproduct and protein feedstuffs

	Feeds[1]															
	BM		WBG		CAN		CIT		DDGS		SBM		SH		SP	
Item, % CP	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
uRUP[2]	89.1	8.82	30.1	6.64	21.2	4.86	27.8	12.3	44.8	0.20	19.7	11.4	35.4	3.86	81.3	4.93
wcRUP[3]	89.1	8.35	30.0	7.33	21.2	4.86	27.8	11.6	44.5	2.52	19.7	12.9	34.0	4.40	81.1	4.88
lcRUP[4]	83.1	7.99	26.5	9.15	20.7	2.24	27.8	4.32	42.0	3.76	---	---	33.4	4.74	75.8	3.45
acRUP[5]	86.2	8.40	29.4	6.66	20.6	4.56	27.1	10.6	43.9	2.44	---	---	33.9	4.10	78.5	4.63

[1]BM = bloodmeal from 3 sources; WBG = wet brewers grains from 2 sources; CAN = canola meal from 3 sources; CIT = citrus pulp from 2 sources; DDGS = corn dried distillers grains with solubles from 3 sources; SBM = soybean meal from 3 sources; SH = soy hulls from 3 sources; and SP = SoyPass® from 1 source.

[2]uRUP = uncorrected RUP.

[3]wcRUP = wet residue corrected RUP using DNA as a microbial marker.

[4]lcRUP = lyophilized residue corrected RUP using DNA as a microbial marker.

[5]acRUP = average corrected RUP using DNA as a microbial marker.

Figure 3.1. Relationship between bacterial CP contamination and NDF concentration of wet in situ residues of alfalfa (ALF), bloodmeal (BM), wet brewers grains (WBG), canola meal (CAN), citrus pulp (CIT), corn silage (CS), corn dried distillers grains with solubles (DDGS), grass hay (GH), soybean meal (SBM), soy hulls (SH), and SoyPass® (SP)

