5-1-2017

IN VITRO APPARENT RUMINAL DIGESTIBILITY OF DIETS CONTAINING CORN DISTILLERS GRAIN WITH DIFFERENT QUANTITIES OF CRUDE FAT

David Eldridge Williams
Southern Illinois University Carbondale, davidewilliams1986@gmail.com

IN VITRO APPARENT RUMINAL DIGESTIBILITY OF DIETS CONTAINING CORN DISTILLERS GRAIN WITH DIFFERENT QUANTITIES OF CRUDE FAT

by

David Williams
B.S., Animal Science, Southern Illinois University, 2015

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

M.S., Animal Science

Department of Animal Science

in the Graduate School

Southern Illinois University Carbondale

May 2017

THESIS APPROVAL

IN VITRO APPARENT RUMINAL DIGESTIBILITY OF DIETS CONTAINING CORN DISTILLERS GRAIN WITH DIFFERENT QUANTITIES OF CRUDE FAT

By

David Williams

A Thesis Submitted in Partial

Fulfillment of the Requirements

for the Degree of

Masters of Science

in the field of Animal Science

Approved by:

Dr. Rebecca Atkinson, Chair

Dr. Gary Apgar

Dr. Jon Schoonover

Graduate School

Southern Illinois University Carbondale

December 12, 2016

AN ABSTRACT OF THE THESIS OF

David Williams for the Master of Science degree in Animal Science, presented on December 12, 2016 at Southern Illinois University Carbondale.

TITLE: IN VITRO APPARENT RUMINAL DIGESTIBILITY OF DIETS CONTAINING CORN DISTILLERS GRAIN WITH VARYING LEVELS OF CRUDE FAT

MAJOR PROFESSOR: Dr. Rebecca Atkinson

Four dual-flow continuous fermenters were used in a Latin square design to determine the apparent ruminal digestibility and ruminal characteristics of diets containing dried distillers grains plus solubles (DDGS) at various levels of fat content. Fermenters were randomly assigned to one of the following treatments: 1) 40% DDGS containing 4.82% fat content (40 LOW); 2) 40% DDGS plus corn oil to obtain 7.5% fat (40 MED); 3) 40% DDGS plus corn oil to obtain 10.5% fat (40 HIGH); or 4) 70% DDGS plus corn oil to obtain 7.5% fat (70 MED). Rumen fluid was collected at the beginning of each period from two ruminally cannulated Angus cows previously adapted to the 40LOW treatment. Each period consisted of 10 days with a seven day adaptation period followed by three days of sample collection. Calories per gram of diet increased as percent fat increased and calories per gram was greater at the 70% inclusion of DDGS compared to 40% inclusion of DDGS at all levels of fat content. However, level of fat in the diet did not influence ($P \geq 0.35$) apparent ruminal digestibility of DM, NDF, ADF, CP or total calories. Similarly, inclusion rate of DDGS had no influence ($P \geq 0.35$) on nutrient digestibility. Ammonia concentrations were greatest ($P = 0.0002$) for 70 MED compared to the other treatments. However, treatment had no impact ($P \geq 0.16$) on volatile fatty acid production with the exception of propionate which increased ($P =0.05$) as the level of DDGS increased from 40 to 70% inclusion rate. This data would suggest that level of fat content of

DDGS has no negative influence on apparent ruminal digestibility and select ruminal characteristics. From an economic perspective, higher fat DDGS should have a higher price differential, but lower fat DDGS can still be an effective protein and energy substitute.

ACKNOWLEDGMENTS

I would like to thank the staff and faculty both in the Animal Sciences department, as well as at the University Farms at Southern Illinois University Carbondale. Especially Dr. Rebecca Atkinson for hard work and dedication through this whole process. I would also like to specifically thank Dr. Gary Apgar and Dr. Jon Schoonover for serving on my committee.

I would also like to express my gratitude to Brandy Strohl M.S., Darcy Hastings, and Dr. Stephanie Clark for their assistance in the laboratory and for their encouragement throughout this process. I would like to thank Colten Collier for his assistance with feeding the cannulated cows that were used for this experiment and his patience with work scheduling.

Most of all I would like to thank my family, especially my wife Charity, whose support and encouragement made this possible. Lastly, I would like to express my gratitude to my Lord and Savior Jesus Christ, "Indeed, I count everything as loss because of the surpassing worth of knowing Christ Jesus my Lord. For his sake I have suffered the loss of all things and count them as rubbish, in order that I may gain Christ " (Philippians 3:8 ESV)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

LIST OF ABBREVIATIONS

Distillers Grains (DG)

Distillers Grains plus Solubles (DGS)

Wet Distillers Grains (WDG)

Dried Distillers Grains Plus Solubles (DDGS)

Wet Distillers Grains Plus Solubles (WDGS)

Rumen Degradable Protein (RDP)

Rumen Undegradable Protein (RUP)

Ammonia (NH_3)

Volatile Fatty Acids (VFA)

Non-Protein Nitrogen (NPN)

Microbial Protein (MP)

Nitrogen (N)

Crude Protein (CP)

Megacalorie (Mcal)

Gross Energy (GE)

Digestible Energy (DE)

Total Digestible Nutrients (TDN)

Metabolizeable Energy (ME)

Net Energy (NE)

Net Energy for Maintenance (NE_m)

Net Energy for Gain (NE_g)

Net Energy for Lactation (NE_l)

Dry Matter (DM)

Average Daily Gain (ADG)

Body Weight (BW)

Sulfur (S)

Dry Matter Intake (DMI)

Gain To Feed Ratio (G:F)

Potential of Hydrogen (pH)

Dry Grind Processing (DCP)

Kilogram (KG)

Hot Carcass Weight (HCW)

Hour (h)

Millimeter (mm)

Celsius (C)

Carbon Dioxide (CO_2)

CHAPTER 1

LITERATURE REVIEW

Distillers Grains (DG) are a byproduct of ethanol production from corn and have been utilized by the livestock industry for over a century with the first study of DG being published in 1907 (Weiss, et al., 2007). Distillers grains were initially used solely as a protein source to supplement; however, DG are currently being used as both a protein and energy source in ruminant diets. With an ever-increasing demand for beef throughout the world there is also a correlated demand for improved efficiency in order to meet these growing demands while keeping feed cost low. Distillers grains are an attractive option as a feed source due to the fact that DG is currently about half the price of other traditional protein sources such as soybean meal and corn gluten meal while still containing 30% crude protein, as well as 10% fat, making DG a good energy source as well (Uwituze et al., 2014). The increase in ethanol production has greatly increased the supply of DG and thus, the interest in increasing the use of DG as a feedstuff. However, with multiple ethanol plants producing DG there is a variance in nutrient composition and quality leading to issues when balancing rations utilizing DG. Additionally, the form of DG varies from plant to plant. These various forms include dried distillers grains (DGS), wet distillers grains (WDG), dried distillers grains plus solubles (DDGS), and wet distillers grains plus solubles (WDGS). Annual production of distillers grains (on a dry basis) was about 1 million tons in 1998, about 10 million tons in 2006, and was estimated to reach 16 million tons by 2010. (Weiss, et al., 2007). However, by 2011 production had increased to 42.59 tons and production of DG in 2014-2015 was 44.2 million tons with 33 million tons used domestically and 11 million tons exported (Wisner, 2015). Of the 33

million tons that was used domestically, the beef industry used 17.80 million tons to feed cattle (Wisner, 2015).

