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Ethanol Byproduct Feeds: Determining Accurate Fiber Content, Nutrient Composition and Variability, Storing with Low-Quality Forages, and Fiber Utilization in Finishing Diets

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ETHANOL BYPRODUCT FEEDS: NUTRIENT COMPOSITION AND

VARIABILITY, DETERMINING ACCURATE FIBER CONTENT, STORING WITH

LOW-QUALITY FORAGES, AND FIBER UTILIZATION IN FINISHING DIETS

By

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Ethanol byproduct feeds: determining accurate fiber content, nutrient composition and

variability, storing with low-quality forages, and fiber utilization in finishing diets

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The growing ethanol industry has produced vast quantities of distillers grains plus

solubles (DGS) in the wet (WDGS) and dry forms and Sweet Bran wet corn gluten feed

(SB). Previous research has demonstrated that these byproduct feeds result in improved

feeding values compared to grass in growing diets and corn in finishing diets, with

positive economic returns. Four experiments were conducted to evaluate dry matter

determination methods and variability of nutrient composition for WDGS, determining

the accurate method for measuring NDF in corn and DGS, compare feeding WDGS

mixed with straw as either fresh or ensiled, and evaluate fiber digestibility and

metabolism characteristics for feeding WDGS and SB in finishing diets. Drying wet

byproduct feeds at 60ºC for 48 h was similar to toluene distillation, but these were

different compared to drying at 105ºC for 3, 8, or 24 h, vacuum oven drying, and Karl

Fischer titration. Mean composition of WDGS was 31.0% CP, 11.9% fat, 0.84% P, and

0.77% S (DM basis). Variation of CP and P was small. Dry matter and fat varied more

across ethanol plants than within and across days. Variation in S was greater in period 1,

but decreased in subsequent periods and variation was similar within days compared to

across days. Grinding corn samples through a 1-mm screen Tecator Cyclomill and using

two doses of alpha-amylase during the relux process results in the most accurate NDF

values. Using a pre-fat extraction step prior to the traditional NDF procedure results in more accurate NDF values for DGS. Increasing the level of WDGS from 30 to 45% DM and mixing this with straw resulted in increased ADG and G:F and feeding these mixtures as ensiled also resulted in improved ADG and G:F compared to feeding them as fresh mixes. Steers fed SB at 35 or 88% DM consumed more DM and NDF compared to feeding 35% WDGS. Feeding a *Lactobacillus buchneri* direct-fed microbial did not affect DM or NDF digestibility for feeding diets containing 35% SB or WDGS, but did increase digestibility for feeding 88% SB. Monitoring accurate DM and nutrient composition of DGS, and accurately determining NDF content of corn and DGS makes for useful information in evaluating fiber utilization of byproduct feeds in growing and finishing diets. Feeding WDGS stored with straw results in greater cattle performance compared to the fresh mix and using the DFM in 88% SB diets improves digestibility.

Keywords: cattle, distillers grains, fiber, gluten feed, storage

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INTRODUCTION

Feeding byproduct feeds from the ethanol industry to livestock animals has become very common over the last 30 years. Livestock producers continue to evaluate how much of these byproduct feeds to use in their diets and the differences between these feeds for animal production. This founded the need for conducting research across the U.S., particularly at the University of Nebraska-Lincoln. Feeding distillers grains and gluten feed to beef cattle has resulted in improved ADG and G:F, but this varies depending on the production process, drying of the byproducts, and dietary inclusion. Nutrient composition and dry matter variation is a concern for livestock producers because of the consistency of the products and correct determination of dry inclusion in diets and dry purchase price, respectively. The fiber content in byproducts may be highly digestible, which could contribute to the improved feeding value compared to grass and corn observed in previous experiments. Therefore, it is important to account for all fiber constituents in diets, including corn fiber. A review of literature on feeding distillers grains and gluten feed to growing and finishing beef cattle was conducted to better understand nutrient composition consistency, feeding value and economic advantage, and fiber components and digestibility.

REVIEW OF LITERATURE

Ethanol and byproducts

Ethanol industry and milling processes

The ethanol industry has grown substantially in recent years and is predicted to be maintained in the years to come (NCGA, 2006). Ethanol is produced by fermenting cereal grain starch into ethanol. This process emits carbon dioxide and produces byproduct feeds that can be fed to livestock. There are two main processes for producing ethanol, the dry milling and wet milling processes. Most of the growth in the ethanol industry has come from the building or expansion of dry milling plants.

Dry milling ethanol plants generally use corn for their processes, but sometimes use grain sorghum or wheat. For the sake of discussion, corn will be referred to as the grain source. Corn contains about two-thirds starch, which is fermented to ethanol; thus, only one-third of the product remains (Klopfenstein et al., 2008). The nutrient composition in these byproduct feeds is concentrated about three fold compared to corn. Distillers grains with solubles (DGS) are the main byproduct feeds produced. These can be marketed in the original wet form (~33% DM, WDGS), dried partially and called modified (~48% DM, MDGS), or dried (~90% DM, DDGS). Ethanol plants must purchase and install expensive driers and use natural gas or electricity for heat in order to dry the products into the modified or dry forms. Therefore, the biological and economic value of each of these byproduct feeds may be different.

The main goal for wet milling plants is to produce human food grade products such as corn oil and high fructose corn syrup (Stock et al., 2000). Therefore, the production of ethanol is lower on the priority list for these plants compared to dry mill

plants. Wet mill plants produce corn gluten meal that can be marketed at a higher price to the poultry and pet food industries. The product that is produced in the wet mills and marketed to ruminant animals is corn gluten feed (CGF), which is a blend of corn bran and steep. Some plants may dry the corn bran in order to add proportionally more steep to the ending product. Corn germ meal can be added as well. These products can range from 40 to 60% DM, depending on the amount of drying and are referred to as wet corn gluten feed (WCGF). These WCGF can also be dried into dry corn gluten feed (DCGF). The biological feeding value of these products will be discussed later.

Fiber in corn and byproducts

To understand the composition of the resulting byproducts from ethanol production, it is important to understand the composition of the corn entering the milling processes. The corn kernel by weight comprises of 82.9% endosperm, 11.1% germ, 5.3% pericarp, and 0.8% tip cap (Watson, 2003). The pericarp is the outer coating that protects the corn kernel from the environment. In fact, as corn kernel weights increase, the weight distribution to the pericarp and tip cap decrease and the endosperm increases (Bressani and Mertz, 1958), indicating less fiber. The authors suggested these differences were largely due to different corn hybrids. Starch content in corn is of the most interest for corn producers and ethanol plant owners. However, for the purpose of this research, fiber is also of interest. Most of the NDF in the corn kernel comes from the pericarp (Watson, 2003). Gaspar et al. (2007) stated that the pericarp contains 35% hemicellulose, 18% cellulose, and 20% of a combination of starch and small amounts of protein, oil, and lignin. Watson (2003) estimated that the pericarp contained slightly less than 90% NDF, with the other components as starch and protein. This source also stated that the tip cap

contains about 80% NDF. Corn producers likely believe that most of the NDF in corn comes from the pericarp, which it does. However, the germ contains about 20% NDF on a DM basis (Watson, 2003). Although the germ only comprises 10 to 12% of the dry weight in the corn kernel (Watson and Ramstad, 1987), it contains 81 to 86% of the total oil in corn (Wolf et al., 1952). The fiber in the pericarp and germ total to 9 to 10% NDF in corn, with very small amounts of NDF coming from the endosperm. Corn also contains about 65% starch (primarily from the endosperm), 9% CP (primarily in the endosperm and germ), and 4% fat. These nutrient components can be difficult to process and degrade to expose the fiber for accurate determination of NDF content (Mertens, 2002).

The endosperm and germ can be fed to non-ruminant animals including humans due to high pancreatic amylase production that hydrolyses starch into glucose in the stomach, but non-ruminants cannot readily digest fiber. The whole corn kernel can be fed to swine and poultry because the pericarp is proportionally small by weight of the whole kernel. However, the pericarp (i.e. the corn bran) becomes more concentrated in WCGF and WDGS. Ruminant livestock can readily digest fiber in the rumen due to the high population of fibrolytic rumen microbes, but non-ruminants do not have these specific bacteria. There are small amounts of fiber digested in the large intestine of ruminant and non-ruminant livestock. Therefore, the fiber in byproducts becomes a viable feed source for producing meat and milk with cattle.

Nutrient composition of byproducts

In general, the protein, fiber, and fat content in corn DGS and CGF is greater than the corn it is produced from. Both products have little to no starch (i.e. less than 5% of

DM). Nutrient composition of DGS is approximately 31% CP (70% undegradable intake protein), 11.9% ether extract, 33% NDF, 4.5% ash, 0.84% P, and 0.77% S (Buckner et al., 2010). Nutrient composition of CGF is approximately 18 to 24% CP (depending on the amount of added steep liquor; 30% undegradable intake protein), 3.5% ether extract, 37 to 48% NDF, 5% ash, 0.9% P, and 0.5% S (Stock et al., 2000). The range in CP and NDF is dependent on the ratio of corn bran to steep that wet mill plants blend together.

There are two different types of WCGF produced. The first product, consisting of wet corn bran blended with steep, is more widely produced. Corn bran is lower in CP and higher in NDF compared to steep. Because the corn bran in this product is wet, less steep is added to the bran as compared to the second product, making for lower CP and higher NDF. This first CGF product contains 15 to 18% CP, 44 to 48% NDF, and 40 to 42% DM, and will be referred to as traditional WCGF. The second WCGF is produced by drying the wet corn bran and adding a greater proportion of steep to the bran compared to the first WCGF product. This product may also contain solvent-extracted germ meal (SEM). Germ meal is what remains after fat (corn oil) has been extracted with a solvent from the germ of the corn kernel. Due to a greater proportion of steep and SEM, this second WCGF contains more CP (20 to 25%), less NDF (37 to 42%), and is higher in DM (60%) than the traditional WCGF. This product is produced by Cargill, Inc. and will be referred to as its trademark name, Sweet Bran (SB). Dry CGF can be made from either of these two products by drying to 10% moisture.

Although the nutrient composition of DGS and CGF is somewhat different, one commonality is their high content of fiber (NDF). Neutral detergent fiber is comprised of hemicellulose, cellulose, and lignin, but the NDF in WCGF and WDGS is largely

hemicellulose and cellulose, which are highly digestible. The NDF in DGS and CGF is primarily corn bran which comes from the pericarp of the corn kernel. A small portion of the NDF in byproduct feeds can originate from the corn germ. All of the fiber that is in the germ of corn remains in WDGS, but it may or may not be in WCGF depending on the process. The improvement in feeding value of these byproduct feeds relative to corn may be due to their highly digestible fiber, as DeHaan (1983) stated that the fiber in corn bran was 87% digestible in the rumen in an in-vitro setting. In comparison, digestion of starch is 92 to 99% total tract and 78% of this occurs in the rumen (Waldo, 1973; Owens et al., 1986).

Nutrient composition and variation of DGS

Although WDGS has become a common feedstuff in the livestock industry, there has been concern about the consistency of its nutrient composition (Babcock et al., 2008). Three nutrients commonly measured in WDGS are CP, fat, and S. Measuring DM is important in wet feeds because they are marketed and sold on an as-is basis. The nutrient content of wet byproducts is only worthwhile on a DM basis. Therefore, knowing the DM content of these products is important to calculate a DM cost from the as-is price. The variability of DM may be just as critical as the plant's average DM. If a cattle producer pays for their byproducts based on the plant's average DM, this may be inaccurate if the DM of a specific byproduct load is different. Kaiser (2005) reported CV of 2.8 to 3.8% within each plant for WDGS samples collected from 3 ethanol plants. Holt and Pritchard (2004) observed DM variation for DGS samples collected across ethanol plants with CV of 6.8 and 4.7% for WDGS and MDGS, respectively.

Measuring CP content in feeds is relatively simple and inexpensive compared to measuring other nutrients. Holt and Pritchard (2004) observed a SD of 1.4 and Kaiser (2005) reported a SD of 1.5 to 1.6 for CP content of WDGS samples. Samples of DDGS have been measured for CP content as well. Spiehs et al. (2002) and Akayezu et al. (1998) observed ranges in CP content for DDGS of 28.7 to 31.6% and 27.7 to 32.3%, respectively.

Fat content in DGS is important to measure because if large amounts of fat are fed, then feed intakes may decrease (Vander Pol et al., 2009). Spiehs et al. (2002) and Akayezu et al. (1998) reported fat ranges for DDGS within ethanol plants of 10.2 to 11.7% and 8.8 to 12.4%, respectively. Holt and Pritchard (2004) also showed differences in fat content in WDGS and DDGS among ethanol plants, ranging from 10.4 to 14.2%.

Knowing the P content in DGS is important because excess P is excreted in manure. The more concerning aspect is being able to calculate P manure excretion for nutrient mass balance purposes in feedlot operations. Holt and Pritchard (2004) observed a range of 0.49 to 0.78% P for WDGS and DDGS. However, Spiehs et al. (2002) reported an average of 0.89% P for DDGS, and Kaiser (2005) reported averages of 0.8 to 0.9% P within ethanol plants. Kaiser (2005) also reported SD for P of 0.1 to 0.2 for WDGS.

The NRC (1996) suggested the maximum tolerable S level was 0.40% for potential occurrence of polioencephalomalacia (PEM), thus making S in WDGS important if it is high and/or variable. Spiehs et al. (2002) reported a range for S means with 12 ethanol plants for DDGS of 0.33 to 0.74% and CV ranging from 6.4 to 40.8%. Holt and Pritchard (2004) also reported high variability in S levels for DDGS, but the variability was not quantified. These data suggest that S content in DGS possibly varies

more than other nutrients analyzed. However, a more comprehensive analysis is needed

for determining nutrient variability in WDGS.

Determination of NDF content

Understanding the NDF content in feeds and being able to quantify this amount is

important for ruminant nutrition. However, some other nutrients in feeds may hinder the

accurate determination of NDF content. The NDF procedure was originally developed by

Van Soest and Wine (1967) for the analysis of total fiber in forages. Determining NDF

was primarily important for forages until the last 20 years. Now, determining the NDF

content in byproduct feeds may be just as important because the production of ethanol

and byproducts are more prevalent. Although corn was fed in finishing diets 20 years

ago, corn was considered all concentrate with no fiber. Now that byproduct feeds

containing little starch have been produced and have been fed in both finishing and

growing diets, this changed the recent needs for analyzing NDF content in feeds. Protein,

ether extract, and starch can hinder accurate determination of NDF. Protein can be

complexed with lignins in numerous feeds, but protein can be dissolved by using sodium

sulfite in the NDF procedure (Van Soest, 1994). Although CGF and DGS contain little

starch, DGS can contain large amounts of ether extract (9 to 13%). Therefore,

determining accurate NDF in byproduct feeds is important because fat can hinder the

filtering capability of the fiber residue. Using a solvent to extract fat that interferes with

fiber content may be a possibility. Because byproduct feeds are increasingly important in

ruminant diets, knowing the NDF content in the corn that the byproducts originated from

becomes important. However, large amounts of starch, both easily degradable and

resistant, can be hard to digest easily by only using neutral detergent solution (Mertens,

2002). The small amounts of starch remaining in byproduct feeds may be of the resistant starch type because the readily available starch was removed during fermentation. Mertens (2002) suggested that heat-stable α-amylase can be used in the NDF procedure to break down more remaining starch in feeds. These findings suggest that new developments may be needed to measure NDF in high starch or high fat feeds.

Use and economic trends for distillers grains

Ethanol byproducts have become a popular commodity to use in finishing diets by feedlot owners in Nebraska. In a survey conducted by Waterbury et al. (2009), 91.2% of all of the cattle operations in Nebraska were using byproduct feeds in 2008. Of these operations, wet byproducts were primarily fed to finishing cattle. The main byproducts fed to finishing cattle were WDGS and MDGS at 53 and 29%, respectively, of total byproducts fed. In a feedlot nutritionists survey (Vasconcelos and Galyean, 2007), nutritionists responded that DGS was the primary byproduct fed in finishing diets. This represented 70% of the total number of cattle on feed that consumed DGS due to the number of consultants that responded and the number of cattle and cattle operations they represent.

As the ethanol industry continues to grow, more dry mill plants are built and more DGS are produced (NCGA, 2006). This creates a greater supply of DGS that is available for purchase by livestock operations. A simple economic concept is one that as supply increases and demand decreases, then the price decreases. In the last 4 years, more supply of DGS has been produced, which the price of DGS would be expected to decrease. However, the price of DGS is very reflective of the price of corn. Therefore, if corn prices increase, DGS prices typically increase as well. Regardless of corn prices, cattle on

feed in feedlots in the Midwest tend to decrease after June when cattle are sent to market and before receiving new calves in the fall. This results in fewer cattle on feed in the summer months and less demand for DGS in finishing diets. Although there is less demand for DGS in the summer, supply of DGS remains the same, thus DGS prices decrease (Erickson et al., 2008).

The seasonal price decrease of DGS provides an opportunity for cattle producers to purchase DGS in the summer months at lower costs. Ideally, cattle producers would like to purchase feeds when they are cheap and feed them when needed. Many times, this period of need is during the winter months for cow/calf owners when forage supply is limited and cattle may need some supplementation (Janovick et al., 2004). For feedlot owners, this may be at any time of the year when feedlot cattle occupancy is large and demand is great. The price of WDGS will typically be less than that of DDGS (Waterbury and Mark, 2008) because DDGS can be sold to poultry and swine producers as a feed source, thus demand will remain greater for DDGS. The price of WDGS is also cheaper than DDGS because of the cost of drying the DDGS. Purchasing WDGS provides an opportunity for cattle producers to buy a high protein, high energy feedstuff to feed to finishing cattle and cattle grazing poor quality forages that need supplementation.

One problem with storing WDGS is the consistency of the product because it is very soft and spongy. Wet distillers grains plus solubles does not stack and instead spreads out. This product will also spoil quickly in an aerobic environment due to its high moisture content, particularly in the hot summer months. Therefore, research has been conducted to test how much low quality, dense forages are needed to mix with WDGS

and store in either a concrete bunker silo or in anaerobic silo bags under pressure

(Erickson et al., 2008). Straw and moderate quality grass hay at 12.5 and 15% DM,

respectively, were determined to be the minimum inclusions needed to mix with WDGS

in silo bags with 300 psi of pressure without breaking the bags. The required inclusions

of forages increase when WDGS is stored in concrete bunkers. For instance, 35 to 40%

grass hay or straw hay (DM basis) is needed to mix with WDGS in a bunker silo. An

inclusion of 30% corn stalks (DM basis), a bulky roughage, is needed in bunker silos.

Using low quality forages to mix with high quality WDGS perhaps aids in utilizing these

forages better as compared to feeding them alone, as these mixtures of low quality

forages with WDGS are readily consumed in cattle diets.

Producers can harvest or purchase poor quality forages, such as straw, corn stalks,

and poor grass hay. These harvested forages are used as winter feeds, and can be used as

an excellent sources of roughage in finishing diets. Straw and corn stalks are interesting

forages because they already exist after wheat and corn, respectively, have been

harvested. They can be produced at a relatively cheap price for cattle producers because

the only cost in harvesting these residues is the expense of running the baling equipment.

Therefore, feedlot owners can buy readily available, cheap forages that can be used in

finishing diets as roughages. Low quality forages may also be used in mixing with the

high feeding value byproduct of WDGS. One option is to mix low quality forages with

WDGS and store this in a bunker or bag. However, this does not eliminate spoilage

concerns related to WDGS. Covers can be used on top of the WDGS mixtures to decrease

spoilage (Christensen et al., 2010). Using plastic or solubles as covers are feasible options

that decrease the amount of spoilage. Amount of spoilage decreases in a 3 m bunker from

3.9 to 4.9% to 0.6 and 2.0% for plastic and solubles covers, respectively. Therefore, cattle producers have an opportunity to purchase WDGS during the summer months, store them with low quality forages, and feed this mixture to cattle at a relatively cheap price when supplementation is needed.

Distillers grains to growing cattle

Feeding ensiled WDGS with low quality roughages to growing cattle

Although storing WDGS with low quality forages may be economically advantageous, the feeding value of these mixtures has been only minimally researched. Wilken et al. (2009) evaluated feeding 30% WDGS (DM basis) mixed with chopped corn stalks as either mixed fresh daily or from bags that had been mixed and ensiled. Steers were fed individually using the Calan gate system. Steers fed the fresh mix had decreased DMI, ADG, and G:F compared to the ensiled mix. This suggested that the stored mix had a better feeding value that the fresh mix. The problem with this experiment was that in order to accurately evaluate energy value differences between the fresh and ensiled mixtures, cattle should have been fed to have similar DMI. From a research perspective, keeping DMI similar between the mixture types would create an opportunity to evaluate energy differences accurately by measuring ADG or G:F.

Peterson et al. (2009) attempted to feed the fresh and ensiled mixes at similar DMI. They evaluated feeding 35 and 45% WDGS (DM basis) combined with wheat straw and feeding it as fresh commodities mixed on the d of feeding or feeding them after the mixture had been ensiled and stored in a silo bag. Steers were fed individually using the Calan gate system. Steers fed 45% WDGS had decreased DMI, similar ADG, and increased G:F compared to steers fed 35% WDGS. This suggested that the mixture has a

greater feeding value when WDGS is included at greater inclusions. Steers fed the fresh mixes had greater ADG and G:F than steers fed the ensiled mixes. One problem with this study was the source of WDGS in the fresh mixes was not the same source as the WDGS in the ensiled mixes. Therefore, an experiment was needed to evaluate cattle performance when the same source of WDGS was fed as either fresh or ensiled with a low quality forage at similar intakes.