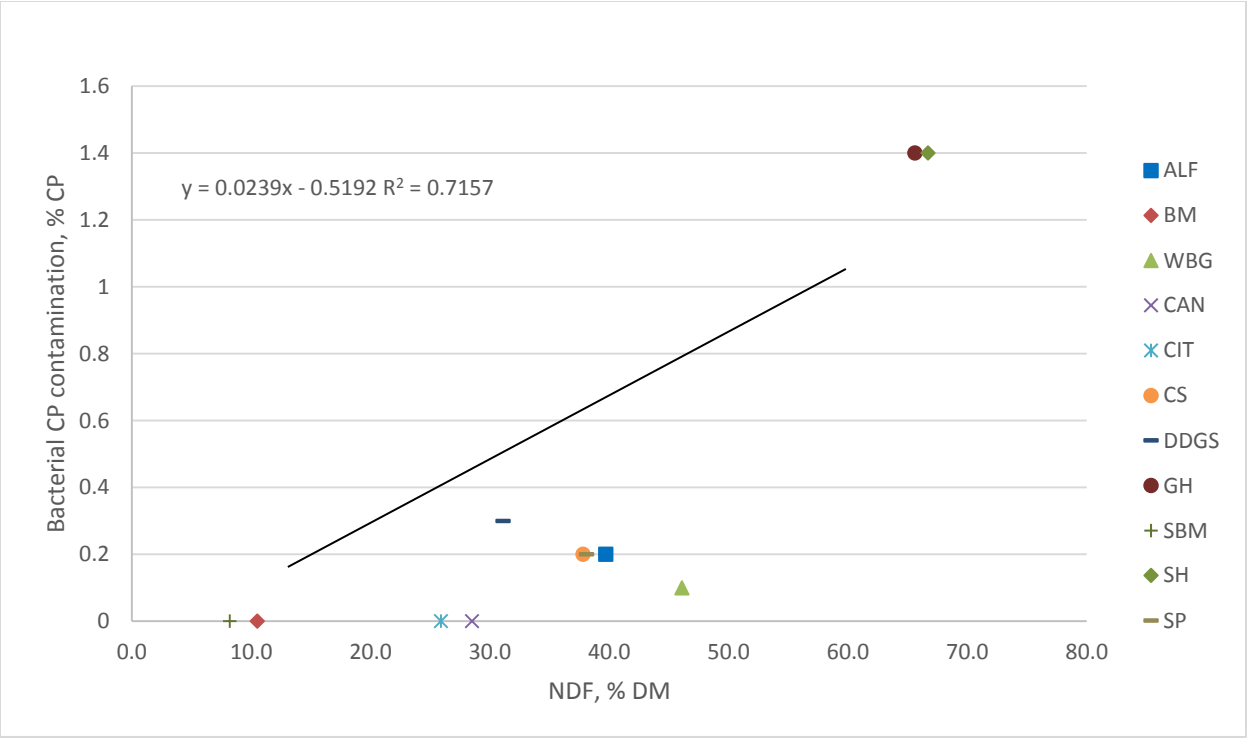


Figure 3.2. Relationship between bacterial CP contamination and NDF concentration of lyophilized in situ residues of alfalfa (ALF), bloodmeal (BM), wet brewers grains (WBG), canola meal (CAN), citrus pulp (CIT), corn silage (CS), corn dried distillers grains with solubles (DDGS), grass hay (GH), soy hulls (SH), and SoyPass® (SP)

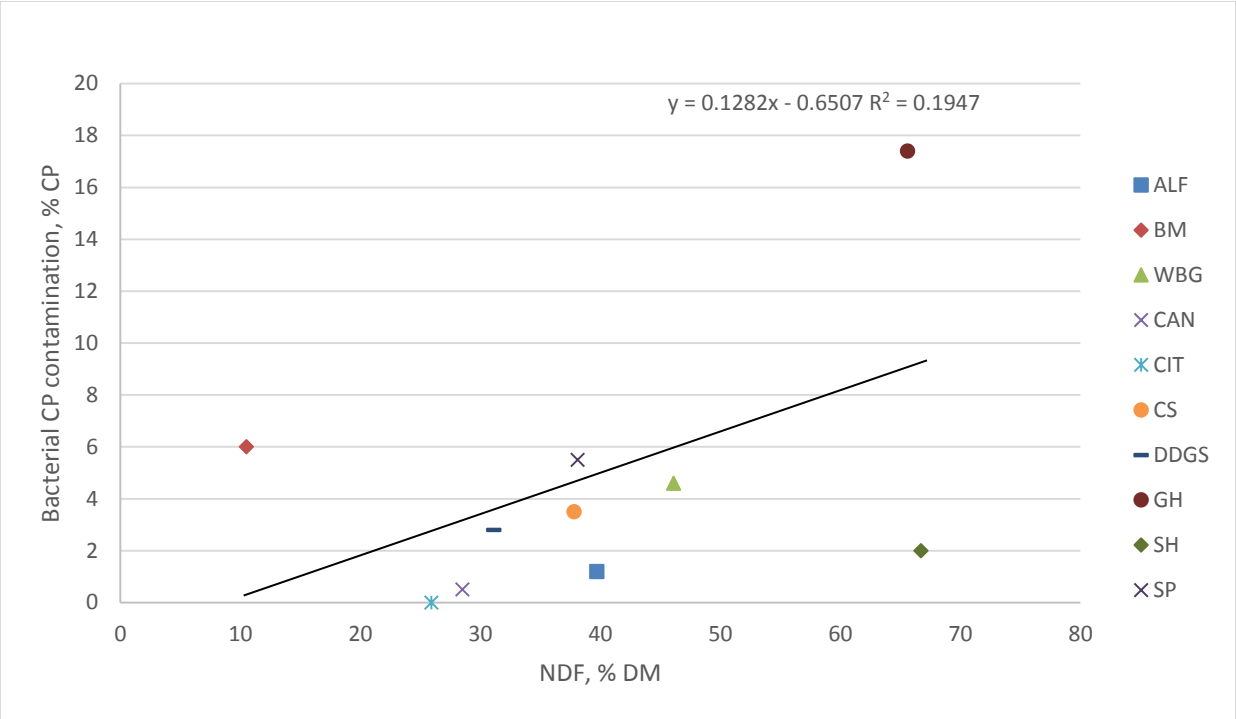
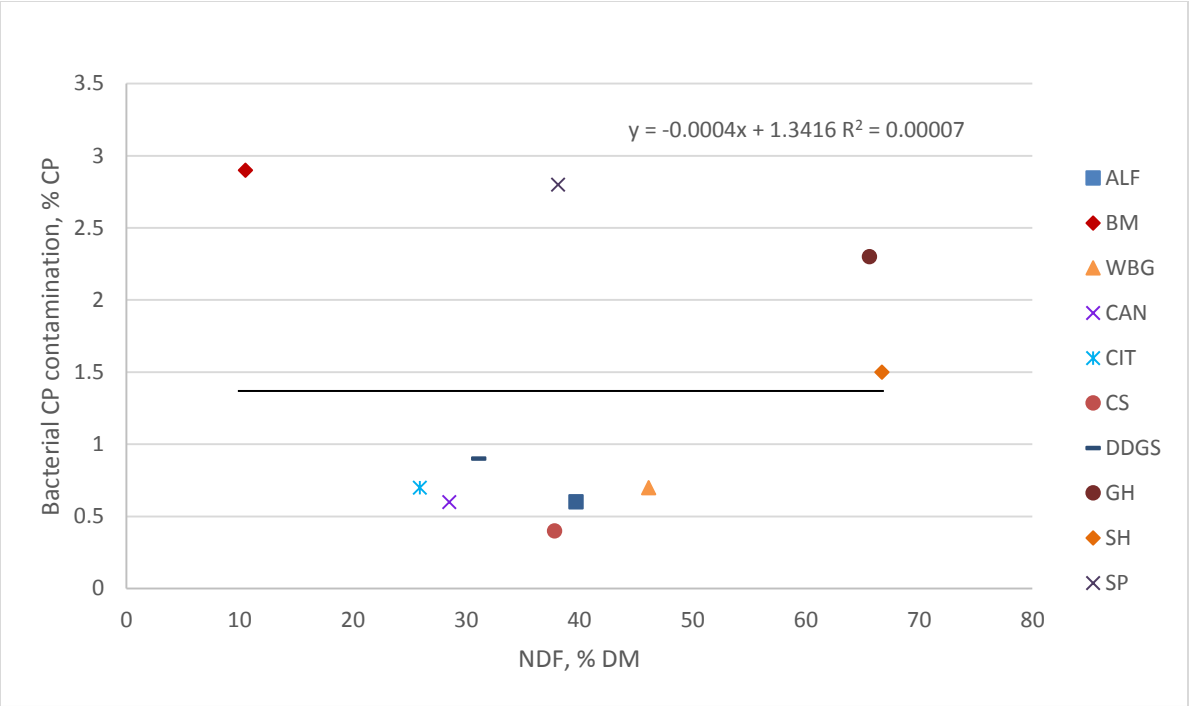