Ruminant Protein Requirements

When formulating rations for ruminant animals nutritionists look at two different values for protein; rumen degradable protein (RDP) and rumen undegradeable protein (RUP). Both of these forms play a different role in ruminant nutrition. The RDP portion of the protein can be broken down inside the rumen and used for the synthesis of microbial protein. The RUP portion escapes the rumen and can be processed for use by the animal in the small intestine.

Soluble portions of proteins can be degraded in the rumen by proteolytic bacteria producing NH_3, VFA, carbon dioxide, and other metabolites (Church, 1976). These amino acids and nitrogenous bases such as pyrimidines and purines are metabolized and microorganisms are capable of using NPN compounds for a portion of their metabolic requirements (Wegner et al., 1941; Person and Smith., 1943; Church, 1976). The ammonia is used mainly for microbial protein (MP) synthesis. However, some is absorbed into the portal vein and then almost entirely removed by the liver (Reynolds, 1995). Most is then converted to urea or used to synthesize glutamine from glutamate (Reynolds and Kristensen 2014; Parker et al., 1995; Reynolds, 1995; Nieto et al., 2002). Ammonia is absorbed across both the epithelium of the rumen and sections of the gastrointestinal tract (Reynolds and Kristenson, 2014). The urea produced by the liver is partially excreted in the urine, the remainder is recycled through either saliva or direct transfer across the epithelial tissue by blood. The urea is then degraded to NH_3 via

microbial urease, the resulting N can be used for microbial protein synthesis or be absorbed as NH_3 (Reynolds and Kristenson, 2014).

The protein content of DDGS is variable and this variation is most likely due to the differences in processing methods used in ethanol production (Spiehs et al., 2002). The amount of RUP present in DG had been suggested to range from 56-72% of CP (NRC, 2000; Archibeque et al., 2008; Kelzer et al., 2010b). Castillo-Lopez et al. (2014) observed a RUP level of 64% during an *in vivo* study of DDGS. This would suggest that the level of RUP is greater than that of most corn grains which contain around 58.8% RUP (NRC, 2001). This observation may suggest that when heat is applied during processing of DDGS, the protein is made less available for ruminal degradation due to an increase in RUP (Castillo-Lopez et al., 2014). This theory is also supported by the findings of Kelzer et al. (2010) who observed a RUP concentration of 33.2% CP in DDGS that was not subjected to any kind of heat treatment and a RUP of 56.3% CP in DDGS that had been subjected to heat exposure. However, it should be noted that although there is an increased amount of RUP in the heat-treated DDGS, the effects of heat treatment might lower the availability of the RUP due to the occurrence of the malliard reaction. It also should be noted that in a feedlot setting animals are fed a high concentrate ration. Therefore, the accelerated passage rate that occurs with this type of diet could lower the availability of RUP due to inadequate time in the digestive tract for protein to be properly digested and absorbed.

Ruminant Energy Requirements

Energy is required for all bodily functions of the ruminant animal, from functions needed to simply remain alive to functions directly associated with production. The

energy requirement of the animal will vary based on many factors including age, sex, size, health, and the environment the animal is in (Cooke, 2010). Unlike protein and mineral requirements, energy requirements cannot be quantified using weight and scales but must be measured in reference to a known standard. The most accepted standard is that of the calorie, which is defined as the amount of energy needed to raise the temperature of 1 gram of water by 1 degree Celsius. Due to the amount of energy consumed and utilized by cattle on a daily basis it is helpful to use the term megacalorie (Mcal), which is equal to 1 million calories (Cooke, 2010).

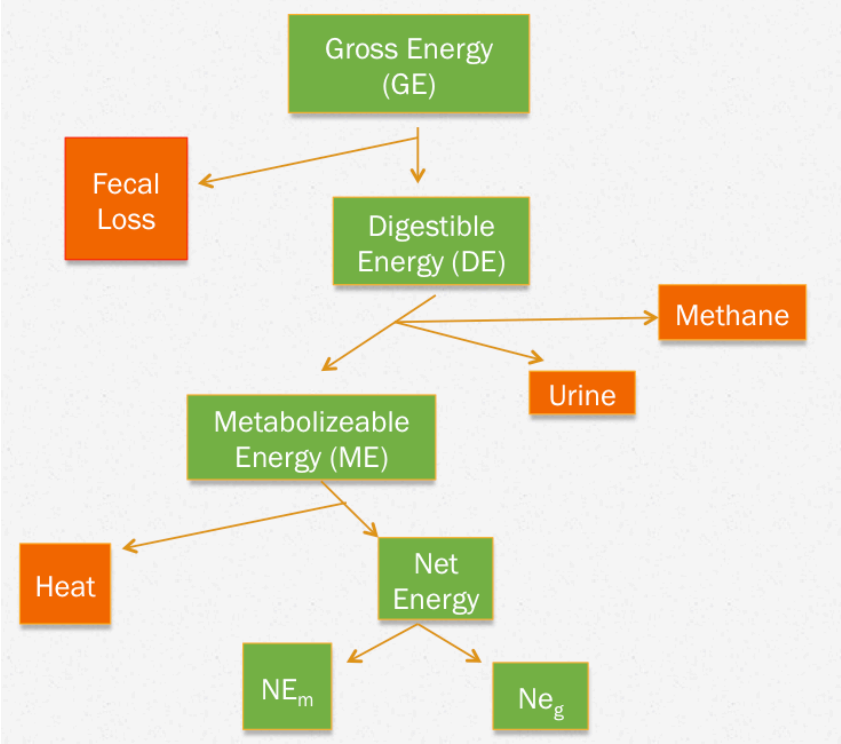

Figure 1: Breakdown of Energy Utilization in the Ruminant Animal.

There are a variety of measures available to evaluate energy requirements of ruminant animals, as well as the value of energy in specific feed rations. Gross energy

(GE) is defined as the energy released as heat when a feedstuff is combusted. While this measurement defines the amount of energy in the feedstuff, it does nothing to tell us of what portion of that energy will be available to the animal (Cooke, 2010). Digestible energy (DE) is the term that refers to the energy remaining after energy is lost in feces. This term better serves the ruminant nutritionist as the majority of energy lost is due to fecal loss. The energy that is actually retained in the animal is referred to as "digested" energy. It is important to note that DE does not account for energy lost due to urination, digestion, and metabolism (Cooke, 2010).

