EFFECTS OF TREATING SORGHUM WET DISTILLER'S GRAINS WITH SOLUBLES WITH AN ENZYME COMPLEX ON NUTRIENT DIGESTIBILITY AND PERFORMANCE IN FINISHING BEEF STEERS

by

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ABSTRACT

Two experiments were conducted to determine the effects of treating sorghum wet distiller's grains with solubles (SWDGS) with an enzyme complex, or enzyme complex-buffer combination on diet digestibility and feedlot performance. Experimental treatments were as follows; 1) untreated SWDGS (**Control**), 2) addition of an enzyme complex to SWDGS (**Enzyme**); and 3) addition of enzyme complex and limestone buffer to SWDGS (**E+B**). Sorghum WDGS, regardless of treatment, was included at 45% DM (DM basis) of a steam-flaked corn-based finishing diet. The enzyme complex (Biozyme, Inc., St. Joseph, MI) was a proprietary blend containing both exogenous and endogenous hemicellulase, cellulase, and amylase enzymes, and was added at rate of 6 L/dry ton and 6.2 L/dry ton of SWDGS in Experiment 1 and 2; respectively. In the first experiment, six crossbred beef steers (initial BW = 577 ± 6.9 kg) were used to evaluate the effects of treating SWDGS with an enzyme complex or buffered enzyme complex on digestibility of nutrients. No differences ($P > 0.28$) in DMI, or digestibility of DM, OM, and starch between treatments were detected. Steers fed E+B treatment had higher ($P = 0.07$) ruminal pH than Control or Enzyme steers. Neutral detergent fiber digestibility tended ($P = 0.15$) to be greater for E+B fed steers. In a second experiment, 54 beef steers (initial BW = 370 ± 9 kg) were fed in a finishing study evaluating the same dietary treatments as the first

experiment. No difference ($P > 0.31$) in DMI, ADG, or G:F was detected for the entire feeding period when Enzyme fed cattle were compared to Control. In contrast to the first experiment, DMI for the feeding period was lower ($P = 0.03$) for E+B fed cattle compared to Enzyme. There was no difference ($P = 0.39$) in ADG during the entire feeding period between Enzyme and E+B fed cattle; however, G:F was greater ($P = 0.05$) for E+B fed cattle compared to Enzyme. Standard carcass measurements did not differ ($P > 0.43$) between Enzyme and Control treatments. Most standard carcass measurements did not differ ($P > 0.42$) between E+B and Enzyme treatment, however, E+B cattle had less rib fat ($P = 0.05$) and had lower ($P = 0.02$) yield grades. Furthermore, the E+B fed cattle tended to have ($P = 0.06$) larger ribeye areas than Enzyme cattle. In conclusion, while treating SWDGS with a buffered enzyme complex containing primarily fibrolytic enzymes effects had positive on NDF digestibility, no corresponding improvements in cattle performance were detected.

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LIST OF ABBREVIATIONS

CDDG- corn dried distiller's grains
CDDGS- corn dried distiller's grains with solubles
CDG- corn distiller's grains
CDS- condensed distiller's solubles
CWDG- corn wet distiller's grains
CWDGS- corn wet distiller's grains with solubles
DDG- dried distiller's grains
DDGS- dried distiller's grains with solubles
DG- distiller's grains
DGF- dry gluten feed
DIP- degradable intake protein
DMB- dry matter basis
DRC- dry-rolled corn
FPU- filter paper units
HMC- high moisture corn
IU- international units
MWDGS- modified wet distiller's grains with solubles
REA- Ribeye area
SDDGS- sorghum dried distiller's grains with solubles
SDG- sorghum distiller's grains
SFC- steam-flaked corn
SWDG- sorghum wet distiller's grains
SWDGS- sorghum wet distiller's grains with solubles
TMR- total mixed ration
UIP- undegradable intake protein
WDG- wet distiller's grains
WDGS- wet distiller's grains with solubles
WGF- wet gluten feed
YG- yield grade

CHAPTER I

REVIEW OF LITERATURE

Introduction

A by-product is a secondary product resulting from the harvesting or processing of a principle commodity. They have little direct value as a human edible food; however, many by-products have substantial value as animal feedstuffs. The feeding of by-products in the form of crop residues has been practiced for hundreds of years. The feeding of by-products such as distiller's grains has become more common recently, due to factors such as changing regulatory pressures, economic considerations, and waste technology (Grasser et al., 1995).

By-products of grain milling have become increasingly important to the livestock industry over the past ten years because their production has increased dramatically (MacDonald, 2011). According to the Renewable Fuels Association (RFA, 2014), by-products of the ethanol industry have quickly become one of the largest contributors to the U.S. livestock feed supply. Roughly one-third of every bushel of grain that enters the ethanol process is returned to the livestock feed supply, most often in the form of distiller's grains, but also as gluten feed and gluten meal. In the 2013 marketing year, the ethanol industry produced 35.5

million metric tons (mmt) of high quality feed, expected to rise to 37.8 mmt in the 2014 marketing year (RFA, 2014).

Other industries that produce by-products that can be used as animal feedstuffs include the food and fiber industries (Grasser et al, 1995). A few noteworthy by-products from these industries include almond hulls, dried beet pulp, wet brewer's grains, wet citrus pulp, pressed citrus pulp, whole cottonseed, and rice bran. All are by-products of the food industry except for whole cottonseed, a by-product of the fiber industry. In 1992 these by-products accounted for 27% of the total feed concentrate moved within the state of California (Grasser et al., 1995). Current research has the primary focus to improve utilization of by-products as an animal feed resource to coincide with an environmental focus in the livestock industry today.

Importance of By-product Utilization

Ruminants have the unique capacity to utilize fiber, compared to monogastrics. The rumen provides an environment in which microbes, some of which produce the enzyme cellulase, can thrive. Cellulase breaks down cellulose, the most abundant plant constituent, comprising 20-40% of the dry matter of all plants (Van Soest 1982; Oltjen and Beckett, 1996). This ability makes a large number of by-products, such as distiller's grains (DG), particularly suitable for ruminant diets (Grasser et al., 1995). Furthermore, on a DM basis distiller's grains contain approximately 36% fiber, making them a valuable potential energy source for ruminant animals (Stock et al., 2000). The usage of

by-products as feedstuffs in ruminant diets not only benefits the animals by potentially providing vital nutrients, it also serves an important environmental purpose (Grasser et al, 1995; Oltjen and Beckett, 1996; Mirzaei-Aghsaghali and Maheri-Sis, 2008).

Demand for food and fiber increases as the human population increases, resulting in increased generation of by-products. Assuming that global production of by-products parallels the projected growth rate of cereal grains, the generation of by-products will increase by up to 40% by 2020 (Fadel, 1999). Based on this projection, by-products would become an increasing waste problem if not fed to livestock (Fadel, 1999). Disposal of by-products can present an environmental issue as dumping or burning creates potential water and air pollution problems (Mirzaei-Aghsaghali and Maheri-Sis, 2008). Feeding by-products to livestock not only serves as a means to utilize them, but has also allowed by-products to become a vital part of sustainable agriculture, turning a potential waste product into milk and meat for human consumption (Oltjen and Beckett, 1996). Furthermore, feeding by-products reduces the livestock industry's dependence on cereal grains that can be used directly as human edible food (Mirzaei-Aghsaghali and Maheri-Sis, 2008; Wilkinson, 2011). Although it is not necessary to use cereal grains in livestock feeding, in systems that do not, productivity and efficiency can be decreased (Wilkinson, 2011).

By better characterizing by-product nutrient profiles, and implementing strategies to improve nutrient utilization, these potential negative effects associated with replacement of cereal grains with by-products may be reduced.

Furthermore by maximizing utilization of nutrients, pollution problems associated with nutrient excretion can be minimized. While multiple research studies have been performed in these areas regarding distiller's grains, composition can vary greatly (Lardy, 2007; Buckner et al., 2011). Therefore, further research is warranted to better characterize the value of distiller's grains in livestock feeding, and possibly improve their utilization.

By-Products of the Grain Milling Industry

Within the grain milling industry are the wet and dry milling industries; which produce different primary products and by-products. The by-products wet and dry gluten feed (WGF and DGF, respectively) are derived from the wet milling process of grain, primarily corn (Stock et al., 2000). By-products of the dry milling of grains, primarily corn and sorghum, to produce ethanol are generally referred to as distiller's grains (DG) or condensed distiller's solubles (CDS). Distiller's grains can be further categorized as dry distiller's grains (DDG), dry distiller's grains with solubles (DDGS), wet distiller's grains (WDG), wet distiller's grains with solubles (WDGS), and modified wet distiller's grains with solubles (MWDGS), based on amount of drying and addition of CDS (Stock et al., 2000; Lardy, 2007; MacDonald, 2011). Briefly, the differing processes of wet and dry grain milling will be described.

Wet vs. Dry Milling. The wet milling process of grain involves removal of crop residue, fines, and broken kernels through screening (Stock et al., 2000). The grain is then steeped for 40 to 48 h before going through a series of grinds,

differential separations, and centrifuges to separate the kernel fractions. Liquid recovered from the steeping process contains some transferred nutrients and has value as a feedstuff; it is concentrated down and sold as steep liquor. The primary component to be isolated is starch, sold as-is or converted to a wide variety of products such as corn syrup or high-fructose corn sweetener; the corn germ is also separated and processed to extract corn oil. The remaining portion of the kernel is termed bran and can be dried down to form DGF or mixed back with the steep liquor to form WGF (Stock et al., 2000; MacDonald, 2011).

The dry milling process of grain (primarily corn and sorghum) for ethanol production involves conversion of starch to ethanol (Stock et al., 2000; MacDonald, 2011). The grain source is ground and added yeasts ferment starch, producing ethanol. The fermented mash is distilled, removing the ethanol, and leaving behind what is called whole stillage. The whole stillage is either centrifuged or screened to separate out the coarse grain particles or WDG. Wet DG can be sold as-is or dried and sold as DDG. The remaining liquid fraction, called thin stillage, can either be sold as-is or partially evaporated to produce CDS. A portion of CDS can be added back to WDG or DDG to produce WDGS or DDGS (Stock et al., 2000; MacDonald, 2011). Nutrient composition of the various dry milling by-products varies widely, with CP ranging from 20-35%, and NE_g (Mcal/kg) from 1.47-2.05 on a DM basis (DMB; Lardy, 2007).