Figure 3.3. Relationship between bacterial CP contamination and NDF concentration of combined wet and lyophilized in situ residues of alfalfa (ALF), bloodmeal (BM), wet brewers grains (WBG), canola meal (CAN), citrus pulp (CIT), corn silage (CS), corn dried distillers grains with solubles (DDGS), grass hay (GH), soy hulls (SH), and SoyPass® (SP)



CHAPTER IV

SUMMARY AND CONCLUSIONS

Inclusion of corn dried distillers grains with solubles (DDGS) as a byproduct in dairy diets has become increasingly popular with the surge of the ethanol industry, due to it's benefit to the animal as an economically low-cost feed. The primary goal of ethanol production companies is to produce ethanol, therefore, the attention and consistency of the DDGS byproduct feed is a secondary concern for most ethanol producers. This causes DDGS to not only be inconsistent across production sites, but also inconsistent within a site from batch to batch variation. This issue has caused interest in understanding not only the chemical composition of DDGS, but also the digestibilities of the components in dairy cattle.

This research focused on evaluating the chemical composition, mineral composition, fatty acid profile, amino acid profile, and both in vitro protein and fiber digestibility of DDGS from 7 different production sites across Michigan, South Dakota, and Nebraska. Newly developed assays by Ross et al. (2013) and Combs (2013) to predict digestibilities of the protein fraction and the fiber fraction, respectively, were used in this study. Generally speaking, variability existed among all components and digestibilities of DDGS, although these differences were numerically small among chemical, mineral, fatty acid, and amino acid compositions therefore making them impractical to consider when formulating dairy cow rations. Interestingly, fiber and protein digestibilities varied significantly across DDGS sources. Total-tract neutral detergent fiber digestibility ranged from 60.0 to 70.5 ± 1.593 % DM across samples. Rumen-undegradable protein (RUP) and the digestibility of this RUP ranged from 65.3 to 89.1 ± 2.772 % CP, and 56.4 to 77.5 ± 2.404 % CP, respectively. The variation in fiber and

protein digestibility among production sites should be further explored for application of these digestibility differences in formulation decisions.

