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COMPARISON OF WET OR DRY DISTILLERS GRAINS PLUS SOLUBLES TO CORN AS AN ENERGY SOURCE IN FORAGE BASED DIETS AND *IN VITRO* FORAGE STANDARD DEVELOPMENT BASED ON *IN VIVO* DIGESTIBILITIES UTILIZING BROME HAY, PRAIRIE HAY AND MEADOW HAY.

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By

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Advisors: Terry Klopfenstein and Galen Erickson

Four growing experiments were conducted to evaluate the use of dry rolled corn (DRC) and either dry (DDGS) or wet distillers grains plus solubles (WDGS) as energy sources in high forage diets. In Exp. 1, steers were fed a blend of sorghum silage and alfalfa hay and supplemented 1 of 4 inclusions of WDGS, DDGS or a MIX (67% WDGS: 33% Straw). In Exp. 2, Exp. 3 and Exp. 4, steers were fed diets including WDGS, DDGS or DRC at different inclusions, replacing sorghum silage and grass hay. In Exp. 1, WDGS, DDGS and MIX produced greater ending BW, ADG and G:F compared to the basal diet. Increasing distillers grains improved ADG and G:F in forage based diets. Data from Exp. 2 - 4 were pooled and ADG was regressed against inclusion thereby allowing the energy value of WDGS to be calculated relative to that of DRC. The energy value of WDGS was 137% and 136% of DRC when fed at 15 and 30% of the diet DM, respectively.

In vitro digestibility and *in vivo* digestibility estimates are highly correlated but absolute values differ. Therefore, our objective was to determine *in vivo* digestibilities of 5 forages and use these forages as standards for *in vitro* digestibility procedures when *in vivo* estimates are needed for unknown forage samples. Eight *in vitro* runs were

conducted using 5 hay standards and 6 forage samples with unknown *in vivo* digestibilities in order to predict actual *in vivo* values from *in vitro* estimates. Runs were evaluated using either regression equations or standard mean adjustment. Using regression equations increase forage*run variation. Forage*run variation decreased using the standard mean adjustment. Standard mean adjustment appears to be a valid method to adjust IVOMD values and estimate *in vivo* digestibility.

DEDICATION

I would like to dedicate this part of my life to my husband Jeff Ahern and my mother Meg Harry. Words cannot describe how much the two of you mean to me. First, to my husband, thank you for being there for all of my schooling including the beginning way back in 2004 before graduate school. I know there have been many long nights and many times you have helped me with my research when I needed someone to keep me company. I could not have gotten through the tough times without you and loved sharing all my triumphs with you. Thank you for being my rock through these years. You have no idea how much it has meant to me.

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INTRODUCTION

World population has reached approximately seven billion. The challenge for the foreseeable future will be to find a way to feed the world's population. Grain production contributes a large portion of food to the people around the world directly and indirectly. Rice, wheat, oats, barley, flour, and cereal are just a few examples of direct grain usage. Further processing of grain has yielded many different consumer products, such as starch products, sweeteners, syrups, ethanol and proteins. Ethanol production dates back to the early 1800s when it was used for fuel and direct consumption. Today, the majority of ethanol is used as fuel. Cereal grains high in starch are typically used in ethanol production. While wheat and rice also have a high starch content, corn prevails in usage due to its availability (Kalscheur et al., 2012). The starch content of corn is approximately 66%, 4.0% oil, 8% protein and 11.2 percent fiber (DM basis) (NRC, 1996). Steady growth in the ethanol industry began in the early 1900s, with a significant boom in the last 10 years. In 2011, the ethanol industry produced 13.9 billion gallons of ethanol, using 5 billion bushels of corn (RFA, 2014).

Fermentation of the sugar derived from the starch produces ethanol. The products remaining, often referred to as byproducts, are used for livestock feeds (e.g. distillers grains plus solubles, corn gluten feed and corn gluten meal). With 39 million metric tons of livestock feed and 13.9 billion tons of ethanol produced in 2012 (RFA, 2014). There are two primary processes used to produce ethanol: wet milling and dry milling. According to Rendleman et al. (2007) 25% of ethanol produced comes from the wet

milling process, which also produces corn bran, corn germ meal, corn gluten meal, corn oil and steep liquor (Stock et al., 2000). Wet corn gluten feed (WCGF) is comprised of corn bran and steep liquor combined at varying ratios. Wet corn gluten feed can be dried, pelleted, shipped overseas or shipped wet or dry directly to feedlots (Stock et al, 2000).

The other 75 % of ethanol production is from dry milling plants, which yield slightly more ethanol at 2.8 gallons per bushel compared to 2.7 gallons from wet milling (Rendleman et al., 2007). One bushel of corn produces approximately 2.8 gallons of ethanol, 18 pounds of dried distillers grains plus solubles (DDGS) and 18 pounds of carbon dioxide (RFA, 2014). Distillers grains (DG) have become a commonly used commodity in the cattle industry. Distillers can be used in the feedlot or in cow-calf and stocker cattle diets (Erickson et al., 2007).

Due to the fact that corn is roughly two-thirds starch, the nutrient content of DG is 3 times that of corn. Distillers grains can be used as either a protein or energy source in cattle diets. Use of DG is especially pertinent in cow-calf operations, however the energy value of the byproducts used to supplement has yet to be well defined in high forage diets. Supplementing these byproducts allows producers to increase stocking levels of pastures without purchasing additional land. Protein content of distillers grains plus solubles (DGS) provides enough metabolizable protein (MP) to allow grazing of low quality forage. Rapid fermentation of starch from corn causes a decrease in pH and challenges the rumen microbial balance (Vander Pol et al., 2008). The change in the rumen environment when corn is included in the diet inhibits forage digestion. The starch content in DGS is low, however the fat content (~11%) fed at high levels may inhibit forage digestion (Lodge et al., 1997). Energy value of DGS has been well researched in

concentrate diets and shows a large amount of variation between wet and dry. Studies conducted utilizing DGS determined that wet DGS (WDGS) is about 135% and DDGS is about 112% the energy value relative to corn (Bremer et al., 2011). Loy et al. (2008) conducted a study comparing dry-rolled corn (DRC) and DDGS at 2 supplementation levels and determined the energy value of DDGS to be between 118-130% that of corn, in a forage based diet. The purpose of this literature review is to discuss the production, feeding value, use and relative value of byproducts used in forage-based diets.

GRAIN MILLING

The primary cereal grain used in the Midwest for fermentation is corn, due to its availability. There are two types of grain milling production systems in the United States, each has a different process resulting in production of ethanol, the primary product of dry milling, and secondary byproducts. Approximately 25% of ethanol is produced by wet mills and the remainder of ethanol produced is through dry-mill ethanol plants (Rendleman et al., 2007).

Wet Milling. The wet milling process is more involved and produces more products. Wet mill plants utilize No. 2 corn or better due to the fact that products for human consumption are produced along with ethanol (Stock et al., 2000). Wet milling is capable of producing high fructose corn sweetener, corn syrups used as a sugar substitute (Stock et al., 2000).

Briefly, corn utilized during wet milling is screened, cleaned (Figure 1) and steeped in a dilute sulfurous dioxide: water mixture for approximately 48 hours. Heavy steep water is removed and corn is coarsely ground. Germ floats toward the top of the slurry. Germ is separated, dried and the oil removed via solvent extraction. After germ

removal the remaining slurry is ground more thoroughly (Stock et al., 2000). At this point the starch and corn gluten meal (CGM) are removed. The starch is centrifuged and can be made into high fructose corn syrup, ethanol, sold as-is or be used for other fermentation processes. Wet bran remains after starch and corn gluten meal are eliminated. Bran is combined with steep water and, depending on the company, solvent extracted meal to produce wet corn gluten feed (Stock et al., 2000).

Dry Milling. Dry milling contrasts that of wet milling due to the fact that the primary purpose is production of fuel grade ethanol. In the U. S., the primary cereal grains utilized during dry milling are corn and sorghum, however wheat, barley, beets, sugar cane or a combination of grains may be used (Stock et al., 2000). The by-products resulting from the dry milling process are condensed distillers solubles, DDGS, modified DGS, WDGS and carbon dioxide.

Prior to the initial step of dry milling, grain is cleaned to remove any field waste (ICM, 2012). Grain is then sent through a hammer mill and ground into a coarse meal (Figure 2). Meal is then cooked and stored in slurry tanks. After water is added to milled grain, pH is adjusted to approximately 5.8. Alpha-amylase is added to convert the starch into sugar for fermentation. The slurry is heated for 30 to 45 min at 82 to 88^{o}C, which aids in reduction of viscosity (ICM, 2012). Primary liquefaction involves the slurry being pumped through a pressurized jet cooker at 105^{o}C and being held for 5 min. Slurry is managed at high temperatures to control growth of bacteria. The slurry mixture is then sent through a vacuum or atmospheric flash condenser allowing cooling. Secondary liquefaction follows flash cooling of the slurry, where it is held at 82 to 87^{o}C for 1 to 2 h. This step allows the alpha-amylase enzyme to break down starch into short chain

dextrins. In the secondary stage a second enzyme, called gluco-amylase, is added to the slurry mixture while being pumped into fermentation tanks (ICM, 2012).

Following addition of gluco-amylase and transfer to fermentation tanks, the slurry mixture is referred to as mash. Gluco-amylase is an enzyme that breaks down short chain dextrins into simple sugars (ICM, 2012). At this point yeast is added to the mash. The sugars are utilized by the yeast and the byproducts remaining after saccharification fermentation are ethanol and carbon dioxide. The carbon dioxide may be used in many products after it has been captured. In order for carbon dioxide to be utilized it must be purified and then compressed. Carbon dioxide can be sold as-is, processed in carbonated beverages or used in the meat industry to flash freeze (ICM, 2012).

Fermentation of the mash continues for 50 to 60 h which yields a mixture of solids, yeast and approximately 15% ethanol. In order to obtain the ethanol, the fermented mash must go through a distillation process (ICM, 2012). The mash is pumped into a heated multi-column distillation system. These columns utilize the difference in boiling points of ethanol and water (78.37 and 100^{o}C, respectively) to boil and fraction off the ethanol. The resulting product is approximately 95% ethanol by volume or 190-proof after leaving the distillation columns. Residual residue from distillation is called stillage and retains the non-fermentable solids and water that is pumped from the bottom of the distillation columns and moved to the centrifuges for further processing (ICM, 2012).

The 190-proof ethanol is pumped into molecular sieves (ICM, 2012). Molecular sieves are specialized tanks which contain molecular beads that adsorb water molecules remaining in the ethanol stream, allowing the ethanol to pass through unaffected. Post

molecular sieving product is now 99% ethanol or 198-proof. Ethanol is then pumped into an on-site storage tank where the ethanol is denatured and stored until it can be shipped by rail or tanker truck. Denaturing of the final product is federally mandated to leave ethanol unsuitable for human consumption (ICM, 2012).

Stillage solids removed from the distillation process, containing grain, yeast and water, are sent through a centrifuge for grain recovery. The process separates the stillage into thin stillage which is approximately 10% DM and wet cake also called wet distillers grains (WDG) (Stock et al., 2000). Thin stillage is sent through a series of evaporators where water is removed. The remaining product is typically called syrup or corn distillers solubles (CDS). The corn distillers solubles can be sold alone or added back to the WDG before being sent through a dryer system. The CDS added back to WDG produces a product called wet distillers grains plus solubles (30 to 40% DM). After the drying process, the remaining product is either modified distillers grains plus solubles (MDGS) at 40 to 45% DM or DDGS at 88 to 90% DM. All combinations or individual feed parts can be sold to local animal producers (e.g. feedlots and dairies) (ICM, 2012).

Biorefineries. Locations of biorefineries are spread across the United States. The greatest concentration of ethanol plants reside in the Midwestern and Central Plains of the United States, coinciding with the Corn Belt. Approximately 200 plants are operational as of November 6, 2012 (RFA, 2012). The number of existing ethanol plants has more than doubled since 2005, the top 5 states being Iowa, 41; Nebraska, 27; Minnesota, 22; South Dakota and Illinois, 15; and Kansas and Indiana, 14 (RFA, 2012). Livestock producers in these states have are able to utilize byproducts. The level of

byproduct inclusion into feedstuffs, by producers in the Central Plains, is largely based on research conducted using DGS and WCGF.

Wide spread drought in 2011 and 2012 has impacted corn crop production causing corn price to rise (USDA, 2012). Increased demand for corn, due to increased production of biorefineries over the years, also drove corn prices up. Increased corn price and decreased corn production caused several ethanol plants to temporarily shut down in 2012, leaving room to speculate on the sustainability of byproduct production in the future. However, current corn price has declined and essentially all ethanol plants are operational.

NUTRIENT VARIATION AND COMPOSITION

The leading grain utilized in the dry milling process is corn. The corn kernel consists of the outer pericarp layer, secondary endosperm layer, internal germ and the tip cap. During the primary liquefaction phase of dry milling, the pericarp of the corn is broken down when soaked in hot water at a pH of 5.8, exposing the endosperm of corn and allowing further processing to occur. The endosperm makes up about two thirds of the corn kernel and contains the starch used during ethanol production. After endosperm extraction, the remaining one third of the kernel consists of pericarp, germ and tip cap, making up a large portion of WDG. Removal of the starch from the kernel increases the nutrients remaining 3-fold. Residual nutrients are comprised of fat, fiber (NDF), phosphorus (P) and protein (CP) (Stock et al., 2000). The original corn kernel contains 4% fat, 12% NDF, 0.3% P and 8% CP (NRC, 1996). Nutrients remaining in stillage contain approximately 12% fat, 36% NDF, 0.9% P and 30% CP (Buckner et al., 2011).

Due to slight manufacturing differences between biorefineries, there is variation between WDG produced from these biorefineries. However, this variation is not only between plants, but loads of WDG within the same ethanol plant. There are many possible explanations for variability in WDG, whether it occurs with a change in acidity during distillation, the amount of CDS added back to WDG or the length of time or temperature used for drying (Spiehs et al., 2002; Buckner et al., 2011). Variation in the nutrient composition poses a challenge when attempting to summarize the nutritional value of DGS. However, according to Beylea et al. (2004), variation in nutrient composition is not due to the initial corn brought into the plant. United States Grains Council guide to DDGS (3rd Edition, 2012) suggests processing of grain plays a significant role in the variation of nutrient composition.

Variation. According to U.S. Grains Council guide to DDGS (3rd Edition, 2012) variation among 32 U.S. corn DDGS sources were 28.7-32.9% CP, 8.8-12.4% fat and 3.0-9.8% ash, a few of the most variable nutrients in DDGS. Dry matter variation occurring in DG and DGS is expected due to the differing products available (e.g. DDGS, MDGS, WDGS and CDS). Spiehs et al. (2002) conducted a study analyzing 118 DDGS samples collected from 10 ethanol plants. Between 1997 and 1999, 2 plants utilized in this study were located in South Dakota and 8 others were located in Minnesota. Nutrient content of DDGS ranged from 87.2-90.2% DM, 28.1-31.6% CP, 35.4-49.1% NDF, 8.2-11.7% fat and 5.2-6.7% ash. An overall average of 88.3% DM, 28.2% CP and 42.1% NDF was determined from this study.

Holt et al. conducted a study in 2004 evaluating 4 regional dry milling ethanol plants. Sampling was completed over 3 mo in 2002, consisting of a 4 d collection period

each month. Findings concluded that DDGS fluctuated from 89.4-90.9% DM, 30.7-36.7% CP, 37.3-48.9% NDF, 10.4-14.2% fat, 0.35-0.69% sulfur (S) and 3.9-4.2% ash. Wet distillers grains plus solubles ranged from 29.5-36.5% DM, 34.4-36.6% CP, 36.1-48.2% NDF, 11.0-13.1% fat, 0.36-0.40% S and 2.8-4.2% ash. One explanation for the variation in DGS is due to byproduct production differences between biorefineries. A similar study conducted by Buckner et al. (2011) determined nutrient composition of WDGS and MDGS. Six dry milling ethanol plants were utilized. Sampling, at these mills, occurred every 4 mo over a 5 d collection period where 10 samples were collected per d. Samples were analyzed for DM, CP, P and S. Buckner et al. 2011, determined an average DM of 32.5% for WDGS and 45.2% for MDGS. Nutrient composition ranged from 29.6-34.0% CP, 10.2-13.3% fat, 0.74-0.93% P and 0.67-1.06% S. Crude protein and fat from this experiment suggest greatest variation between plants, which is to be expected due to different processing methods. The fat variation may be due to the amount of distillers solubles added to the distillers grains. Sulfur variation between plants was also apparent. However, minimal variation existed within plant and day of sampling. Evidence suggests that should be closely monitored due to potential feeding hazards for cattle (Suttle, 2010).

DISTILLERS GRAINS AS A PROTEIN AND ENERGY SOURCE

Traditional use of distillers grains is based on the nutrient composition of the byproduct. Due to the CP content of distillers, the byproduct was initially utilized as a protein source. The protein from DGS is approximately 65% undegradable intake protein (UIP) as a percent of CP (National Research Council, 2000), so DGS are a great source of MP. When overfed UIP, cattle recycle and use the excess MP to produce urea. In the past,

ammonia was used commercially to produce urea. Urea is a non-protein nitrogen source that contains 46.7% nitrogen. One pound of feed grade urea provides as much nitrogen as approximately 1.28 kg of protein (Stanton and Whittier, 1998). When comparing the price of urea to other protein sources such as soybean meal, combining 6.14 kg of urea and 39.3 kg of corn produce similar energy values and equal protein content to 45.5 kg of soybean mean (44% CP; Stanton and Whittier, 1998). The cost of the urea-corn mixture is typically less expensive than other protein sources. However, this process is expensive and costs producers 2 to 2.5 times the price of corn (Babcock et al., 2008). Distillers grains plus solubles provided a less expensive protein source than other options at the time, and could replace corn in the diet. In order to supply sufficient protein, 15 to 20% of the diet DM must be distillers grains (Erickson et al., 2007).

Extensive research has been conducted on the efficacy of distillers grains as a protein source. While research has shown that feeding 15 to 20% of the diet DM distillers grains offers adequate protein, studies have shown that feeding above those levels (i.e. 20 to 40% of the diet DM) begins to provide energy to the diet. Several research studies have been conducted comparing the energy value of WDGS and DDGS to corn in finishing diets. These studies indicate that both WDGS and DDGS have a greater energy value relative to that of corn (Bremer et al., 2011). When comparing WDGS to DDGS studies have indicated that WDGS have a greater (131-143%) energy value relative to corn (Bremer et al., 2011).

Energy Source (Concentrate Diets). Early in the 1980s researchers began investigating the feeding value of distillers byproducts in finishing diets. Farlin (1981) found that replacing corn with WDG at 25, 50 and 75% in a finishing diet resulted in

more energy per kilogram of DM than the corn the WDG replaced. This increased feeding value was despite the fact that the starch, which at the time was perceived to be the main energy source, had been removed from the product. Firkins et al. (1985) conducted 8 trials utilizing WDG and DDG and found that when feeding at 50% of diet DM, ADG and G:F met or exceeded that of a corn based finishing diet.

Logically, based on nutrient content, the energy value of distillers grains would be approximately 118% the energy value relative to corn. However, Larson et al. (1993) conducted a study utilizing yearling and calves on a finishing trial, over a 2 year period, to determine the feeding value of WDGS. Animals were fed 5.2, 12.6 and 40% (DM basis) WDGS or 79% DRC. Cattle fed 5.2% WDG, 12.6% WDGS and 79% DRC received similar levels of protein, and diets containing 40% WDGS exceeded metabolizable protein requirements. Wet distillers grains plus solubles averaged 135% the energy value of corn (Larson et al., 1993).

Ham et al. (1994) conducted 5 studies using DDGS and WDGS to compare the feeding value in feedlot diets. Distillers grains plus solubles were included in the diet at 40% (DM basis), replacing corn. Cattle fed WDGS and DDGS were more efficient than cattle on the corn control diet. However, when comparing WDGS and DDGS, cattle fed WDGS were more efficient than cattle fed DDGS. Relative to corn, WDGS were 147% the feeding value and DDGS were 124%. While it has yet to be determined why there is a difference between wet and dry DGS, subsequent research studies follow this trend.

Numerous studies have been conducted to determine the most efficient level at which DGS can be fed in feedlot diets. Bremer et al. (2011) combined 14 experiments in a meta-analysis, in which varying levels of WDGS were fed in finishing diets. Results

showed that feeding WDGS resulted in increased ADG and G:F compared to that of cattle on a traditional corn-based finishing diet. The meta-analysis included WDGS and DDGS at 10, 20, 30, 40 and 50% (DM basis) and a quadratic response was observed for feed efficiency (Bremer et al., 2011). Optimum feeding level for WDGS was between 30 to 40% (DM basis) and 10 to 20% for DDGS on a DM basis (Bremer et al., 2010). While calculated energy values for WDGS and DDGS from these studies were above 100% of corn, the average for DDGS was 125% the value of corn. Results from the meta-analysis, diets containing DRC, high moisture corn (HMC) or DRC:HMC combination being replaced with 15 to 40% WDGS indicated the feeding value relative to corn was approximately 131-143% (Bremer et al., 2011).

