EXPLORING SYNERGIES BETWEEN BIOFUEL COPRODUCTS FOR FINISHING BEEF CATTLE

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ABSTRACT

Two experiments were conducted to evaluate the effects of feeding condensed distillers solubles (**DS**) and crude glycerin alone or in combination on performance of finishing beef cattle and *in vitro* fermentation. In both experiments, dietary treatments consisted of a steam-flaked corn (**SFC**) based-diet with 0% DS or crude glycerin (**CON**), 10% condensed distillers solubles (**CDS**), 10% crude glycerin (**GLY**), or a combination of 5% DS and 5% crude glycerin (**C+G**) included on a DM basis. All treatment diets contained 15% (DM basis) wet distillers grains plus solubles (**WDGS**). In Exp. 1, crossbred steers (n=250; initial BW = 322 ± 15 kg) were used in a randomized complete block finishing trial. Growth performance and gain efficiency were not different ($P > 0.10$) across all treatments. Treatment had no effect ($P > 0.10$) on carcass weight, marbling score, yield grade, LM area, or % grading USDA choice. In Exp. 2, ruminal fluid was collected from 2 ruminally cannulated steers to evaluate *in vitro* fermentation characteristics. No differences ($P = 0.43$) were observed for dry matter disappearance (**DMD**) across all treatments. The GLY and C+G treatments had decreased ($P = 0.02$ and $P = 0.05$, respectively) neutral detergent fiber disappearance (**NDFD**), while the CDS treatment tended to have decreased ($P = 0.06$) NDFD compared to CON. Concentrations of NH_3 decreased ($P < 0.04$) with GLY and C+G treatments compared to CON. Total gas production decreased ($P < 0.01$) for the C+G treatment compared to other treatments. Likewise, the C+G treatment also had decreased ($P < 0.07$) percent of CO_2 than other treatments. Percent CH_4 decreased ($P = 0.01$) for the C+G treatment compared to the CON and CDS treatments, but did not differ ($P = 0.13$) from the GLY treatment. The CDS treatment had increased ($P < 0.02$) total VFA compared to the CON or C+G

treatments. Treatments had no effect ($P > 0.17$) on concentrations of acetate, propionate, and butyrate, but valerate concentrations were the greatest ($P < 0.04$) for the CON treatment. Concentrations of isobutyrate increased ($P < 0.05$) for the CON and C+G treatments compared to the GLY treatment and tended to increase ($P < 0.10$) compared to the CDS treatment. Including DS or glycerin in the substrates decreased ($P < 0.08$) isovalerate compared to CON. Feeding additional byproducts such as DS and crude glycerin alone or in combination in a finishing diet did not alter live animal performance or carcass characteristics; however, feeding a combination of the two byproducts may alter *in vitro* fermentation.

Key words: corn wet distillers grains, condensed distillers solubles, crude glycerin

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DS = condensed distillers solubles
CSM = cottonseed meal
DDG = dried distillers grains
DGS = distillers grains plus solubles
DDGS = dried distillers grains plus solubles
DMD = dry matter dissapearance
DRC = dry-rolled corn
EE = ether extract
MDGS = modified distillers grains plus solubles
NDFD = neutral detergent fiber dissapearance
HMC = high-moisture corn
LG = long grind
peNDF = physically effective neutral detergent fiber
PSPS = Penn State particle separator
SFC = steam-flaked corn
SG = short grind
VFA = volatile fatty acid
WCGF = wet corn gluten feed
WDG = wet distillers grains
WDGS = wet distillers grains plus solubles

CHAPTER I

REVIEW OF LITERATURE

Introduction

Fermentation of cereal grains has been used for centuries to produce beverage alcohol (Klopfenstein et al., 2008). The demand for using agricultural crops for liquid fuels spiked in the 1970s with the disruption of the supply of oil from the middle-east, as well as the phase-out of lead as an octane booster in gasoline (Bothast and Schlicher, 2005). Today, with limited oil reserves and a high dependence on foreign oil, the United States' need for alternative energy sources have been a highly focused area, with biofuels remaining as an attractive option (Bothast and Schlicher, 2005). As the production of biofuels continues to increase in the U.S., so does the quantity of associated byproducts that are derived from the production of these fuels. The byproducts are secondary products that are of no use to the producers of biofuels, but may be used as animal feed.

Condensed distillers solubles (**DS**) are a liquid byproduct produced from the dry-grind ethanol process (Stock et al., 2000). The DS is often added back to the solid fraction byproducts to produce distillers grains plus solubles (**DGS**). The amount of DS added to distillers grains is dependent on the ethanol plant's capacity to store the liquid DS. When supply of DS exceeds storage availability, DS is available to livestock producers as a standalone feed ingredient (Pesta et al., 2015). Condensed distillers solubles have consistently shown to improve performance when fed to beef cattle (Titlow et al., 2013; Hughes et al., 2013; Pesta et al., 2015). These performance benefits may be

attributed to DS being moderate in CP (20-30%) and high in fat (9-25%), which makes DS a suitable feed ingredient for beef cattle (Lardy, 2007).

Crude glycerin is the liquid byproduct of catalyzed transesterification reactions of triglycerides to produce biodiesel. Crude glycerin is often used in animal feeds due to its low cost, and its ability to control dust and reduce sorting of dietary components of mixed rations (Drouillard, 2008). Several experiments have shown that crude glycerin improves performance when fed at low levels (less than 8% DMB) to growing and finishing cattle (Hales et al., 2013; Parsons et al., 2009). The increase in performance may be related to the fermentation of crude glycerin in the rumen, as it is fermented entirely to propionate (Parsons et al., 2009). With an increase in the production of these byproducts, and their relatively low cost compared to corn grain, a better understanding of how DS and crude glycerin might be fed in combination to finishing cattle is needed.

Ethanol and Distillers Coproducts

Ethanol Production Processes

Producing alcohol from grain involves the fermentative conversion of starch to alcohol by yeast (Stock et al., 2000). According to the Renewable Fuels Association (RFA, 2016) Ethanol Industry Outlook, 94% of ethanol plants in the United States utilize field corn as a feedstock for ethanol production. Corn is the most economical grain source for ethanol production in the United States as starch in corn accounts for 70-72% of the kernel weight on a dry weight basis (Bothast and Schlicher, 2005). Ninety percent of ethanol in the United States is produced by a dry-grind production process (RFA, 2016). The dry-grind method is designed to utilize the entire corn kernel during the fermentation process (Rausch and Belyea, 2006). The whole kernels are ground and

mixed into water with added amylase and heated. After the heating process, glucoamylase and yeast are added and the mash is fermented resulting in a material consisting of ethanol, water, and solids that were not fermented (Rausch and Belyea, 2006). The fermented mash is processed using various adsorption techniques to remove the alcohol from the large volume of water and residual dry matter (Stock et al., 2000). The alcohol is blended with a small amount of gasoline to produce fuel-grade ethanol (Rausch and Belyea, 2006).

Coproducts of Dry-Grind Ethanol Production

When grain is fermented into ethanol, the remaining slurry after the distillation process is called whole or spent stillage and typically contains 5 to 10% DM (Stock et al., 2000). Whole stillage is obtained from the bottom of the distillation unit and is centrifuged to separate the thin stillage from the wet grains (Rausch and Belyea, 2006). The solid fraction may be sold as wet distillers grains (**WDG**) or dried to produce dried distillers grains (**DDG**). The liquid that remains after the separation of the solids is called thin stillage, and contains small grain particles and yeast cells that constitute up to 40% of the DM (Stock et al., 2000). The thin stillage is concentrated using an evaporator to produce DS (Rausch and Belyea, 2006). The DS may be marketed as a separate ingredient or added back to the grains to produce wet distillers grains plus solubles (**WDGS**), or if dried, dried distillers grains plus solubles (**DDGS**; DiCostanzo et al., 2015). If an ethanol plant adds all of the DS back to the distillers grains, the distillers grains plus solubles contain roughly 81% distillers grains and 19% solubles (Klopfenstein et al., 2007).

Distillers Grains plus Solubles

Dried or wet distillers grains plus solubles are commonly included in cattle finishing diets as a protein or additive energy source (Klopfenstein et al., 2008). Distillers grains are added to feedlot diets as a protein source to potentially replace other natural sources of protein or urea. Wet distillers grains fed less than 15-20% DM of the diet serve as a protein source in the diet, whereas greater than 20% DM basis inclusion can be used to replace grains as an energy source (Klopfenstein et al., 2007).

Grain processing method and inclusion rate can have an effect on the performance of cattle consuming WDGS. Corrigan et al. (2009) reported an interaction between corn processing method (dry-rolled corn; **DRC** vs. steam-flaked corn; **SFC**) and WDGS fed at 0, 15, 27.5 and 40% (DM) for shrunk final BW, carcass adjusted final BW, ADG, and G:F. In response to increasing WDGS level, shrunk final BW, carcass adjusted BW and ADG increased quadratically ($P < 0.05$) in SFC-based diets with the greatest ADG for steers fed 15% WDGS. Dry matter intake differed ($P < 0.01$) across all corn processing methods with SFC having the least. A linear increase of G:F occurred as WDGS inclusion increased for DRC ($P < 0.01$) and HMC ($P < 0.05$) but no differences were detected for the SFC treatments.

Luebbe et al. (2012) fed SFC-based diets containing 0, 15, 30, 45, and 60% WDGS (DMB). A quadratic response ($P < 0.01$) occurred for dry matter intake with increasing levels of WDGS, with the maximum occurring at 15 and 30% inclusion. Final BW, ADG and G:F decreased linearly ($P < 0.01$) as level of WDGS increased. May et al., (2010) fed SFC-based diets with 0, 15, and 30% WDGS (DMB) and found that steers consuming 0% WDGS tended to have a greater DMI ($P = 0.10$) and ADG ($P = 0.05$) than

cattle consuming diets containing WDGS. Cattle consuming 15% WDGS had G:F values similar to that of the 0% WDGS diet. May et al, (2010) also investigated intake and apparent total tract digestibility of steers consuming SFC-based diets containing 0 or 15% WDGS. Feeding steers 15% WDGS increased ($P < 0.05$) CP and NDF intake, and tended ($P < 0.10$) to decrease the intake of starch. Apparent total tract digestion of DM, OM, CP, NDF, and starch did not differ ($P \geq 0.25$) across treatments. The results from this research illustrate that WDGS can be included in SFC-based finishing diets up to 15% (DM basis) with comparable performance to diets without WDGS. Following the base assumption that byproducts are purchased at 95% the price of corn, including WDGS at moderate amounts in SFC-based diets may decrease feed costs without negatively effecting cattle performance (Klopfenstein et al., 2007).

Lipids in Feedlot Cattle Diets Containing Ethanol Byproducts

Fat is generally added to feedlot cattle diets as it can improve palatability, feed efficiency, partially alleviate heat stress, and may influence nutrient partitioning (Zinn and Jorquera, 2007). Lipids can be classified as saturated or unsaturated fatty acids with the difference being in the bonding structure of the carbons in the fatty acid molecules (Bremer et al., 2011). When esterified lipids are consumed by ruminants, they are hydrolyzed extensively by microbial lipases into free fatty acids and glycerol (Jenkins, 1993). Unsaturated free fatty acids formed by lipolysis are hydrogenated to their saturated counterparts by the rumen microbes; this is known as biohydrogenation (Polan et al., 1964).

Nutritionally, lipids are a concentrated source of energy that is three times the net energy value of corn (Zinn and Jorquera, 2007). The energy value of fat is largely

dependent on its extent of intestinal digestibility (Zinn et al., 2000). Post-ruminal digestibility of saturated fatty acids is low compared to unsaturated fatty acids, but due to biohydrogenation, only about 35% of unsaturated fatty acids consumed by the animal pass to the small intestine in the unsaturated form. (Plascencia et al., 1999). Absorption of fatty acids is dependent on the formation and surface area of bile salt micelles in the intestine. Increasing concentrations of unsaturated fatty acids in the small intestine stimulate the formation of micelles and may aid in the absorption of saturated fatty acids (Zinn et al., 2000). When a rumen-protected canola oilseed fat was fed in combination with yellow grease, Zinn et al. (2000) reported that for every 1% increase in the proportion of C18:1 entering the small intestine, the digestibility of C18:0 increased by 1%.