Estimating the RUP content of feeds can be difficult due to varying protein contents in different feeds, as well as the intricate microbial community present in the rumen that can affect estimates of RUP. Rumen microorganisms adhere strongly to feed particles, and have the ability to withstand the rinsing of in situ bags post-rumen incubation, leading to overestimates of RUP content. Using microbial markers, researchers can attempt to quantify the extent of microbial contamination on a wide range of feedstuffs. This research used DNA as a microbial marker using droplet digital polymerase chain reaction to quantify this contamination as a percent of the in situ residue CP content. Feedstuffs used in this study included alfalfa hay, bloodmeal, wet brewers grain, canola meal, citrus pulp, corn silage, DDGS, grass hay, soybean meal, soy hulls and SoyPass®, all of which having a unique chemical composition.

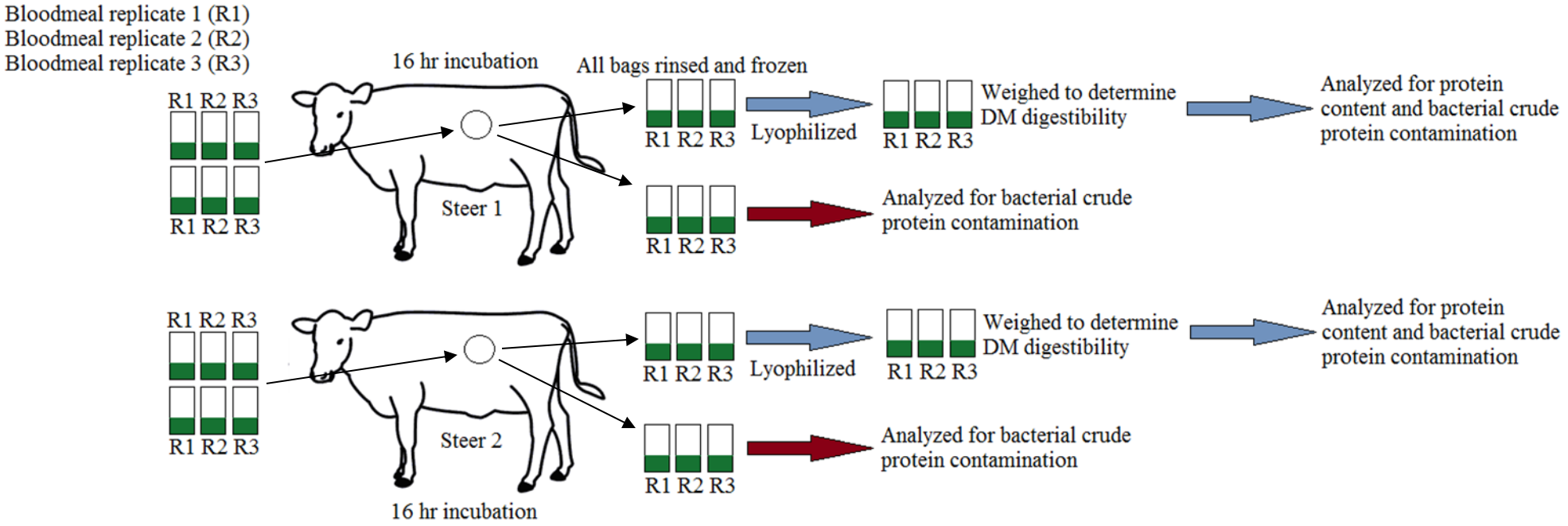
Dry matter digestibility ranged from 3.86 to 82.5 ± 22.4 % across samples, the lowest being bloodmeal and the highest being soybean meal. Rumen-undegradable protein content ranged from 19.7 to 89.1 ± 25.3 % CP, the lowest being soybean meal, and the highest being bloodmeal. These results are in agreeance that the least digestible feed would have the highest concentration of bypass protein. Estimates of bacterial CP (BCP) contamination increased with increasing NDF concentration, leadind us to accept our hypothesis, although the form of the residue may affect the estimate of the degree of BCP.

Further research should be done using DNA as a microbial marker to estimate BCP contamination of both wet and lyophilized residues post-ruminal incubation in order to accurately predict the RUP content of feeds used in dairy diets.