Distillers grains in forage diets

Distillers grains provide cattle producers with an opportunity to feed a high protein, high energy feedstuff cheaper than corn (Waterbury and Mark, 2008). This product can often be economical for producers that need to supplement cows or calves grazing forages when the protein and energy content in the forages does not meet the animal's needs. To evaluate the energy value of DDGS, Loy et al. (2008) supplemented DDGS at 0.21 and 0.81% of BW (10 and 33% of diet DM) and observed increases in ADG and G:F for heifers compared to feeding DRC. Therefore, they concluded that DDGS had 130 and 118% the energy value of DRC at 10 and 33% of diet DM, respectively. Nuttelman et al. (2009) evaluated feeding WDGS compared to DRC in forage diets and observed a 130% greater energy value for WDGS compared to DRC at 25% DM inclusion due to increased ADG and G:F. In a follow-up study, Nuttlemen et al. (2010) observed greater energy values of 149 to 142% for WDGS compared to that of DRC in forage diets at 15 to 35% of diet DM, respectively, due to increased ADG and G:F. These authors concluded that DGS not only provides protein in high forage diets, but has a greater energy value than DRC. It appeared that WDGS had a greater energy value than DDGS, but they were not compared directly in the same experiment.

Ahern et al. (2011) evaluated feeding 15 and 30% WDGS or DDGS in comparison to 22 and 50% DRC (DM basis). Inclusion of DRC was greater than DGS because the authors predicted DGS would have a greater energy value than DRC and they wanted cattle to have similar ADG at the same level (low or high) of DGS and DRC. They succeeded as ADG was similar between DGS and DRC and feeding values were 114 and 119% for DDGS and WDGS, respectively, compared to DRC. Increasing the inclusion of WDGS, DDGS, or DRC also increased ADG and G:F. Therefore, the calculated energy values for DDGS and WDGS are not always the same across different experiments. Distillers grains plus solubles is a higher energy feed source than DRC in high forage diets for cattle.

Distillers grains and gluten feed to finishing cattle

Feeding value of gluten feed and distillers grains in finishing diets

The biological feeding values of dry and wet CGF are not the same. Drying CGF reduces its energy value compared to WCGF (Green et al., 1987; Ham et al., 1995). The following discussion will describe the energy value of WCGF compared to corn. Stock et al. (2000) summarized 7 experiments that fed the traditional WCGF product in replacement of either DRC or HMC. Similar G:F and net energy for gain were observed for traditional WCGF compared to the corn as ADG and G:F was affected by less than 1%. Traditional WCGF can be fed in finishing diets (less than 25%) as a protein source in replacement of supplemental protein. However, there is little energy value differences for traditional WCGF compared to DRC or HMC and the WCGF would likely only be fed at inclusions greater than 20% if it was priced at the feedlot cheaper than corn.

A statistical meta-analysis was conducted on multiple experiments where SB was evaluated compared to DRC or HMC (Klopfenstein et al., 2007). Increasing linear effects were observed for DMI, ADG, and G:F, indicating that as inclusions of SB increased in the diet (up to 40% DM), performance measurements increased as well. This translated into an improved feeding value (112%) for SB compared to corn and improved carcass quality grade. Economics of feeding WCGF will be discussed later.

The feeding value of WDGS was evaluated in another statistical meta-analysis by Klopfenstein et al. (2008). Inclusion of WDGS was 0 to 50% of diet DM and WDGS replaced either DRC or HMC or a combination of both corns. They observed a quadratic effect for DMI and ADG. Inclusions of WDGS at 20 to 30% DM resulted in the greatest DMI and ADG. Greater ADG values were observed when WDGS was fed in diets at 40 to 50% DM compared to feeding the corn only diets. Feed efficiency increased quadratically as inclusion of WDGS increased. This resulted in a feeding value of 145 to 126% for WDGS at 10 to 50% DM inclusion, respectively, compared to feeding corn only. Carcass fat thickness and quality grade resulted in increasing quadratic trend as ADG. Cattle not only gain more weight and convert feed to weight gain more efficiently when fed WDGS, but cattle also finish with adequate fat depth and marbling.

Both WDGS and DDGS have been evaluated in the same experiments previously (Ham et al., 1994; Sarturi et al., 2010). However, there was no previous experiment that had evaluated WDGS, MDGS, and DDGS in the same trial until Nuttelman et al. (2010). These authors concluded that increasing quadratic trends resulted when feeding MDGS and DDGS at 20 to 40% diet DM, similar to previous experiments. The improvement in ADG, G:F, and feeding value decreased as DM content in the DGS increased. At diet

DM inclusions of 20, 30, and 40%, the feeding value improvement for WDGS, MDGS, and DDGS was 43, 27, and 9%, respectively, greater than feeding corn alone. These authors suggested that drying DGS reduced their feeding values, similar to the reduced feeding values observed for drying CGF.

Economic scenarios for feeding DGS and CGF in finishing diets

Profitability of cattle in a feedlot depends on numerous variables. The predominate variables are the purchase price of feeder cattle, the price of corn used in diets, and yardage costs that are reflective of days on feed. Including byproducts, especially wet byproducts, in diets increased the number of variables that had to be accounted for in determining cattle feeding profitability. These additional variables include the purchase price of the byproducts (most likely on an as-is basis), the DM content of the byproducts to determine purchased DM price, hauling cost to transport the byproducts from the ethanol plants to the feedlot, and additional hauling costs associated with feed truck use at the feedlot due to feeding wetter diets. If cattle ADG is greater for feeding byproducts and feeder cattle and fat cattle weights remain the same, then cattle do not require as many days in the feedlot to reach market weight. Therefore, an economic spreadsheet model was developed to consider all of these variables for cattle profitability (Buckner et al., 2008).

If WDGS, MDGS, and DDGS are priced at 90% the price of corn (freight on-board, at the plant) on an equal DM basis and hauled 60 miles or less to the feedlot, then the economic advantage would be greatest for WDGS and least for DDGS. These returns were greatest at 30 to 40% diet DM for WDGS and MDGS and at 20% diet DM for DDGS. Although each of these scenarios for feeding WDGS, MDGS, and DDGS

resulted in more profit than a corn only diet, feeding WDGS resulted in up to $50 / head additional profit compared to feeding corn. Because there are many dry milling plants in Nebraska, the hauling distance is often within 60 to 100 miles to transport DGS from an ethanol plant to a feedlot. Therefore, the additional cost that feedlot owners incur to transport the water in WDGS or MDGS is minimal compared to the benefit of greater cattle ADG and G:F when fed these DGS compared to DDGS or corn. The improvement in ADG and G:F results in fewer days on feed and less total pounds of feed consumed.

The previous scenario is only one example of potential economic returns when feeding DGS. If DGS are priced less than 90% the price of corn, then more profit is returned and greater dietary inclusions can be fed to reach optimum economic inclusion. The closer a feedlot is to an ethanol plant, the more profit is expected (Buckner et al., 2008).

Feeding traditional WCGF at 90% the price of corn and hauling the WCGF 60 miles to the feedlot resulted in economic returns of less than $1/ finished steer fed compared to feeding a blend of DRC and HMC with no byproducts. This similar economic return is primarily due to the similar feeding value of traditional WCGF compared to corn. Even though traditional WCGF was priced less than corn, hauling this wet product to the feedlot offset any beneficial purchase price. Depending on supplemental protein prices, traditional WCGF can be economically advantageous at lower inclusions (less than 20% DM) due to its high protein content compared to corn. Because ADG and G:F linearly increased when feeding SB, the number of days to reach a common fat cattle weight decreased, thus decreasing yardage costs. When SB was priced at 90% the price of corn and hauled 60 miles to a feedlot, economic returns

increased with increasing dietary inclusion of SB. If the price paid for SB decreased then returns increased, and if hauling distance from the wet mill to a feedlot increased then returns decreased.

Components of wet corn gluten feed on cattle performance

As previously discussed, there are differences in cattle performance when fed the two different types of WCGF. These differences are likely due to the combinations of corn bran, steep, and SEM, which will be discussed.

Corn bran is primarily a source of fiber (80% NDF) with very little fat or starch, suggesting that it would have a low energy value. Including corn bran in DRC based finishing diets has increased DMI (Scott et al., 1997; Adams et al., 2004; Sayer et al., 2010). The increase in DMI could be due to one of two reasons. Cattle consume more DM when dietary inclusion of fiber increases, possibly including when corn bran is added to diets. Previous research indicated that increasing the amount of roughage (i.e. fiber) in finishing diets promoted greater DMI, possibly due to more "scratch factor" in the rumen from fiber that stimulates appetite (Galyean and Defoor, 2003) and decreased ruminal acidosis. The second reason may be due to a dilution in dietary energy content. Including corn bran in diets mainly contributes fiber with nearly no fat or starch, therefore, diets contain less energy. Therefore, it was not surprising that including corn bran in finishing diets resulted in either no impact or a decreased effect on ADG, resulting in decreased G:F (Adams et al., 2004; Sayer et al., 2010).

Steep liquor, often in combination with distillers solubles from the wet milling industry, called steep, has a greater energy value than corn bran and corn that it replaces in finishing diets. Steep is very low in NDF, but contains 45% CP (Blanchard, 1992) and

has greater net energy than corn (Stock et al., 2000). Replacing DRC in diets with steep

at 15% DM without any corn bran increased DMI (Scott et al., 1997). This affect

declined at 30% DM inclusion and DMI was not statistically different from the corn

control diet, suggesting that acidosis was adequately controlled at the 15% inclusion.

When ruminal acidosis occurs, DMI decreases due to this large load of acid in the rumen

and negative appetite stimulation (Cooper et al., 1999). Feeding steep in combination

with corn bran at total inclusions of 22.5% (Herold et al., 1998) or 45 and 60% (DM

basis; Sayer et al., 2010) increased DMI compared to the corn only diet. They suggested

that including corn bran in diets containing steep continued to aid in the control of

acidosis and increased DMI. In each of these 3 experiments, including steep alone or in

combination with corn bran increased ADG and G:F. The increase in ADG and G:F

suggested that steep has a greater energy value than the corn that it replaced.

The results from feeding corn bran or steep alone or in combination with each

other, explains the performance differences observed when cattle are fed WCGF

compared to corn only. The increase in DMI is likely due to greater dietary fiber content

from corn bran, and the increase in ADG and G:F is likely due to including steep.

Because steep is the component of WCGF that contributes to greater weight gains and

feed efficiency, the more steep that can be included in diets containing corn bran, the

greater the performance response will likely be. Hence, the reason as to why the two

WCGF products result in different performance responses. The traditional WCGF

product that results in the same energy value as corn has less steep relative to corn bran.

SB has about 112% the energy value of corn and has more steep. This product also

contains a small amount of SEM.

Solvent extracted germ meal may be blended in the SB product. SEM contains about 50% NDF, 24% CP, and a small portion of fat. This NDF is high in hemicellulose and cellulose with little lignin, thus making it more digestible than typical grasses that are high in NDF and have more lignin. Herold et al. (1998) evaluated feeding 9% SEM or 19% SEM with 19% steep (DM basis) and observed no differences in DMI, but ADG increased for the combination treatment. Feeding SEM in both of these diets improved G:F, suggesting SEM has a greater energy value than the corn it replaces. They conducted another study in which SEM was fed in addition to a combination of corn bran and steep (Herold et al., 1998). Although DMI and ADG were not affected, the SEM improved G:F. These results suggested that SEM provided an added benefit to a combination of corn bran and steep in terms of improved G:F and greater energy values. The improved energy value of SB may be due to more steep because the corn bran is dried and the SEM that is added to the mixture compared to traditional WCGF.

Feeding high levels of wet corn gluten feed

A potential opportunity exists to feed greater than 40 or 50% byproducts (DM basis) in finishing diets, particularly if corn becomes expensive. In theory, byproducts provide minimal amounts of fermentable starch that could cause acidosis. In fact, WCGF and WDGS provide a readily form of fiber from corn containing mostly hemicellulose and cellulose with little lignin that can be utilized in finishing diets. Feeding WCGF at dietary levels greater than 40% DM provides an opportunity to feedlot managers to feed more byproducts but with less risk than feeding WDGS due to lower fat and S content. Wet corn gluten feed has a lower fat content (3.5%) that would be less susceptible to decreasing cattle intakes and performance compared to WDGS that has a fat content of

12%. Sulfur content in WCGF is also less than that of WDGS (0.5% vs 0.7%), suggesting less risk of PEM.

Corn based diets containing 50, 70, and 90% traditional WCGF (DM basis) were fed to finishing cattle to evaluate greater inclusions of WCGF on performance (Firkins et al., 1985). An increase in DMI was observed with the greater inclusions of WCGF compared to corn, similar to the meta-analysis. No statistical differences were observed in G:F for feeding greater inclusions of traditional WCGF, but ADG increased in a quadratic relationship. The authors suggested that feeding traditional WCGF at intermediate levels (50 to 70%) resulted in optimum cattle performance. However, feeding the 90% traditional WCGF diet resulted in ADG and G:F compared to feeding cattle an all corn diet. Therefore, if traditional WCGF can be priced and hauled to a feedlot cheaper than corn, then feeding 90% traditional WCGF becomes economically advantageous.

A diet containing all SB, at 86.6% DM inclusion, was compared to a 50:50 SB (44%) and DRC (43%) based diet and an all DRC diet to determine differences in cattle performance and degree of carcass finish (Richards et al., 1996). Dry matter intake decreased as SB inclusion increased, but ADG increased quadratically. Daily weight gain was greatest for cattle fed the 50:50 SB and DRC diet, but cattle fed the all SB diet resulted in similar ADG as cattle fed the DRC diet. Increased G:F resulted for cattle fed the SB diets. Therefore, an improved feeding value resulted from feeding SB compared to corn, regardless of SB inclusion level. Although the high SB diet contained low dietary fat and starch, degree of finish (i.e. fat thickness) was similar for cattle fed this diet compared to the SB and DRC combination diet and the DRC diet. This research indicated

that SB can be fed at high inclusion levels in the diet and will improve cattle performance while maintaining fat deposition compared to feeding only corn in diets. However, this high level of SB in finishing diets with no corn has not been evaluated for ruminal metabolism.

Metabolism characteristics from feeding wet corn gluten feed

A few metabolism experiments have evaluated ruminal metabolism and digestion effects of feeding WCGF based diets relative to corn control diets. These experiments have mainly evaluated DM and NDF digestibility, ruminal pH, and ruminal VFA concentrations. Scott et al. (1998), Bierman et al. (1999), Sayer et al. (2010) evaluated feeding diets containing WCGF or corn bran in combination with steep compared to a traditional DRC control diet. No significant differences in DMI were observed in these experiments from feeding the WCGF based diets compared to the corn control diets. However, Scott et al. (1998) and Sayer et al. (2010) observed numerical DMI increases for feeding corn bran alone or in combination with steep compared to the corn diets. These differences were not observed statistically likely due to the feeding of individual cattle as there can be large individual intake differences and these differences were not overcome by large replications in metabolism studies. Two experiments, McCoy et al. (1997) and Montgomery et al. (2004), observed greater DMI when feeding traditional WCGF at 45% in a DRC based diet and 40% in a SFC based diet (DM basis), respectively, compared to their DRC and SFC control diets. McCoy et al. (1997) fed a 90% traditional WCGF diet with no corn and observed numerically greater DMI compared to a DRC only diet.

Effects of DM digestibility of WCGF containing finishing diets were similar to those effects observed for DMI. Dry matter digestibility was lower for cattle fed WCGF or corn bran in combination with steep compared to a DRC control diet in the experiments of McCoy et al. (1997), Scott et al. (1998), Bierman et al. (1999), and Sayer et al. (2010). Montgomery et al. (2004) observed an increase in DM digestibility when feeding 40% WCGF compared to a SFC control diet. Although feeding WCGF generally reduced DM digestibility in these trials, DMI either increased statistically or numerically. Therefore, similar amounts of DM were digested in the WCGF containing diets and the corn diets within each of these trials. Feeding WCGF or corn bran in combination with steep in diets resulted in increased NDF digestibility compared to corn control diets (McCoy et al., 1997; Montgomery et al., 2004; Sayer et al., 2010). This included the 90% WCGF diet with no corn fed by McCoy et al. (1997). This improved NDF digestibility suggests that a large proportion of the fiber in WCGF is digested because it has a greater NDF content than the corn it replaces. Fiber utilization is an asset for cattlemen because cattle can digest the high fibrous byproduct feeds, unlike non-ruminant livestock such as poultry and swine.

Incidence of acidosis, as measured by rumen pH, is a consideration to evaluate when feeding byproducts in finishing diets. Prior to the last 20 years, high concentrate diets containing corn were fed that were high in starch. Therefore, comparing byproduct diets to corn based diets on the incidence of acidosis (rumen pH) is important. Montgomery et al. (2004) fed 40% WCGF in a SFC based diet and observed increased average pH compared to the SFC control diet. Sayer et al. (2010) fed 30% corn bran, 30% corn bran plus 15% steep, and 45% corn bran plus 15% steep (DM basis) and

observed increased average rumen pH for each of these byproduct diets compared to a

DRC based control diet. These increases in rumen pH are likely due to a replacement of

fermentable starch (i.e. corn) with byproducts that are high in fiber. Adding steep alone in

finishing diets with no corn bran resulted in different effects. Blanchard (1992) stated that

steep may contain 25% lactic acid and steep typically has a pH of 4.0. Therefore, it is

logical that when steep was included in DRC based diets at 15 or 30% DM, maximum

and average rumen pH were reduced compared to the DRC control diet (Scott et al.,

1998). Krehbiel et al. (1995) evaluated rumen pH changes to dosing cattle ruminally with

7.9 kg of 100% WCGF, 50% WCGF: 50% DRC, or 100% DRC. Cattle dosed with only

DRC spent a greater amount of time with a rumen pH below 5.6 than cattle dosed with

only WCGF or WCGF in combination with DRC. Rumen pH levels for cattle fed only

DRC did not return to baseline levels (observed prior to dosing) within 24 h of after being

dosed with DRC, suggesting long lasting acidotic effects due to the addition of corn.

They attributed this decrease in pH to a greater production of organic acids and VFA that

were also observed. Because rumen pH declined some from dosing with WCGF, the

authors suggested that WCGF may help control the incidence of acidosis but it does not

eliminate these risks.

The production of VFA is important in ruminant livestock production because

VFA are the primary source of energy for the ruminant animal. Volatile fatty acids are

readily produced in the rumen from carbohydrates and can be easily absorbed across the

rumen wall into the bloodstream. Proportionally, cattle fed high forage diets produce

more acetate than cattle consuming high grain diets. Cattle that consume high grain diets,

particularly with starch as a substrate, produce more propionate relative to acetate

production. The production of VFA is difficult to determine because researchers would have to determine VFA concentrations, total amount of rumen volume, and VFA absorption. Determining rumen volume and the flow of VFA out of the rumen are the difficult tasks to conduct. Therefore, researchers primarily evaluate the concentration of VFA and the acetate to propionate ratio (A:P) from ruminal spot samples. Similar total VFA concentration and molar proportions of acetate and propionate were observed by Scott et al. (1998) for cattle fed diets containing 15% corn bran plus 15% steep and 15% corn bran plus 30% steep (comparable to WCGF) compared to a DRC based control diet. However, Sayer et al. (2010) observed increased molar proportions of acetate and decreased molar proportions of propionate when feeding 30% corn bran or 45% corn bran plus 15% steep compared to a DRC control diet. Their A:P ratios were greater for feeding the byproduct diets compared to the DRC control diet, suggesting less propionate present for metabolism.

Metabolism characteristics for feeding wet distillers grains plus solubles

A few experiments have been conducted to evaluate feeding WDGS compared to a DRC based diet. The effects of feeding WDGS on DMI in a metabolism trial may not be indicative of what would be observed in a commercial large pen setting, due to individual intake variation. Therefore, it is logical that differences in DMI across metabolism trials may not be consistent. For instance, Corrigan et al. (2009) observed increased DMI when feeding 40% WDGS compared to a DRC diet, but Vander Pol et al. (2009) observed similar DMI for the same comparison. Ham et al. (1994) observed statistically lower DMI when feeding a 40% WDGS diet consisting of 25% wet distillers grains and 15% distillers solubles in a metabolism trial. They also observed a numeric

DMI decrease when 40% wet distillers grains with no solubles was fed in another metabolism trial. Bremer et al. (2010) observed a numeric decrease in DMI when feeding 56% WDGS compared to a DRC based diet. These results indicated that the effects of feeding WDGS in diets in metabolism trials on DMI are inconsistent. Although the effects of DMI were inconsistent in these trials, all of the experiments observed increased NDF intake because the WDGS diets has twice the amount of NDF content as the corn diets.