Energy Source (Forage Diets). Extensive research has been done evaluating the energy value of WDGS relative to corn in feedlot diets. Distillers grains plus solubles also provide energy in forage based diets. Dry DGS is commonly used as a supplement in grazing and confinement situations (Griffin et al., 2012). Studies conducted using WDGS mixed with low quality forage show similar results. Experiments conducted utilizing ensiled or fresh mixes of WDGS:straw resulted in improved final BW, DMI, ADG and G:F. Peterson et al. (2009) fed steers either 35:65 or 45:55 mix of ensiled or fresh WDGS:straw. Steers were more efficient as the inclusion of WDGS increased in the diet: G:F was 0.092 with 35% WDGS and 0.120 with 45% WDGS ($P = 0.03$), DMI 4.49 kg and 4.10 kg ($P = 0.03$), respectively. There was no difference in final BW ($P = 0.19$) or DMI ($P = 0.74$) between ensiled or fresh WDGS:straw mixes. However, ADG ($P = 0.08$) and G:F ($P = 0.09$) had a tendency to be different. Wilken et al. (2009) conducted an experiment comparing CDS and WDGS ensiled with cornstalks fed to growing calves at

differing levels. Cattle consuming the ensiled WDGS:straw mix increased in final BW ($P < 0.01$), ADG ($P < 0.01$), and G:F ($P < 0.01$) relative to the WDGS:stalks mixed fresh daily. Cattle fed WDGS were more efficient ($P < 0.01$) and had an increase in ADG ($P < 0.01$) compared to those fed CDS. Buckner et al. (2010) conducted an experiment ensiling WDGS and straw at ratios of 30:70 and 45:55. Increasing concentration of WDGS ensiled with straw increased final BW ($P <0.01$), ADG ($P <0.01$), and G:F ($P <0.01$) (Buckner et al., 2010). Data from these experiments suggest that mixes of a low quality forage with WDGS increase palatability of the mixtures and that digestion increases due to the increase in ADG and G:F.

Griffin et al. (2012) analyzed data from 20 forage-based growing studies conducted using pasture grazing of confinement systems. Supplementation in pasture studies linearly increased ADG and ending BW ($L^2 < 0.01$). Average daily gain increased and total intake responded quadratically to increasing levels of DDGS supplementation ($Q^2 <0.01$) in confinement situations (Griffin et al., 2012). Confinement studies had a greater response to DDGS supplementation than pasture studies. Data suggest large differences in the response to DDGS supplementation potentially due to DDGS nutrient composition. The UIP content of DDGS is an excellent source of protein for growing calves. Differentiating the difference in response between protein or energy is a challenge due to the potential to increase microbial production with energy supplementation (Griffin et al., 2012).

The energy value of WDGS relative to corn on average is between 130 and 143% (Bremer et al., 2011), however this research has been conducted with finishing diets. Loy et al. (2008) conducted a growing study comparing DRC, DDGS and DRC with corn

gluten meal (DRC + CGM) fed at 0.21% (LOW) or 0.81% (HIGH) of BW daily of 3 times weekly. There was a supplement x concentration interaction for ADG and G:F ($P < 0.01$). Data demonstrated improved ADG and G:F with DDGS or DRC + CGM supplementation ($P < 0.01$) compared to feeding DRC alone (Loy et al., 2008). Heifers supplemented at the LOW level with DDGS had greater ADG and G:F ($P < 0.01$) than either DRC or DRC + CGM. This is likely due to the low level of starch and energy density of fat, undegraded protein and corn fiber.

An experiment (MacDonald et al., 2007) conducted utilized grazing heifers supplemented with dry distillers grains (DDG), CGM or corn oil (OIL). Corn gluten meal and OIL were fed to the same UIP and fat equivalent to that of DDG. Cattle supplemented DDG showed a linear increase in ADG ($P < 0.01$) and CGM tended to increase ADG ($P = 0.14$) at a slower rate than DDGS. Supplementation of OIL did not affect ADG ($P = 0.25$) and ADG tended to be than that of DDG ($P = 0.09$). MacDonald et al. (2007) stated that an associative effect relative to protein and fat available from DDG may cause the additional gain seen in cattle supplemented with DDG. Several experiments conducted by Corrigan et al. (2009) examined the effects of feeding different levels of DDG and differing proportions of CDS added back to DDG. As expected, as inclusion of DDG included increased ADG increased, however a DDG level x CDS level interaction occurred ($P < 0.01$). Fat digestibility was greater ($P = 0.02$) in steers fed DDG containing 22.1% CDS versus 0% CDS.

Bremer et al. (2011) determined an energy value of 112% for DDGS and 130-143% WDGS in concentrate diets. Few experiments comparing DDGS and WDGS to determine the energy value relative to corn in forage based diets have been conducted.

Wilken et al. (2009) conducted an experiment comparing DDGS and MDGS in wet or dry forage based diets. There was no significant difference between type of byproduct (dry or modified DGS) for ending BW ($P = 0.94$), DMI ($P = 0.69$), ADG ($P = 0.94$) or G:F ($P = 0.83$). Forage type (wet or dry forage) was significant for ending BW, DMI, ADG or G:F ($P < 0.01$). Results from this experiment determine that type of distillers grains (modified or dry) does not have a significant impact on performance of growing calves (Wilken et al., 2009).

Based on the study conducted by Loy et al. (2008), the feeding value of DDGS appears to be greater in relation to DRC in forage based diets. The study compared DRC and DDGS at 2supplementation levels and determined the energy value relative to DRC to be between 18 to 30% greater than corn (Loy et al., 2008). There have been many experiments conducted evaluating the efficacy of supplementing DGS in forage based diet, though the energy value or type of DGS being used in high forage diets has not been as widely researched as feedlot situations.

OBJECTIVES

Distillers grains plus solubles are an excellent feed source that provide protein and energy when fed to cattle. The energy value of DGS has been well defined in concentrate diets with WDGS having a greater energy value than DDGS. However, the energy value of any type of DGS is greater than corn. Few comparisons, for energy value, between wet and dry DGS in forage diets have been made. The initial objective of the following experiments was to compare DRC, DDGS and WDGS as energy sources in forage based diets and determine the energy value of DGS relative to DRC. The second objective was to determine the differences in growth performance between WDGS and DDGS, and to

determine if forage DMI can be reduced by feeding WDGS mixed with low quality forage.

Review of Literature-Part II

In Vivo and In Vitro Use of Forage

INTRODUCTION

Forages are defined by several factors. Due to the wide range of species, one major factor used in forage definition is nutrient content. Whether legume or grass, forages play a major role in growing and finishing cattle diets. Forage consumption by cattle in Nebraska occurs, primarily, in the western part of the state. The Nebraska Sandhills consist mainly of native grass species of the warm and cool season varieties. These native grasses aid in feeding Nebraska cattle whether the situation involves a grazing cow/calf herd, backgrounding calves before entering the feedlot or feeding harvested forage in confinement. Understanding digestibility of forages helps predict cattle performance. However, the vast range of forage species found in Nebraska makes it hard to describe specific nutritive values for each type of grass.

Prediction of forage digestion is one method to estimate energy content, which then is utilized to formulate diets fed to grazing animals. Change in forage throughout the growing season makes determining the nutrient profile of a specific forage important. Forages are affected not only by year-to-year variation, but also early season to late season variation. Variation causes problems with accurate ration calculations for grazing and growing animals. Properly formulating a diet is aided by the use of NRC (2000) models. However, CP, degradability and TDN must be known. *In vivo* digestibility of forages can help account for these variations in forages. Nonetheless studies can be costly and time consuming. *In vitro* evaluation of forages provides a prompt and less expensive approach to evaluating nutrient digestibility, allowing more data to be evaluated in a timely manner. Estimation of *in vivo* digestibility of specific forages using different

calibration data sets has been ongoing since the early 1960s (Weiss, 1994). The use of regression equations aids in reducing the prediction error associated with *in vivo* digestibility estimation. According to Weiss (1994), many studies have shown strong statistical correlations ($R > 0.9$) between *in vivo* and *in vitro* digestibility data (e.g., Tilley and Terry, 1963; Troelsen, 1970; Valdes and Jones, 1987; Aufrère et al., 1992). While a strong correlation is important in order to compare *in vivo* to *in vitro* digestibilities, correlation does not mean that *in vivo* digestibility is equal to IVDMD. The purpose of this literature review is to discuss forage quality, forage digestion and the comparison between *in vivo* and *in vitro* digestibilities.

FORAGE QUALITY

There are 2 main classifications of forages: legumes and grasses. For the purposes of this review, the focus will be on grasses. Grass is considered a herbaceous plant that can be defined by long linear leaves that serve as the structure and for metabolic usage. These grasses are jointed fibrous stems that tend to develop toward the mid to late growing part of the season. Forage degradability is dependent largely on quality; this can be affected by several factors ranging from plant maturity to processing method. Forage quality can be defined as the extent to which a feed elicits a productive response, for instance daily gain. Factors that can influence forage quality range from the plant form (grass or legume), maturity of the plant, environmental factors such as temperature and water, harvest and storage effects and a few others that will not be discussed. Attributes of high quality forage relate to nutrient recovery and high nutrient concentration. Forage quality is also dependent upon animal intake, digestibility and animal efficiency (Paterson et al., 1994).

Composition. Understanding the general cell structure of grasses aids in determination of forage digestion. Grasses consist mainly of cell solubles, which are readily digestible, hemicellulose, cellulose, pectin and lignin. Neutral detergent fiber can be defined as the hemicellulose, cellulose and lignin portion of forage and ADF is the cellulose and lignin. Acid detergent fiber content directly affects the digestibility of forage. Typically, as ADF content increases, cell solubles decrease, and the extent of and the rate at which the forage can be digested decreases (Nelson, 1994). Analysis of forage samples taken in the Sandhills of Nebraska in the mid-late season of pasture growth would show the increase in ADF. As forages mature throughout the growing season, the plant structure alters slightly, plant structure strengthens as the plant grows and uses the cell solubles to aid in building cell walls in turn increasing the NDF and ADF content of the forage (Nelson, 1994). Gustad et al. (2006) conducted a study utilizing Sandhills native range showing NDF content increasing from 61.5% in mid-June, to 76.0% NDF in mid-August. Geisert et al. (2007) conducted an *in vivo* study on 5 forages ranging in maturity and found a similar increase in NDF content of brome hay from 69.3% NDF for immature brome hay to 78.3% NDF mature brome hay.

The plant leaf:stem ratio is directly responsible for changing NDF, ADF and lignin content. Digestibility and CP decrease as the plant ages and NDF, ADF and lignin increase. Depending on the grass type, either C_3 or C_4 plants, the highest nutritive quality falls in late spring or early summer for cool season grasses and late spring to early summer for warm season grasses. Pastures with mixes of grass types will have 2 seasonal peaks (Cogswell and Kamstra, 1976). Cogswell and Kamstra (1976) compared 2 warm season and 2 cool season forages from June through September and showed an overall

decrease in CP and digestibility and increased ADF content. Johnson et al. (1998) evaluated the seasonal changes on dietary composition and digestion on cattle grazing mixed grasses in the Northern Plains. Samples linearly decreased in CP and *in vitro* organic matter disappearance (IVOMD), while NDF, ADF and RUP increased linearly throughout the growing season.

Gunter et al. (1995) conducted a study evaluating diet quality of midgrass prairie rangeland or plains bluestem pastures from mid-May through mid-October. Diet samples were obtained through the use of either esophageally fistulated steers or ruminally and duodenally fistulated calves. Diet samples obtained compared midgrass and bluestem on an OM-basis. Diet samples collected in mid-May were between 57.4 and 65.5% NDF, increasing to 80.8 to 77.9% NDF in mid-August. A decrease in % NDF was observed in mid-October, however this decrease was most likely due to late season regrowth. As expected, the same was observed for % ADF, with midgrass being at 33.8 and bluestem 33.9 mid-May and rising to 43.4 and 41.8 in mid-August.

Maturity of grasses directly affects forage quality by decreasing quality as the growing season prolongs. Environmental factors can cause deviations from expected forage quality. For example, forage quality improves due to fall regrowth of the plant. Temperature and precipitation tend to be the leading factors that influence forage quality over any other environmental or plant factors. Typically, as the temperature increases, the plant grows which causes the cell solubles to be utilized making the cell wall structure, thus changing the NDF, ADF and lignin content and decreasing forage quality (Nelson et al., 1994).

Harvest and Storage. According to Rees (1982) and Rotz and Abrams (1988), under optimal drying conditions at harvest, initial forage DM losses can be between 15 to 18%. Rain damage can increase losses up to 30% (Rotz and Abrams, 1988). Several things can be done to impact forage quality following harvest, including grinding and pelleting, and storage of forage to prevent degradation. Grinding and pelleting forages tends to decrease digestibility of the forage due to quicker passage rates through the digestive system (Berger et al., 1994). However, there is a tendency to increase animal performance due to greater forage intake (Berger et al., 1994).

Storing forages as silage affects forage quality. Good silage should preserve nutrients in forage and reduce the variation in nutrient supply and quality. In general, fermentation of forage is due to the conversion sugars from the forage plant wall into lactic acid. Lactic acid bacteria utilize the highly available non-structural carbohydrates (sugars), mainly glucose and sucrose. The production of lactic acid decreases the pH of the forage and stops bacterial activity on the forage (Rotz and Muck, 1994). Harvesting and storing silage is one way to collect a large amount of high quality forage for an in vivo digestion experiment.

FORAGE DIGESTIBILITY

Determining forage digestibility relies on multiple factors such as voluntary forage intake, appropriate diet sampling and reliable digestibility estimation and determination. Accurate forage intake begins the process and if handled incorrectly, provides misleading information for further forage evaluation. Diet sampling may affect precise management of forages collected as well. If either forage intake or diet sampling is inaccurate, estimating forage digestibility is challenging. However, forage digestibility

is determined using *in vivo* or *in vitro* estimations. Weiss et al. (1994) explained that *in vitro* procedures are highly correlated with *in vivo* digestibilities. Even though *in vitro* and *in vivo* digestibilities are highly correlated, values are not necessarily equal.

Intake. Measurement of forage intake can be conducted through several venues, either using grazing animals or confined animals. One precise way of measuring forage intake is through confinement. Selected forage, harvested previously, is offered to the animals and orts are collected. Forage, orts and feces remaining can be analyzed for nutrients such as NDF, CP and OM and differences calculated between the forage offered and forage refused (orts). Typically, in digestion studies, animals are fed individually and multiple animals are included to account for animal variation. In order to prevent limitations, cattle are fed *ad libitum* (Burns et al., 1994). Cattle fed at a level much greater than *ad libitum* often become selective, thus sort feed.

Burns et al. (1994) explained empirical estimates have been developed through sets of equations employing regression techniques that estimate quantity of forage consumed. In determining regression equations for beef cattle, live weight and daily gain are variables used in the model developing the empirical estimates. Equations for dairy cattle are markedly more complicated due to variables including milk production, time of calving and month of lactation. Empirical evaluation, while useful, does not provide information on animal variation and is affected by environmental factors (Burns et al., 1994). Environmental factors cannot be accounted for in either confinement situations or empirical estimates.

There have been several advances in intake measurement in confinement situations (i.e., electronic gates, computerized chew meters, continuous-reading mangers,

ingestive mastication etc.) (Burns et al., 1994). The development of electronically gated systems allow cattle to feed individually. Electronic gates, such as the Calan gate system (American Calan, Inc., Northwood, NH), allow cattle to eat from an individual feed bunk, but houses cattle together permitting socialization. Animals housed in such systems are exposed to more natural influences (i.e., temperature) than typical confinement situations (Burns et al., 1994). While using electronic gates are advantageous, a few drawbacks are cost, initial animal training, circuit boards functioning properly, etc. (Burns et al., 1994).

Forage intake for grazing animals is difficult to accurately estimate due to many factors, such as grazing selectivity (Burns et al., 1994). Measuring forage intake of confined or grazing animals can be done directly or indirectly. Direct methods include weighing animals prior to and post grazing and weighing forage mass differences. Indirect methods of forage intake estimates include fecal excretion, forage and diet digestibility or empirical estimates. Fecal excretion can be estimated through total fecal collection or through the use of a marker. Dosing animals with a marker requires both daily dosing and fecal collection at certain time points or pulse dosing of inert markers. Either direct or indirect fecal collection of grazing animals increases handling time of animals. Frequent disruption of grazing can alter intake and increases animal stress (Burns et al., 1994).

Diet Sampling. Accurate diet sampling for grazing animals poses a challenge in digestibility studies not only due to animal selectivity, but mixed forages in grazed pastures. Diet sampling can be done in several manners, such as by clipping or hand plucking or use of animals surgically altered with esophageal or rumen fistulas (Burns et al., 1994). Experts debate the accuracy of either sampling method. Hand clipping

techniques eliminate the use of animals reducing sample contamination by animal, animal handling and care and overall cost. However, clipping or plucking does not factor in the animal grazing habits and clipped samples may not be representative. Clipping requires little equipment, but it is important to cover large sampling quadrants. Once a representative sample has been collected, digestibility of diets can be determined through in vitro or in situ techniques or the plant chemical characteristics. Past procedures indicate that lignin is the most common internal marker utilized. Empirical equations use estimates of daily animal requirements developed for grazing animals, which is a back calculation from animal response (Burns et al., 1994).

Use of live fistulated cattle for diet sampling provides a realistic sample of forages that would be selected by grazing cattle. Esophageally fistulated animals have been used since the early 1800s by Bernard and Pavlov (Van Dyne and Torell, 1964). In terms of determining forage digestibility for grazing animals, use of ruminally or esophageally fistulated animals to obtain diet samples has been shown to be the most feasible way to evaluate intake. Van Dyne and Torell (1964) consider diet samples collected from fistulated animals provide the most realistic sample relative to forage intake. However, esophageally fistulated animals pose a restriction on the amount of time for collection, due to constriction of the fistula. An advantage to the use of ruminally cannulated animals is the amount of sample that can be collected at each sampling point (Cochran and Galyean, 1994).

Care must be employed during collections from either type of fistulated animal to prevent sample contamination. Saliva contamination of the diet sample poses issues when determining DM digestibility, however the saliva must not be drained off due to possible

changes to the DM digestibility (Burns et al., 1994; Musgrave et al., 2013). Before diet samples can be collected, careful rumen evacuation must be conducted to ensure all rumen contents have been removed. Following rumen evacuation, fistulated cattle are allowed to graze for a set time prior to diet sampling (Cochran and Galyean, 1994). During an experiment, fistulated cattle graze normally and a collection period should have a minimum of 4 collection d to obtain diet samples (Cochran and Galyean, 1994).

In vivo Digestibility. Cochran and Galyean (1994) define digestibility as the fraction of feedstuff or dietary nutrient lost between ingestion and excretion. *In vivo* digestibility is measured through quantification of initial feedstuff or nutrient consumed by the animal and calculating the amount excreted in the feces. The digested portion is lost and is the difference between initial amount fed and the amount excreted. Intake must be determined by weighing feed (i.e., feed bunks or by hand). When feed bunks are used the bunks may be suspended on load cells. Feed bunks are useful because the load cells measure the total amount of feed consumed, the number of meals throughout the day and the amount of feed consumed at each meal. In order to accurately measure digestibility, measuring feed intake and refusal is imperative. A major component to digestion trials is accurate collection of sample for nutrient analysis at a later time.

Establishing *ad libitum* intake prior to initiation of *in vivo* studies is important (Burns et al., 1994). Determining *ad libitum* intake requires a minimum of 2 days prior to the beginning of the digestion trial (Cochran and Galyean, 1994). Feeding animals at a percentage below that of *ad libitum* reduces the amount of refusals or eliminates them all together. Restricting feed can limit passage rate of feedstuff and can potentially negatively affect digestibility. According to Cochran and Galyean (1994), to ensure that

the level of intake established is actually consumed and is physiologically valid, animals are slightly restricted as a percentage of *ad libitum* intake. Cochran and Galyean (1994) suggest feeding at 90 to 95% of ad libitum intake. Feeding at this rate also prevents the animal from sorting feed. Performing the experiment in this manner reduces the amount of orts to analyze and quantify (Schneider and Flatt, 1975). In the event that refusals remain, orts need to be collected, weighed and retained. A representative sub sample of the feed and refusals need to be analyzed. In grazing situations, feed intake cannot be controlled and must be analyzed using internal markers of feed. Cochran and Galyean (1994) explain that internal markers are inherent dietary constituents (i.e. ADF, ADIN and lignin) that are resistant to digestion.

***Fecal Collection*.** Fecal collection is an important factor in vivo studies. Fecal collections can be handled several ways, including total fecal collection, use of external markers or analysis of internal markers. Total fecal collection determines total tract DM or OM "apparent" digestion through measurement of fecal output. Fecal bags can be used in confinement situations or grazing. In a metabolism study, using fecal bags can be avoided by using stalls without slats. Fecal bags must be emptied frequently to prevent feces loss and to avoid animal soreness at the tail and over the withers. Fecal matter collected must be weighed and subsampled for nutrient analysis. Subsampling can be done in 1 of 2 ways: saving a percentage of daily fecal excrement or a fixed daily weight of feces (Cochran and Galyean, 1994). Schneider and Flatt (1975) suggest keeping complete fecal collection from sheep or 4 to 20% of daily excretion for cattle.

External markers aid in estimation of fecal output. External markers can be dosed continuously, frequently or in single pulse dose methods (Cochran and Galyean, 1994).

Single pulse dosing has been shown to be less accurate and the amount of fecal sampling is more intensive (Galyean et al., 1987). Continuous dosing allows for fecal grab samples to be taken and analyzed for marker concentration, allowing an estimation of fecal matter excreted. Using external markers on grazing animals can disrupt grazing behavior, which may lower intake. Animals must be adapted to handling to avoid a decrease in intake (Cochran and Galyean, 1994). Internal markers can be utilized in a similar manner as external markers if intake is known. When intake is not known, external markers are required. Concentration of the marker is determined and is estimated based on nutrient concentration changes between the original feed sample and fecal output (Cochran and Galyean, 1994).