Nutrient Composition of Wet Distiller's Grains

Corn and sorghum grain consist of two-thirds starch on a DMB, after fermentation and removal of starch approximately one-third of the original grain is recovered in the whole stillage (Stock et al. 2000; Klopfenstein et al., 2008). Removal of starch therefore causes an approximate three-fold concentration in the remaining nutrients: protein, fat, fiber, and minerals. In corn and sorghum DG (CDG and SDG, respectively) CP increases from approximately 10 to 30%, fat from 4 to 12% and NDF from 12 to 36% (Stock et al. 2000; Klopfenstein et al., 2008).

Corn vs. Sorghum Distiller's Grains. Although similar in nutrient composition, reviews by Klopfenstein et al. (2008) and Owens (2008) suggest that corn WDG (CWDG) is superior to sorghum WDG (SWDG) as a feedstuff. However, several studies have found no significant differences between the two types of DG in terms of animal performance and carcass characteristics, and digestibility of nutrients (Al-Suwaiegh et al., 2002; Vasconcelos et al., 2007; Depenbusch et al., 2009; May et al., 2010).

Al-Suwaiegh et al. (2002) evaluated the nutritional value of DG from the fermentation of corn or sorghum. Sixty steers were individually fed three dry-rolled corn (DRC) based finishing diets for 127 days containing no WDG (control), or 30% CWDG or 30% SWDG, replacing DRC. Steers fed CWDG and SWDG had similar ADG and G:F ($P = 0.19$ and $P = 0.25$, respectively). Standard carcass measurements did not differ ($P \geq 0.37$) between DG source, with the exception of fat thickness which tended ($P = 0.08$) to be higher SWDG fed steers compared to CWDG fed steers (Al-Suwaiegh et al., 2002).

Similarly, Depenbusch et al. (2009) used diets containing 15% CDG or SDG. Two hundred and ninety-nine crossbred yearling steers were fed for approximately 114 d comparing seven dietary treatments using steam-flaked corn (SFC) based finishing diets. Treatments included a control ration containing no WDGS, 15% sorghum WDGS (SWDGS) with 0 or 6% alfalfa hay, 15% sorghum DDGS (SDDGS) with 0 or 6% alfalfa hay, 15% corn WDGS (CWDGS) with 6% alfalfa hay, and 15% corn DDGS (CDDGS) with 6% alfalfa hay. Performance and digestibility results were similar ($P \geq 0.09$) for DMI, ADG, G:F, carcass characteristics and apparent total tract digestibility of DM and OM between the two types of DG (Depenbusch et al., 2009).

Vasconcelos et al. (2007) compared SWDGS and CWDGS fed at 10% of dietary DM. Beef steers (n=200) were fed increasing levels of SWDGS (0, 5, 10, 15% of DM) and one level of CWDGS (10% of DM). The WDGS replaced SFC in a high concentrate diet. Neither ADG or G:F differed ($P = 0.63$ and $P = 0.42$, respectively) between the diets containing 10% SWDGS and 10% CWDGS during the 133 d feeding period (Vasconcelos et al., 2007). It should be noted however, that the SWDGS used was not 100% sorghum based as it contained 34.5% corn DDG (CDDG) as a means for the plant to extend supplies and reach target moisture content of the final product. Often times ethanol plants can offer a CDG and SDG combination, this allows them to meet supply quotas and maintain moisture content. However, this adds to the variability of the product received by livestock facilities.

May et al. (2010) conducted two experiments using beef steers to evaluate the effects of DG source on feedlot cattle performance, carcass characteristics, and total tract nutrient digestibility. In experiment 1, 224 Angus and Angus crossbred steers were used and seven dietary treatments were applied in SFC-based diets. Treatments were: no WDG (control), or diets containing 15% CWDG, 15% SWDG, 15% Blend (50:50 CWDG and SWDG), 30% CWDG, 30% SWDG, and 30% Blend. No differences on final BW (P =0.13), ADG (P > 0.10), and G:F (P > 0.10) were observed. Marbling score, HCW, LM area, and YG did not differ (P > 0.10) among treatments. In Experiment 2, 36 steers were used to evaluate total tract digestibility of nutrients for the control, 15% CWDG and 15% SWDG treatments from Experiment 1. Apparent total tract digestion of DM, OM, CP, NDF, and starch did not differ ($P \geq 0.25$) among treatments (May et al., 2010).

In contrast to Depenbusch et al. (2009) and May et al. (2010), Lodge et al. (1997) reported apparent OM digestibility was lower for SWDG compared to CWDG when fed to sheep at 80% of dietary dry matter completely replacing DRC. Conflicting results may be partially explained by DG completely replacing corn grain, and DG fed at a higher level (80% versus 15% of dietary DM).

In a review of DG research with beef cattle, Owens (2008) reported the NE_g of SDG remained below that of the grain it replaced, which contrasts his findings with CDG. Higher lignin content in NDF of SDG compared to corn may explain the lower NE_g values (Owens, 2008). Al-Suwaiegh et al. (2002) and May et al. (2010) support this theory by reporting the ADF content of CWDGS to be

lower (25.3 and 15.7%, respectively) than SWDGS (28.3 and 25.3%, respectively). Al-Suwaiegh et al. (2002) calculated the NE_g of the two types of distiller's to be 2.00 Mcal/kg and 1.87 Mcal/kg, respectively. However, this difference was diluted out in the total mixed ration (TMR) as calculated NE_g of the TMR was not different ($P = 0.15$) for the CWDGS (1.43 Mcal/kg) and SWDGS (1.39 Mcal/kg) diets when DG were included at 15% of dietary DM (Al-Suwaiegh et al., 2002). Conversely, May et al. (2010) found a significant difference ($P \leq 0.05$) in the calculated NE_g between diets containing CWDG versus SWDG at inclusion levels of 15 and 30% (DM basis).

Another factor potentially explaining observed differences in NE between corn and sorghum WDG is lipid content. Lipids are an important energy source providing 2.25 times more calories than carbohydrates (starch and fiber). Nutrient content analysis of CWDG and SWDG in studies comparing the two showed that CWDG had higher in lipid content than SWDG; averaging the results of four of the studies mentioned previously, lipid content of CWDG was approximately 13.8% on a DMB while the lipid content of SWDG was approximately 11.7% (DMB; Lodge et al., 1997; Al-Suwaiegh et al., 2002; Depenbusch et al., 2009; May et al., 2010).

While previous studies have shown no differences in performance or digestibility when comparing CWDG to SWDG at a low inclusion rate (≤ 15% DMB), some researchers suggest SWDG is inferior to CWDG as a feedstuff. Factors that could potentially cause a discrepancy between the two types of DG

include digestible fiber and lipid content of SWDG compared to CWDG. Level of inclusion and corn processing method (DRC vs. SFC) could also play a role.

Use of WDG in Cattle Feeding

Several factors make WDG an attractive ingredient in beef cattle diets. As discussed, the nutrient profile of WDG makes it a valuable source of both protein and energy (from fiber and fat). In addition, replacement of grain with a fibrous feedstuff such as WDG can potentially mitigate certain metabolic disorders. Furthermore, its physical attributes aid in improving diet palatability. However, availability and composition of the by-product can vary from region to region.

High CP concentrations in DG may allow for replacement of a portion of or the entire supplemental CP source, decreasing the overall cost of the diet (May et al., 2010). An important consideration when utilizing WDG as a protein source (< 15% inclusion) is that undegradable intake protein (UIP) content approaches 60% which is high compared to other feedstuffs (Lardy, 2007; Vasconcelos et al., 2007). Maximum efficiency of microbial protein synthesis is achieved with an appropriate balance of degradable intake protein (DIP) and UIP and is directly related to rumen efficiency (Van Soest, 1982). Incorporating urea to correct DIP deficiencies in diets containing WDG has yielded varied results and may not generate any positive effects on animal performance (Vasconcelos et al., 2007). The absence of performance improvements in response to supplemental DIP in diets containing WDG reflect the ruminant's natural ability to recycle nitrogen in the form of urea back to the rumen (Van Soest, 1982; Church, 1988).

At levels of inclusion > 15%, WDG serves not only as a protein source, but an energy source as well, replacing dietary grain (Klopfenstein, 2001). When WDG are being added to the diet at inclusion levels where grain is being replaced starch content of the diet decreases while fiber content increases. Starch is the predominant driving factor in the metabolic disorder known as acidosis. It is therefore theorized that inclusion of WDG as an energy source in place of starch could mitigate the occurrence of acidosis (Klopfenstein et al., 2008). In addition to its contributions as an energy and CP source the moisture content and physical characteristics of WDG make it an effective "conditioning agent" when preparing a TMR. These characteristics aid in palatability, reduced sorting of less palatable ingredients, and allow for the replacement of molasses or other liquid feed products (Klopfenstein et al., 2008; May et al., 2010).

With the relatively high fiber content of DGS, it is plausible that DGS could serve as a replacement for roughages in finishing diets. Depenbusch et al. (2009) investigated this theory by evaluating the performance of cattle fed diets containing DGS with and without added roughage. While the complete removal of alfalfa hay improved digestibility ($P = 0.01$), it resulted in reduced DMI, ADG, and HCW ($P = 0.01$). Consequently, complete removal of hay in diets containing DGS may not result in maximum feedlot performance (Depenbusch et al., 2009).

Another factor in the use of WDG in cattle diets is availability and transportation costs. High costs associated with moving water limit the transport of high moisture by-products to within an approximately 100-mile radius from the processing plant (Grasser et al. 1995; Klopfenstein, 2001). Figure 1.1 and 1.2

show the relationship between the location of ethanol plants and the cattle feeding industry in the U.S. (USDA, 2002; RFA, 2014). It is clear that while ethanol plants are concentrated in the Midwest, cattle are more concentrated in the southern Grain Plains. More recently, plants are being constructed in the southern Great Plains, making by-products such as WDG more available and cost effective to producers in the area (Cole et al., 2006).

Inclusion of Distiller's in Livestock Diets. Two main factors influencing inclusion rate include the use of WDGS as a protein or energy source. Furthermore, the use of SFC vs. DRC is another important consideration (Cole et al., 2006; Owens, 2008). Influence of WDG inclusion level on dietary fat and sulfur content and potential effects on DMI and the occurrence of polioenchepalomalacia should also be noted (Buckner et al., 2011). When being used as a source of CP in the diet, DG (wet or dry) are commonly fed at levels between 10-15%. At levels greater than 15% inclusion, DG begin replacing corn (or other grains) as an energy source in the diet. Lardy et al. (2007) recommended inclusion rates of WDG in backgrounding and finishing diets at 25-30% DMB as optimum when DRC is used, although they can be used at levels up to 40%.