There is currently a complex paradox with feeding WDGS in finishing diets. Cattle ADG and G:F increases when feeding WDGS, but the effects of feeding WDGS on ruminal metabolism are inconsistent as diet digestibility often decreases. Dry matter digestibility decreased in the trials of Corrigan et al. (2009) and Bremer et al. (2010) from 81% for the DRC diets to 76% for the 40 to 56% WDGS diets. However, Vander Pol et al. (2009) and Ham et al. (1994) observed similar DM digestibility for cattle fed 40% WDGS compared to DRC control diets. All of these experiments observed statistically similar NDF digestibility, but NDF digestibility values were numerically greater for feeding WDGS compared to DRC. Fiber digestion could have improved due to less dietary starch in the WDGS diets and increased rumen pH. However, dietary fat increased in the WDGS diets, which could hinder NDF digestion but these data do not indicate so. Not only were NDF digestibilities similar for cattle fed WDGS and the corn control diets, but cattle fed the WDGS diets digested twice as much total fiber because dietary fiber content was two-fold greater than the corn diets. No differences were observed in DM or NDF digestibility of corn bran when incubated in in-situ bags for 22 or 48 h (Corrigan et al., 2009; Bremer et al., 2010, respectively) in the rumen of cattle fed

WDGS or DRC. These digestibility values were low at 28.9% DM digestibility and 26.7% NDF digestibility. This suggests that the fiber from WDGS (corn bran) may be largely digested post-ruminally or may not be representative of rumen digestion. Total tract dietary NDF digestibility values from these experiments were two to three times greater than the ruminal in-situ corn bran NDF digestibility values. This suggests that the in-situ NDF digestibility values were artificially low maybe due to plugged pores of the bags or a large proportion of the NDF in WDGS is utilized post-ruminally. However, Vander Pol et al. (2009) reported 56 and 71% pre-duodenal diet NDF digestibility for DRC and WDGS fed steers, respectively. There can be inherent errors associated with measuring in-situ or total tract digestibility. One factor possibly contributing to these differences may be particle size as corn bran has a larger size than the fiber associated within WDGS. The corn bran fiber in WDGS is also smaller than that in WCGF. These differences in particle size may affect the rate and extent of ruminal digestibility and rate of passage.

Ruminal pH measures from feeding WDGS have been generally lower compared to feeding DRC control diets. The experiments of Ham et al. (1994), Corrigan et al. (2009), and Vander Pol et al. (2009) observed decreased ruminal pH, whether it was average, minimum, or maximum pH. A decrease in rumen pH would normally suggest that cattle would be more susceptible to ruminal acidosis. However, these authors did not indicate any clinical incidences related to acidosis as cattle intakes did not decrease. Diets containing WDGS should result in greater rumen pH due to less fermentable starch, but WDGS contains a rapid source of organic acid originating from the milling process. Diets containing greater amounts of fat have greater rumen pH as well, whether it be from

tallow or corn oil (Vander Pol et al., 2009; Bremer et al., 2010). These three experiments

in addition to Bremer et al. (2010) observed a decrease in pH variance or pH change

when feeding WDGS compared to feeding DRC control diets. Although cattle have

decreased rumen pH when consuming WDGS diets, the change in pH from feeding

WDGS is less than that from feeding DRC control diets. Therefore, the affects of feeding

WDGS on ruminal metabolism remains perplexing due to decreased pH values but

increased ADG and G:F in a feedlot setting.

The affects of feeding WDGS compared to DRC control diets on ruminal VFA

concentrations might help explain the benefit in feeding WDGS. Vander Pol et al. (2009)

observed a decrease in acetate and an increase in propionate molar concentrations.

Corrigan et al. (2009) also observed an increase in propionate concentration. This

calculated into a decrease in the A:P ratio. These data suggested that with more

propionate and less acetate concentrations in the rumen, more energy is available for

body growth and cattle should be more efficient due to the propionate. These effects on

VFA concentrations have not repeated well across other experiments. For instance, Ham

et al. (1994) observed a decrease in acetate and propionate concentrations and a decrease

in A:P from feeding 40% WDGS compared to a DRC control diet. Bremer et al. (2010)

reported no changes in the concentrations of acetate or propionate and A:P ratio from

feeding 56% WDGS compared to DRC. Ham et al. (1994) suggested that adding water to

diets in the WDGS form may increase particle size, which would decrease rate of passage

in the rumen. Changing the rate of passage in the rumen can affect digestibility and VFA

production (Van Soest, 1994). They also suggested that total VFA production cannot be

determined from measuring VFA concentration. Therefore, energetic efficiency cannot

be predicted due to not knowing total VFA production. Feeding WDGS and SB in the same metabolism trial has not been evaluated to directly compare rumen metabolism and digestibility.

Fiber for cattle

Fiber in finishing diets

Forages (i.e. fiber) have been included in high concentrate finishing diets and are commonly referred to as roughages. One reason for using forages in finishing diets is providing a scratch factor in the rumen to stimulate intake and rumination. Forages may help offset any ruminal disturbances due to feeding a high starch diet and provide a safety net for consulting nutritionists by decreasing the risk of acidosis in cattle. Fiber is not a required nutrient for ruminants (Van Soest, 1994), but when the inclusion of forages is increased in finishing diets, intakes increase. Increasing the inclusion of forage from 8 to 24% DM as a blend of alfalfa hay and corn silage in HMC or SFC based diets resulted in increased DMI (Gill et al., 1981). However, ADG and G:F did not increase similarly to DMI. Affects on ADG and G:F were dependent on grain type. Intakes are increased likely due to the physical characteristics of forages such as bulk density and content of NDF (Defoor et al., 2002). They suggested that ADG and G:F do not increase for cattle consuming greater dietary inclusions of forages because diet NEg decreases due to a decrease in grain inclusion.

Two nutritionists' surveys have reported roughage level and source in finishing diets (Galyean and Gleghorn, 2001; Vasconcelos and Galyean, 2007). These surveys reported a range of 4.5 to 13.5% DM inclusion of forages used in finishing diets that were fed to cattle in many commercial feedlots in the Plains states. The main forages used

were alfalfa hay and corn silage, representing about 70% of the surveyed feedlots. Other fiber sources used were cottonseed hulls, sudangrass hay, and a mixture of other hay sources.

As previously stated, forages have been used in finishing diets to offset any acidosis challenges with high starch inclusions. However, the use of forages perhaps changes with the inclusion of byproduct feeds that contain little starch. Farran et al. (2006) fed 0, 3.75, or 7.5% alfalfa hay in finishing diets containing 0 or 35% WCGF. When no WCGF was fed, increasing the inclusion of alfalfa hay in diets resulted in increased DMI and ADG with no effects on G:F. They suggested that increasing hay inclusions in diets containing no byproducts promoted an increase in intakes but did not affect efficiency, similar to Gill et al. (1981). Increasing alfalfa hay in diets containing 35% WCGF resulted in increased DMI and ADG, but G:F decreased. The authors concluded that although ADG increased for including alfalfa hay in WCGF diets, some cattle producers may wish to feed less hay to maintain G:F.

Benton et al. (2007) fed 30% WDGS diets containing alfalfa hay, corn silage, and corn stalks at a conventional level, one-half that level, and these were compared to a diet with no forage. The conventional level was equal to 8% alfalfa hay and the one-half level was equal to 4% alfalfa hay. Corn silage and corn stalks were included in other diets at levels to have equal amounts of forage NDF compared to the conventional and one-half levels of alfalfa hay. When the conventional forage levels were fed, DMI and ADG increased compared to the lower levels and no forage. However, cattle fed the 3% corn stalks diet had equal performance to the conventional diets. When forages were removed, G:F improved, but DMI, ADG, and profit decreased compared to diets containing corn

stalks or conventional levels of alfalfa hay or corn silage. The authors concluded that it was not beneficial to remove forages from finishing diets containing WDGS, but lower quality forages that are often cheaper than alfalfa can be used when feeding WDGS. These low quality forages can be used because supplemental protein is not needed due to the inclusion of WDGS. The authors suggested that it is logical to formulate finishing diets with forages based on the NDF content in the forages rather than forage DM inclusion.

Fiber characteristics relative to digestibility

Beef cattle are ruminants and consume forages containing cellulose. Cellulose is glucose units bonded together using β-bonds. One advantage to feeding cattle compared to non-ruminant livestock is feeding forages because cattle have rumen bacteria that digest carbohydrate β-bonds. One disadvantage to feeding forages to beef cattle is lower digestibility compared to feeding cereal grains. Obtaining 60% in-vivo digestibility with forages is considered acceptable for cattle maintenance (NRC, 1996), but digestibility of cereal grains are commonly 80% or more (Owens et al., 1986). The main digestibility difference between forages and grains is due to the speed and extent of exposing and degrading carbohydrates as this process occurs more rapidly with grains compared to forages.

Breaking down forage particles and utilizing their substrates (i.e. VFA) for energy largely depends on rumen microbial attachment. As particle size decreases with a feedstuff, greater utilization of that feed occurs for animal production purposes. Decreasing particle size occurs multiple ways: chewing, rumination, microbial attachment and breakdown (Van Soest, 1994). Although a decrease in forage particle size

aids in the utilization of those particles, the time those particles reside in the rumen for digestion decreases. This concept is referred to as rumen retention time, and the rate that particles leave the rumen is called passage rate. As rumination occurs and particle size decreases, the particles are more physically dense and hydration increases (Van Soest, 1994). As particle density and hydration increase, ruminal passage rate of the particles increases. Different types of forages can be more physically dense by nature. For example, alfalfa hay has greater density than wheat straw hay. Conceptually, rumen retention time is the opposite of passage rate. The longer the particles can reside in the rumen (i.e. retention time), the more time the particles have to be digested in the rumen.

Forage particles are divided into two fractions: cell solubles and cell wall. When cell solubles are consumed, they are nearly 100% digestible (Van Soest, 1967). The cell wall requires microbial attachment, chewing, and rumination for digestion in the rumen. The cell wall of the plant cell is what constitutes fiber in forages. Fiber is typically defined as neutral detergent fiber (NDF), which consists of hemicellulose, cellulose, and lignin. Fiber provides structural support for plants to stand up and grow. Therefore, it is logical that the NDF content of forages increase as forages mature (Van Soest, 1994). An increase in NDF content in forages includes increases in hemicellulose, cellulose, and lignin content. The rank of digestibility for the three NDF components from greatest to lowest is hemicellulose, cellulose, and lignin (Akin et al., 1974 and 1975). These authors classified these fiber components as morphological tissue types. Hemicellulose is the mesophyll and phloem tissue of plants that is digested faster. Cellulose is the bundle sheaths and epidermal cells that are slower to digest than hemicellulose. Total tract digestion of hemicellulose is 60 to 70% and cellulose is about 60% (Van Soest, 1994).

However, digestibility of hemicellulose and cellulose can be different between grasses and alfalfa. Lignin in NDF is the lignified vascular bundles and sclerenchyma tissues that are relatively indigestible and affect the availability of other cell wall carbohydrates for digestion. For instance, Mertens (1973) stated that lignin content in NDF is closely related with indigestible forage residue. Hanley et al. (1992) suggested that forage lignin content is inversely proportional to the amount of digestible hemicellulose in that forage. They also stated that cellulose indigestibility is proportional to mean retention in the rumen, and rumen retention time is closely related to forage lignification. Therefore, it is logical that as forage plants grow and mature and lignin content increases, the digestibility of that forage decreases (Van Soest, 1994). Not only does NDF digestibility decrease as forages mature, but DM digestibility also decreases because there is less cell solubles present in the forage to digest quickly in the rumen.

In general, the greater daily DMI is for cattle, the more feed there is available for digestion and thus for animal production. Voluntary feed intake is largely limited in high forage diets by rumen gut fill and distention (Allen, 1996). Rumen gut fill and distention is largely proportional to fiber mass of the forage (Balch and Campling, 1962). Therefore, one can conclude that voluntary feed intake is related to the NDF content in the forage, as DMI likely increases with decreased forage NDF content. Content of NDF in forages cannot be used alone in predicting cattle feed intake. Particle size of the forage before mastication, particle fragility, and rate and extent of NDF digestion affect forage intake. Although an increase in feed intake is observed as a positive for beef production, there is a consequence to this increased intake: increased ruminal passage rate. As previously stated, the faster the passage rate is, the lower the rumen retention time is for the forage

to reside in the rumen. Decreased forage retention time results in lower extent of digestibility.

Although the rumen is the primary location for digesting fiber, the large intestine is capable of digesting some fiber (Mertens and Ely, 1979; Van Soest, 1994). Van Soest (1994) suggested that the intestines of the ruminant animal were similar to the intestines of the nonruminant animal. Limited research has been conducted evaluating fiber digestibility in the large intestine of ruminants. However, Kass et al. (1980) evaluated feeding 0 to 60% alfalfa to young growing pigs and observed increased growth of the colon and cecum, indicating greater utilization of fiber in the large intestine. Mertens and Ely (1979) observed that when forages were fed in the pelleted form, less fiber was digested in the rumen and more fiber was available in the large intestine. Although more fiber was available in the large intestine, less total fiber was digested total tract with the pelleted forage, indicating poorer fiber utilization in the large intestine compared to the rumen.

The utilization of fiber in concentrate diets fed to ruminant livestock can be affected differently than that fiber consumed by grazing ruminants. When cattle consume concentrate feeds, the pH of the rumen decreases due to an increase in starch utilization and an increase in lactate and VFA production (Van Soest, 1994). However, fibrolytic rumen microorganisms that utilize cellulose and hemicellulose are inhibited at pH levels below 6 either due to the pH level or the competition with starch (Grant and Mertens, 1992). Several of the microbes that utilize forage can utilize starch, resulting in a competition for microbial use. A larger proportion of the decrease in fiber utilization in finishing diets may be due to this competition affect and less on decreased rumen pH.

However, Hofman (1988) classified concentrate consuming ruminants as intermediates between nonruminants with pregastric digestion and forage grazing ruminants. He observed that forage fed ruminants have longer intestines, but concentrate fed ruminants have proportionally more large intestine than small intestine compared to forage fed ruminants. This large intestine proportionality difference in favor of concentrate fed ruminants suggests that more fiber can be used in the large intestine than we might hypothesize with no complexing factors related to starch like there is in the rumen.

Direct fed microbials

Direct fed microbials (DFM) have been defined as a source of live, naturally occurring microorganisms that include cultures of fungi or bacteria. The purpose of using DFM has been to improve livestock performance. They may affect cattle metabolism or behavior, but ultimately if they do not affect cattle performance then they have little value. For instance, feeding a lactic acid bacteria, *L. acidophilus*, in a yogurt to young Holstein dairy calves resulted in more rumination in the calves at 30 d of age than calves fed a placebo yogurt (Nakanishi et al., 1993). They suggested that this bacterium promoted rumen development. However, there were no implications on dairy calf production. The effects of using DFM should be focused on performance enhancement.

Many experiments have evaluated the efficacy of DFM with stressed, newly received feedlot beef calves. In a review by Fox (1998), seven experiments indicated an improvement in calf performance when feeding a bacterial DFM containing live cultures. This summary indicated a 13.2% increase in ADG, a 2.5% increase in DMI, and a 6.3% increase in G:F. However, in a review by Krehbiel et al. (2003), five other experiments

showed no effects when feeding a bacterial DFM to newly weaned or newly received feedlot calves.

Specifically, *Lactobacillus buchneri* has been used to ensile and treat alfalfa haylage (Kung et al., 2003). When the alfalfa haylage was exposed and sampled, the haylage contained more acetic and propionic acids compared to non-DFM treated haylage. This DFM also decreased haylage pH and increased lactic acid production when it was initially ensiled, making it more aerobically stable. The *Lactobacillus buchneri* treated alfalfa haylage was fed in dairy rations (Kung et al., 2003). The authors observed no effects on DMI and milk composition, but milk production increased from feeding the DFM treated alfalfa haylage compared to haylage with no DFM. This suggests that *Lactobacillus buchneri* may improve cattle performance in some situations. Weinberg et al. (2007) used a lactic acid bacteria with wheat or corn silage in an in-vitro setting with or without added corn starch to measure DM and NDF digestibility. The authors observed decreases in NDF digestibility for these silages as starch level increased. When no starch was added, DM and NDF digestibility increased for using the lactic acid bacteria compared to not using it. However, using *Lactobacillus buchneri* in finishing diets containing byproducts that are high in fiber content on rumen metabolism has not been evaluated. Using this DFM with stored WDGS mixed with a low quality forage has also not been evaluated.

These findings suggest that further research was needed to nutritive factors associated with ethanol byproduct feeds. Therefore, this research was conducted as a cooperative effort between the Nebraska Corn Board Association, Lallemand Animal

Nutrition, and the University of Nebraska-Lincoln. The objectives of this research reported herein are as follows:

1) Evaluate different methods for determining DM content in wet byproducts; and determine nutrient composition and variation for WDGS and MDGS samples obtained from several ethanol plants.

2) Determine accurate NDF values for corn and DGS using different analytical techniques.

3) Evaluate the effects of feeding two levels of WDGS mixed with straw and fed as a fresh mix or as an ensiled mix on cattle performance; and determine the value of using *Lactobacillus buchneri* inoculum in the ensiled mixes on cattle performance.

4) Evaluate feeding WDGS and SB at the same inclusion and SB with no corn in finishing diets with or without top-dressing the diets with *Lactobacillus buchneri* on digestibility, rumen pH, and VFA concentrations.

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Nutrient Variability for Distillers Grains plus Solubles and Dry Matter Determination of Ethanol Byproducts[1]

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ABSTRACT

Three experiments were conducted to evaluate nutrient content and DM determination methods of dry milling byproducts. In Exp. 1, nutrient composition was determined for wet distillers grains plus solubles (WDGS) and modified distillers grains plus solubles (MDGS) from 6 ethanol plants with 10 samples collected per day, across 5 d, and sampling was repeated over 4 separate mo. Mean composition was 31.0% CP, 11.9% fat, 0.84% P, and 0.77% S (DM basis). Coefficients of variation for DM content were greater for some plants than others and variation occurred within and across days. Variability was small for CP and P whereas fat differed among ethanol plants. Large variation in means and CV were observed for S in period 1, but variation subsequently decreased. Coefficients of variation for S were similar for samples collected within the same day and across days. In Exp. 2, samples of WDGS, MDGS, Dakota Bran Cake, and distillers solubles were used to determine DM content by drying samples at 105ºC for 3, 8, and 24 h and 60ºC for 24 and 48 h, vacuum oven drying, toluene distillation, and Karl Fischer titration. Compared to toluene distillation, drying at 105ºC resulted in less DM ($P \leq 0.10$), and vacuum drying and Karl Fischer titration resulted in greater DM ($P < 0.01$). In Exp. 3, additional WDGS, MDGS, and wet grains with no solubles were used to determine DM with oven drying at 60ºC for 48 h, 105ºC for 3 h, or toluene distillation. Drying at 60ºC for 48 h was similar to toluene distillation ($P \leq 0.60$).

Keywords: distillers grains, dry matter, laboratory methods, nutrient composition, variation

INTRODUCTION

Although wet distillers grains plus solubles (**WDGS**) has become a common feedstuff in the livestock industry, there is concern about its nutrient composition and consistency (Babcock et al., 2008). Three nutrients commonly measured in WDGS are DM, fat, and S. Price paid for WDGS on a DM basis may be problematic if the DM content is less than expected or is incorrectly determined. If large amounts of high fat WDGS are fed, then cattle intakes may decrease if dietary fat is greater than 8% DM (Vander Pol et al., 2009). The NRC (1996) suggested the maximum tolerable S level was 0.40% for potential occurrence of polioencephalomalacia, thus making S in WDGS important if it is high and/or variable. Little research has been reported on nutrient variability with WDGS.

Methods to determine the DM of feeds are widely used in the agriculture industry. Given the variation in moisture, understanding these methods is of particular importance when considering wet ethanol byproducts (50 to 70% moisture). Dry matter content of feeds is typically defined as the material remaining after heating the sample in an oven for a fixed period of time, with the calculated loss of weight assumed to be water. This method is used most commonly as it is rapid and inexpensive. However, Mo and Tjornhom (1978) determined volatile organic substances are also lost and additional side reactions may occur for wet, fermented forages during the oven drying process. Toluene distillation offers an alternative method to determine DM content of feed through direct but separate removal of moisture (Brahmakshatriya and Donker, 1971). However, no published research exists for comparing DM methods in wet byproducts.

Our objectives were to determine nutrient composition plus variability for WDGS from several ethanol plants across many days, and to compare drying methods to toluene distillation for determining DM of wet byproducts.

MATERIALS AND METHODS

Exp. 1

Six ethanol plants in Nebraska agreed to sample distillers grains plus solubles. Four plants produced WDGS and 2 plants produced modified distillers grains plus solubles (**MDGS**), but the samples will be generally referred to as DGS to maintain confidentiality. A collected sample represented a semi-truck load of DGS a cattle producer would receive. Samples were collected from 4 to 5 locations in the DGS pile to be loaded on a semi-truck or from the loader that filled the truck. These samples were combined, mixed thoroughly, and a 250 to 500 g sub-sample was collected and placed into a plastic, air-tight bag and frozen. Ten samples were taken across a d for 5 consecutive d, with 50 total samples during the wk. This was repeated over 4 sampling mo (periods) throughout a yr, totaling 200 samples per ethanol plant and 1200 samples in the dataset. Samples were shipped frozen overnight following the sampling period to the UNL ruminant nutrition laboratory for analysis.