Total digestible nutrients (TDN) is a measurement that is similar to DE. However, TDN also accounts for the digestible protein content. This is important as protein also has an energy value. TDN is the only energy measurement that can be calculated on a weight basis (Cooke, 2010). Metabolizable energy (ME) accounts for energy lost through the production of urine and gasses during digestion. As a result, ME is the best measure of the amount of available energy provided to the animal by a specific feedstuff. Generally, ME represents 82% of DE due to the fact that losses ascribed to urine and gas are similar amongst most feeds (Cooke, 2010). Net energy (NE) takes into account the heat lost during the digestion process and adjusts the value of ME to compensate. The value then represents the amount of energy that was retained and used by the animal. Within the parameters of NE are 3 subsets, which include NE for maintenance (NE_m), NE for gain (NE_g), and NE for lactation (NE_L). Energy requirements for cattle can be managed using TDN which is the most simple method, or by using the NE system for calculation (Cooke, 2010).

Considering that every biological function carried out within an animal's body requires the expenditure of energy, proper nutrition is key for many functions within the scope of cattle production. Basic maintenance functions of the animal include things such as heartbeat, brain activity, respiration, and all other functions (Cooke, 2010). The physical activity of the animal also falls under maintenance requirements. The maintenance requirement of an animal is equal to the amount of energy needed to maintain the current state of the animal with no loss or gain of weight. This value is typically 70% of the ME requirement of mature cows, 90% for mature bulls, and at least 50% of the ME required for growing animals (Cooke, 2010).

Growth of animals requires the cells within bone, muscle, fat, and organ tissue to grow and multiply within the animal. Every step of this process requires an expenditure of energy. Energy is also needed to produce the specific hormones that will act on target tissues to stimulate said growth (Cooke, 2010). One of the most important aspects of animal production is health. In order to maintain a healthy state energy must be used in order to synthesize cells and substances needed by the immune system to create antibodies. Cattle should also be at optimum health levels in order to achieve a greater response to vaccination procedures (Cooke, 2010).

Perhaps the largest demand for energy comes during the reproductive stage of an animal's life cycle. During gestation, energy is needed to maintain the synthesis of hormones needed to facilitate the reproductive process and to modulate the communication between the fetus and dam (Cooke, 2010). During lactation, moderate amounts of energy are needed in order to synthesize milk, as well as, the amount of milk fat, protein, and lactose needed during this process. While cattle can draw on reserves of

energy if needed, excessive use of energy reserves is detrimental to both reproduction and health (Cooke, 2010).

Energy is used in a specific order in beef cattle. This order exists to preserve the most critical functions of the animal and to make the best use of energy in times when intake is inadequate for the level of output. The first use of energy is to meet the needs of vital functions (maintenance, health, replacement of lost tissues), in growing animals the growth requirement can also be considered as a vital bodily function. If the amount of energy needed for the animals vital functions is satisfied, the remaining energy can be used for other needs such as lactation in heifers and cows and reproduction in both bulls and cows. Any remaining energy is kept as a reserve, which can be mobilized if needed (Cooke, 2010).

During times of inadequate energy supply, reproduction is the first thing that will be compromised as the animal will look after itself and current offspring before supporting any new pregnancy (Cooke, 2010). If energy availability is reduced even further, the animal will stop supporting lactation. If the energy deficit becomes so great that the animal can no longer support vital functions, muscle tissue will be broken down in order to provide energy. At this time, impairment of the immune system and a reduction in physical activity will begin and if the deficit continues, the animal will eventually die (Cooke, 2010).

Distillers Grains As a Supplement

Supplementing DDGS to steers grazing native range during the forage growth season increases final body weight at levels of 0.2% body weight on a DM basis (Martinez-Perez et al., 2014). However, the authors observed that increasing

supplementation to 0.6% body weight did not further increase final body weight basis (Martinez-Perez et al., 2014). A linear relationship was noted between supplemental intake of DDGS and ADG, which was consistent with Morris et al., (2006) who also observed a linear increase in ADG when feeding grazing yearling steers supplemental DDGS at 0.00, 0.26, 0.57, 0.77, and 1.03% of BW. Supplementation of DDGS also corrected an energy gap that occurred with the forage diet, this is likely due to the levels of readily digested fiber and fat that is supplied by the DDGS, the protein levels in the DDGS likely also aided in bridging this energy gap. After energy needs are met the cattle respond to the additional protein supplementation, this increased MP may have contributed to the higher ADG that was shown with DDGS supplementation (Martinez-Perez et al., 2014). Distillers grains may be supplemented up to 0.4% without affecting forage intake. However, if there is a lack of forage, levels above 0.4% can be used to compensate for the lower levels of forage intake (Martinez-Perez et al., 2014).

Distillers grains can also be effectively used as a supplement in back grounding diets to effectively increase ADG and feed conversion when compared to other sources such as barley (Yang et al., 2012). Four protein sources were utilized to determine growth rate and feed efficiency. Corn DDGS improved growth rate and feed efficiency by an average of 9% and 8% compared to wheat DDGS but was similar to canola meal (Yang et al., 2012).

Supplementing DDGS to cattle at a rate higher than 50% of the ration can increase CP, oil, and S to excessive concentrations, this can decrease performance and inhibit fatty acid deposition (Ferrell et al., 2008; Depenbusch et al,. 2009; Gunn et al., 2009). However, if DG is being used to supplement cattle that have been early weaned

these effects may not be as negative. Early weaned cattle have increased CP requirements and also consume less DM. Thus, the energy needed to dispose of the excess N that will be present from the feeding of greater amounts of DDGS may actually decrease excessive fat accumulation that is a common occurrence with early-weaned cattle effectively overcoming the issues that occur with feeding levels greater than 50% in older cattle (Yang et al., 2012).

Distillers Grains In Feedlot Rations

With the availability of DG increasing and price being lower than other energy/protein sources, much research is being and has been conducted to evaluate DG as a source in feedlot rations (Gunn et al., 2009). It has been shown that finishing diets can contain up to 40% WDGS while seeing an improvement in ADG, DMI, and G:F compared to corn based diets (Larson et al.,1993; Ham et al., 1994). Feeding of up to 40% DGS in a finishing diet does not appear to have a negative influence on performance; however, a decreased performance has been observed on finishing heifers fed DG at a rate exceeding 45% DM (Gordon et al., 2002; Gunn et al., 2009). Gunn et al., (2009) conducted a study in which 5 diets of DDGS where fed: 1) 25% DM DG; 2) 50% DM DG; 3) 25% DG with added corn protein to be isonitrogenous to 50% DG; 4) 25%DG with added vegetable oil to be isocaloric to 50% DG; 5) 25% DG with added corn protein and vegetable oil to be both isonitrogenous and isocaloric to 50% DG. It was observed that steers being fed the diets containing elevated protein, elevated fat, and elevated protein and fat together had lower ADG compared to steers fed the ration containing 25% DM of DDGS (Gunn et al., 2009). This resulted in a lower final body weight for steers consuming those rations, steers fed the diets that were isonitrogenous

and isonitrogenous/isocaloric at 50% also showed a decrease in G:F (Gunn et al., 2009). These results are in standing with another study conducted by Buttrey et al., (2015) who observed a reduction in G:F when steers where fed a diet of 35% DGS equating to an 11.2% reduction in efficiency and 0.14 kg loss in daily body weight gained. The reduction in performance seen with high amounts of DGS is believed to be either excessive CP present in the diet; the poor efficiency in which the excess protein is converted into fat, or the microbial protein being of lesser value than the protein being degraded in the rumen (Owens et al., 2005). In the study conducted by Gunn et al. (2009), there was no difference in dressing percentage between diets of 25% DDGS and 50% DDGS. However, hot carcass weight (HCW) and final body weight (BW) did differ in that steers fed the 25% DDGS diet had heavier HCW than those fed the diet containing both extra protein and fat, as well as the diet containing only elevated protein alone but there was no difference in HCW between the 25% DDGS diet and the diet containing elevated fat only (Gunn. et al., 2009). However, Atkinson et al., (2007) observed no significant adverse impacts on performance at levels up to 70% inclusion of DDGS in diets fed to angus steers in a feedlot setting. This difference between studies suggests that location of DG production and concomitant nutrient differences therein plays a role in how much can be fed in feedlot diets.