Several studies have shown that optimum level of inclusion in diets based on SFC to be no more than 15% (Daubert et al., 2005; Depenbusch et al., 2009). Daubert et al. (2005) fed 637 heifers for 58 d to determine the optimal amount of SWDGS in SFC-based finishing diets. Dietary treatments consisted of six concentrations of SWDGS (0, 8, 16, 24, 32, and 40% DMB). While DMI decreased linearly ($P < 0.01$) with increasing SWDGS, ADG and feed efficiency

responded in a quadratic manner ($P < 0.01$ and <0.01, respectively) peaking at 8% and 16% inclusion, respectively. Ribeye area (REA) decreased linearly ($P < 0.02$) while average yield grade (YG) increased linearly ($P < 0.02$) as SWDGS inclusion increased. However, grid-based carcass values were not significantly different across treatments. Regression analysis of efficiency data indicated optimum level of inclusion of SWDGS was approximately 15%; although, diets containing up to 24% SWDGS yielded efficiencies equal or superior to diets containing no SWDGS (Daubert et al., 2005). Similar to the study by Duabert et al. (2005), Depenbusch et al. (2009) also reported that optimal inclusion of WDGS in SFC-based diets is 15% (DMB). Average daily gain and G:F were not different ($P = 0.82$ and $P = 0.92$) for cattle consuming diets containing 0% WDGS or 15% WDGS. Furthermore, HCW did not differ ($P = 0.47$) between 0% and 15% inclusion levels (Depenbusch et al., 2009).

Luebbe et al. (2012) conducted a study using six hundred crossbred steers fed dietary treatments including two control diets containing no DG and DRC or SFC, or SFC-based diets with four CWDGS concentrations (15, 30, 45, 60% DMB). Dry matter intake, ADG, G:F, and HCW decreased linearly ($P <0.01$) as level of CWDGS was increased in the diet. Numerical values for performance data between cattle fed 0% or 15% CWDG were similar. Based on the results, there are no apparent differences between cattle fed SFC-based finishing diets containing 0 or 15% WDG (DMB). A companion study using ruminally and duodenally cannulated steers found a linear decrease ($P < 0.01$) in ruminal and total tract OM digestibility with increasing CWDGS concentration. Total tract

digestibility of starch decreased quadratically ($P < 0.01$) with increasing concentration of CWDGS, peaking at 15% inclusion (Luebbe et al., 2012). Similarly, May et al. (2010) reported a significant reduction ($P \leq 0.05$) in G:F with 15% or 30% CWDGS inclusion (DMB) compared with no DG in the diet. This was accompanied by lower ($P \leq 0.05$) HCW for steers fed CWDGS, and reduced ($P \leq 0.05$) HCW as inclusion rate increased (May et al, 2010).

The results of these studies suggest optimal inclusion of WDGS in SFC-based diets is approximately 15%, in contrast to an optimal inclusion of 25-30% when used in DRC-based diets (Lardy, 2007).

Use of WDG in SFC vs. DRC-based Diets. Previous research indicates an interaction between corn processing method and DG (Cole et al., 2006; Owens, 2008; MacDonald, 2011). Corrigan et al. (2009) evaluated the effects of corn processing method and CWDGS inclusion level on feedlot performance of finishing steers. Beef steers (n=480) were fed 12 diets for 168 d. A 3 x 4 factorial design testing corn processing method (DRC, high moisture corn (HMC), or SFC) and WDGS inclusion level (0, 15, 27.5 or 40% DMB). Final shrunk BW linearly increased ($P < 0.10$) as WDGS inclusion increased for steers fed DRC. In contrast, there was a quadratic effect ($P < 0.01$) on final shrunk BW as WDGS inclusion increased for SFC fed steers, peaking at 15% inclusion. Average daily gain also increased linearly ($P < 0.01$) for steers fed DRC and decreased quadratically ($P < 0.05$) for steers fed SFC, again peaking at 15% inclusion. Inclusion level of WDGS in SFC had no significant effects ($P > 0.10$) on carcass characteristics with the exception of YG. Yield grade quadratically decreased (*P*

< 0.05) peaking at 15% inclusion as WDGS inclusion increased in SFC-based diets (Corrigan et al., 2009).

This phenomenon may be explained, in part, by greater starch availability in SFC vs. DRC (Huntington, 1997; Armbruster, 2006). When WDG replaces DRC energy availability is increased in the diet generally causing an improvement in animal performance. However, when WDG replaces a more readily available energy source, such as SFC, effect on performance is generally negative at higher inclusion levels (> 15% DMB; Cole et al., 2006; Owens, 2008; MacDonald, 2011).

Fat and Sulfur Content of WDG. When including a high lipid ingredient such as WDG in a ration, it is important to consider the effect of inclusion rate on TMR dietary fat content, as there is potential for DMI to decrease as dietary fat content increases (Huffman et al., 1992; Vander Pol et al., 2009). Huffman et al. (1992) reported a decrease in DMI with increasing levels of dietary fat. In a finishing trial, 88 steers and 176 heifers were used to evaluate the effects of forage and fat level on performance. Dry rolled corn-based diets contained 0 or 7.5% forage and 0, 2, 4, or 6% tallow. Ether extract content (% DM) for the 0% forage diets was 5.10, 6.95, 8.80, and 10.65% for the 0, 2, 4, and 6% added tallow diets, respectively. The 7.5% forage diets contained (% DM) 4.83, 6.68, 8.53, and 10.38 ether extract. Dry matter intake decreased linearly ($P < 0.01$) as level of tallow increased, but reached a plateau ($P < 0.03$) at the 4% tallow level (Huffman et al., 1992).

Vander Pol et al. (2009) conducted an experiment to determine the effect of feeding WDGS or supplemental fat on finishing performance. Sixty heifers were individually fed six DRC:high moisture corn-based finishing diets for 113 d. Dietary treatments were 0% corn oil (control), 2.5% corn oil, 5% corn oil, 0% WDGS (control), 20% WDGS or 40% WDGS. Dietary ether extract (% DMB) for each treatment was 3.98, 6.37, 8.76, 3.98, 6.39, and 8.80%, respectively. Dry matter intake tended (P = 0.13) to decrease as level of fat increased (Vander Pol et al., 2009). Results of these studies indicate a decrease in DMI as level of dietary fat increases but source of fat may play a role in the extent of the decrease.

According to the NRC (1996) the maximum tolerance level for dietary sulfur content is 0.40%. Above this level there is a potential for occurrence of polioencephalomalacia, a disorder of the central nervous system. Buckner et al. (2011) evaluated nutrient composition of WDGS from six Midwestern ethanol plants by taking ten samples per day across five days, in four separate months. Mean analyzed sulfur content was 0.77% (DMB). If WDG are included at higher levels, such as when being used as an energy source (15-40%), TMR sulfur content should be monitored to prevent polioencephalomalacia.

Fibrolytic Enzymes

Inclusion rate and corn processing method are not the only focus of DG research. Certain feed additives have come to light and may be utilized to improve performance of animals consuming high amounts of distiller's grains.

External enzymes used in feeding systems can contain cellulases, hemicellulases, and alpha-amylases extracted from a various array of microbes. Those of fungal origin (*Trichoderma longibrachiatum*, *Aspergillus niger*, and *Aspergillus oryzae*) and bacterial origin (*Bacillus* spp.) are the most widely used (Beauchemin et al., 2004a).

Activity of enzymes produced can vary widely depending on the microbial strain selected, growth substrate, and culture conditions employed for enzyme production (Beauchemin et al., 2004a). Enzyme products are relatively concentrated and purified, containing specific enzyme activities, thus they do not contain live cells. Furthermore, these products may contain both exogenous and endogenous enzymes. Endogenous enzymes are those that are naturally produced within the animal while exogenous enzymes are derived from outside sources. Enzymes can be applied to livestock diets in both granulated and liquid forms (Beauchemin et al., 2004a).

Mode of Action. Cellulase and hemicellulase convert the major structural polysaccharides cellulose and hemicellulose into soluble sugars (Beauchemin et al., 2004a). The major cellulases involved in cellulose hydrolysis are: endocellulase (endoglucanase, endo-β-1, 4-glucanase, carboxymethyl cellulase or β-1,4-glucan glucanohydrolase), exocellulase (exoglucanase, exo-β-1,4-glucanase, cellulose β-1,4-cellobiosidase), and β-glucosidase (cellobiosidase or β-1,4-glucan glucanohydrolase). The endoglucanases and exoglucanases act synergistically to hydrolyze cellulose producing cellulobiose, which β-glucosidase cleaves releasing glucose. In the secondary walls of plant cells, hemicellulose is

characterized by a linear xylan core polymer, consisting of repeating units of β-1,4 linked xylose. The main enzymes involved in degrading the xylan core polymer to soluble sugars (xylose) are xylanases and β-1,4-xylosidase (Beauchemin et al., 2004a).

Differences in the impact on the efficacy of cell wall degradation may vary because of relative proportions and activities of enzymes in specific products (Beauchemin et al., 2004a; Beauchemin et al., 2004b; Adesogan, 2005). It should also be noted that these products are generally not tested under conditions normally found within the rumen (pH ~ 5.9, 39.5^{o}C). Therefore, the dosage recommended by the manufacturer may not result in a positive response in vivo. Any enzyme additive candidate should be tested in vitro under conditions simulating the appropriate rumen environment (Beauchemin et al., 2004a; Beauchemin et al., 2004b; Adesogan, 2005). In addition to breaking down the cellulosic and hemicellulosic components of feedstuffs, adding exogenous enzymes to a diet increases bacterial attachment, stimulates ruminal microbial populations, and acts synergistically with enzymes produced by the microbes. These additive effects increase enzyme activity in the rumen, enhancing digestibility of the diet (Beauchemin et al., 2004b).

Use of Fibrolytic Enzymes in Livestock Diets

Recent studies have shown that adding exogenous fibrolytic enzymes to ruminant diets can increase milk production of dairy cows and weight gain of growing beef cattle as a result of enhanced feed digestion (Beauchemin et al.,

1997; Yang et al., 2000). Studies have demonstrated that enzymes are effective when used in forage-based diets and also in high concentrate diets (Lewis et al., 1996; Beauchemin et al., 1997; Beauchemin et al. 1999; McAllister et al., 1999). However, several factors such as enzyme-feed specificity, level of enzyme application, and method of application can influence efficacy (Lewis et al., 1996; McAllister et al., 1999; Yang et al., 2000; Beauchemin et al., 2004a; Adesogan, 2005).