Along with energy and protein, ethanol byproducts can provide a moderate amount of fat when included in cattle diets (Lardy, 2007). The level of fat can increase from 4% in corn to 12% during the distillers process (Klopfenstein et al., 2008). The lipid profile of ethanol byproducts is highly unsaturated, typically consisting of greater than 60% linoleic acid (Schingoethe et al., 2009). Experiments conducted by Vander Pol et al. (2009) evaluated WDGS as a fat source compared to corn or other sources of supplemental fat in DRC/HMC-based finishing diets. Heifers were fed dietary treatments with varying fat sources (corn oil or WDGS) and levels to determine the effects on feedlot performance. The corn oil was fed at 0, 2.5, and 5% and the WDGS was fed at 0, 20, and 40% of diet DM. Diets were formulated such that the 2.5% corn oil and the 20% WDGS, and the 5% corn oil and the 40% WDGS diets contained the same amount of ether extract (**EE**). Results from this trial showed that DMI tended ($P = 0.13$) to decrease

linearly as corn oil or WDGS increased. As supplemental corn oil increased, ADG decreased linearly ($P = 0.04$) and G:F also tended to decreased linearly ($P = 0.10$) compared to the control. As WDGS increased in the diet, ADG and G:F remained similar ($P > 0.20$) between treatments.

Vander Pol et al. (2009) also conducted a metabolism experiment to evaluate the effects of feeding WDGS, a composite, or supplemental corn oil in finishing diets on feeding behavior, digestion, duodenal fatty acid profiles, and metabolism of lipids. Treatments consisted of dry-rolled corn-based finishing diets containing 40% inclusion of WDGS, a 2.5:1 composite of corn bran and corn gluten meal, a 7.2:2.8:1 composite of corn bran, corn gluten meal and corn oil, and two diets with 0 or 5% corn oil. Fat added as corn oil was 70% digested and fat added as WDGS was 81% digested. Unsaturated fatty acids were higher (30.9%) in duodenal contents of cattle fed WDGS than steers fed corn oil (10.8%). These data indicate that the fatty acids in WDGS are not hydrogenated to the same extent in the rumen as fatty acids in supplemental corn oil and increased the lipid digestibility in WDGS compared with corn oil. The increase in fat digestion of WDGS could be explained by an increase in unsaturated fatty acids entering the intestine that stimulate bile salt micelle formation, which aid in digestion (Zinn et al., 2000).

Condensed Distillers Solubles

Protein and Lipids in Condensed Distillers Solubles

Generally, condensed distillers solubles contains 20 to 30% CP (Lardy, 2007), and the majority of the protein comes from yeast cells that have been heated during distillation and condensation. Heat denatures yeast cells and makes them resistant to lyses and degradation by rumen microbes, therefore DS contains high amounts of rumen

bypass protein (Klopfenstein et al., 2008). Several studies have been conducted to investigate using DS to replace other forms of crude protein fed to cattle. Trenkle and Pingel (2004) fed yearling steers to evaluate the effects on feedlot performance and carcass characteristics when DS was used to replace a portion of DRC and supplemental urea in finishing diets. Dietary treatments consisted of DS fed at 0, 4, 8, and 12% (DMB), with urea only included in the 0% DS diet to balance for CP. No differences ($P > 0.05$) for DMI, ADG, or feed efficiency were observed when DS replaced a portion of DRC and urea in the diet. Similarly, carcass characteristics were not affected ($P > 0.05$) by including DS in the diet. The results suggest that DS can provide enough energy and protein to replace corn and urea in finishing diets without negatively affecting performance or carcass characteristics (Trenkle and Pingel, 2004).

Simroth-Rodriguez et al. (2013) evaluated the effects of replacing supplemental N from urea or cottonseed meal (**CSM**) with DS on growth performance and carcass attributes of feedlot steers. The control diet was SFC-based with urea and CSM providing supplemental CP. Dietary treatments consisted of 10% DS replacing urea or CSM and 20% DS replacing urea or CSM. Diets were formulated to contain equal fat, and CP values increased as DS inclusion increased. Dry matter intake and ADG were greater ($P < 0.06$) for steers when DS replaced urea. Feed efficiency was not different ($P > 0.10$) between the control or when DS replaced urea or CSM. Dry matter intake and ADG remained similar ($P > 0.10$) across DS inclusion; however, feed efficiency decreased ($P = 0.02$) when 20% DS was fed compared to 10% (DMB). Carcass traits were not different between the control and DS treatments ($P > 0.49$), but LM area increased ($P = 0.07$) and HCW, fat thickness, YG, and empty body fat decreased ($P < 0.05$) when DS replaced

CSM. The results from this trial suggest that DS is more effective at replacing CP supplied by urea than CSM (Simroth-Rodriguez et al., 2013).

Similar to other ethanol byproducts, DS provides a moderate amount of fat in cattle diets. Fat in DS can be variable and range from 9 to 25% (Lardy, 2007). The lipid profile of DS is slightly more saturated than other sources of fat and may not be biohydrogenated to the extent of other sources of lipids (Bremer et al., 2011). Bremer et al. (2010) investigated the effects of dietary fat source on fat metabolism characteristics of steers fed feedlot finishing diets. Treatments were five DRC-based diets with different lipid sources. A control diet contained no fat source and the remaining diets contained fat in the forms of corn oil, tallow, DS, and WDGS. All diets containing added fat were formulated to contain 8.5% fat (DM basis). Lipid digestibilities were greater than 89% for all the fat treatments, indicating that intestinal fat absorption efficiencies were not reduced by high-fat diets. Total tract DM digestibility was greatest ($P < 0.10$) for DS and lowest for WDGS. Average ruminal pH was lowest ($P < 0.10$) for DS and highest for corn oil, and the time of pH below 5.6 was greatest ($P < 0.10$) for DS and least for the corn oil and tallow treatments. Feeding DS increased ($P < 0.10$) total fatty acid digestibility compared to other treatments. Rumen volatile fatty acid (VFA) proportion of acetate was greatest ($P < 0.10$) for the corn oil and WDGS treatments and the least for DS, resulting in numerically lower acetate to propionate ratio for the DS treatment. Fat profiles and DMI were similar for DS and corn oil; however, DS fat does not appear to limit ruminal fermentation compared to corn oil (Bremer et al., 2010).

Ruminal Characteristics of Condensed Distillers Solubles

The inclusion of DS acts differently in the rumen compared to other distillers byproducts. These differences can be attributed by its liquid physical form, protein structure, and fatty acid profile (Fron et al., 1995). Larson et al. (1993) evaluated ruminal fermentation characteristics and bypass of thin stillage. Steers were fed a DRC-based finishing diet and offered thin stillage alone that was marked with Co-EDTA to estimate the percentage of thin stillage that bypassed ruminal fermentation. Based on the amount of Co-EDTA measured in the rumen, roughly 53% of the thin stillage bypassed the rumen, suggesting that the undegraded protein and fat in distillers solubles could allow more nutrients to be passed to the small intestine (Larson et al., 1993).

Fron et al. (1995) conducted an *in vitro* and live animal experiment to determine the impact of DS on rumen metabolism and amylolytic and lactilytic microbial populations. Concentrations of fumarate, malate, and succinate were identified in several sources of DS. The *in vitro* trial measured the disappearance rate of added lactic acid from mixed rumen contents when water, thin stillage, or DS were added. Adding DS to the incubations increased ($P < 0.07$) the concentration of L-lactate, as well as the VFA concentrations of butyrate and propionate. In the metabolism trial, ruminally cannulated steers were fed DRC-based finishing diets with or without 15% DS (DM basis). The DS replaced corn when included in the diet. Dietary treatment had no effect ($P > 0.10$) on rumen pH and pH remained between 5.3 and 5.8 throughout the sampling period. Concentrations of amylolytic and lactilytic bacteria were greater ($P < 0.05$) for cattle consuming DS. Nisbet and Martin (1990) demonstrated that growth and lactate uptake of *Selenomonas ruminantium* are increased in the presence of fumarate and malate. This is

supported by Fron et al. (1995) who determined that DS can enhance capacity of the microbial population to utilize lactic acid by increasing the numbers of lactilytic bacteria in the rumen.

Condensed Distillers Solubles in Feedlot Diets

Several experiments have been conducted to evaluate the feeding value of DS in feedlot diets (Pesta et al., 2015; Titlow et al., 2013; Harris et al., 2014; Hughes et al., 2013). Pesta et al. (2015) evaluated the effects of including increased levels of DS in finishing diets on performance and carcass characteristics of beef steers. Condensed distillers solubles was included at 0, 9, 18, 27, or 36% (DM basis). The addition of DS replaced a proportion of urea and corn (1:1 blend of DRC and high-moisture corn; **HMC**). Dietary crude protein, ether extract and sulfur increased as level of DS increased in the diet. Intake decreased linearly ($P < 0.01$) as DS level increased, which was likely due to an increase in dietary fat, sulfur, or energy density of the diet. A quadratic response was observed for ADG ($P = 0.01$) and G:F ($P < 0.01$) with maximum weight gain at 20.8% DS inclusion, and a maximum G:F at a 32.5% DS level. These values were calculated using the first derivative of the quadratic response. An increase ($P = 0.01$) in final carcass-adjusted BW was observed for the 18% DS treatment, and decreased in the 27 and 36% treatments. Hot carcass weight increased quadratically ($P = 0.01$) with the greatest HCW reported in cattle fed 18% DS. Dressing percent increased linearly ($P = 0.03$) as DS inclusion increased. The data suggests that DS may be included in finishing up to 27% DM in DRC:HMC-based diets (Pesta et al., 2015).

Titlow et al. (2013) investigated the effects of corn processing method (DRC or SFC) and DS level (0, 15, or 30%; DM basis) on performance and carcass characteristics

of finishing steers. Dry matter intake for both SFC and DRC fed cattle decreased ($P < 0.04$) as DS level increased. A processing method × DS level interaction ($P < 0.05$) was noted for carcass adjusted final BW, ADG, and F:G. There was a quadratic increase ($P < 0.01$) in final BW and ADG for DRC-based diets. Average daily gain increased at the 15% DS level and decreased at the 30% level compared to the 0% DS treatment for both corn processing methods. Feed:gain decreased quadratically ($P < 0.03$) as DS level increased in DRC diets. Final BW and ADG increased linearly ($P = 0.01$) as DS level increased in the SFC treatments. Fat thickness increased linearly ($P = 0.02$) for SFC diets, but for DRC-based diets no differences ($P = 0.88$) occurred across DS treatments. There was a linear increase ($P = 0.01$) for yield grade in SFC diets due to the increase in fat thickness, but no difference in yield grade were detected for DRC treatments. The linear increase in ADG for steers fed SFC-based diets and DS up to 30% suggests that DS may be fed at levels greater than 30% (DM; Titlow et al., 2013). Harris et al. (2014) fed SFC diets with 0, 9, 18, 27 or 36% DS (DM basis) replacing SFC and urea in the treatment diets. Dietary crude protein, fat, Ca, P, and S increased as inclusion of DS increased. Dry matter intake decreased linearly ($P < 0.01$) as level of DS increased. A quadratic effect ($P < 0.01$) was observed for daily gain and feed efficiency with the maximum ADG and minimum F:G occurring when DS is fed at 27% (DM). A quadratic difference ($P < 0.01$) in HCW occurred as DS increased, in which HCW peaked at 27% DS inclusion. Marbling score, calculated YG, and LM area tended to increase quadratically ($P < 0.10$) as level of DS increased. This study suggests that SFC can be replaced with DS up to 36% without negatively effecting performance or carcass traits of finishing steers (Harris et al., 2014).