One of the concerns with DG is that although the protein content is actually higher, the starch content is lower than that of corn (Bedwell et al., 2008). One of the key quality markers in beef is the presence and consistency of intramuscular fat, referred to as "marbling". Research conducted by Smith and Crouse, (1984) observed that glucose is used first as the carbon source for marbling adipose tissue while acetate is used for

subcutaneous fat deposition, with less than 20% of the carbon needed for fat synthesis of intramuscular adipose tissue being provided by acetate and lactate while approximately 70% of needed carbon is provided by glucose. However, these percentages change as cattle increase in size. As body fat percentage increases, less of the carbon needed for fat synthesis is sourced from glucose and an increasing amount is sourced from acetate. Therefore, while starch is certainly an important factor in the marbling of feedlot cattle, it appears to be of even more importance to provide adequate starch during early stages of life to ensure optimal development of marbling early on (Smith and Crouse, 1984).

There does appear to be some impact on the marbling scores of cattle fed greater than 23% DM DDGS in their diets (Reinhardt et al., 2007). No difference was noted in USDA grades, however a diet containing 25% DDGS showed a greater marbling score than diets with higher concentrations of DDGS (Gunn. et al., 2009). The reduction in marbling scores may be due to a decrease of dietary starch, which is an issue with diets high in DG, and that such diets promote subcutaneous fat disposition whereas diets higher in starch tend to promote intramuscular fat deposition instead (Smith and Crouse, 1984; Choat et al., 2003). Another concern is that high WDGS diets lower the digestibility of starch from other sources on top of not providing the needed starch for beef cattle (Pingel and Trenkle, 2006). This is a notable concern as an audit conducted in 2000 showed 1/3 of all carcass's reported nationally received a marbling score of "small" (McKenna et al., 2002). With this in mind, an additional decrease in marbling scores would greatly decrease the number of animals grading choice or better resulting in a great deal of financial dames when animals are fed diets containing 50% DDGS (Gunn et al., 2009).

Amat et al. (2014) evaluated the health effects of feeding DDGS in a feedlot-finishing ration. It has been thought that since the majority of starch is no longer present in DDGS due to removal during processing (Weigel et al., 1997; Stein and Shurson, 2009) that there would be a reduction in the instance of ruminal acidosis with feedlot rations where DDGS is included in the ration (Larson et al., 1993). However, several studies have been conducted to evaluate the reduction of ruminal acidosis and no notable mitagatory effects have been observed in rumen pH levels when portions of various other rations where replaced with DG (Beliveau and Mckinnon, 2009). Another study went so far as to conduct histopathological examinations on the rumen tissue of cattle fed both corn and wheat DDGS and also observed no significant differences in the occurrence of histopathological lesions across the various diets used (Amat et al., 2014). Additionally the authors observed that there was no increased occurrence of cystitis in the group fed the DDGS diets.

With liver abcessation being one of the largest concerns in feedlot cattle, most likely due to high concentrate diets (Brink et al., 1990; Nagaraja and Chengpappa, 1998). It was hypothesized that a decreased occurrence would be noted with DG inclusion. This was due to the previously mentioned line of thinking that a reduction in ruminal acidosis, which is believed to be linked to liver abcessation, would be noted based on the lower levels of starch. However, in the study conducted by Amat et al. (2014) no such reduction was noted in animals fed diets ranging from 20-60% corn or wheat DDGS. These studies provide a strong argument that DG has no significant impact on health when used in a finishing ration.

Low cost and high availability make DG an attractive option in many phases of the beef production cycle. However, the variation in results among different research studies is a cause for concern. Producers will be unlikely to explore further use of DG if researchers cannot offer some explanation for these varied results.

Variations in Composition

There are a variety of reasons that could explain the difference seen from study to study involving DG. These variations can become and issue as the published concentrations are often assumptions (Belyea et al., 2010). There are two different processes used for ethanol extraction from corn, dry grind processing and wet mill processing (Franceschin et al., 2008). While wet milling is the most efficient process for ethanol production as far as extracting the most from each component (AMG, 2013), wet milling requires a significant amount of equipment and thus is much more expensive (Belyea et al., 2010). Therefore, dry milling is by far the most used process in today's ethanol plants (Franceschin et al., 2008). To grasp the many ways in which variation can occur it is helpful to first understand the process by which the ethanol is extracted from corn.

The dry grind process (DGP) can be broken down into five steps:

1. Grinding, cooking and liquefaction.
2. Saccharification and fermentation.
3. Distillation and dehydration.
4. Water evaporation and recycling.
5. Drying of the non-fermentable fraction (Franceschin et al., 2008).

The corn is first milled down at an average rate of 41,900 kg/h to a particle size of less than 2 mm so that water will be able to penetrate and is mixed in a slurry tank with approximately 68,500 kg/h of water. The slurry is heated using steam at 110^{o} C to facilitate sterilization and the cleaving of hydrogen bonds to aid in the absorption of water. Increased surface area is created as the starch granules swell. This part of the process is referred to as gelatinization due to the consistency of the mixture (Franceschin et al., 2008).

In the next step of the process which is called "liquefaction" alpha amylase enzyme acts on the exposed molecules resulting in a random breaking of alpha 1, 4 glucosidic amylose and amylopectin linkages effectively decreasing viscosity (Franceschin et al., 2008). The mash is then added to a backset stream and cooled to 35^{o}C in preparation for the fermentation process. Saccharification and fermentation occur at the same time. Near complete hydrolysis of starch and oligosaccharides into glucose molecules is facilitated by glucoamylase enzyme with yeast acting as a catalyst for the reaction (Franceschin et al., 2008).

This process creates a large amount of carbon dioxide, which is mostly purged. The remaining CO_2 is removed in a separate step after reheating of the mixture and just prior to distillation. The gas stream that is being purged is also directed through an absorption column in order to collect any ethanol present in the stream. The distillation process occurs using three columns each with a different pressure setting. The broth is split into two of the columns, which distill the mixture to an ethanol content of around 50%, the product is then sent through the final column, which produces a distillate with 92% ethanol purity. The distillate is later sent through a molecular sieve in order to be

dehydrated to the industry standard for fuel, which is 99.8% ethanol (Franceschin et al., 2008).