Analyses for DM, CP, fat, P, and S content were conducted in duplicate. If coefficient of variation was greater than 5%, then the analysis was repeated and the new results were used. Based on results from Exp. 1 and 2, DM was conducted using a 60°C oven for 48 h because this method is statistically similar to toluene distillation. After drying, samples were ground through a 1-mm screen (Wiley Mill; Thomas Scientific, Swedesboro, NJ) prior to nutrient analysis. Crude protein was calculated from % nitrogen

using a LECO nitrogen analyzer (AOAC, 1999; method 990.03). Phosphorus and S were determined by wet ashing with nitric and perchloric acids and analyzed colorimetrically (AOAC, 1999; methods 968.08, 965.17; Tinsdale et al., 1985). Fat was determined by extraction with petroleum ether under pressure in filter bags (AOCS, 1998; method Am 5-04). Fat, P, and S were performed at a commercial laboratory (Ward Laboratories, Inc., Kearney, NE).

Data were summarized by d, ethanol plant, and sampling period to compare mean nutrient values. Coefficients of variation were calculated to evaluate variability within d, across d, and within plants. A CV was calculated each d (10 samples/ d) within each ethanol plant and sampling period. These 5 CV per ethanol plant and period were then averaged and this CV value will be expressed as "within d variation". Average nutrient content was calculated per d. These daily averages (5 d) within each period and ethanol plant were used to calculate a CV and will be expressed as "across d variation". Statistical analysis on the within d variation CV within period for each nutrient were conducted using Proc Mixed procedure of SAS (Version 8.02, SAS Inc., Cary, NC), which used the within d CV from each d as the experimental unit. This procedure was used to evaluate average ethanol plant nutrient composition by using average daily nutrient composition as the experimental unit. Probabilities less than or equal to 0.05 were considered significant.

Exp. 2

Four different types of high moisture, byproduct feeds were used to evaluate drying methods for determining DM content. These feed samples included WDGS (31-35% DM; Abengoa Bioenergy, York, NE), MDGS (42-48% DM; Husker Ag, Plainview,

NE), Dakota Bran Cake (**Dbran**, 50-54% DM; POET Nutrition, Sioux Falls, SD), and distillers solubles (**solubles**, 25-35% DM; Abengoa Bioenergy). Random grab samples were obtained from the piles (representing one semi-truck load) of wet byproducts that were being fed to cattle at the University of Nebraska-Lincoln Agricultural Research and Development Center research feedlot near Mead, NE. These samples were mixed together (totaling 2.5 kg) and sub-sampled for each analysis of DM.

Methods for determining DM included drying samples in a 60ºC forced air oven for 24 or 48 h, 105ºC for 3, 8, or 24 h, using a vacuum oven, toluene distillation, and Karl Fischer titration. The 105ºC and 60ºC oven methods were conducted by weighing 5 g as-is sample into dry aluminum pans (8 replications). Weights were recorded on the same samples at 3, 8, and 24 h for the 105ºC oven and at 24 and 48 h for a different set of samples in the 60ºC oven. A vacuum oven analysis (AOAC, 1999; method 934.01) was conducted on each sample type (3 replicates). Samples were weighed (5 g as-is) into pre-weighed moisture tins and placed on a vacuum oven tray. Trays were placed in a 70ºC vacuum oven, the door was sealed, and the vacuum was applied at 50 mm Hg. After 4 h, the vacuum was turned off and tins were removed from the tray and allowed to cool in a dessicator and then weighed. In addition, a Karl Fischer titration (AOAC, 1999; method 2001.12; Thiex and Van Erem, 2002) was conducted in duplicate on all samples. Toluene distillation (AOAC, 1999; method 925.04) was conducted in duplicate on every sample. A 25 g as-is sample was weighed into a 250 mL Pyrex round-bottom flask and toluene was added to cover the sample. Toluene was rinsed down the sides of the condenser into the collection trap and the trap was filled until toluene ran over into the flask. Heat was provided to the flask so the toluene would boil within 10 min, at which point the 90 min

reflux began. Moisture measurements were obtained at 30, 45, 60, 75, and 90 min and the condenser was rinsed with toluene at 45, 60, 75, and 90 min. An aliquot of the distilled liquid was collected via glass syringe. Two mL of this liquid plus 0.2 mL 2-Ethylbutyrate solution (0.365 g 2-ethylbutyrate in 100 mL of ddH_2O) were analyzed for volatiles using gas chromatography based on methodology described by Erwin et al. (1961). Specific organic compounds were not identified but were summed to equal total volatiles.

Exp. 3

A follow-up study on drying methods was conducted to evaluate DM content for 27 WDGS, 22 MDGS, and 14 wet grains with no solubles (wet grains; POET Nutrition) samples. Weekly subsamples of these byproducts were collected from June 2006 through December 2008 when they were being fed at the University of Nebraska-Lincoln Agricultural Research and Development Center research feedlot near Mead, NE. The samples were composited by as-is weight to make a monthly composit sample.

Dry matter was analyzed in duplicate with toluene distillation, 60ºC oven drying for 48 h, and 105ºC oven drying for 3 h. Toluene distillations were conducted as previously stated, but were refluxed for 75 min because this was determined as the maximum time needed to recover all potential moisture. The 60ºC and 105ºC oven DM methods were conducted with 20 and 1 g as-is samples, respectively. If the sample coefficient of variation was greater than 5% within each method, then the analysis was repeated.

To determine amount of volatiles lost by drying in the 60ºC oven for 48 h, 3 WDGS, 1 MDGS, and 1 wet grains samples were analyzed by toluene distillation comparing the as-is samples to oven dried samples that were reconstituted to their

original moisture content with distilled H_2O. The amount of volatiles in the distilled liquid was analyzed using a gas chromatograph as previously stated.

Data were analyzed using the MIXED procedure of SAS for each experiment. Dry matter method and byproduct type were considered fixed effects and interactions between these were tested for significance ($P < 0.05$). Simple effects are reported regardless of significant interactions to illustrate DM for each byproduct type.

RESULTS AND DISCUSSION

Exp. 1

Average DM for WDGS and MDGS were 32.5 and 45.2%, respectively. Due to confidentiality, actual DM contents by plant are not disclosed. Therefore, DM values were converted to a 100% basis. Regardless of type of distillers grain, the DM content varied between plants (data not shown), which emphasizes the importance of producers knowing the DM of the product purchased. Coefficients of variation (independent of the mean) were different across ethanol plants and within sampling periods (50 samples per plant per period), but this variation remained relatively small as only 3 values were above 4% (4.0, 4.7, 7.1) for 24 CV calculated. This variation is similar to Kaiser (2005) who reported CV of 2.8 to 3.8% within each plant for WDGS samples collected from 3 ethanol plants. Differences in mean DM by ethanol plant can be understood due to plant production systems. Holt and Pritchard (2004) observed greater DM variation for samples collected across ethanol plants with CV of 6.8 and 4.7% for WDGS and MDGS, respectively. In the current experiment, variability in DM was also observed within d as the CV were different by plant for periods 2, 3, and 4 ($P \leq 0.03$, Table 1); however, CV were less than or equal to 3.1%, which are relatively small. The average CV across all 20

d of sampling within each ethanol plant ranged from 1.05 to 2.35%. Across d variation

for DM was also small with CV less than 3% within each ethanol plant (data not shown).

While DM content of byproducts is very important, CV less than 5% may not be of

practical significance. Some of this variation is likely attributable to sampling accuracy.

The concentration of CP for WDGS was 31.0% (DM basis) across all ethanol

plants and sampling periods, which was greater than 29.5% reported by NRC (1996).

Mean CP by plant ranged from 30.1 to 32.2% ($P < 0.01$, Table 2), but CP within plant

and by period only differed by 2 percentage units of the plant's overall mean. This range

in CP content observed among plants was similar to the range reported by Spiehs et al.

(2002) and Akayezu et al. (1998) of 28.7 to 31.6% and 27.7 to 32.3%, respectively, for

dry distillers grains with solubles (DDGS). In the current experiment, CP contents were

different ($P < 0.01$) by ethanol plant, which can be expected due to production

differences. However, variation within sampling period for each ethanol plant remained

small with CV less than 3% (except for 1 value at 3.9%; data not shown). Although the

within d variation CV were different by ethanol plant for periods 1, 3, and 4 ($P \leq 0.01$),

these values were generally less than 2.7% (only 1 value at 3.72%). These resulted in SD

of 0.3 to 1.3. When calculating the average of CV obtained within each of the 20 d of the

4 periods of sampling, these values were less than 2%, suggesting little within d variation

across sampling periods. Across d variation within plant was small as well with CV

below 1.6% (data not shown). The SD observed for CP within plant and period were

slightly less than the 1.4 observed by Holt and Pritchard (2004) and the 1.5 to 1.6

observed by Kaiser (2005). Soybean meal is considered a consistent feedstuff. However,

the NRC (1996) reported a CP average of 51.8% for soybean meal with a SD of 3.45 for

786 samples tested. We did not consider the SD reported in this experiment for WDGS highly variable because they were less than the SD observed for soybean meal.

The average fat content for all of the samples was 11.9% (DM basis), which was greater than 10.3% reported by the NRC (1996). Although the mean fat content by ethanol plant and averaged across periods ranged from 10.9 to 13.0% (Table 3), the variation appeared to be largely dependent on the ethanol plant and not the sampling periods as plant means were different from each other ($P < 0.01$). Spiehs et al. (2002) and Akayezu et al. (1998) reported fat ranges for DDGS within ethanol plants of 10.2 to 11.7% and 8.8 to 12.4%, respectively. Holt and Pritchard (2004) also showed differences in fat content in WDGS and DDGS among ethanol plants, ranging from 10.4 to 14.2%. These data suggest there are processing differences from plant to plant that influence fat levels (which may relate to the amount of distillers solubles that are added to the distillers grain). The within d CV for fat were generally less than 5% (only 1 value was greater, 6.6%). Across d variation was similar to within d variation as CV were 1.2 to 4.5% within plant (data not shown). Practically, because the mean fat concentration among plants differed more than the CV associated within each plant's mean, producers should monitor the average fat content of WDGS from their ethanol plant and be less concerned with load to load variation in fat. Dietary fat content is important to know because DMI can decrease when dietary fat is greater than 8% (Vander Pol et al., 2009).

The average P content for all of the samples was 0.84% (DM basis) and P content was different among plants with a range of 0.78 to 0.91% ($P < 0.01$, Table 4). The NRC (1996) reported 0.32% P for WDGS, and Holt and Pritchard (2004) observed a range of 0.49 to 0.78% P for WDGS and DDGS. However, Spiehs et al. (2002) reported an

average of 0.89% P for DDGS, and Kaiser (2005) reported averages of 0.8 to 0.9% P within ethanol plants. In the current study, minimal within d variation was observed for P as the CV were 1.1 to 3.4%, resulting in SD of 0.01 to 0.03. This measure of within d variation was only different ($P < 0.01$) for period 2, suggesting consistent within d P variation among plants. Across d variation was small for P as CV by ethanol plant were 1.2 to 2.8% (data not shown). Kaiser (2005) reported SD for P of 0.1 to 0.2 for WDGS. The NRC (1996) reported an average of 0.73% P with a SD of 0.20 for 352 soybean meal samples and 0.07% P average with a SD of 0.25 for 3,516 corn grain samples analyzed. The SD of P in WDGS in the current experiment was much less, suggesting P variability was small compared to soybean meal and corn. No toxicity for P is likely in ruminants at dietary concentrations up to 1%, so P content of WDGS is not an issue for the animal. However, the amount of P is important to know to balance diets for Ca and prevent urinary calculi (NRC, 1996). The P content of WDGS is also important to accurately assess total diet P for nutrient management plans and spreading manure on crop fields.

Average S content for all of the samples was 0.77% (DM basis), which is greater than 0.40% as reported by the NRC (1996). Sulfur values were numerically greater in period 1 than the other 3 periods (Table 5). One of the plants in period 1 had an average S of 1.06%, with ranges of 0.90 to 1.26% and CV of 6.17% within d. Another plant in period 1 had an average S of 0.71%, but the CV was 36.3% (0.26 SD) due to a range of 0.44 to 1.72% S for individual samples. After period 1 sampling, results were presented to managers of the ethanol plants. We observed lower S means and CV for plants in subsequent periods. Specifically, means by plant for all samples ranged from 0.71 to 0.84% ($P < 0.01$) and CV by plant and within periods 2, 3, and 4 were 2.2 to 12.8%,

resulting in SD equal to or less than 0.10 (data not shown). Spiehs et al. (2002) reported a range for S means with 12 ethanol plants of 0.33 to 0.74% and CV ranging from 6.4 to 40.8%. Variation within d for S appeared to be greater than any other nutrient and generally resulted in CV less than 6.2% (1 value at 12.9%) and were different ($P \leq 0.02$) among plants for periods 2 and 3. Across d variation within plant was numerically similar to within d variation as CV were 1.9 to 7.7%, with 1 value at 13.3% (data not shown). Holt and Pritchard (2004) reported high variability in S levels for DDGS, but the variability was not quantified. These data suggest S values should be routinely monitored as increases in S of WDGS can lead to nutritional challenges for cattle (NRC, 1996), especially when feeding more than 30% of diet DM.

Exp. 2

No interactions of type of byproduct and time dried at temperatures of 105ºC or 60ºC ($P = 0.58$) were observed for DM content. All samples dried in the 105ºC oven linearly decreased ($P < 0.01$) in DM over time as average DM content was 40.5, 39.7, and 38.8% for 3, 8, and 24 h, respectively. A lower ($P = 0.06$) DM content was obtained for drying samples in a 60ºC oven for 48 h (41.3%) compared to 24 h (41.7%; Table 6). A greater ($P < 0.01$) DM content was observed when a vacuum oven was used for WDGS (35.2%) and MDGS (45.0%) compared to all other methods. Vacuum drying also resulted in a greater DM (54.4%; $P < 0.01$) for Dbran compared to toluene distillation (53.7%) or oven drying at 60ºC (54.0 and 53.7% for 24 and 48 h, respectively). No differences were observed for solubles between toluene distillation and vacuum drying ($P = 0.74$). This suggests the vacuum oven removes more apparent moisture with some samples than others.

The DM determined from toluene distillation was 33.2, 43.3, 53.7, and 35.9% for WDGS, MDGS, Dbran, and solubles, respectively. There were no differences in DM for WDGS ($P \geq 0.36$) and Dbran ($P \geq 0.18$) between methods of toluene distillation and oven drying at 60ºC for 24 or 48 h, and DM was also not different ($P = 0.21$) for solubles using toluene distillation and the 60ºC oven for 24 h. In many commercial laboratories, drying in the 105ºC oven for 3 h is the preferred method for determining DM because results can be obtained within the same day. However, in this experiment, we observed that oven drying at 105ºC for 3 h or longer resulted in a lower ($P \leq 0.10$) DM content compared to that determined by toluene distillation or oven drying at 60ºC for 48 h. This effect was observed for all four byproduct types and suggests that volatile compounds are lost in addition to water. Thiex and Van Erem (1999) also discovered that drying samples in ovens at greater temperatures, 135ºC compared to 104ºC, underestimated DM content (underestimates DM) for haylage and corn silage samples.

Dry matter results from the Karl Fischer analysis were 37.3, 45.6, 54.8, and 35.7% for WDGS, MDGS, Dbran, and solubles, respectively, but no statistical comparisons to other methods were conducted because only 2 replicates were used with this procedure. However, DM estimates obtained from the Karl Fischer method were numerically greater for WDGS and MDGS than all other methods and were greater for Dbran and solubles compared to oven drying. This discrepancy may be because the accuracy of the Karl Fischer method depends upon the accuracy of the calibration standard (Thiex and Van Erem, 2002). In addition, Thiex and Van Erem (1999) reported higher correlation coefficients and slope (closer to 1) for dry hay than those for haylage and corn silage when comparing methods of oven drying at 104 or 135°C to Karl Fischer,

suggesting poor DM comparisons between oven drying and Karl Fischer titration with wetter feeds (< 70% DM). The American Feed Industry Association (2007) did not recommend the Karl Fischer moisture test for determining DM for dry distillers grains plus solubles, but instead recommended drying the samples in a 105°C oven for 3 h. They stated using this oven method resulted in small biased DM contents with acceptable CV and a small economic risk with minimal labor costs.

Exp. 3

No significant interactions were observed for DM between drying method and byproduct sample type ($P = 0.84$). Type of sample had a significant effect ($P < 0.01$) on DM content, but drying method of toluene distillation and oven drying were not different ($P = 0.20$). However, DM estimates determined from toluene distillation (38.4%) were numerically closer to the values obtained from drying samples in a 60ºC oven for 48 h (38.5%) compared to DM values observed from drying samples at 105ºC for 3 h (38.0%, Table 7). These results are similar to that observed in Exp. 2, in which oven drying at 60ºC for 48 h resulted in closer numeric values to toluene distillation than oven drying at 105ºC for 3 h.

When the toluene distillation procedure was performed and the liquid was collected for volatiles analysis in a GC, acetic acid was detected at the same time as residual toluene. Therefore, the contamination of toluene with volatiles was evaluated by conducting toluene distillation and determining the amount of toluene in the GC collected in the water with moistened corn bran (30% DM) that contains no volatiles. The amount of toluene was very small (0.08% of DM) which suggests a large proportion of this peak was acetic acid.

The original byproduct samples contained 0.74% volatiles (of DM), and the reconstituted samples contained 0.17% volatiles (of DM) after oven drying at 60°C for 48 h. This suggests that a large proportion of the volatiles contained in wet byproducts are lost in oven drying processes, which results in underestimating DM content. However, drying wet byproducts at 60°C for 48 h was the most similar in DM content to toluene distillation. Therefore, this suggests that drying wet byproducts at 60°C for 48 h causes some volatiles to be lost. However, it is likely that samples are also not completely dry. The net result is that oven drying at 60°C for 48 h is similar to toluene distillation.

Drying wet samples at 60°C for 48 h to determine DM content may be an inexpensive and safe method for obtaining accurate estimates of DM for wet corn milling byproducts. Determining DM analysis using 105°C for 3 h gave average values that were 0.79 percentage units (2%) lower than toluene distillation. Nutritionists and producers should be aware of and account for this discrepancy if this method is used by their commercial laboratory. Values for DM determined in 60°C ovens for 48 h were within 0.06 percentage units of toluene distillation.

IMPLICATIONS

Nutrient composition for WDGS samples were: 31.0% CP, 11.9% fat, 0.84% P, and 0.77% S. Dry matter content should be known and periodically checked as this is important when purchasing wet feed. Fat content should be known if a producer changes their source of WDGS as greater differences were observed across plants than within the same plant. Sulfur content should also be routinely monitored as significant variation was observed within the same day and across days of collection. Compared to toluene distillation, drying in a 105°C oven underestimated DM content for wet byproducts,

while the vacuum oven and the Karl Fischer titration overestimated DM content. Drying

in a 60°C oven for 48 h was similar to toluene distillation and is the recommended

method to obtain accurate DM values.

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Table 1. CV%[1] for DM[2] by ethanol plant and sampling period.

	Ethanol Plant						
	A	B	C	D	E	F	F-test of CV[3]
Period 1	1.27	3.23	2.42	1.99	1.07	2.03	0.14
Period 2	0.88^{ab}	0.80^{a}	1.99^{ab}	3.09^{b}	0.89^{ab}	2.52^{b}	<0.01
Period 3	1.41^{a}	1.26^{a}	2.90^{b}	1.28^{a}	1.52^{a}	1.55^{a}	<0.01
Period 4	0.62^{a}	3.07^{b}	2.07^{ab}	1.62^{ab}	2.31^{ab}	1.61^{ab}	0.03
Avg CV[4]	1.05	2.09	2.35	2.00	1.45	1.93	

[1] CV represents an average of the CV calculated within d for each d (5 d) by each period.

[2] DM was determined by drying samples in a 60°C oven for 48 h.

[3] CV F-test represents the F-test detected for ethanol plant differences in CV within each period.

[4] Avg CV represents the average of the 20 CV calculated within each of the 20 individual d of the 4 sampling periods.

[abc] Means in the same row without a common superscript differ ($P < 0.05$).

Table 2. Average[1] and CV%[2] for CP by ethanol plant and sampling period.

| | Ethanol Plant | | | | | | | |
	A	B	C	D	E	F	F-test of CV[3]	Avg CP F-test[4]
Period 1	$30.9 (1.29^a)$	$34.0 (3.72^c)$	$30.5 (1.69^{ab})$	$30.3 (2.46^{ab})$	$30.7 (1.45^{ab})$	$29.6 (2.72^{bc})$	0.01	
Period 2	30.8 (1.24)	30.9 (1.24)	30.4 (1.34)	30.2 (1.68)	32.4 (1.15)	31.0 (1.25)	0.15	
Period 3	$31.2 (0.96^a)$	$31.9 (0.92^a)$	$30.8 (1.38^{ab})$	$30.6 (0.99^a)$	$30.8 (1.79^b)$	$29.4 (1.57^b)$	0.01	
Period 4	$31.5 (0.93^a)$	$32.0 (2.06^b)$	$32.0 (1.12^a)$	$31.4 (1.00^a)$	$30.9 (1.25^a)$	$30.4 (1.00^a)$	<0.01	
Avg mean and CV[5]	$31.1^c (1.11)$	$32.2^d (1.99)$	$30.9^{bc} (1.38)$	$30.6^b (1.53)$	$31.2^c (1.41)$	$30.1^a (1.64)$		<0.01

[1] Average represents each ethanol plant's average for the 50 samples (10 samples/ d for 5 d) collected within each period. Averages represented as a % of DM.

[2] CV (presented in parenthesis) represents an average of the CV calculated within d for each d (5 d) by each period.