Digestibility Calculations. Characteristics of the nutrients in the initial diet fed, actual nutrients of diet consumed and feces remaining need to be analyzed to determine forage digestibility. Following digestion trials, calculations must be made to determine relative digestibility. The following are examples of common formulas used either in total fecal collection or marker fecal grab sample to calculate digestion. The following formula is utilized for a specific nutrient when orts do not have to be accounted for (Cochran and Galyean, 1994):

1. $\%\text{Nutrient Digestion} = \frac{\text{Nutrient Consumed (kg)} - \text{Nutrient in feces (kg)}}{\text{Nutrient Consumed (kg)}} \times 100$

Digestibility is determined when significant levels of feed have been refused and that feed differs in nutrient composition from initial feed offered. The formula used to determine the digestion coefficient for a specific nutrients is as follows (Cochran and Galyean, 1994):

2. %Nutrient digestion = $\frac{\text{Nutrient Fed (kg)} - \text{Nutrients Refused (kg)} - \text{Nutrient in feces (kg)}}{\text{Nutrient fed (kg)} - \text{Nutrient Refused (kg)}} \times 100$

When intake is known and an external marker was used either continuously or frequently dosed, the equation for fecal output can be calculated as such (Cochran and Galyean, 1994):

3. Fecal DM Output (g/d) = $\frac{\text{Marker Dose (g/d)}}{\text{Concentration of Marker in Feces (g/g of DM)}}$

Fecal output can be determined using equation 3 when external or internal markers were used. Values determined from equation 3 can then be used in either equation 1 or 2. In order to use equation 3 for an internal marker, the amount of that marker consumed by the animal must be known (concentration of marker in diet x amount of diet consumed). However, when intake is unknown, the following equation must be utilized (Cochran and Galyean, 1994):

4. % Nutrient Digestion = $100 - 100 \times \frac{\%\text{ Marker in Feed} \times \%\text{ Nutrient in Feces}}{\%\text{ Marker in Feces} \times \%\text{ Nutrient in Feed}}$

In vivo considerations and sources of error. During any experiment sources of error must be considered. While grazing situations provide more accurate data on selectivity, confinement allows researchers to control more error. Not only must the researcher be cognizant of analytical and technical errors, but the researcher must take animal variation, environmental and experimental design into account (Cochran and Galyean, 1994). Error from any aspect of an experiment is additive, from collection of samples to laboratory analysis. Donefer (1966) stated that variation among laboratories was 3 to 6 times greater than within laboratories. Consistency among laboratories and researchers is imperative. Variation among animals is the largest source of variation.

Galyean et al. (1976) conducted an experiment using a 4 x 4 Latin square on high concentrate diets the mean for total track OM digestion for the animal was 4.8 times greater than that for the mean for total tract OM based on the collection period. Based on animal variation it is important to carefully select animals based on uniformity, whether physiological or genetic (Cochran and Galyean, 1994). The primary researcher must consider the amount of time of animal confinement, restraint of the animal and the housing temperature (Cochran and Galyean, 1994).

In vitro Digestibility. Accurate prediction of forage digestibility is imperative to diet formulation and animal performance. Conducting *in vivo* studies is time consuming, costly and involves the harvest of a large amount of forage. Harvesting forage to accurately represent what the animal would naturally select while grazing is a limiting factor. In the same manner, hand clipping samples involves a large amount of manual labor and does not conserve time. There are several ways to estimate digestibility. However, precision and accuracy of these predictions is dependent on laboratory techniques. Precision is a measure of laboratory variation and accuracy is based on how close the estimate is to the actual value. When the actual value of the feed is not the primary focus many laboratories focus on the precision of an experiment. Determination of *in vivo* digestibility is crucial for calculation of diet and supplement formulation. One approach to determining forage digestibility is through the use of *in vitro* techniques (Weiss, 1994).

Use of *in vitro* dry matter disappearance was first reported in 1919 by Waentig and Gierisch (Hungate, 1966). Ruminal contents mixed with a specific buffer solution are incubated with feeds, from that *in vitro* dry matter disappearance (IVDMD) is defined. In

order to replicate the rumen environment as closely as possible, it is important to recreate the digestive process in the ruminant animal. A significant issue for early *in vitro* experiments was control of inoculum pH. As expected, digestion of the forage occurs and volatile fatty acids (VFA) are produced. With no way to control or absorb VFAs in an *in vitro* system, pH decreases in the tube. Experiments conducted in the early 1940s and 1950s led to improvements on the *in vitro* system, however maintaining a constant pH was still a struggle. McDougall (1948) conducted experiments evaluating sheep saliva and published data on the mineral composition. Utilization of this buffer in *in vitro* systems allowed for long term incubation (72 to 96 h) of tubes (Weiss, 1994).

Multiple *in vitro* systems were developed during this time period and it was important to define specific criteria for *in vitro* system evaluation (Warner, 1956). These criteria were as follows:

1. The maintenance of a normal microbiological population.
2. The maintenance of normal rates of digestion.
3. The ability to predict *in vivo* results.

Establishing a relationship between *in vivo* and *in vitro* digestibility is imperative to forage evaluation. Several *in vivo -in vitro* relationships were established in the late 1950s by Baumgardt et al. (1958) and Walker (1959), however precision and accuracy of these procedures were low (Weiss, 1994). Tilley and Terry (1963) established a 2-stage procedure which is still used in laboratories with minor modifications.

Two-stage method. Initially, the Tilley and Terry (1963) method entailed utilizing a small amount of feed (0.5 g) and adding a mixture of ruminal fluid (10 ml) and a buffer solution (40 ml) in small flasks, keeping the system anaerobic and incubating the flasks

for 48 h. Fermentation was discontinued using mercuric chloride. Samples were centrifuged and the residue was subjected to acid-pepsin digestion for another 48 h. Post acid-pepsin digestion, the sample was centrifuged and the residue was dried and weighed to determine IVDMD. Blanks were included in analysis to correct for DM remaining in the inoculum. Tilley and Terry (1963) reported several different grasses and legumes could be used to predict *in vivo* digestibilities from IVDMD values with a high degree of accuracy. Modifications to the original procedure have been introduced in order to increase accuracy and precision of the *in vitro* system (Weiss, 1994). The inoculum in the Tilley and Terry (1963) procedure was modified to use a 50:50 mixture of rumen fluid and McDougall's buffer (1948). In order to prevent nitrogen limitation urea was added to the McDougall's buffer at 1 g urea L^{-1} of McDougall's buffer (1948). The incubation system consists of a water bath heated to 39^{o}C using a water pump circulator to prevent temperature variation throughout the water bath. During the 48 h incubation tubes are swirled every 12 h. After incubation, forage samples are subjected to a pepsin digestion. During this step, 6 ml of 20% hydrochloric acid and 2 ml of a 5% pepsin solution are added to the tubes. The *in vitro* system allows laboratories to analyze a large number of samples in a relatively small amount of time. It is important for the laboratories to maintain a high level of precision, however the greatest source of variation affecting precision is in the inoculum (Barnes, 1967).

In vitro digestibility variation. Inoculum variation is largely due to the donor animal. This variation stems from animal to animal variation, animal species, animal diet and collection time. Collecting rumen fluid from a minimum of 2 animals is important due to animal variation. Management of animal feed is important, with lower quality

forage decreasing the precision of *in vitro* organic matter disappearance (IVOMD). Diets containing a higher level of CP tended to have less variation (Weiss, 1994). Time of collection is important relative to time of feeding; Williams (1988) suggests collecting rumen fluid between 8 and 12 h post feeding. In order to keep laboratory precision high it is important to keep *in vitro* analysis collection times constant (Weiss, 1994).

While precision of *in vitro* experiments are important to finding the relative difference between samples, precision does not ensure accuracy of the experiment. Factors that may affect accuracy when determining *in vivo* and *in vitro* digestibility are DMI and diet composition (Tyrrell and Moe, 1975; Hoover, 1986). According to Weiss (1994), many studies have shown strong statistical correlations ($r > 0.90$) between *in vivo* and *in vitro* digestibility data (e.g., Tilley and Terry, 1963; Alexander and McGowan, 1966; Troelsen, 1970; McLeod and Minson, 1974; Aerts et al., 1977; Valdes and Jones, 1987; Givens et al., 1989; Genizi et al., 1990; Navaratne et al., 1990; Aufrère et al., 1992). While a strong correlation is important in order to compare *in vivo* to *in vitro* digestibilities, correlation does not mean that *in vivo* digestibility is equal to IVDMD. Regression equations have been introduced as a way to adjust these data. Forages samples, with unknown *in vivo* digestibility values, can be used in *in vitro* analysis and adjusted using regression equations developed from *in vivo* digestibilities values (Weiss, 1994). Laboratories have determined calibration equation techniques to produce these regression equations. There are 3 different methods to develop these equations.

Calibration techniques. In the first method, laboratories establish digestibility coefficients for both *in vitro* and *in vivo* digestibility using an assorted population of feeds. This option is time consuming and involves more labor and expenses and would

not be feasible for many laboratories. This method would only apply to feeds relative to a small geographic area (Weiss, 1994). The second method includes a large set of various feeds that have known *in vivo* digestibilities, which are used as a calibration set. The calibration set of feeds are analyzed using the *in vitro* analysis along with the feeds with unknown *in vivo* digestibilities. Following *in vitro* analysis the calibration set digestibilities are regressed against *in vivo* values, generating a regression equation. This equation is used to regress *in vitro* data of the unknown feed samples, creating an adjusted *in vitro* value relative to the calibration set of *in vivo* values. The final regression method is an indirect calibration set. *In vivo* estimates are made from the analysis of samples with a known IVDMD value determined at a commercial laboratory. These samples are then used in an *in vitro* analysis at an independent laboratory. Following completion of *in vitro* analysis, data are used to generate an equation using IVDMD from the independent laboratory to estimates of *in vitro* data from the commercial laboratory (Weiss, 1994). In order for proper function of this calibration set, the commercial lab must have an accurate *in vitro-in vivo* equation (Weiss, 1994). According to Weiss (1994), separate equations are needed for: 1) legumes and grasses; 2) corn silage; 3) concentrate feeds and 4) low quality roughages. Due to confounding variables from each *in vitro* run, each laboratory should develop its own equations. Table 1 provides sample equations from Weiss (1994), however these equations should not be used directly by laboratories.

The 2-stage method of collecting *in vitro* digestibilities has its limitations. While precision is needed during *in vitro* experimentation, accuracy tends to be of most importance relative to cost and time management. Certain factors must be evaluated prior

to *in vitro* experimentation. Confounding effects must be estimated before the experiment is conducted, for instance the donor animal should be fed a diet similar to the forage in question. For larger laboratories it may be pertinent for donor animals to be fed a diet predominately consisting of forage, but not lacking in specific minerals and CP (Weiss, 1994). Another limitation of the 2-stage method of analysis is length of time samples are required to incubate. According to the original Tilley and Terry (1963) 2-stage procedure, a 48 h fermentation time is required followed by a 48 h pepsin digestion. Due to differences in forage types, there will not be one specific time point that works for all forages. Weiss (1994) explains that a dairy cow with a high production rate will turnover forage samples at a much quicker rate and thus 48 h fermentation may overestimate *in vivo* digestibility. On the other hand, 48 h fermentation may underestimate an animal such as a beef cow fed at maintenance (Weiss, 1994).

Despite the fact that recommendations have been made not to include standards within *in vitro* procedures (Genizi et al., 1990; Ayres 1991) there is an importance of developing forages with known *in vivo* digestibilities in these procedures. These forages must be included in the 2-stage method of an *in vitro* analysis in order to determine *in vivo-in vitro* regression equations for determination of digestibility for unknown forages (Weiss, 1994).

OBJECTIVES

Forage digestibility is difficult to predict. The use of *in vivo* evaluation can be time consuming and costly, while accurately predicting the true value of a forage. *In vitro* techniques allow laboratories to evaluate a larger sample size, however digestibility estimates are not equal to *in vivo* estimates. Researchers are able to obtain *in vivo*

estimates from different *in vitro* runs utilizing regression equations from *in vitro* data produced from standard forages with known *in vivo* digestibilities (Weiss, 1994). Precision and accuracy of *in vivo* and *in vitro* experiments is imperative to predict animal performance. The objective of these experiments was to determine *in vivo* digestibility of 5 differing forages and use these forages as standards in *in vitro* digestibility procedures to estimate *in vivo* values for unknown forage samples.

LITERATURE CITED

Ayres, J. and B. Michalet-Doreau. 1988. Comparison of methods for predicting digestibility of feeds. Anim. Feed Sci. Technol. 20:203-218.

Babcock, B.A., D.J. Hayes and J.D. Lawrence. 2008. Using distillers grains in the U.S. and International Livestock and Poultry industries: Midwest Agribusiness Trade Research & Information Center, Ames, IA. http://www.ddgs.umn.edu/prod/groups/cfans/@pub/@cfans/@ansci/documents/asset/cfans_asset_415605.pdf. Accessed June 13, 2012.

Barnes, R.F. 1967. Collaborative *in vitro* rumen fermentation studies on forage substrates. J. Anim. Sci. 26:1120-1130.

Baumgardt, B.R., J.L. Cason, and R.A. Markley. 1958. Comparison of several laboratory methods as used in estimating the nutritive value of forages. J. Anim. Sci. 17:1205 (Abstr.).

Belyea, R.L., K.D. Rausch and M.E. Tumbleson. 2004. Composition of corn and distillers dried grains with solubles form dry grind ethanol processing. Bioresource Technology 94(2004): 293-298.

Berger, L.L., G.C. Fahey, Jr., L.D. Bourquin and E.C. Titgemeyer. 1994. Modification of forage quality after harvest. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Bremer, V.R., A.K. Watson, A.J. Liska, G.E. Erickson, PAS, G. Cassman, K.J. Hanford and T.J. Klopfenstein. 2011 Effect of distillers grains moisture and inclusion level in livestock diets on greenhouse gas emissions in the corn-ethanol-livestock life cycle. Prof. Anim. Sci. 27:449-455.

Buckner, C.D., M.F. Wilken, J.R. Benton, PAS, S.J. Vanness, V.R. Bremer, T.J. Klopfenstein, P.J. Kononoff and G.E. Erickson, PAS. 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27:57-64.

Buckner, C.D., T.J. Klopfenstein, G.E. Erickson, W.A. Griffin and J.R. Benton. 2010. Comparing ensiled or fresh mixed wet distillers grains with solubles with straw at two inclusions in growing calf diets. Nebraska Beef Report. MP93:42-43.

Burns, J.C., K.R. Pond and D.S. Fisher. 1994. Measurement of forage intake. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Cochran, R.C. and M.L. Galyean. 1994. Measurement of *in in vivo* digestion by ruminants. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Cogswell, C. and L.D. Kamstra. 1976. The stage of maturity and its effect upon the chemical composition of four native range species. J. Range Manage. 29(6):460-463.

Corrigan, M.E., T.J. Klopfenstein, G.E. Erickson, N.F. Meyer, K.J. Vander Pol, M.A. Greenquist, M.K. Luebbe, K.K. Karges and M.L. Gibson. 2009. Effects of level of condensed distillers solubles in corn dried distillers grains on intake, daily body weight gain, and digestibility in growing steers fed forage diets. J. Anim. Sci. 87:4073-4081.

Donefer, E. 1966. Collaborative *in vivo* studies on alfalfa hay. J. Anim. Sci. 25: 1227-1231.

Erickson, G.E., V.R. Bremer, T.J. Klopfenstein, A. Stalker and R. Rasby. 2007. Feeding of corn milling co-products to beef cattle. UNL Extension Publication.

Farlin, S.D. 1981. Wet distillers grains for cattle. Anim. Nutr. Health. 36:35.

Firkins, J.L., L.L. Berger and G.C. Fahey, Jr. 1985. Evaluation of wet and dry distillers grains and wet and dry corn gluten feeds for ruminants. J. Anim. Sci. 60:847-860.

Galyean, M.L., L.J. Krysl and R.E. Estell. 1987. Marker-based approaches for estimation of fecal output and digestibility in ruminants. F.N. Owens (ed.) Feed intake by beef cattle. Oklahoma State Univ. MP 121:96-113.

Genizi, A., A. Goldmand, A. Yulzari and N.G. Seligman. 1990. Evaluation of methods for calibrating *in vitro* digestibility estimates of ruminant feeds. Anim. Feed Sci. Technol. 29:265-278.

Griffin, W.A., V.R. Bremer, T.J. Klopfenstein, L.A. Stalker, L.W. Lomas, J.L. Moyer and G.E. Erickson. 2012. A meta-analysis evaluation of supplementing dried distillers grains plus solubles to cattle consuming forage-based diets. Prof. Anim. Sci. 28:306-312.

Gunter, S.A., F.T. McCollum III, R.L. Gillen, and L.J. Krysl. 1995. Diet quality and ruminal digestion in beef cattle grazing midgrass prairie rangeland or plains bluestem pasture throughout the summer. J. Anim. Sci. 73:1174-1186.

Gunter, S.A., F.T. McCollum III and R.L. Gillen. 1997. Forage intake by and site and extent of digestion in beef cattle grazing midgrass prairie rangeland or plains bluestem pasture throughout the summer. J. Anim. Sci. 75:490-501.

Gustad, K.H., T.J. Klopfenstein and G.E. Erickson. 2006. The use of dried distillers grains to extend range capacity. Univ. Neb. MS Thesis.

Ham, G.A., R.A. Stock, T.J. Klopfenstein, E.M. Larson, D.H. Shain and R.P. Huffman. 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. 72:3264.

Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755-2766.

Hungate, R.E. 1966. The rumen and its microbes. Academic Press, New York, NY.

Kalscheur, K.F., A.D. Garcia, D.J. Schingoethe, F.D. Royon and A.R. Hippen. 2012 Feeding biofuel co-products to dairy cattle. In: Biofuel co-products as livestock feed. http://www.fao.org/docrep/016/i3009e/i3009e.pdf . Accessed July 1, 2013.

Johnson, J.A., J.S. Caton, W. Poland, D.R. Kirby and D.V. Dhuyvetter. 1998. Influence of season on dietary composition, intake and digestion by beef steers grazing mixed-grass prairie in the northern Great Plains. J. Anim. Sci. 76:1682-1690.

Lardy, G. 2007. Feeding co-products of the ethanol industry to beef cattle. Extension Publication AS-1232.

Larson, E.M., R.A. Stock, T.J. Klopfenstein, M.H. Sindt and R.P. Huffman. 1993. Feeding value of wet distillers by products for finishing ruminants. J. Anim. Sci. 71:2228-2236.

Lodge, S.L., R.A. Stock, T.J. Klopfenstein, D.H. Shain, and D. W. Herold. 1997b. Evaluation of wet distillers composite for finishing ruminants. J. Anim. Sci. 75:44-50.

Loy, T.W., J.C. MacDonald, T.J. Klopfenstein and G.E. Erickson. 2007. Effect of distillers grains or corn supplementation frequency on forage intake and digestibility. J. Anim. Sci. 85:2625-2630.

Loy, T.W., T.J. Klopfenstein, G.E. Erickson, C.N. Macken and J.C. MacDonald. 2008. Effect of supplemental energy source and frequency on growing calf performance. J. Anim. Sci. 86:3504-3510.

MacDonald, J.C., T.J. Klopfenstein, G.E. Erickson and W.A. Griffin. 2007. Effects of dried distillers grains and equivalent undegradable intake protein or either extract on performance and forage intake of heifers grazing smooth bromegrass pastures. J. Anim. Sci. 85:2614-2624.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43:99-109.

Merchen, N.R. and L.D. Bourquin. 1994. Processes of digestion and factors influencing digestion of forage-based diets by ruminants. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Musgrave, J. A., K.L. Gillespie, S.K. Moore, L.A. Stalker, T.J. Klopfenstein, C.J. Schneider, J.L. Harding, and B.L. Nuttelman. 2013. Influence of pre-collection diet and preparation technique on nutrient composition of masticate samples. Nebraska Beef Report. MP98:49-50.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Nelson, C.J. and L.E. Moser. 1994. Plant factors affecting forage quality. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Paterson, J.A., R.L. Belyea, J.P. Bowman, M.S. Kerley, and J.E. Williams. 1994. The impact of forage quality and supplementation regimen on ruminant animal intake and performance. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Peterson, M.M., M.K. Luebbe, R.J. Rasby, T.J. Klopfenstein, G.E. Erickson, L.M. Kovarik. 2009. Level of wet distillers grains plus solubles and solubles ensiled with wheat straw for growing steers. Nebraska Beef Report. MP92:35-36.

Rees, D.V.H. 1982. A discussion of sources of dry matter loss during the process of haymaking. J. Agric. Eng. Res. 27:469-479.

Rendleman, C. and H. Shapouri. 2007. New technologies in ethanol production. USDA. http://usda.gov/oce/reports/energy/aer842_ethanol.pdf. Accessed June 12, 2013.

RFA. 2012. 2012 Ethanol Industry Outlook: Accelerating Industry Innovation. Renewable Fuels Association. http://ethanolrfa.3cdn.net/d4ad995ffb7ae8fbfe_1vm62ypzd.pdf. Accessed June 20th, 2013.

Rotz, CA, and S.M. Abrams. 1988. Losses and quality changes during alfalfa hay harvest and storage. Trans. ASAE 31:350-355.

Rotz, C.A. and R.E. Muck. 1994. Changes in forage quality during harvest and storage. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Schneider, B.H., and W.P. Flatt. 1975. The evaluation of feeds through digestibility experiments. The Univ. of Georgia Press, Athens.

Spiehs, M.J., M.H. Whitney and G.C. Shurson. 2002. Nutrient database for distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639-2645.

Stanton, T.L. and J. Whittier. 1998. Urea and NPN for cattle and sheep. Colorado State University Extension Fact Sheet No. 1,608. Reviewed 2006. http://www.ext.colostate.edu/pubs/livestk/01608.pdf. (Accessed17 March 2014.)

Stock, R.A., J.M. Lewis, T.J. Klopfenstein and C.T. Milton. 2000. Review of new information on the use of wet and dry milling feed by-products in feedlot diets. J. Anim. Sci. 77:1-12.

Suttle, N.F. 2010. Mineral Nutrition of Livestock. 4th ed. CAB International, Cambridge, MA.

Tilley, J.M.A. and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18:104-111.

Tyrrell, H.F. and P.W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58:1151-1163.