Rate of Application and Enzyme-feed Specificity. Beauchemin et al. (1997) conducted a study to establish whether fibrolytic enzymes influenced performance of feedlot cattle fed high-grain diets and compared grain sources. Fifty-six crossbred steers were fed a basal diet consisting of 95.1% concentrate and 4.9% silage for an average of 124 days. Dietary treatments included corn or barley-based diets with no added enzyme as a control, or grain treated with two differing enzyme complexes. Enzyme complexes were applied to the concentrate at a rate of 4.0L/ton DM. One enzyme contained higher xylanase and lower cellulase activity while the other contained lower xylanase but higher cellulase activity. Measured activities were as follows 1) 5500 international units (IU) of xylanase and 188 filter paper units (FPU) of cellulase/kg of dietary DM (Enzyme 1) and 2) 2200 IU of xylanase and 383 FPU of cellulase/kg dietary DM (Enzyme 2).

Enzyme 1 inclusion improved ($P < 0.05$) feed conversion of the barley-based diet by 11% compared to the control. Neither enzyme treatment improved ($P > 0.10$) feed conversion of the corn-based diet. Dry matter intake and ADG

were not different ($P > 0.10$) from the addition of fibrolytic enzymes to the diets (Beauchemin et al., 1997). Results of this study indicate the importance of enzyme combination relative to specific feed ingredients.

Barley-based diets were used with a fibrolytic enzyme solution of cellulases and xylanases in backgrounding and finishing studies (McAllister et al., 1999). In the backgrounding experiment, a single enzyme complex was applied to the barley silage directly at a rate of 0, 1.25, 3.5, and 5.0 L/ton of TMR (82.5% barley silage, 12.5% rolled barley). Feed intake tended to increase quadratically ($P > 0.11$) throughout the entire feeding period. Average daily gain tended ($P = 0.06$) to increase quadratically as enzyme concentration increased from day 0 to 56, however cumulative performance was similar between treatments ($P = 0.16$). A non-linear response to increasing application rate suggests higher levels of enzyme may not improve performance. In the finishing experiment cattle were fed the enzyme at a rate of 0 L/ton or 3.5 L/ton applied to the TMR directly (70% barley-ryegrass silage and 30% steam-rolled barley). Treating the TMR with 3.5 L/ton of enzyme increased ($P < 0.01$) ADG by 10% (McAllister et al, 1999). Reasons for a poor response to higher levels may include negative feedback inhibition, blocking of binding sites, or prevention of substrate colonization (Adesogan, 2005).

Methods of Enzyme Application. Yang et al. (2000) evaluated various application methods of an enzyme product and subsequent effects on feed intake, milk production, and digestibility. A proprietary enzyme blend containing relatively high xylanase and low cellulase activities was applied at a rate of 50 mg/kg total

diet DM, regardless of delivery method. Forty-three Holstein cows were fed one of three diets for 15 weeks. The basal diet contained 24% corn silage, 14% chopped hay, and 62% steam-rolled barley based-concentrate (Yang et al., 2000). Treatments were a control treatment with no enzyme, enzyme sprayed daily onto the TMR, or enzyme applied to the grain during steam rolling. Total tract DM digestibility was highest for the cows fed treated concentrate and lowest for cows fed the control; the treated TMR was intermediate ($P < 0.05$). Organic matter digestibility was not different between the treated grain and treated TMR, but digestibility was higher compared to control ($P < 0.05$). Milk production was higher for the cows fed the treated grain; no difference was noted between the control and treated TMR ($P > 0.05$). Adding a fibrolytic enzyme increased digestibility of the diet when applied to the TMR or grain. However, an increase in milk yield occurred only when the enzyme was added to the grain (Yang et al., 2000).

Lewis et al. (1996) used five ruminally cannulated steers to examine method of delivery of an enzyme mixture containing cellulases and xylanases. Steers were fed a basal diet consisting of 70% grass hay and 30% barley on a DM basis. The enzyme mixture was applied at a rate of 1.65 mL/kg of forage or concentrate DM and measured activities were 23,000 hydroxyethyl cellulase, 5,800 xylanase, 55 cellobiase, and 83 glucose oxidase IU/mL. Enzyme was applied as follows: 1) no enzyme (control), 2) to forage 24 h before feeding, 3) to forage 0 h before feeding, 4) to barley 0 h before feeding, and 5) infused ruminally 2 h after feeding. Total tract digestibility of DM, NDF, and ADF tended

to be higher ($P < 0.10$) when the enzyme was added to forage 24 or 0 h before feeding compared to the control (Lewis et al., 1996). It seems logical that a response was elicited when the enzyme was applied to the forage and not the concentrate as it was applied at the same rate to 70% and 30% of the diet, respectively, resulting in a higher dosage rate when delivered via the forage.

Enzyme Application in Commercial Settings. In a commercial feedlot setting, 1200 heifers were used to evaluate the effects of using a fibrolytic enzyme mixture in a high concentrate diet on intake and growth rate (Beauchemin et al., 1999). The basal diet was comprised of 88% dry-rolled barley, 4.2% supplement, and 7.8% barley silage. Two dietary treatments were used: 1) enzyme added to the dry-rolled barley and 2) a control with no added enzyme. Enzyme was applied at a rate of 1.40 L/ton (as-fed basis) resulting in calculated enzyme activities per kilogram of dietary DM of 33.4 IU xylanase and 66.3 IU cellulase. Intake was not affected by treatment ($P > 0.05$), however ADG was greater ($P < 0.01$) for heifers fed the ration containing enzyme treated barley (1.53 kg/d vs 1.40 kg/d; Beauchemin et al., 1999).

Results of current research indicate fibrolytic enzymes can be used to increase diet digestibility and performance of dairy cows and growing beef cattle. Method of delivery and level of application should be considered when adding enzymes to a diet. Furthermore, enzyme-substrate specificity should be taken into account to select the enzyme product most likely to be effective in a particular diet. In a feedlot setting, enzymes may be applicable in both high

forage backgrounding diets as well as high concentrate finishing diets (Beauchemin et al., 1997; Beauchemin et al., 1999; and McAllister et al., 1999).

Utilizing Enzymes in Distiller's Grains. As previously discussed, many by-products, including DG, commonly used in feedlot diets are high in dietary fiber. Use of fibrolytic enzymes may improve digestibility of these by-products, and subsequently, positively affect animal performance.

A possible issue with SDG is a less digestible fiber fraction compared to CDG (Owens, 2008), resulting in less available energy. Optimizing fiber digestibility is key to extracting the most energy, and therefore value, out of SDG and the addition of fibrolytic enzymes may enhance digestion. Based on the findings of several recent studies examining the effects of fibrolytic enzymes in high-concentrate diets, it was hypothesized that adding a mixture of fibrolytic enzymes to SWDGS will increase digestibility of the fiber fraction resulting in more energy available to the animal, thus increasing performance. Therefore, the objectives of this research were to:

1) Examine the effects of treating SWDGS with an enzyme blend or buffered enzyme blend on digestibility characteristics of nutrients in finishing steers
2) Examine the effects of treating SWDGS with an enzyme blend or buffered enzyme blend on performance and carcass characteristics in finishing steers

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Figure 1.1 Distribution of ethanol plants in the United States (RFA, 2014)