[3] CV F-test represents the F-test detected for ethanol plant differences in CV within each period.

[4] Avg CP F-test represents the F-test detected for ethanol plant differences in average CP.

[5] Avg mean and CV represents the average mean over the 4 sampling periods and the average of the 20 CV calculated within each of the 20 individual d of the 4 sampling periods.

[abcd] Means in the same row without a common superscript differ ($P < 0.05$).

Table 3. Average[1] and CV%[2] for fat by ethanol plant and sampling period.

	Ethanol Plant						CV F-test[3]	Avg Fat F-test[4]
	A	B	C	D	E	F		
Period 1	12.5 (2.31^a)	10.8 (6.55^c)	12.7 (3.03^{ab})	12.4 (3.66^{ab})	11.5 (2.80^{ab})	11.5 (4.99^{bc})	0.01	
Period 2	11.7 (1.76^a)	10.7 (2.03^a)	13.1 (3.52^{abc})	11.7 (2.80^{ab})	11.8 (5.70^c)	11.7 (4.49^{bc})	0.02	
Period 3	12.1 (1.32)	11.3 (2.39)	13.3 (2.59)	12.4 (2.02)	10.2 (2.23)	12.4 (1.96)	0.38	
Period 4	11.9 (1.64^a)	11.3 (2.83^b)	13.0 (2.25^{ab})	12.3 (1.99^{ab})	10.3 (2.74^b)	12.4 (1.52^a)	0.03	
Avg mean and CV[5]	12.1^b (1.76)	11.0^a (3.45)	13.0^c (2.85)	12.2^b (2.62)	10.9^a (3.37)	12.0^b (3.24)		<0.01

[1] Average (% of DM) represents each ethanol plant's average for the 50 samples (10 samples/ d for 5 d) collected within each period.

[2] CV (presented in parenthesis) represents an average of the CV calculated within d for each d (5 d) by each period.

[3] CV F-test represents the F-test detected for ethanol plant differences in CV within each period.

[4] Avg fat F-test represents the F-test detected for ethanol plant differences in average fat.

[5] Avg mean and CV represents the average mean over the 4 sampling periods and the average of the 20 CV calculated within each of the 20 individual d of the 4 sampling periods.

[abc] Means in the same row without a common superscript differ ($P < 0.05$).

Table 4. Average[1] and CV%[2] for P by ethanol plant and sampling period.

	Ethanol Plant						CV F-test[3]	Avg P F-test[4]
	A	B	C	D	E	F		
Period 1	0.83 (2.11)	0.79 (3.39)	0.87 (2.23)	0.85 (2.34)	0.80 (2.11)	0.78 (2.55)	0.53	
Period 2	0.84 (1.49^{ab})	0.76 (1.37^{ab})	0.90 (2.85^{c})	0.87 (2.47^{bc})	0.80 (1.07^{a})	0.80 (3.13^{c})	<0.01	
Period 3	0.84 (1.25)	0.78 (1.77)	0.92 (2.76)	0.87 (1.54)	0.74 (1.79)	0.86 (1.46)	0.14	
Period 4	0.86 (1.36)	0.79 (2.39)	0.93 (2.13)	0.89 (1.63)	0.80 (2.73)	0.86 (1.28)	0.15	
Avg mean and CV[5]	0.84^{c} (1.55)	0.78^{a} (2.23)	0.91^{e} (2.49)	0.87^{d} (2.00)	0.78^{a} (1.93)	0.82^{b} (2.11)		<0.01

[1] Average (% of DM) represents each ethanol plant's average for the 50 samples (10 samples/ d for 5 d) collected within each period.

[2] CV (presented in parenthesis) represents an average of the CV calculated within d for each d (5 d) by each period.

[3] CV F-test represents the F-test detected for ethanol plant differences in CV within each period.

[4] Avg P F-test represents the F-test detected for ethanol plant differences in average P.

[5] Avg mean and CV represents the average mean over the 4 sampling periods and the average of the 20 CV calculated within each of the 20 individual d of the 4 sampling periods.

[abcde] Means in the same row without a common superscript differ ($P < 0.05$).

Table 5. Average[1] and CV%[2] for S by ethanol plant and sampling period.

	Ethanol Plant						CV F-test[3]	Avg S F-test[4]
	A	B	C	D	E	F		
Period 1	0.71 (12.88)	0.72 (5.60)	0.83 (5.53)	1.06 (6.17)	0.81 (5.20)	0.90 (5.50)	0.13	
Period 2	0.76 (7.21[b])	0.74 (4.06[a])	0.72 (4.82[ab])	0.69 (3.25[a])	0.76 (3.29[a])	0.82 (3.69[a])	0.02	
Period 3	0.67 (4.95[bc])	0.75 (3.11[a])	0.73 (6.38[c])	0.78 (3.81[ab])	0.75 (3.97[ab])	0.89 (2.96[a])	0.01	
Period 4	0.69 (3.49)	0.73 (3.15)	0.76 (3.98)	0.76 (4.82)	0.72 (3.50)	0.77 (3.50)	0.44	
Avg mean and CV[5]	0.71[a] (7.13)	0.74[ab] (3.98)	0.76[ab] (5.18)	0.82[c] (4.51)	0.76[b] (3.99)	0.84[c] (3.91)		<0.01

[1] Average (% of DM) represents each ethanol plant's average for the 50 samples (10 samples/ d for 5 d) collected within each period.

[2] CV (presented in parenthesis) represents an average of the CV calculated within d for each d (5 d) by each period.

[3] CV F-test represents the F-test detected for ethanol plant differences in CV within each period.

[4] Avg S F-test represents the F-test detected for ethanol plant differences in average S.

[5] Avg mean and CV represents the average mean over the 4 sampling periods and the average of the 20 CV calculated within each of the 20 individual d of the 4 sampling periods.

[abc] Means in the same row without a common superscript differ ($P < 0.05$).

Table 6. Average percent DM of four different ethanol byproducts[1] evaluated by different methods[2].

Sample	60°C		105°C			Toluene	Vacuum	F-test
	24 h	48 h	3 h	8 h	24 h			
WDGS	33.2^d	33.0^{cd}	32.7^c	32.2^b	31.6^a	33.2^d	35.2^e	<0.01
MDGS	44.1^f	43.7^e	42.9^c	42.2^b	41.3^a	43.3^d	45.0^g	<0.01
DBran	54.0^e	53.7^d	52.8^c	52.1^b	51.3^a	53.7^d	55.4^f	<0.01
Solubles	35.6^{de}	34.9^d	33.5^c	32.2^b	31.1^a	35.9^{de}	35.8^e	<0.01

[1] WDGS = wet distillers grains plus solubles, MDGS = modified wet distillers grains plus solubles, DBran = Dakota Bran Cake (POET Nutrition), Solubles = distillers solubles. Analysis included 8 replicates for oven drying methods and toluene distillation and 3 replicates for vacuum drying per sample.

[2] 60°C = oven drying at 60°C for 24 or 48 h, 105°C = oven drying at 105°C for 3, 8, or 24 h, Toluene = toluene distillation, Vacuum = vacuum oven drying. No interactions resulted between drying method and byproduct type ($P \geq 0.58$). Drying method differed within byproduct type ($P < 0.01$).

[abcdefg] Means within the same row with different superscripts differ ($P \leq 0.10$).

Table 7. Average percent DM of three different ethanol byproducts[1] evaluated by different methods[2].

Sample	60ºC, 48 h	105ºC, 3 h	Toluene	F-test
WDGS	33.3[b]	32.6[a]	33.5[b]	0.01
MDGS	49.7	49.0	49.5	0.60
Wet Grains	32.6	32.3	32.2	0.88

[1] WDGS = wet distillers grains plus solubles, MWDGS = modified wet distillers grains plus solubles, Wet Grains = wet distillers grains with no solubles. Analysis included 27 samples for WDGS, 22 for MDGS, and 14 for Wet Grains.

[2] 60°C, 48 h = oven drying at 60°C for 48 h, 105°C, 3 h = oven drying at 105°C for 3 h, Toluene = toluene distillation. No interaction resulted for drying method and byproduct sample on percent DM ($P = 0.84$).

[ab] Means within the same row with different superscripts differ ($P \leq 0.10$).

Determining NDF for Corn and Distillers Grains plus Solubles using Different Analytical Techniques[3]

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ABSTRACT

Six experiments were conducted to evaluate methods for measuring NDF in corn and distillers grains plus solubles (DGS). In Exp. 1 and 2, dry-rolled corn (DRC) and high-moisture corn (HMC) samples were used to evaluate the addition of sodium sulfite (SS) and alpha-amylase (AMY) on NDF content. Although there was an interaction between sample and analytical treatment in Exp. 1 and 2, using SS decreased NDF values and was used throughout subsequent experiments. Adding two doses of AMY decreased NDF values compared to one dose in Exp. 2, but NDF values remained greater than expected, especially for DRC. In Exp. 3, the same corn hybrid processed as DRC and HMC was compared to a steam-flaked corn (SFC) sample and one to four doses of AMY were used to determine corn NDF. No interaction resulted and the DRC hybrid continued to have the greatest NDF value compared to HMC and SFC. Adding two doses of AMY or more decreased NDF values compared to using one dose. However, using more than two doses AMY did not decrease NDF compared to two doses, and this method was used in Exp. 4 and 5. Corns from Exp. 3 were used in Exp. 4 and an interaction resulted between techniques as corns were treated with combinations of AMY and amyloglucosidase (GLU) doses and pressurized with steam and heat in an autoclave (AUT). Using AUT and GLU did not decrease corn NDF content compared to using AMY alone. No corn NDF values in Exp. 4 were considered acceptable with the use of GLU or AUT. Four DRC samples were ground through a 1-mm screen Wiley mill (Wiley) or Tecator Cyclomill (Cyclo) in Exp. 5. Although an interaction resulted between sample and grinder type, using the Cyclo with SS and two doses of AMY resulted in acceptable NDF values (9 to 10%). Five DGS samples were used in Exp. 6 to evaluate

the traditional NDF beaker method with 100 mL NDF solution, 200 mL NDF solution, and using a pre-fat solvent extraction step prior to the traditional method on NDF content for DGS. Using the pre-fat extraction process decreased NDF values for DGS compared to the traditional methods. We recommend grinding through a Cyclo and using two doses of 0.5 mL AMY and SS for corns and using the pre-fat extraction process for DGS appears to accurately measure NDF.

Keywords: corn, distillers grains, fiber

INTRODUCTION

Corn is widely used across many industries for human consumption, livestock consumption, and ethanol production due to its highly digestible starch content. Beef, swine, and poultry in the U.S. are finished on high concentrate diets containing corn. However, the traditional practices for finishing beef cattle have changed over the last 20 yr with greater inclusions of ethanol byproduct feeds (Klopfenstein et al., 2008). The dry milling ethanol industry uses the starch in corn (approximately 66% of DM) to produce ethanol. The nutrients besides starch remain in distillers grains plus solubles (DGS), and are concentrated approximately 3-fold compared to corn (Klopfenstein et al., 2008).

The traditional NDF procedure was developed to measure the fiber content of forages and included a 1-mm grind size and 0.5 g sample weight (Van Soest and Wine, 1967). Unfortunately, measuring NDF content accurately may be difficult in high starch or high fat feeds. Therefore, the use of alpha-amylase and a solvent, respectively, may help this process (Van Soest, 1994; Mertens, 2002). Corn processing may enable the starch in corn to be more available and easier to hydrolyze, making the NDF easier to

measure. Therefore, our objective was to determine the NDF content in corn grain and DGS using the traditional beaker procedure but with new modifications.

MATERIALS AND METHODS

Six experiments were conducted to evaluate different methodologies for determining NDF content in corn and DGS. Heat stable alpha-amylase (AMY; 20,350 LU/mL, ANKOM Technology, Macedon, NY) and sodium sulfite (SS; crystalline, 98.6% assay, Fisher Scientific, Pittsburgh, PA) were used to digest starch and protein, respectively. All analyses were conducted at the University of Nebraska-Lincoln ruminant nutrition laboratory (Lincoln, NE). A lab corrected DM was conducted on all samples by weighing 0.5 g of sample (in duplicate) into a pre-weighed and pre-dried aluminum pan and drying in a 105 ºC oven for 16 h, followed by weighing the dried sample plus aluminum pan. The NDF procedures used neutral detergent solution obtained from Midland Scientific Inc. (Davenport, IA). Following the NDF digestion process, the NDF residue was filtered on pre-dried and pre-weighed Whatman grade 541 filters (12.5 cm diameter, Fisher Scientific) then dried for 16 h in a 105ºC oven and the dried filter and residue were weighed. All analytical treatments were conducted in triplicate for each sample tested within each experiment, and the individual observation within method was considered an experimental unit.

Exp. 1

To initially evaluate corn NDF content, a sample of dry-rolled corn (DRC) and high-moisture corn (HMC) were compared using starch degrading enzymes and protein degrading sulfite. One sample of each DRC and HMC were obtained on the same day in November, 2007 from the University of Nebraska-Lincoln Agricultural Research and

Development Center research feedlot near Mead, NE. Samples were dried at 60ºC for 48

h and ground through a 1-mm screen (Wiley Mill; Thomas Scientific, Swedesboro, NJ).

Samples were weighed (0.5 g) into tall-form 600 mL glass beakers and 100 mL of NDF

solution (Midland Scientific, Inc.) was added. Following 1 h of reflux, residue was then

filtered. Three treatments included: 1) adding 0.5 mL AMY at reflux initiation with no SS

(1 AMY -SS), 2) adding 0.5 mL AMY at reflux initiation plus weighing 0.5 g SS into the

beakers (1 AMY +SS), and 3) adding 0.5 mL AMY at reflux initiation and 50 min after

reflux initiation plus weighing 0.5 g SS into the beakers (2 AMY +SS).

Exp. 2

Number of AMY doses was evaluated as a means to hydrolyze starch and

measure NDF content. Corns from Exp. 1 were weighed into beakers, refluxed, and

filtered similar to Exp. 1. Three treatments included: 1) adding one dose of 0.5 mL AMY

at reflux initiation (1 AMY), 2) adding two doses of 0.5 mL AMY one at reflux initiation

and a second at 50 min post reflux initiation (2 AMY), and 3) adding three doses of 0.5

mL AMY at reflux initiation and 30 and 50 min post reflux initiation (3 AMY). All

treatments included weighing 0.5 g SS into the beakers with the corn.

Exp. 3

To eliminate any starch or fiber differences related to corn hybrids, corns of

different processing methods and AMY enzyme treatment were evaluated for NDF

content. Samples of the same corn hybrid (Golden Harvest H-8562) were processed as

DRC or HMC and obtained in June 2006 from the UNL research feedlot indicated

previously. A steam-flaked corn (SFC) sample, not of the same hybrid but used for

comparison, was also obtained from the research feedlot at the same time. Corns were

dried, ground, and weighed into beakers, refluxed, and filtered similar to Exp. 1 and 2.

Four treatments included: 1) adding one dose of 0.5 mL AMY at reflux initiation (1

AMY-0.5 mL), 2) adding two doses of 0.5 mL AMY one each at reflux initiation and 50

min post reflux initiation (2 AMY-0.5 mL), 3) adding four doses of 0.5 mL AMY one

each at reflux initiation and 20, 35, and 50 min post reflux initiation (4 AMY-0.5 mL),

and 4) adding two doses of 1.0 mL AMY one at reflux initiation and a second at 50 min

post reflux initiation (2 AMY-1 mL). All treatments included weighing 0.5 g SS into the

beakers with the corn.

Exp. 4

Combinations of AMY and amyloglucosidase (GLU) enzymes and treating the

samples with heat and steam using an autoclave were evaluate other methods to

accurately measure NDF content by hydrolyzing starch. The corn samples from Exp. 3

were prepared, weighed, refluxed, and filtered similarly to previous experiments with

four treatments. Treatment one included adding 0.5 mL AMY at reflux initiation,

refluxing for 30 min, cooling beakers until solution reached 50ºC and adding 0.5 mL

GLU and allowed to sit for 10 min then reflux again for 30 min and adding 0.5 mL AMY

10 min before removing beakers from the end of the reflux step (AMY-GLU-AMY-0.5

mL). Treatment two included adding 1 mL AMY at reflux initiation and 50 min post

reflux initiation. Beakers were then removed and when the solution reached 50ºC, 1 mL

GLU was added and allowed to sit for 10 min before filtering (AMY-AMY-GLU-1 mL).

Treatment three included adding steam and heat at 121ºC for 30 min to corn samples

residing in NDF beakers in an autoclave (AUT) then refluxing with 1 mL AMY at reflux

initiation (AUT-AMY-1 mL). Treatment four included using the same AUT process,

refluxing, adding two doses of 0.5 mL AMY at reflux initiation and 50 min post reflux initiation, let solution cool to 50 ℃, and add 0.5 mL GLU before filtering (AUT-AMY-AMY-GLU-0.5 mL). All treatments included weighing 0.5 g SS into the beakers with the corn.

Exp. 5

Two grind sizes of corn were used to determine NDF content by allowing for maximum AMY activity. Samples of DRC including the same sample from Exp. 1 and 2, the same sample from Exp. 3 and 4, and two corn samples obtained from Poet Nutrition (Poet 1 and Poet 2; Sioux Falls, SD). Corns were dried, weighed, refluxed, and filtered similarly to all previous experiments. Two treatments included: 1) grinding samples through a 1-mm screen Wiley Mill (Wiley) or 2) grinding samples through a 1-mm Tecator Cyclotec sample mill (American Instrument Exchange, Haverhill, MA; Cyclo). Both treatments included weighing 0.5 g SS into the beakers with the corn and using two doses of 0.5 mL AMY at reflux initiation and 50 min post reflux initiation.

Exp. 6

An experiment was conducted to accurately determine NDF content of high-fat (> 5% fat) dried DGS (DDGS) samples using different amounts of NDF solution and a pre-fat extraction method. Five DDGS samples (POET Nutrition) with differing amounts of solubles added to the grains portion were ground through a 1-mm screen Willey. These samples were represented as 0, 33, 67, 100, and 110% (0DDGS, 33DDGS, 67DDGS, 100DDGS, and 110DDGS) the normal incorporation of solubles to grains. Three analytical treatments were evaluated for these samples: 1) the traditional Van Soest and Wine (1967) method explained in Exp. 1 plus an acetone rinse at filtering, 2) the same as

method 1 but with 200 mL of NDF solution, and 3) using a bi-phasic fat extraction method described by Bremer et al. (2010a) then rinsing the non-lipid residue into a 600 mL tall form beaker with 100 mL NDF solution and applying an acetone rinse at filtering. All treatments included weighing 0.5 g SS into the beakers and adding 0.5 mL AMY at reflux initiation.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (Version 8.02, SAS Inc., Cary, NC) for each experiment. Sample type and analytical treatment were considered fixed effects and interactions between these were tested for significance. When no significant interactions were observed ($P > 0.05$), main effects of sample type and analytical treatment are presented. When significant interactions were observed ($P \leq 0.05$), simple effects are presented.

RESULTS AND DISCUSSION

Exp. 1

An interaction resulted for NDF content between corn sample and analytical treatment ($P < 0.01$; Table 1). This suggests that the use of SS and AMY were not consistent in extracting non-fibrous materials from different corn samples. In general, using SS to extract protein complexed with NDF resulted in decreased NDF values when AMY level remained constant. This agrees with Van Soest (1994) who stated that protein can be complexed with lignin in numerous feeds and the protein can be dissolved using SS, resulting in a lower NDF value. Therefore, we decided to continue using 0.5 g SS in any NDF procedure we conducted.

When including SS, there continued to be an interaction ($P < 0.01$) between corn sample and number of AMY doses. With HMC, increasing the number of AMY doses from one to two decreased ($P < 0.01$) the NDF content from 17.20 to 8.85% DM. This agreed with the NRC (1996) that stated the NDF content in corn is 9 to 10% of DM, dependent on bushel weight. However, increasing the number of AMY doses resulted in increased ($P < 0.01$) NDF content in the DRC sample from 12.30 to 14.27% DM. Neither of these NDF results for DRC appeared to be an acceptable value for corn NDF. When filtering these DRC samples, the filters appeared to retain some visual granular material which appeared to be non-fiber material, perhaps the germ or endosperm in corn.

Exp. 2

An interaction resulted between analytical technique (number of AMY doses) and corn sample ($P < 0.01$; Table 1). Increasing the number of AMY doses from 1 to 2 decreased the NDF content for both DRC (26.81 vs. 12.63%, respectively) and HMC (16.45 vs.10.16%, respectively). This decrease in NDF values was likely due to a decrease in the removal of starch for measuring fiber. Mertens (2002) suggested that starch in feeds can be difficult to hydrolize by only using NDF solution, and AMY can be used in the NDF procedure to help this process. No differences in NDF content resulted between dosing AMY 2 or 3 times within each corn type ($P \geq 0.50$). However, the NDF value for DRC was numerically greater than HMC with 2 doses of AMY, and was statistically greater ($P = 0.02$) than HMC when 3 doses of AMY were used. These results suggest that more corn starch is degraded when AMY doses increase from 1 to 2 with smaller changes from 2 to 3 doses.

The NDF values for HMC, when dosing 2 (10.16%) or 3 (10.05%) times with AMY, are similar to those stated in the NRC (1996). The NDF values reported for DRC in this experiment were numerically greater than those for HMC, and these values for DRC continued to be greater than expected. When filtering these DRC samples, the filters still contained some granular material as stated in Exp. 1. The results from Exp. 1 and 2 indicate that starch complexes and interferes with the fiber in corn which likely results in incomplete starch removal (greater NDF values).