USDA. 2012. Crop production down in 2012 due to drought, USDA reports. http://www.nass.usda.gov/Newsroom/2013/01_11_2013.asp Accessed June 1, 2013.

U.S. Grains Council. 2012. A guide to Distiller's Dried grains with Solubles (DDGS) Third Edition: U.S. Grains Council. http://www.ddgs.umn.edu/prod/groups/cfans/@pub/@cfans/@ansci/documents/asset/cfans_asset_417244.pdf. Accessed June 2, 2013.

Vander Pol, K.J., G.E. Erickson, T.J. Klopfenstein, M.A. Greenquist and T. Robb. 2006. Effect of dietary inclusion of wet distillers grains on feedlot performance of finishing cattle and energy value relative to corn. Nebraska Beef Rep. MP88-A:51.

Vander Pol, K.J., M.A. Greenquist, G.E. Erickson, T.J. Klopfenstein and T. Robb. 2008. Effect of corn processing in finishing diets containing wet distillers grains on feedlot performance and carcass characteristics of finishing steers. Prof. Anim. Sci. 24:439-444.

Van Dyne, G.M., and D.T. Torell. 1964. Development and use of esophageal fistula: A Review. J. Range Manage. 17(1):7-19.

Van Soest, P.J. 1982 Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca, NY.

Walker, D.M. 1959. The *in vitro* digestion of roughage dry matter. J. Agric. Sci. (Camb.) 53:192-197.

Warner, A.C.I. 1956. Criteria for establishing the validity of *in vitro* studies with rumen micro-organisms in so-called artificial rumen systems. J. Gen. Microbiol. 14:733-748.

Weiss, W.P. 1994. Estimation of digestibility of forages by laboratory methods. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Wilken, M.F., M.K. Luebbe, G.E. Erickson, T.J. Klopfenstein and J.R. Benton. 2009. Feeding corn distillers solubles or wet distillers grains plus solubles and cornstalks to growing calves. Nebraska Beef Report. MP92:30-32.

Wilken, M.F., T.L. Mader, G.E. Erickson and L.J. Johnson. 2009. Comparison of dry distillers or modified wet distillers grains plus solubles in wet or dry forage-based diets. Nebraska Beef Report. 2009:33-34.Williams, A.G. 1988. Factors affecting the formation of polysaccharide-degrading enzymes by rumen micro-organisms. Anim. Feed Sci. Technol. 21:191-195.

Table 1. Sample equations for converting IVDMD values (two-stage method) to in vivo OM digestibility (all values expressed as g/kg, DM basis).[1]

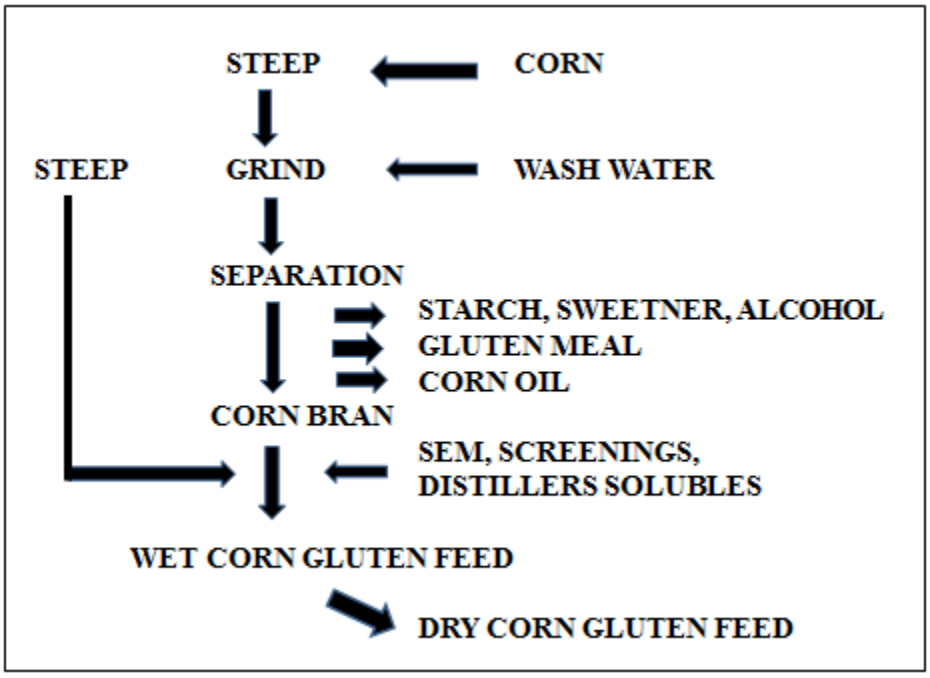
Feed	Intercept	Slope	SE_p[2]	Reference
C-3 grasses	124	0.82	22.7	Aerts et al., 1977
C-3 grasses	5.2	1.01	14.6	Terry et al., 1978
C-3 grasses	-136	1.20	18.5	Omed et al., 1989
C-3 grasses	172	0.71	24.0	Moss and Givens, 1990
C-4 grasses	115	0.83	24.0	McLeod and Minson, 1969
C-4 grasses	-125	1.27	37.8	Navaratne et al., 1990
Legumes	-4.1	1.02	16.0	Terry et al., 1978
Legumes	-9.8	1.03	19.4	Omed et al., 1989
C-3 grasses and legumes[3]	-10.1	0.99	23.1	Tilley and Terry, 1963
C-3 grasses and legumes[3]	-48.2	1.08	19.3	Omed et al., 1989
Corn silage	29.3	0.58	21.1	Aufrère et al., 1992
Concentrates	-26.6	1.10	50.1	Omed et al., 1989

[1] *In vivo* = a + b * IVDMD.

[2] Standard error of prediction.

[3] Equation for predicting digestible DM, not OM.

Figure 1. Wet milling process[1].



[1] www.agmrc.org

Figure 2. Dry milling process[1].

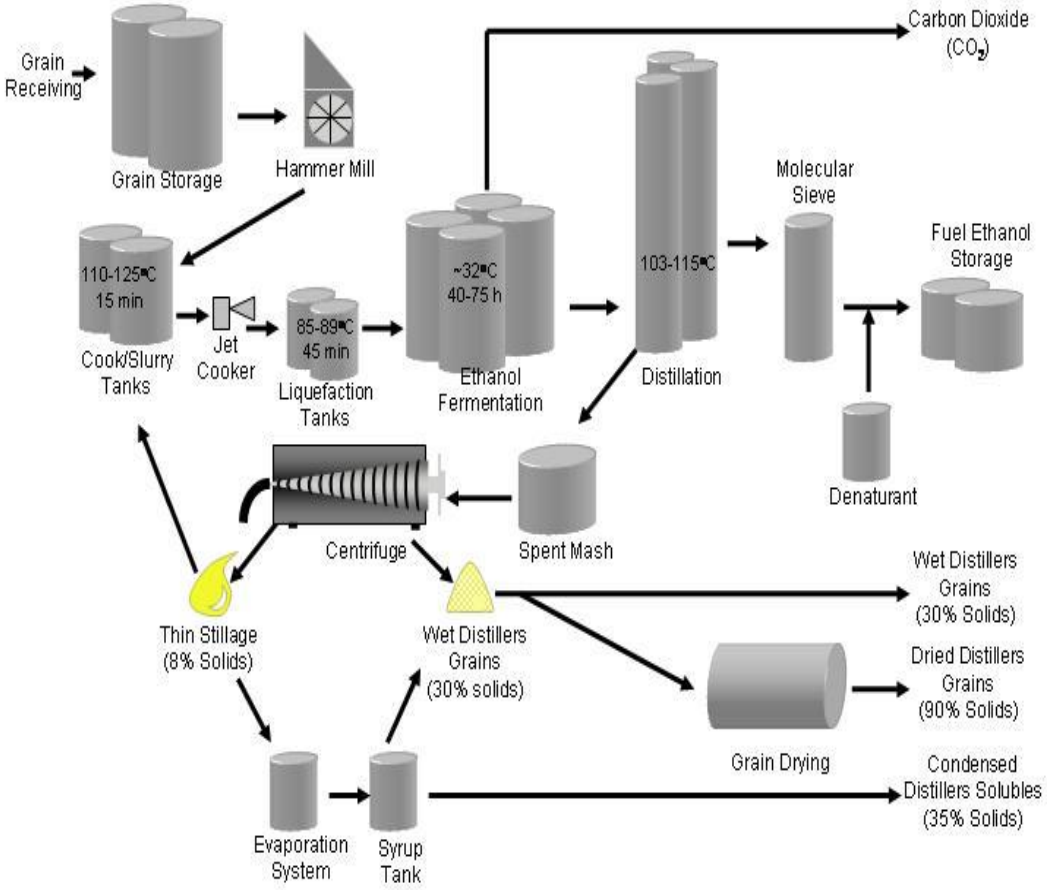


FIGURE 1: FUEL ETHANOL AND CO-PRODUCTS PRODUCTION

*Dry mill ethanol production process presented at http://www.icminc.com/ethanol/production_process and subsequently modified by CFIA

[1] www.icminc.com

Chapter II

Comparison of wet or dry distillers grains plus solubles to corn as an energy source in forage based diets.

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ABSTRACT:

Four experiments were conducted comparing wet or dry distillers grains plus solubles (WDGS or DDGS) to corn as an energy source in forage based diets. In Exp. 1, 93 individually fed steers (268 ± 14 kg) were fed a 60:40 blend of sorghum silage and alfalfa hay, respectively and supplemented at 0, 0.33, 0.67 or 1.0% of BW either WDGS, DDGS or a MIX (67% WDGS and 33% ground wheat straw) per steer daily. In Exp. 2, 160 steers (286 ± 19 kg) were blocked into two weight groups, assigned to 1 of 10 pens (16 steers/ pen) and fed diets including either 25% WDGS or 33.6% dry rolled corn (DRC) in a sorghum silage, grass hay and supplement diet. In Exp. 3, 60 individually fed steers (231± 14 kg) were fed DRC at 22.0, 41.0, or 60.0%, or WDGS at 15.0, 25.0, and 35.0% of the diet (DM basis). In Exp. 4, 120 individually fed steers (247 ± 10 kg and 317 ± 28 kg) were fed diets including DDGS, WDGS or DRC, with sorghum silage, grass hay and supplement. In Exp. 2, Exp. 3 and Exp. 4, diets were formulated to meet RDP and MP requirements. In Exp. 1 WDGS and DDGS produced greater ending BW and DMI compared to the mix supplement ($P = 0.05$). Increasing amounts of distillers grains increased ADG ($P < 0.01$) in forage based diets. Wet grains mixed with straw numerically reduced DMI without affecting G:F. By design, steers in Exp. 2 had similar DMI and ADG across treatments ($P = 0.72$ and 0.11, respectively). Cattle in Exp. 3 consuming WDGS gained more than DRC ($P < 0.01$). Average daily gain increased with increasing levels of DRC and WDGS ($P < 0.01$). Each block of steers in Exp. 4, by design, had similar DMI and ADG ($P = 0.89$ and 0.81 respectively) across treatments. Using regression analysis, data from Exp. 2, Exp. 3 and Exp. 4 were pooled to generate ADG at differing inclusions allowing energy value of WDGS to be calculated relative to

DRC. The energy value of DGS fed at 15% of diet DM was 137% and fed at 30% of the diet DM was 136% relative to DRC.

Key words: beef, calf, dry distillers grains, forage-based diet, wet distillers grains

INTRODUCTION:

Expansion of the corn milling industry to make ethanol has led to increased usage of distillers byproducts. Previous research (Bremer et al., 2011) conducted explored the benefit of utilizing distillers byproducts in finishing diets in place of corn. However, the energy value of distillers byproducts in high forage diets is not as well defined. Furthermore, research has shown dry distillers grains plus solubles (DDGS) supplementation in forage-based diets decreases forage DMI (Loy et al., 2007; Loy et al., 2008). Supplementation strategies allow producers to increase carrying capacity of pastures without acquisition of additional land. A study compared dry-rolled corn (DRC) and DDGS at two supplementation levels in a forage based diet and determined the energy value relative to DRC to be 118-130% that of corn (Loy et al., 2008). In contrast with forage-based diets, energy value of distillers grains plus solubles (DGS) in concentrate diets has been well researched. A meta-analysis based on prediction equations developed from 20 feedlot cattle finishing experiments suggests greater energy value for wet distillers grains plus solubles (WDGS; 130 to 143% between 20-40% inclusion diet DM) than DDGS (112% for any inclusion diet DM; Bremer et al., 2011). Nuttelman et al. (2011) conducted an experiment directly comparing WDGS and DDGS in concentrate diets. Feeding values calculated from G:F, resulted in WDGS and DDGS being 146 and 109% the energy value of corn, respectively, supporting values found by

Bremer et al. (2011). Few direct comparisons between wet and dry DGS in forage diets have been made.

The objective of Exp. 1 was to determine the differences in performance between WDGS and DDGS, and to determine if forage DMI can be reduced by feeding WDGS mixed with low quality forage. Results from Exp. 1 lead to the objective Exp. 2, Exp. 3 and Exp. 4 to compare DRC, DDGS and WDGS as energy sources in forage based diets and determine the energy value of DGS relative to DRC.

MATERIALS AND METHODS

Four experiments were conducted at the University of Nebraska-Lincoln Agricultural Research and Development Center research feedlot near Ithica, NE for which animal use procedures were reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee.

Exp. 1

Ninety-three crossbred steer calves (268 $\pm$ 14 kg) were used in a generalized randomized blocked experiment design to evaluate growth performance between different types of distillers grains. Upon arrival at the feedlot in October 2006, steers were individually identified and vaccinated for prevention of *Haemophilus somnus* with 2cc Somubac (Pfizer Animal Health, New York, NY*)* for prevention of bovine viral diarrhea, infectious bovine rhinotracheitis, parainfluenza-3 and bovine respiratory syncytial virus with 2cc BovaShield Gold 5 (Pfizer Animal Health) and given an injectable parasiticide using 6.5cc Dectomax Injectable (Pfizer Animal Health). Approximately 21 days post arrival, steers were revaccinated for prevention of pinkeye with 2cc Piliguard Pinkeye 1 (Durvet Animal Health Products, Blue Springs, MO) and

for prevention of *Clostridium Chauvoei*, *Cl. Septicum*, *Cl. Novyi*, *Cl. Sordellii*, *Cl. Perfringens* and types C and D *Haemophilus Somnus* with 5 cc Ultrabac7/Somubac (Pfizer Animal Health). At receiving, steers were received on bromegrass pastures (21 d) until revaccination and then steers were managed on cornstalks, for approximately 23 d until being moved to the individual feeding fed barn for a 30 d gate training to the Calan gate system (American Calan, Inc., Northwood, NH). Prior to the start of the experiment in December, steers were limit fed a diet consisting of 47.5% alfalfa hay, 47.5% wet corn gluten feed, and 5.0% supplement (DM basis) at 2.0% BW (5.4 kg of DM) for 5 d (Watson et al., 2013), then weighed on 3 consecutive days (d -1, d 0 and d1; Stock et al., 1983). The 3 d BW were averaged (268 kg) and used as initial BW for performance calculations. Steers were stratified by BW and assigned randomly to one of three supplemental treatments based on an average of d -1 and d 0 BW for each block. The experiment was conducted from December 13, 2006, through April 4, 2007.

Steers were fed a control diet consisting of 60% sorghum silage and 40% alfalfa hay, and supplemented one of four levels of supplement: 0, 0.33, 0.67 or 1.0% of BW distillers grains/ steer daily (DM basis). Treatments included DDGS, WDGS, and a mix that was 67% WDGS and 33% ground wheat straw (MIX). Supplementation was adjusted to changes in BW using percentage of BW fed (0, 0.33, 0.67 and 1.0% respectively) and 1 d interim weights every 28 d. The MIX diet was stored in silo bags for 30 d prior to initiation of the trial. Limestone was provided in the dry supplement to ensure a minimum 1.2:1 ration of Ca:P.

For cattle fed MIX, the WDGS:Straw mix was fed to allow cattle to consume the assigned level of distillers grains. For example, cattle fed the MIX at 0.33% BW

received 0.9 kg of WDGS and 0.5 kg of wheat straw equaling 1.4 kg of supplement (DM basis). Distillers grains (Abengoa Bioenergy, York, NE) and MIX were fed on top of the base diet to encourage total consumption of the supplement. The control diet was used to simulate a similar response in performance that is typically expected from steers in a grazing phase of production. The control diet was mixed every 2 to 3 d. Both DDGS and WDGS utilized in this study were delivered to the feedlot as needed.

Steers were individually fed for 84 d using Calan electronic gates. Steers were limit fed a mix of 47.5% alfalfa hay, 47.5% wet corn gluten feed, and 5.0% supplement for 5 d following the conclusion of the feeding period to reduce variation due to gut fill (Watson et al., 2013). After limit feeding, cattle were weighed 3 consecutive days following the end of the feeding period. The average of the 3 d weights were used as the ending BW. Individual weigh backs were collected weekly and a sample of refused feed was taken and DM was determined using a 60° C forced air oven for 48 h (AOAC, 1999; method 4.2.03). Bunks were evaluated daily and necessary adjustments were made to base diet delivery.

In order to obtain accurate DMI, all feed samples were sampled weekly and analyzed for DM using a 60^{o}C forced air oven for 48 h (AOAC, 1999; method 4.2.03). Representative sub-samples of dietary ingredients were collected (Table 1) and analyzed for NDF (Van Soest et al., 1991; Mertens et al., 2002), CP and S (LECO Corp., St. Joseph, MI; AOAC, 1999; method 990.03). Ash was determined using a muffle furnace for 6 h at 600^{o}C (AOAC, 1999; method 4.1.10) and OM based on ash content. Byproducts utilized were analyzed for fat content using the fat procedure described by Bremer et al. (2010) and NDF (Van Soest et al., 1991; Mertens et al., 2002; Buckner et

al., 2013) content was determined utilizing the subsequent sample following fat extraction.

Statistical Analysis

Growth performance data from Exp. 1 were evaluated as a generalized randomized blocked experimental design using PROC GLIMMIX (Version 9.3, SAS Inst. Inc., Cary, NC). Individual animal was the experimental unit. Block was used as a fixed effect assigned by weight, and probabilities less than or equal to α 0.05 were considered significant. The model for Exp. 1 included block, control, energy source within control (DDGS, WDGS, and MIX) and level of dietary treatment within control supplemented (0.33, 0.67 or 1.0% of BW) analyzed as a 3 x 3 + 1 factorial design. The interaction between energy source and level of supplementation were analyzed for linear and quadratic effects using orthogonal contrasts including the forage control diet for 0% inclusion.

Exp. 2

One hundred sixty crossbred steers (286 ± 19 kg) were used in a generalized randomized blocked experimental design, 67 day growing trial, to compare the energy value of WDGS to DRC in a forage-based diet. Upon arrival at the feedlot in October 2007, steers were individually identified and vaccinated for prevention of *Haemophilus somnus* with 2cc Somubac (Pfizer Animal Health), for prevention of bovine viral diarrhea, infectious bovine rhinotracheitis, parainfluenza-3 and bovine respiratory syncytial virus with 2cc BovaShield Gold 5 (Pfizer Animal Health) and given an injectable parasiticide using 6.5cc Dectomax Injectable (Pfizer Animal Health). Approximately 21 days post arrival, steers were revaccinated for prevention of pinkeye

with 2cc Piliguard Pinkeye 1 (Durvet Animal Health Products) and for prevention of *Clostridium Chauvoei, Cl. Septicum, Cl. Novyi, Cl. Sordellii, Cl. Perfringens* and types C and D *Haemophilus Somnus* with 5 cc Ultrabac7/Somubac (Pfizer Animal Health, New York, NY). At receiving, steers were grazed on bromegrass pastures until revaccination and then steers were managed on cornstalks for approximately 123 d until being brought to feedlot pens. Prior to experiment initiation, steers were limit fed a diet containing 47.5% alfalfa hay, 47.5% wet corn gluten feed and 5.0% supplement for 5 d, then weighed on 2 consecutive days (d 0 and d1). These BW were averaged (286 kg) and used as initial BW for performance calculations. Calves were blocked into 2 weight groups (6 heavy and 4 light), stratified by BW within block, and assigned randomly to pens based on d 0 BW. Pens were assigned randomly within block to 1 of 2 dietary treatments (10 pens / treatment) with 16 steers / pen. The experiment was conducted from March 4, 2008, through May 9, 2008.

Dietary treatments included sorghum silage fixed at 35% for both treatments and grass hay adjusted according to level of WDGS at 25% (Abengoa Bioenergy, York, NE) or DRC at 33.6% (Table 2). The nutrient profile for dietary ingredients included in this experiment can be found in Table 3. Diets were mixed daily. Diets were formulated using the NRC (1996) model and were formulated to meet energy and MP requirements for a targeted gain of 1.0 kg/day. Supplements for both diets included urea to meet degradable intake protein (DIP) requirements. To prevent a performance response due to protein, Soypass® was included in the diet containing DRC to provide undegradable intake protein (UIP) to meet the MP requirements. For diet formulation, WDGS was assumed to

contain 130% the energy value of DRC (Loy et al., 2008). Limestone was provided in the dry supplement to ensure a minimum 1.2:1 ratio of Ca:P.

Bunks were evaluated daily and managed so that intakes were equal across both treatments for paired pens. Feed refusals were collected weekly and DM of the feed refused was determined using a 60ºC forced air oven. Feed refusals (DM) were used to accurately calculate DMI. Steers were fed for 67 d and limit fed a diet containing of 47.5% alfalfa hay, 47.5% wet corn gluten feed and 5.0% supplement for 5 d (Watson et al., 2013), then weighed on 2 consecutive days (Stock et al., 1983).