APPENDIX I

PROCESS OF IN SITU INCUBATION OF FEEDSTUFFS AND DNA EXTRACTION

Appendix I. An example of the analysis of DM digestibility, RUP, and bacterial crude protein contamination on bloodmeal, where replicate represents samples from different sources



APPENDIX II

CALCULATION OF MICROBIAL PROTEIN USING DNA AS A MARKER

1) **The abundance of target DNA per µL of ddPCR master mix is calculated**
    a) All sample results from droplet digital PCR (QX200 Droplet Digital PCR System, Bio-Rad Laboratories, Inc, Hercules, CA) are manually gated using Quantasoft Analysis Pro software (Bio-Rad Laboratories, Inc, Hercules, CA) in the 2D amplitude using the heat map setting
    b) Abundance/µL are recorded for all samples from the Quantasoft Analysis Pro software (Bio-Rad Laboratories, Inc, Hercules, CA)
    c) Abundance/µL is multiplied by volume of initial master-mix solution/sample (22 µL)
    d) DNA concentration (ng/µL) is multiplied by volume of DNA added to 22 µL master-mix solution
    e) Step ‘d’ is divided by step ‘c’ in order to attain abundance/ng DNA in master-mix solution

2) **The concentration of DNA in in situ residue samples is calculated**
    a) The DNA concentration of the residue elution sample is calculated by spectrophotometry (DS-C Cuvette Spectrophotometer, DeNovix, Inc. Wilimngton, DE) to attain ng/µL DNA
    b) DNA (ng/µL) is multiplied by volume of elution sample from the DNA extraction process, then divided by the residue weight used from extraction in ng (DM basis) to attain DNA/ng residue

c) Abundance/ng DNA from step ‘e’ in calculation 1 is then multiplied by DNA/ng residue from step ‘b’ in calculation 2 to attain abundance/ng residue

d) Abundance/ng residue is then multiplied by 10^9 to attain abundance/g residue

3) The abundance of bacterial crude protein (BCP) per gram of microbial pellet is calculated

a) The CP content of the microbial pellet residue is divided by the abundance/g residue of the microbial pellet samples. This value is divided by 1 to attain abundance/g BCP

b) The abundance/g BCP of the microbial pellet is divided by the abundance/g residue of the sample to attain g of BCP per g DM sample

4) The corrected RUP as percent CP is calculated

a) The average g BCP/g DM of the sample is multiplied by the residue weight of the in situ bags (g) to attain g BCP of the residue

b) The residue BCP (g) is subtracted from the residue CP (g) to attain g of corrected residue CP

c) The corrected residue CP (g) is divided by the initial CP (g), then this value is subtracted from 1 to attain % corrected RDP

d) The % corrected RDP is subtracted from 100 to attain corrected % RUP

APPENDIX III

POSTER PRESENTATION FROM ADSA ANNUAL MEETING, 2017

Evaluation of chemical composition and in vitro protein and fiber digestibility of corn dried distillers grains with solubles originating from seven sources

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INTRODUCTION

Corn dried distillers grains with solubles (DDGS) contain approximately 30 % fiber and 30 % protein, making this feed a relatively good source of nutrients for dairy cattle. Understanding the source variation and digestibility coefficients of nutrients contained in DDGS will improve our ability to formulate diets to optimize milk production. Corn milling co-products are often a cost effective source of bypass protein for lactating dairy cattle but the concentration of rumen undegradable protein (RUP), the digestibility of RUP (dRUP) and NDF may vary by production process and facility. In vitro laboratory assays that attempt to assess these exist and may be useful in understanding the variation across production facilities. An in vitro lab assay (Ross et al., 2013) to estimate RUP and dRUP is now commercially available and may be useful in describing differences in these methods between sites of production of DDGS. Additionally, researchers have recently developed an in vitro lab assay that attempts to estimate total tract fiber digestibility (TTNDFD) from forages when fed to lactating cows but data is limiting on byproducts (Lopes et al., 2015a,b).

OBJECTIVES

The objectives of this study were to characterize chemical composition and evaluate differences in digestibility of DDGS originating from seven different dry milling facilities across Michigan, South Dakota and Nebraska.