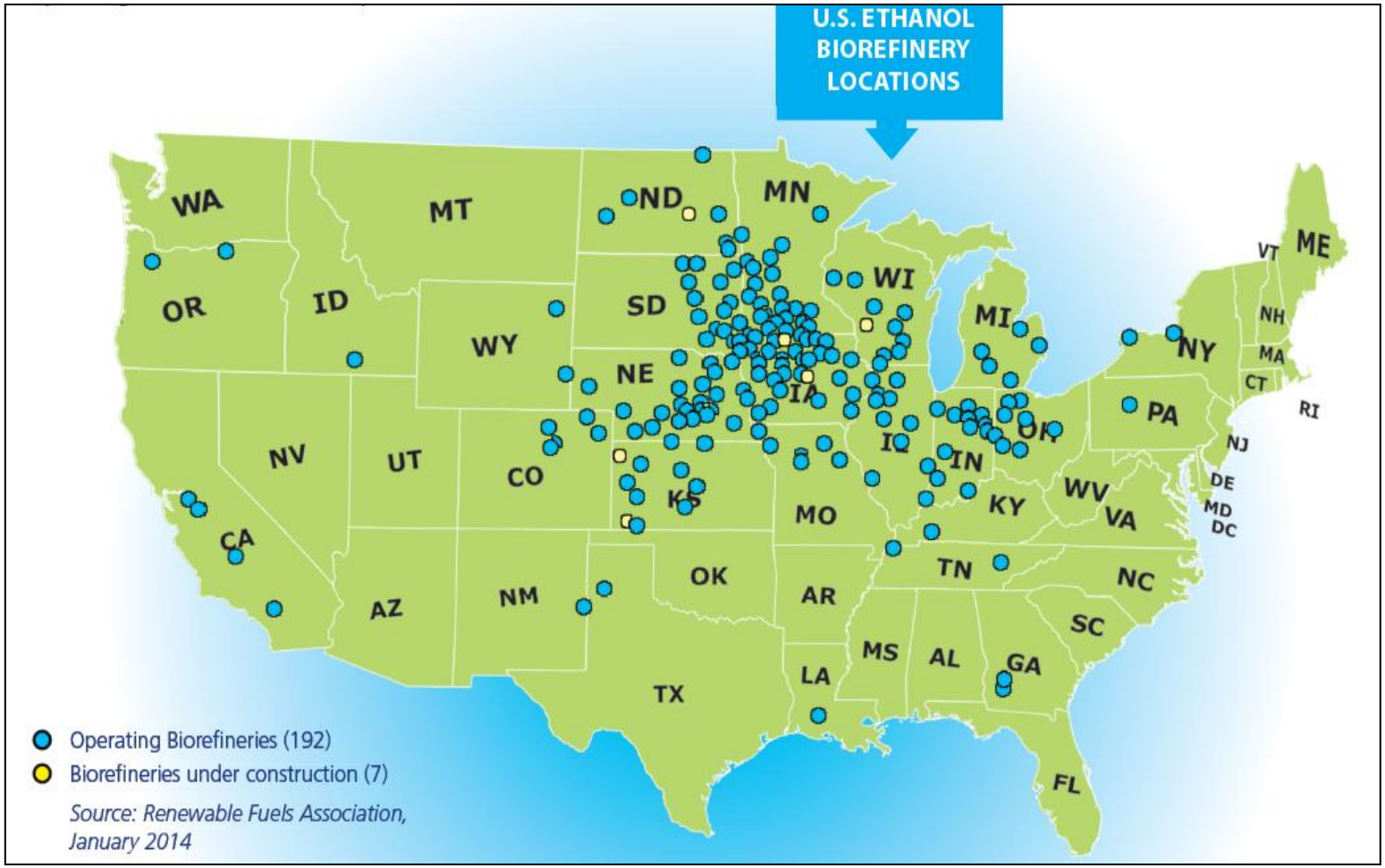
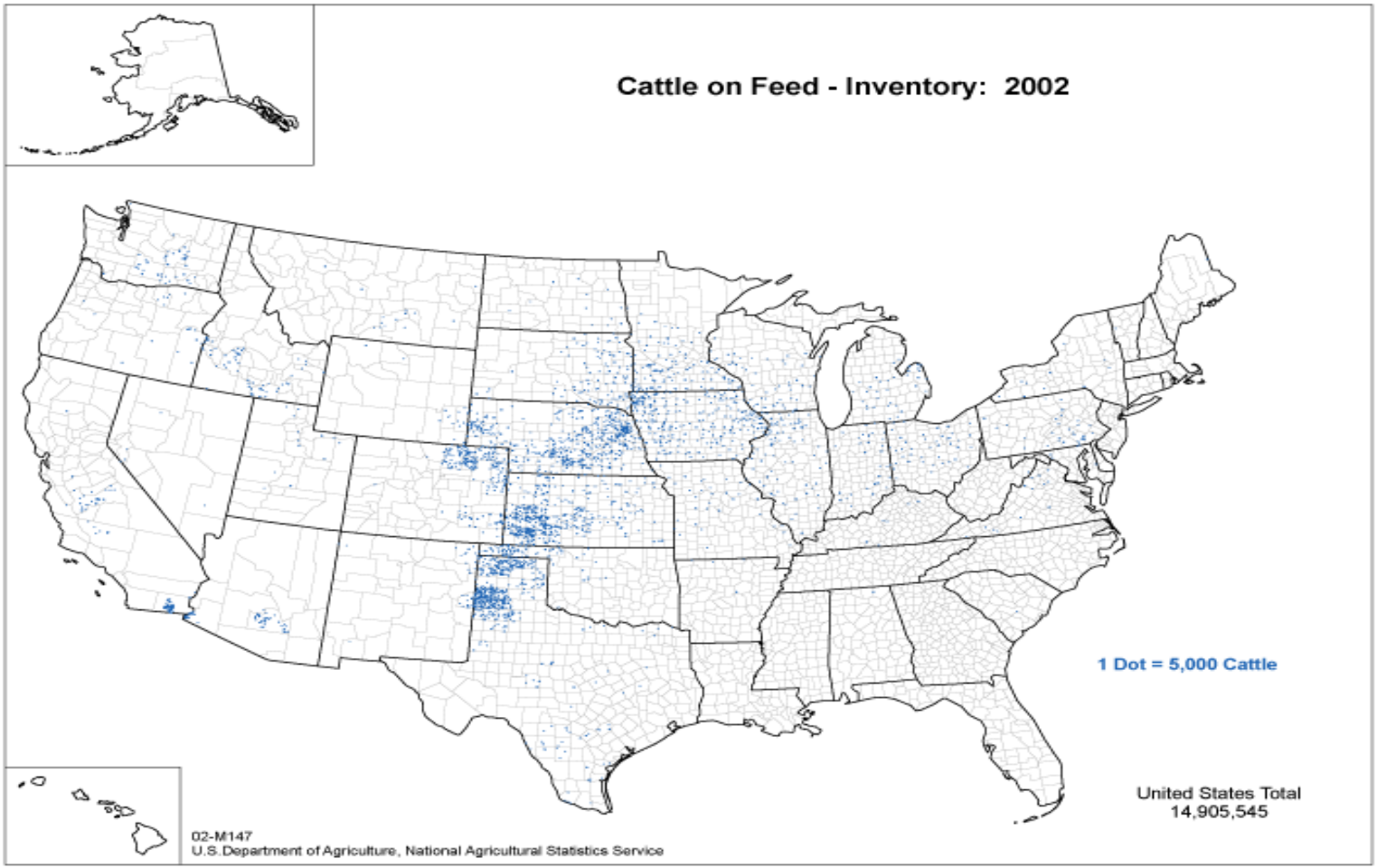


Figure 1.2 Distribution of fed cattle in the United States (USDA, 2002)



CHAPTER II

EFFECTS OF AN ENZYME COMPLEX ON DIET DIGESTIBILITY IN BEEF STEERS FED SORGHUM WET DISTILLER'S GRAINS WITH SOLUBLES-BASED DIETS

Introduction

Ethanol production in the southern Great Plains differs from that in the Midwest as plants in the southern Great Plains use sorghum grain as well as corn grain due to drought tolerance of sorghum. Reviews by Owens (2008) and Klopfenstein et al. (2008) suggest that sorghum WDGS (SWDGS) are inferior to corn WDGS (CWDGS) as a feedstuff. This difference may be partially due to a less digestible fiber fraction (Owens, 2008). Application of fibrolytic enzymes to SWDGS could potentially degrade the less digestible fiber and increase NDF and ADF digestibility (MacDonald, 2011). Results of a study conducted by Lewis et al. (1996) showed a tendency for increased total tract digestibility of DM, NDF, and ADF when a cellulase/xylanase enzyme mixture was added to forage 24 or 0 h before feeding compared to the control. Yang et al. (2000) observed an increase in total tract DM digestibility when an enzyme mixture containing cellulases and xylanases was added to the concentrate portion of a diet formulated for lactating dairy cows. Furthermore, digestibility of OM increased when enzyme was added to the concentrate portion or total mixed ration (TMR). Results of these studies indicate positive responses in fiber digestibility when exogenous fibrolytic enzymes are supplemented to cattle fed both forage and concentrate-based diets, however minimal research exists on buffering enzymes to maintain activity at an optimal pH for fiber digestion. The objective of this study was to evaluate

the effects of treating SWDGS with an enzyme complex containing primarily fibrolytic enzymes or buffering the enzyme complex on nutrient digestibility in cattle fed diets containing SFC.

Materials and Methods

Experimental Design

Six ruminally and duodenally cannulated steers (initial BW = 577 ± 6.9 kg) were used in a 3 x 3 repeated Latin square design. The basal diet consisted of 38% steam-flaked corn, 7.5% alfalfa hay, 4.5% premixed supplement (Table 2.1). Three dietary treatments were evaluated: 1) untreated SWDGS as a control (**Control**), 2) SWDGS with an enzyme complex added (**Enzyme**), and 3) buffered SWDGS with an enzyme complex added (**E+B**). Sorghum WDGS were treated with 10% limestone (E+B) and incorporated in the diet at 50% DM basis (DMB) to provide a 45% SWDGS and 5% limestone mixture. Sorghum WDGS were incorporated at 45% (DMB) in the Control and Enzyme diets and because the E+B SWDGS was treated with limestone, limestone was added to the Control and Enzyme diets (5%, DMB). The enzyme complex (Biozyme, Inc., St. Joseph, MI) was a proprietary blend both exogenous and endogenous hemicellulase, cellulase, and amylase enzymes. It was added at rate of 6 L/dry ton of SWDGS and diluted in water (84.6 L/dry ton of SWDGS) to aid in even distribution of the enzyme complex during treatment. Prior to study initiation, 100 tons of SWDGS were received and placed into an agricultural bag (Ag-Bag, St. Nazianz, WI).

Treated SWDGS was prepared once weekly and stored for a minimum of six days before being incorporated into diets.

Steers were housed individually in covered outdoor pens (3.6 m^2) during the entire course of the study. Steers were allowed *ad-libitum* intake and fed twice daily at 0800 and 1700h during three, 21 d periods. Periods consisted of 16 d of diet adaption followed by 5 days of sample collection using chromic oxide as an external marker to estimate fecal output. Boluses containing 7.5 g of chromic oxide were placed in rumen via the rumen cannula at 0800 and 1700 h during the last 13 d of each period. Diet, feed refusal, ruminal, and fecal samples were obtained during the collection period for further analysis. Diets were mixed bi-weekly and stored at 1^{o}C. Dry matter content of dietary ingredients was assessed weekly by drying in a forced-air oven at 60^{o}C for 48h and used to adjust dietary as-fed composition.

Sample Collection

Diet/Orts Samples

Diet and feed refusal samples were collected on d 17 through 21. Feed refusals were removed from each bunk just prior to the 0800 h feeding, weighed, and a 10% subsample was collected. If total refusal was less than 1 kg all of the orts were collected. Weight of determined feed refusals was deducted from DM delivery to calculate DMI. Diet and feed refusal samples were composited daily and stored at 4^{o}C until analyzed.

Ruminal and Fecal Samples

Ruminal fluid samples were collected on d 21 at 0700, 1100, 1300, and 1700 h. Ruminal fluid samples were taken from the ventral portion of the rumen and strained through four layers of cheesecloth. Sample pH was measured using a portable pH meter (Oakton II Series, Eutech Instruments, Singapore) and 3 subsamples (50 mL aliquots) were collected. Samples were stored at -4^{o}C until analyzed. For total tract digestion measurements, 250g of fecal matter was collected on d 18 through 21 at 0800, 1100, 1300, and 1700 h. Samples were frozen at -4^{o}C until analyzed.

Sample Preparation

Diet/Orts Samples

Subsamples of the diet and feed refusals were taken and dried in a forced-air oven for 48 h at 60^{o}C. Samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. Subsequently, approximately half of each sample was ground through a 0.5mm screen using a Cyclotec 1093 (Foss, Eden Prairie, MN).

Rumen Sample Preparation

One ruminal fluid aliquot from each time point was prepared for determination of VFA and ammonia concentration. Thawed samples were centrifuged at 5,000 x *g* for 30 minutes at 4^{o}C and four, 2 mL aliquots were harvested from the supernatant. Two aliquots were combined with 0.5 mL of 25% meta-phosphoric acid with 2-ethylbutyrate and two were combined with 0.5 mL of 25% meta-phosphoric acid for the VFA and ammonia assays, respectively.