The products that cannot be used in the fermentation process are referred to as "whole stillage" which consists of suspended grain solids, solid and liquid material, which have been dissolved, and water. These products are sent to a centrifuge where they are processed into two products; a wet cake consisting of 35% solids by weight and thin stillage containing 8% solids by weight (Franceschin et al., 2008). Some of these products are recycled while the rest are sent to an evaporator. The evaporators remove moisture resulting in mixture containing 35% solids by weight, which is referred to as syrup. The syrup is mixed with the wet cake from the previous step and the resulting mixture is dried down to 90% DM. This final product is DG (Franceschin et al., 2008).

While wet milling of DG does still occur, dry grinding is the most used process. Still it should be noted that there is a significant difference seen in the final product from these two methods. Belyea et al. (2010) compared the compositional data of four dry grind facilities and two wet mill facilities. It was observed that DG from dry grind processing contained higher concentrations of fiber, protein, and fat. This difference may be a result of chemicals that are added to the dry grind process in order to attain peak fermentation conditions (Belyea et al., 2010).

Aside from variations caused by processing methods, a great deal of variation can occur based on the variety of corn being processed (Belyea et al., 2010). In respect to ethanol yields it was observed that a variance of 23% existed within various maize hybrids most likely because of variation in starch composition (Singh and Graebar 2005). Particle size of the maize also changes the effectiveness of hydrolysis and fermentation.

Also screen size inside the grinder, moisture content of the maize, knife sharpness, and the presence of foreign matter all play a role in particle size and these conditions can affect changes even within batches from the same plant (Belyea et al., 2010). Within the various stages of processing other factors can affect final concentrations. Differences in solids concentration, as well as operating temperatures and the exact level of additives used all play a role. Also factors such as water quality and composition/amount of backset can have an effect (Belyea et al., 2010).

There are several addition steps that occur after the fermentation process is complete that can cause variations in concentration. The centrifugation process that whole stillage is subjected to is not perfect and after centrifugation the products can vary in both proportion and concentration (Raush and Belyea, 2006). After thin stillage is sent through the evaporators the resulting distillers solubles often vary in both proportion and concentration (Belyea et al., 1998). The process by which the wet grains (syrup) and distillers solubles are combined to form wet distiller grains is difficult to control resulting in variance (Belyea et al., 2010) and the final drying step can have a significant impact on protein quality (Swietkiewicz and Koreleski, 2008). Spiehs et al. (2002) observed a variance in amino acid content among different plants. Most notable was lysine, which had values ranging from 2.9% to 25.7% and methionine ranging from 0.49% to 0.69% (Spiehs et al., 2002).

With all of these factors in place it would seem necessary that, to ensure accuracy when formulating rations, plants should conduct a complete chemical analysis at least once a year in order to account for any variations due to changes in corn crops being used for processing (Spiehs et al., 2002). It would also appear necessary that when using DG

for ration formulation a analysis from the specific plant being sourced should be used when evaluating nutritive values of DG for each specific ration.

Because ethanol processing varies from plant to plant, it is necessary to establish a predication equation to estimate DE of DG containing different levels of fat. Knowing the DE of DG will allow for ethanol plants to market and price commodities according to energy value. Furthermore, it will allow nutritionists to formulate least cost rations. Therefore, the purpose of this study was to determine the DE of DDGS with varying levels of corn oil *in vitro* prior to conducting an *in vivo* trial.

MATERIALS AND METHODS

Continuous Culture System

A dual-flow continuous culture apparatus (Stern and Hoover, 1990) was used in a Latin Square design. The temperature of the fermenter contents were maintained at 38°C ± 1.0˚C and the pH recorded.

The fermenter inoculum was obtained from two ruminally cannulated Angus cows who were consuming a concentrate diet containing DDGS. Whole rumen contents were strained under pressure through 8 layers of cheesecloth and used within 30 minutes. Strained ruminal fluid from each cow was mixed and 1200 mL of mixed ruminal fluid was added to each fermenter along with pre-warmed buffer (300 mL per fermenter) (Weller and Pilgrim, 1974) with urea omitted, was used to inoculate the fermenter system. The average fermenter volume was 1,654 mL and the liquid dilution rate was 0.12 h^{-1} using the buffer of Weller and Pilgrim (1974) with urea omitted. The solids dilution rate was 0.055 h^{-1}, which produced a means solids retention time of 18 hours. The pH and temperature was recorded prior to each feeding and the fermenters were fed 2 times daily at 0700 and 1900.

Diets and Feeding

All forage was ground through a 2-mm screen in a Thomas Wiley Mill (Thomas-Scientific Philadelphia, PA) prior to mixing with concentrates and feeding. A total of 100 g of each diet was fed to each fermenter daily. Fermenters were fed 50 g two times a day at 0700 and 1900 to prevent stirring problems. Fermenters were randomly assigned to one of the following treatments: 1) 43.8% corn, 40% DDGS, and 14.3% hay (40LOW); 2)

40LOW + 1.0% corn oil (40MED); 3) 40LOW + 2.14% corn oil; or 4) 12.7% corn, 68.7% DDGS, 14.4% hay + 1.76% corn oil (70 MED). All diets contained limestone and soyhulls and were balanced to meet or exceed NRC (2000) requirements for a feedlot steer (Table 1).

Sampling and Analyses

Each fermentation period was 10 d, with a 7 d adaptation period followed by 3 d for sampling. During sampling periods, effluent was continuously collected and held at 4°C in a cold water bath to limit bacterial fermentation. Total effluent from each 24 h within the 3 d sampling period was mixed, and a 1 L subsample was taken and composited each day and stored at -20°C, providing 3 L of total composite effluent. Effluent was lyophilized (Virtis bench Top, Gardiner, NY) prior to analysis.

Laboratory Analysis

Feed and ruminal contents were analyzed for DM and ash (AOAC, 1990), and N content (LECO Model Fp-528 Nitrogen analyzer; LECO Corp., St. Joseph, MI). Neutral and acid detergent fiber contents of feed and effluent was determined using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY).

Samples for VFA analysis were mixed with 1 ml of freshly prepared 25% metaphosphoric acid, centrifuged (IEC Centra GP8R, Needham Heightsm, MA) at 20,000 × g at 4° C for 20 min and supernant fluid was collected and stored -20° until further analysis. Samples for VFA analysis were prepared as described by Jenkins (1987) using 2-ethylbutyric acid as an internal standard. A Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Inc.,Columbia, MD) equipped with a flame-ionization detector and 30-m SP-2560 fused silica capillary column (Restek Stabil

WAX DA column, Bellefonte, PA) were utilized for VFA analysis. The helium carrier gas was maintained at a linear velocity of 23 cm/s. The oven temperature was programmed to 65° C for 3 min, increases to 12° C/min to a final temperature of 225° C which was held for 9 min. The column temperature was maintained at 65° C and flame ionization detector temperature at 225° C. Ruminal ammonia N concentrations were determined by the phenol-hypochlorite procedure (Broderick and Kand, 1980).