MATERIALS/METHODS

- Feedstuffs evaluated included DDGS from 7 different locations throughout Michigan, South Dakota and Nebraska. Two to four batches from each location were obtained over a 1-month period for a total of 25 samples.
- Samples analyzed for chemical composition, RUP and dRUP (Ross et al., 2013), TTNDFD (Combs, 2013), and color by Cumberland Valley Analytical Services (Hagerstown, MD).
- Data analyzed as a complete randomized design assuming experimental unit was production site.

Key Abbreviations

TTNDFD: Total tract NDF digestibility
NDF: Neutral detergent fiber
DM: Dry matter
RUP: Rumen-undegradable protein
dRUP: Rumen-undegradable protein digestibility

DDGS-1: DDGS from Caro, MI (n = 4)
DDGS-2: DDGS from Groton, SD (n = 4)
DDGS-3: DDGS from Chancellor, SD (n = 4)
DDGS-4: DDGS from Sioux Falls, SD (n = 3)
DDGS-5: DDGS from Mitchell, SD (n = 4)
DDGS-6: DDGS from Big Stone, SD (n = 4
DDGS-7: DDGS from Nebraska (n = 2)

RESULTS

Table 1. Chemical composition and fatty acid profile of DDGS from 7 sources across Michigan, South Dakota and Nebraska