There can be starch and fiber differences in corn due to corn hybrid differences. Watson (2003) stated that as corn bushel weights increase (due to changes in corn hybrids), the weight of the endosperm increases and the pericarp decreases. The pericarp contains slightly less than 90% NDF (Watson, 2003), which is mostly hemicellulose and cellulose (Gasper et al., 2007). Therefore, when corn hybrids contain less pericarp, the NDF content of the corn should decrease.

Exp. 3

In order to accurately compare NDF content in corn processing types, we needed to obtain the same corn hybrid processed as DRC and HMC to analyze for NDF. No interaction ($P = 0.93$) was observed between analytical treatment and corn type (Table 2). Although a statistical difference was not observed between corn samples ($P = 0.47$), greater NDF values were observed for the DRC hybrid for every AMY treatment. Averaged across all treatments, the NDF value for the DRC sample was 1.33% and 1.27% units greater than the HMC and SFC samples, respectively. When the same corn hybrid is used, but is processed differently, the NDF value should be similar, particularly with the DRC and HMC in this experiment. Due to numerical differences for corns

between processing types, these data suggest a difference in the effect of AMY when corn processing method changes.

Increasing the dose of AMY from one to two at 0.5 mL decreased ($P < 0.01$) the corn NDF content measured. The NDF values were 21.82 and 13.08% for one and two AMY doses, respectively. However, increasing the doses of AMY from two to four did not further decrease ($P = 0.53$) NDF. We hypothesized that increasing the dosing amount of AMY from 0.5 mL to 1.0 mL would hydrolyze more starch and lower the NDF content. However, no difference ($P \geq 0.63$) was observed for dosing AMY twice at 1 mL compared to dosing AMY two or four times at 0.5 mL. In this experiment, increasing the AMY dose from one to two appeared to digest more starch and result in a more accurate corn NDF value, but increasing AMY beyond 2 doses at 0.5 mL did not appear to digest more starch. Regardless of AMY dosage, we continued to visually observe granular material remaining on the filters that did not appear fibrous. Therefore, we concluded that the NDF results from this experiment were not indicative of true fiber content.

Exp. 4

An interaction resulted between corn sample and analytical treatment of enzymes in combination with AUT ($P < 0.01$; Table 3). Using different combinations of AMY and GLU in treatments AMY-GLU-AMY-0.5 and AMY-AMY-GLU-1 resulted in mixed results for the corn samples. Neither enzyme combination treatment appeared to be superior at reducing NDF values. Using an enzyme treatment alone decreased NDF values for the DRC and SFC samples ($P < 0.01$) compared to AUT. We suspected that using GLU in combination with AMY would degrade the difficult glucose bonds in the non-reducing ends of starch to result in acceptable NDF values. However, the NDF

values observed in this experiment were not as acceptable (not as close to 9 to 10%) as the values in Exp. 3 in which two doses of AMY were used in a continuous refluxing process.

We hypothesized that using AUT would help degrade starch and make it more available for enzyme utilization, similar to how SFC is processed with steam and pressure. Using AUT lowered the NDF content for HMC compared to the DRC and SFC samples ($P < 0.01$), but the NDF values for HMC remained above (15.28 and 12.13% for AUT-AMY-1 and AUT-AMY-AMY-GLU-0.5, respectively) acceptability. However, AUT did not make starch more available in the DRC and SFC samples as NDF values remained above 30 and 15% for the AUT-AMY-1 and AUT-AMY-AMY-GLU-0.5 treatments, respectively. We concluded that using pressure, steam, and heat from an autoclave was unsuccessful in degrading starch in corn. Therefore, we continued to use two doses of AMY to hydrolyze starch in our experiments.

Exp. 5

An interaction resulted between DRC samples and grind size for NDF content ($P < 0.01$; Table 4). This interaction is due to the marginal decrease being different among samples for grinding through the Cyclo compared to the Wiley. The NDF values observed for all four DRC samples ground through the Wiley (13.77 to 17.66%) were considered above acceptability. We continued to visually observe granular material residing on the filters that did not appear to be fiber similar to previous experiments.

When the DRC samples were ground through the Cyclo, not only did the samples result in lower NDF values ($P < 0.01$), but no visual granular material remained on the filters. Three of the four samples resulted in NDF values of 9.74 to 10.60% DM, which

we considered acceptable for corn. We realize the Poet 2 DRC sample resulted in an NDF value of 7.56% DM, but this sample replicated very well (SD = 0.1). This difference may be due to corn hybrid differences as hybrids can vary in starch and fiber content dependent on bushel weights (Watson, 2003).

After conducting these five experiments to determine an analytical procedure that accurately measured the NDF content in corn, we realized how researchers may observe differing NDF results if corn processing and enzyme treatments are different. Dairy One Forage Analysis Laboratory (Ithaca, NY; 2010) summarized 263 corn samples from 2000 to 2010 and reported an average NDF value of 18.90% DM, with a normal range of 12.99 to 24.81% using the ANKOM method and dosing with AMY and weighing SS into the machine. These numbers remain above what the NRC (1996) stated and what we observed. We believe the NDF analyses obtained for corn samples using an ANKOM filter bag machine are not accurate. If unwanted starch remains in filter bags, then NDF values would be greater than expected.

In the traditional NDF beaker system, we observed that dosing once with 0.5 mL AMY was not sufficient at degrading starch and two doses were needed at reflux initiation and 50 min post reflux initiation to allow time for the enzyme to work at its full potential. Finally, this analytical procedure is not accurate unless the corn samples have been broken up and ground fine enough (i.e. through a 1-mm screen Cyclo) to degrade the complex between corn starch and corn fiber. These combined techniques result in corn NDF values that are correct and comparable to the NRC (1996).

Exp. 6

The NDF content for DDGS decreased as the ratio of solubles to distillers grains increased, regardless of analytical treatment (Table 5). This was to be expected as solubles contain very little NDF (2 to 8% DM; Bremer et al., 2010b). However, an interaction resulted between DDGS sample and analytical technique ($P < 0.01$), which was due to inconsistent results between the traditional and added NDF solution treatments. This indicated that adding twice as much NDF solution to the procedure did not decrease NDF content for all of the samples. We hypothesized that the additional NDF solution would be useful in solublizing additional fat from the DDGS samples compared to the traditional procedure but this did not occur.

As expected, fat content increased as level of solubles was added to the distillers grains ($P < 0.01$; 7.1 to 13.9% fat). Solubles typically contain 18 to 28% fat, and Bremer et al. (2010b) observed 23.6% fat. Therefore, we believed it would be logical to use a pre-fat extraction process (Bremer et al., 2010a) before the traditional NDF method to decrease interacting factors between fat and fiber. Using this procedure resulted in decreased ($P < 0.01$) NDF content for each DDGS sample. A decrease of 4.5 to 5.9% units was observed when using the pre-fat extraction step before measuring NDF compared to the traditional NDF procedure. The measured NDF content of these DDGS with the pre-fat procedure was 26.69 to 37.29% DM, but was variable due to solubles inclusion.

Dairy One Forage Laboratory (2010) analyzed 4,794 DGS samples for NDF content in an ANKOM filter bag machine and determined an average NDF content of 33.85% DM with a normal range of 29.28 to 38.43%. This range can be due to varying levels of solubles added to the distillers grains and incomplete removal of fat from the

filter bags for NDF analysis. Therefore, we feel it is appropriate to measure NDF in high-fat feeds using the traditional beaker method coupled with the pre-fat extraction process because NDF solution alone cannot solublize large quantities of fat in feeds.

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Table 1. NDF[1] content of dry-rolled corn (DRC) or high-moisture corn (HMC) samples obtained in November 2007 when treated with different doses of alpha-amylase (AMY) and sodium sulfite (SS) in Exp. 1 and additional doses of AMY in Exp. 2.

| | Treatments for Exp. 1[2] | | | | | | | |
| | 1 AMY -SS | | 1 AMY +SS | | 2 AMY +SS | | | |
	DRC-Nov 2007	HMC-Nov 2007	DRC-Nov 2007	HMC-Nov 2007	DRC-Nov 2007	HMC-Nov 2007	SEM[3]	Inter[4]
NDF	33.58[f]	21.81[e]	12.30[b]	17.20[d]	14.27[c]	8.85[a]	0.59	<0.01

| | Treatments for Exp. 2[5] | | | | | | | |
| | 1 AMY | | 2 AMY | | 3 AMY | | | |
	DRC-Nov 2007	HMC-Nov 2007	DRC-Nov 2007	HMC-Nov 2007	DRC-Nov 2007	HMC-Nov 2007	SEM[3]	Inter[4]
NDF	26.81[d]	16.45[c]	12.63[ab]	10.16[a]	13.58[b]	10.05[a]	0.97	<0.01

[1] Values expressed on a % of DM basis.

[2] Where 1 AMY -SS = adding 1 dose of 0.5 mL AMY at reflux initiation with no SS, 1 AMY +SS = adding 1 dose of 0.5 mL AMY at reflux initiation and weighing 0.5 g SS into beakers with corn before reflux process, 2 AMY +SS = adding 2 doses of 0.5 mL AMY at reflux initiation and 50 min post reflux initiation and weighing 0.5 g SS into beakers with corn before reflux process.

[3] Each treatment mean represents 3 replicates (n).

[4] Where Inter = *P*-value for F-test of interaction between corn sample and analytical treatment.

[5] Where 1 AMY = adding 1 dose of 0.5 mL AMY at reflux initiation, 2 AMY = adding 2 doses of 0.5 mL AMY at reflux initiation and 50 min post reflux initiation, 3 AMY = adding 3 doses of 0.5 mL AMY at reflux initiation and 30 and 50 min post reflux initiation. All three treatments included weighing 0.5 g SS in beakers with corn.

[abcdef] Means in the same row without a common superscript differ ($P < 0.05$).

Table 2. NDF[1] content of the same corn hybrid processed as dry-rolled corn (DRC) or high-moisture corn (HMC) and a steam-flaked corn (SFC) sample obtained in June 2006 when treated with different doses of alpha-amylase (AMY) in Exp. 3[2].

	Corn Type[3]				Analytical Treatment[4]				
	DRC hyb	HMC hyb	SFC	P-value[5]	1 AMY-0.5 mL	2 AMY-0.5 mL	4 AMY-0.5 mL	2 AMY-1 mL	P-value[6]
NDF	15.86	14.53	14.59	0.47	21.82[b]	13.08[a]	12.20[a]	12.88[a]	<0.01

[1] Values expressed on a % of DM basis.

[2] F-test of interaction between corn sample and analytical treatment P-value = 0.93.

[3] Where DRC hyb = Golden Harvest H-8562 hybrid processed as DRC, HMC hyb = Golden Harvest H-8562 hybrid processed as HMC, SFC = corn processed as SFC.

[4] Where 1 AMY-0.5 mL = adding 1 dose of 0.5 mL AMY at reflux initiation, 2 AMY-0.5 mL = adding 2 doses of 0.5 mL AMY at reflux initiation and 50 min post reflux initiation, 4 AMY-0.5 mL = adding 4 doses of 0.5 mL AMY at reflux initiation and 30 and 50 min post reflux initiation, 2 AMY-1 mL = adding 2 doses of 1 mL AMY at reflux initiation and 50 min post reflux initiation. All four treatments included weighing 0.5 g sodium sulfite in beakers with corn.

[5] P-value for F-test differences among corn samples. SEM = 0.85; each treatment mean represents 16 replicates (n).

[6] P-value for F-test differences among analytical treatments. SEM = 0.98; each treatment mean represents 12 replicates (n).

Table 3. NDF[1] content of the same corn hybrid processed as dry-rolled corn (DRC) or high-moisture corn (HMC) and a steam-flaked corn (SFC) sample obtained in June 2006 when treated with different doses of alpha-amylase (AMY), amyloglucosidase (GLU), and pressurizing with steam and heat in an autoclave (AUT) in Exp. 4.

AT[2]:	AMY-GLU-AMY-0.5			AMY-AMY-GLU-1			AUT-AMY-1			AUT-AMY-AMY-GLU-0.5			
Corn[3]:	DRC hyb	HMC hyb	SFC	DRC hyb	HMC hyb	SFC	DRC hyb	HMC hyb	SFC	DRC hyb	HMC hyb	SFC	Inter[4]
NDF	15.16^{de}	14.02^{cd}	12.02^{ab}	13.81^{bcd}	12.36^{abc}	11.26^{a}	32.87^{g}	15.28^{de}	35.50^{h}	20.53^{f}	12.13^{ab}	16.35^{e}	<0.01

[1] Values expressed on a % of DM basis.

[2] Where AT = analytical treatment; AMY-GLU-AMY-0.5 mL = adding 1 dose of 0.5 mL AMY at reflux initiation, reflux for 30 min, sit aside beakers until solution reached 50ºC and add 0.5 mL GLU and let sit for 10 min then reflux again for 30 min and add 0.5 mL AMY 10 min prior to the end of the reflux process; AMY-AMY-GLU-1 = adding 2 doses of 1 mL AMY at reflux initiation and 50 min post reflux initiation, sit aside beakers until solution reached 50ºC and add 1 mL GLU, let beakers sit for 10 min prior to filtering; AUT-AMY-1 = using an AUT at 121ºC for 30 min, start reflux process, and add 1 mL AMY at reflux initiation; AUT-AMY-AMY-GLU-0.5 = using an AUT at 121ºC for 30 min, start reflux process, add two doses of 0.5 mL AMY at reflux initiation and 50 min post reflux initiation, sit aside beakers until solution reached 50ºC and add 0.5 mL GLU, let beakers sit for 10 min prior to filtering. All four treatments included weighing 0.5 g sodium sulfite in beakers with corn.

[3] Where DRC hyb = Golden Harvest H-8562 hybrid processed as DRC, HMC hyb = Golden Harvest H-8562 hybrid processed as HMC, SFC = corn processed as SFC.

[4] Where Inter = P-value for F-test of interaction between corn sample and analytical treatment. SEM = 0.64; each treatment mean represents 3 replicates (n).

abcdefgh Means in the same row without a common superscript differ (P <0.05).

Table 4. NDF[1] content of four dry-rolled corn (DRC) samples ground through a 1-mm screen in a Wiley Mill (Wiley) or a Tecator Cyclomill (Cyclo) using two doses of alpha-amylase (AMY) in Exp. 5.

Corn[2]:	DRC hyb		DRC-Nov 2007		DRC-Poet 1		DRC-Poet 2			
Grind[3]:	Wiley	Cyclo	Wiley	Cyclo	Wiley	Cyclo	Wiley	Cyclo	SEM[4]	Inter[5]
NDF	13.77^c	10.60^b	16.70^d	10.38^b	17.66^d	9.74^b	14.85^c	7.56^a	0.55	<0.01

[1] Values expressed on a % of DM basis.

[2] Where DRC hyb = Golden Harvest H-8562 hybrid processed as DRC, DRC-Nov 2007 = DRC sample obtained in November 2007, DRC-Poet 1 = Corn sample 1 obtained from Poet Nutrition and processed as DRC, DRC-Poet 2 = Corn sample 2 obtained from Poet Nutrition and processed as DRC.

[3] Where Wiley = ground sample through a 1-mm screen Wiley Mill, Cyclo = ground sample through a 1-mm screen Tecator Cyclomill. All treatments included dosing twice with 0.5 mL AMY at reflux initiation and 50 min post reflux initiation, and weighing 0.5 g sodium sulfite in beakers with corn.

[4] Each treatment mean represents 3 replicates (n).

[5] Where Inter = P-value for F-test of interaction between corn sample and analytical treatment.

[abcd] Means in the same row without a common superscript differ (P <0.05).

Table 5. NDF and fat[1] content of dried distillers grains plus solubles (DDGS) with different ratios of grains to solubles when using the traditional NDF procedure with 100 mL NDF solution, 200 mL of NDF solution, or conducting a pre-fat extraction followed by the traditional NDF procedure with 100 mL NDF solution[2].

| | Analytical Treatment[3] | | | |
Sample	100 mL NDF	200 mL NDF	Pre-fat NDF (100 mL)	Fat
0DDGS	43.40[k]	41.61[j]	37.29[i]	7.1
33DDGS	38.07[i]	37.93[i]	32.72[fg]	9.2
67DDGS	33.58[g]	34.82[h]	28.96[c]	10.8
100DDGS	31.32[de]	32.61[fg]	27.51[b]	12.8
110DDGS	31.79[ef]	30.69[d]	25.69[a]	13.9

[1] Values expressed on a % of DM basis.

[2] Interaction with an F-test resulted in a *P*-value of < 0.01 between DDGS sample and analytical treatment. SEM = 0.59; each treatment mean represents 3 replicates (n).

[3] Where 100 mL NDF = using the traditional Van Soest and Wine (1967) procedure with 100 mL NDF solution, 200 mL NDF = using the traditional method with 200 mL NDF solution, Pre-fat NDF (100 mL) = conducting a pre-fat exctraction on the samples and rinsing the residue into beakers with 100 mL NDF solution. All treatments included weighing 0.5 g sodium sulfite in beakers with corn, dosing with 0.5 mL alpha-amylase at reflux initiation, and rinsing filters with acetone.

[4] Where 0DDGS = 0% of traditional amount of solubles added to distillers grains, 33DDGS = 33% of traditional amount of solubles added to distillers grains, 67DDGS = 67% of traditional amount of solubles added to distillers grains, 100DDGS = 100% of traditional amount of solubles added to distillers grains, 110DDGS = 110% of traditional amount of solubles added to distillers grains.

Ensiled or Fresh Mixed Wet Distillers Grains with Solubles with Straw at Two Inclusions in Growing Calf Diets[5]

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ABSTRACT

A growing trial was conducted to evaluate feeding wet distillers grains with solubles (WDGS) with wheat straw as either fresh or ensiled mixes on steer performance. Sixty crossbred steers (BW = 231 ± 18.2 kg) were individually fed in a 2 x 3 factorial arrangement of treatments for 80 d. The diets included 2 levels of WDGS at 30 or 45% (diet DM) blended with wheat straw and 3 storage types for feeding the mixtures as fresh, ensiled without an innoculum, or ensiled with a *Lactobacillus buchneri* innoculum. The fresh mixture was mixed every other d and the ensiled mixtures were mixed and stored for 45 d before trial initiation. Steers were pair-fed by BW for the storage types of mixture fed. No significant interactions were observed between WDGS level and type of feeding mixture. Feeding 45% WDGS to steers resulted in greater DMI, ADG, and G:F compared to feeding 30% WDGS. Steers fed the ensiled mixes had greater ADG and G:F compared to the fresh mix, resulting in a 21.5% greater feeding value for the ensiled mixtures. Feeding an increased level of WDGS from 30 to 45% of diet DM increased ADG and G:F, and ensiling WDGS with straw improved the feeding value of that mix.

Keywords: cattle, distillers grains, storage

INTRODUCTION

Cattle on feed numbers typically decrease in the summer months in the midwest, resulting in a decreased demand for purchasing wet distillers grains with solubles (WDGS; Erickson et al., 2008). This provides an opportunity for cattle producers to purchase a high energy, high protein byproduct to feed to cattle at later times in the year. Wet distillers grains plus solubles has 119 to 150% the energy value of DRC in high forage diets (Nuttelman et al., 2010; Ahern et al., 2011). Mixing WDGS with a low

quality forage can aid in the storage of this wet byproduct feed. Mixture ratios needed for storing WDGS with forages are different depending on the forage and storage method (i.e. silo bags or bunker; Erickson et al., 2008). However, the feeding value of stored WDGS with forages has not been accurately determined. Previous research has attempted to evaluate feeding WDGS with straw or corn stalks (Peterson et al., 2009; Wilken et al., 2009), but the feeding value of these mixes could not be accurately determined due to differences in DMI and source of WDGS. In a review by Krehbiel et al. (2003), mixed effects resulted from using a variety of direct fed microbials (DFM) in receiving or growing calf diets on nutrient metabolism and calf performance. Therefore, the objective of this experiment was to accurately determine the feeding value of WDGS at 30 or 45% diet DM mixed with straw as fresh commodities, or mixed and stored with or without a *Lactobacillus buchneri* direct-fed microbial.

MATERIALS AND METHODS

Sixty crossbred, steer calves (BW = 231 ± 18.2 kg) were used in a 2 x 3 factorial arrangement of dietary treatments to evaluate feeding WDGS mixed with straw fed fresh or after storage. Treatments included two mixture ratios of WDGS and straw and three storage types. Ratios were 30% WDGS (Abengoa Bioenergy, York, NE) with 70% straw or 45% WDGS with 55% straw (DM basis). The composition of WDGS was 32% DM, 32.4% CP, 32.7% NDF, 12.8% fat, and 0.90% S. The composition of straw was 88% DM, 5.4% CP, 80.4% NDF, 0.9% fat, and 0.12% S. Three storage types of each mixture included mixed fresh every other d, ensiled and stored without a microbial inoculum, or ensiled and stored with a microbial inoculum. The mixtures for the ensiled treatments had been mixed and stored in silo bags at 300 psi pressure for 70 d before experiment

initiation. The same source of WDGS was used in the fresh mix and the ensiled mixes as

WDGS was stored by itself in a silo bag under no pressure at the same time that the

mixtures were stored for use in the fresh mix. Therefore, no WDGS composition or cattle

performance differences should be due to WDGS storage. The inoculum was applied to

provide 500,000 colony forming units (CFU) of *Lactobacillus buchneri* strain 40788

(Lallemand Animal Nutrition North American, Milwaukee, WI) per gram of as-is

mixture at time of storage. All of the mixtures were fed at 97.5% of diet DM with 2.5%

dry supplement. The dry supplement was formulated to supply steers with 0.05% beef

trace mineral, 0.0625% tallow, and 0.015% vitamin A, D, and E. Diets were formulated

to meet or exceed NRC (1996) requirements for metabolizable protein, degradable intake

protein, Ca, and P by providing urea and limestone in the supplement. Limestone was

included at 1.23 and 1.28% of diet DM for 30 and 45% WDGS mixtures, respectively.