In the experiments previously discussed, dietary fat concentrations increased as level of DS increased, which clouds interpretation on the independent effects of dietary fat and energy density of DS. Hughes et al. (2013) investigated the effects of feeding DS with WDGS in SFC-based finishing diets. Treatments included DS concentrations (0, 7.5, or 15% of diet DM) in diets containing 0 or 15% WDGS (represented by 0/0, 0/15, 7.5/15, and 15/15 DS/WDGS, respectively). The WDGS and DS replaced portions of SFC, cottonseed meal, yellow grease, and urea. Diets were formulated to contain equal fat and crude protein values across all treatments. Dry matter intake tended to increase ($P = 0.12$) with increasing levels of DS, but DMI was not different between the control and the average of the remaining treatments ($P = 0.58$). Average daily gain was greater ($P < 0.01$) for cattle consuming WDGS and DS than the control diet (0/0) but ADG was not different ($P > 0.43$) among DS treatments. Overall, feeding DS improved ($P < 0.01$) gain efficiency compared to a control, but no differences ($P > 0.10$) in F:G were observed within DS treatments. Hot carcass weight ($P = 0.02$) and fat thickness ($P = 0.09$) were greater for cattle consuming WDGS and DS and no other differences ($P > 0.10$) were observed for other carcass measurements. These results suggest that the NEg of WDGS and DS were 100 and 85% the NEg of steam-flaked corn, respectively. Wet DGS is an effective replacement for SFC in finishing diets at 15% (DM basis) and DS may be included up to 15% (DM) in combination with WDGS in feedlot diets balanced for fat without negatively effecting performance or carcass traits.

Crude Glycerin

Biodiesel and Glycerin Production

Vegetable oils were first used as diesel fuel in the 1930s, but typically only in emergency situations. At that time, an abundance of cheap petroleum fuels made it economically infeasible to use an alternative diesel fuel from biological sources (Ma and Hanna, 1999). The fuel crisis during the late 1970s sparked the interest in using vegetable-oil derived fuels and methods in refining biodiesel started being investigated (Knothe, 2005).

Transesterification of triglycerides by methanol is the most common method of producing biodiesel in the United States. The coproduct of this process is crude glycerin (Van Gerpen and Knothe, 2005). Glycerol is insoluble in biodiesel and is easily removed by settling or centrifugation. The glycerol leaving the separator is only about 50% glycerol, with the remaining fraction consisting of methanol, the catalyst and soap. The glycerol is refined by adding acid to split the soaps into free fatty acids (FFA) and salts. The FFA are not soluble in glycerol and will separate, making them easy to remove and recycle. The salts remain with the glycerol, but some may precipitate out. The methanol is removed by evaporation, which yields crude glycerin with a purity of ~85%. The impurities remaining typically consist of salt, ash, lipid, methanol, and water and can be highly variable depending on the production process (Van Gerpen and Knothe, 2005).

Crude glycerin is a sweet, viscous liquid that can be used in animal diets to increase palatability, control dust, and aid in preventing sorting of dietary ingredients in a total mixed ration (Drouillard, 2008). According to the Association of American Feed Control Officials (AAFCO, 2016), crude glycerin cannot be fed to animals until it

contains at least 80% glycerin and not more than 15% water, 0.5% methanol, and 5 ppm heavy metals. In addition, it may contain up to 8% salt and it may not be included at greater than 15% of complete feed for ruminants (AAFCO, 2016).

Ruminal Fermentation and Metabolism of Crude Glycerin

Crude glycerin does not contain a similar nutrient composition to that of coproducts from ethanol production. Values for protein and fat are low, and macro minerals can range from 4 to 165 ppm with the exception of sodium, which is typically just over 1% (Thompson and He, 2006). Using glycerin as a feed ingredient for cattle depends largely on how well the animals are able to utilize the glycerin in the rumen (Hess et al., 2008). Glycerin has three fates in the rumen: passage (13%), fermentation (44%) and absorption (43%; Krehbiel, 2008). Of the glycerin that is fermented, it has consistently resulted in a shift in the VFA profile towards propionate at the expense of acetate in the rumen (Werner Omazic et al., 2015).

Parsons and Drouillard (2010) fed steers SFC-based finishing diets containing 0, 2, and 4% crude glycerin (DM) to determine the effects on rumen fermentation. Apparent total tract digestibilities of DM, OM, starch, CP and fat were similar across all treatments ($P > 0.51$). Digestibility of NDF decreased linearly ($P < 0.01$) as glycerin level increased. Ruminal pH increased linearly ($P < 0.06$) from 5.61 in control steers to 5.67 and 5.71 for 2 and 4% glycerin, respectively. Butyrate, valerate, and acetate concentrations decreased linearly ($P < 0.06$) as crude glycerin increased. These changes in VFA profiles may relate to changes in fiber digestion that were reported.

Long et al. (2015) investigated the effects of feeding 0, 8, or 16% crude glycerin (DM basis) on ruminal metabolism characteristics. Feeding glycerin did not affect ($P \geq$

0.19) *in situ* DM or NDF digestibility. As expected, concentrations of propionate increased linearly ($P = 0.01$) with increasing dietary glycerin. As glycerin levels increased, butyrate decreased ($P = 0.03$), which was similar to the findings by Parsons and Drouillard (2010). Differences in fiber digestibility between the experiments by Long et al. (2015) and Parsons and Drouillard et al. (2010) may be due to the extent of glycerin inclusion (high vs. low, respectively) or differences in diet composition as dietary components can influence the microbial population that utilize glycerin (Hess et al., 2008).

Ramos and Kerley (2012) included 0, 5, 10 and 20% crude glycerin with whole corn diets in continuous cultures to measure fermentation characteristics. Dry matter and OM digestibility decreased linearly ($P < 0.05$) as glycerin increased, but there was no effect of glycerin on CP and NDF digestibility ($P = 0.20$ and 0.65, respectively). Total concentrations of VFA and ammonia did not change ($P > 0.05$) due to crude glycerin level, but concentrations of propionate increased linearly ($P < 0.01$) and acetate decreased linearly ($P < 0.01$) as glycerin inclusion increased. Microbial efficiency increased quadratically ($P = 0.01$) as glycerin increased, whereas microbial N flow did not differ ($P = 0.36$). These results suggest that including crude glycerin in cattle diets can decrease the acetate:propionate ratio, but due to the reduction in DM and OM digestibility, lower inclusions of glycerin (less than 10% DM) may be optimum.

Supplementing crude glycerin to cattle has been shown to partially inhibit rumen lipolysis, the prerequisite for rumen biohydrogenation. Edwards et al. (2012) used mixed populations of ruminal bacteria incubated for 48 h *in vitro* with 6 or 20% (vol/vol) glycerin. Concentrations of free fatty acids for the 6 and 20% glycerin samples were

reduced ($P < 0.05$) by 80 and 86%, respectively when compared to the nonsupplemented controls. Since free fatty acids are a result of rumen lipolysis, this data suggests that supplementing glycerol near 8 to 15% of the diet DM (equivalent to the 6% vol/vol treatment) may effectively reduce rumen lipolysis, and thus reducing biohydrogenation of fats and allow more unsaturated fatty acids to pass to the small intestine for absorption.

Effects of Crude Glycerin on Feedlot Cattle Performance

Crude glycerin is an affordable option as an alternative feed source and several studies have evaluated the use of glycerin in diets for beef cattle. Parsons et al. (2009) fed crossbred heifers SFC-based finishing diets containing 0, 2, 4, 8, 12 or 16% crude glycerin (DM basis) to evaluate its effects on performance and carcass traits. A linear decrease ($P < 0.01$) in DMI was reported as glycerin concentration increased. This response may be due to an increase in propionate concentration as glycerin increased. Propionate is a proposed chemical satiety factor in feedlot cattle that may influence intake (Grovum, 1988). Both ADG and G:F responded quadratically ($P \leq 0.05$) and ADG and G:F was optimal when glycerin was fed at 2% of the diet. Glycerin increased ($P < 0.05$) final BW and HCW quadratically when fed at 2, 4, and 8% of the diet and reduced final BW and HCW when fed at 12 and 16%. Feeding increasing levels of glycerin linearly decreased ($P < 0.05$) ribeye area, marbling scores and subcutaneous fat. Additionally, glycerin tended to decrease ($P = 0.08$) percentage of cattle grading USDA choice or higher. The results from this trial suggest that crude glycerin can improve feedlot performance and yield leaner carcasses when fed at less than 8% on a DM basis.

Hales et al. (2013) conducted a similar experiment in which crude glycerin was used to replace SFC in growing diets to determine the effects on cattle performance.

Glycerin was included at 0, 2.5, 5, 7.5, and 10% of diet DM. Final BW tended to respond quadratically ($P = 0.09$) in which it increased from 0 to 7.5% and decreased from 7.5 to 10% glycerin. Average daily gain also responded quadratically ($P = 0.04$), with the maximum ADG peaking at 7.5% glycerin inclusion. No differences ($P > 0.23$) among treatments were detected for DMI. Feed efficiency decreased linearly ($P = 0.05$) with increasing levels of glycerin. These results are similar to that of Parsons et al. (2009) where less than 8% inclusion of glycerin was optimal for gain; however, DMI does not appear to be affected in growing diets like in the finishing diets of Parsons et al. (2009).

Given the known effect of glycerin reducing ruminal biohydrogenation of fatty acids *in vitro* and diets discussed did not contain supplemental fat indicates a limitation in the feeding value of glycerin that may be overcome. Buttrey et al. (2015) investigated crude glycerin concentrations in SFC-based diets containing 3% yellow grease on animal performance and carcass characteristics. Glycerin was included at 0, 2.5, 5, and 10% dietary DM, replacing SFC and diets contained similar amounts of ether extract. No differences ($P > 0.01$) for DMI, ADG, or G:F were reported across all treatments. It is unclear as to why these results in performance do not agree with that of other published studies. Hot carcass weight and dressing percentage tended to respond quadratically ($P = 0.10$) by decreasing from 2.5 to 5% and increasing at 10% inclusion. Fat thickness decreased linearly ($P < 0.01$) as glycerin increased, which resulted in a linear decrease ($P = 0.04$) in calculated YG. No differences ($P > 0.21$) were detected for marbling score and LM area. Buttrey et al. (2015) concluded that feeding crude glycerin with supplemental fat from yellow grease did not improve feedlot performance, but decreased fat thickness and YG similarly to Parsons et al. (2009).

Glycerin in Feedlot Diets Containing Ethanol Byproducts

Due to the expansion of the renewable fuels industry and the subsequent increase in the availability of the byproducts that come from these fuels, it may be beneficial to investigate the use of byproducts from both ethanol and biodiesel production together in finishing diets and data is limited on this subject. Schneider et al. (2010) fed diets containing 15% WDG with 0 or 2% crude glycerin replacing steam-flaked corn. They found no differences in feedlot performance or carcass characteristics. Buttrey et al. (2012) fed 4% crude glycerin in diets containing 20% WDGS in SFC or DRC-based diets. Including WDGS in steam-flaked corn diets containing crude glycerin tended to increase final BW ($P = 0.10$) and G:F ($P = 0.08$) compared to the SFC-based diets containing no WDGS.

The research by Schneider et al. (2010) and Buttrey et al. (2012) yielded different results in performance from cattle consuming crude glycerin with ethanol byproducts. One reason for these differences could be due to the byproducts used in these experiments. The solubles fraction of the WDGS fed by Buttrey et al. (2012) may have had an unexplained interaction with the crude glycerin, as the WDG fed by Schneider et al. (2010) did not contain solubles. These unknown differences in performance warrant further investigation.