Samples for ammonia analysis were refrigerated at 4°C for a minimum of thirty minutes before further analysis. Samples for VFA determination were centrifuged a second time after a minimum of 30 minutes of refrigeration at 4°C, again at 5,000 x *g* for 30 minutes at 4°C. Samples were then passed through a 0.45 µm polyethersulfone syringe filter into gas chromatography vials. Vials were then capped and stored at 4°C until analyzed.

Fecal Samples

Fecal samples were thawed and composited by steer within period (25 g from each sample) then dried in a forced-air oven for 48 h at 60°C for determination of DM content. Dried fecal samples were ground in a Wiley mill (Thomas Scientific) to pass a 1-mm screen. Subsequently, approximately half of each sample was ground through a 0.5mm screen using the Cyclotec 1093 (Foss). Chromium (Cr) concentration of fecal samples was determined by atomic absorption spectrophotometry (PerkinElmer model 3110, PerkinElmer, Waltham, MA) using an air acetylene flame according to Williams et al. (1962).

Nutrient Analysis

Dry and Organic Matter

Laboratory DM determination was performed on diet, feed refusal, and fecal samples by drying in a forced-air oven for 12 hours at 100°C, and OM was determined by ashing samples at 500°C for six hours.

Starch

Starch content was determined using a spectrophotometer (Power Wave-XS Spectrometer, BioTek US, Winooski, VT) after converting starch to glucose using an enzyme kit (Megazyme International Ireland Ltd., Wicklow, Ireland; method 996.11; AOAC, 2003). Samples were weighed into 50mL conical tubes and 0.2mL of aqueous ethanol (80% v/v) was added to each before vortexing. Immediately after 3mL of thermostable α-amylase was added and samples were incubated in a 100^{o}C water bath for 12 minutes with vortexing at 4, 8, and 12 minutes. Samples were then incubated at 50^{o}C with 4mL of sodium acetate buffer and 0.1mL amyloglucosidase. After removal from the water bath, samples were diluted to 30 or 50mL with deionized water for low or high starch samples, respectively. Samples were mixed thoroughly before centrifugation at 3,000 rpm for 10 minutes. After centrifugation duplicate aliquots (0.1mL) of the diluted solution were transferred to glass test tubes where 3mL of GOPOD reagent buffer (Megazyme International Ireland Ltd.) were added and samples were incubated at 50^{o}C for 20 minutes. Approximately 250µL from each test tube was distributed on a 96-well plate and absorbance was read at 510nm. Standards and blanks were included for quality control.

Neutral and Acid Detergent Fiber

Concentration of NDF was determined using the procedures of Van Soest et al. (1991) with sodium sulfite and amylase (Ankom Inc., Fairport, NY). Samples were weighed into filter bags (Ankom Inc.) and refluxed for 75 minutes at 100^{o}C in approximately 2L of NDF solution (Ankom Inc.), followed by three rinse cycles. Rinses involved 100^{o}C deionized water and amylase, and as a final

step to insure no residual fat was present, bags were saturated in acetone for five minutes. Determination of ADF content was performed non-sequentially using the procedures of Van Soest et al. (1991). Samples were weighed into filter bags (Ankom Inc.) and refluxed for 75 minutes at 100^{o}C in approximately 2L of ADF solution (Ankom Inc.), followed by three rinse cycles. Rinses involved 100^{o}C deionized water, and as a final step to insure no residual fat was present bags were saturated in acetone for five minutes.

Volatile Fatty Acids and Ammonia

After initial preparation, rumen fluid samples were analyzed for VFA and ammonia concentration. Samples were analyzed for VFA concentration using gas chromatography (Varian 3900, Varian Inc., Palo Alto, CA) according to procedures of Erwin et al. (1961). Ammonia concentration was determined using the method described by Broderick and Kang (1980) and quantified using a spectrophotometer (Power Wave-XS, BioTek US) at 550 nm.

Statistical analyses

Dry matter intake, nutrient digestibility, VFA and ammonia data were analyzed as a repeated Latin square design using the GLM procedure in SAS (SAS Inst Inc., Cary, NC) with steer as experimental unit. The model included the fixed effects of square, period within square, animal within square, and treatment. Rumen pH data were analyzed as repeated measures using the GLIMMIX procedure in SAS (SAS Inst Inc., Cary, NC) with time point as the experimental unit. The model included the fixed effects of square, period, and treatment and

the random effect of animal. Effects were considered significant at a *P*-value of ≤ 0.10, with tendencies declared at *P*-values between 0.10 and 0.15. Treatment means were separated using LSMEANS with PDIFF option.

Results and Discussion

Although the enzyme complex contained starch digesting enzymes, the results and discussion will focus primarily on fiber digestion characteristics. Nutrient composition of the treatment diets are shown in Table 2.1 Treatment of SWDGS with enzyme alone resulted in a 6% decrease in NDF content of the TMR. However, ADF content of the TMR was not affected by either treatment.

Dry matter intake and nutrient digestibility data are shown in Table 2.2. Dry matter intake was not affected (*P* = 0.54) by Enzyme or E+B treatment. Similarly, both Yang et al. (2000) and Lewis et al. (1996) reported no difference in intake with enzyme supplementation in concentrate and forage-based diets. Total tract digestion of DM, OM, and starch was not affected by treatment (*P* = 0.36, 0.28, and 0.81, respectively). Contrastingly, Yang et al. (2000) reported an increase in DM and OM digestibility when a fibrolytic enzyme complex was added to the grain portion only or to the complete diet. However, it should be noted that this effect was measured in dairy cattle with different production requirements and intakes compared to beef cattle. Organic matter (74.6%) and starch digestibility (96.6%) were not different (*P* = 0.28 and *P* = 0.81, respectively) across treatments. These results are similar to those reported in a study by Luebbe et al. (2012) where reported OM and starch digestibility were

78.6% and 98.2%, respectively, while using diets similar to those used in the current study (45% WDGS, 39.5% SFC vs. 45% WDGS, 38% SFC, respectively). The slightly lower values observed during the current study may be due to the difference in WDGS source, as Luebbe et al. (2012) used primarily corn-based (< 15% sorghum) compared to SWDGS in the current study.

Steers fed the E+B treatment tended (P = 0.15) to have greater NDF digestibility than the steers fed Enzyme or Control diets. Total tract NDF digestibility for E+B fed cattle was 54.5%, which is higher than observed by Luebbe et al. (48.5%; 2012).

Ruminal pH was higher with the E+B diet (P = 0.07) compared to Control (5.54 vs. 5.36), while ruminal pH of Enzyme fed cattle was intermediate (5.47; Table 2.3). Fiber digestion is optimized at a ruminal pH of ≥ 6.0, as fiber digesting cellulolytic bacteria thrive at a higher pH (Van Soest, 1982). This may partially explain the increase in NDF digestibility for the E+B fed steers. In contrast, Enzyme fed steers tended (P = 0.15) to have greater ADF digestibility than steers fed E+B or Control. Limited research exists on enzyme treatment of distiller's grains, therefore, the differing effects on fiber digestibility between the Enzyme and E+B treatments cannot be readily explained.

Average ammonia concentration across treatments was 9.96 mg/dL. Control fed cattle had a higher (P = 0.06) ruminal ammonia concentration than Enzyme fed cattle (11.77 vs. 7.85 mg/dL), while ruminal ammonia concentration for E+B fed cattle was intermediate (10.25 mg/dL). No differences (P = 0.53) were detected in total volatile fatty acid (VFA) concentration across treatments.

Interestingly, total VFA concentration was higher than reported by Luebbe et al. (2012), 135.86 millimoles vs. 116.9 millimoles, when a similar diet (45% WDG, DMB) was used. Furthermore, no differences ($P > 0.29$) were found in the molar proportions of acetate, propionate, isobutyrate, butyrate, isovalerate, or valerate. Acetate to propionate ratio did not differ ($P = 0.35$; 1.65) across treatments. We expected the E+B fed cattle to have increased total VFA and acetate production based on greater NDF digestibility of steers fed the E+B diet compared to control and enzyme fed cattle, however, this was not observed.

Conclusions

We hypothesized that fiber digestibility would improve with the addition of an enzyme mixture containing exogenous and endogenous fibrolytic enzymes. While no significant improvements in fiber digestibility were detected, results suggest that treating SWDGS with buffered enzymes tends to improve NDF digestibility of a SFC-based finishing diet. Further research is warranted to examine the effects of treating SWDGS with an enzyme mixture or buffered mixture on animal performance. Further research should include evaluating the method of application, rate of application, and enzyme specificity to better assess the potential benefits of enzyme complexes containing primarily fibrolytic enzymes to improve digestibility of SWDGS before they are dismissed.

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Table 2.1 Ingredient and nutrient composition of experimental diets

	Dietary Treatment[a]		
Ingredient, % (DMB)	**Control**	**Enzyme**	**E+B**
Steam-flaked Corn	38.0	38.0	38.0
Alfalfa Hay	7.5	7.5	7.5
Sorghum WDGS[b]	45.0	45.0	50.0
Limestone	5.0	5.0	0.0
Supplement[c]	4.5	4.5	4.5
Nutrient, % (DMB)			
DM	48.8	47.8	48.5
OM	89.2	90.0	90.2
Starch	29.5	30.2	29.4
CP	19.2	20.1	20.1
NDF	21.2	20.0	24.4
ADF	14.8	15.1	14.9

[a]Diets contained 45% SWDGS (DMB) with Control = untreated SWDGS, Enzyme = SWDGS with an enzyme complex added, E+B = buffered SWDGS with an enzyme complex added. The enzyme complex was added at a rate of 6 L/ dry ton.

[b]Limestone was added to the E+B treatment at a rate of 10% per ton (DMB), therefore 50% dietary inclusion of E+B treated SWDGS provided 45% SWDGS and 5% limestone.

[c]Provided vitamins and minerals to meet or exceed the NRC (1996) requirements. Monensin and Tylosin were provided at a rate of 436 mg/hd/d and 97 mg/hd/d, respectively.