Urea was provided in the 30% WDGS mixtures at 0.93% diet DM. The remainder of the

diet contained fine ground corn at 0.2125 and 1.0925% for 30 and 45% WDGS mixtures,

respectively.

Upon arrival in October 2008, steers were individually identified and vaccinated

with Pyramid 4 (for prevention of IBR, BVD, BRSV, and PI3, Fort Dodge Animal

Health, Overland Park, KS) and Somubac (for prevention of haemophilus somnus, Pfizer

Animal Health, New York, NY) and given an injectible parasiticide (Dectomax, Pfizer

Animal Health). Approximately 14 d following initial processing, steers were

revaccinated with Pyramid 4 (Fort Dodge Animal Health), Vision 7 (for prevention of

clostridium chauvoei, septicum, novyi, sordellii, perfringens Types C & D infections,

Intervet-Schering Plough, Millsboro, DE), and injected with Piliguard Pinkeye Triview

(for prevention of pinkeye, Intervet-Schering Plough). Before experiment initiation in December 2008, steers were limit-fed a diet containing 50% alfalfa hay and 50% Sweet Bran (Cargill Inc, Blair, NE; DM basis) at 2.0% of BW (4.6 kg DM) for 5 d, then weighed on 3 consecutive d (d -1, 0, and 1). These weights were averaged (231 kg) and used as initial BW for cattle performance calculations. Steers were stratified by BW based on d -1 and 0 BW and dietary treatments were assigned randomly to bunks (10 steers/ treatment). The experiment was conducted from December 12, 2008 to March 5, 2009 at the University of Nebraska-Lincoln Agricultural Research and Development Center research feedlot near Mead, NE. Animal use procedures were reviewed and approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Steers were individually fed using Calan electronic gates (American Calan Inc., Northwood, NH). Steers were trained to the gates for 28 d before experiment initiation. Steers that were assigned to the fresh mixes were fed ad libitum. Steers fed the ensiled mixes were matched by similar BW to steers fed the fresh mixes and were fed the same DMI at 0700 h and fed for 84 d. At the end of the experiment, steers were limit-fed the same common diet fed at the beginning of the trial for 5 d at 2.0% of BW to limit gut fill effects. Body weights were obtained on 3 consecutive d and averaged and used as ending BW (270 kg) for ADG and G:F calculations.

Feed samples and feed refusals were collected weekly and analyzed for DM at 60ºC for 48 h to obtain accurate DMI. Dried feed samples were composited by mo and analyzed for CP using combustion (AOAC, 1999; method 990.03), fat using a biphasic solvent extraction (Bremer et al., 2010), and S using combustion (Leco, 2010).

Growth performance data were analyzed using the Proc Mixed procedure of SAS (Version 8.02, SAS Inc., Cary, NC) as a CRD, with steer as the experimental unit. Treatment effects were analyzed as a 2 x 3 factorial and if interactions were significant (alpha $\leq$ 0.05), then simple effects are reported. If interactions were not significant (alpha > 0.05) then main effects are reported.

RESULTS AND DISCUSSION

No interactions ($P \geq 0.10$; Table 1) were observed between ratio of WDGS to straw and type of mixture. Therefore, only main effects are presented. Steers consumed more DM ($P = 0.05$) when they were fed 45% WDGS (4.4 kg) compared to 30% WDGS (4.2 kg), suggesting the 45% WDGS diet was more palatable, provided less rumen fill, had greater rumen passage rate, and/or had greater total-tract digestibility due to decreased inclusion of wheat straw. This intake effect is similar to Ahern et al. (2011) who observed increased DMI when WDGS was increased in grass hay diets. Steers fed 45% WDGS had greater ending BW, ADG, and G:F ($P < 0.01$) compared to feeding 30% WDGS. Daily gain increased from 0.37 kg/d to 0.55 kg/d and G:F increased from 0.088 to 0.125 when WDGS increased from 30 to 45%, respectively. Peterson et al. (2009) observed an increase in G:F for feeding an increased level of WDGS from 35 to 45%. These data suggest that by increasing dietary inclusion of WDGS, steer ADG and G:F increased. In the current experiment, a 42% improvement in G:F was calculated due to increasing WDGS from 30 to 45% inclusion and removing 15% of the straw.

By design, DMI was not affected by storage type ($P = 0.99$). Although DMI was kept constant for steers fed different storage types, increased ADG and G:F ($P \leq 0.02$) resulted from feeding the ensiled mixes compared to feeding the fresh mixes. In the first

trial of a series of 3, Wilken et al. (2009) observed an increase in ADG, DMI, and G:F for feeding the ensiled mix of 30% WDGS with corn stalks compared to the fresh mix. In the second trial by Peterson et al. (2009), no differences were observed in ADG or G:F when feeding fresh or ensiled WDGS mixed with straw, but a different source of WDGS was fed in the 2 mixes. These data suggest that if WDGS is mixed and ensiled with a low quality forage, then the feeding value of the mixture improves. In the current study, this improvement calculates to a 21.5% greater G:F for mixing and storing WDGS with straw. A 4.4% numerical improvement in G:F was observed when the mixes were ensiled with the *Lactobacillus buchneri* inoculant, however, this was not significant ($P = 0.46$). The lack of an effect in using this DFM in diets was similar to the results observed in the review by Krehbiel et al. (2003), in which DFM were fed to newly weaned or received feedlot calves and resulted in no differences. However, using a lactic acid bacteria in high forage or silage experiments has increased in-vitro DM and NDF digestibilities compared to not using the bacteria (Weinberg et al., 2007), but these results decreased when starch was added. The improvement in NDF digestibility is likely a result from increased hemicellulose or cellulose digestion as these fiber components are 60 to 70% digestible and lignin is nearly indigestible (Van Soest, 1994). In the current experiment, the mixes do not undergo a true ensiling process similar to silages and change in pH. The mixes are already at a low pH due to the WDGS and contain little starch to undergo anaerobic fermentation. Conversely, silages contain more starch, undergo anaerobic fermentation, produce lactic acid, and decrease in pH (McDonald et al., 1991).

There should not be any fermentation differences between the fresh and ensiled mixes because the WDGS fed in the fresh mix was stored in an anaerobic bag similar to

the stored mix. Therefore, the improvements in ADG and G:F suggest changes in composition and/or a digestibility improvement of the straw portion of the mixes. The improved cattle performance suggests an improved rate or extent of NDF digestion. Swelling of the straw fiber particles may result from ensiling, thus, increasing particle size, rumen retention time, and extent of digestion. Feeding ensiled mixes previously resulted in increased DMI compared to the mixes fed fresh (Wilken et al., 2009). These data suggest that not only palatability increases (indicated by increased DMI) for ensiling mixes of WDGS with a low quality forage, but digestion improves as well which increases ADG and G:F. Using *Lactobacillus buchneri* in the ensiled mixes did not result in significant differences, perhaps due to low pH conditions. However, a numeric increase in G:F was observed for using the inoculant, possibly due to an improvement in hemicellulose or cellulose digestion, as Weinberg et al. (2007) observed increased NDF digestibility for forages or silages using a lactic acid bacteria. A ferulic acid esterase enzyme is produced from *Lactobacillus buchneri* which has the potential to improve fiber digestion in the rumen (Nsereko et al., 2008).

Feeding an increased level of WDGS from 30 to 45% DM increased cattle growth and G:F, indicating an improved feeding value either due to the increased WDGS or decreased straw content. Storing WDGS with straw resulted in improved cattle performance compared to feeding the mixture fresh, possibly suggesting an improvement in fiber digestion of the straw for storing.

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Table 1. Steer performance when fed mixes of wet distillers grains with solubles (WDGS) and straw fed fresh or ensiled with or without an inoculum.

	WDGS: Straw Mix[1]				Storage Type[2]					Inter[5]
	30:70	45:55	SEM[3]	P-value	Fresh	Ensil-No Inoc	Ensil-W/Inoc	SEM[4]	P-value	
Initial BW, kg	231	231	3	0.97	231	230	231	4	0.96	0.99
Ending BW, kg	262	278	4	<0.01	265[a]	271[b]	274[b]	5	0.43	0.71
DMI, kg/d	4.2	4.4	0.1	0.05	4.3	4.3	4.3	0.1	0.99	1.0
ADG, kg	0.37	0.55	0.02	<0.01	0.40[a]	0.49[b]	0.50[b]	0.03	0.02	0.16
G:F[6]	0.088	0.125	0.004	<0.01	0.093[a]	0.111[b]	0.116[b]	0.005	<0.01	0.10

[1] Main effects for WDGS and straw mixtures. Where 30:70 = 30% WDGS and 70% straw, 45:55 = 45% WDGS and 55% straw (DM basis). Mixtures were fed at 97.5% of diet DM with 2.5% of diet DM as a dry supplement.

[2] Main effects for the storage type of mixture fed. Where Fresh = mixture fed as fresh mixed every other d, Ensil-No Inco = mixture fed as ensiled with no inoculum, Ensil-W/Inoc = mixture fed as ensiled with inoculum. Inoculum provided 500,000 colony forming units (CFU) of *Lactobacillus buchneri* strain 40788 (Lallemand Animal Nutrition North American, Milwaukee, WI) per gram of as-is mixture.

[3] Each treatment mean represents 30 steers (n).

[4] Each treatment mean represents 20 steers (n).

[5] Where Inter = *P*-value for F-test of interaction between WDGS and straw mix and storage type.

[6] Calculated as total gain over total DMI.

[ab] Means in the same row without a common superscript differ ($P < 0.05$).

Fiber Digestibility and Rumen Metabolism for Diets Containing Wet Distillers Grains with Solubles or Wet Corn Gluten Feed and using a *Lactobacillus Buchneri* Direct-Fed Microbial[7]

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ABSTRACT

A metabolism trial was conducted to evaluate feeding wet distillers grains plus solubles (WDGS) and Sweet Bran wet corn gluten feed (SB) in finishing diets with or without a *Lactobacillus buchneri* direct-fed microbial (DFM) on digestibility and rumen characteristics. Seven ruminally cannulated steers were used in a 6 x 7 unbalanced Latin square design trial and were fed dietary treatments arranged as a 3 x 2 factorial. Diets contained 35% WDGS (35WDGS) or 35 or 88% SB (35SB and 88SB, respectively) in replacement of dry-rolled corn and were top-dressed daily with or without 1×10^9 CFU's of the DFM. Steers consumed more DM from feeding 35SB or 88SB than 35WDGS and the greatest amount of NDF was consumed from feeding 88SB. Tendencies for interactions resulted for DM and NDF digestibilities. Steers fed 88SB tended to have lower DM digestibility compared to 35WDGS and 35SB. However, NDF digestibility tended to increase from top-dressing the 88SB diet with the DFM. Digestibility of NDF may have increased from feeding 88SB because ruminal pH was greater compared to 35WDGS and 35SB, due to no inclusion of corn grain. Ruminal pH was the lowest for steers fed 35SB. Molar proportion of propionate was least from feeding 88SB, likely due to no inclusion of grain. In-situ NDF digestibility of corn bran and SB were greater when the samples were incubated in steers fed a 70% brome grass hay and 30% corn and supplement diet compared to 35WDGS, 35SB, and 88SB. Digestibility of DM and NDF were similar for feeding 35WDGS and 35SB, but feeding 88SB resulted in decreased DM digestibility and increased ruminal pH. Feeding the DFM may be beneficial in improving DM and NDF digestibility in diets containing limited starch and moderate levels of fiber (88SB).

Keywords: cattle, distillers grains, gluten feed

INTRODUCTION

Feeding beef cattle wet corn gluten feed (Sweet Bran[®], SB, Cargill, Blair,

Nebraska) and wet distillers grains plus solubles (WDGS) up to 40% diet DM results in

increased ADG and G:F in dry-rolled corn (DRC) and high-moisture corn diets (HMC;

Klopfenstein et al., 2007 and 2008). Feeding values for SB were 112% and WDGS were

125 to 148% of corn (Klopfenstein et al., 2007 and 2008). The improved feeding values

may be due to decreased dietary starch and risk of ruminal acidosis and increased fat

content contributing to a greater energy value. However, the effects of SB and WDGS on

total-tract digestibility and the NDF in these byproducts remain in question.

Total-tract digestibility for WCGF or WDGS based diets has greater, similar, and

lower results compared to corn control diets (Scott et al., 1998; Corrigan et al., 2009;

Vander Pol et al., 2009; Sayer et al., 2010). Feeding WCGF or WDGS in the replacement

of corn in finishing diets would be expected to result in greater rumen pH due to less

starch. However, results for average, minimum, and maximum pH have not been

consistent across trials for feeding WCGF or WDGS compared to corn (Corrigan et al.,

2009; Vander Pol et al., 2009; Sayer et al., 2010). The improved feeding value for WDGS

may be due to greater inclusion of dietary fat from WDGS, more unsaturated fatty acids

reaching the duodenum (Vander Pol et al., 2009; Bremer et al., 2010a), or large amounts

of UIP content that is recycled and used as energy (Klopfenstein et al., 2008).

These byproducts contain large amounts of NDF (28 to 40%) that contain little

lignin and can be highly digestible. The fiber component in SB and WDGS (corn bran),

largely hemicellulose and cellulose, may be part of the reason for improved feeding

values compared to corn. DeHaan (1983) reported 87% fiber digestion of corn bran in an in-vitro setting. Direct-fed microbials (DFM) may improve NDF digestibility for cattle fed moderate amounts of fiber (Weinberg et al., 2007). Using a DFM in these byproduct containing finishing diets may improve the digestibility of the diets and thus improve cattle performance. Therefore, we conducted a metabolism trial to evaluate feeding SB and WDGS containing diets with or without a DFM on digestibility, rumen pH, and rumen VFA concentrations.

MATERIALS AND METHODS

Seven ruminally cannulated steers (BW = 361 kg) were used in a 6 x 7 unbalanced Latin square to evaluate feeding SB or WDGS in finishing diets and top-dressing a DFM on intake, digestibility, and ruminal pH. Surgical and post-surgical care procedures followed those reviewed and approved by the University of Nebraska Institutional Animal Care Program. The experiment was conducted from June 6, 2008 to October 26, 2008. Treatments were arranged in a 3 x 2 factorial design with 3 diets containing 35% WDGS (35WDGS), 35% SB (35SB), or 88% SB (88SB; DM basis; Table 1). Byproducts replaced equal DM amounts of DRC. The 88SB diet contained no DRC. The composition of WDGS (Abengoa Bioenergy, York, Nebraska) was 31.4% DM, 36.4% NDF, 33.1% CP, 12.2% fat, and 0.93% S (DM basis). The composition of SB was 59.6% DM, 38.3% NDF, 24.9% CP, 3.3% fat, and 0.52% S (DM basis). The other 2 factors included top-dressing the diets at feeding with or without a DFM consisting of 1 x 10^9 CFU's of *Lactobacillus buchneri* strain 40788 (Lallemand Animal Nutrition North American, Milwaukee, WI). All diets included 7% alfalfa hay and 5% dry supplement. Limestone was provided in the supplement to meet or exceed 1.2:1 Ca: P

and fine ground corn was used as a carrier. The supplement was formulated to meet or exceed MP requirements (NRC, 1996) and provide monensin (320 mg/steer daily; Elanco Animal Health, Indianapolis, IN), thiamine (150 mg/steer daily; International Nutrition, Omaha, NE), and tylosin (90 mg/steer daily, Elanco Animal Health). The supplement formulated for the 88SB diet contained the most limestone and that amount was used in the other two diets. Diets were mixed twice weekly and stored at 5ºC until feeding.

Steers were housed in individual pens with slotted floors, in a 25ºC temperature-controlled room. Six, 21-d periods were used that contained a 12 d adaptation period followed by a 9-d collection period. Steers were maintained as two groups and 3 of the steers were given the DFM for each of the first 3 periods and the other 4 steers were given the DFM for each of the last 3 periods. Steers maintained continued to consume their respective diets from period 3 during a 2-wk period to allow time for the effects of the DFM to be minimized. Steers were individually fed in pens once daily at 0800 h. Feed ingredients were sampled on the d of mixing during the collection periods and composited by period for subsequent chemical analysis. Feed refusals were collected daily at 0750 h and discarded from d 1 to d 16 of each period. Refusals were collected daily for the 5 d collection periods, weighed, sampled (10% of wet weight), and retained for digestibility measurements. Wireless pH probes (Dascor, Escondido, CA) were submersed in the rumen from d 13 to d 21. Chromic oxide was used as an indigestible marker to measure fecal output and calculate total-tract digestibility. One gelatin bolus containing 7.5 g of Cr_2O_3 was administered through the ruminal cannula of each steer twice daily at 0800 and 1800 h on d 13 through d 20 of each period, with two doses given

at 0800 h on d 13. Fecal grab samples were obtained from the rectum from d 17 to d 21 at 0800, 1300, and 1800 h and composited daily on an equal wet weight basis.

Ruminal fluid was collected before feeding (0800 h) and at 1300 and 2200 h on d 21 and frozen (-20˚C) immediately. To conduct VFA, ruminal fluid samples were thawed and centrifuged at 5,000 x g for 10 min. Two mL of the supernatant was deproteinized with 0.5 mL of 25% metaphosphoric acid containing 2-ethylbutyrate (0.2904 g in 100 mL), and 2-ethylbutyrate was used as an internal standard. Individual VFA were separated and analyzed utilizing gas chromatography (Erwin et al., 1961; Hewlett-Packard, Avondale, PA). Ruminal pH measurements included average, minimum, and maximum pH, magnitude of pH change, pH variance, time spent below pH 5.6, and area of pH below 5.6 (time below x magnitude below).

Daily composites for fecals and period composites for SB and WDGS were freeze-dried using a Virtis Freezemobile model 25 SL (The VirTis Company, Gardiner, NY) and retained. Dry fecal samples were ground through a 1-mm screen Wiley mill (Thomas Scientific, Swedesboro, NJ) and composited by period on an equal dry weight basis. Dry fecal composites were analyzed for chromium concentration using atomic absorption spectrophotometry (Varian Spectra AA-30; Williams et al., 1962) to determine total daily fecal output. Alfalfa hay, DRC, SB, WDGS, and feed refusals were dried at 60°C in a forced air oven for 48 h to calculate accurate DMI. Freeze-dried SB and WDGS, and oven dried alfalfa hay were ground through a 1-mm screen Wiley mill (Thomas Scientific). Oven dried DRC and feed refusals were ground through a 1-mm screen Tecator Cyclotec sample mill (American Instrument Exchange, Haverhill, MA). All feed samples were measured for ash (AOAC, 1999; method 942.05), CP using

combustion (AOAC, 1999; method 990.03), fat using a biphasic solvent extraction (Bremer et al., 2010b), S using combustion (Leco, 2010), and NDF. Neutral detergent fiber was determined for SB and alfalfa hay using the procedure described by Van Soest and Wine (1967) with 0.5 g sodium sulfite (SS) and 0.5 mL alpha-amylase (AMY) for SB. Content of NDF was determined for DRC and feed refusals using the method described by Buckner et al. (2010) and for WDGS using the pre-fat extraction method described by Buckner et al. (2010). Fecal NDF was determined by weighing 0.5 g sample into 50 mL in-vitro tubes, adding 10 mL of 5% HCl acid solution and 2 mL of 5% pepsin, incubating overnight in a 37ºC water bath, rinsing solution and sample into a 600 mL tall-form beaker followed by the procedure described by Van Soest and Wine (1967) using 0.5 g SS, 0.5 mL AMY and rinsing the filter with acetone.

In-situ bags (10 x 20 cm; ANKOM Technology, Macedon, NY) were used to estimate rumen NDF digestibility. Samples of corn bran, solvent extracted germ meal (GERM), SB, and WDGS were weighed into the bags to estimate ruminal NDF digestibility. Bags were incubated at 8, 16, 24, 48, 72 h in the rumen of the steers for each period (3 bags/ sample/ steer/ time point) on d 15 to 17. The bags were placed in the rumen at different time points so that all of the bags could be removed at the same time. Bags removed from the rumen and non-incubated bags (0 h) were machine washed (39°C) using 5 rinse cycles. Each cycle consisted of 1 min of agitation and 2 min of spin. Bags were dried at 60°C for 48 h, weighed, and air equilibrated for 24 h and weighed. Subsamples of the residue were weighed into tall-form glass beakers with 0.5 g SS to measure NDF using the Van Soest and Wine (1967) procedure. Modifications to the procedure included using 0.5 mL AMY and rinsing filters with acetone. Because we

observed low rumen digestibility values, we incubated corn bran and GERM at 0, 12, 24, 36, 48, and 72 h in the rumen of 2 steers fed a mixed diet to determine potential digestibility extent in the rumens of cattle not hindered by low pH or starch. The mixed diet contained 70% brome grass hay, 20% DRC, and 10% of a blend of soybean meal and vitamin and mineral premixes.