The WDGS utilized in this study were delivered to the feedlot as needed throughout the experiment. In order to obtain accurate DMI, all feed samples were sampled weekly and analyzed for DM using a 60^{o}C forced air oven for 48 h (Buckner et al., 2011). Representative sub-samples of dietary ingredients were collected (Table 3) and analyzed for NDF (Van Soest et al., 1991; Mertens et al., 2002; Buckner et al., 2013), CP and S (LECO Corp.; AOAC, 1999; method 990.03). Ash was determined using a muffle furnace for 6 h at 600^{o}C (AOAC, 1999; method 4.1.10) and OM based on ash content. Byproducts utilized were analyzed for fat content using the ether extract procedure described by Bremer et al. (2010) and NDF (Mertens et al., 2002; Buckner et al., 2013) content was determined utilizing the subsequent sample following fat extraction.

Statistical Analysis

Growth performance data from Exp. 2 were evaluated as a generalized randomized blocked experimental design using PROC MIXED (Version 9.2, SAS Inst. Inc., Cary, NC). Pen was the experimental unit in Exp. 2 (10 pens / treatment) and the pens were blocked by weight (6 heavy and 4 light). Block was used as a fixed effect, and

probabilities less than or equal to α 0.05 were considered significant. The model included block and dietary treatment and block * dietary treatment interaction. Data for Exp. 2 were analyzed as an unstructured treatment design with 2 energy sources (DRC and WDGS).

Exp. 3

Sixty crossbred calves (231 ± 14 kg) were utilized in a completely randomized design to evaluate the energy value of WDGS relative to that of DRC in forage based diets for growing steers. Steers arrived at the feedlot in October, 2008. Calves were received, identified and given similar vaccinations as Exp. 1 and Exp. 2. Approximately 14 d after initial processing, steers were revaccinated with the same products used in Exp. 1 and Exp. 2. Calves were received onto brome grass pastures and after revaccination steers were managed on corn stalks until being trained to the individual feeding barn in a similar manner to Exp. 1 using the Calan gate system (American Calan, Inc.). Prior to experiment initiation, steers were limit-fed the same diet as in Exp. 1 at 2.0% of BW (4.6 kg of DM) for 5 d, then weighed on 3 consecutive days (d -1, 0 and 1). The 3 d BW were averaged (231 kg) and used as initial BW for performance calculations. Calves were stratified by BW, and assigned randomly to 1 of 6 treatments based on the average BW from d -1 and d 0. This experiment was conducted from December 12, 2008, through March 4, 2009.

Dietary treatments were arranged as a 2 x 3 factorial design. The two factors were energy source (WDGS and DRC) and level of inclusion. Inclusions of WDGS were included at 15.0, 25.0, or 35.0% (DM basis) and 22.0, 41.0 and 60.0% for diets containing DRC (LOW, MEDIUM, and HIGH; DM basis). Dietary treatments contained

30% sorghum silage and various levels of grass hay depending on the inclusion level of WDGS (Abengoa Bioenergy, York, NE) or DRC (Table 4). The nutrient profile for dietary ingredients included in this experiment can be found in Table 5. Dry supplement was included in diets at 2.2 to 5.5% of the diet DM to provide sufficient urea to all diets to meet or exceed the DIP requirements and to supply limestone to meet a 1.2:1 minimum Ca:P ratio as determined by the NRC (1996). Soypass® was included in the low and intermediate levels of DRC treatments to meet or exceed the MP requirements (NRC, 1996). Inclusion of urea and Soypass® was to prevent a protein response rather than an energy response between WDGS and DRC. Based on data from Loy et al. (2008) feeding value for WDGS is 130% the energy value of DRC in forage based diets. The WDGS energy value of 130% was used to determine the inclusion level of DRC so the diets would be isocaloric. Therefore, DRC was included at 22.0, 41.0, or 60.0% of the diet DM for treatments containing DRC. In order to keep intakes similar between DRC and WDGS treatments, calves were pair-fed within level (LOW, MEDIUM or HIGH) based on initial body weight.

Steers were individually fed ad libitum for 84 d using Calan electronic gates (American Calan, Inc.). The WDGS utilized in this study was delivered as needed. Bunks were evaluated daily so that intakes were equal across treatments for paired animals. Feed refusals were collected weekly and DM of refused feed was determined. Cattle were limit fed a mixture of 47.5% wet corn gluten feed, 47.5% alfalfa hay and 5.0% supplement for 5 d prior to and following the feeding period to reduce variation due to gut fill (Watson et al., 2013). Calves were consecutively weighed on the final 3 d (-1, 0 and 1) of the limit feeding period and the average of each 3 d weight was used for ending

BW (Stock et al., 1983). In order to obtain accurate DMI, all feed samples were sampled weekly and analyzed for DM using a 60^{o}C forced air oven for 48 h (Buckner et al., 2011). Representative sub-samples of dietary ingredients were collected (Table 5) and analyzed for NDF (Van Soest et al., 1991; Mertens et al., 2002; Buckner et al., 2013), CP and S (LECO Corp.; AOAC, 1999; method 990.03). Ash was determined using a muffle furnace for 6 h at 600^{o}C (AOAC, 1999; method 4.1.10) and OM based on ash content. Byproducts utilized were analyzed for fat content using the fat extract procedure described by Bremer et al. (2010) and NDF (Van Soest et al., 1991; Mertens et al., 2002; Buckner et al., 2013) content was determined utilizing the subsequent sample following fat extraction.

Statistical Analysis

Growth performance data from Exp. 3 were evaluated as a completely randomized design using PROC MIXED (Version 9.2, SAS Inst. Inc., Cary, NC). Individual animal was the experimental unit for Exp. 3. Probabilities less than or equal to α 0.05 were considered significant. Data for Exp. 3 were analyzed as a 2 x 3 factorial design, with 2 different energy sources and 3 inclusions (LOW, MEDIUM or HIGH) Model effects included energy source, energy source inclusion level and interactions of these factors. When no significant interactions ($P > 0.05$) were observed, main effects of energy source and level of energy source fed were presented. Main effects of level of energy source were analyzed for linear and quadratic effects using orthogonal contrasts.

Exp. 4

One hundred twenty crossbred steers, in 2 weight blocks (247 ± 10 kg and 317 ± 28 kg) were used in an 84 d growing trial to compare the energy value of DDGS and

WDGS to DRC in a forage based diet. Upon arrival at the feedlot in October 2009, steers were individually identified and vaccinated similar to Exp. 1, Exp. 2 and Exp. 3. Approximately 14 d after initial processing, steers were revaccinated similarly to Exp.1, Exp. 2 and Exp. 3. Steers were received onto brome grass pastures, revaccinated and then managed on corn stalks. Before trial initiation, steers were brought to the individually fed barn for Calan electronic gate training (American Calan, Inc.). Steers were limit fed the same diet as in Exp. 1 and Exp. 3 at 2.0% of BW (4.9 and 6.3 kg of DM) for 5 d (Watson et al., 2013), then weighed on 3 consecutive days (d -1, 0 and 1; Stock et al., 1983). Initial 3 d BW were averaged (247 and 317 kg based on start date) and used as initial BW for performance calculations. Calves were blocked into 2 weight groups based on start date, stratified by BW within block and assigned randomly to 1 of 6 diets or the control. The first block of this experiment was conducted from December 18, 2009 through March 11, 2010 and the second block was conducted from April 14, 2010 through July 9, 2010.

Animals were randomly paired within block, into groups of 3 based on BW and fed either the low or high levels of each diet. Dietary treatments consisted of DDGS, WDGS or DRC replacing a 60:40 blend of grass hay and sorghum silage (Table 6). Dry distillers grains plus solubles and WDGS were fed at either 15 or 30% (Green Plains, Ord, NE) or DRC fed at either 22 or 50% (DM basis). Byproduct inclusion in the diets replaced a 1:1 ratio (DM basis) of 60:40 blend of grass hay and sorghum silage mixture. All diets contained a supplement that included urea to meet DIP requirements. Soypass® was used in the control diet and DRC treatments to provide UIP to meet the MP

requirement (NRC, 1996). The nutrient profile for dietary ingredients included in this experiment can be found in Table 7.

Diets were formulated using the NRC (1996) model to meet energy and MP requirements. Diets were calculated to contain the same amount of energy with 83% TDN for DRC and assuming DGS contains 108% TDN based on Exp. 2 and Loy et al. (2008). Gain was predicted at 0.79 kg / day for the low inclusion level at 15% and 1.08 kg / day for the high inclusion level at 30% DGS. Dry rolled corn diets were formulated to equal ADG relative to the DGS treatments which calculated to 22 and 50% corn for low and high inclusion, respectively. Bunks were evaluated daily and managed based on the animal within each group of 3 eating the least as a percentage of BW. Feed refusals were collected weekly and DM of refused feed was determined.

All feed samples were collected weekly and analyzed for DM in a 60^{o}C forced air oven for 48 h (Buckner et al., 2011) to determine accurate DMI. Representative sub-samples of dietary ingredients were collected (Table 1) and analyzed for NDF (Mertens et al., 2002; Buckner et al., 2013), CP and S (LECO Corp., St. Joseph, MI; AOAC; 1999; method 990.03). Ash was determined using a muffle furnace for 6 h at 600^{o}C (AOAC, 1999; method 4.1.10) and OM based on ash content. Byproducts utilized were analyzed for fat content using the ether extract procedure described by Bremer et al. (2010) and NDF (Mertens et al., 2002; Buckner et al., 2013) content was determined utilizing the subsequent sample following fat extraction.

Statistical Analysis

Growth performance data from Exp. 4 were evaluated as a randomized complete block design plus a control using PROC MIXED (Version 9.2, SAS Inst. Inc., Cary, NC).

Individual animal was the experimental unit for Exp. 4 and steers were blocked based on trial initiation and weight. Block was used as a fixed effect, and probabilities less than or equal to α 0.05 were considered significant. The model included level of energy source inclusion and type of energy source. Data for Exp. 4 were analyzed as a 2 x 3 + 1 factorial design, with 2 feeding levels (15 and 22% LOW or 30 and 50% HIGH; DGS and DRC for LOW and HIGH, respectively) and 3 different energy sources. Model effects included energy source, energy source inclusion level (LOW or HIGH), and interactions of these factors. When no significant interactions ($P > 0.05$) were observed, main effects of energy source and level of energy source fed were presented. Main effects of level of energy source were analyzed for linear and quadratic effects using orthogonal contrasts including the forage control diet for 0% inclusion.

Pooled Analysis

Data from the 3 experiments containing both DRC and WDGS were pooled in order to predict the energy value of WDGS relative to DRC. Using regression analysis, estimates were made for the amount of DRC in the diet to provide equal ADG to 15 and 30% WDGS. The regression analysis was used to estimate ADG at differing levels. This analysis was needed in order to use the same NE adjuster values for both the DRC and DGS diets. Block et al. (2006) reported that NE adjuster values changed with rate of ADG, with values declining as ADG increased. In order to prevent an over prediction of ADG the NE adjuster values had to be equal for both DRC and DGS diets. To facilitate the comparison of energy values of DRC and DGS, it was necessary to do the evaluation at equal ADG.

Dry rolled corn and WDGS replaced both grass hay and sorghum silage as the inclusion increased. The change in level of DRC or WDGS determined the calculated change in both hay and sorghum silage. This allowed the calculation of amounts of hay and silage in each of the three diets (Tables 1, 2 and 3). Because DDGS was not included in Exp. 2 or Exp. 3, there were not sufficient observations for DDGS and therefore no DDGS data were included in the pooled data.

Statistical Analysis

Pooled data were analyzed using the Glimix procedure of SAS (SAS Inst. Inc., Cary, NC). Model effects included trial (Exp. 2, Exp. 3 and Exp. 4), type of energy source (DRC or WDGS), block within trial and inclusion within energy source (15 or 30% WDGS and 27.74 or 54.71% DRC). Inclusion of energy source was treated as a covariate. Regression analysis produced the following equations used to predict ADG at differing levels: DRC (y = 0.02 (± 0.02)x + 1.59 (± 0.12)); WDGS (y = 0.04 (± 0.02)x + 1.61 (± 0.12)).

RESULTS

Exp. 1

There were no type * level interactions observed with the exception of DMI (Figure 1) therefore the main effects of growth performance will be presented (Table 8 and 9). Supplementing wet or dry DGS or MIX linearly ($P < 0.01$) increased DMI. Dry matter intake was similar for cattle supplemented with MIX and WDGS ($P = 0.94$). Cattle supplemented with DDGS had greater DMI compared to MIX and WDGS ($P < 0.01$). As expected cattle fed the control (0% BW) consumed the least at 6.15 kg DMI.

No difference between supplementation type for ending BW ($P = 0.12$), ADG ($P = 0.20$), or G:F ($P = 0.55$) was observed (Table 8). However, cattle supplemented with WDGS and DDGS tended to have heavier ending BW compared to MIX ($P = 0.12$), suggesting the MIX fed cattle gained at a slower rate. Because cattle were supplemented a set percentage of BW and forage was fed ad libitum, the proportion of DGS in the WDGS diets was slightly greater than that in the DDGS diet. The G:F values were not different ($P = 0.55$; Table 8).

When comparing levels of supplementation, ending BW ($P < 0.01$) exhibited a linear increase of 40.9 kg from the 0.33% BW level of supplementation to the 1.0% BW level of supplementation (Table 9). Additionally, ADG ($P < 0.01$) increased linearly with the 0 level of supplementation gaining 0.70 kg/d and the 1.0% BW level of supplementation gaining 1.20 kg/d. Gain efficiency improved linearly ($P < 0.01$) with increasing levels of distillers grains supplementation.

Exp. 2

There were no block * dietary treatment interactions. Therefore, only the main effects of growth performance due to dietary treatment are presented. There were no differences for ending BW ($P = 0.07$; Table 10). By design, DMI was similar between treatments ($P = 0.72$). Although not different ($P < 0.11$), ADG and G:F were numerically improved for WDGS compared to the DRC diet (Table 10).

Exp. 3

There were no type * level interactions ($P > 0.81$; Table 11). Therefore, only the main effects of growth performance due to energy source and level are presented. There was no difference ($P > 0.13$; Table11) for ending BW. By design, DMI was identical

among treatments ($P = 1.00$). Cattle consuming diets containing WDGS gained 0.10 kg more per d than cattle consuming diets with DRC ($P < 0.01$; Table 11). Gain efficiency was also improved for cattle consuming WDGS ($P < 0.01$) due to greater ADG and constant DMI.

Ending BW responded quadratically ($P < 0.01$) with increasing level of energy with the LOW level being the lightest at the conclusion of the experiment (Table 12). Dry matter intake was not different among levels ($P = 0.18$). There was a quadratic response for ADG with the MEDIUM and HIGH levels of DRC and WDGS gaining 0.22 and 0.31 kg per day more compared to LOW, respectively. Consequently, feed efficiency was improved with increased level of either DRC or WDGS ($P < 0.01$).

Exp. 4

There were no interactions between inclusion (low or high) and type of feed (DRC, DDGS or WDGS). By design, type of feed (DRC, DDGS or WDGS) did not impact initial BW, ending BW, DMI, ADG or G:F (Table 13). The main effect of inclusion level is shown in Table 14. Ending BW and ADG increased linearly as the level of energy increased in the diet, while G:F linearly increased ($P < 0.01$). This linear improvement was expected, since as the amount of grain or byproduct included increased, so did the level of energy. Intake was not different between levels ($P = 0.64$).

Pooled Analysis

The predicted DRC inclusions at 15 and 30% DGS were 27.74 and 54.71% (Figure 2). Predictions for the DRC inclusions were done by regressing DGS inclusion by ADG. Using the observed ADG at 15% inclusion DGS we evaluated the regression lines and determined DRC inclusion at the same ADG. The NRC DRC diet equivalent to 15%

WDGS was evaluated with the NRC model. Net Energy adjuster of 103.2 was needed to predict the observed gain. Based on Loy et al. (2008), the DRC was given an energy value of 83% TDN. The same NE adjuster was used with the 15% WDGS diet. The energy value of the WDGS was changed until the ADG for that diet (0.97 kg/d) was achieved. That energy value was 113.5% TDN which is 137% the value of DRC.

The same process was used to estimate the TDN content of the DGS when fed at 30% of diet dry matter. In this case, the DRC diet contained 54.71% DRC and a NE adjuster of 96.8 was needed to predict the ADG of 1.22 kg/d. The energy value of the WDGS was 112.7% TDN which is 136% the value of DRC.

DISCUSSION

Pooled Analysis

Relative to Exp. 2, Exp. 3 and the study conducted by Loy et al. (2008), calves showed improved ADG and G:F with DGS usage compared to feeding DRC and Soypass®. The improvement in ADG and feed efficiency occurred even though the same ADG was targeted between DGS and DRC diets. This is likely due to the low level of starch and energy density of fat, undegraded protein and corn fiber in DGS. In Exp. 4, no difference was observed for ADG or G:F; this was due to targeting equal ADG between DGS and DRC prior to the initiation of the experiment in a similar manner to Exp. 2 and Exp. 3. Firkins et al. (1985) conducted 8 trials utilizing WDG and DDG in finishing diets and found that when feeding at 50% diet DM animal performance met or exceeded that of corn based finishing diet, reiterating that DG has a greater energy value relative to corn. Additionally, increasing inclusion of distillers grains supplemented in Exp. 1, Exp. 3 and Exp. 4, increased ending BW, ADG and G:F. According to Loy et al. (2008),

similar results were observed when heifers were fed greater levels of DDGS and DRC plus corn gluten meal compared to DRC. Greater inclusions of DGS in forage based diets elicits a greater growth performance response.

Griffin et al. (2012) found that ADG increased and total intake responded quadratically to increasing levels of DDGS supplementation similar to DMI in Exp. 1. Conversely, data suggest large differences in the ADG response to DDGS supplementation (Griffin et al., 2012). Griffin et al. (2012) discussed that nutrient composition of distillers evaluated from several different studies (Spiehs et al., 2002; Buckner et al., 2011) have shown variation between batch production and plant, CP ranged from 28.7 to 34.0% and fat ranged from 8.8 to 13.3%. Crude protein is composed of degradable intake protein (DIP) and undegradable in take protein (UIP) and NPN. Undegradable intake protein is an important factor in cattle diets, more specifically growing calves. MacDonald et al. (2007) determined the UIP content of DDGS to be between 15 to 20% on a DM basis. In a review conducted by Klopfenstein (1996) evaluating UIP supplementation in growing cattle, he discussed that at greater inclusions of supplemented UIP, gain increases. The increased gain is due to the UIP meeting a metabolizable protein deficiency plus added energy from the type of supplement included in the diet (Klopfenstein, 1996). Differentiating the difference in response between protein and energy is a challenge due to the potential to increase microbial production with energy supplementation and not being able to determine whether MP has come from microbial residue or protein supplementation (Griffin et al., 2012).

Rapid fermentation of starch in DRC based diets decreases rumen pH, which, when coupled with other mechanisms of starch and fiber digestion, can affect fibrolytic

activity in the rumen (Fieser and Vanzant, 2004). Farlin (1981) found that replacing corn with WDG at 25, 50 and 75% in a finishing diet resulted in more energy per kilogram of DM than corn being replaced in the diet. This was despite the fact that the starch, which is an excellent energy source, had been removed from the product. The increased feeding value of DGS in relation to DRC is attributed to the decreased negative associative effects on fiber digestion that are observed with increasing levels of starch, as well as the higher fat content of the WDGS. Loy et al. (2008) used the NRC (1996) model to predict actual cattle performance. Due to under predicted cattle performance at lower rates of gain, NE adjusters, within the model, were increased above 100%. Adjustments made to the NE adjusters are used for energy (TDN) calculations in the experiment conducted by Loy et al. (2008). Loy et al. (2008), suggested the TDN concentration, predicted using the NRC (1996), of DDGS declined as the level of DDGS inclusion increased. Loy et al. 2008) theorized that the decline in energy was due to the DDGS fat content as the inclusion of DDGS increased in the diet. Loy et al. (2007) theorized that this increase in fat concentration due to greater inclusion of DDGS had negative impacts on ruminal fibrolytic activity. MacDonald et al. (2007) conducted an experiment utilizing grazing heifers supplemented with DDG, CGM or corn oil (OIL). Cattle supplemented DDG showed a linear increase in ADG whereas OIL did not affect ADG. MacDonald et al. (2007) stated that an associative effect relative to protein and fat available from DDG may cause the additional gain observed in cattle supplemented with DDG.

Several experiments conducted by Corrigan et al. (2009) examined the effects of feeding different levels of DDG and differing proportions of condensed distillers solubles (CDS) added back to DDG. As expected, as inclusion of DDG increased, ADG increased.

Steers responded quadratically to the 2 greatest CDS levels when supplemented with DDG at 0.5 and 0.75% of BW. However, ADG decreased at the greatest CDS level and when supplemented with DDG at 1.0% of BW, suggesting that the fat inclusion in the diet had a limiting effect on digestibility. Wilken et al. (2009) conducted an experiment comparing ensiled CDS and WDGS fed to growing calves at differing levels. Similar to our experiments, as level of byproduct increased, final BW, ADG and G:F increased. Laboratory analysis performed on the feed ingredients showed fat to be greater for CDS than WDGS (Wilken et al., 2009). Cattle fed WDGS had improved G:F and compared to those fed CDS at the same inclusion (Wilken et al., 2009). Conclusions from these experiments indicate that fat available from byproducts may affect growth performance at increased inclusions (Corrigan et al., 2009; Wilken et al., 2009). Fat combined with UIP are excellent energy sources (Loy et al., 2008). Hess et al. (2008) suggests that total fat should not exceed 2-3% of the diet DM to prevent any negative associative effects. The quadratic response, observed in Exp. 3, may be attributed to fat exceeding 3% of the diet DM when feeding WDGS at 35%. With the exception of the 35% inclusion of WDGS in Exp. 3, fat did not exceed 3% of the diet DM in the other experiments. Increased UIP and fat concentration relative to corn is additive when fed to growing cattle consuming a forage based diet, however fat inclusions exceeding 3% can have a negative effect on forage digestion. (MacDonald et al., 2007; Hess et al., 2008; Corrigan et al., 2009).