Table 2.2 Effect of enzyme treated and buffered enzyme treated sorghum wet distiller's grains with solubles on dry matter intake and total tract digestibility of nutrients

	Dietary Treatment[a]				*P*-values[b]		
Variable	**Control**	**Enzyme**	**E+B**	**SE**	**Treatment**	**Period**	**Animal**
DMI, kg	11.3	10.7	10.8	0.35	0.54	0.24	0.03
Total Tract Digestion, %[c]							
DM	69.7	67.9	70.3	0.68	0.36	0.25	0.55
OM	75.3	73.1	75.3	0.61	0.28	0.36	0.42
Starch	96.6	95.9	97.2	0.75	0.81	0.40	0.60
NDF	40.2[b]	39.9[b]	54.5[a]	3.18	0.15	0.41	0.52
ADF	53.6[b]	64.1[a]	50.9[b]	2.80	0.15	0.26	0.28

[a]Diets contained 45% SWDGS (DMB) with Control = untreated SWDGS, Enzyme = SWDGS with an enzyme complex added, E+B = buffered SWDGS with an enzyme complex added. The enzyme complex was added at a rate of 6 L/ dry ton.

[b]Effects were considered significant at a *P*-value of ≤ 0.10, with tendencies declared at *P*-values between 0.10 and 0.15. Superscripts differ (*P*-value of ≤ 0.10).

Table 2.3 Effect of enzyme treated and buffered enzyme treated sorghum wet distiller's grains with solubles on fermentation characteristics

	Dietary Treatment[a]				*P*-values[b]		
Variable	**Control**	**Enzyme**	**E+B**	**SE**	**Treatment**	**Period**	**Animal**
pH	5.36[a]	5.47[ab]	5.54[b]	0.08	0.07	≤0.01	-
NH_3, mg/dL	11.77[a]	7.85[b]	10.25[ab]	0.81	0.06	0.59	≤0.01
Volatile Fatty Acids[c]							
Total	129.94	134.16	143.48	4.88	0.53	0.59	0.20
Acetate	48.06	48.66	52.20	1.19	0.32	0.80	0.07
Propionate	35.68	35.54	31.73	1.14	0.29	0.58	0.08
Isobutyrate	0.47	0.61	0.51	0.05	0.53	0.08	0.20
Butyrate	11.5	11.09	11.29	0.37	0.88	0.58	0.23
Isovalerate	1.97	1.69	2.18	0.18	0.54	0.59	0.17
Valerate	2.28	2.42	2.09	0.15	0.72	0.84	0.80
A:P[d]	1.55	1.56	1.85	0.10	0.35	0.56	0.07

[a]Diets contained 45% SWDGS (DMB) with Control = untreated SWDGS, Enzyme = SWDGS with an enzyme complex added, E+B = buffered SWDGS with an enzyme complex added. The enzyme complex was added at a rate of 6 L/dry ton.

[b]Effects were considered significant at a *P*-value of ≤ 0.10, with tendencies declared at *P*-values between 0.10 and 0.15. Superscripts differ (*P*-value of ≤ 0.10).

[c]Total Volatile fatty acid (VFA) concentration is reported in millimoles. Concentration of individual VFAs is reported in moles/100 moles.

[d]A:P= Acetate to Propionate ratio

CHAPTER III

CATTLE PERFORMANCE AND CARCASS CHARACTERISTICS OF BEEF STEERS FED SORGHUM WET DISTILLER'S GRAINS WITH SOLUBLES TREATED WITH AN ENZYME COMPLEX

Introduction

Reviews by Owens (2008) and Klopfenstein (2008) suggest that feeding sorghum-based wet distiller's grains (SWDG) results in minimal differences in animal performance compared to corn-based (CWDG). Several studies have shown no differences between CWDG and SWDG at an inclusion level of 30% or less on a DM basis (DMB) in dry-rolled corn or steam-flaked corn-based diets (DRC and SFC, respectively; Al-Suwaiegh et al., 2002; Depenbusch et al., 2009; May et al., 2010). Observed differences between SWDG and CWDG may be due to a higher ADF and lower fat content of SWDG versus CWDG. Furthermore, studies have demonstrated usage of wet distiller's grains (WDG) in DRC-based diets commonly used in the Midwest improves animal performance; however these same results have not been noted in SFC-based diets used in the southern Great Plains (Cole et al., 2006; Owens, 2008; MacDonald, 2011). This phenomenon may be explained, in part, by greater starch availability in SFC vs. DRC (Huntington, 1997; Armbruster, 2006). When WDG replaces DRC, energy availability is increased in the diet causing an improvement in animal performance. However, when WDG replaces a more readily available energy source, such as SFC, effect on performance is generally negative at higher inclusion levels (> 15% DMB; Cole et al., 2006; Owens, 2008; MacDonald, 2011).

Applying enzyme technologies to increase the digestibility of fibrous components of WDG may lead to increased animal performance when WDG is

fed with SFC. One method may be to treat WDG with fibrolytic enzymes, which solubilize fiber. While performance improvements of cattle fed forage-based diets supplemented with fibrolytic enzymes is logical, effects on performance in cattle fed concentrate based diets is more questionable. Several studies have demonstrated improvements in milk production and growth performance of dairy and beef cattle fed high concentrate diets when fibrolytic enzymes were supplemented (Beauchemin et al., 1997; Beauchemin et al., 1999; McAllister et al., 1999; Yang et al., 2000); however, minimal research exists on buffering enzymes to potentially promote activity at an optimal pH for fiber digestion. The objective of this study was to evaluate the effects of treating SWDG with solubles (SWDGS) with an enzyme complex containing primarily fibrolytic enzymes or buffering the enzyme complex on the performance and carcass characteristics of cattle fed SFC-based finishing diets.

Materials and Methods

Experimental Design

Seventy-one yearling crossbred steers were received at the Texas A&M AgriLife Research Feedlot on June 21, 2013. Upon receiving, steers received an individual ID tag and were weighed and sorted into pens. Steers weighing within ± 1 SD of mean body weight were considered eligible for the experiment. Primarily black cattle were chosen for the study to decrease variation within the group. Therefore, 54 beef steers (initial BW = 357 ± 8 kg) were used in a randomized complete block design and fed for an average of 161 days. Steers

were blocked by weight into a light and heavy group. Steers were randomly assigned to treatment and pen, with an equal number of treatment observations within a pen.

At processing, steers received 17 mL of fenbendazole (Safeguard, Merck Animal Health, Millsboro, DE) for treatment of internal parasites and were fitted with a Calan head gate key. Steers were fed individually using Calan electronic gates (American Calan, Northwood, NH) and trained to the system while being adjusted to a finishing ration over the course of approximately one month. Prior to initiating the trial and feeding finishing diets, the steers were weighed and processed again on d -1. Processing included: 1) placement of numbered, colored ID tags in left ear in accordance to pen number and Calan gate number 2) vaccination against respiratory viruses (Bovi-Shield Gold 5, Zoetis, Kalamazoo, MI) 3) vaccination against Clostridial spp. (Vision 7, Merck Animal Health) 4) treatment for internal parasites with fenbendazole (Safeguard, Merck Animal Health) and 5) implantation with 200 mg of trenbolone acetate and 40 mg of estradiol (Revalor-XS, Merck Animal Health) in the caudal aspect of the right ear.

Steers were weighed again the following day before feeding and the average of the two consecutive weights was used as the initial BW. Experimental diets were initiated on this day and delivered at an amount determined from the previous three days average DMI. Cattle were progressed to an amount allowing for *ad libitum* intake. Bunks were managed to contain 0.5-1 lbs of refused feed

each morning. Determined feed refusals were dried and dry weight was deducted from dry matter delivered to calculate DMI.

Steers were housed in six partially covered outdoor pens (7 x 29.4 m) equipped with nine Calan gates per pen and fed once daily beginning at approximately 0730h, diets were mixed daily immediately prior to feeding. A sample of each diet was collected weekly for determination of DM and to calculate DM delivery. Dry matter content of each dietary ingredient was assessed weekly by drying in a forced-air oven at 60^{o}C for 72h and used to adjust dietary as-fed composition.

The basal diet consisted of 38% steam-flaked corn, 7.5% alfalfa hay, 4.5% premixed supplement (Table 3.1). Three dietary treatments were evaluated: 1) untreated SWDGS as a control (**Control**), 2) SWDGS with an enzyme complex added (**Enzyme**), and 3) buffered SWDGS with an enzyme complex added (**E+B**). Sorghum WDGS were treated with 10% limestone (E+B) and incorporated in the diet at 50% DM basis (DMB) to provide a 45% SWDGS and 5% limestone mixture. Sorghum WDGS were incorporated at 45% (DMB) in the Control and Enzyme diets and because the E+B SWDGS was treated with limestone, limestone was added to the Control and Enzyme diets (5%, DMB). The enzyme complex (Biozyme, Inc., St. Joseph, MI) was a proprietary blend with both exogenous and endogenous hemicellulase, cellulase, and amylase enzymes. It was added at rate of 6.2 L/dry ton of SWDGS and diluted in water (73 L/dry ton of SWDGS) to aid in even distribution of the enzyme complex during treatment. Treated SWDGS was prepared once prior to the beginning of

the study and again in the middle of the feeding period (d 84). Prepared SWDGS was packed and stored in agricultural bags (Ag-Bag, St. Nazianz, WI).

Cattle were weighed prior to the morning feeding every 35 days from d 1 to 140. Final weights occurred on d 148 (heavy block) and d 174 (light block) before transportation to a Tyson Fresh Meats facility 47 kilometers away in Amarillo, TX. Standard carcass measurements were collected by trained personnel from West Texas A & M Beef Carcass Research Center.

Statistical analyses

Data were analyzed for homogeneity of variance using Levene's and Bartlett's tests in SAS (SAS Inst Inc., Cary, NC). Feedlot performance and carcass data were analyzed as a completely randomized block design using the MIXED procedure of SAS with steer as experimental unit. The model included fixed effects of treatment and random effects of pen, and pen by treatment interaction. Means were separated using preplanned contrasts Control vs. Enzyme and Enzyme vs. E+B. Contrasts were considered significant at a *P*-value of ≤ 0.05, with tendencies declared at *P*-values between > 0.05 and ≤ 0.15.

Results and Discussion

Although the enzyme complex contained starch digesting enzymes, the results and discussion will focus primarily on fiber digestion characteristics. Two steers were removed from the study due to digestive illness unrelated to treatment, and one steer due to inconsistent feed intakes unrelated to treatment.

Nutrient composition of dietary treatments and SWDGS are shown in Tables 3.1 and 3.2. Treatment with enzyme alone had minimal effects on fiber content, however, treatment of SWDGS with a buffered enzyme resulted in a 19% reduction in NDF and ADF compared to Control. However, these effects were diluted out when analyzing the total mixed ration (TMR).