Statistical Analysis

All digestion data were analyzed using MIXED procedure of SAS (SAS 9.3 Inst., Inc., Cary, NC) using the model for a Latin square design. The model included treatment and period with fermenter specified in the RANDOM statement of SAS. Ruminal fermentation data (NH3 and VFA) were analyzed using MIXED procedure of SAS (SAS 9.3 Inst., Inc., Cary, NC) for repeated measures. The model included period, treatment, and time as well as treatment × time interactions. The RANDOM statement of SAS included the interaction of period × time within fermenter. An autoregressive covariance structure (AR1 of the MIXED procedure of SAS) was determined to be most appropriate based on Akaike's Information Criterion. There were no interactions so only treatment means are reported. Comparisons of main effects were determined using least square means and Fisher's protected LSD ($P = 0.05$) and tendency set at $P \leq 0.10$.

RESULTS AND DISCUSSION

We observed no significant difference ($P \geq 0.35$) across treatments on percent digestibility of DM, NDF, ADF, CP, or Calories (Table 2). The current *in vitro* study observed a maximum digestibility of 64.9% for NDF and 57.5% for ADF (Table 2). However an *in vivo* study conducted by Leupp et al., (2009a) observed greater levels of both NDF and ADF digestibility when steers were fed 45 and 60% DDGS (72.7 to 73% and 71.6-73.8% respectively) in a 70% concentrate diet. The differences in results between the sourced *in vivo* studies and the current *in vitro* study may be explained by the ability to precisely control the passage rate during an *in vitro* study when utilizing a dual-flow fermentation system. In contrast to the current study, Leupp et al. (2009b) observed a linear increase in apparent ruminal digestibility of protein with increasing levels of DDGS containing 9.8% fat in steers fed moderate quality forage. However, Leupp et al (2009a) also observed a decreased percentage of ruminal digestibility of CP (16.8-9.8%) compared to the current *in vitro* study.

Fat digestibility was greater ($P = 0.01$) in the 40LOW treatment compared to all other treatments (Table 2). There was an increase ($P = 0.01$) in digestibility of fat when comparing 40MED (4.1% fat) to 70MED (5.8% fat) and 40HIGH (5.2% fat) to 70MED (5.8% fat), respectively. This is in agreement with Corrigan et al. (2014) who observed an increase (79-86.3%) in fat digestibility when higher levels of CDS where added to DG, effectively raising the fat content from 6.9% to 13.3% respectively. There is limited *in vitro* data to compare to.

Although no significant differences ($P \geq 0.10$) were seen in *in vitro* rumen pH between diets containing 40% DDGS at 4.82,7.5 or 10.5% fat (Table 3), pH did increase (P=0.0002) for the 70 MED diet compared to the 40LOW, 40MED, and 40 High treatments. During the current study, buffer was used as a means to control flow rate, which in turn controlled the retention time of the fermenters. This is a likely reason why this increase in pH was observed. However, the fact that the pH level remained at 6.0 for more than 15 hours each day further explains why no difference was observed in apparent digestibility of DM, NDF, and ADF. A greater (P = 0.0002) concentration of ammonia was observed in the 70MED treatment compared to all other treatments. This increase is likely due to the fact that the 70MED treatment contained approximately 5% more CP than the other diets. However, it should be noted that since this study was done *in vitro* there was no opportunity for N recycling with the other treatments. This is a possible explanation for the fact that most *in vivo* studies surveyed showed no increase in ammonia concentration with increasing amounts of DG.

In the current study propionate concentration was greater (P = 0.05) in the 70MED treatment compared to all other treatments (Table 3). There was no other significant difference (P = 0.72) in VFA concentrations among different treatments. The increase in propionate production would indicate that energy efficiency was increased in the 70MED treatment compared to the other treatments.

Implications

Increasing dietary concentration of DDGS from based on diets does not affect *in vitro* rumen digestibility of DM, CP and fiber. While higher fat content of DDGS should be seen as having added value, the lower fat DDGS still is useful as both an energy and a protein source. This study suggests that evaluating DDGS with crude fat differences of 4.82, 7.5 and 10.5 should provide enough variation to develop a prediction equation for estimating DE values for DDGS with varying levels of caloric density. This equation should provide a basis for establishing differential pricing of DDGS based on energy content.

The author would like to point out that as this study was done *in vitro,* we are only able to speculate as to the impacts that these treatments had on ruminal fermentation of nutrients. An *in vivo* trial should be conducted in order to ascertain total tract digestibility of nutrients. However, based on the current research, nutritionists would need to be provided with a batch specific analysis for every load of DDGS being used in ration formulation. This would ensure that that energy and protein requirements of livestock are being met and that there are no deficiencies or excess of nutrients. If a prediction equation were to be formulated then processing plants could standardize temperatures used in the production process, level of additive, centrifugation time and speed, amount of syrup mixed back with wet distillers after centrifugation, and temperature of the final drying process to create a more uniform product.

Table 1. Ingredients and analyzed composition of treatments diets.

Item	Treatment Diets[1]			
	40LOW	40MED	40HIGH	70MED
Ingredient, % OF DM				
Corn	43.8	43.8	43.8	12.7
DDGS	40.0	39.0	37.9	68.7
Hay	14.3	14.3	14.3	14.4
Limestone	1.8	1.8	1.8	2.2
Soyhulls	0.2	0.2	0.2	0.2
Corn Oil	0	1.0	2.14	1.76
Chemical Composition, %				
DM	88.2	88.3	88.4	89.1
CP	16.9	16.7	16.3	22.5
NDF	24.9	24.6	24.3	30.4
ADF	14.2	14.1	13.9	18.2
Fat	3.2	4.1	5.2	5.8
Calories	4472.06	4517.25	4568.76	4711.03

[1] Treatments: 40LOW = 40% DDGS and 4.82% Fat, 40MED = 40% DDGS and 7.5% Fat, 40HIGH = 40% DDGS and 10.5% Fat, 70MED = 70% DDGS and 7.5% Fat.

Table 2. Effects of various levels of fat from distillers grains on *in vitro* apparent ruminal digestibility.

	Treatment Diets[1]					
Digestibility, % of intake	40LOW	40MED	40HIGH	70MED	SEM	*P*-value
DM	34.1	40.4	36.5	33.2	2.90	0.35
NDF	54.2	64.9	62.3	63.9	4.38	0.36
ADF	44.5	49.7	47.6	57.5	9.07	0.77
CP	28.9	38.1	32.3	37.8	4.11	0.37
Fat	58.5[b]	75.9[a]	76.1[a]	86.8[a]	4.87	0.01
Calories	42.3	48.5	44.6	42.3	3.21	0.51

ab Denotes significant difference among treatments (P > 0.05).
[1] Treatments: 40LOW = 40% DDGS and 4.82% Fat, 40MED = 40% DDGS and 7.5% Fat, 40HIGH = 40% DDGS and 10.5% Fat, 70MED = 70% DDGS and 7.5% Fat.