Quadratic effects of ADG and G:F observed in Exp. 3 may be due to the higher fat content in WDGS being fed at an increased level compared to the DRC diet. Another explanation could be due to the way the brome hay was utilized when compared to that of Exp. 4. Solely reducing the brome hay in the diet in Exp. 3 versus reducing the blend of

sorghum silage:brome hay in Exp. 4 may have impacted the NDF content present in the DRC diet. Brome hay, in Exp. 2, was treated in the same manner as Exp. 3, however only numerical differences were observed. The pooled data are evaluated as such to account for variation among studies. The energy values for DDGS determined previously (Loy et al., 2008) were 130% when DDGS was fed at 10% of diet dry matter and 118% when fed at 33% of diet DM. The energy value from our experiments is slightly greater than determined by Loy et al. (2008) at 137% energy value fed at 15% diet DM and 136% energy value relative to corn fed at 30% of the diet DM. The difference in energy value may be attributed to supplementing cattle at a percent of BW versus feeding a fixed amount in the diet. Cattle were supplemented either 0.21 or 0.81% of BW which is a relatively small inclusion of DDGS in the diets of Loy et al. (2008) compared to DGS inclusion in our experiment (pooled data). The number of observations included in the pooled data suggest greater accuracy in the predicted energy values relative to Loy et al. (2008).

Wet versus Dry DGS

Data from Exp. 1 suggest that there is no difference between type (WDGS or DDGS) of supplementation. As the level of supplementation increased so did ADG, suggesting that calves were meeting MP requirements. Protein content and energy of the supplement fed were determined to be the cause for increased ADG in response to DDGS supplementation (MacDonald et al., 2007). According to MacDonald et al. (2007), approximately one third of the gain response is due to the protein deficiency being met through DDGS supplementation. Due to animal requirements established using the NRC (1996), steers utilized in Exp. 1 were on the lighter in BW than for cattle evaluated in the

meta-analysis conducted by Griffin et al. (2012). Griffin et al. (2012) discussed several factors that may affect gain response in young cattle that tend to have lighter BW. One factor that may affect gain response is that lighter cattle require a greater level of metabolizable protein per kilogram of BW compared to heavier cattle. A second factor to consider is the energy response for lighter cattle, as these animals are more efficient than heavier cattle.

Bremer et al. (2011) combined 14 experiments in a meta-analysis, in which varying levels of WDGS were fed in finishing diets. Results showed that feeding WDGS increased ADG and G:F, compared to that of cattle on a traditional corn based finishing diet. Studies used in the meta-analysis (Bremer et al., 2011) utilized WDGS and DDGS at 10, 20, 30, 40 and 50% (DM basis) analysis showed a quadratic response in feed efficiency. Optimum feeding level for WDGS (Bremer et al., 2011) was between 30 to 40% (DM basis) and 10 to 20% for DDGS DM of diet (Bremer et al., 2011). The energy value of WDGS and DDGS was between 143-130% and 112% respectively, relative to corn (Bremer et al., 2011). Contrary to Exp. 1-4, Bremer et al. (2011) found that as moisture content of DGS decreased so did the energy value.

Without a direct comparison in all four experiments, we cannot conclude that WDGS has more energy in forage diets than DDGS. However, data from Exp. 1 and Exp. 4 show there is no difference in energy value between WDGS and DDGS. There were no statistical differences in growth performance between DDGS and WDGS. Wilken et al. (2009) conducted an experiment comparing DDGS and modified distillers grains plus solubles (MDGS) ensiled with wet (corn silage) or dry (oat hay : oat straw mix and DRC) forage. Wilken et al. (2009) found no interaction between forage type and byproduct

type. Similar to Exp. 1, there was no statistical difference in ending BW, DMI, ADG or G:F between DDGS and MDGS. Growth performance from this experiment (Wilken et al., 2009) also support evidence from Exp. 1 and Exp. 4 that energy value of DDGS and WDGS is not different in forage based diets. This experiment (Wilken et al., 2009) reiterates that type of distillers (modified or dry) does not have a significant impact on performance of growing calves.

Low Quality Forage : WDGS mixes

Increased inclusion of byproducts in forage based and finishing diets have increased the amount of byproducts kept on site. Byproduct storage can be done in a bunker or silo bags, similar to silage. Storing WDGS has been successful by mixing the byproduct with low quality forages (Adams et al., 2008). The MIX treatment included in Exp. 1 shows that wet byproduct can be stored over a period of time and used to supplement growing steers with minimal efficiency losses relative to feeding DGS. Researchers conducted experiments utilizing ensiled mixes of WDGS:straw that have shown improved ending BW, ADG and G:F compared to forage based diets. Steers fed a mix, either 35:65 or 45:55, of ensiled or fresh WDGS:straw were more efficient as WDGS increased in the diet (Peterson et al., 2009). For cattle fed WDGS at 35% of the diet, G:F was 0.092 and 0.120 with 45% WDGS, ADG 0.44 kg and 0.50 kg, respectively (Peterson et al., 2009). Buckner et al. (2010) conducted a similar experiment using ensiled WDGS and straw at ratios of 30:70 and 45:55. Increasing level of WDGS ensiled with straw increased ending BW, ADG, and G:F (Buckner et al., 2010). Feeding the MIX supplement in Exp. 1 decreased DMI without affecting G:F compared to supplementation with DDGS or WDGS. Palatability of WDGS : straw mixes increased as the inclusion of

DGS increased compared to mixes with lower DGS inclusions (Peterson et al., 2009; Buckner et al., 2010). Therefore the level of WDGS : straw in the MIX was included in Exp. 1 to demonstrate that similar growth performance, relative to feeding DGS, can be achieved when using stored WDGS. Data from Exp. 1 suggest that mixes of WDGS and straw (67:33 blend) will store, be palatable, and will reduce grazed forage intake.

IMPLICATIONS

These experiments reiterate that distillers grains (dry or wet) have a high energy value relative to supplemented corn in forage-based diets. The moisture content of DGS does not affect the energy value relative to DRC in a forage based diet, however inclusion of DGS responds quadratically after reaching 35% of the diet DM. The energy density of fat, undegradable protein and corn fiber are the possible reasons contributing to greater energy value compared to corn as a supplement.

LITERATURE CITED

Adams, D.R., M. F. Wilken, B.L. Nuttelman, L.M. Kovarik, J.R. Benton, M.A. Greenquist, G.E. Erickson, T.J. Klopfenstein and R.J. Rasby. 2008. Evaluation of storage methods for wet distillers grains plus solubles with added forages. Nebraska Beef Report. MP91:23-25.

AOAC. 1999. Official Methods of Analysis. 16th ed. AOAC, Arlington, VA.

Block, H. C.,T. J. Klopfenstein and G. E. Erickson. 2006. Evaluation of average daily gain prediction by level one of the 1996 National Research Council beef model and development of net energy adjusters. J. Anim. Sci. 84:866-876.

Bremer, V.R., A.K. Watson, A.J. Liska, G.E. Erickson, PAS, G. Cassman, K.J. Hanford and T.J. Klopfenstein. 2011. Effect of distillers grains moisture and inclusion level in livestock diets on greenhouse gas emissions in the corn-ethanol-livestock life cycle. Prof. Anim. Sci. 27:449-455.

Buckner, C.D., T.J. Klopfenstein, G.E. Erickson, W.A. Griffin and J.R. Benton. 2010. Comparing ensiled or fresh mixed wet distillers grains with solubles with straw at two inclusions in growing calf diets. Nebraska Beef Report. MP93:42-43.

Buckner, C.D., M.F. Wilken, J.R. Benton, PAS, S.J. Vanness, V.R. Bremer, T.J. Klopfenstein, P.J. Kononoff and G.E. Erickson, PAS. 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27:57-64.

Buckner, C.D., T.J. Klopfenstein and G.E. Erickson. 2013. Evaluation of modification to the neutral detergent-fiber analysis procedure for corn and distillers grains plus solubles. Prof. Anim. Sci. 29:252-259.

Corrigan, M.E., T.J. Klopfenstein, G.E. Erickson, N.F. Meyer, K.J. Vander Pol, M.A. Greenquist, M.K. Luebbe, K.K. Karges and M.L. Gibson. 2009. Effects of level of condensed distillers solubles in corn dried distillers grains on intake, daily body weight gain, and digestibility in growing steers fed forage diets. J. Anim. Sci. 87:4073-4081.

Farlin, S.D. 1981. Wet distillers grains for cattle. Anim. Nutr. Health. 36:35.

Fieser B.G. and E.S. Vanzant. 2004. Interactions between supplement energy source and tall fescue hay maturity on forage utilization by beef steers. J. Anim. Sci. 82:307-318.

Firkins, J.L., L.L. Berger and G.C. Fahey, Jr. 1985. Evaluation of wet and dry distillers grains and wet and dry corn gluten feeds for ruminants. J. Anim. Sci. 60:847-860.

Griffin, W.A., V.R. Bremer, T.J. Klopfenstein, L.A. Stalker, L.W. Lomas, J.L. Moyer and G.E. Erickson. 2012. A meta-analysis evaluation of supplementing dried distillers grains plus solubles to cattle consuming forage-based diets. Prof. Anim. Sci. 28:306-312.

Klopfenstein, T.J. 1996. Need for escape protein by grazing cattle. Anim. Feed Sci. Technol. 60:-199.

Loy, T. W., J. C. Macdonald, T. J. Klopfenstein, and G. E. Erickson. 2007. Effect of distillers grains or corn supplementation frequency on forage intake and digestibility. J. Anim. Sci. 85:2625-2630.

Loy, T. W., T. J. Klopfenstein, G. E. Erickson, C. N. Macken, and J. C. MacDonald. 2008. Effect of supplemental energy source and frequency on growing calf performance. J. Anim. Sci. 86:3504-3510.

MacDonald, J.C., T.J. Klopfenstein, G.E. Erickson and W.A. Griffin. 2007. Effects of dried distillers grains and equivalent undegradable intake protein or ether extract on performance and forage intake of heifers grazing smooth bromegrass pastures. J. Anim. Sci. 85:2614-2624.

Mertens, D.R., M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, K. Frank, D. Gambin, M. Garkie, B. Gildemeister, D. Jeffress, C-S. Jeon, D. Jones, D. Kaplan, G-N. Kim, S. Kobata, D. Main, W. Moua, B. Paul, J. Roberston, D. Taysom, N. Thiex, J. Williams and M. Wolf. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing beakers or crucibles: collaborative study. J. Assoc. Off. Anal. Chem. 85:1217-1240.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Nuttelman, B. L., W. A. Griffin, G. E. Erickson and T. J. Klopfenstein. 2011. Comparing dry, wet or modified distillers grains plus solubles on feedlot cattle performance. Nebraska Beef Report. MP94:50-52.

Peterson, M.M., M.K. Luebbe, R.J. Rasby, T.J. Klopfenstein, G.E. Erickson, L.M. Kovarik. 2009. Level of wet distillers grains plus solubles and solubles ensiled with wheat straw for growing steers. Nebraska Beef Report. MP92:35-36.

Stock, R., T. Klopfenstein, D. Brink, S. Lowry, D. Rock and S. Abrams. 1983. Impact of weighting procedures and variation in protein degradation on measured performance of growing lambs and cattle. J. Anim. Sci. 57:1276-1285.

Vander Pol, K.J., G.E. Erickson, T.J. Klopfenstein, M.A. Greenquist and T. Robb. 2006. Effect of dietary inclusion of wet distillers grains on feedlot performance of

finishing cattle and energy value relative to corn. Nebraska Beef Report. MP88-A:51.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583.

Watson, A.K., B.L. Nuttelman, T.J. Klopfenstein, L.W. Lomas and G.E. Erickson. 2013. Impacts of a limit-feeding procedure on variation and accuracy of cattle weights. J. Anim. Sci. 91:5507-5517.

Wilken, M.F., M.K. Luebbe, G.E. Erickson, T.J. Klopfenstein and J.R. Benton. 2009. Feeding corn distillers solubles or wet distillers grains plus solubles and cornstalks to growing calves. Nebraska Beef Report. MP92:30-32.

Wilken, M.F., T.L. Mader, G.E. Erickson and L.J. Johnson. 2009. Comparison of dry distillers or modified wet distillers grains plus solubles in wet or dry forage-based diets. Nebraska Beef Report. MP92:33-34.

Table 1. Nutrient composition of dietary ingredients fed to growing steers evaluating the performance differences between DDGS, WDGS or MIX supplementation Exp. 1 (DM basis)

Nutrient	WDGS[1]	DDGS[1]	MIX[1]	Alfalfa Hay	Sorghum Silage
DM	32.69	92.25	42.33	87.13	33.85
OM	96.01	95.69	94.03	91.35	91.58
CP	30.32	29.69	20.48	17.91	7.90
NDF	34.73	28.87	51.50	52.37	57.40
Fat	11.46	11.12	8.12	--	--
S	0.73	1.06	0.62	0.23	0.13

[1]WDGS- Wet Distillers Grains plus Solubles; DDGS- Dry Distillers Grains plus Solubles; MIX-67% WDGS and 33% Ground Wheat Straw

Table 2. Diet (DM basis), fed to growing steers evaluating the energy value of WDGS relative to DRC in Exp. 2

	Diet Treatment	
Ingredients	DRC[1]	WDGS[1]
DRC	33.60	--
WDGS	--	25.00
Grass Hay	26.41	39.05
Sorghum Silage	35.00	35.00
Supplement		
Urea	0.90	0.30
Soypass®	3.35	--
Limestone	0.24	0.24
Salt	0.30	0.30
Selenium	0.01	--
Trace minerals premix	0.05	0.05
Vitamin ADE premix	0.15	0.15
Tallow	0.12	0.02

[1]DRC- Dry Rolled Corn; WDGS- Wet Distillers Grains plus Solubles

Table 3. Nutrient composition of dietary ingredients fed to growing steers evaluating the energy value of WDGS relative to DRC in Exp. 2 (DM basis)

Nutrient	WDGS[1]	DRC[1]	Grass Hay	Sorghum Silage
DM	33.73	87.65	86.07	32.53
OM	95.63	98.93	92.69	89.73
CP	31.73	9.51	7.70	8.02
NDF	35.63	14.21	74.48	63.41
Fat	10.98	2.98	--	--
S	0.95	0.13	0.15	0.13

[1]WDGS- Wet Distillers Grains plus Solubles; DRC- Dry Rolled Corn

Table 4. Diet (DM basis), fed to growing steers evaluating the energy value of WDGS relative to DRC in Exp. 3

	WDGS[1]			DRC[1]		
Item	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
WDGS	15.0	25.0	35.0	--	--	--
DRC	--	--	--	22.0	41.0	60.0
S.Silage[1]	30.0	30.0	30.0	30.0	30.0	30.0
Grass hay	52.8	42.8	32.8	42.5	24.6	6.8
Supplement						
Urea	0.8	0.8	0.8	1.0	1.3	1.6
Soypass®	--	--	--	3.0	1.5	--
Limestone	0.99	0.99	0.99	0.97	1.08	1.18
Salt	0.30	0.30	0.30	0.30	0.03	0.30
Selenium	--	--	--	0.01	0.01	0.01
Trace minerals premix	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin ADE premix	0.02	0.02	0.02	0.02	0.02	0.02
Tallow	0.05	0.05	0.05	0.14	0.11	0.08

[1] WDGS – Wet Distillers Grains plus Solubles; DRC – Dry Rolled Corn; S. Silage – Sorghum Silage

Table 5. Nutrient composition of dietary ingredients fed to growing steers evaluating the energy value of WDGS relative to DRC in Exp. 3 (DM basis)

Nutrient	WDGS[1]	DRC[1]	Grass Hay	Sorghum Silage
DM	33.67	87.22	85.21	35.73
OM	95.25	99.00	92.75	90.46
CP	31.32	9.05	8.43	6.82
NDF	36.08	13.34	77.3	58.82
Fat	12.08	3.87	--	--
S	0.80	0.12	0.14	0.11

[1]WDGS- Wet Distillers Grains plus Solubles; DRC- Dry Rolled Corn

Table 6. Diet (DM basis), fed to growing steers evaluating the energy value of DDGS and WDGS relative to DRC including a control in Exp. 4

	Diet Treatment						
	Control[1]	DRC[1]		DDGS[1]		WDGS[1]	
Ingredients	60:40	22	50	15	30	15	30
Grass Hay	56.52	43.08	26.26	49.5	40.5	49.5	40.5
Sorghum Silage	37.68	28.72	17.44	33.0	27.0	33.0	27.0
DRC	-	22.0	50.0	-	-	-	-
DDGS	-	-	-	15.0	30.0	-	-
WDGS	-	-	-	-	-	15.0	30.0
Supplement							
Urea	0.65	1.05	1.51	1.13	1.13	1.13	1.13
Soypass®	3.80	3.70	3.45	-	-	-	-
Limestone	0.82	0.943	0.943	0.943	0.943	0.943	0.943
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Trace minerals premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin ADE premix	0.015	0.15	0.015	0.15	0.015	0.15	0.15
Tallow	0.141	0.151	0.157	0.061	0.061	0.061	0.061

[1]Control- 60% Grass Hay and 40% Sorghum Silage; DRC- Dry Rolled Corn; DDGS- Dry Distillers Grains plus Solubles; WDGS- Wet Distillers Grains plus Solubles

Table 7. Nutrient composition of dietary ingredients fed to growing steers evaluating the energy value of DDGS and WDGS relative to DRC in Exp. 4 (DM basis)

Nutrient	WDGS[1]	DDGS[1]	DRC[1]	Brome Hay	Sorghum Silage
DM	36.33	88.80	86.48	87.61	35.31
OM	95.41	95.40	98.78	93.16	90.59
CP	31.02	30.23	8.68	8.46	6.83
NDF	35.69	40.68	11.36	69.18	67.14
Fat	11.44	10.78	3.52	--	--
S	0.66	0.69	0.12	0.14	0.11

[1]WDGS- Wet Distillers Grains plus Solubles; DDGS- Dry Distillers Grains plus Solubles; DRC- Dry Rolled Corn

Table 8. Growth performance characteristics of growing steers being supplemented DDGS, WDGS or MIX for 113 d in Exp. 1

Item	DDGS[1]	WDGS[1]	MIX[1]	SEM	*P*-Value
Initial BW, kg	269	269	268	1.5	0.44
Ending BW, kg	366	362	355	5.5	0.12
ADG, kg/d	1.13	1.09	1.03	0.06	0.20
G:F	0.149	0.158	0.155	0.01	0.55

[1]DDGS- Dry Distillers Grains plus Solubles; WDGS-Wet Distillers Grains plus Solubles; MIX- 67% WDGS and 33% Straw blend
[2]DMI found Figure 1

Table 9. Growth performance characteristics evaluating growing steers being supplemented at 4 differing levels of DDGS[1], WDGS[1] or MIX[1] for 113 d in Exp. 1

Item	0[2]	0.33[2]	0.67[2]	1.0[2]	SEM	Linear	Quadratic
Initial BW, kg	270	268	268	269	1.5	0.33	0.75
Ending BW, kg	330	351	360	372	5.5	<0.01	0.70
ADG, kg/d	0.70	0.98	1.08	1.20	0.06	<0.01	0.78
G:F	0.113	0.140	0.148	0.174	0.01	<0.01	0.23

[1]DDGS- Dry Distillers Grains plus Solubles; WDGS- Wet Distillers Grains plus Solubles; MIX- 67% WDGS and 33% Straw blend

[2]Supplement level as a percentage of BW

[3]DMI found Figure 1

Table 10. Growth performance characteristics of growing steers being fed DRC or WDGS for 67 d in Exp. 2

	DRC[1]	WDGS[1]	SEM	*P*-value
Initial BW, kg	286	286	0.45	0.48
Ending BW, kg	369	375	2.7	0.07
DMI, kg/d	8.13	8.05	0.32	0.72
ADG, kg	1.24	1.31	0.04	0.11
G:F	0.151	0.163	0.01	0.25

[1]DRC- Dry Rolled Corn; WDGS- Wet Distillers Grains plus Solubles

Table 11. Growth performance characteristics of growing steers being fed DRC or WDGS for 83 d in Exp. 3

	DRC[1]	WDGS[1]	SEM	*P*-value
Initial BW, kg	232	231	2.7	0.82
Ending BW, kg	316	323	3.2	0.13
DMI, kg/d	7.18	7.18	0.11	1.0
ADG, kg	1.00	1.10	0.02	<0.01
G:F	0.140	0.153	0.003	<0.01

[1] DRC – 22, 44 or 60% Dry Rolled Corn; WDGS – 15, 25 or 35% Wet Distillers Grains plus Solubles

Table 12. Growth performance characteristics evaluating growing steers being fed at 3 differing levels of WDGS[1] or DRC[1] for 83 d in Exp. 3

	LOW[2]	MEDIUM[2]	HIGH[2]	SEM	*P*-value	Linear	Quadratic
Initial BW, kg	230	232	232	3.2	0.93	__	__
Ending BW, kg	304	325	331	3.6	<0.01	0.28	<0.01
DMI, kg/d	7.09	7.32	7.14	0.13	0.38	0.35	0.18
ADG, kg	0.87	1.09	1.18	0.03	<0.01	0.10	<0.01
G:F	0.123	0.161	0.165	0.004	<0.01	0.02	<0.01

[1]DRC- Dry Rolled Corn; WDGS- Wet Distillers Grains plus Solubles

[2] LOW – 15% WDGS or 22% DRC; MEDIUM – 25% WDGS or 44% DRC; HIGH– 35% WDGS or 60% DRC

Table 13. Growth performance characteristics of growing steers being fed DRC, DDGS or WDGS for 84 d in Exp. 4