Performance results are reported in Table 3.3. Final BW did not differ (P = 0.51) for Enzyme steers compared to Control, nor did final BW differ (P = 0.31) for E+B steers compared to Enzyme. McAllister et al. (1999) found no difference in final BW of finishing steers fed diets with or without an enzyme complex, however, these results are not directly comparable as McAllister et al. (1999) evaluated barley-based diets. Dry matter intake was not different (P = 0.35) for Enzyme fed cattle compared to Control for the entire feeding period. However, there was a tendency for increased (P = 0.11) DMI for Enzyme fed cattle from d 36 to 70 compared to Control. Several studies involving the treatment of the TMR or proportions of the TMR with enzyme complexes have also found no difference in DMI between cattle receiving enzyme treated diets compared to control (Beauchemin et al., 1997, Beauchemin et al., 1999; McAllister et al., 1999; Yang et al., 2000).

In contrast to the results of the digestibility trial, DMI for cattle consuming the E+B diet was lower (P = 0.03) throughout the entire feeding period compared to Enzyme treatment. From d 0 to 35 DMI tended to be lower (P = 0.13) for E+B cattle compared to Enzyme treatment, and from d 36 to 70 and d 71 to 105 DMI was lower ($P \leq 0.04$) for E+B cattle compared to Enzyme fed cattle. This may

have been due to the differences in storage time of the SWDGS treatments, as SWDGS prepared during the metabolism trail was used within a period of one week versus several months for the feeding trial. During the performance trial all SWDGS molded slightly and the E+B treatment was observed to be more prone to spoiling. Average DMI across treatments was 9.2 kg/d, which is considerably lower than the 11.4 kg/d DMI reported by Luebbe et al. (2012). Furthermore, Al-Suwaiegh et al. (2002) reported 11.1 kg/d DMI when cattle were fed diets containing 30% SWDG (DMB). Conversely, Daubert et al. (2005) fed diets containing 40% SWDG (DMB) and observed a DMI of 8.3 kg/d. From the results of these studies it can be concluded that the average DMI of 9.2 kg/d observed in the present study, while not abnormal, is slightly lower than other reports.

Cattle fed the Enzyme treatment had greater (P = 0.04) average daily gain and tended to have an improved (P = 0.10) G:F from d 36 to 70 compared to Control. Cumulative ADG and G:F, however, were not different (P = 0.83 and 0.31; respectively) between Enzyme fed cattle and Control. Average daily gain was lower (P = 0.05) for E+B fed cattle compared to Enzyme treatment from d 36 to 70, however there was no difference (P = 0.39) in ADG between the E+B and Enzyme treatment for the entire feeding period. While no differences were seen in ADG for the entire feeding period, E+B fed cattle had greater (P = 0.05) G:F for the entire feeding period compared to Enzyme. These results were similar to those reported by McAllister et al. (1999).

Standard carcass measurements were not affected (P > 0.43) by treatment when Enzyme fed cattle were compared to Control (Table 3.4).

McAllister et al. (1999) and Beauchemin et al. (1997) also observed no differences in standard carcass measurements when cattle where fed diets supplemented with enzyme complexes compared to control. There was no difference (*P* = 0.47 and 0.55) in HCW or dressing percent between E+ B and Enzyme treatments. However, cattle fed the E+B diet had less (*P* = 0.05) rib fat and lower yield grades (*P* = 0.02) than cattle fed the Enzyme treatment. The E+B cattle tended to have greater (*P* = 0.06) ribeye area (REA) compared to cattle fed the Enzyme treatment. Limited to no research exists on enzyme treatment of distiller's grains and its effects on carcass merit, therefore the tendency for greater REA when comparing the E+B and Enzyme treatments is not readily explainable.

Conclusions

Analysis of SWDGS showed an improvement in NDF and ADF content when SWDGS was treated with a limestone buffer and enzyme complex. However, these effects were diluted when the treated SWDGS were incorporated into the diet, as NDF and ADF content of the total diet remained relatively similar.

Results of the current study demonstrate that the addition of an enzyme complex alone or in conjunction with a limestone buffer to SWDGS does not result in improved feedlot performance. However, addition of a buffered enzyme complex had positive effects on carcass characteristics compared to enzyme complex alone. When treating the SWDGS before a feeding trial, it is unknown as to how prolonged exposure to the SWDGS affects the enzyme or vice versa.

Further research is warranted to evaluate method and rate of application, enzyme specificity, and ingredient storage method before dismissing the potential benefits of using enzymes to improve digestibility of SWDGS.

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Table 3.1 Ingredient and nutrient composition of experimental diets

	Dietary Treatment[a]		
Ingredient, % (DMB)	**Control**	**Enzyme**	**E+B**
Steam-flaked Corn	38.0	38.0	38.0
Alfalfa Hay	7.5	7.5	7.5
Sorghum WDGS[b]	45.0	45.0	50.0
Limestone	5.0	5.0	0.0
Supplement[c]	4.5	4.5	4.5
Nutrient (DMB)			
DM, %	51.76	50.38	49.71
NDF, %	26.2	27.6	25.5
ADF, %	20.5	23.0	20.9
CP, %	23.7	26.0	24.2
Fat, %	6.4	7.2	6.1
Ca, %	1.99	2.40	2.34
P, %	0.57	0.63	0.58
S, %	0.36	0.39	0.38
Na, %	0.20	0.22	0.21
NE_m, Mcal/kg	1.72	1.63	1.69
NE_g, Mcal/kg	1.10	1.01	1.08

[a]Diets contained 45% SWDGS (DMB) with Control = untreated SWDGS, Enzyme = SWDGS with an enzyme complex added, E+B = buffered SWDGS with an enzyme complex added. The enzyme complex was added at a rate of 6 L/dry ton.
[b]Limestone was added to the E+B treatment at a rate of 10% per ton (DMB), therefore 50% dietary inclusion of E +B treated SWDGS provided 45% SWDGS and 5% limestone.
[c]Provided vitamins and minerals to meet or exceed the NRC (1996) requirements. Monensin and Tylosin provided at a rate of 381 mg/hd/d and 85 mg/hd/d respectively.

Table 3.2 Nutrient composition of treated sorghum wet distiller's grains with solubles

	Treatment[a]		
Nutrient[b]	**Control**	**Enzyme**	**E+B**
DM, %	35.09	33.44	34.07
NDF, %	36.80	35.10	29.70
ADF, %	33.90	33.40	27.40
CP, %	40.90	42.60	37.20
Fat, %	11.30	11.40	9.80
Ca, %	0.36	0.35	4.51
P, %	0.88	0.94	1.00
S, %	0.60	0.61	0.60
NE_m, Mcal/kg	1.14	1.16	1.47
NE_g, Mcal/kg	0.70	0.70	0.90

[a]Control = untreated SWDGS, Enzyme = SWDGS with an enzyme complex added, E+B = buffered SWDGS with an enzyme complex added. The enzyme complex was added at a rate of 6 L/dry ton.

[b]Monthly composites were collected throughout the feeding period and composited into one sample for nutrient analysis.

Table 3.3 Effect of enzyme treated and buffered enzyme treated sorghum wet distiller's grains with solubles on growth performance

	Treatment[a]				*P*-Value[b]	
Variable	**Control**	**Enzyme**	**E+B**	**SE**	**C vs. E**	**E vs. E+B**
N	18	18	18	-	-	-
Cattle removed[c]	0	1	2	-	-	-
Average DOF	161	161	161	-	-	-
Initial wt[d], kg	354	358	358	8	0.29	0.91
Final wt[d], kg	568	578	562	10	0.51	0.31
DMI, kg/d						
d 0 to 35	8.6	8.8	7.9	0.37	0.63	0.13
d 36 to 70	9.6	10.6	8.8	0.39	0.11	0.01
d 71 to 105	10.0	10.3	8.9	0.43	0.65	0.04
d 0 to end	9.3	9.8	8.6	0.33	0.35	0.03
ADG, kg/d						
d 0 to 35	1.53	1.68	1.59	0.11	0.33	0.54
d 36 to 70	1.35	1.67	1.36	0.10	0.04	0.05
d 71 to 105	1.64	1.48	1.30	0.10	0.28	0.21
d 0 to end	1.25	1.27	1.19	0.06	0.83	0.39
G:F						
d 0 to 35	0.17	0.19	0.20	0.01	0.33	0.49
d 36 to 70	0.14	0.16	0.15	0.01	0.10	0.59
d 71 to 105	0.16	0.14	0.14	0.01	0.08	0.99
d 0 to end	0.13	0.12	0.14	0.01	0.31	0.05

[a]Diets contained 45% SWDGS (DMB) with Control = untreated SWDGS, Enzyme = SWDGS with an enzyme complex added, E+B = buffered SWDGS with an enzyme complex added. The enzyme complex was added at a rate of 6 L/dry ton.
[b]Effects were considered significant at a *P*-value of ≤ 0.05, with tendencies declared at *P*-values between 0.05 and 0.15.
[c]Performance from cattle removed was not included in statistical analyses.
[d]Adjusted for 4% shrink

Table 3.4 Effect of enzyme treated and buffered enzyme treated sorghum wet distiller's grains with solubles on carcass traits

	Treatment[a]				*P*-Value[b]	
Variable	**Control**	**Enzyme**	**E+B**	**SE**	**C vs. E**	**E vs. E +B**
HCW, kg	356	363	356	6	0.43	0.47
Dress, %	62.7	63.0	63.3	0.48	0.81	0.55
Rib fat, cm	1.02	1.02	0.76	0.04	0.67	0.05
KPH, %	1.9	1.9	1.8	0.13	0.89	0.42
REA, cm^2	86.8	87.0	93.4	2.29	0.96	0.06
Yield grade	2.5	2.6	1.9	0.18	0.63	0.02
Marbling score[cd]	40	39	38	1.69	0.77	0.56

[a]Diets contained 45% SWDGS (DMB) with Control = untreated SWDGS, Enzyme = SWDGS with an enzyme complex added, E+B = buffered SWDGS with an enzyme complex added. The enzyme complex was added at a rate of 6 L/dry ton.

[b]Effects were considered significant at a *P*-value of ≤ 0.05, with tendencies declared at *P*-values between 0.05 and 0.15.

[c]Marbling Score: 30 = Small; 40 = Slight; 50 = Moderate traces

[d]Corresponding quality grade = Choice, Select, and Select for Control, Enzyme, and E+B fed cattle, respectively.