	Feedstuffs								
	DDGS-1	DDGS-2	DDGS-3	DDGS-4	DDGS-5	DDGS-6	DDGS-7		
Item	Mean	Mean	Mean	Mean	Mean	Mean	Mean	SEM	*P*-value
% DM	89.5[a]	89.2[ab]	88.7[b]	88.9[b]	89.0[ab]	88.7[b]	87.2[c]	0.197	<0.01
CP, % DM	29.6[b]	30.7[a]	30.6[a]	30.9[a]	31.2[a]	31.2[a]	31.2[a]	0.323	0.03
Sol Protein, % DM	3.98[c]	4.40[ab]	3.90[c]	3.83[c]	4.20[ab]	4.80[a]	5.75[a]	0.252	<0.01
NPN, % N	0.05[c]	0.05[c]	0.04[c]	0.04[c]	0.04[c]	0.04[c]	0.04[c]	0.006	0.95
AD1CP, % DM	1.86[b]	1.69[b]	2.57[a]	2.00[b]	1.64[b]	1.81[b]	2.10[ab]	0.152	<0.01
ND1CP, % DM	2.26[abc]	2.03[c]	2.81[a]	2.28[abc]	2.04[c]	2.11[bc]	2.81[ab]	0.206	0.09
ADF, % DM	12.2[bc]	10.8[c]	15.2[ab]	11.3[bc]	10.1[c]	12.1[bc]	13.2[a]	0.415	<0.01
NDF, % DM	31.9[bc]	29.4[c]	30.2[bc]	31.8[abc]	29.6[c]	30.8[abc]	32.7[a]	0.522	<0.01
aNDFom, % DM	31.3[ab]	29.0[c]	29.9[bc]	31.2[ab]	29.20[c]	30.3[bc]	32.3[a]	0.531	0.01
Lignin, % DM	1.76[b]	2.10[ab]	2.32[a]	2.34[a]	1.85[b]	2.09[ab]	1.71[b]	0.151	0.08
Sugar, % DM	4.42[ab]	4.45[ab]	4.53[ab]	5.60[a]	3.80[c]	5.90[a]	5.60[ab]	0.467	0.06
Starch, % DM	5.23[bc]	7.28[a]	6.00[bc]	4.80[bc]	6.80[ab]	5.03[bc]	3.05[c]	0.652	0.02
Crude fat, % DM	6.26[b]	5.74[b]	5.95[b]	7.43[a]	5.53[b]	6.04[b]	7.52[a]	0.267	<0.01
Ash, % DM	5.55[a]	5.53[a]	5.65[a]	5.21[a]	5.69[a]	5.62[a]	5.14[a]	0.094	0.01
Ca, % DM	0.05[bc]	0.05[bc]	0.10[a]	0.06[b]	0.08[a]	0.03[c]	0.05[bc]	0.006	<0.01
P, % DM	0.85[ab]	0.80[c]	0.81[bc]	0.85[abc]	0.87[a]	0.85[c]	0.90[a]	0.015	<0.01
Mg, % DM	0.35[b]	0.36[b]	0.37[b]	0.37[b]	0.40[a]	0.37[b]	0.35[b]	0.007	<0.01
K, % DM	1.33[a]	1.26[b]	1.27[ab]	1.32[ab]	1.28[ab]	1.30[ab]	1.28[ab]	0.021	0.34
S, % DM	1.16[ab]	1.15[ab]	1.24[a]	1.04[ab]	1.22[ab]	1.01[b]	0.73[c]	0.072	0.01
Na, % DM	0.24[ab]	0.26[a]	0.26[a]	0.18[bc]	0.26[a]	0.23[ab]	0.12[c]	0.019	<0.01
Cl, % DM	0.20[c]	0.20[bc]	0.20[abc]	0.22[bc]	0.23[a]	0.22[abc]	0.16[d]	0.007	<0.01
Fe, mg/kg	76.8[bc]	88.0[ab]	87.0[ab]	90.3[a]	87.8[ab]	74.3[c]	90.0[ab]	4.689	0.16
Mn, mg/kg	15.3[d]	19.0[c]	25.8[a]	18.7[c]	22.3[b]	15.5[d]	19.5[c]	0.533	<0.01
Zn, mg/kg	83.3[c]	55.3[d]	58.0[d]	66.7[bc]	63.0[cd]	57.8[d]	75.5[b]	3.226	<0.01
Cu, mg/kg	3.00[b]	3.00[b]	3.25[b]	3.33[b]	3.50[ab]	3.50[ab]	4.50[a]	0.287	0.13
Color Analysis	57.1[bc]	54.9[c]	49.2[e]	58.5[b]	55.9[bc]	55.6[bc]	57.1[bc]	0.923	<0.01
Total FA, % DM	6.70[a]	6.06[b]	6.16[b]	6.08[b]	6.58[ab]	6.36[ab]	6.08[ab]	0.179	0.13
C14:0, % TFA	0.06[a]	0.06[a]	0.06[a]	0.06[ab]	0.07[a]	0.07[a]	0.06[a]	0.000	<0.01
C15:0, % TFA	0.03[b]	0.03[b]	0.03[b]	0.03[b]	0.03[b]	0.03[b]	0.03[ab]	0.000	0.04
C16:0, % TFA	14.8[ab]	15.0[ab]	15.2[a]	15.2[a]	14.9[ab]	14.7[b]	14.7[ab]	0.052	<0.01
C16:1c9, % TFA	0.13[a]	0.13[a]	0.13[a]	0.13[a]	0.09[b]	0.13[a]	0.13[a]	0.007	0.03
C17:0, % TFA	0.08[a]	0.09[a]	0.09[a]	0.09[a]	0.07[b]	0.09[a]	0.09[a]	0.004	0.08
C18:0, % TFA	2.06[b]	2.12[a]	2.20[a]	2.21[a]	2.30[a]	2.25[a]	2.22[ab]	0.011	<0.01
C18:1t, % TFA	1.54[ab]	1.55[ab]	1.52[b]	1.57[ab]	1.60[a]	1.55[ab]	1.54[ab]	0.027	0.48
C18:1c, % TFA	1.45[b]	1.42[b]	1.45[b]	1.44[b]	1.46[b]	1.46[b]	1.46[b]	0.025	0.86
C18:2c9c12, % TFA	54.6[a]	54.3[a]	53.8[a]	53.9[a]	53.2[a]	53.3[ab]	53.8[a]	0.057	<0.01
C20:0, % TFA	0.41[a]	0.42[a]	0.43[a]	0.43[a]	0.42[ab]	0.41[ab]	0.40[b]	0.002	<0.01
C20:1c11, % TFA	0.29[a]	0.29[a]	0.28[a]	0.28[a]	0.26[a]	0.27[a]	0.25[a]	0.002	<0.01
C18:3c9c12c15, % TFA	1.45[a]	1.48[a]	1.54[a]	1.55[a]	1.48[a]	1.48[a]	1.50[a]	0.007	<0.01
C20:2n6, % TFA	0.06[a]	0.06[a]	0.06[a]	0.06[a]	0.04[b]	0.06[a]	0.05[ab]	0.003	0.02
C22:0, % TFA	0.18[a]	0.19[a]	0.20[a]	0.20[a]	0.14[b]	0.18[a]	0.19[a]	0.011	0.03
C24:0, % TFA	0.27[a]	0.28[ab]	0.29[a]	0.28[ab]	0.27[ab]	0.28[ab]	0.27[ab]	0.003	<0.01
Unknown, % TFA	0.43[a]	0.39[a]	0.46[a]	0.42[a]	0.58[a]	0.41[a]	0.41[a]	0.035	0.02