Table 3. Effects of various levels of fat from distiller's grains on ruminal characteristics of rations designed for developing heifers.

	TREATMENT DIETS[1]					
	40LOW	40MED	40HIGH	70MED	SEM	*P*-VALUE
pH	6.49[b]	6.53[b]	6.48[b]	6.69[a]	0.03	0.0002
Ammonia, mg/dL	0.87[b]	1.01[b]	0.85[b]	4.74[a]	1.00	0.0002
Total VFA, mM	35.21	38.33	36.76	34.22	2.68	0.72
VFA, mol/100 mol						
Acetate	37.04	35.49	34.07	35.61	1.06	0.33
Propionate	46.01[b]	47.13[b]	49.46[b]	51.04[a]	1.17	0.05
Isobutyrate	0.52	0.41	0.44	0.45	0.03	0.19
Butyrate	14.94	15.34	14.51	11.20	1.28	0.16
Isovalerate	0.60	0.67	0.59	0.70	0.04	0.24
Valerate	0.90	0.96	0.92	1.00	0.07	0.73

ab Denotes significant difference among treatments (P > 0.05).
[1] Treatments: 40LOW = 40% DDGS and 4.82% Fat, 40MED = 40% DDGS and 7.5% Fat, 40HIGH = 40% DDGS and 10.5% Fat, 70MED = 70% DDGS and 7.5% Fat.

LITERATURE CITED

Amat S., J. J. McKinnon, E. Simko, S Hendrick. 2014. Evaluation of feeding corn or wheat dried distillers' grains with solubles on animal health of finishing feedlot steers. Can. J. Anim. Sci. 2014 94:525-531.

Amg. 2013. Corn Milling: Wet vs. Dry. [Online] Available: http://blog.amg-eng.com/corn-wet-milling-vs-dry-milling. Accessed January 2016

AOAC. 1990. Official methods of analysis(15th ed.) Arlington, VA: Association of Official Analytical Chemists.

Archibeque, S. L., H. C. Freetly, and C. L. Ferrell. 2008. Feeding distillers grains supplements to improve amino acid nutriture of lambs consuming moderate-quality forages. J. Anim. Sci. 86:691–701.

Atkinson, R. L., P. M. Walker, S. W. Reader, J. M Carmack, K. Ajuwon, S. L. Lake B. R. Wiegand, L. A. Forster. 2007. Effect of Low Fat Corn Distiller's Grains Fed at 40 and 70% Inclusion on Performance and Meat Quality of Steers. PAS 28: 41-55

Bedwell, P. S., D. B. Faulkner, D. W. Shike, D. F. Parrett, L. L. Berger, F. A. Ireland, and T. G. Nash. 2008. Effects of source of energy on performance, ultrasonic carcass, and economic characteristics of early-weaned heifers. Prof. Anim. Sci. 24:451–459.

Belyea, R., Eckhoff, S., Wallig, M., Tumbleson, M., 1998. Variability in the nutritional quality of distillers solubles. Bioresour. Technol. 66, 207–212.

Beliveau, R. M. and McKinnon, J. J. 2009. Effect of graded levels of wheat-based dried distillers' grains with solubles on rumen fermentation in finishing cattle. Can. J. Anim. Sci. 89:513-520

Belyea, R.L., K.D. Rausch, T.E. Clevenger, V. Singh. D.B. Johnston, M.E. Tumbleson. 2010. Sources of variation in composition of DDGS. Anim. Feed Sci. and Tech. 159:122-130.

Brink , D. R., S.R. Lowry, R.A. Stock, J.C. Parrott. 1990. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. J. Anim. Sci. 68: 1201-1207.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determinations of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64-75.

Buttrey E.K., F.T. McCollum, III, K.H. Jenkins, J.M. Patterson, B.E. Clark, M.K. Luebbe, T.E. Lawrance, J.C. MacDonald. 2015. Use of dried distillers grains throughout a beef production system: Effects on stocker and finishing performance, carcass characteristics, and fatty acid composition of beef. J Anim. Sci. 2012.90:2381-2393.

Castillo-lopez, E., T.J. Klopfenstein, S.C. Fernando, P.J. Kononoff. 2014. In vivo determination of rumen undegradeable protein of dried distillers grains with solubles and evaluation of duodenal microbial crude protein flow. J. Anim. Sci. 2013.91: 924-934.

Choat, W. T., C. R. Krehbiel, G. C. Duff, R. E. Kirksey, L. M. Lauriault, J. D. Rivera, B. M. Capitan, D. A. Walker, G. B. Donart, and C. L. Goad. 2003. Influence of grazing dormant native range or winter wheat pasture on subsequent finishing

cattle performance, carcass characteristics, and ruminal metab- olism. J. Anim. Sci. 81:3191–3201.

Church, D.C. 1976. Digestive physiology and nutrition of ruminants. Vol. 1. Digestive physiology. O & B books, Corvallis, Or.

Cooke, R.F. 2010. Energy nutrition for cattle. Beef cattle library, Orgegon state university.

Depenbusch, B. E., C. M. Coleman, J. J. Higgins, and J. S. Drouillard. 2009. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. J. Anim. Sci. 87:2653–2663.

Ferrell, C., S. Shackelford, and V. Varel. 2008. Effects of distillers grains on feedlot performance of crossbred steers. J. Anim. Sci. 86 (E-Suppl. 2):583.

Franceshin, G., A. Zamboni, F. Bezzo, A. Bertucco. 2008. Ethanol from corn: A technical and economical assessment based on different scenarios. Chem. Eng, Res. Design. 86:488-498.

Gordon, C. M., J. S. Drouillard, J. Gosch, J. J. Sindt, S. P. Mont- gomery, J. N. Pike, T. J. Kessen, M. J. Supizio, M. F. Spire, and J. J. Higgins. 2002. Dakota gold brand dried distillers grains with solubles: Effects on finishing performance and car- cass characteristics. Pages 27–29 in Kansas State University Cattleman's Day Report of Progress No. 890. Kansas State Univ., Manhattan.

Gunn P.J., A.D. Weaver, R.P. Lemenager, D.E. Gerrard, M.C. Claeys, S.L. Lake. 2009. Effects of dietary fat and crude protein on feedlot performance, carcass

characteristics, and meat quality in finishing steers fed differing levels of dried distillers grains with solubles. J. Anim. Sci. 87: 2882-2890

Ham, G. A., R. A. Stock, T. J. Klopfenstein, E. M. Larson, D. H. Shain, and R. P. Huffman. 1994. Wet corn distillers byprod- ucts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. 72:3246–3257.

Jenkins T.C. (1987): Effect of fats and fatty acid combinations on ruminal fermentation in semicontinuous in vitro cultures. J. Anim. Sci. 64, 1526–1532.

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

Larson, E. M., R. A. Stock, T. J. Klopfenstein, M. H. Sindt, and R. P. Huffman. 1993. Feeding value of wet distillers byproducts for finishing ruminants. J. Anim. Sci. 73:2931–2939.