Item	DRC[1]	DDGS[1]	WDGS[1]	SEM	*P*-value
Initial BW, kg	282	283	282	3.5	0.97
Ending BW, kg	365	364	362	4.0	0.87
DMI, kg/d	7.23	7.36	7.18	0.10	0.42
ADG, kg	0.99	0.97	0.96	0.03	0.78
G:F	0.137	0.132	0.135	0.01	0.61

[1]DRC- 22 or 50% Dry Rolled Corn; DDGS- 15 or 30% Dry Distillers Grains plus Solubles; WDGS- 15 or 30% Wet Distillers Grains plus Solubles

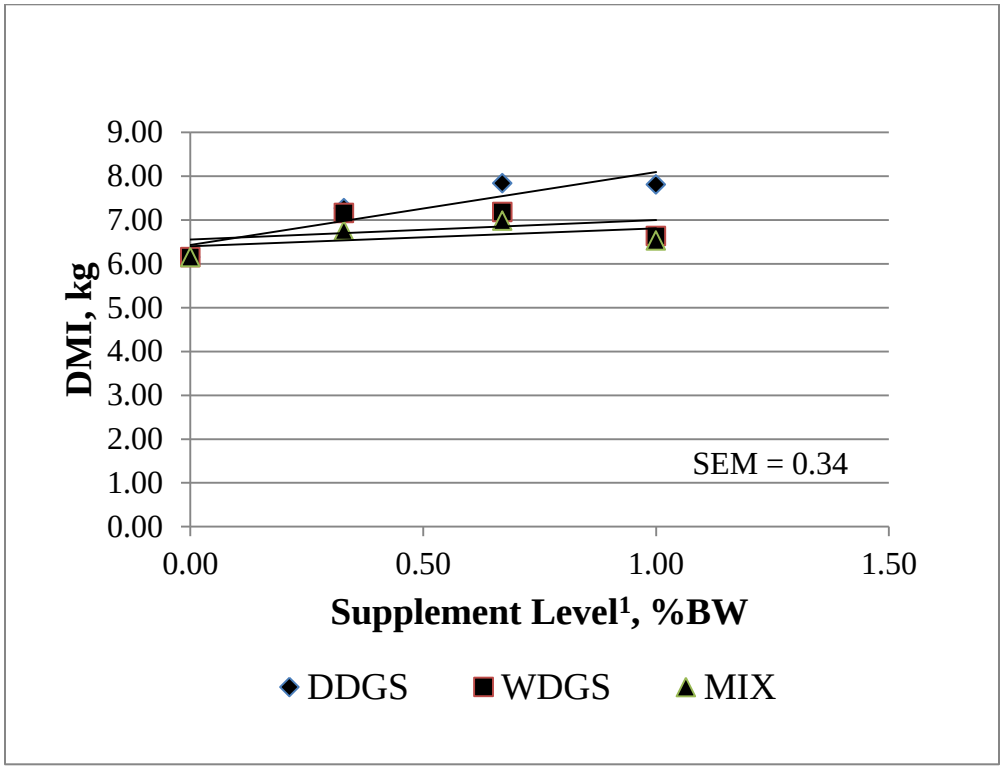
Table 14. Growth performance characteristics evaluating growing steers being fed at 3 differing levels of DRC[1], DDGS[1] or WDGS[1] for 84 d in Exp. 4

Item	Control[2]	Low[2]	High[2]	Linear	Quadratic
Initial BW, kg	283	282	282	0.92	0.89
Ending BW, kg	337	354	373	<0.01	0.69
DMI, kg/d	6.95	7.05	7.45	<0.01	0.17
ADG, kg	0.65	0.85	1.08	<0.01	0.63
G:F	0.093	0.122	0.146	<0.01	0.52

[1]DRC- Dry Rolled Corn; DDGS- Dry Distillers Grains plus Solubles; WDGS- Wet Distillers Grains plus Solubles

[2]Control- 60:40 Blend of Sorghum Silage: Grass Hay, Low – 15% DDGS, WDGS or 22% DRC, High – 30% DDGS, WDGS or 50% DRC

Figure 1. Effect of supplement level on dry matter intake (DMI), kg on growing steers being supplemented DDGS, WDGS or MIX for 113 d Exp. 1



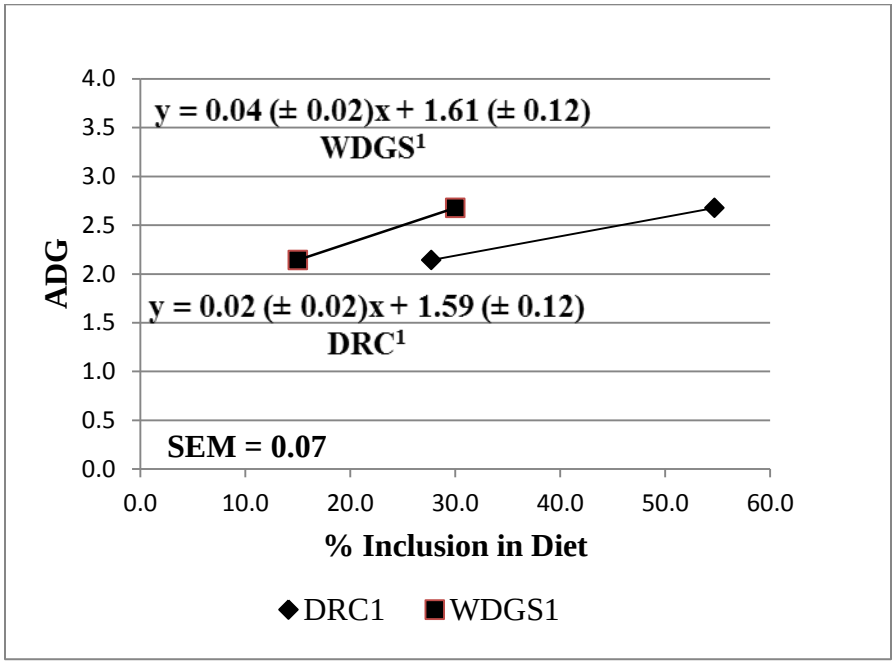
[1]Supplement level (0, 0.33, 0.67, 1.0, %BW): DDGS- Dry Distillers Grains plus Solubles; WDGS- Wet Distillers Grains plus Solubles; MIX- 67% WDGS, 33% Straw blend

[2] DDGS vs MIX *P* =0.004

[3] DDGS vs WDGS *P* =0.005

[4] MIX vs WDGS *P* =0.94

Figure 2. Regression analysis of pooled data for growing steers evaluating the energy value of WDGS relative to DRC



[1]DRC- 22 to 57% inclusion Dry Rolled Corn; WDGS- 15 to 30% inclusion Wet Distillers Grains plus Solubles

Chapter III

In vitro forage standard development based on *in vivo* digestibilities utilizing brome hay, prairie hay and meadow hay.

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ABSTRACT:

Three experiments were conducted to determine *in vivo* digestibility of forages and to compare those forages to *in vitro* digestibility. The objective of these experiments was to develop a calibration set of forages with known *in vivo* digestibilities. In Exp. 1, 8 crossbred yearling steers (323 kg) were used in a 5 x 5 Latin rectangle designed to determine *in vivo* DM digestibility (DMD) and OM digestibility (OMD) of 5 forages. Treatments for Exp. 1 included immature alfalfa, mature alfalfa, immature brome, mature brome and prairie hay. In Exp. 2, 6 crossbred yearling steers (252 kg) were used in a 2 x 3 switchback design to determine *in vivo* DMD and OMD. Treatments for Exp. 2 included low quality brome or immature meadow hay.

Experiment 3 was conducted using 5 forages from Exp. 1 and 2 as standards for *in vitro* digestibility procedures to adjust *in vitro* digestibility values and to estimate *in vivo* values for forage samples with unknown *in vivo* values. The 5 hays included immature brome grass, mature brome grass, prairie hay from Exp. 1 and low quality brome hay and immature meadow hay from Exp. 2. In Exp. 3, eight *in vitro* runs were conducted using the Tilley and Terry two-stage method, including the 5 hay standards and 6 forage samples with unknown *in vivo* digestibilities in order to predict actual *in vivo* values from *in vitro* experimentation. The 6 forage samples were included to demonstrate the in vitro variation using forages with a significant range in digestibility. *In vitro* OMD (IVOMD) of the 5 standard forages was regressed against the *in vivo* OMD values found in Exp.1 and Exp. 2. Using the regression equations generated, the 6 forages with unknown *in vivo* values were adjusted. *In vitro* OMD variation increased ($P < 0.01$) when evaluating forage*run interaction (F-value = 24.70), compared to that of the unadjusted IVOMD

values for the 6 unknown forages (F-value = 19.41). The mean of the 5 standard forages for IVOMD and *in vivo* OMD were determined and the difference between *in vivo* OMD and IVOMD was calculated and used to adjust the same 6 forages. *In vitro* OMD variation for standard mean adjustment (F-value = 19.40) was similar to IVOMD (F-value = 19.41). Standard mean adjustment had the greatest effect on decreasing variation within run (F-value = 14.84). Results from this experiment suggest that the 5 forages can be included as calibration standards in IVOMD determination experiments. However, regression analysis may not be the most accurate process to produce *in vivo* OMD estimates for forages with unknown *in vivo* values.

Keywords: forage quality, *in vivo* digestibility, *in vitro* digestibility, regression analysis

INTRODUCTION

Due to the wide range of species and classifications, there are several factors that define forages. Nutrient content is one factor used in defining forages. Prediction of forage digestibility or energy content is critical to formulate diets. Structural changes in forages throughout the growing season make estimations of digestibility a challenge. Forages are affected not only by year-to-year variation, but also early season to late season variation. Variation causes problems with accurate diet formulations for grazing and growing animals. Properly predicting the appropriate diet is aided by the use of NRC models (2000), however, CP and TDN must be known. Forage estimation of *in vivo* digestibility is challenging (Cochran and Galyean, 1994). Determining intake in grazing animals is difficult (Burns et al., 1994). *In vivo* evaluation of forages help in determining these variations in forages, however, studies can be costly and time consuming. *In vivo* digestion experiments have been conducted to produce forage samples with known *in*

vivo digestibilities to be utilized in laboratory settings. *In vitro* digestibility experiments have been tested since the early 1900s and have been found to be highly correlated with *in vivo* digestibility values (Weiss, 1994). *In vitro* evaluation of forages would allow for large number of forages to be quickly and inexpensively evaluated.

Estimation of *in vivo* digestibility of specific forages using different calibration data sets has been ongoing since the early 1960s. Researchers are able to adjust for variation using regression equations produced from *in vitro* data (Weiss, 1994). The objective of these experiments was to determine *in vivo* digestibility of 5 forages differing in digestibility and use these forages as standards in *in vitro* digestibility procedures to estimate *in vivo* values for unknown forage samples.

MATERIALS AND METHODS

Two *in vivo* digestibility experiments were conducted at the University of Nebraska. Experiment 1 was conducted at the University of Nebraska West Central Research and Extension Center. Experiment 2 was conducted at the University of Nebraska-Lincoln in the Animal Science Complex in Lincoln, Nebraska. Animal use procedures were reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee.

Exp. 1

Experimental Design, Animals and Diet. Eight crossbred yearling steers (323 kg) were used in a Latin rectangle designed to determine *in vivo* DM digestibility (DMD) and OMD of 5 forages. The steers were individually fed using the Calan gate system (American Calan, Inc., Northwood, NH). There were 5 periods and steers were assigned randomly to treatment each period. Experimental periods consisted of 16-d adaptation

followed by a 5-d collection within period. Steers were fed *ad libitum* during the adaptation period. During the collection period, steers were fed at 95% of their established ad libitum intake. Steers were fed once daily at 0800 h following feed refusal collection. Throughout the collection period, feed and feed refusals (orts) were collected, weighed and sampled for later analysis.

Experiment 1 treatments consisted of 5 different chopped hays: immature alfalfa, mature alfalfa, immature smooth bromegrass (Ibrome1), mature smooth bromegrass (Mbrome1) and prairie grass hay (Prairie1; Table 1). According to Weiss (1994), separate equations are needed for: 1) legumes and grasses; 2) corn silage; 3) concentrate feeds and 4) low quality roughages, when evaluating IVOMD adjustment equations. For the purposes of the experiment only Ibrome1, Mbrome1 and Prairie1 will be evaluated for IVOMD. The purpose of determining IVOMD of these 3 grazed forages is to regress IVOMD values against in vivo OMD values, use the forages as standards and develop a regression equation. Prior to the initiation of the experiment, hays were chopped using a tub grinder with a 10-cm screen. To prevent contamination and spoilage of hays after grinding, hays were stored separately in an enclosed building.

Orts were obtained prior to feeding during the collection period. Steers were fed once daily in the morning following collection of feed refusals. Diet samples and feed refusals were collected during collection weeks and composited on a weighted average. Orts and diet samples were dried using a 60^{o}C forced air oven for 48 h (AOAC, 1999; method 4.2.03) and saved for laboratory analysis at a later date. Dry matters were calculated and recorded. Feed refusals and diet samples were analyzed in the laboratory for CP, DM, OM, IVDMD, NDF and ADF.

Total Fecal Collection. Total fecal collection was conducted. Harnesses and fecal bags were fitted to steers on d 16 at 1700 h. Fecal bags were removed at 0700 h and 1700 h each day of the collection period and feces was emptied. Fecal bags were weighed prior to placement on the steers after each collection. The complete fecal collection was weighed by steer after the 1700 h collection. Feces were subsampled for analysis in the laboratory. Subsampled feces were dried in a 60^{o}C force air oven for 48 h (AOAC, 1999; method 4.2.03). Fecal composites were analyzed for CP, DM, OM and NDF.

Laboratory Analysis. Following the digestibility experiment, dried diet, feed refusal and composited fecal samples were ground through a 2-mm screen using a Wiley Mill. A subsample of the composite was taken and ground using a Wiley Mill through 1-mm screen for laboratory analysis. Ash was determined using a muffle furnace for 6 h at 600^{o}C (AOAC, 1999; method 4.1.10) and OM calculated based on ash content (Table 1). Samples from were analyzed for CP (LECO Corp., St. Joseph, MI; AOAC, 1999; method 990.03). Neutral detergent fiber and ADF was determined (Van Soest et al., 1991; Mertens et al., 2002). Feed samples were analyzed for IVDMD and IVOMD (Tilley and Terry, 1963).

In vivo and In vitro Digestibility. Experiment 1 determined *in vivo* digestibility using steer intake and fecal excretion. Specific nutrient digestibility (DM, OM and NDF) were determined using the following equation (Cochran and Galyean, 1994):

$$\%\text{Nutrient digestion} = \left[\frac{\text{Nutrient Fed (kg)} - \text{Nutrients Refused (kg)} - \text{Nutrient in Feces (kg)}}{\text{Nutrient fed (kg)} - \text{Nutrient Refused (kg)}}\right] \times 100$$

The 5 forages from Exp. 1 were used for *in vitro* analysis to determine IVDMD and IVOMD. *In vitro* DMD and IVOMD were determined using the Tilley and Terry method (1963). The inoculum in the Tilley and Terry (1963) procedure was modified to

use a 50:50 mixture of rumen fluid and McDougall's buffer (1948). In order to prevent nitrogen limitation, urea was added to the McDougall's buffer at 1 g urea L^{-1} of McDougall's buffer (1948). Two crossbred yearling steers were used for rumen fluid collection. Donor steers used in Exp. 1 were fed a smooth brome grass hay diet once daily at 1.5% of BW at 0700 h. Equal volumes of rumen fluid were collected from each steer and mixed together prior to preparation with McDougall's buffer (1948). Inoculum was kept anaerobic through continual flush of CO_2 into the mixture

Forage samples (0.5 g) were incubated for 48 h in 100 ml tubes with 50 ml of inoculum. Sample tubes were flushed with CO_2 as inoculum was added to keep the environment anaerobic. The incubation system consisted of a water bath heated to 39^{o}C using a water pump circulator to prevent temperature variation throughout the water bath. During the 48 h, incubation tubes were swirled every 12 h. Forage samples were subjected to a pepsin digestion. During this step, 6 ml of 20% hydrochloric acid and 2 ml of a 5% pepsin solution were added to the tubes. Tubes were swirled to ensure the additional solutions added were mixed properly. Following addition of acid pepsin mixture, tubes were incubated for another 24 h. Subsequent analysis of tubes included filtering tubes, using 541 Whatman filter paper (Fisher Scientific, catalog # 09-851D). Filter plus residue was dried in 100^{o}C forced air oven for a minimum of 6 h, weighed and incinerated in a 600^{o}C muffle furnace for 6 h. *In vitro* DMD and IVOMD were calculated from the residues.

Forage samples from Exp. 1 were evaluated in 21 separate *in vitro* runs to evaluate regression equations developed from *in vivo* and *in vitro* comparisons. The intended purpose of these *in vitro* experiments was to develop regression equations based

on the *in vivo* values determined from the digestibility experiment on the 5 forages. The objective of Exp. 1 was to produce 5 standard hays for *in vitro* use. Following Exp. 1, alfalfa forage samples were removed from the calibration data set because legume inclusion created greater variation in regression equations due to extrapolation of data (Weiss, 1994). Removal of alfalfa from Exp. 1 decreased the range of digestibility in the regression equations. The purpose of Exp. 2 was to add supplemental forage samples to extend the range of forage digestibilities.

***Statistical Analysis*.** Nutrient composition for *in vitro* and *in vivo* data were analyzed using the PROC MIXED procedure of SAS (Version 9.2, SAS Inst. Inc., Cary, NC). Model effects included period and hay as fixed effects and steer as a random effect. The REG procedure of SAS (Version 9.2, SAS Inst. Inc., Cary, NC) tested the regression of *in vivo* and *in vitro* digestibility and tested slope of regression equations. A protected F-test was used to evaluate treatment mean differences. Least square means were separated using Least Significant Difference method when an overall significant treatment ($P < 0.05$) F-test was detected. The IVDMD values from each of the separate runs were regressed against the *in vivo* DMD. The slope of the regression lines were compared for equal slopes. Run differences were tested (Version 9.2, SAS Inst. Inc., Cary, NC).

Exp. 2

Experimental Design, Animals and Diet. Six crossbred yearling steers (252 kg) were used in a switchback design experiment to determine *in vivo* DMD and OMD of 2 forages. Animals were housed in the metabolism research area of the University of Nebraska-Lincoln Animal Science complex in a climate controlled room at 21°C. Cattle

were in individual pens with free access to water and salt blocks. The experiment had three, 21-d periods and steers were assigned randomly to treatment for each period. Experimental periods consisted of a 15-d adaptation followed by a 6-d collection period. Steers were fed ad libitum during the first 7-d of adaptation. From d 8 to 21, steers were fed at 95% ad libitum. Throughout the collection period, feed and feed refusals (orts) were collected, weighed and sampled for later analysis.

Experiment 2 treatments were comprised of low quality smooth bromegrass (Brome2) and immature meadow hay (Meadow2; Table 2). Prior to the initiation of the experiment, hays were chopped using a tub grinder with a 10-cm screen. To prevent contamination and spoilage of hays after grinding, hays were stored separately in an enclosed building. Based on analyses done prior to Exp. 2, Meadow2 hay and Brome2 hay were deficient in degradable intake protein (DIP). In order to make sure DIP was not limiting, hays were top dressed with a 50% urea solution. Based on the DIP potential (DIPP) calculations,

Meadow2 Calculations:

MP Content of Hay
MP (g/kg) = (UIP (g/kg) * 0.8) + (TDN (g/kg) * 0.13 * 0.8 *0.8)
MP (g/kg) = (1.724 * 0.8) + (570 * 0.13 * 0.8 * 0.8)
MP = 48.3232 g/kg
DIP potential (DIPP)
DIPP (g/kg) = (0.13 * TDN (g/kg)) – DIP (g/kg)
DIPP (g/kg) = (0.13 * 570) – 74.8576
DIPP = *-0.7576 g/kg*
Total MP = 48.3232 g/kg

Brome2 Calculations:

MP Content of Hay
MP (g/kg) = (UIP (g/kg) * 0.8) + (TDN (g/kg) * 0.13 * 0.8 *0.8)
MP (g/kg) = (1.854 * 0.8) + (500 * 0.13 * 0.8 * 0.8)
MP = 43.0832 g/kg

DIP potential (DIPP)

DIPP (g/kg) = (0.13 * TDN (g/kg)) – DIP (g/kg)

DIPP (g/kg) = (0.13 * 500) – 80.546

DIPP = *-15.546 g/kg*

Total MP = 43.0832 g/kg

Meadow2 hay was top dressed with 6.27 ml/kg of the 50% urea solution and Brome2 hay with 2.24 ml/kg of the 50% urea solution fed daily.

Orts were obtained prior to feeding during the collection period. Steers were fed once daily in the morning following collection of feed refusals. Diet samples and feed refusals were collected during collection weeks and composited on a weighted average (DM basis). Orts and diet samples were dried using a 60^{o}C forced air oven for 48 h (AOAC, 1999; method 4.2.) and saved for laboratory analysis at a later date. Dry matters were calculated and recorded. Feed and orts were analyzed for CP, DM, OM, IVDMD, IVOMD.

Total Fecal Collection. Total fecal collection was conducted d 16 to 21. Harnesses and fecal bags were fitted to steers on d 15 at 1700 h. Fecal bags were removed at 0700 h and 1700 h each day of the collection period and feces were emptied. Fecal bags were weighed prior to placement on the steers after each collection. The complete fecal collection was weighed by steer after the 1700 h collection then placed in a 22.7 kg mixer. Feces were subsampled for analysis in the laboratory. Subsampled feces were dried in a 60^{o}C forced air oven for 72 h. Once dried, fecal samples were ground through a Wiley Mill with a 2-mm screen. Daily fecal samples were then composited by steer by week and ground using a Wiley Mill with a 1-mm screen prior to laboratory analysis. Fecal composites were analyzed for CP, DM, OM and NDF.