Summary

Minimal research exists on feeding DS and crude glycerin in combination to finishing cattle. It is known that DS contains substrates that stimulate the growth and function of *Selenomonas ruminantium*, which is the main fermenter of glycerin (Nisbet

and Martin, 1990; Krehbiel, 2008). Feeding DS with glycerin has the potential to increase the population of *Selenomonas ruminantium* in the rumen, which may increase fermentation of added glycerin subsequent molar proportions of propionate, the most energetically favorable VFA. Glycerin has the ability to reduce biohydrogenation of fatty acids in the rumen (Edwards et al., 2012), and Bremer et al. (2010) demonstrated that lipids in DS are not protected from biohydrogenation like that of lipids in WDGS. Feeding glycerin with DS may synergistically aid in the reduction of biohydrogenation of the fats provided by DS. Since the NE value of dietary fat is largely a function of its intestinal digestibility, this reduction in biohydrogenation may allow for more fatty acids to pass to the small intestine for absorption and increase the energy value of the lipids provided by DS (Zinn et al., 2000).

Given these factors, we propose that synergy with combinations of DS and crude glycerin may improve growth performance while maintaining carcass quality compared to feeding these byproducts independently; therefore, our objectives for this research were to:

1) Determine the effects on *in vitro* fermentation when condensed distillers solubles and crude glycerin are fed alone or combination in finishing diets.

2) Determine the effects of feeding condensed distillers solubles and crude glycerin alone or in combination on performance and carcass characteristics of finishing steers.

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CHAPTER II

EFFECTS OF FEEDING CORN CONDENSED DISTILLERS SOLUBLES AND CRUDE GLYCERIN ALONE OR IN COMBINATION IN FINISHING CATTLE DIETS ON *IN VITRO* FERMENTATION CHARACTERISTICS

Introduction

Glycerin is a liquid byproduct of biodiesel production and may be used in animal feeds to control dust and prevent segregation of dietary components of mixed rations (Drouillard, 2008). Glycerin is an attractive feed ingredient for ruminants as it has consistently shown to improve volatile fatty acid (VFA) profiles of the rumen by reducing acetate and increasing propionate, the more energetically favorable VFA (Trabue et al., 2007; Ramos and Kerley, 2012; Long et al, 2015). This shift in acetate:propionate was demonstrated by Rèmond et al. (1993) who found that about half of glycerin consumed by ruminants passes out of the rumen or is absorbed across the rumen epithelium. The remaining glycerin is fermented entirely to propionate by *Selenomonas ruminantium,* which is a common species of the rumen flora (Rèmond et al., 1993). Condensed distillers solubles (**DS**) is a flowable liquid derived from ethanol production (Stock et al., 2000). Laboratory analysis by Fron et al. (1995) identified concentrations of succinate, fumarate, and malate in several sources of DS. Nisbet and Martin (1990) found that growth of *S. ruminantium* are increased in the presence of fumarate and malate; therefore, it is hypothesized that by combining DS with glycerin in cattle diets, the substrates provided by the DS would stimulate the growth of *S. ruminantium*, thus increasing the fermentation of glycerin into propionate. The objective

of this experiment was in investigate the effects of combining DS and glycerin in cattle diets on *in vitro* fermentation characteristics compared to including these byproducts independently.

Materials and Methods

Treatments and Substrates

The treatment substrates used in this experiment consisted of SFC-based finishing diets containing 15% wet distillers grains plus solubles (**WDGS**) and 8.5% corn stalks (DM basis) with: no additional byproducts (**CON**), 10% condensed distillers solubles (**CDS**), 10% glycerin (**GLY**), or a combination of 5% DS and 5% glycerin (**C+G**). Diet samples were dried at 55°C for 48 h and ground through a 2 mm screen (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific, Swedesboro, NJ). Filter bags (F57 25 µm porosity, Ankom Technology, Macedon NY) were presoaked in acetone, labeled with a solvent resistant permanent marker, and weighed. Treatment substrate (500 mg) was added to each bag. The bags were weighed in quadruplicate (4 bags/treatment) and sealed using an impulse sealer (American International Electric, model AIE-200) Four blank bags were incubated. All bags were fixed with a plastic-coated wire weight to ensure that the bags stayed submerged during incubation.

In Vitro Fermentation Procedure

Ruminal fluid was collected from two ruminally fistulated steers approximately 4 h post-feeding. The diet of the donor steers consisted of 54.75% steam-flaked corn (**SFC**), 25% wet corn gluten feed (**WCGF**), 10% ground corn stalks, 4% supplement, 0.80% urea, 1.8% limestone, and 3.65% corn oil. Ruminal fluid was placed directly into pre-warmed insulated containers to maintain temperature during transport to the

laboratory. The ruminal fluid from both steers was strained through four layers of cheese cloth, mixed, and purged with carbon dioxide. McDougall's buffer (McDougall, 1948) was added to achieve a 2:1 buffer to ruminal fluid. The pH was recorded of rumen fluid (pH = 5.52) and the inoculum (pH = 6.76) using an electronic pH meter (sympHony H10p, VWR, Radnor, PA) prior to incubation. Inoculum (150 mL) was added to 250 mL fermentation flasks (Ankom Technology, Fairport, NY) containing the substrate bags. The fermentation flasks were flushed with carbon dioxide, capped with a pressure module (Ankom Technology) and placed into a circulating water bath set at 39°C. The flasks were incubated for 48 h and gas production was recorded every 30 min using the Ankom RF Gas Production System (Ankom Technology). Following incubation, flasks were removed from the water bath and pH was recorded. Inoculum (50 mL) was collected from each flask, poured into sealed conical tubes, and immediately frozen for subsequent VFA and ammonia analysis. The substrate bags were removed from the flasks and immediately rinsed with cold water to stop fermentation.

Laboratory Analysis

Non-incubated substrates were dried at 100°C for 12 h to determine initial DM. After *in vitro* fermentation, the substrate bags were dried at 55°C for 16 h and then weighed to determine *in vitro* dry matter disappearance (**DMD**) and saved for NDF analysis. The dry residue weight (corrected for the blanks) was subtracted from the dry substrate weight and divided by the dry weight of substrate to calculate DMD. Initial and post-incubation substrates were analyzed for NDF to determine *in vitro* NDF disappearance (**NDFD**). Samples were analyzed for NDF using an Ankom fiber analyzer with sodium sulfite and amylase (Model 200/220, Ankom Technology). Initial substrates

were presoaked in acetone twice for 10 min prior to analysis as EE content was greater than 5%. Neutral detergent fiber values from the initial samples were used to calculate NDF content in the pre-incubation substrates. Values for post-incubation residual NDF were divided by the amount of NDF calculated to be in the samples prior to incubation to determine NDFD.

Ruminal fluid samples were prepared by adding 25% wt/wt metaphosporic acid with 2-ethyl butyrate and analyzed for VFA using the procedures of Erwin et al. (1961) with a Varian 3900 GC (Varian Inc. Palo Alto, CA) equipped with a flame ionization detector and a 30-m × 0.25-mm Supelco fused silica capillary column (Sigma-Aldrich, St. Louis, MO). Helium gas was used as the carrier for VFA analysis. Ammonia concentrations were determined using a spectrophotometer (PowerWave-XS Spectrometer, Bio Tek US, Winooski, VT) at a wavelength of 550 nm as described by Broderick and Kang (1980).

Statistical Analysis

Data from the *in vitro* experiment were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC). The model statement included treatment as the fixed effect and replicate as the random effect. The LSMEANS statement with PDIFF option was used to separate treatment means. Effects were considered significant at $P \leq 0.05$, with tendencies being declared at $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

In Vitro Disappearance

Results from the *in vitro* fermentation experiment are presented in Table 2.1. No differences ($P = 0.43$) were observed for DMD. Quinn et al. (2011) reported no

differences for DMD when DS concentrations increased with proportions of distillers grains in SFC-based diets. These data differ from Chen et al. (1976), who observed a decrease in DMD of orchardgrass when 1.25 mg of DS was included during the fermentation, suggesting that DS may have a negative impact on DMD of high forage diets compared to high concentrate diets. The CON treatment tended to increase (P = 0.09) NDFD compared to the other treatments. There is limited data on the effect of DS on *in vitro* NDFD. The current trial does not agree with previous *in vitro* research on glycerin inclusion. Ramos and Kerley (2012) reported a linear decrease in DMD and no difference in NDFD when glycerin was included in a high concentrate substrate containing 0, 5, 10, and 20% glycerin (DMB). These data suggest that glycerin has different effects on *in vitro* disappearance of DM and NDF depending on diet type and glycerin inclusion.

Ammonia Concentration and Gas Production

Ammonia (NH_3) concentrations decreased ($P < 0.04$) with GLY and C+G treatments compared to CON. Lee et al. (2011) reported a decrease in NH_3 concentration *in vitro* when glycerin was included with corn (5:1 corn to glycerin) compared to corn alone. The result of the current trial are similar to that of metabolism trials by Gilbery et al. (2006) and Sasikala-Appukuttan et al. (2008) who reported no differences in NH_3 concentration of rumen fluid when DS was included at less than 15% of diet DM. In the current experiment, total gas production for the 48 h incubation decreased ($P < 0.01$) for the C+G treatment compared to other treatments. Quinn et al. (2011) reported an increase in total gas production when level of DS increased in distillers grains in high concentrate diets *in vitro*. Lee et al. (2011) observed a decrease ($P \leq 0.001$) in total gas production for

glycerin and a 5:1 corn to glycerin blend compared to corn alone. In contrast, Avila et al. (2011) reported no differences in total gas production when glycerin was included at concentrations of 0 or 21% DM as a replacement for barley grain in finishing diets.

Inoculum pH and Volatile Fatty Acid Concentration

The C+G treatment had a greater ($P < 0.01$) inoculum pH after 48 h of incubation compared to the other treatments. Rico et al. (2012) reported no differences in pH when dried glycerin replaced dietary starch at 0, 3, 5, and 8% of the diet DM during a continuous culture experiment, which would agree with the current experiment. Metabolism trials conducted by Bremer et al. (2010) and Ham et al. (1994) reported that inclusion of DS in finishing diets lowered ruminal pH compared to DRC-based controls, this is contrary to the CDS treatment results in the current trial. The increase in inoculum pH for the C+G treatment may be explained by a decrease in total VFA concentration; however, the total VFA concentrations for the CON and C+G treatments were similar ($P = 0.94$) which is not readily explained. Including DS at 10% DMB increased ($P < 0.02$) total VFA compared to the CON or C+G treatments. The results for total VFA are not consistent with previous research. Ham et al. (1994) and Bremer et al. (2010) reported no differences for total VFA when DS was included compared to a DRC-based control. Concentrations of isobutyrate tended to increased ($P < 0.10$) for the CON and C+G treatments compared to the CDS and GLY treatments. Including DS or glycerin in the substrates decreased ($P < 0.08$) isovalerate compared to the control, whereas valerate concentrations were the greatest ($P < 0.04$) for the CON treatment compared to other treatments. Valerate increased for CON ($P < 0.04$) and decreased ($P < 0.02$) for the C+G treatment compared to CDS and GLY treatments.

In previous research, DS has consistently shown to decrease ruminal acetate when included in ruminant diets (Glibery et al., 2006; Sasikala-Appukuttan et al., 2008; Bremer et al., 2010). Glycerin fed to ruminant animals also has repeatedly shown to alter ruminal VFA profiles by decreasing acetate and increasing propionate (Rèmond et al., 1993; Wang et al., 2009; Krueger et al., 2010; Lee et al., 2011; Ramos and Kerley, 2012; Rico et al., 2012; Long et al., 2015). Concentrations of acetate, propionate, and butyrate did not differ ($P > 0.17$) among treatments in the current *in vitro* experiment. The donor steers did not have access to either byproduct prior to the *in vitro* experiment; therefore the microbes had no prior exposure to DS or glycerin. Rèmond et al. (1993) reported that diet type, or duration of adaptation of the microbes to glycerin did not affect the fermentation of glycerin. This can be explained by Hobson and Mann (1961), who determined that glycerin fermentation occurs rapidly by the bacteria *Selenomonas*, which is a common species of the rumen flora. Differences in VFA profiles of the current experiment and of those previously published may be explained by variation in experimental conditions, which can differ within *in vitro* trials and between *in vitro* and *in vivo* experiments.