Table 2. Rumen-undegradable protein (RUP) content and digestibility of RUP (dRUP) of DDGS from 7 sources across Michigan, South Dakota and Nebraska (Ross, D. A., M. Gutierrez-Botero, and M. E. Van Amburgh. 2013. Development of an in vitro intestinal digestibility assay for ruminant feeds. Cornell Nutr. Conf. Pp. 190-202. East Syracuse, NY)

	Feedstuffs								
Item	DDGS-1	DDGS-2	DDGS-3	DDGS-4	DDGS-5	DDGS-6	DDGS-7	SEM	*P*-value
RUP									
% CP	68.0[c]	65.9[c]	66.7[c]	65.3[c]	77.3[a]	89.1[a]	84.7[ab]	2.772	<0.01
dRUP									
% RUP	57.5[c]	56.4[c]	56.7[c]	57.7[c]	68.0[b]	77.5[a]	71.9[ab]	2.404	<0.01

RESULTS

Table 3. NDF content of DDGS from seven different sources across Michigan, South Dakota and Nebraska at various in vitro incubation times (Combs, D. K. 2013. TTNDFD: A new approach to evaluate forages. Pages 113-125 in Proc. 2013 Cornell Nutr. Conf. Dept. Anim. Sci., Cornell Univ., Ithaca)

	Feedstuffs								
	DDGS-1	DDGS-2	DDGS-3	DDGS-4	DDGS-5	DDGS-6	DDGS-7		
Item	Mean	Mean	Mean	Mean	Mean	Mean	Mean	SEM	*P*-value
24 h NDF digestibility									
% DM	20.8[c]	18.8[c]	18.9[c]	20.0[bc]	18.9[c]	19.0[bc]	16.0[c]	0.600	0.01
% NDF	66.2[a]	64.9[a]	63.1[a]	64.1[a]	64.6[a]	62.7[a]	49.1[b]	1.494	<0.01
30 h NDF digestibility									
% DM	21.8[bc]	20.3[cd]	21.4[bc]	22.6[a]	21.1[bcd]	20.0[cd]	19.6[d]	0.377	<0.01
% NDF	69.7[a]	70.3[a]	71.4[a]	72.5[a]	72.4[a]	66.1[b]	60.6[c]	0.889	<0.01
48 h NDF digestibility									
% DM	24.8[a]	22.9[b]	23.6[ab]	24.2[ab]	23.9[ab]	24.1[ab]	24.0[ab]	0.559	0.43
% NDF	79.1[ab]	79.2[ab]	78.8[b]	77.7[bc]	81.7[a]	79.6[ab]	74.1[c]	0.938	0.01
240 h NDF digestibility									
% DM	26.1[abc]	24.2[c]	25.3[abc]	26.9[ab]	25.4[abc]	25.7[abc]	27.5[a]	0.477	0.01
% NDF	83.3[a]	83.5[a]	84.3[a]	86.2[a]	86.8[a]	84.9[a]	85.3[a]	0.403	<0.01
TTNDFD, % DM	64.1[ab]	66.6[a]	65.9[ab]	67.0[a]	70.5[a]	64.3[ab]	60.0[b]	1.593	<0.05

DISCUSSION

- As expected dried distillers grains with solubles (DDGS) were observed to contain approximately 30 % fiber and 30 % protein and small differences in these concentrations were observed by production site.
- Interestingly, the digestibility of fiber and protein as estimated by two in vitro methods also suggest that differences exist between production site.
- The RUP content and digestibility of this RUP differed by source, suggesting that routine analysis of this assay may be useful in tracking and characterizing this feed for commercial purposes. Research linking in vitro to live animal measures are needed to more accurately understand the implication of these differences.
- Similar to protein, differences by site were also observed in the digestibility of NDF. Also suggesting that routine analysis of this assay may be useful in tracking and characterizing this feed for commercial purposes
- Greatest estimates of total tract NDF digestibility were observed in DDGS originating from plants eliminating a cooking step in the ethanol production process (DDGS-1 through DDGS-6).

CONCLUSIONS

Results indicate that both nutrient composition and digestibility vary across production sites of DDGS. In vitro assays assessing the digestibility of protein and fiber are useful in detecting differences in sources of DDGS. Application of these estimates in formulations decisions should be further explored.