Laboratory Analysis. Following the digestibility experiments, dried diet and feed refusal composited samples were ground through a 2-mm screen using a Wiley Mill. A subsample of the composite was taken and ground using a Wiley Mill through 1-mm screen for laboratory analysis. Ash was determined using a muffle furnace for 6 h at 600^{o}C (AOAC, 1999; method 4.1.10) and OM calculated based on ash content (Table 2). Samples were analyzed for CP (LECO Corp., St. Joseph, MI; AOAC, 1999; method 990.03). Neutral detergent fiber and ADF were determined (Van Soest et al., 1991; Mertens et al., 2002). Feed samples were analyzed for IVDMD and IVOMD (Tilley and Terry, 1963).

In vivo and In vitro Digestibility. Experiment 2 determined *in vivo* digestibility using steer intake and fecal excretion as outlined in Exp. 1. The 2 forages from Exp. 2 were used for *in vitro* analysis to determine IVDMD and IVOMD as outlined in Exp. 1. Inoculum was collected for use in the *in vitro* procedure in a similar manner to Exp. 1. Donor steers utilized in Exp. 2 received 6.8 kg of a 70.5% grass hay, 23.3% dry distillers grains plus solubles (DDGS), 5.8% dry rolled corn (DRC) and 0.36% supplement on an DM basis at 1600 h. The purpose of Exp. 2 was to add supplemental forage samples to extend the range of forage digestibilities.

Statistical Analysis. Nutrient composition of *in vitro* DMD and OMD and *in vivo* DMD, OMD, and indigestible ADF (IADF) data were analyzed using Glimmix procedures of SAS (Version 9.2, SAS Inst. Inc., Cary, NC) to detect differences in digestibility by treatment. Model effects include treatment and period as a fixed effect and calf as a random effect. The REG procedure of SAS was used to plot data of *in vivo* and *in vitro* digestibility to detect differences in digestibility by period.

Exp. 3

The 5 forage samples from Exp. 1 and Exp. 2 (Ibrome[1], Mbrome[1], Prairie1, Brome[2] and Meadow[2]) were included in 8 separate *in vitro* experiments conducted by 1 technician. Experiment 3 utilized 5 forages from the previous 2 experiments to comprise a calibration data set, thus creating laboratory standards. The *in vitro* technique used for Exp. 3 is the same as outlined for Exp. 1 (Tilley and Terry, 1963). Four tubes were utilized per sample type in each *in vitro* analysis. Donor steers utilized in Exp. 3 received 6.8 kg of a 70.5% grass hay, 23.3% DDGS, 5.8% DRC and 0.36% supplement on an DM basis at 1600 h. Forage samples with unknown *in vivo* values were included in each of the 8 *in vitro* runs. These 6 forage samples were included to demonstrate the run-to-run IVOMD variation and the differences between two procedures used to adjust the *in vitro* values. These forage samples included 2 diet samples from cows on pasture in the Sandhills of Nebraska in late June and early August 2010, 2 diet samples from steers on brome pasture in Eastern Nebraska from late May and early August 2008 and 2 corn stalk parts (husk and leaf) harvested in 2010. Estimated *in vivo* values were calculated for the forage samples without known *in vivo* values.

Statistical Analysis. Nutrient composition of *in vitro* OMD data for the 6 forage samples with unknown *in vivo* values were analyzed using the MIXED procedure of SAS (Version 9.2, SAS Inst. Inc., Cary, NC). Model effects included forage, run and the forage*run interaction. A protected F-test was used to evaluate treatment mean differences. Least square means were separated using Least Significant Difference method when an overall significant treatment ($P < 0.05$) F-test was detected.

RESULTS AND DISCUSSION

For the purposes of this experiment, the alfalfa samples from Exp. 1 are excluded because separate regression equations should be developed for legumes. *In vitro* DMD differed between the forage samples used in Exp. 1 and Exp. 2 by design. Maturity of grasses directly affects forage quality by decreasing digestibility as the growing season progresses (Nelson et al., 1994). Forage IVOMD (Table 3) from Exp. 1 ranged from 57.5% Ibrome1, 55.9% Mbrome1 to 53.5% Prairie1. Decreased IVOMD was expected as the maturity of brome hay increased. Prairie1 was selected to represent low quality forage for the regression analysis. Forage IVOMD (Table 4) in Exp. 2 was 47.4 and 58.8% for Brome2 and Meadow2 hay respectively. Similar changes are seen in nutrient composition for forages as maturity increases. According to Johnson et al. (1998), mixed grass samples linearly decreased in CP and IVOMD as maturity increased while NDF, ADF, lignin and RUP increase. This change in forage quality has been well documented (Kamstra et al., 1968; Cogswell and Kamstra, 1976; Lardy et al., 1997).

In Exp. 1, *in vivo* OMD was greatest for Ibrome1 ($P < 0.001$) and lowest for Prairie1 (Table 3). By design *in vivo* OMD of Ibrome1, Mbrome1 and Prairie1 hays were different ($P < 0.01$). Differences were observed in DMI between Ibrome1and Mbrome1($P = 0.02$), with both Ibrome1 and Mbrome1 being different than Prairie1. No differences were observed for Ibrome1, Mbrome1 or Prairie1 when evaluated for IVDMD or IVOMD ($P = 0.15$ and $P = 0.14$, respectively).

As expected, *in vivo* OMD in Exp. 2 was greater for Meadow2 ($P < 0.01$) compared to Brome2 (Table 4). The difference seen between Meadow2 and Brome2 digestibility is likely due to the selection of these forages based on maturity of the

sample. Based on data from in vivo digestion we expected IVDMD and IVOMD to be different for the Brome2 and Meadow2 ($P < 0.01$ and $P < 0.01$, respectively).

In vivo versus In vitro Digestibility. The use of regression equations aids in reducing the prediction error associated with *in vivo* digestibility estimation. According to Weiss (1994), many studies have shown strong statistical correlations ($r > 0.9$) between *in vivo* and *in vitro* digestibility data (Tilley and Terry, 1963; McLeod and Minson, 1974; Genizi et al., 1990). While a strong correlation is important in order to compare *in vivo* to *in vitro* digestibilities, correlation does not mean that *in vivo* digestibility is equal to IVDMD. Conducting a digestibility experiment in pastures is challenging. In order to formulate diets and supplementation for grazing livestock more accurately a calibration set of forages would be needed to more accurately predict *in vivo* digestibility (Lardy et al., 2004; Patterson et al., 2006). Another benefit of the *in vitro* system is adjusting for variation between *in vitro* runs to predict *in vivo* OM digestibility (OMD) from *in vitro* OM disappearance (IVOMD). Precision and accuracy of *in vivo* and *in vitro* experiments is imperative to predict animal performance using the NRC (Patterson et al., 2006).

Data from Exp. 1, a comparison of 21 *in vitro* runs which include the alfalfa hays, were evaluated for forage*run variation and found there was a significant difference ($P < 0.001$) between *in vitro* run. This difference in variation occurs within laboratory between *in vitro* runs when using the same procedure. Regression equations change based on run-to-run variation (e.g. regression equation Run 1 of 21: $y = 0.6048x + 14.133$; Run 13 of 21: $y = 0.469x + 25.42$), therefore regression equations must be generated for each *in vitro* analysis. While no differences were observed ($P = 0.99$) between the slopes of each *in vitro* regression included in the analysis, R^2 ranged from 0.54 to 0.97. Combining the

21 run data set together produced a significant (R^2 = 0.83) correlation between *in vivo* and *in vitro* digestibility using the 5 original forages. On average, *in vitro* digestibility values ran 6.4 percentage units higher than *in vivo* DMD, approximately an 11% difference between *in vivo* and *in vitro* digestibility. This reiterates that *in vitro* digestibility and *in vivo* digestibility are highly correlated, but not equal (Weiss, 1994).

Due to run-to-run variation between *in vitro* runs, generating a separate regression equation using these standard forages for each run is necessary. Weiss (1994) expressed that a universal equation for *in vitro* experiments could not be used and each lab must create individual regression equations. Generating a different regression equation for each *in vitro* analysis is important to account for variation occurring within run, such as technician error, animal variation and analytical technique variation. Tilley and Terry (1963) stress that a minimum of 2 differing feeds should be included in *in vitro* analysis to increase accuracy of *in vivo* digestibility estimates. Inclusion of a standard set of feeds is suggested to be an important factor in accurately predicting *in vivo* digestibility of unknown forages (McLeod and Minson, 1969a, b, 1974).

Removal of the alfalfa hays from the standard set generated in Exp. 1 reduced the range of forage digestibility. Decreasing the range of digestibility used in a regression equation creates a problem when adjusting samples with unknown *in vivo* digestibility values, thus adjustments begin to extrapolate outside the regression range and reduce accuracy of *in vivo* estimate. Harvesting enough forage with high digestibility is a limiting factor in determining *in vivo* digestibilities (Weiss, 1994). Forages used in Exp. 2 were utilized due to preliminary IVDMD analysis suggesting high quality forage (Meadow2) and poor quality forage (Brome2). The 5 forages from Exp. 1 and Exp. 2

(Ibrome1, Mbrome1, Prairie1, Brome2 and Meadow2) were included in 8 *in vitro* analyses with 6 forages samples with unknown *in vivo* digestibilities.

Initially, standard samples were regressed as outlined in Exp. 1. However, when forage samples with unknown *in vivo* digestibility values were tested, a significant increase in forage*run variation occurred once unknown forages were adjusted using regression analysis (Figure 1). This suggests the accuracy of the adjusted *in vivo* estimates decreased. Regression equations generated by 3 different laboratories differed for forages with known *in vivo* digestibilities (Genizi et al., 1990). Variation occurred within a single laboratory testing 2 different water baths, however technician and inoculum utilized in the experiment were the same. Genizi et al. (1990) reported that the residual standard error was 0.0002 higher for unadjusted data compared to the adjusted data. Conclusions from Genizi et al. (1990) imply that data adjusted with regression equations may not reduce variation between *in vitro* and *in vivo* estimates. Genizi did suggest the use of adjustment equations if consistent variation between *in vivo* and *in vitro* digestibility occurs between similar feeds.

Due to significant increase ($P < 0.001$) (Table 5) in forage by run variation using regression analysis to adjust unknown forage samples, a new method of adjustment was tested. Comparing the forage*run variation for unadjusted IVOMD (U-IVOMD) values (F-value = 19.41) to that of forage by run variation of regression equation adjusted IVOMD (RE –IVOMD) values (F-value = 24.70), variation should decrease. The increase in variation lead to the development of the standard mean forage adjustment of IVOMD (SM-IVOMD). Instead of creating regression equations using the individual standard forage samples, the standard mean of the *in vivo* OMD values and the standard

mean of the 5 standard forages for IVOMD were determined. *In vitro* OMD of the averaged standard forages was then subtracted from standard mean *in vivo* OMD. The difference was then used to adjust the forage samples with unknown *in vivo* values. The F-value is used to evaluate the variance of the group mean. Forage*run variation when using the SM-IVOMD technique (F-value = 19.40) is similar to U-IVOMD values (*F*-value = 19.41) and appears to be a more accurate technique for forage adjustment compared to RE-IVOMD adjustment. Run effect is improved utilizing the SM-IVOMD (*F*-value = 14.84) compared to U-IVOMD (*F*-value = 45.98) or RE –IVOMD adjustment (*F*-value = 15.70). Decreasing the run effect is important in accurate estimation of *in vivo* OMD values.

Accuracy in the prediction of *in vivo* or *in vitro* values is based on the ability to predict a value that is as close to the actual value as possible. Obtaining accurate data in a laboratory setting is a challenge especially when conducting *in vitro* analyses. One way to reduce variation is by having each run conducted by the same technician. The 8 *in vitro* runs from Exp. 3 were evaluated in order to determine the standard error of the mean (SEM) and least significant difference (LSD) between the 6 forages with unknown *in vivo* values within each run. Estimated IVOMD values from these 8 runs were within a SEM of 0.41 to 1.10 and LSD range of 1.53 to 3.26. Standard error of the mean and LSD are linked to the sample set (e.g. 6 forage samples with unknown *in vivo* digestibilities). The LSD range from the 6 forage sample set reiterates that being able to detect differences between samples can vary from 1.5 to 3 units. The LSD and SEM indicate that because of run-to-run variation multiple runs must be evaluated to accurately predict *in vivo* estimates.

Using the regression equation determined from the average IVDMD and average *in vivo* DMD values from the 21 runs, including the 5 forages used in Exp. 1, we were able to estimate that there was approximately a 5 unit greater difference between *in vitro* and *in vivo* estimates. However, a 5 unit difference in *in vivo* DMD greatly affects performance of the grazing animal. In order to illustrate the importance of accurate prediction of *in vivo* estimates, a 545 kg lactating cow was evaluated using the NRC (2000). Inputs were set for a lactating cow that was 60 d pregnant and 150 d into lactation consuming a forage-based diet. The TDN of the forage was set at 56%. Dry matter intake was 12 kg / d and NEm balance = 0.27 Mcal. At a TDN of 56% and a positive NEm balance, the lactating cow would gain 1 BCS in 663 d. However, if the TDN of the forage is adjusted to 51%, a 5 unit difference, DMI remains at 12 kg / d and the NEm balance = -1.78 Mcal. With a negative NEm balance the lactating cow would lose 1 BCS in 102 d. Data from an experiment conducted by Watson et al. (2012), evaluated yearling steers grazing smooth bromegrass pastures consuming 8.4 kg / d. Values from Watson et al. (2012) were used in the NRC (2000) to further demonstrate the effect of a 5 unit difference in *in vivo* digestibility estimates. Total digestible nutrients of the smooth bromegrass were set to 65% and ADG = 0.59 kg. When the TDN was changed to 60%, ADG decreased to 0.38 kg. Variation in *in vivo* estimations based on *in vitro* regression analysis can greatly affect predicted performance of grazing cattle. Accurately predicting the digestibility is important to prediction of gains or BCS changes for cattle on pasture.

IMPLICATIONS

Results from this experiment suggest that the 5 forages (Ibrome1, Mbrome1, Prairie1, Brome2 and Meadow2) can be included as standards in IVOMD determination

experiments. However, regression analysis may not be the most accurate process to produce *in vivo* OMD estimates for forages with unknown *in vivo* values. Using the standard mean analysis decreases run-to-run variation and forage*run variation is similar. Standard mean analysis of unknown forage IVOMD values is less variable than regression analysis and should be used for IVOMD adjustments. Inclusion of standard forages allows the technician to at the very least estimate whether the *in vitro* analysis has run properly.

LITERATURE CITED

AOAC. 1996. Official Methods of Analysis 16th Ed.. AOAC, Arlington, VA.

AOAC. 1999. Official Methods of Analysis. 16th ed. AOAC, Arlington, VA.

Burns, J.C., K.R. Pond and D.S. Fisher. 1994. Measurement of forage intake. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Buckner, C.D., M.F. Wilken, J.R. Benton, PAS, S.J. Vanness, V.R. Bremer, T.J. Klopfenstein, P.J. Kononoff and G.E. Erickson, PAS. 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27:57-64.

Cochran, R.C. and M.L. Galyean. 1994. Measurement of *in vivo* digestion by ruminants. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Geisert, B.G. 2007. Development of a set of forage standards to estimate *in vivo* digestibility of forage and prediction of forage quality of diets consumed by cattle grazing Nebraska Sandhills range pastures. Univ. Neb. PhD Dissertation.

Genizi, A., A. Goldmand, A. Yulzari and N.G. Seligman. 1990. Evaluation of methods for calibrating *in vitro* digestibility estimates of ruminant feeds. Anim. Feed Sci. Technol. 29:265-278.

Johnson, J.A., J.S. Caton, W. Poland, D.R. Kirby and D.V. Dhuyvetter. 1998. Influence of season on dietary composition, intake and digestion by beef steers grazing mixed-grass prairie in the northern Great Plains. J. Anim. Sci. 76:1682-1690.

Kamstra, L.D., D.L. Schentzel, J.K. Lewis and R.L. Elderkin. 1968. Maturity study with Western Wheatgrass. J. Range Manage. 21:235-239.

Lardy, G.P., D.C. Adams, T.J. Klopfenstein, D. Clark and J. Lamb. 1997. Seasonal changes in protein degradabilities of Sandhills native range and sub-irrigated meadow diets and application of a metabolizable protein system. Nebraska Beef Report. MP80:3-5.

Lardy, G.P., D.C. Adams, T.J. Klopfenstein and H.H. Patterson. 2004. Building beef cow nutritional program with the 1996 NRC beef cattle requirements model. J. Anim. Sci. 82(Esuppl.):E83.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43:99-109.

McLeod, M.N. and D.J. Minson. 1969a. Sources of variation in the *in vitro* digestibility of tropical grasses. J. Br. Grassl. Soc. 24: 244-249.

McLeod, M.N. and D.J. Minson. 1969b. The use of the *in vitro* technique in the determination of the digestibility of grass/legume mixtures. J. Br. Grassl. Soc. 24:296-298.

McLeod, M.N. and D.J. Minson. 1974. Predicting the organic matter digestibility from *in vivo* and *in vitro* determination of DMD. J. Br. Grassl. Soc. 29:17-20.

McLeod, M.N. and D.J. Minson. 1976. The analytical and biological accuracy of estimating the dry matter digestibility of different legume species. Anim. Feed. Sci. Technol. 1:61-72.

Mertens, D.R., M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, K. Frank, D. Gambin, M. Garkie, B. Gildemeister, D. Jeffress, C-S. Jeon, D. Jones, D. Kaplan, G-N. Kim, S. Kobata, D. Main, W. Moua, B. Paul, J. Roberston, D. Taysom, N. Thiex, J. Williams and M. Wolf. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing beakers or crucibles: collaborative study. J. Assoc. Off. Anal. Chem. 85:1217-1240.

Nelson, C.J. and L.E. Moser. 1994. Plant factors affecting forage quality. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Patterson, H.H., D.C. Adams, T.J. Klopfenstein and G.P. Lardy. 2006. Application of the 1996 NRC to protein and energy nutrition of range cattle. Prof. Anim. Sci. 22:307-317.

Tilley, J.M.A. and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18:104-111.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583.

Watson, A.K., T.J. Klopfenstein, W.H. Schacht, G.E. Erickson, D.R. Mark, M.K. Luebbe, K.R. Brink and M.A. Greenquist. 2012. Smooth bromegrass pasture beef growing systems: Fertilization strategies and economic analysis. Prof. Anim. Sci. 28:443-451.

Weiss, W.P. 1994. Estimation of digestibility of forages by laboratory methods. In: Forage quality, evaluation and utilization. ASA, CSSA, SSSA, Madison, WI.

Table 1. Nutrient composition of dietary ingredients fed to growing steers evaluating the *in vivo* digestibility of 3 forages in Exp. 1 (DM basis)

Nutrient	Ibrome[1]	Mbrome[1]	Prairie[1]
DM	89.8	90.1	87.3
OM	92.6	92.2	91.0
CP	9.3	7.5	7.9
NDF	66.7	69.6	68.3
ADF	40.0	43.7	43.4

[1]Ibrome- immature bromegrass; Mbrome- mature bromegrass; Prairie- prairie hay

Table 2. Nutrient composition of dietary ingredients fed to growing steers evaluating the *in vivo* digestibility of 2 forages in Exp. 2 (DM basis)

Nutrient	Brome[1]	Meadow[1]
DM	89.2	90.7
OM	92.4	89.2
CP	8.2	7.6
NDF	71.4	60.0
ADF	45.4	40.1

[1]Brome- mature bromegrass; Meadow- meadow hay

Table 3. In Vivo and In Vitro digestibility of 3 different hays fed to 8 yearling steers used in a 5 x 5 Latin rectangle Exp. 1

	Diet				
Variable	Ibrome[2]	Mbrome[2]	Prairie[2]	SEM	*P*-value
In vivo					
DMI[3], kg[1]	6.1[a]	5.9[ab]	5.5[c]	0.2	0.02
DMD[3], %	50.9[a]	48.9[ab]	45.9[c]	1.7	<0.01
OMD[3], %	57.4[a]	55.4[ab]	52.9[c]	1.5	<0.01
In vitro					
DMD[3], %	59.1[a]	53.9[ab]	52.8[b]	1.4	0.15
OMD[3], %	62.3[a]	57.9[ab]	49.8[b]	2.5	0.14

[1] DM basis

[2]Ibrome - immature bromegrass; Mbrome - mature bromegrass; Prairie - prairie hay

[3]DMI - dry matter intake; DMD - dry matter digestibility; OMD - organic matter digestibility

[a,b,c]Least square means within row without common superscripts differ ($P < 0.05$)

Table 4. In Vivo and In Vitro digestibility of 2 different hays fed to 6 yearling steers in a 2 x 3 switchback design Exp. 2

	Diet			
Variable	Brome[2]	Meadow[2]	SEM	*P*-value
In vivo				
DMD[1], %	51.3	55.0	0.67	<0.01
OMD[1], %	50.9	60.3	0.73	<0.01
In vitro				
DMD[1], %	47.6	56.3	0.59	<0.01
OMD[1], %	47.4	58.8	0.73	<0.01

[1]DM basis
[2]DMD- dry matter digestibility; OMD- organic matter digestibility
[3]Brome[2]- low quality smooth bromegrass; Meadow[2]- immature meadow hay

Table 5. Comparison of IVOMD adjustment analysis on IVOMD variation. Exp. 3

	F-Value			
Effect	U-IVOMD[1]	RE-IVOMD[2]	SM-IVOMD[3]	*P*-Value
Forage	644.7	689.2	644.3	<0.001
Run	46.0	15.7	14.8	<0.001
Forage*Run	19.4	24.7	19.4	<0.001

[1] U-IVOMD- unadjusted *in vitro* organic matter digestibility
[2] RE-IVOMD- regression equation adjusted *in vitro* organic matter digestibility
[3] SM-IVOMD- standard mean adjusted *in vitro* organic matter digestibility

Figure 1. Organic Matter Digestibility Regression Equations from Exp. 3