Conclusion

The results from this experiment suggest that combining 5% DS and 5% glycerin (DMB) in SFC-based finishing diets may alter fermentation *in vitro* compared to the other treatments. A reduction in fermentation would explain the lower gas production values and total VFA concentrations of the C+G treatment, which are the end-products of ruminal fermentation. There is little data to compare to for combining DS and glycerin in feedlot cattle diets, and the *in vitro* results contrasted with previous research. Therefore,

further research is warranted to determine the effects of feeding DS in combination with glycerin on ruminal fermentation.

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Table 2.1 Effect of treatment on *in vitro* inoculum pH, dry matter disappearance (DMD), neutral detergent fiber disappearance (NDFD), ammonia concentration, gas production, and volatile fatty acid (VFA) profile after 48 h of incubation.

	Treatments[1]					
Item	**CON**	**CDS**	**GLY**	**C+G**	**SEM**	***P*-value[2]**
pH[3]	6.61[a]	6.63[a]	6.62[a]	6.67[b]	0.005	<0.01
DMD, %	69.60	71.69	69.82	68.99	1.43	0.43
NDFD, %	32.13[d]	28.17[e]	27.05[e]	27.88[e]	1.36	0.09
NH_3, mg/dL	18.80[a]	18.54[a]	17.54[b]	17.00[b]	0.32	<0.01
Total gas production, mL	174.8[a]	170.4[a]	152.9[a]	111.0[b]	10.35	<0.01
Total VFA, m*M*	94.98[a]	105.16[b]	100.64[a,b]	94.68[a]	3.68	0.05
VFA, mol/100 mol						
Acetate	50.88	52.53	51.77	51.66	0.49	0.19
Propionate	23.16	23.18	23.61	23.91	0.30	0.29
Butyrate	13.80	13.50	13.66	13.14	0.20	0.17
Valerate	2.75[a]	2.59[b]	2.63[b]	2.48[c]	0.05	<0.01
Isobutyrate	1.45[d]	0.87[e]	0.75[e]	1.41[d]	0.21	0.09
Isovalerate	7.89[a]	7.25[b]	7.51[a,b]	7.34[b]	0.16	0.04
A:P[4]	2.20	2.27	2.20	2.17	0.04	0.34

[1]Treatment substrates consisted of SFC-based finishing diets with no additional liquid byproducts (CON), 10% condensed distillers solubles (CDS), 10% glycerin (GLY), or a combination of 5% DS and 5% glycerin (C+G).
[2]Treatment means without a common superscript differ [a,b,c] ($P \leq 0.05$); Treatment means without a common superscript tend to differ [d,e] ($P \leq 0.10$).
[3]The pH of the inoculum was determined after 48 h of incubation.
[4]A:P = acetate to propionate ratio.

CHAPTER III

EFFECTS OF FEEDING CORN CONDENSED DISTILLERS SOLUBLES AND CRUDE GLYCERIN ALONE OR IN COMBINATION ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF FINISHING STEERS

Introduction

The production of biofuels like ethanol and biodiesel continue to increase in the U.S., leading to an increase in production of associated byproducts that can be used as animal feeds (U.S. Energy Information Administration, 2016; Stock et al., 2000). Glycerin is a byproduct of biodiesel production and is produced through catalyzed transesterification reactions of triglycerides with the use of methanol (Ma and Hanna, 1999). Feeding glycerin has shown to improve growth performance and feed efficiency in feedlot ruminants. Gunn et al. (2010) determined glycerin can be fed to finishing lambs up to 15% of diet DM to improve feedlot performance. Parsons et al. (2009) fed SFC-based finishing diets up to 16% glycerin (DMB) to finishing heifers and reported an increase in average daily gain (ADG) and feed conversion when glycerin was fed up to 8% of diet DM. Hales et al. (2013) reported similar results with steers fed glycerin up to 10% (DMB) in SFC-based growing diets, with increased final BW and ADG in cattle consuming 7.5% glycerin. Overall, a threshold of 8% glycerin inclusion was reported to be optimal for growing and finishing cattle.

Condensed distillers solubles (**DS**) are a liquid byproduct derived from the dry-grind ethanol process (Stock et al., 2000). The DS contains moderate amounts of crude protein (20 to 30%) and significant fat (9 to 25%; Lardy, 2007). Pesta et al. (2015)

observed a decrease in feed intake and an increase in daily gain and G:F when DS was included up to 36% (DM basis) in dry-rolled:high-moisture corn-based finishing diets. Titlow et al. (2013) also reported a decrease in DMI and an increase in ADG and G:F when cattle consumed DS at 15 or 30% (DMB) in finishing diets containing steam-flaked corn (SFC) or dry-rolled corn (DRC). Condensed distillers solubles can be fed in combination with other byproducts as well. Hughes et al. (2013) reported an increase in ADG and G:F when DS was fed at 7.5 or 15% (DMB) in combination with 15% wet distillers grains plus solubles (WDGS). Similarly, Pesta et al. (2015) reported including DS in diets at 20% (DMB) with modified distillers grains plus solubles (**MDGS**) or a blend of MDGS and wet corn gluten feed (**WCGF**) resulted in an increase in ADG and G:F. Limited research exists that explores the synergies of feeding multiple liquid byproducts in finishing diets. Given reported improvements in feedlot performance when fed with DGS and WCGF, we hypothesized that feeding DS and glycerin in combination may increase growth performance while maintaining carcass quality compared to feeding them separately. Therefore, the objective of this experiment was to determine the effects on cattle performance and carcass characteristics when feeding DS and glycerin alone or in combination to finishing beef steers.

Materials and Methods

All procedures involving live animals were approved by the West Texas A&M University-CREET Animal Care and Use Committee (approval # 01-04-14).

Animals

Two-hundred fifty crossbred beef steers were fed until harvest in two separate groups at the Texas A&M AgriLife/USDA-ARS Research Feedlot near Bushland, TX.

The first group of cattle (n = 124; initial BW = 327 ± 15 kg) were received on May 17, 2014 and allowed 24 h of rest before initial processing, in which steers were given an individual identification ear tag, vaccinated for virus and clostridial protection (Vista 5, Vision 7; Merck Animal Health, Summit, NJ), received an antiparasitic (Safeguard; Merck Animal Health), and were given a long-acting growth implant (Revalor-XS; 200 mg trenbolone acetate and 40 mg estradiol; Merck Animal Health). The second group (n = 126; arrival BW = 275 ± 10 kg) arrived on December 11, 2014 and following a 24 h rest period, cattle were tagged with an individual identification number and metaphlaxis was administered (Micotil; Elanco Animal Health, Greenfield, IN). Calves were vaccinated with a modified live virus vaccine (Pyramid 5 + Presponse; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), a clostridial-tetanus toxoid (Covexin 8; Merck Animal Health) and were given a parasiticide (Cydectin; Boehringer Ingelheim Vetmedica Inc.). Bulls were castrated with a restrictive rubber band (Callicrate Bander; No-Bull Enterprises LLC, St. Francis, KS). Calves in the second group were given a growth implant (Component E-S; 200 mg progesterone USP, 20 mg estradiol benzoate, and 29 mg tylosin tartrate; Elanco Animal Health) 14 d after arrival and were fed a growing diet for approximately 50 d prior to the experiment. Cattle in the second group were initially weighed at the beginning of the trial (initial BW = 317 ± 10 kg) and on d 30 of the experiment, were reimplanted with a long-acting implant (Revalor-XS; 200 mg trenbolone acetate and 40 mg estradiol; Merck Animal Health). The differences in processing methods of both groups were due to differences in cattle type and arrival BW. At trial initiation, cattle were individually weighed for two consecutive days and weights were averaged to determine the initial BW (average initial BW group 1 and 2 = 322 ± 15

kg). Steers were blocked by initial BW into one of four weight blocks (4 blocks per treatment). Within each block, animals were randomly assigned to treatment and pen (8 head per pen, 8 pens per treatment total). Cattle were housed in 6 × 28-m uncovered fly ash or soil surfaced feedlot pens that allowed 48 cm of bunk space per animal. Steers had *ad libitum* access to feed and clean water throughout the feeding study. For intermediate weights, cattle were weighed by pen (Digitol Truckmate; IND310; Mettler Toledo, Columbus, OH; readability ± 2.3 kg) prior to feeding in 30 d intervals until harvest to monitor weight gain and feed efficiency. Six animals were removed from study due to injury or death unrelated to treatment.

Treatments and Diets

Four dietary treatments were used in this randomized complete block design experiment. Treatment diets (Table 3.1) consisted of a SFC-based finishing diet with: a negative control containing no additional byproducts (**CON**), 10% condensed distillers solubles (**CDS**), 10% glycerin (**GLY**), and 5% DS and 5% glycerin (**C+G**). Adding DS or glycerin to the diet replaced a portion of SFC. Yellow grease and urea were adjusted to balance for CP, fat and energy content across treatments. Nutrient compositions of the treatment diets are represented in Table 3.2. The treatment byproducts were applied during the step-up phases as cattle were transitioned from a high forage starter diet to a high grain finishing diet, in which SFC replaced alfalfa hay.

Feed Delivery and Sampling

Cattle were fed their respective diet once daily at 0700 h. Bunks were scored 30 min prior to feeding and were adjusted so < 0.45 kg/hd DM remained in the bunk at the time of scoring. Diets were mixed in a stationary feed mixer (184-10B, Roto-Mix, Dodge

City, KS; EZ 3400, Digi-Star, Fort Atkinson, WI, readability ± 0.91 kg) and conveyed into a feed truck (354-12B, Roto-Mix; EZ 3200, Digi-Star, readability ± 2.3 kg) for delivery to pens. Individual feed ingredients were sampled weekly for DM determination. Weekly ingredient samples were composited by month and sent to a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX) for nutrient analysis. Diet samples were collected weekly from several locations within each bunk immediately after feeding and composited by treatment for DM analysis. If feed was refused, the orts were collected, weighed and analyzed for DM to subtract from daily DMI. Feed refusals were collected when excess feed or fines were present, or when feed was deemed soiled due to precipitation or animals defecating in the bunk. Feed remaining in the bunks on the morning of weigh days were also removed, weighed, and analyzed for DM to accurately calculate DMI for each 30-d weigh periods.

Carcass Evaluation

Cattle were determined to be ready for harvest when greater than 50% of the animals were visually estimated to grade USDA choice or higher. Steers were split by weight block (2 blocks per treatment) with heavy blocks shipping first (group 1, 148 DOF; group 2, 145 DOF) and lighter blocks shipping at a later date (group 1, 196 DOF; group 2, 179 DOF). Cattle were on feed for an average of 167 d. Animals were transported to a commercial abattoir (Tyson Fresh Meats, Amarillo, TX) for harvest. Carcass data were collected by trained personnel from the West Texas A&M University Beef Carcass Research Center. Hot carcass weight (HCW) and incidences of abscessed livers were determined on the day of harvest and longissimus muscle (LM) area, marbling score, subcutaneous fat thickness, and USDA quality and yield grades were

determined after a 48 h chill. Dressing percent (DP) was calculated by dividing HCW by the pen average final BW.