Intake and digestibility data were analyzed as a 6 x 7 unbalanced Latin square design with a 3 x 2 factorial treatment arrangement using the Proc Mixed procedure of SAS (Version 8.02, SAS Inc., Cary, NC). Ruminal pH and VFA data were analyzed as a repeated measure with a cholesky covariance structure. Period and treatment were included in the model as fixed effects and steer was a random effect. Interactions between diet and DFM were tested. If no significant interactions were observed ($P > 0.05$), then main effects of diet type and DFM supplementation are presented. If significant interactions were observed ($P \leq 0.05$), then the simple effects of DFM within diet are presented. However, some tendencies (P between 0.05 and 0.15) for interactions may have been biologically important, therefore, selected variables have simple effects presented. In-situ data were analyzed within sample type as a 3 x 6 factorial design for incubation time and the finishing diet that bags were incubated in. No significant interactions were observed ($P > 0.10$); therefore, only results for incubation time are presented. Rate of dissapearance for samples incubated in cattle fed the mixed diet was determined using the non-lag model described by Boucher et al. (2007).

RESULTS AND DISCUSSION

No significant interactions between diet and DFM resulted for DM or NDF intake ($P \geq 0.97$, Table 2). Feeding 35SB and 88SB resulted in a 1.0 to 1.5 kg/d greater DMI (P

< 0.01) than feeding 35WDGS. Feeding WDGS compared to DRC has previously

increased DMI (Corrigan et al., 2009), maintained DMI (Vander Pol et al., 2009), and

decreased DMI (Ham et al., 1994) in metabolism trials. We concluded from these studies

that feeding WDGS to a few steers (in metabolism trials) has inconsistent results for DMI

compared to feeding DRC. This DMI variation can be due to small numbers of

observations, potentially large variation in individual steer intake, or differing effects

from the type or source of WDGS. Feeding SB or the components of WCGF (corn bran,

steep, and/or SEM) has resulted in similar or increased DMI compared to feeding corn

(McCoy et al., 1997; Scott et al., 1998; Sayer et al., 2010). In all of these experiments, the

authors observed increases in NDF intake due to greater NDF in the diets. The increased

DMI for feeding SB compared to WDGS in the current experiment could be due to

greater fiber content, larger fiber particle size in SB, or less acidosis. Because the 88SB

diet had the greatest NDF content, intake of NDF was the greatest ($P < 0.01$) for this diet

(3.60 kg/ d). These steers consumed a greater proportion of fiber from byproducts

(specifically SB) compared to 35WDGS and 35SB. The 35% byproduct diets provided a

greater proportion of fiber from alfalfa hay than 88SB. Steers fed 35SB consumed more

NDF (2.51 kg/ d) than steers fed the 35WDGS diet (2.05 kg/ d) primarily due to greater

DMI. Top-dressing diets with the DFM increased DMI ($P = 0.04$) and tended to increase

NDF intake ($P = 0.10$).

Tendencies for interactions resulted for DM ($P = 0.08$) and NDF ($P = 0.15$)

digestibility between diet and DFM (Table 3). Feeding 35SB with or without the DFM

and 35WDGS without the DFM resulted in greater DM digestibility compared to feeding

88SB ($P \leq 0.04$). Feeding 35WDGS with the DFM resulted in numerically greater DM

digestibility than feeding 88SB. Feeding moderate levels (20 to 50% diet DM) of WDGS and SB (or the components of SB, i.e. corn bran, steep, SEM) has resulted in different effects for DM digestibility compared to feeding corn (McCoy et al., 1997; Scott et al., 1998; Bierman et al., 1999; Corrigan et al., 2009; Vander Pol et al., 2009; Bremer et al., 2010a). Therefore, it is not surprising that feeding 35WDGS and 35SB in the current experiment resulted in similar DM digestibility.

The DM digestibility results from feeding diets containing corn (35WDGS and 35SB) compared to no corn (88SB) are logical. Although the fiber in SB is largely hemicellulose and cellulose with little lignin (because corn has little lignin; Gaspar et al., 2007), fiber is less digestible than starch from corn. Lignin is nearly indigestible and hemicellulose and cellulose are 60 to 70% digestible for forages (Van Soest, 1994). However, digestibility measures of fiber in forages may not be indicative of fiber components in byproduct feeds, particularly when fed in finishing diets. Starch digestibility of cereal grains is commonly 80% or more (Owens et al., 1986). Therefore, the fiber in WDGS and SB should be less digestible than the starch in the corn it replaces, making the 88SB diet less digestible in DM. An improvement in DM digestibility resulted, however, for top-dressing the 88SB diet with the DFM compared to not using the DFM ($P = 0.05$). All of the 35WDGS and 35SB diets and the 88SB diet with no DFM resulted in similar NDF digestibilities. Although not significant, top-dressing the DFM on the 88SB diet resulted in numerically greater NDF digestibility compared to feeding 88SB without the DFM and 35WDGS and 35SB with the DFM. These differences were perhaps not detected statistically due to limited replications and more sources of measurement error for NDF digestibility compared to DM digestibility. Measuring NDF

digestibility requires determining the NDF content in the feed offered, feed refusals, and fecal samples, in addition to measuring chromium and DMI.

No significant interactions between diet and DFM resulted for any ruminal pH variables ($P \geq 0.42$; Table 4). Average, maximum, and minimum pH were least for steers fed 35SB, intermediate for 35WDGS, and greatest for 88SB ($P \leq 0.01$). Increased pH has been observed for feeding 30 to 45% WCGF based diets compared to corn diets (Montgomery et al., 2004; Sayer et al., 2010). However, some measures of ruminal pH decreased from feeding 40% WDGS compared to DRC (Ham et al., 1994; Corrigan et al., 2009; Vander Pol et al., 2009). Therefore, we hypothesized that feeding 35SB would result in greater ruminal pH measures than feeding 35WDGS, but the current experiment resulted in the opposite pH results. A possible reason for this result is due to greater DMI (greater starch intake) for cattle consuming 35SB compared to 35WDGS. No differences were observed among diets for ruminal pH change and variance ($P \geq 0.18$). Steers fed 88SB had the least ($P < 0.01$) time (125 min/d) and area (0 min*pH units < 5.6/d) below pH 5.6. Additionally, steers fed 35WDGS had decreased area below pH 5.6 (453 min*pH units < 5.6/d) compared to steers fed 35SB (672 min/d[3], $P < 0.01$). These trends indicated that steers fed the 88SB diet exhibited little risk of experiencing subacute acidosis. Top-dressing diets with the DFM tended to decrease average ($P = 0.14$) and minimum ($P = 0.08$) ruminal pH compared to not top-dressing with the DFM. No differences in pH change, pH variance, or time and area below pH 5.6 resulted from top-dressing with the DFM ($P \geq 0.42$).

Lactobacillus buchneri strain 40788 was ensiled with alfalfa haylage and resulted in increased rumen pH and an increase in milk production when fed to dairy cows (Kung

et al., 2003). Using a lactic acid bacteria in high forage or silage experiments with low starch has increased in-vitro DM and NDF digestibilities compared to not using the bacteria (Weinberg et al., 2007). A ferulic acid esterase enzyme is produced from *Lactobacillus buchneri* which has the potential to improve fiber digestion in the rumen (Nsereko et al., 2008). *Lactobacillus buchneri* appears to aid in NDF digestibility in greater pH environments, which is the situation for steers fed 88SB. This diet provided more NDF than 35SB and 35WDGS, with less corn starch resulting in greater rumen pH (Table 4). Feeding 88SB may have provided a better ruminal environment for the DFM in regards to fiber digestibility than the other diets with lower rumen pH. The population of fibrolytic rumen microorganisms is inhibited when rumen pH levels decrease below 6 due to the pH level or the competition with starch (Grant and Mertens, 2002). The 88SB diet contained no corn starch and average pH levels were above 6, possibly causing an increase in the activity of fibrolytic bacteria.

No interactions resulted for molar proportions of acetate or propionate or the ratio of acetate-to-propionate (A:P; $P \geq 0.25$; Table 5). Molar proportion of acetate was similar regardless of diet or DFM ($P \geq 0.41$). Propionate molar proportions tended to be greater for feeding 35WDGS or 35SB compared to 88SB ($P = 0.08$). These results are logical because the 88SB diet contains less starch than 35WDGS and 35SB. Starch promotes more propionate production compared to feeds containing fiber (Van Soest, 1994). This indicates that steers that consume 88SB should be less efficient at producing BW gain than steers that consumed WDGS or SB at 35% with corn included in the diets. Acetate to propionate ratios were unaffected by diet or DFM ($P \geq 0.34$) likely due to large error (SEM = 0.22). An interaction was observed between diet and DFM for total VFA

concentrations ($P = 0.02$; data not shown). The 35WDGS and 35SB diets, regardless of

DFM, and the 88SB diet without the DFM resulted in similar total VFA concentrations

(data not shown; average = 131 mM). Feeding 88SB with the DFM resulted in the lowest

total VFA concentration (111 mM). These interaction results with the DFM are difficult

to explain biologically. Concentrations of individual VFA are of importance, but actual

VFA production cannot be determined from these data.

No interactions resulted between incubation time and the finishing diet fed to the

steers the samples were incubated in for NDF disappearance of corn bran, SEM, SB, or

WDGS ($P \leq 0.11$). Disappearance of NDF from corn bran, SB, and WDGS was greatest

($P \leq 0.05$) when samples were incubated in the rumen of cattle fed 88SB (data not

shown). Corn bran, SEM, SB, and WDGS disappearance of NDF at 72 h was 33.5, 78.2,

44.4, and 52.7%, respectively (Table 6). Corn bran comes from the pericarp and tip cap

of the corn kernel, which contains 90 and 80% NDF, respectively (Watson, 2003). This

NDF is mostly hemicellulose and cellulose (Gaspar et al., 2007). Vander Pol et al. (2009)

observed ruminal NDF digestibility of 71% for cattle fed a 40% WDGS diet. Therefore,

we expected in-situ NDF disappearance values for corn bran, SB, and WDGS to be

greater than what we observed. The in-situ technique may not be appropriate in finishing

diets due to coating of in-situ bags with starch or fat that can hinder disappearance. The

NDF disappearance from corn bran and WDGS at 24 h was 25.6 and 43.8%, respectively.

Therefore, the NDF disappearance for these samples should be approximately 2 to 3

times greater at 72 h compared to 24 h, but they were not. This suggests there is a

problem with the in-situ technique in finishing diets more than rumen conditions. The

disappearance of GERM and WDGS NDF in the washout bags (0 h) was 11.0 and 15.0%,

respectively. These values are large compared to corn bran because the particle size of GERM and WDGS is small. Fiber that is washed out of the bags at 0 h is assumed to be 100% digested. Bremer et al. (2010a) reported corn bran NDF disappearance values at 48 h of 31.6 and 24.7% from incubating corn bran in the rumen of cattle fed corn and WDGS diets, respectively, which is consistent with our values. Disappearance of NDF from corn bran, SEM, and SB at 72 h was 88.1, 93.8, and 85.9%, respectively, when the samples were incubated in steers fed the mixed diet (Table 7). Rate of digestion for corn bran, SEM, and SB were 2.36, 5.27, and 2.93%/ h, respectively in the mixed diets. Digestibility of NDF in rumen in-situ bags is likely not indicative of total-tract digestibility because bags may reside in the rumen for a different length of time than if the feed was fed and normal rumen passage rate was observed and the rumen in-situ bag technique does not account for hind gut fermentation. We believe the results observed from incubating the byproducts in the rumen of cattle fed the mixed diet are logical. This suggests there is a confounding effect of incubating samples in in-situ bags into the rumen of cattle fed finishing diets and measuring NDF disappearance.

Based on these data, DMI is greater for feeding SB in finishing diets compared to feeding WDGS. Steers consumed more NDF when fed 88SB, which resulted in improved NDF digestibility when the diet was top-dressed with the DFM of *Lactobacillus buchneri*. These effects were due to either increased rumen pH and/or low levels of dietary starch. Although DM digestibility and propionate molar proportions were lower for steers fed 88SB compared to feeding 35SB or 35WDGS, there is less risk of acidosis with the 88SB diet.

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Table 1. Composition and nutrient analysis for finishing diets containing 35% wet distillers grains plus solubles or Sweet Bran and 88% Sweet Bran.[1]

	Treatment[2]		
Ingredient	35WDGS	35SB	88SB
Dry-rolled corn	53	53	--
Wet distillers grains plus solubles	35	--	--
Sweet Bran	--	35	88
Alfalfa hay	7	7	7
Dry Supplement	5	5	5
Fine ground corn	2.822	2.822	2.822
Limestone	1.764	1.764	1.764
Sodium chloride	0.30	0.30	0.30
Trace mineral premix[3]	0.05	0.05	0.05
Potassium chloride	0.02	0.02	0.02
Rumensin-80 premix[4]	0.019	0.019	0.019
Vitamin A-D-E premix[5]	0.015	0.015	0.015
Tylan-40 premix[6]	0.01	0.01	0.01
Nutrient Composition[7]			
CP	17.7	14.9	23.3
Fat	6.2	3.0	2.8
NDF	23.8	24.5	38.4
S	0.41	0.26	0.47

[1] Values expressed on a % DM basis.

[2] Where 35WDGS = 35% wet distillers grains plus solubles, 35SB = 35% Sweet Bran, 88SB = 88% Sweet Bran.

[3] Premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu, 0.3% I, 0.05% Co.

[4] Premix contained 176 g monensin•kg^{-1}.

[5] Premix contained 1500 IU vitamin A, 3000 IU vitamin D, 3.7 IU vitamin E•g^{-1}.

[6] Premix contained 88 g tylosin•kg^{-1}.

[7] Based on analyzed nutrients for each ingredient.

Table 2. Steer intake from feeding wet distillers grains plus solubles or Sweet Bran in finishing diets.

	Diet[1]					DFM[2]				Inter[5]
	35WDGS	35SB	88SB	SEM[3]	*P*-value	No DFM	W/ DFM	SEM[4]	*P*-value	
DM Intake, kg/d	8.61[a]	10.15[b]	9.63[b]	0.42	<0.01	9.16[a]	9.77[b]	0.40	0.04	0.99
NDF Intake, kg/d	2.05[a]	2.51[b]	3.60[c]	0.12	<0.01	2.66	2.78	0.11	0.10	0.97

[1] Where 35WDGS = 35% wet distillers grains plus solubles, 35SB = 35% Sweet Bran, 88SB = 88% Sweet Bran (DM basis).

[2] Where No DFM = diets were not top dressed with a direct-fed microbial, W/ DFM = diets were top dressed daily with 1 x 10^9 CFU's of *Lactobacillus buchneri* strain 40788 (Lallemand Animal Nutrition North American, Milwaukee, WI).

[3] Each treatment mean represents 14 replications (n).

[4] Each treatment mean represents 21 replications (n).

[5] Inter = *P*-value for F-test of interaction between diet and DFM.

[abc] Means in the same row within the same main effect without a common superscript differ ($P < 0.05$).

Table 3. Steer intake and total-tract digestibility from feeding wet distillers grains plus solubles or Sweet Bran in finishing diets.

	Treatment[1]						SEM[2]	Inter[3]
	35WDGS-	35WDGS+	35SB-	35SB+	88SB-	88SB+		
DM Digestibility, %	79.2[c]	77.7[bc]	79.4[c]	79.0[c]	73.7[a]	76.2[b]	0.96	0.08
NDF Digestibility, %	68.2[ab]	64.8[a]	68.1[a]	65.8[a]	69.0[ab]	72.3[b]	1.76	0.15

[1] Where 35WDGS- = 35% wet distillers grains plus solubles with no DFM, 35WDGS+ = 35% wet distillers grains plus solubles with the DFM, 35SB- = 35% Sweet Bran with no DFM, 35SB+ = 35% Sweet Bran with the DFM, 88SB- = 88% Sweet Bran with no DFM, 88SB+ = 88% Sweet Bran with the DFM. Diets were top-dressed with or without the DFM containing 1 x 10^9 CFU's of *Lactobacillus buchneri* strain 40788 (Lallemand Animal Nutrition North American).
Table 2. Performance measurements and carcass characteristics for treatments [ab].
[2] Each treatment mean represents 7 replications (n).

[3] Inter = *P*-value for F-test of interaction between diet and DFM.

[abc] Means in the same row without a common superscript differ ($P < 0.05$).

Table 4. Rumen pH variables for feeding wet distillers grains plus solubles or Sweet Bran in finishing diets.

	Diet[1]					DFM[2]				Inter[5]
	35WDGS	35SB	88SB	SEM[3]	*P*-value	No DFM	W/ DFM	SEM[4]	*P*-value	
Average pH	5.38^b	5.13^a	6.07^c	0.06	<0.01	5.57	5.47	0.05	0.14	1.00
Maximum pH	6.00^b	5.76^a	6.60^c	0.07	<0.01	6.14	6.10	0.06	0.66	0.57
Minimum pH	5.01^b	4.82^a	5.52^c	0.04	<0.01	5.16^b	5.08^a	0.04	0.08	0.60
pH change	0.99	0.94	1.06	0.04	0.18	0.97	1.03	0.04	0.20	0.81
pH variance	0.04	0.05	0.05	0.01	0.75	0.04	0.05	0.01	0.23	0.42
Time < 5.6 pH, min/d	1160^b	1261^b	125^a	75	<0.01	816	881	59	0.49	0.98
Area < 5.6 pH, min/d[3]	453^b	672^c	0^a	52	<0.01	370	370	42	1.00	0.91

[1] Where 35WDGS = 35% wet distillers grains plus solubles, 35SB = 35% Sweet Bran, 88SB = 88% Sweet Bran (DM basis). Inclusion of byproducts replaced equal DM portions of dry-rolled corn.

[2] Where No DFM = diets were not top dressed with a direct-fed microbial, W/ DFM = diets were top dressed daily with 1 x 10^9 CFU's of *Lactobacillus buchneri* strain 40788 (Lallemand Animal Nutrition North American, Milwaukee, WI).

[3] Each treatment mean represents 126 analyses (n).

[4] Each treatment mean represents 189 analyses (n).

[5] Inter = *P*-value for F-test of interaction between diet and DFM.

[abc] Means in the same row within the same main effect without a common superscript differ (*P* < 0.05).

Table 5. Rumen VFA concentrations for feeding steers wet distillers grains plus solubles or Sweet Bran in finishing diets.

	Diet[1]					DFM[2]				Inter[5]
	35WDGS	35SB	88SB	SEM[3]	*P*-value	No DFM	W/ DFM	SEM[4]	*P*-value	
Acetate, mol/100mol	51.8	49.9	51.3	1.5	0.41	51.3	50.6	1.3	0.58	0.25
Propionate, mol/100mol	30.9[b]	32.6[b]	26.4[a]	2.4	0.08	29.1	30.9	2.1	0.42	0.45
Acetate:propionate	2.06	1.68	1.98	0.22	0.34	1.96	1.85	0.19	0.62	0.35

[1] Where 35WDGS = 35% wet distillers grains plus solubles, 35SB = 35% Sweet Bran, 88SB = 88% Sweet Bran (DM basis).

[2] Where No DFM = diets were not top dressed with a direct-fed microbial, W/ DFM = diets were top dressed daily with 1×10^9 CFU's of *Lactobacillus buchneri* strain 40788 (Lallemand Animal Nutrition North American, Milwaukee, WI).

[3] Each treatment mean represents 42 analyses (n).

[4] Each treatment mean represents 63 analyses (n).

[5] Inter = *P*-value for F-test of interaction between diet and DFM.

[abc] Means in the same row within the same main effect without a common superscript differ ($P < 0.05$).

Table 6. Disappearance % of NDF for corn bran, solvent extracted germ meal, Sweet Bran (SB), and wet distillers grains plus

solubles (WDGS) when incubated in the rumen of steers fed finishing diets containing 35% WDGS or SB or 88% SB.

	Incubation Hour						SEM[1]	*P*-value
	0	8	16	24	48	72		
Corn Bran	1.7	19.9	22.5	25.6	30.4	33.5	1.0	<0.01
Solvent extracted germ meal	8.3	55.4	62.0	67.6	74.7	78.2	1.7	<0.01
Sweet Bran	0.1	28.3	33.0	34.1	39.9	44.4	1.8	<0.01
Wet distillers grains plus solubles	15.9	35.7	39.7	43.8	49.3	52.7	1.3	<0.01

[1] Each mean represents 63 replications (n).

Table 7. Disappearance % of NDF for corn bran, solvent extracted germ meal, and Sweet Bran when incubated in the rumen of steers fed a mixed diet containing 70% brome grass hay, 20% dry-rolled corn, and 10% protein supplement.

	Incubation Hour						NDFd[1]	SEM[2]	*P*-value
	0	8	16	24	48	72			
Corn Bran	12.2	28.3	46.7	55.0	75.9	88.1	2.36	1.1	<0.01
Solvent extracted germ meal	17.1	53.3	74.2	81.2	87.2	93.7	5.27	1.5	<0.01
Sweet Bran	16.0	38.1	54.8	70.8	78.4	85.9	2.93	1.6	<0.01

[1] NDFd = NDF disappearance rate calculated as %/h.

[2] Each mean represents 6 replications (n).