Leupp, J.L., G.P. Lardy, K.K. Karges, M.L. Gibson, J.S. Caton. 2009a. Effects of increasing level of corn distillers grains with solubles on intake, digestion, and ruminal fermentation of steers fed seventy percent concentrate diets. J. Anim. Sci. 87:2906–2912.

Leupp, J.L., G.P. Lardy, K.K. Karges, M.L. Gibson, J.S. Caton. 2009b. Effects of increasing levels of corn distillers dried grains with solubles to steers offered moderate-quality forage. J. Anim. Sci. 87:4064–4072.

Martinez-perez, M.F., D. Calderon-Mendoza, A. Islas, A.M. Encinias, F. Loya-Olguin, S.A. Soto-Navarro. 2014. Effect of corn dry distiller grains plus solubles supplementation level on performance and digestion characteristics of steers grazing native range during forage growing season. J. Anim. Sci. 91: 1350-1361

McKenna, D. R., D. L. Roebert, P. K. Bates, T. B. Schmidt, D. S. Hale, D. B. Griffin, J. W. Savell, J. C. Brooks, J. B. Morgan, T. H. Montgomery, K. E. Belk, and G. C. Smith. 2002. Na- tional Beef Quality Audit-2000: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. J. Anim. Sci. 80:1212–1222.

Morris, S. E., J. C. MacDonald, D. C. Adams, T. J. Klopfenstein, R. L. Davis, and J. R. Teichert. 2006. Effects of supplementing dried distillers grains to steers grazing summer sandhill range. Pages 30–32 in 2006 Nebraska Beef Cattle Report. MP88-A. Univ. Nebraska, Lincoln.

Nagaraja, T. G. and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci. 76: 287-298.

Nieto, R., T. Obitsu, A. Fernandez-Quintela, D. Bremner, E. Milne, A. G. Calder, and G. E. Lobley. 2002. Glutamine metabolis in ovine splanchnic tissues: Effects of infusion of ammonium bicarbonate or amino acids into the abomasum. Br. J. Nutr. 87:357–366.

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

NRC. 2001. Nutrient Requirements of Dairy Cattle, 7th ed. Nat. Acad. Press, Washington, DC.

Owens, F. N., and R. A. Zinn. 2005. Corn grain for cattle: Influence of processing on site and extent of digestion. pp. 78-85. Southwest Nutr. Conf., Univ. of Arizona. http://animal.cals.arizona.edu/swnmc/2005/index.htm

Parker, D. S., M. A. Lomax, C. J. Seal, and J. C. Wilton. 1995. Metabolic implications of ammonia production in the ruminant. Proc.Nutr. Soc. 54:549–563

Pearson, R. M., and J. A. B. Smith. 1943. The utilization of urea in the bovine rumen. 2. The conversion of urea to ammonia. Biochem. J. 37:148-153.

Pingel, D., and A. Trenkle. 2006. Digestibility of diets with corn grain and urea replaced with corn distillers grains or solubles. A. S. Leaflet R2069. Iowa State Univ., Ames.

Rausch, K.D. and R.L. Belyea, 2006. The future of coproducts from corn processing. Appl. Biochem. Biotechnol. 12, 47–86.

Reinhardt, C. D., A. DiCostanzo, and G. Milliken. 2007. Distillers byproducts alters carcass fat distribution of feedlot cattle. J. Anim. Sci. 85(Suppl. 2):132. (Abstr.)

Reynolds, C. K. 1995. Quantitative aspects of liver metabolism in ruminants. Pages 351 371 in Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Proc. 8th Int. Symp. Rumin. Physiol. W. van Engelhardt, S. Leonhard-Marek, G. Breves, and D. Giesecke, ed. Ferdinand Enke Verlag, Stuttgardt, Germany.

Reynolds, C.K. and N.B. Kristenson. 2014. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. J. Anim. Sci. 86: 293-305.

Singh, V. and J.V. Graeber, 2005. Effect of corn hybrid variability and planting location on dry grind ethanol production. Am. Soc. Agric. Eng. 48, 709–714.

Smith, S. B., and J. D. Crouse. 1984. Relative contributions of ac- etate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. J. Nutr. 114:792–800.

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

Stein, H. H. and G.C. Shurson. 2009. Board-invited review: The use and application of distillers dried grains with solubles in swine diets. J Anim. Sci. 87:1292-1303

Stern, M. D. and W. H. Hoover. 1990. The dual flow continuous culture system. In: Continuous Culture Fermentors: Frustration or Fermentation? Workshop Handbook for NE ADSA ASAS Regional Meeting, Chazy, NY.

Swietkiewicz, S. and J. Koreleski, 2008. The use of distillers dried grains with solubles (DDGS) in poultry nutrition. World's Poultry Sci. Assoc. 64, 257–265.

Uwituze, S., G.L. Parsons, M.K. Shelor, B.E. Depenbusch, K.K. Karges, M.L. Gibson, C.D. Reinhardt, J.J. Higgins, J.S. Drouillard. 2014. Evaluation of dried distillers grains and roughage source in steam flaked corn finishing diets. J. Anim. Sci. 8: 258–274.

Wegner, M.I., A.N. Booth, G. Bohstedt, E.B. Hart. 1941. The utilization of urea by ruminants as influenced by the level of protein in the ration. J. Dairy Sci. 24: 835-844.

Weigel, J.C., D. Loy, L. Kilmer. 1997. Feed co-products of the dry corn milling process. Iowa State University, Iowa Corn Promotion Board, Iowa Department of Agriculture, Renewable Fuels Association, National Corn Growers Association. [Online] Available: http://www.iowacorn.org/forms/drymillbook.pdf. Accesed November 2015.

Weiss, B., M. Eastridge, D, Shoemaker, N. St-Pierre. 2007. Distillers Grains. Ohio State University, Ohio State University Extension Factsheet

Weller, R. A. and A. F. Pilgrim. 1974. Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous in vitro fermentation system. Br. J. Nutr. 32:341-351.

Wisner, R. 2015. Estimated U.S. Dried Distillers Grains with Solubles (DDGS) Production & Use. [Online] Available: http://www.extension.iastate.edu/agdm/crops/outlook/dgsbalancesheet.pdf. Accessed June 2016.

Yang, W.Z., L. Xu, C. Li, K.A. Beauchemin. 2012. Short Communication: Effects of supplemental canola meal and various types of distillers' grains on growth performance of backgrounded steers. Can. J. Anim. Sci. 93: 281-286.

VITA

Graduate School
Southern Illinois University

David E. Williams
allamericancowboy86@siu.edu

Southern Illinois University Carbondale
Bachelor of Science, Animal Science, May 2015

Thesis Title:
TITLE: IN VITRO APPARENT RUMINAL DIGESTIBILITY OF DIETS CONTAINING CORN DISTILLERS GRAIN WITH DIFFERENT QUANTITIES OF CRUDE FAT

Major Professor: Dr. Rebecca Atkinson