Statistical Analysis

No differences were detected between groups of cattle; therefore data from both groups were pooled for statistical analysis. Live animal performance and carcass characteristics were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Pen was the experimental unit and block was included in the random statement. Differences in least square means that resulted from the treatments were evaluated using the PDIFF option. Frequency distributions of carcasses within specific quality grade categories as well as abscessed livers were analyzed as binomial proportions using the GLIMMIX procedure of SAS (SAS Institute). The mean differences were considered significant at $P \leq 0.05$ and tendencies were declared at $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

Live Animal Performance

No treatment × period interactions were observed ($P > 0.82$) for live animal performance; therefore, only treatment means across the entire duration of the experiment will be discussed. Final BW, ADG, DMI, and G:F were not different ($P > 0.82$) across all treatments (Table 3.3). These data contrast with previous research where DS was fed alone in finishing diets and improvements in feedlot performance were observed compared to controls (Hughes et al., 2013; Titlow et al., 2013; Pesta et al., 2015). Pesta et al. (2015) reported an increase in final BW, ADG, and G:F and a decrease in DMI when DS replaced corn grain (1:1 blend of HMC and DRC) up to 36% (DMB). However, CP,

fat, and sulfur were not balanced across treatments as values of CP, fat and sulfur sequentially increased with increasing levels of DS, which may explain the differences in performance. Hughes et al. (2013) determined that including DS at 7.5 or 15% (DMB) in combination with 15% (DMB) wet distillers grain plus solubles (WDGS) increased ADG and G:F compared to a negative control. This study used SFC-based finishing diets balanced for CP and fat. In the current trial, feedlot performance remained similar between the DS treatment and the control. The inconsistent results between this study and previous research are not readily explained.

The effect on live animal performance of the GLY treatment is supported by the work of Parsons et al. (2009) where glycerin was included in SFC-based finishing diets up to 16% (DMB). The authors concluded that increased ADG and G:F plateaued at 8% glycerin inclusion. In the current trial, glycerin was included at 10% (DMB), which agrees with Parsons et al. (2009) when feeding glycerin above 8% of the diet, feedlot performance remains similar. The results also concur with Buttrey et al. (2015) in which no differences were reported for final BW, DMI, ADG, and G:F when glycerin was included up to 10% (DMB) in SFC-based diets containing supplemental fat provided by yellow grease.

The C+G treatment was similar to the other treatments for live animal performance; however, the reason for this is unclear. Glycerin was included at 5% of diet DM, which is below the 8% threshold described by Parsons et al. (2009). The fat content in the diet provided by DS, yellow grease and WDGS may have diminished the effects of feeding low levels glycerin on performance, as Parsons et al. (2009) did not provide supplemental fat in the diets used whereas Buttrey et al. (2015) included yellow grease.

The C+G treatment in the current trial contained 5% DS (DM basis). Little data is published that has investigated feeding DS at inclusion levels less than 7.5% (DM basis), so the low level of DS (5% DM basis) may not have been enough to impact performance.

Carcass Characteristics

Dietary treatment had no effect ($P > 0.33$) on carcass traits measured in this experiment (Table 3.4). These data do not agree with results from previous research feeding DS or glycerin independently in finishing diets. Titlow et al. (2013) reported a linear increase in HCW, fat thickness, and yield grade when DS was included in SFC-based diets at 15 or 30% inclusion (DM basis) compared to the control. Hughes et al. (2013) reported an increase in HCW and fat thickness when DS was included at 7.5 and 15% (DM basis) in SFC-based diets compared to the control. Parsons et al. (2009) determined that including glycerin in SFC finishing diets decreased marbling score and subcutaneous fat thickness compared to a control. Buttrey et al. (2015) also reported a decrease in fat thickness when level of glycerin increased compared to the control, which resulted in a linear decrease in calculated yield grade for cattle consuming the glycerin treatments. It is unknown as to why the CDS and GLY treatments in the current study had similar carcass characteristics which contrasts previous research. Numerically the CDS treatment had the greatest subcutaneous fat thickness and the GLY treatment had the least (1.35 vs. 1.20 cm), which follows previous research.

Conclusion

There is limited data available investigating the effects of combining byproducts from both ethanol and biodiesel production. It is known that including DS and low levels of glycerin in feedlot cattle diets alone can improve animal performance without negatively effecting carcass characteristics. We hypothesized similar results from feeding

a combination of the two byproducts; however, results from this trial suggest that there is no additional performance benefit from including both DS and glycerin at 5% diet DM. Generally, byproducts can be purchased at a cheaper price than corn, so it may be possible to replace SFC with these byproducts without negatively effecting feedlot performance or carcass traits to reduce feed costs. The limitations of this would be the ability of the feedlots to handle liquid feed ingredients and the availability of DS, as it is often added back to the solid fraction to produce distillers grains plus solubles.

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Table 3.1 Ingredient composition of treatment diets.

	Dietary treatments[1]			
Item, % DM basis	**CON**	**CDS**	**GLY**	**C+G**
Steam-flaked corn	69.35	60.60	58.85	59.75
Corn wet distillers grains plus solubles	15.00	15.00	15.00	15.00
Ground corn stalks	8.50	8.50	8.50	8.50
Yellow grease	2.00	0.95	2.40	1.70
Corn condensed distillers solubles	-	10.00	-	5.00
Glycerin	-	-	10.00	5.00
Supplement[2]	3.00	3.00	3.00	3.00
Limestone	1.15	1.15	1.15	1.15
Urea	1.00	0.80	1.10	0.90

[1]Dietary treatments consisted of a SFC-based finishing diet with: no additional liquid byproducts (CON), 10% DS (CDS), 10% glycerin (GLY), or 5% DS and 5% glycerin (C+G).

[2]Formulated to meet or exceed the vitamin and mineral recommendations by the NRC (2000) and provide 26.7 mg/kg of monensin (Rumensin, Elanco Animal Health) and 6.0 mg/kg of tylosin (Tylan, Elanco Animal Health). Formulated for label use, intakes were lower than expected.

Table 3.2 Nutrient composition of treatment diets.

	Dietary treatments			
Nutrient values[2]	**CON**	**CDS**	**GLY**	**C+G**
DM, %	69.56	62.28	68.11	65.22
CP, %	14.30	14.40	13.90	14.00
Ether extract, %	6.02	6.14	6.15	6.17
NDF, %	15.29	15.81	16.80	15.98
Ca, %	0.49	0.52	0.49	0.51
P, %	0.30	0.42	0.29	0.35
S, %	0.17	0.21	0.18	0.20
Na, %	0.15	0.26	0.36	0.31
NEm, Mcal/kg	2.21	2.16	1.96	2.07
NEg, Mcal/kg	1.32	1.30	1.17	1.23

[1]Dietary treatments consisted of a SFC-based finishing diet with: no additional liquid byproducts (CON), 10% DS (CDS), 10% glycerin (GLY), or 5% DS and 5% glycerin (C+G).
[2]Values based off proximate analysis of individual ingredients.

Table 3.3 Effect of treatments on feedlot performance.

	Dietary treatments					
Item	**CON**	**CDS**	**GLY**	**C+G**	**SEM**	***P*-value**
Initial shrunk BW, kg[1]	322	322	322	322	12.96	1.00
Final shrunk BW, kg[1]	600	603	604	604	7.62	0.91
Period DMI, kg/d						
d 0 to 29	8.02	8.02	8.05	7.78	0.24	0.99
d 30 to 59	8.92	9.15	9.34	9.14	0.36	0.98
d 60 to 89	10.00	10.20	10.04	10.01	0.36	0.98
d 90 to 119	10.39	10.35	10.18	9.86	0.36	0.91
d 120 to 149	10.39	10.14	10.35	10.02	0.36	0.91
d 0 to end[2]	9.80	9.89	9.87	9.90	0.26	0.94
Period ADG, kg/d						
d 0 to 29	1.75	1.81	1.71	1.76	0.15	0.92
d 30 to 59	1.98	2.05	2.27	2.16	0.15	0.61
d 60 to 89	1.74	1.74	1.70	1.71	0.15	0.98
d 90 to 119	1.85	1.89	1.84	1.79	0.15	0.92
d 120 to 149	1.60	1.48	1.64	1.53	0.15	0.81
d 0 to end[2]	1.69	1.69	1.69	1.72	0.05	0.94
Period G:F, kg/kg						
d 0 to 29	0.219	0.226	0.209	0.223	0.01	0.83
d 30 to 59	0.223	0.225	0.244	0.238	0.002	0.87
d 60 to 89	0.202	0.199	0.195	0.199	0.01	0.96
d 90 to 119	0.179	0.184	0.182	0.183	0.01	0.91
d 120 to 149	0.154	0.146	0.159	0.153	0.01	0.90
d 0 to end[2]	0.179	0.179	0.178	0.183	0.004	0.82

[1]A 4% shrink was applied to BW.

[2]Average days on feed from d 0 to end (slaughter) were 167.

Table 3.4 Effects of treatments on carcass characteristics.

	Dietary treatments					
Item	**CON**	**CDS**	**GLY**	**C+G**	**SEM**	***P*-value**
HCW, kg	386	387	389	386	5.80	0.94
DP[1]	64.36	64.12	64.37	63.95	0.40	0.52
Marbling score[2]	401	422	419	412	11.41	0.51
Subcutaneous fat, cm	1.24	1.35	1.20	1.22	0.06	0.33
LM area, cm^2	89.66	92.33	91.56	90.46	2.29	0.85
Calculated YG	2.90	2.89	2.80	2.83	0.16	0.97
Choice or greater, %[3]	53.97	64.52	65.00	53.23	0.38	0.40
Abscessed livers, %	6.35	9.38	10.00	9.52	0.67	0.89

[1]DP = dressing percent; calculated by dividing HCW from final shrunk BW.
[2]Marbling score: 400 = Small 00.
[3]Includes carcasses that graded USDA choice, premium choice and prime.

APPENDIX I

EFFECTS OF ROUGHAGE INCLUSION AND PARTICLE SIZE ON DIGESTION AND RUMINAL FERMENTATION CHARACTERISTICS OF BEEF STEERS

Introduction

Roughages are commonly fed in ruminant diets to maintain rumen health; however, they are included at lower levels in finishing diets due to lower energy values and digestibility characteristics (Mertens, 1997; Allen, 1997). Finishing animals receive higher energy diets for growth efficiency; therefore it is important to understand minimum roughage inclusion without negatively affecting rumen function. Previous research has shown that increasing dietary roughage in feedlot diets decreases dry matter digestibility (Hales et al., 2014; Benton et al., 2015). Although roughage may depress digestibility and dilute the energy content of the diet, roughage is still included in finishing diets to aid in ruminal buffering and prevention of digestive upset (Allen, 1997).

Mertens (1997) described physically effective NDF (**peNDF**) as the roughage's ability to stimulate rumination. Therefore, increasing the physical effectiveness of the roughage source can aid in maintaining a higher ruminal pH by stimulating salivary buffer secretions via chewing activity (Allen, 1997). Approximately one-half of the bicarbonate that enters the rumen comes from saliva during eating and ruminating (Owens, 1998), therefore rumination time should increase the rumen buffering capacity. Fiber can vary in its effectiveness in stimulating rumination, primarily due to differences in coarseness, digestibility and particle size (Allen, 1997). Yang and Beauchemin (2006) reported an increase in rumination time with monitoring halters as larger particles of corn

silage was fed to dairy cows. Likewise, Park et al. (2015) reported an increase in visual chewing activity as dietary peNDF increased in beef cattle diets.

Overall, the effects of roughage inclusion and particle length on digestion and ruminal fermentation have been more thoroughly researched in dairy cattle compared to beef. Limited data exists that investigates whether a higher roughage inclusion can be replaced by a lower roughage inclusion with a larger particle size in finishing diets. Further research is needed to measure these effects on rumination and ruminal fermentation characteristics of finishing cattle. Therefore, the objectives of this experiment were to: 1) determine the effects of roughage inclusion and grind size in finishing diets on digestibility characteristics, rumination activity, and ruminal fermentation characteristics of beef steers and 2) determine if roughage of a larger particle size may be included at a lower level compared to a higher level with a smaller particle size in relation to peNDF and rumination behavior.

Materials and Methods

All procedures involving live animals were approved by the West Texas A&M University/CREET Institutional Animal Care and Use Committee (approval # 01-08-15).

Animals and Treatments

Four ruminally cannulated beef steers (initial BW = 631 ± 19 kg) were used in a 4 × 4 Latin square experiment. Each experimental period lasted 18-d, with 14-d for adaptation and 4-d for sample collection. Animals were fed SFC-based finishing diets (Table A.1) once daily at 0700-h. Dietary treatments consisted of roughage inclusion and grind size that were arranged as a 2 × 2 factorial. Roughage inclusion treatments were corn stalks fed at 5 or 10% of diet DM. The grind size treatments were corn stalks that were ground through a commercial tub grinder equipped with a 7.62 cm screen once

(long-grind; **LG**) or twice (short-grind, **SG**). The treatment diets are describes as: 5% SG corn stalks (**5SG**), 5% LG corn stalks (**5LG**), 10% SG corn stalks (**10SG**) or 10% LG corn stalks (**10LG**). Steers were housed individually in 2 × 18 m partially covered outdoor pens throughout the study. Animals were weighed at the beginning and end of each 18 d period. Each animal was fitted with a collar (HR Tag; SCR Dairy, Madison, WI) that measured rumination minutes continuously via a sensory microphone that detected the passage of a feed bolus. One animal was removed from the third period due to cannula issues unrelated to treatment.

Sampling

Diet samples and orts were collected on d 14 through 18. Diet samples and feed refusals were collected, weighed, and subsampled for nutrient analysis. Fecal output was estimated by dosing a 5-g bolus of chromic oxide twice daily (0700 and 1900 h) via the rumen cannula on d 10 through 18. Fecal samples were collected at 0600 and 1800 h on d 15 and 17, and at 1200 and 2400 h on d 16 and 18. Fecal samples were wet composited across the entire collection period by animal. Three 250-mL aliquots were prepared from the wet composite, and frozen at -4°C. Ruminal fluid samples also were collected on the same schedule, and strained through 4 layers of cheese cloth. Sample pH was immediately measured using a portable pH meter (VWR symphony, model H10P, Radnor, PA) and three 50-mL aliquots were retained and frozen at -4°C. Sampling was conducted in this manner so that the rumen cannula was only opened twice daily, with twelve hours between, to reduce the amount of oxygen that entered the rumen environment, and so that the rumen environment may stabilize between each sampling time-point. Diet samples were collected weekly and separated using the Penn State

Particle Separator (**PSPS**) as described by Heinrichs and Kononoff (2002) by wet-sieving. Physically effective NDF was estimated by multiplying the quantity (as a percentage of the total sample) of sample larger than 1.18 mm in particle size by the NDF content of that sample as described by Mertens (1997).

Laboratory Analysis

Diet and ort samples were dried at 55°C for 48 h and fecal aliquots were lyophilized (Labconco, Kansas City, MO). Diet, ort, and fecal samples were ground using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen, and a subsample of this was ground through a Cyclotec mill (Cyclotec CT 193, Foss, Hoganas, Sweden) to pass through a 0.5-mm screen for the starch analysis. Laboratory DM of diet, ort, and fecal samples were determined by drying at 100°C for 24 h and organic matter (OM) was determined by ashing samples at 500°C for 6 h. Ether extract (EE) of diet and orts were determined using petroleum ether in an automated EE extraction system (Ankom XT15 Extraction System, Ankom Technology, Fairport, NY). Because the EE content was greater than 5%, diet and ort samples were submerged in acetone twice for 10 min before concentration of NDF and ADF were determined using an Ankom fiber analyzer (Model 200/220, Ankom Technology). Total nitrogen of samples were analyzed at a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX). Starch content was determined using a PowerWave-XS Spectrometer (Bio Tek US, Winooski, VT) after converting starch to glucose with an enzyme kit (Megazyme International Ireland Ltd., Wicklow Ireland). Chromium concentrations of feces were determined by atomic absorption (AAAnalyst 200, PerkinElmer, Inc., Waltham, MA) and fecal output (g/d) was calculated by dividing the amount of chromium dosed by marker

concentration in the feces as described by Merchen (1988). Volatile fatty acid concentrations of rumen fluid samples were determined using a Varian 3900 GC (Varian Inc., Palo Alto, CA) according to the procedures of Erwin et al. (1961). Ruminal fluid samples were also analyzed for ammonia concentration using procedures outlined by Broderick and Kang (1980) and quantified using a PowerWave-XS Spectrometer (Bio Tek US) at a wavelength of 550 nm.

Statistical Analysis

Data were analyzed as a Latin square with a factorial arrangement of treatments using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). The model included fixed effects of roughage grind, inclusion and grind × inclusion. For VFA, NH_3, and ruminal pH, the model statement also included the effect of time, grind × time, inclusion × time, and grind × inclusion × time. Animal was included as a random term and the LSMEANS statement was used to separate treatment means. Significance was declared at $P \leq 0.05$ and trends at $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

Particle Separation

Particle separation and peNDF of treatment diets are represented in Table A.2. An effect of both grind size and inclusion was observed for all particle sieves. A grind × inclusion interaction ($P = 0.04$) was observed for particles that were retained on the 7.87 mm sieve. The 10LG treatment had a greater ($P < 0.01$) percentage of particles retained on the 7.87 mm sieve compared to other treatments. The 10% inclusion treatments had an increased ($P < 0.01$) percentage of particles retained in the top three sieves and a

decreased ($P < 0.01$) percentage of particles less than 1.18 mm compared to the 5% inclusion treatments.

A grind × inclusion interaction ($P = 0.04$) was observed for estimated physically effective neutral detergent fiber (peNDF). The 10LG treatment had a greater ($P < 0.01$) percentage of estimated peNDF compared to other treatments, while the 5SG treatment had the least ($P < 0.02$). The 10SG treatment was intermediate with the 5LG treatment having similar values to the 5SG and 10SG treatments. The difference in peNDF for the 10LG treatment was due to the increase of particles retained in the top 3 sieves, and a higher NDF content than the other diets. These data suggest that a lower inclusion of roughage with a larger particle size provides similar peNDF to that of a higher inclusion of roughage with a smaller particle size.

Nutrient Intake and Digestibility

Roughage inclusion and grind size had no effect ($P > 0.16$; Table A.3) on DM, OM, starch, or nitrogen intake. Regardless of particle size, diets including 10% corn stalks tended to increase ($P = 0.07$) NDF intake and increased ($P = 0.01$) ADF intake compared to 5% corn stalks. Increasing concentrations of roughages in finishing diets typically increase DMI of feedlot cattle, possibly due to the roughage diluting higher energy feeds (Galyean and Defoor, 2003). In the current trial, the similarities in DMI may be attributed to the replacement of roughage with WCGF, a high-fiber byproduct feed compared to using SFC. Differences in NDF and ADF intake were expected between the 5% and 10% roughage diets as the fiber content in the corn stalks were greater than the WCGF that it replaced.

Including roughage at 10% of diet DM increased ($P \leq 0.05$) fecal output of NDF, ADF, and starch, and tended to increase ($P = 0.06$) fecal output of DM. An effect of grind size was also observed for ADF fecal output, as feeding SG corn stalks tended to increase ($P = 0.08$) fecal output of ADF compared to the LG treatments. Diets containing 5% roughage tended to increase ($P < 0.09$) DM and NDF total tract digestibility. These results agree with Hales et al. (2014) who reported DM digestibility decreased linearly with increasing levels of alfalfa up to 14% of diet DM in DRC-based finishing diets, as well as a tendency for a quadratic decrease in NDF digestibility as roughage increased. Likewise, Benton et al. (2015) reported a decrease in DM and NDF digestibility with increasing inclusions of roughage in finishing diets containing a DRC:HMC blend. In the current trial, feeding roughage at 5% of diet DM tended to increase ($P = 0.09$) starch apparent total tract digestion. This does not agree with that of Kreikemeier et at. (1990), who reported that a higher inclusion (5 vs 15% DMB) of alfalfa tended to increase *in situ* starch digestion of steam-rolled wheat. The authors speculate that starch digestion relative to forage inclusion is dependent on an increase in microbial growth and turnover, as well as passage rate. Differences in starch digestion reported by Kreikemeier et at. (1990) and the current trial may be due to grain type, roughage quality, or post-ruminal starch digestion.

Rumination and Fermentation Characteristics

Diets containing LG corn stalks increased ($P < 0.01$; Table A.4) rumination time compared to diets containing the SG corn stalks. Furthermore, diets containing 10% inclusion of roughage also had increased ($P < 0.01$) rumination time compared to the 5% inclusion treatments. These data concur with Yang and Beauchemin (2006) who reported

a linear increase in rumination time (min/d) with increasing peNDF by increasing chop length of corn silage fed to dairy cows. Park et al. (2015) also reported an increase in total number of chews in relation to increasing peNDF content of the diets. Given that the peNDF content measured in the experimental diets of the current trial increased with roughage inclusion and grind size, we expected to see an increase in rumination time relative to peNDF, as peNDF of a feed is the physical properties of fiber that stimulate chewing activity, as described by Mertens (2002).

Rumen pH increased ($P = 0.02$) for cattle fed the LG corn stalks when compared to the SG corn stalks. Shain et al. (1999) reported no differences in rumen pH for finishing steers consuming different grind sizes of either alfalfa or wheat straw as a roughage source. Likewise, Yang and Beauchemin (2006) reported no differences in ruminal pH of dairy cows consuming corn silage with different chop lengths. The similarity in pH between treatments in both trials could be due to roughage digestibility. The differences in ruminal pH between grind sizes in the current trial may be related to increasing physically effective NDF in the diet that maintained pH by stimulating salivary buffer secretion via rumination activity (Allen, 1997). Inclusion rate of roughage also increased ($P < 0.01$) rumen pH for cattle consuming 10% roughage compared to the 5% roughage treatments. Sindt et al. (2003) reported a linear increase in ruminal pH as roughage level increased from 0 to 6% (DMB) in SFC-based diets. The increase in ruminal pH relative to roughage inclusion may be due to increased chewing activity, or by a reduction in VFA concentration in the rumen. Rumination minutes and pH remained similar over time across treatments (Figure A.1). Rumination minutes and ruminal pH peaked at similar times relative to feeding. As rumination increased, pH increased,

suggesting that chewing activity aids in buffering of the rumen. All treatments went below a pH 5.6, the benchmark for subacute acidosis as described by Owens (1998), for a similar period of time.

An inclusion effect on ruminal NH_3 was observed in which diets containing 10% roughage had increased ($P = 0.04$) ruminal NH_3 compared to the 5% roughage treatments. However, Sindt et al. (2003) reported a quadratic effect of ruminal NH_3, which increased for 2% alfalfa, and plateaued at 6% alfalfa inclusion. Since NH_3 is the most common base found in the rumen, the increase in NH_3 for the 10% inclusion diets may partially explain the increase in rumen pH and the decrease in total VFA concentration. Higher ruminal ammonia concentrations could have come from higher inclusions of urea in the 10% inclusion diets as described by Owens (1998), which could increase ruminal pH values as well.

Including higher roughage amounts in ruminant diets often results in a VFA profile shift to acetate from propionate. Total VFA increased ($P < 0.01$) for the 5% roughage diets compared to the 10% roughage diets. Proportions of acetate increased ($P < 0.01$) and propionate decreased ($P < 0.01$) for diets containing 10% roughage compared to the 5% inclusion treatments. These values resulted in an increased ($P < 0.01$) A:P for the 10% inclusion treatments compared to the 5% roughage diets. Likewise, Sindt et al. (2003) reported a linear increase in A:P with increasing levels of alfalfa hay fed to finishing cattle. In the current study, the LG diets also had increased ($P = 0.02$) acetate compared to the SG treatments. The A:P decreased ($P = 0.01$) for the SG diets compared to the LG diets. In contrast, Shain et al. (1999) observed an increase in acetate for a shorter particle size of alfalfa compared to a longer particle size (2.54 vs 12.7 mm) but

reported no differences in acetate for similar particle sizes of wheat straw. The authors speculate that animals consuming wheat straw ruminated more, which suggest that roughage quality may have effected VFA concentration and particle size did not. A grind × inclusion interaction ($P < 0.01$) was observed for valerate, in which the 10LG treatment had decreased ($P < 0.01$) valerate compared to other treatments. An effect of inclusion × time ($P < 0.02$) was observed for both propionate and A:P. Propionate increased after feeding and were maintained until 17-h post feeding in cattle consuming the 5% inclusion diets, whereas propionate concentrations began to decline after 11-h post feeding for cattle consuming 10% roughage. These data suggest an increase in rumination time for higher inclusions of roughage may increase salivary buffer flow and make ruminal conditions more favorable for fiber digestion, as described by Allen (1997).

Conclusion

The results from this experiment suggest that the 5LG diet had similar peNDF values to that of the 10SG diet. Cattle consuming the LG corn stalks ruminated longer per day than cattle consuming the SG corn stalks, suggesting that particle length increases rumination time regardless of inclusion rate. Feeding roughages with a larger particle size may improve rumen buffering capacity, as we observed an increase in pH by including roughage of a larger particle size. We also observed an increase in DM, NDF, and starch digestion with including roughage at 5% of the diet DM compared to an inclusion of 10%, suggesting a lower inclusion of roughage is optimal for diet digestibility even with a larger particle size. Likewise, VFA profiles were more energetically favorable with 5% inclusion of roughage compared to the 10% inclusion, as propionate increased for in 5% roughage diets. These data suggest that it may be optimal from an energy and

digestibility standpoint to include roughage in finishing diets at 5% DMB, but by feeding a larger particle size, the appropriate amount of peNDF and rumination time may be maintained for cattle consuming finishing diets.

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Table A.1 Ingredient and nutrient composition of treatment diets.

	Dietary treatments[1]			
Item, % DMB	**5SG**	**10SG**	**5LG**	**10LG**
Steam-flaked corn	54.40	57.70	54.58	54.71
Wet corn gluten feed	29.95	23.38	30.05	25.21
Short-grind corn stalks[2]	5.10	9.35	-	-
Long-grind corn stalks[2]	-	-	5.05	9.88
Supplement[3]	3.97	3.66	3.99	3.98
Urea	0.51	0.74	0.51	0.78
Limestone	2.53	1.77	2.26	1.80
Corn oil	3.55	3.40	3.56	3.63
Analyzed composition, % DMB				
DM	79.8	81.3	79.8	81.4
OM	93.7	93.5	94.3	93.9
CP	13.6	13.2	13.4	13.5
NDF	19.6	20.5	20.4	21.4
ADF	9.3	10.6	9.1	10.3
Ether extract	5.9	5.7	6.0	6.3

[1]Dietary treatments: 5SG = 5% inclusion of short-grind corn stalks, 10SG = 10% inclusion of short-grind corn stalks, 5LG = 5% inclusion of long-grind corn stalks, 10LG = 10% inclusion of long-grind corn stalks.
[2]Short grind corn stalks = passed through a commercial tub grinder twice, long grind corn stalks = passed through a commercial tub grinder once; Tub grinder was equipped with a 7.6 cm screen.
[3]Supplement was formulated to meet or exceed vitamin and mineral requirements established by the NRC, 2000 and provided 35.6 mg/kg of Monensin (Rumensin, Elanco Animal Health, Greenfield, IN) and 7.9 mg/kg of Tylosin (Tylan, Elanco Animal Health).

Table A.2 Particle separation and estimated physically effective neutral detergent fiber (peNDF) of dietary treatments.

	Dietary Treatments[1]					*P*-value[2]		
Item	**5SG**	**10SG**	**5LG**	**10LG**	**SEM**	**Grind**	**Inclusion**	**G × I[3]**
Sieve screen size, mm	---------- Retained/screen, % ----------							
19.05	2.1	3.6	3.3	4.2	0.40	0.02	<0.01	0.39
7.87	41.6[a]	43.1[a]	41.3[a]	49.4[b]	1.56	0.07	<0.01	0.04
1.18	21.5	20.3	21.0	18.5	0.50	0.02	<0.01	0.18
Particles less than 1.18	34.7	33.0	34.5	28.0	1.31	0.06	<0.01	0.08
Particles greater than 1.18	65.3	67.0	65.5	72.0	1.31	0.06	<0.01	0.08
Estimated peNDF, % DM[4]	12.8[a]	13.8[b]	13.3[ab]	15.4[c]	0.27	<0.01	<0.01	0.04

[1]Dietary treatments: 5SG = 5% inclusion of short-grind corn stalks, 10SG = 10% inclusion of short-grind corn stalks, 5LG = 5% inclusion of long-grind corn stalks, 10LG = 10% inclusion of long-grind corn stalks.
[2]Means without a common superscript differ [a,b,c] ($P \leq 0.05$).
[3]G × I = Grind × Inclusion.
[4]Calculated by multiplying the particles greater than 1.18 mm (as a percent of the total sample) by the NDF content of the sample.

Table A.3 Effect of roughage grind size and inclusion on intake, fecal output, and apparent total tract digestibility of finishing beef steers.

	Dietary treatments[1]					*P*-value		
Item	**5SG**	**10SG**	**5LG**	**10LG**	**SEM**	**Grind**	**Inclusion**	**G × I[2]**
No. of observations	4	4	3	4	-	-	-	-
Intake, kg/d								
DM	11.1	11.5	10.7	11.0	0.40	0.16	0.23	0.99
OM	10.4	10.7	10.1	10.4	0.37	0.22	0.28	0.98
NDF	2.2	2.4	2.2	2.4	0.10	0.92	0.07	0.99
ADF	1.0	1.2	0.9	1.1	0.07	0.20	0.01	0.95
Starch	5.3	5.5	4.9	5.2	0.26	0.18	0.35	0.86
Nitrogen, g/d	241.7	242.6	227.6	238.9	9.56	0.21	0.38	0.44
Fecal output, kg/d								
DM	1.6	2.0	1.5	1.7	0.15	0.18	0.06	0.27
OM	1.1	1.2	1.2	1.3	0.13	0.66	0.11	0.34
NDF	0.6	0.9	0.6	0.8	0.10	0.39	0.04	0.32
ADF	0.5	0.7	0.4	0.5	0.07	0.08	0.03	0.30
Starch	0.03	0.06	0.04	0.05	0.009	0.89	0.05	0.23
Nitrogen, g/d	41.3	46.3	40.4	41.6	3.35	0.34	0.30	0.52
Apparent TT digestibility, %[3]								
DM	85.7	82.5	85.6	84.9	1.28	0.32	0.09	0.26
OM	89.2	86.9	88.3	87.8	0.97	0.99	0.11	0.25
NDF	71.1	61.3	71.1	68.5	3.79	0.28	0.08	0.28
ADF	55.8	41.8	58.4	56.6	7.40	0.20	0.24	0.36
Starch	99.4	98.9	99.1	99.1	0.18	0.73	0.09	0.22
Nitrogen	82.9	82.3	80.9	82.6	1.14	0.59	0.38	0.25

[1]Dietary treatments: 5SG = 5% inclusion of short-grind corn stalks, 10SG = 10% inclusion of short-grind corn stalks, 5LG = 5% inclusion of long-grind corn stalks, 10LG = 10% inclusion of long-grind corn stalks.
[2]G × I = Grind × Inclusion.
[3]TT = total tract.

Table A.4 Effect of roughage grind size and inclusion on rumination and ruminal fermentation of finishing beef steers.

	Dietary treatments[1]					*P*-value[2]		
Item	**5SG**	**10SG**	**5LG**	**10LG**	**SEM**	**Grind**	**Inclusion**	**G × I[3]**
No. of observations	4	4	3	4	-	-	-	-
Rumination time, min/d	225	265	283	313	26.20	<0.01	<0.01	0.66
Ruminal pH	5.65	5.83	5.72	5.92	0.06	0.02	<0.01	0.83
NH_3, mg/dL	5.4	6.7	4.8	6.0	1.25	0.29	0.04	0.89
Total VFA, m*M*	101.8	94.4	103.0	88.6	6.07	0.46	<0.01	0.25
VFA, mol/100 mol								
Acetate	40.0	46.9	41.2	49.2	2.07	0.02	<0.01	0.48
Propionate[4]	45.2	38.9	44.1	38.3	2.02	0.16	<0.01	0.75
Butyrate	11.5	10.4	11.2	10.0	1.18	0.63	0.12	0.97
Valerate	3.4[a]	3.7[a]	3.4[a]	2.5[b]	0.83	<0.01	0.19	<0.01
A:P[4]	0.9	1.2	1.0	1.4	1.37	0.01	<0.01	0.39

[1]Dietary treatments: 5SG = 5% inclusion of short-grind corn stalks, 10SG = 10% inclusion of short-grind corn stalks, 5LG = 5% inclusion of long-grind corn stalks, 10LG = 10% inclusion of long-grind corn stalks.
[2]Means without a common superscript differ [a,b,c] ($P \leq 0.05$).
[3]G × I = Grind × Inclusion.
[4]Effect of inclusion × time ($P \leq 0.05$).

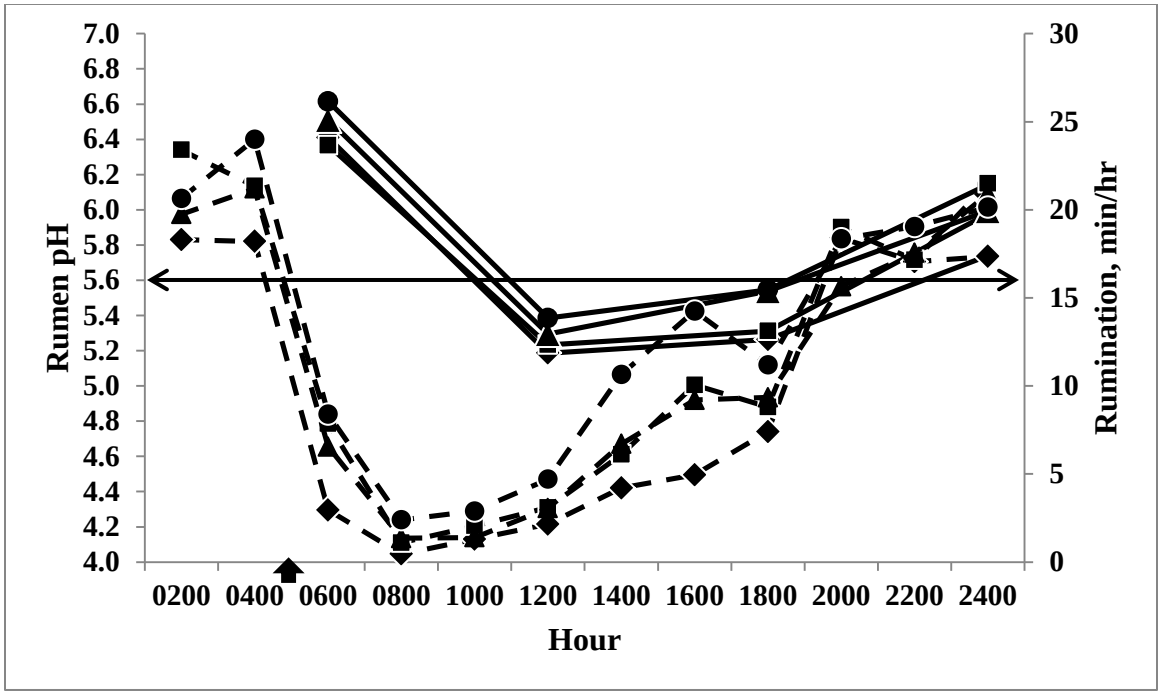

Figure A.1 Effect of roughage grind size and inclusion (5SG, ♦; 5LG, ■; 10SG, ▲; 10LG, ●) on ruminal pH (solid lines) and rumination (dashed lines). Also included is feeding time (↑) and the benchmark for subacute acidosis (↔) as described by Owens (1998).